Isolation and Characterization of Latex from Russian Dandelion

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Abbreviations

AA	Ammonium alginate
BAC	Benzalkonium chloride
CA	Creaming agent
CMC	Critical micellar concentration
DF	Dilution factor
DLVO	Derjaguin-Landau-Verwey-Overbeek
DLS	Dynamic light scattering
ETA	Ethanolamine
ETA-HCl	Ethanolamine-hydrochloride
FE	Flow extraction
HB	*Hevea brasiliensis*
HNRL	High-ammonia natural rubber latex
IUIS	International Union of Immunological Societies
JE	Juice extraction
ME	Mill extraction
MW	Molecular weight
ME	Mono-exponential
MM	Monomodal
NR	Natural rubber
NRL	Natural rubber latex
PTA	Phosphotungstic acid
PDI	Polydispersity index
RE	Raw extract
SN	Sample number
SAL	Sodium alginate
DOC	Sodium deoxycholate
SDS	Sodium dodecyl sulfate
SC	Solids content
SR	Synthetic rubber
TKS	*Taraxacum kok-saghyz*
TCA	Trichloroacetic acid
ZP	Zeta-potential

Abstract

Within this work, natural rubber latex (NRL) was isolated from roots of Russian dandelion and characterized. A process starting from the root to the characterized latex concentrate was described by adapting established concepts for colloidal systems, plant extraction and ordinary latex production. An understanding of the appropriate handling and preparation of the roots prior to extraction was obtained. Maintaining an intact root surface during harvest, followed by moist storage and thorough cleaning was crucial for a successful latex extraction. Different approaches to latex extraction were investigated. A weaker root shredding led to extracts with better processing properties, but significant latex losses during extraction could not be prevented by stronger shredding either. The flow method provided the most suitable extracts in terms of latex quality and processing effort. An adapted, enlarged extraction sequence could be designed and applied. Concentration and purification of raw latex was achieved by a step-by-step process. The application of repeated sequences of creaming, centrifugation and redilution resulted in protocols to produce latex with a solids content (SC) of over 60%. Correlations between solids content, contamination, stability and their effects on latex processing were established in order to develop concepts for dealing with different types of extract or raw latex. During extraction, concentration, purification and storage, latex stabilization was achieved with solutions containing ethanolamine/ethanolamine-hydrochloride (ETA/ETA-HCl), oleates, sulfites and benzalkonium chloride (BAC). Latex could be processed into rubber foam by Synthomer Deutschland GmbH. Further colloidal properties could be determined and related to latex from other rubber-bearing plants, the respective isolation approach and the resulting handling and processing properties. Dynamic light scattering (DLS) showed a bimodal (BM) particle size distribution (PSD) with a weakly pronounced smaller mode and particle sizes increasing with the extent of the purification. Zeta-potential (ZP) measurements confirmed the stability of differently obtained latex concentrates after storage, in the presence of electrolytes and after redilution. In addition, the protein concentration could be significantly reduced.

Zusammenfassung

Im Rahmen dieser Arbeit wurde Naturkautschuklatex (NRL) aus Wurzeln von russischem Löwenzahn isoliert und charakterisiert. Dabei wurde ein Prozess ausgehend von der Wurzel hin zum charakterisierten Latex durch Anpassung von Konzepten für kolloidale Systeme, Pflanzenextraktion und gewöhnliche Latexgewinnung beschrieben. Es wurde ein Verständnis für die geeignete Handhabung und Vorbereitung der Wurzeln vor der Extraktion erhalten. Die Erhaltung einer intakten Wurzeloberfläche während der Ernte, gefolgt von feuchter Lagerung und gründlicher Reinigung war entscheidend für eine erfolgreiche Latexextraktion. Es wurden verschiedene Ansätze zur Latexextraktion untersucht. Ein geringerer Zerkleinerungsgrad der Wurzeln führte zu Extrakten mit besseren Verarbeitungseigenschaften, aber signifikante Latexverluste während der Extraktion konnten auch durch ein stärkeres Zerkleinern nicht verhindert werden. Die Flussmethode lieferte die hinsichtlich Latexqualität und Verarbeitungsaufwand am besten geeigneten Extrakte. Dabei konnte eine angepasste, vergrößerte Extraktionssequenz entworfen und angewendet werden. Die Konzentration und Aufreinigung des Rohlatex wurde schrittweise erreicht. Die Anwendung wiederholter Sequenzen von Aufrahmen, Zentrifugieren und Verdünnen führte zu Protokollen zur Herstellung von Latex mit einem Feststoffgehalt (SC) von über 60%. Korrelationen zwischen Feststoffgehalt, Verunreinigungen, Stabilität und deren Auswirkungen auf die Latexverarbeitung wurden hergestellt, um Konzepte für den Umgang mit verschiedenen Arten von Rohlatex zu entwickeln. Während der Extraktion, Konzentration, Aufreinigung und Lagerung konnte der Latex mit Lösungen basierend auf Ethanolamin/Ethanolaminhydrochlorid (ETA/ETA-HCl), Oleaten, Sulfiten und Benzalkoniumchlorid (BAC) stabilisiert werden. Der Latex konnte von der Synthomer Deutschland GmbH zu Latexschaum verarbeitet werden. Weitere kolloidale Eigenschaften konnten bestimmt und mit Latex aus anderen Kautschukträgern, dem jeweiligen Isolierungsansatz und daraus resultierenden Handhabungs- und Verarbeitungseigenschaften in Beziehung gesetzt werden. Mit dynamischer Lichtstreuung (DLS) wurde eine bimodale (BM) Partikelgrößenverteilung (PSD) mit schwach ausgeprägter klein-eren Mode sowie mit dem Reinigungsgrad zunehmende Partikelgrößen bestimmt. Messungen des Zeta-Potentials (ZP) bestätigten die Stabilität verschiedener Latexkonzentrate nach Lagerung, in Gegenwart von Elektrolyten und nach Verdünnung. Außerdem konnte die Proteinkonzentration signifikant reduziert werden.

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1 Introduction

Natural rubber (NR) is a valuable resource and mainly obtained from the Pará rubber tree, *Hevea brasiliensis* (HB). With its unique properties, NR can be found in numerous important consumer goods and medical devices. Classic NR production has several disadvantages, including labor-intensive harvesting, endangered production due to fungal diseases and allergic reactions to HB proteins. However, no synthetic equivalent has yet been found for many applications [1]. These aspects are discussed in section 2.1 and show the importance of developing alternative sources for high quality NR. To this end, many groups studied the potential of plants containing NR at an early stage. In particular, the Russian dandelion, *Taraxacum kok-saghyz* (TKS) has been identified as a potentially suitable source of NR [2, 3, 4, 1]. In section 2.2 the basic properties of NR and their characterization are discussed. The focus is on NRL, which is a colloidal dispersion of NR in aqueous medium [5]. Correlations between the structure and colloidal properties of NRL and the macroscopic consequences illustrate the importance of controlling and maintaining the dispersed state for certain applications. Knowledge of these properties is necessary for comparisons with latices of different origins and particularly essential for latex isolation. Accord-

ingly, several approaches to latex extraction, concentration and preservation have been already investigated and are compared in section 2.3. Methods for HB latex are well described, but either cannot be used at all or can only be used with restrictions for TKS. Discussion of specific methods for the isolation of TKS latex reveals that both the extraction and concentration need to be further developed. Since an NR concentration of about 60% is often required [2, 3], a detailed description of the isolation and characterization of such a latex is necessary. In addition, the upscaling potential of possible methods needs to be investigated to produce higher amounts of latex for application tests and to estimate future chances of latex isolation. Moreover, section 2.3 emphasises that the success of latex isolation depends on suitable preservation. Studies by our group showed that ETA is able to stabilize TKS latex and replace the volatile and toxic ammonia that was used as a common stabilizer for NRL [2]. This raises the question of whether it can also provide sufficient stability for modified latex isolation methods and more highly concentrated latices.
In view of these aspects, the focus of this work is clearly on the investigation and conception of a holistic isolation process for latex from TKS. For one, the discussed approaches are further developed and correlated with each other, whereby generally applicable concepts and protocols are designed. For another, a detailed physical-chemical characterization of raw, intermediate and end products illustrates the influence of isolation methods on the colloidal properties and the processability of TKS latex. In section 4.1 the results of the latex extraction are presented. First, the preparation of TKS is described, which includes its cultivation, storage and cleaning. Then the conception and success of the actual latex extraction are discussed, with the focus on the promising flow method (FE) [3, 6]. The subsequent purification and concentration is shown in section 4.2. In particular, the application of creaming and centrifugation to TKS extracts is discussed, taking into account the type and amount of extract. At the same time, care is taken to preserve the latex, using suitable stabilizing solutions. These efforts eventually lead to the development of a protocol for isolating latex that meets the necessary requirements for application testing as this is one of the main goals. The results are supported using gravimetry to determine the latex concentration and light microscopy to monitor the appearance of the extracts as well as the interactions of the latex particles. Selected latices are examined in more detail in section 4.3 using DLS and ZP measurements, which provide information on PSD and stability. In addition, the reduction in protein concentration is investigated. If necessary, the methods are adapted for use with TKS latex.

2 Fundamentals

First, sources and characteristics of NR as well as important products of it are presented. Second, the fundamental characteristics of NRL are discussed. They are important for the extraction, concentration and preservation of latex from TKS, which is outlined as a third.

2.1 Sources and Applications of Natural Rubber

2.1.1 Natural Rubber as Valuable Resource

NR is used worldwide as a raw material [7]. It can be found in over 40000 products, including medical devices, consumer products, tires, engineering and industrial goods [7, 4]. These comprise surgical, dental and sanitary gloves, urethral catheters, medical balloons, orthopedic rubber bands, adhesive plasters, feeding bottle teats and medical

ice bags. Commonly used commodity products are, for example, cooking gloves, clothing rubber bands, cosmetic sponge puffs and rubber mattresses [8]. Of the above-mentioned products, however, NR is mainly used for vehicle tires and latex gloves [7]. The development of global rubber consumption is shown in Fig. 2.1. It becomes clear that the total demand for rubber has risen steadily [3]. Since 1980 the difference between synthetic rubber (SR) and NR consumption has decreased and shows the increasing importance of NR products [4].

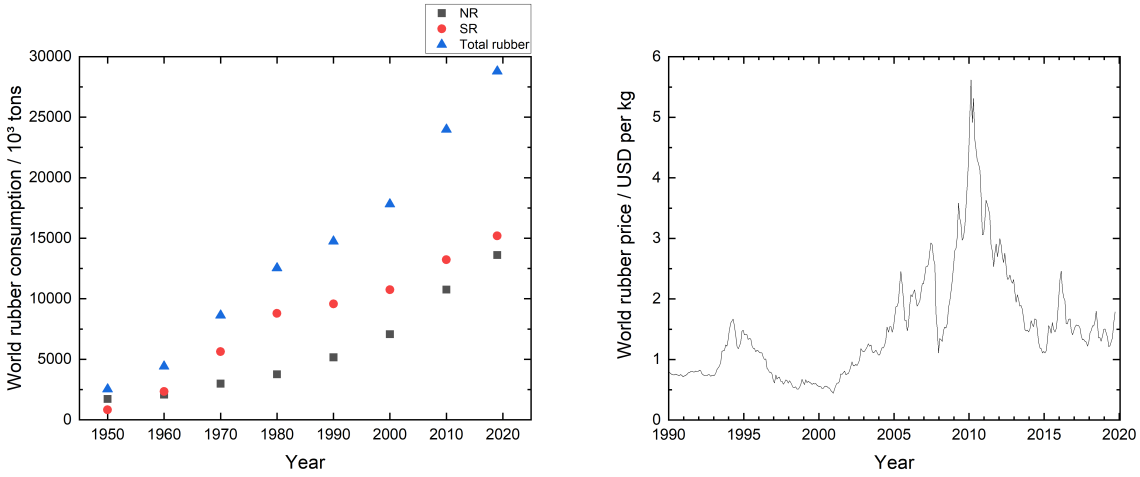


Figure 2.1 World rubber consumption since 1950 according to [9] and world NR price (RSS3 grade) since 1990 according to [10].

The NR price of the recent years is shown in Fig. 2.1. It is governed by the rubber supply, the demand for the final products and the price of the NR alternatives. In addition, supply and demand are influenced by the price of oil from which the synthetic rubber is made of. Vehicle tires have a particularly large impact on NR demand, as car tire manufacturers take over 60-70% of all NR produced. Since China is the world's largest NR user, its economic growth is a measure of the NR price. Similarly, Thailand's NR supply controls NR price as it is the world's largest producer [7, 3]. NR has unique properties. In particular, elasticity, abrasion and impact resistance, efficient heat dissipation and malleability at cold temperatures are important characteristics of NR. It cannot be easily replaced by synthetic alternatives and is therefore of strategic importance for its main uses [4]. NR consists of *cis*-1,4-polyisoprene with a high MW [5]. In contrast, synthetic *cis*-1,4-polyisoprene generally also includes 3,4- and 1,2-units along with *cis*-1,4- and *trans*-1,4-units and is therefore usually viewed more as a copolymer. However, it has been shown that polyisoprene, which

essentially only consists of *cis*-1,4-units, can be synthesized. Despite the structural similarity to NR, synthetic *cis*-1,4-polyisoprene shows clear differences in its physical and mechanical properties [11]. All of these observations show the importance of an abundant source of high quality rubber.

2.1.2 Rubber Bearing Plants

With decreasing economic importance, NR-bearing plants can be divided into three groups. First, there are plants like HB that are currently used to extract NR. It is responsible for more than 99% of rubber from natural sources. The second group are NR-bearing plants of current investigations for future applications. Finally there is the group of around 1000 rubber plants that have not yet been exploited [12].

2.1.2.1 Hevea Brasiliensis

The rubber tree HB is originally native to the Amazon rainforest, but there are also plantations in Southeast Asia, especially in Thailand, Indonesia and Malaysia. The trees can reach heights of more than 40 m, become over 100 years old, require temperatures of 25-30 °C, at least 2000 mm of precipitation per year and high humidity. Therefore, HB can only grow between 15° north and 15° south. After 5-7 years of growth, a tree can produce latex for about 25-30 years. HB is ideal for collecting NR for several reasons. The presence of milk vessels makes it possible to obtain latex by tapping (see section 2.3.1.2). It can be carried out well for many years and no resins are formed during the process. In addition, the polyisoprene produced consists almost exclusively of *cis* double bonds. However, the extraction of NR from HB has several disadvantages. In addition to the above-mentioned requirement for a suitable climate, large areas and a plentiful and cheap labor force are needed. This confirms that NR plantations are uneconomical in highly developed countries [12]. Despite attempts to mechanize the tapping process, harvesting is still labor intensive [1, 13]. Moreover, it has been reported that rubber tree plantations are being replaced by more profitable palm oil plantations due to the increasing demand for biofuels [4]. Fungal diseases, especially the South American Leaf Blight (SALB), can endanger NR production. In addition, the number of allergic reactions to proteins made from HB latex has increased over the past 15 years. These observations make the search

for other NR sources obvious. For that purpose, annual and fast growing plants with high concentrations of NR and a high MW would be desirable [1, 12].

2.1.2.2 Rubber Bearing Plants of Current Investigations

NR-bearing plants that have been used in the past or are currently being investigated for future applications number around 50 species. These include, for example, *Ficus elastica*, *Funtumia castilla*, cassava and tapioca bush [12]. Of special interest are guayule and TKS [1], which are compared to the rubber tree HB in Tab. 2.1.

Table 2.1 Selected characteristics of NR-bearing plants according to [14].

Plant	Source of NR	Production [t/a]	NR content [%]
Rubber tree	Bark	$9*10^9$	30-40
Guayule shrub	Root	10^4	3-12
Russian dandelion	Root	$3*10^3$	0-15

Guayule is continuously studied in the United States and Mexico. Its latex is present in individual milk cells and therefore cannot be extracted by tapping. Since guayule grows in arid deserts that are not suitable for other purposes, the large area is not obstructive [12]. At the same time, the restriction to such regions includes the requirement of highly specific temperatures in winter for a reasonable rubber production [15]. An irreversible, heat-induced chain cleavage was reported about guayule rubber, which means that it is less stable against oxygen and heat than HB rubber [1]. Advantageously, guayule has been reported to be a source of hypoallergenic NR [16]. In addition, its NR has the same MW and the same basic properties as HB rubber. Several studies reported NR yields of around 1000 kg per hectare and year. This overall development has increased interest in guayule rubber, and the Yulex company had early started building low protein latex production, including new sources of hypoallergenic NR gloves and medical items. TKS was used as an NR source in the USSR as early as the 1930s. In 1941, 67000 hectares of Russian dandelion were grown and covered 30% of all rubber consumption in the USSR. During World War II, shortages in NR caused several other countries, including the US, UK, Germany, Sweden and Spain, to start growing Russian dandelions as part of emergency rubber programs. The best yields for fields cultivated in the USA were around 110 kg NR per hectare, the best Russian for 200 kg NR per hectare. Early studies reported

that wild plants would mostly produce 2-3% NR, with a maximum of 5-6% NR. The best accessions of the USSR had 15% NR. The agronomic properties of the Russian dandelion have been described as unfavorable. This was due to labor-intensive and expensive cultivation caused by small seedlings that were surpassed by weeds. Since HB's cheap NR became available again after World War II, all Russian dandelion programs were discontinued. With its high MW, NR from TKS showed very good physical and chemical properties, which manifested themselves in the durability of the corresponding tires. The latter were comparable to ones HB-based tires and better than guayule-based tires [1]. TKS latex is found in the milk tubes of the plant [6]. Therefore TKS latex cannot be tapped and has to be released by cutting the tissue. Another disadvantage of NR from TKS is the potentially higher amount of associated proteins than from HB. This increases the potential for sensitization of people who are already sensitized to NR by HB. Thus, TKS could also be considered for conventional and non-medical applications such as tires [1]. Recently, NR from TKS has attracted attention again [2, 3]. It was found that wild plants contain a maximum of approx. 9% NR and 62 kg NR per hectare could be obtained [17]. Further studies showed TKS with NR contents of up to 24% [3]. In addition, new breeding strategies such as the hybridization of the Russian dandelion with the common dandelion (*Taraxacum officinale*) promised further improvements. Due to the larger size of the dandelion roots, yields of 1200-1800 kg NR per hectare can theoretically be achieved [1]. Currently ESKUSA (Germany) has carried out intensive research on the cultivation of TKS plants for the production of NRL [18]. After all, this thesis resumed the investigations of the Russian dandelion within the framework of the joint project TAKOWIND II granted by the German BMEL.

2.2 Characteristics of Latex

2.2.1 General Classification

Table 2.2 Latex types classified by origin, physical or chemical nature of the dispersed polymer and the electric charge bound to particle surface [5].

Latex type	**Origin**
Natural latex	Found in cells of certain plants or trees, formed by metabolic processes
Synthetic latex	Produced from monomers by emulsion polymerization
Artificial latex	Produced by dispersing bulk polymer in aqueous dispersion medium
Modified latex	Produced by modification of existing latex, e.g. cross-linking of dispersed polymer
Latex type	**Physical nature of dispersed polymer**
Rubber latex	Rubbery at ambient temperature
Resin or plastic latex	Glassy at ambient temperature
Latex type	**Chemical nature of dispersed polymer**
Synthetic latex	Various repeat units such as polyisoprene, polystyrene, styrene-butadiene, acrylonitrile-butadiene, polyvinyl acetate, etc.
Functionalized latex	Modified by incorporation of functional groups, e.g. for enhanced colloidal stability, adhesion to surfaces or reactivity towards reagents
Latex type	**Polarity of charge bound to particle surface**
Anionic latex	Electrochemically analogous to high valent anions
Cationic latex	Electrochemically analogous to high valent cations
Nonionic latex	Uncharged particles

The word latex used to denote milky plant juices from which rubbery substances can be obtained. In modern polymer science, latex refers to a stable colloidal dispersion (see section 2.2.3) of a polymeric substance in an aqueous medium. Latices are systems that consist of at least two phases, namely the dispersing medium, which is water in the case of aqueous latices, and the disperse phase. They are usually

whitish-milky, free-flowing liquids, with fluidity coming from the continuous phase. The viscosity is invariably much lower than that of a polymer solution with a similar polymer content (see section 2.2.5). The disperse phase consists of a large number of separate particles, mostly spherical in shape, which are polymeric in nature and have particle diameters in the range from 10 to 5000 nm (see Section 2.2.2.2). On the one hand, these define the boundaries between colloidal dispersions and true solutions of high molecular units. On the other hand, the particles can be so coarse that they tend to sediment or cream rapidly (see section 2.3.2.2). In practice there is a continuous gradation. One can distinguish latex mainly according to its origin, physical or chemical nature of the dispersed polymer and the electric charge bound to particle surface, which leads to the types described in Tab. 2.2. A further differentiation of ionic latex can be made depending on how the charges are bound to the surface. First, there is adsorption of surface-active species at the interface between the particles and the dispersion medium. Second, the chemical combination of ionic groups at this interface is possible. In the case of anionic latex, there are adsorbed or combined carboxylate, sulfate, or sulfonate anions or mixtures thereof. In contrast to polymer solutions, polymer latices are lyophopic sols and thermodynamically unstable in terms of their flocculated, coagulated or gelled state (see section 2.2.3). The thermodynamic tendency of the molecules of the polymer and the dispersion medium to mix is small, resulting in particles of aggregated polymer molecules that are small on a macroscopic scale but large on a molecular scale [5].

2.2.2 Natural Rubber Latex

The special case of NRL as a natural latex is now being examined more closely. The compositions and chemical structures of HB and TKS latex are presented and compared.

2.2.2.1 Composition

NRL is a stable colloidal dispersion of *cis*-1,4-polyisoprene of high MW in an aqueous medium [5]. Substances contained in fresh latex from HB depend on many factors such as clone, season or tapping frequency and are given in Tab. 2.3 [19]. HB latex contains numerous non-rubber constituents in relatively small portions. Of these,

about 55% are dissolved in the aqueous latex serum. A further 35% of the non-rubber constituents are adsorbed on the rubber particle surface and 10% are suspended in the latex as lutoid phase [19, 11]. The non-rubber constituents are of biological importance with respect to structure and origin of the rubber. In addition, they influence the physical and chemical properties of the latex [11].

Table 2.3 Constituents of fresh NRL with their proportion [19].

Constituent	%
Dry rubber content	30 - 35
Proteinaceous substances	1 - 1.5
Lipids	1 - 2.5
Sugars	1
Inorganic ions	1
Water	Ad 100

TKS latex is present in the milk tubes of the plant. It is made up of rubber, resins, and several other inorganic and organic substances. Their content depends not only on growing season and humidity of the soil and air, but also on the specific location of the plant. The amount of solids increases with increasing age of the plant due to a rising storage of NR. The concentrations were reported to be between 44 and 47% [6]. The rubber content of the latex was approx. 30-40% [6, 4]. In addition, the rubber phase comprises a gel fraction and was approx. 34% [3].

2.2.2.2 Latex Particles

In general, the latex particle is characterized by a core-shell structure (see Fig. 2.2) [20]. One model describes a system with a hydrophobic core made of rubber particles surrounded by an inner lecithin-type phospholipid layer. This layer is attached to the surface of the rubber hydrocarbon and an outer protein layer. At the pH of fresh latex, the lecithin layer is slightly positively charged, while the protein layer holds a negative charge. Therefore, these two layers stick together due to ionic interactions. A more recent approach suggests a rather mixed layer of proteins and phospholipids about 20 nm thick. In this case the constituents are present as separated phases in distinct domains within the layer. The proteins make up 84% of the surface layer, while phospholipids constitute only 16% [19, 20].

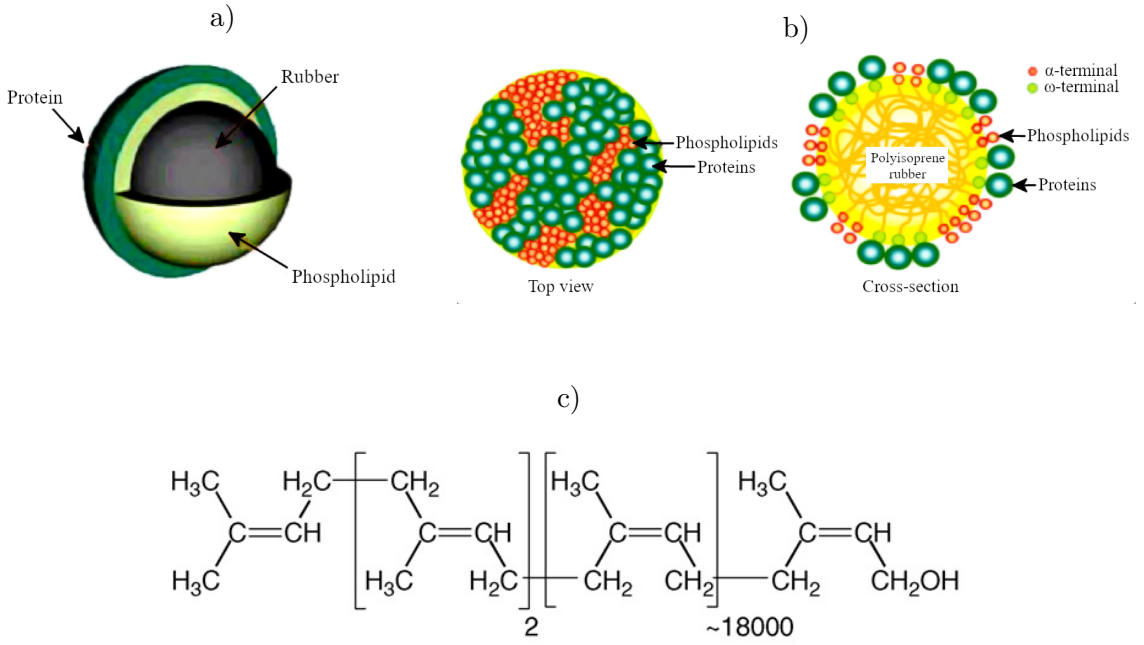


Figure 2.2 a,b) Possible structures of NRL particles according to [20]. c) Chemical formula of polyisoprene [4].

The latex particles in fresh HB range in diameter from 5 to 3000 nm. They are usually spherical, but larger ones in latex from older trees are often pear shaped [11]. Ammonia-preserved HB latices are characterized by a broad bimodal PSD and are relatively polydisperse. The two modes have been identified at 180-200 nm and 700-800 nm, for example [21]. Recent studies confirmed the bimodal distribution with particle diameters of 190-210 nm and 820-900 nm [2]. Very large particles of 5-6 µm are only contained in small quantities [5, 22]. It is believed that they are formed by the coalescence of smaller particles. In addition, there is a strong dependence of the PSD on the type of concentration, preservation, sample preparation, storage and age of the tree [5]. For example, creamed HB latices showed a monomodal (MM) PSD with a diameter of 1000 nm. In this case, no fraction of smaller particles was found, which shows the influence of the concentration method on the PSD [23]. The average MW was reported to be 1310 kDa [4]. Recent studies on TKS latex particles showed a MW of 2180 kDa [4]. Another group found a MW of 4750 kDa with a monomodal PSD, as measured with size exclusion chromatography. Transmission electron microscopy resulted in diameters in the range from 200 to 1000 nm. Furthermore, very small spherical particles of at least 10 nm could be identified. Photon correlation spectroscopy also revealed a monomodal PSD with diameters of 200-700

nm and an average of 320 nm. More than 50% of the particles were found to be between 250-400 nm. The particle size and MW were not influenced by the age of the plants [24]. Further studies confirmed a bimodal PSD with diameters of 130-190 nm and 380-580 nm. The latex was also examined after an extraction and concentration process. DLS found that the bimodal character had disappeared, resulting in a monomodal PSD with diameters of 1040-1260 nm [2]. Tab. 2.4 summarizes the MW and PSD of the discussed latices. Obviously, NRL and its particles generally differ in composition. Thereby, little is known about the specific properties of TKS latex. Especially the influences of the particular types of latex isolation (see section 2.3.1) must be investigated.

Table 2.4 MW and PSD of native and concentrated latex from HB and TKS according to [5, 2, 4, 23, 24]

Latex type	MW [kDa]	PSD	Particle size [nm]
HB, native	1310	BM	180-210 / 700-900
HB, concentrated		MM	1000
TKS, native	2180-4750	MM rsp. BM	200-700 rsp. 130-190 / 380-580
TKS, concentrated		BM	1040-1260

2.2.3 Stability of Latex

Latex stability is important for scientific reasons, as the well defined particles are used in several model systems. In the context of this work, however, the technological impact is far more important. Stability problems are frequently reported in the landscape of water-based polymer dispersions. This is especially true for complex systems that contain surfactants, water-soluble polymers, plasticizers or other particulate materials. The prevention of coagulation still raises poorly understood problems in laboratories and plants [25]. Since unstable dispersions undergo particle aggregation, latex stability is of decisive importance for aqueous coating processes [26]. First, the fundamentals of thermodynamics and kinetics of latex aggregation are discussed. Then the interactions between latex particles as well as the resulting Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and the consequences for latex stabilization are investigated. Subsequently, the electrostatic double layer and the ZP of latex are examined.

2.2.3.1 Aggregation of Latex Particles

As described in section 2.2.1, latex is a lyophobic colloid. In this context, stability refers to a measure for the system to remain unchanged as a colloidal dispersion over time. In terms of thermodynamics, lyophobic colloids usually tend to phase separate due to aggregation and coalescence. The latter includes a loss of interface between the two phases and thus a positive interfacial free energy. The total Gibbs free energy is reduced, which is the driving force for these phenomena [5].

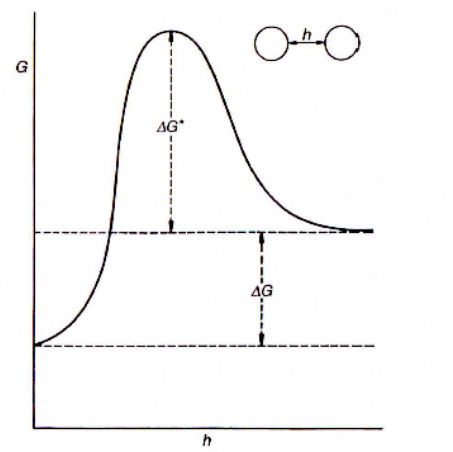


Figure 2.3 Gibbs free energy G of the particle aggregation process as a function of the particle separation h with the difference ΔG between dispersed and aggregated state as well as the corresponding free energy barrier ΔG^* for dispersed particles to reach the aggregated state [5].

Fig. 2.3 shows the corresponding overall scheme. The difference in Gibbs free energy between the dispersed and the aggregated state is denoted as ΔG and is negative for lyophobic colloids. Of great importance is the free energy barrier ΔG^*, which dispersed particles have to overcome in order to reach the aggregated state and is positive for stable sols. Its height is a measure for the sol stability [5]. ΔG^* includes the enthalpic component ΔH^* and the entropic component $-T\Delta S^*$. T denotes the absolute temperature and ΔS^* the increase in entropy of particles with infinite separation for a movement to the top of the barrier. The underlying relationship can be written as follows:

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{2.1}$$

The forces of different parts of the molecular system that stabilize the sol are quantified by ΔH^*. The increase in order within parts of the molecular system during

particle approaches are quantified by $-T\Delta S^*$. Thus, three scenarios can be drawn: If ΔH^* and ΔS^* are positive and $\Delta H^* > \Delta S^*$, there is enthalpic stabilization and destabilization with increasing temperatures. If ΔH^* and ΔH^* are negative and $|\Delta T S^* I| > |\Delta H^*|$, there is entropic stabilization and destabilization at decreasing temperatures. If ΔH^* is positive and ΔS^* negative, there is enthalpic-entropic stabilization and destabilization with increasing temperatures [5]. If the system passes the barrier and reaches the minimum, the process is generally irreversible. For energy barriers far above kT, the minimum is not accessible and the system is kinetically stable [27]. Brownian motion causes random clashes of particles with dispersion medium molecules and brings about close approaches of particles to one another. Overall, this leads to particle aggregation and coalescence. Hence there is not only a thermodynamic force, but also a mechanistic description of particle aggregation and coalescence. Stability is established by barriers between particles that prevent close mutual approach. These are well described by potential energy curves, which include all repulsive and attractive forces, and are discussed in section 2.2.3. If the aggregation is driven by Brownian motion and particles do not interact until they touch, this leads to rapid particle aggregation kinetics [5]. Since the rate-determining step for particle aggregation is the Brownian encounters between two particles, the rate is proportional to the square of the particle concentration. This leads to a second order rate coefficient K, which is a constant [5, 27]:

$$\frac{dN}{dt} = -KN^2 \tag{2.2}$$

with the particle concentration N. In Eq. 2.2, K equals $8\pi Da$ with the diffusion coefficient D of the spherical particles with radius a [5]. Assuming that particles obey the Stokes-Einstein equation, $D = \frac{kT}{6\pi\eta a}$ with k as Boltzmann constant, T is the absolute temperature and η the viscosity of the dispersion medium. Thus K can be written as follows [5]:

$$K = \frac{4kT}{3\eta} \tag{2.3}$$

It shows that K depends on the viscosity and is independent of the particle size [5, 27]. If the kinetics of particle aggregation is retarded, a different second order rate coefficient $K' = \frac{K}{W}$ can be set. The latter describes a particle aggregation process over a longer period of time, where W is defined as the stability factor. Particle aggregation occurs in an all-or-nothing fashion. In contrast, coalescence of the aggregates can also

be incomplete. Therefore, particle aggregation is accelerated by increasing the kinetic energy and the collision frequency of particles and decreasing the energy barrier [5].

2.2.3.2 Interactions between Latex Particles

The colloidal stability of systems like NRL is controlled by attractive and repulsive interactions between the latex particles [19]. It is assumed that factors which influence the colloidal stability are only active at interfaces and do not influence the properties of the bulk particles [25]. In a lyophophic sol, attractive forces are generally referred to as secondary valence forces or van der Waals forces [5]. They are caused by quantum mechanical interactions between atoms of neighboring particles [19]. The attractive forces between particles of a lyophobic sol are therefore rather unspecific, which corresponds to a weak dependence on the molecular structure of the particles [5]. They comprise the electrostatic Keesom, Debye and quantum mechanical dispersion forces [5, 27]. Keesom or orientation forces are attractions between permanent electric dipoles in the molecules. Debye or induction forces are attractions between electric dipoles and those that are induced by other dipoles. London or dispersion forces are attractions between fluctuating dipoles in the atoms of the molecules. All of these types of secondary valence forces are described by an attractive potential of the following form [5]:

$$U(\tau) = -\frac{\beta}{\tau^6} \tag{2.4}$$

with the potential energy $U(\tau)$, the intermolecular or interatomic distance τ and the constant β, which depends on the underlying force. Latex stability is controlled by various repulsion factors. First, there are electrostatic forces that are caused by electric charges, which are bound to the particle surface (see Fig. 2.4) [5].

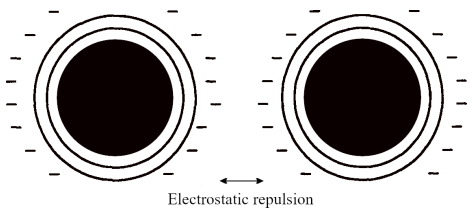


Figure 2.4 Scheme of the electrostatic repulsion between two latex particles according to [19].

These are important for aqueous latices mainly because of the repulsive force by the

counter-ion cloud around each particle. In this case the mutual electrostatic repulsion between same charges is of subordinate importance [5]. Second, there is steric and solvation stabilization as well as particle bridging by polymers [25]. Steric forces are caused by lyophilic macromolecules that are adsorptively or covalently bound to the particle surface [5]. These can be, for example, ionic surfactants or polyelectrolytes [28]. Nonionic block copolymers in which one part is miscible with the disperse phase, but not with the dispersion medium, are also possible. At the same time, the other part of the polymer is miscible with the dispersion medium. Accordingly, there are segments of direct contact with the surface (trains), segments that extend into the dispersion medium (loops), and free ends of the adsorbed polymer that extend into the dispersion medium (tails). The repulsion is based on two possible effects. As polymer strands of two particles approach, their configurational entropy decreases due to the reduced volume available. Second, there is an increased osmotic pressure at sites with higher polymer concentrations [19]. The combination of these steric and electrostatic effects is referred to as electrosteric stabilization, which is shown in Fig. 2.5 [29, 28].

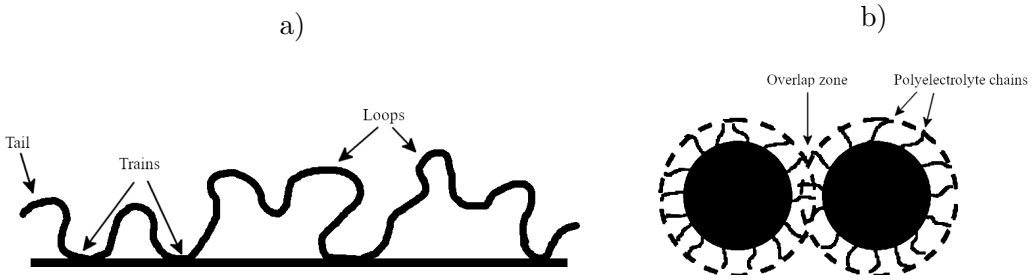


Figure 2.5 a) Conformation of stabilizing macromolecules at particle surface according to [19]. b) Osmotic and entropic contributions to steric stabilization of particles according to [19, 29].

Third, there are exclusion or depletion forces which are caused by lyophilic macromolecules that are dissolved in the dispersion medium [5]. They depend more on osmotic fluctuations of the solvent than directly on particle surface interactions [30]. Solvation forces are caused by the bond between molecules of the dispersion medium and the particle surface [5]. These can be induced, for example, by nonionic polymers through the formation of a solvation layer [19]. Finally, it was shown that the swelling of latex with solvents can also be seen as a factor for colloidal stability. This was explained by the mechanical relaxation of domains within the particles or the polymers [25, 30].

2.2.3.3 DLVO Theory

The discussion of the various factors that influence latex stability inevitably leads to the DLVO theory. It describes the colloidal stability as the result of particle surface interactions on the basis of van der Waals and electrostatic forces [30, 26, 31]. The competition between these attractive and repulsive forces constitutes the stability or instability of colloidal sols. The total interaction potential can be written as the sum of the attractive potential $V_A(h)$ and the repulsive potential $V_R(h)$ [27, 26]:

$$V(h) = V_A(h) + V_R(h) \tag{2.5}$$

Considering two latex particles at the separation h, the van der Waals interaction $V_A(h)$ is [27, 26]:

$$\frac{V_A(h)}{area} = -\frac{H_{121}}{12\pi h^2} \tag{2.6}$$

with the Hamaker constant H_{121} for medium 1, separated by a liquid 2. The double-layer interaction is given by [27, 26]:

$$\frac{V_R(h)}{area} = \frac{64kTc_0^*\Gamma_0^2}{\kappa} exp(-\kappa h) \tag{2.7}$$

with the Boltzmann constant k, the temperature T, the bulk electrolyte concentration c_0^* and the inverse Debye length κ. In Eq. 2.7, Γ_0 defines the term $tanh(\frac{ze_0\phi_0}{4kT})$ with the charge number z, the elementary charge e_0 and the surface potential Φ_0. The total interaction potential can then be written as follows [27]:

$$\frac{V(h)}{area} = -\frac{H_{121}}{12\pi h^2} + \frac{64kTc_0 * \Gamma_0^2}{\kappa} exp(-\kappa h) \tag{2.8}$$

The corresponding interaction force is [27]:

$$\frac{F(h)}{area} = -\frac{H_{121}}{6\pi h^3} + 64kTc_0^*\Pi_0^2 exp(-\kappa h) \tag{2.9}$$

The potential energy curve is given in Fig. 2.6 and comprises two minima. There is a primary minimum at smaller distances and a secondary minimum at larger distances. They are separated by a maximum that represents the barrier against particle coagulation if it is sufficiently high. Reaching the primary minimum means irreversible coagulation. In contrast, flocculation is observed within the secondary minimum.

With energy barriers that are much larger than kT, the system cannot reach the primary minimum and is kinetically stable. With barriers of around kT, a fast approach is possible [27].

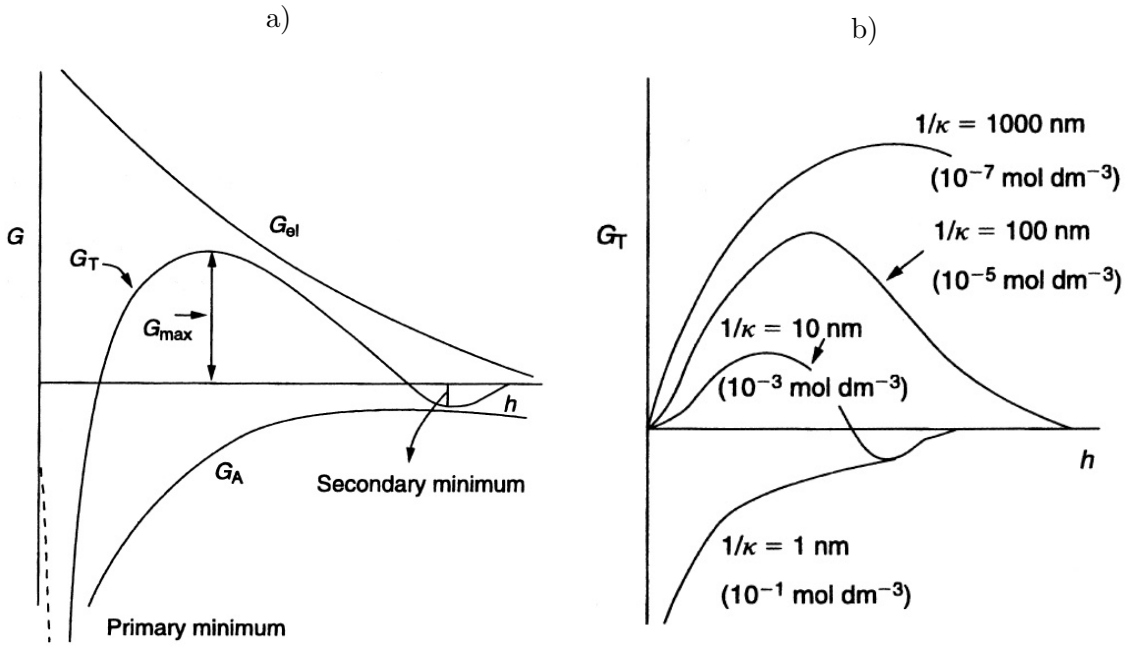


Figure 2.6 a) Energy G of electrostatically stabilized colloidal systems as a function of the separation h between two particles. The attractive energy is represented by G_A, the electrostatic energy of repulsion is G_{el}, the total energy of interaction is G_T and the energy barrier is G_{max}. b) Total energy of interaction G_T as a function of the separation h between two generic particles for different 1:1 electrolyte concentrations. The double layer thickness is represented by the parameter $1/\kappa$. In general, the energy curve also depends on the particle size and shape as well as the Hamaker constant [32].

Knowledge of particle agglomeration leads to the discussion of external factors that can destabilize latex. On the one hand, there are certain processes that require a controlled destabilization of the latex in order to separate the polymer phase from the dispersion medium. On the other hand, latex should generally have sufficient colloidal stability to remain dispersed for periods of time at ambient temperatures [5] and also over a wide range of electrolyte concentrations [29]. Electrolytes influence the stability by screening the repulsive forces [31]. In contrast, the attractive force is not sensitive to the electrolyte concentration. As a result, it is possible to control the colloidal stability by adjusting the ionic strength of the solution [29]. The DLVO theory can be used to explain these electrolyte effects on latex stability [26]. The corresponding energy barrier is shown in Fig. 2.6. As given in Eq. 2.8, the distance dependence

is only governed by the Debye screening length. For small or large distances, the potential curve is controlled by the attractive term. If the electrolyte concentration is low, κ is also low. This constitutes a large energy barrier and usually results in a stable system. At higher values of κ due to high electrolyte concentrations, the barrier is low. This makes the primary minimum accessible and the system is unstable [27]. If the electrolyte concentration reaches a point at which the repulsive forces are completely screened, no further destabilization takes place beyond this so-called critical coagulation concentration [31]. Compounds can even be added in a controlled manner to destabilize latex, for example in the case of determining the rubber content [33]. The main reason for chemical destabilization is the reduced energy barrier between the particles. Destabilizing agents often react with surface-bound groups that contribute to latex stability. These coagulants can be acidic substances, water-soluble electrolytes, hydrophobic and hydrophilic organic liquids, cationic surfactants and water-soluble hydrophilic polymers. In particular, acids react with the surface-bound carboxylate ions. The corresponding uncharged carboxylic acid groups are formed by addition of protons. This leads to a reduced electric charge density, surface potential and counter-charge around the particles. It finally causes a reduced electrostatic barrier between the particles [5]. The DLVO theory can therefore also be used to describe pH effects on latex stability [26]. If destabilization is caused by the addition of chemical agents, it leads to coacervation. Products of the latter can be either gel, coagulum or floccules [5].

2.2.3.4 Electrostatic Double Layer

To apply the DLVO theory, the attractive and repulsive contributions of interactions between particles must be known. The former can be estimated using the Hamaker constant. It is more difficult to determine the surface potential of the particles, which is responsible for the double layer repulsion [27]. Derived from Eq. 2.7, a commonly used term for the electrostatic repulsion of spherical particles can be written [34]:

$$V_R(h) = \epsilon_0 \epsilon_r a \phi_0^2 \ln(1 + exp(-\kappa h)) \tag{2.10}$$

with the particle radius a [34]. Several models have been proposed to describe the surface potential in terms of particle separation in order to calculate the electrostatic double layer repulsion. The first double layer model was set up by Helmholtz in

1879. This concept was based on a charge separation at the interface between metal electrode and electrolyte solution. The charge density ρ on the electrode is compensated for by redistributing oppositely charged ions from the solution. Accordingly, this leads to two oppositely charged layers, which are separated from each other by a distance limited to the radius of the ions, and a single solvation layer. The Outer Helmholtz Plane (OHP) is the boundary defined by a line through the center of these ions. The area inside represents the electrical double layer. This allows the potential in the Helmholtz layer to be calculated using the 1D Poisson equation. Accordingly, the potential is related to the charge distribution [35]:

$$\frac{\delta^2\phi}{\delta h^2} = -\frac{\rho(h)}{\epsilon_r\epsilon_0} \tag{2.11}$$

Unfortunately, this model does not take into account the interactions that occur outside the OHP as well as the thermal motion of ions near the charged surface. The latter shortcoming has been improved by a model proposed by Gouy and Chapman. They outlined a diffuse double layer of counterions of opposite charge. These ions could be described by the Boltzmann distribution [35]:

$$n_i = n_i^0 exp(\frac{-z_i e\phi}{kT}) \tag{2.12}$$

with n_i and z as bulk ion concentration and charge of the ion i. The total charge density per volume can then be written as the sum over all ions [35]:

$$\rho(h) = \sum_i n_i z_i e = \sum_i n_i^0 z_i e exp(\frac{-z_i e\phi}{kT}) \tag{2.13}$$

After combining Eqs. 2.12 and 2.13, the Poisson-Boltzmann equation is obtained, which describes the ion distribution in an electrolyte solution outside a charged interface [35, 27]:

$$\frac{\delta^2\phi}{\delta h^2} = \frac{e}{\epsilon_r\epsilon_0}\sum_i n_i^0 z_i exp(\frac{-z_i e\phi}{kT}) \tag{2.14}$$

At low electrolyte concentrations and in the vicinity of planar and slightly curved surfaces, this theory can predict ionic profiles and the resulting forces. However, it is known for overestimating ion concentrations near highly charged surfaces and multivalent ions [35]. By combining the models presented by Helmholtz and Gouy-Chapman, Stern developed a more realistic description of the double layer (see Fig.

2.7). A compact ion layer according to Helmholtz was combined with the diffuse ion layer in the direction of the bulk solution according to Gouy-Chapman [35]. Movement of the particles relative to the dispersion medium leads to a displacement at the boundary between the two layers. The ZP ζ describes the electrical potential at this boundary [5].

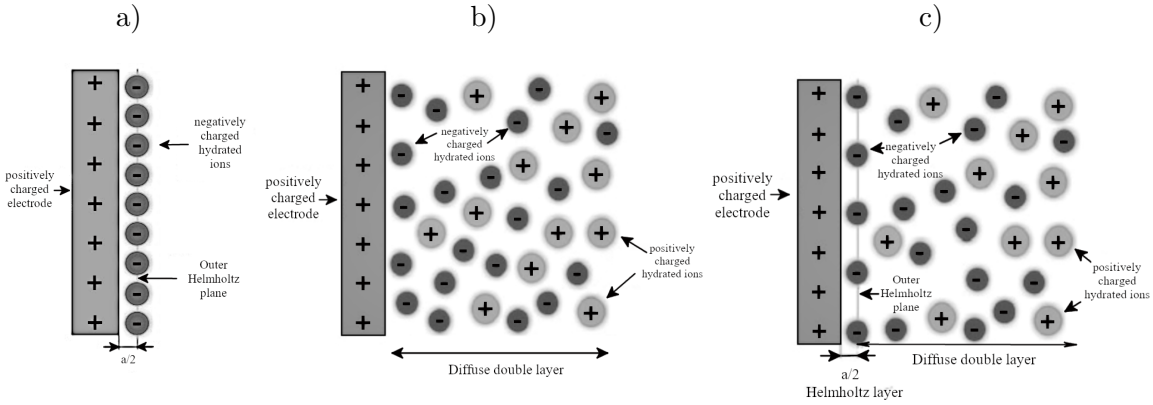


Figure 2.7 Helmholtz model (a), Gouy-chapman model (b) and Stern model (c) according to [35].

2.2.3.5 Zeta Potential

It turned out that the ZP correlates with colloidal stability and can be easily measured using electrokinetic phenomena. These can be classified either according to the applied or induced potential or according to the different stationary and moving phases (see Tab. 2.5) [27].

Table 2.5 Summary of electrokinetic effects [27].

Potential	Stationary (wall)	Moving (colloid particles)
Applied	Electroosmosis	Electrophoresis
Induced	Streaming potential	Sedimentation potential

In an electrophoresis experiment, the movement of suspended colloidal particles is induced by applying an electric field. The opposing viscous force immediately leads to a constant velocity $\vec{v}$ of the particles, which is measured. In this steady state,

Stokes' law can be applied [27, 36]:

$$\vec{v} = \frac{\vec{F}}{6\pi\eta R} \tag{2.15}$$

where $\vec{F}$ is the external force. With small external electric fields $\vec{E}$ the electrophoretic mobility u can be expressed by [27]:

$$u = \frac{\vec{v}}{\vec{E}} \tag{2.16}$$

Assuming an isolated particle with charge q, $\vec{F}$ equals $q\vec{E}$, and the combination of Eqs. 2.15 and 2.16 finally leads to [27]:

$$u = \frac{q}{6\pi\eta R} \tag{2.17}$$

According to the Debye-Hückel theory, the ZP ζ can be written as follows [27]:

$$\zeta = \Phi(R') = \frac{\frac{q'}{R'}}{4\pi\epsilon_r\epsilon_0}(1+\kappa R')^{-1} \tag{2.18}$$

where R' is the effective radius, q' the effective charge, ϵ_0 the relative permittivity and ϵ_0 the dielectric permittivity. Assuming very thick double layers $\kappa R' \ll 1$, field lines are almost not affected by the particles. Then Eq. 2.18 can be expressed by the Hückel equation [27, 36]:

$$\zeta = \frac{3}{2}\frac{\eta u}{\epsilon_r\epsilon_0} \tag{2.19}$$

In the case of $\kappa R' \gg 1$, the Helmholtz-Smoluchowski equation must be used, which differs from the Hückel equation by a factor of 1.5 and reads [27]:

$$\zeta = \frac{\eta u}{\epsilon_r\epsilon_0} \tag{2.20}$$

These derivations constitute the basis for the measurement of the ZP and the discussion of the latex stability in section 4.3.2.

2.2.4 Dynamic Light Scattering

Since the PSD of polymer latices is of great importance for many applications in terms of processability and final properties, it is obvious to choose a suitable determination method [37]. DLS is also known as photon correlation spectroscopy or quasi-elastic light scattering. It defines a spectroscopic method for determining the size distribution of dissolved or suspended particles [38]. DLS is often used for polymer latices with particles in the submicrometer range [37]. The measurement setup is sketched in Fig. 2.8 [39] and is based on the following relationships. Brownian motion causes molecules to move in all directions at different velocities. Rotations or oscillations of molecules or parts thereof are also possible [40]. When a monochromatic incident laser hits the dilute latex sample [41], scattering occurs due to fluctuations in the medium. Differences in the refractive indices of colloidal particles and the dispersion medium lead to a scattering of light [39]. As a result of the Doppler-effect, the light scattering provides a spectrum that is shifted towards the frequency of the incident light [40].

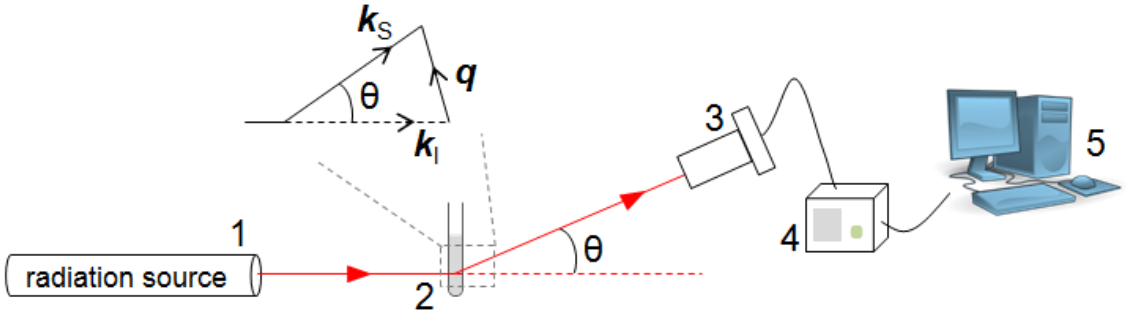


Figure 2.8 Setup of a DLS measurement with radiation source 1, sample 2, detector 3 at angle θ, correlator 4 and computer 5. The scattering vector q is the difference between the propagation vectors of scattered and incident light, respectively k_S and k_I [39].

The scattered intensity reflects the Brownian motion of the particles and is therefore a stochastic signal [42] as shown in Fig. 2.9 [39]. An autocorrelator calculates its time correlation function $g^{(2)}(\tau)$ [42]. It is shown in Fig 2.9. and reads [40]:

$$g^{(2)}(\tau) = \frac{\langle I(0) \cdot I(t) \rangle}{\langle |I|^2 \rangle} \tag{2.21}$$

where $I(t)$ is the scattering intensity and $t = i\tau$ the time between a measurement

pair. The field correlation function $g^{(1)}$ can be derived as [40]:

$$g^{(1)}(\tau) = \exp(-D_0 q^2 \tau) \tag{2.22}$$

where q is the scattering vector and D_0 the diffusion coefficient at infinite dilution. The Stokes-Einstein equation for hard spheres is [40]:

$$D_0 = \frac{kT}{6\pi\eta R_H} \tag{2.23}$$

with Boltzmann's constant k and the viscosity η at the temperature T. Thus the hydrodynamic radius R_H can be determined [40].

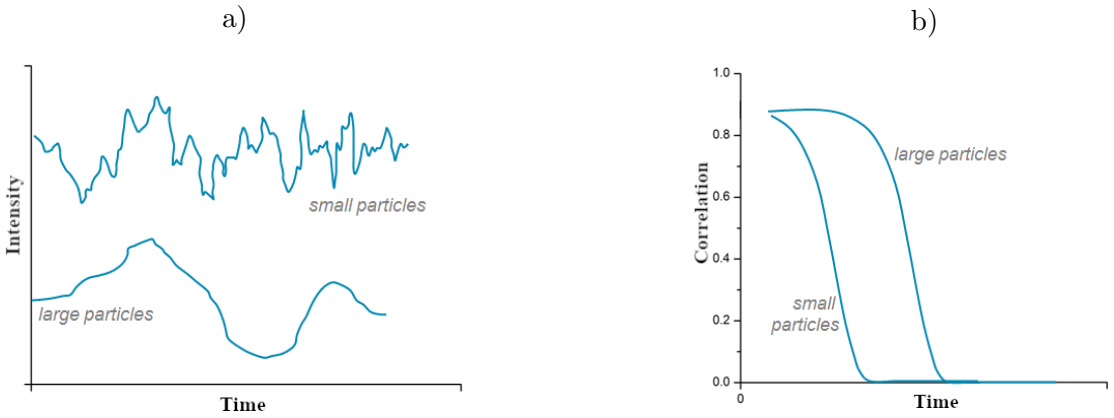


Figure 2.9 Illustration of intensity fluctuations (a) and correlation functions (b) for small and large particles. Since small particles move fast, the signal changes quickly and the correlation does not last for a long time [39].

The inversion of the data is a major difficulty, including mathematically ill-conditioned inversion operations. Several approaches have been used to solve this problem. Among these are mainly the method of cumulants, CONTIN, non-negative least squares, singular value analysis, maximum entropy [43, 44] and MAXAMPER [45]. The CONTIN algorithm in particular is the most commonly used transformation algorithm [46]. The method contained therein is a constrained regularization [47]. Because this method uses a logarithmic scaling, it is not sensitive to the range of diffusion coefficient. In addition, an initial guess is not required and dust is also considered. These circumstances lead to accurate values of the particle size [48]. Another method of analyzing the correlation functions is exponential sampling. Thereby the entire decay distribution function is iteratively reconstructed [46]. Since this method

also has a logarithmic scaling for the diffusion coefficient, it provides relatively accurate estimates of the particle size [48]. Advantages of DLS in general are that it is an absolute method, so no calibration is required. Particles can be measured in the suspended state and are not affected by the measurement. In addition, a DLS measurement is quick [21]. It is therefore a convenient and simple technique to estimate the particle size for numerous colloidal suspensions [43]. Nevertheless, there are some disadvantages. First, it requires low particle concentrations [49]. Of great importance is the fact that the data generated have little information content and the data analysis is non-unique. Therefore the resolution of this technique is limited. However, DLS generally works well for many monomodal or bimodal systems [43]. Difficulties in measuring the PSD for bimodal systems with size ratios greater than approx. 2:1 have been reported [43, 50, 51]. Better results can be achieved if the different fractions are separated and analyzed independently of one another [46]. Attempts to get improved estimates of bimodal systems were promising [52, 53, 54]. It was shown that multi-angle photon correlation improved the accuracy of photon correlation spectroscopy and gave a more reliable PSD than single-angle data analysis [54]. Recent DLS measurement systems have also shown good results by the implementing models and algorithms that have been optimized for multiple narrow modes [52]. All these considerations were ultimately applied to the analysis of the PSD of latex from TKS in section 4.3.1.

2.2.5 Viscosity of Latex

In contrast to polymer solutions, latices show fluidity up to relatively high concentrations. For one, this is due to the tight aggregation of the polymer molecules. For another, it is attributed to the dispersion medium, which is essentially free from polymer. As shown in Fig. 2.10, the viscosity of polymer solutions begins to increase rapidly from the beginning with increasing polymer concentration. Polymer latices show almost the same viscosity as the dispersion medium, until the volume fraction of the polymer reaches a value of between 0.4 and 0.6. Thereafter the viscosity rises strongly with increasing polymer concentration. With polymer latices, much higher polymer concentrations can be obtained with the same viscosity [5]. The production of high SC latex is of great industrial interest. It is associated with high drying rates, energy savings and better mechanical and rheological properties [55]. This can

be important for many applications such as dipping processes, surface coatings and adhesive applications. Much better coverage is obtained in these examples provided it is stable and forms a thin film [5]. Such applications require the viscosity to be kept as low as possible. Although the macroscopic viscosity for SCs of up to 55% in typical industrial products usually remains rather low, the viscosity can rise rapidly beyond this limit. In addition, the latex could be sensitive to shear stress in the reactor or during handling of the latex [56].

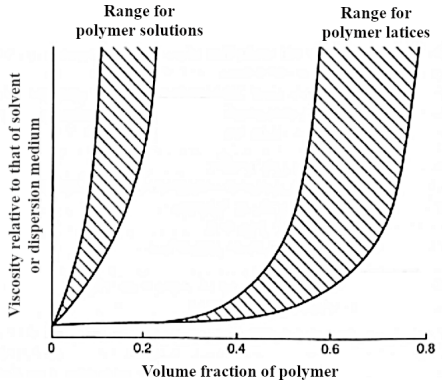


Figure 2.10 Viscosity-concentration relationships for polymer latices and polymer solutions according to [5].

In the following, factors are discussed which influence the concentration-viscosity relationship. The chemical nature, size, size distribution, shape of the polymer molecules themselves and the chemical nature of the dispersion medium have only a weak influence on the latex viscosity. Again, this is caused by the tight aggregation of the polymer molecules. In turn, this leads in only weak interactions between polymer segments and molecules of the dispersion medium, except possibly in the interface area of the particle surface. One factor that affects the latex viscosity is the average size of the polymer particles. The larger the particles are at a certain volume fraction, the lower the viscosity. This is caused by an increased average distance between particles with increasing particle size [5]. In turn, the increased particle distance prevents particles from approaching and forming interparticle bonds. In addition, an increased particle size reduces the surface area per unit volume [57]. Investigations of a monodisperse synthetic latex also confirmed a Newtonian behavior for particle sizes between 99 and 871 nm [58]. Another factor that affects latex viscosity is the PSD of the polymer particles. The broader the PSD for a given average particle size, the lower the viscosity. This is caused by the accommodation of smaller particles in the spaces between larger particles, whereby the effective volume fraction

is reduced [5]. Near the upper limit of the SC, latex viscosity is very sensitive to the PSD. Unfortunately it is still difficult to predict the effect of a complex PSD on viscosity [56]. Studies on synthetic latices have shown that a ratio of 80:10:10 for large:medium:small particles is best in order to obtain latex with the lowest viscosity. In addition, the relatively highest SC or extent of packing was achieved when 80% of the total SC consisted of the larger particle fraction [55]. The viscosity of the dispersion medium can influence the overall latex viscosity, for example through interparticle bridging between polymer molecules. An inclusion of dispersion medium in the particle agglomerates then increases the effective volume fraction of the disperse phase. The shape of polymer particles is expected to affect the latex viscosity. However, it is of less importance considering that most latex particles are spherical [5]. With regard to NRL, latex from various NR-bearing plants showed comparatively higher viscosities for higher particle concentrations. It was found that F. elastica has by far the most viscous latex for any rubber concentration. Guayule follows, while HB latex has the lowest viscosity. In addition, it was reported that latices of various plants differ in the specific rubber concentration at which latex shows a phase shift and transforms into a reversibly aggregated phase. The latter could be described as a whipped cream. It was found that this change occurs at 60% rubber content for HB latex, at 40-45% for Guayule latex and 18% for F.elastica latex [23]. This concentration was referred to as the critical total solids content (TSC). The viscosity increases rapidly with TSC beyond this point. It was explained with the relation to the maximum packing volume of the latex [57]. It is believed that the differences in rheological properties are in part related to the particle size. That said, the relatively smaller particles of HB latex are likely responsible for the lower viscosity. But even larger differences in particle size do not usually lead directly to a noticable difference in viscosity at the phase shift. This is caused by the constant packing density, which is independent of the particle size. Possible reasons are also a different PSD. It was argued that smaller particles could mix with larger ones, thereby inhibiting the flow of the larger particles. The latter was made responsible for the phase shift at lower concentrations [23]. However, this explanation obviously contradicts the previously established relationship of lower viscosity for latex with broader PSD [5]. The components of the latex particle surface, specifically the protein and lipid components of the biomembrane, vary for different NR-bearing plants. Thus both may contribute to the observed viscosity differences. The MW affects the viscosity only to a lesser extent [23]. Another aspect that is particularly important for NRL is that it may have

been concentrated by centrifugation or creaming with the addition of CA. Studies have shown a higher viscosity of creamed latex compared to centrifuged latex, which can be explained by the adsorption of CA molecules on latex particles. In turn, it is believed that this induces the formation of clusters with particles of different sizes. In addition, creamed latex was found to be more pseudoplastic than centrifuged latex [59]. Furthermore it was shown that the re-centrifugation of NRL concentrates does not affect the viscosity [57]. All these observations outline the existing differences between latices from different plants [23] as well the importance of suitable rheological properties of latex [5].

2.2.6 Proteins in Latex

NRL and its products contain proteins that cause latex allergy [60]. Together with the increased use of these products and changes in latex processing in recent years, the extent and severity of symptoms have increased significantly [60, 61]. An immediate type I hypersensitivity can result from the allergy and cause severe anaphylactic reactions [60], upper airway rhinitis, lower airway asthma [61] and contact dermatitis [62]. Based on these observations, the Food and Drug Administration issued a medical alert about using NRL products [60]. Manufacturers have addressed these problems and were able to reduce the number of new cases of HB latex allergy. This has been achieved through certain avoidance tactics and a reduction of the allergen content in NRL products [61]. However, a common problem is the low margin for NRL products compared to the synthetic equivalent. Therefore the costs for reducing the allergenic potential must be correspondingly low [63]. Based on these circumstances it is evident to make efforts to quantify and remove proteins simultaneously with latex isolation. First, HB latex proteins and their removal are studied in order to serve as a model system for adaptation to TKS latex.

2.2.6.1 Proteins of Natural Rubber Latex

Fresh HB latex consists of approx. 1-2% proteins. About 70% of these are soluble, while the remaining 30% are associated with membranes [64]. HB latex can be separated into three main fractions by centrifugation (see Fig. 2.11) [65].

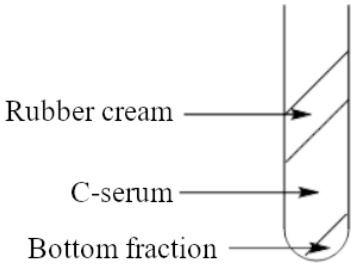


Figure 2.11 Fractionation of latex by high-speed centrifugation according to [65].

Accordingly, a white upper layer, an aqueous phase (C-serum) and a bottom fraction are formed. The upper layer consists of latex particles stabilized by an adsorbed layer of phospholipids and proteins, as described in section 2.2.2.2. This phase generally contains two different proteins which are insoluble in water and present in amounts of approx. 11 mg/g NR. The C-serum is the cytoplasm of the laticifers and contains most of the solutes found in plant cells. Thus, it comprises many different proteins that are connected to cell metabolism. These are, for example, enzymes that are associated with rubber biosynthesis or the respiratory tract. Many hundreds of proteins are present in the C-Serum, usually in amounts of approx. 12 mg/mL. The bottom fraction consists mainly of lutoid particles. It also comprises varying amounts of other organelles and plant cell components with a density greater than that of the C-serum. Finally, there is the B-serum, which denotes the fluid content of the bottom fraction. It can be obtained by freeze-thaw cycles of the bottom fraction of centrifuged latex. The proteins are contained in amounts of approx. 24 mg/mL. Since the protein hevein is responsible for 50-70% of the total B-Serum proteins, an overall smaller amount of different proteins can be found compared to the C-Serum. Proteins present in B- and C-serum are usually water soluble. Upper phase proteins and almost all C-Serum proteins are normally acidic, while B-Serum contains both acidic and basic proteins [65, 64]. The International Union of Immunological Societies (IUIS) found that ten latex proteins are allergenic. Hev b1 and b3 are the two proteins that are located on the latex particle surface. While Hev b1 is rather found on larger particles above 0.4 µm, Hev b3 is usually found on smaller particles. Hev b5, 7, 8, and 9 are in the C-serum. Hev b2, b4, b6, and b10 are in the bottom fraction [64]. In general, the protein spectrum varies in relation to latex preservation and the final product. This is caused by a selective loss of high MW proteins during processing or an ammonia-induced hydrolysis [66]. In addition to HB latex, latex from other NR-bearing plants was also examined for allergenic potential. Guayule has a much lower

protein content than high-ammonia natural rubber latex (HNRL) [61]. It does not contain protein epitopes that cross-react with anti-HNRL protein-specific antibodies [60, 61]. Sensitization of occupationally exposed persons has not been reported and HNRL allergy sufferers can use products without showing allergy symptoms. This led to the widespread assumption that other NR-bearing plants would also not contain protein epitopes that cross-react with anti-HNRL protein antibodies. To test this assumption, the immunology of TKS was recently examined using ELISA and immunoblot methods. Latex from TKS has been shown to contain several proteins that share epitopes with antigenic and allergenic proteins from HB latex. The protein concentrations of native latex and washed rubber particles were measured with the modified lowry method to be 293 µg/mL and 785 µg/mL, respectively. Therefore, latex from TKS cannot be regarded as an allergy-free replacement for HB latex [61]. This is true at least for native TKS latex. Approaches to reduce the protein content through purification are shown in section 4.3.3.

2.2.6.2 Determination of Protein Concentration

The generally proposed protein determination methods exhibit a different accuracy depending on the specific sample properties. The commonly used Lowry method is based on the biuret reaction, which is enhanced by the Folin-Ciocalteau (FC) reagent. The two-step method is tedious and requires a sophisticated technique to get good results [67]. In addition, certain surfactants and buffer that are used for protein solubilization can interfere with the FC reagent [68, 67]. The Kiehldahl method measures the nitrogen content, which is normally a measure of the protein content [69]. This also suggests that any nitrogen-containing molecules other than proteins, such as ETA, will alter the results. The bicinchoninic acid (BCA) assay includes colorimetric detection and quantification of proteins based the biuret reaction. The latter involves the reduction of Cu^{2+} to Cu^{+} by proteins that containing cysteine, tyrosine and/or tryptophan amino acid residues in an alkaline medium. This is followed by the highly sensitive and selective colorimetric detection of Cu^{+} in the presence of BCA. The chelation of two BCA molecules with one Cu^{+} leads to a purple colored reaction product. This complex shows a strong absorbance at 562 nm. The latter increases almost linearly with increasing protein concentrations over a large working range. Since the assay has no true endpoint, samples must be cooled after

incubation. This ensures that the rate of color development after the incubation is sufficiently slow, which enables the parallel analysis of larger sample quantities [67]. An important property of the B- and C-serum proteins for allergenicity tests is their solubility in trichloroacetic acid (TCA). For the purpose of protein quantification, TCA precipitation is used to remove interfering substances of the latex. Although basically all of the C-serum proteins are precipitated by 5% TCA, only about 30% of the B-serum proteins are precipitated this way. The remaining TCA-soluble proteins can be precipitated by 0.2% phosphotungstic acid (PTA). Latex gloves likely contain much larger amounts of B-serum proteins. Therefore a combination of TCA and PTA is proposed to precipitate proteins in latex glove extracts prior to protein quantification [64].

2.2.6.3 Removal of Proteins from Natural Rubber Latex

Different approaches to removing proteins from NRL have been developed to solve the problem of latex allergy. Since the proteins are bound to surface of the latex particles, protein removal can be carried out efficiently in the latex stage (see Fig. 2.12) [8]. The most basic method is repeated centrifugation. In this way, about 50% of proteins can be removed as depicted in Fig. 2.12. This can of course also apply to creaming, which is even more effective, but slower [70].

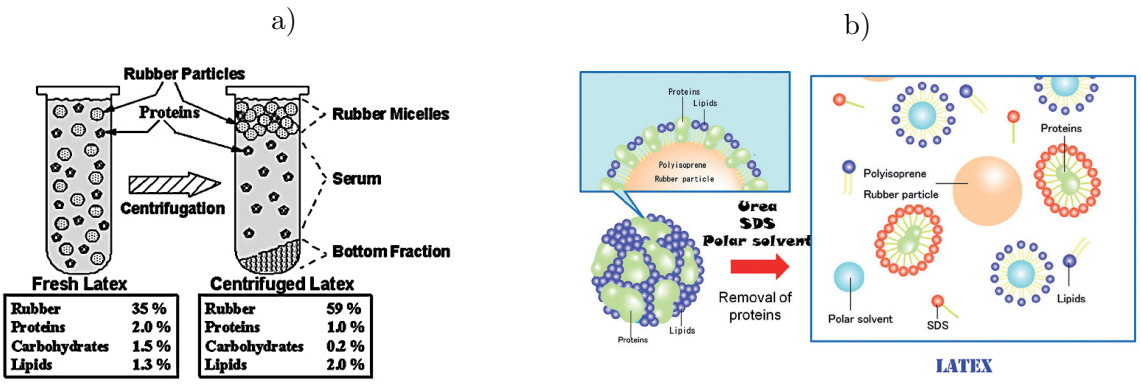


Figure 2.12 Basic principle of protein removal by centrifugation [70] (a) and the according implementation of urea and SDS [8] (b).

Proteins can be denatured with proteolytic enzyme or urea in the presence of sodium dodecyl sulfate (SDS) and migrate from the latex particles into the serum phase. Subsequent centrifugation enables the proteins to be removed from the latex. It was

reported that the combination of denaturation by 0.1% urea with solubilization by SDS and acetone substantially completely reduced the protein content of commercial NRL. This was derived from measurements of the nitrogen content [8]. An optimal urea concentration of approx. 1-2% was found. Higher concentrations reduced the latex stability, which was manifested in a ZP with an absolute value of less than -35 mV. The film forming properties were also negatively influenced [71]. Finally, it must be emphasized that proteins contribute to the stabilizing layer of the latex particles. Their removal during the deproteinization process makes it necessary to maintain stabilization, which can possibly be achieved by adding surfactants. At the same time, such additives can cause difficulties such as excessive foaming and coagulation problems during dipping processes [22]. Furthermore it was reported that treatment of ammonia preserved NRL with 0.20% EDTA-4Na resulted in a decrease in nitrogen content from 0.587% to 0.008% as measured by the Kiehldahl method. The procedure was also based on a denaturation of proteins prior to the serum removal step [72]. Another approach was described as replacing the proteins rather than destroying them. The addition of polyelectrolytes such as polyethylene glycol (PEG) could possibly replace the particle-bound proteins, which could be achieved by a higher affinity for the positively charged inner phospholipid layer. The excess PEG would then be removed by adding compounds with salting out properties. Unfortunately, studies have shown rather poor results. Treatment with PEG could remove approx. 35% extractable proteins from common NRL. Adsorption of proteins to the PEG macromolecules was seen as crucial for the protein reduction [73]. Other groups reported that the use of aluminum hydroxide could reduce the protein content of common NRL by 63% [74]. As part of the development of methods for analyzing latex proteins, it was shown that the proteins bound to latex particle can be effectively solubilized by Triton X-100 and SDS [75]. A purification sequence that included dilution, centrifugation and redilution reduced total serum allergens in common NRL by 53%. Treatment with Triton X-100 or SDS, followed by a sequence of creaming, phase separation and redilution, reduced the allergens in each case by up to 93 or 92%, measured with LEAP-ELISA [76]. These considerations could be used and adapted to isolate TKS latex with reduced protein content, as demonstrated in section 4.3.3.

2.3 Isolation of Latex

2.3.1 Extraction and Preservation of Latex

First, the considerations for latex stability in section 2.2.3 are resumed in a more specific context of preserving TKS roots and their latex. Second, the extraction and concentration of latex is discussed. Established techniques for NRL in general are discussed together with approaches for the special case of TKS latex.

2.3.1.1 Preservation of TKS and Latex

Initially, the focus is on the preservation of TKS plants and their latex before extraction. It was reported that temperatures as low as −12 °C did not adversely affect latex stability for at least 30 days, as shown in Fig 2.13. However, lowering the temperature to −18 °C resulted in a strong decrease in latex stability [6]. In general, when the dispersion medium is frozen, a third phase occurs in the latex. A pure frozen dispersion medium occurs rather than latex particles being dispersed in crystalline dispersion medium. This reduces the available volume of dispersion medium for the particles and the collision frequency increases. The particles can become trapped in crystals or forced into strong contact and high pressure. The latter leads to particle aggregation and coalescence. This type of destabilization is not visible until the latex is thawed. In particular, it is not known to what extent coacervation occurs during freezing and after thawing. Therefore it is seen more as a freeze-thaw-destabilization [5]. Nevertheless, freezing is an widely used method for preserving vegetables and can be used successfully under certain conditions [77, 78]. At temperatures of 15 °C the latex stability decreases over time. This drop is increasingly pronounced at higher temperatures, as shown in Fig. 2.13. The negative influence of higher temperatures can initially be explained by the evaporation of water. In addition, rotting and putrefaction are enhanced [6]. Given these considerations, it was possible to store roots in piles covered with bast and straw for up to 6 months. Up to 93% of the roots showed a stable latex [6]. Furthermore, a strong influence of the humidity within the piles on the storage success was reported. Accordingly, a large latex loss due to coagulation was observed in the upper pile layers. It was also recommended to store roots in alternating layers of ground earth and straw [6].

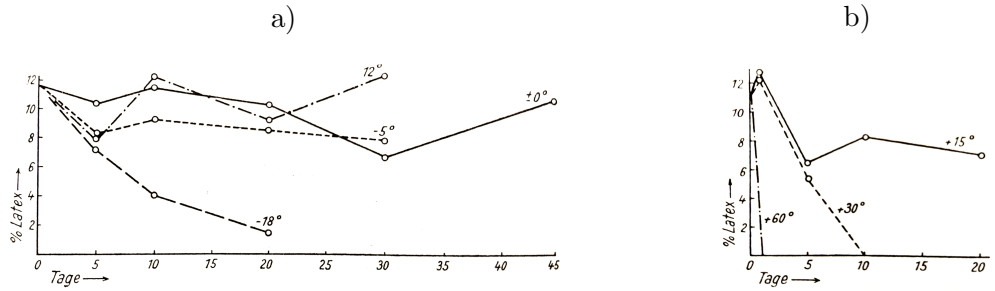


Figure 2.13 Latex yield of TKS in % as a function of the time in days for lower (a) and higher (b) temperatures in °C according to [6].

As suggested by colleagues from the Institute of Plant Sciences (University of Regensburg), a storage of TKS in liquid media might be suitable. This could comprise ethanol and mixtures of sorbitol and glycerine as humectants. In addition, the use of sodium azide, ascorbic acid and buffered solutions in general was suggested.
In the following, the preservation of latex after extraction is discussed. When latex is extracted, a strong dilution occurs. Apart from that, the extracts also absorb many other substances such as electrolytes and dirt particles. This represents a completely changed environment and a disturbed equilibrium of the latex. These circumstances carry the risk of coagulation [6]. Spontaneous coagulation can be explained considering that NRL alone is already a good source for microorganism growth. This is due to the presence of nutritious non-rubber substances such as proteins, sugars and amino acids [19, 79]. Microorganisms use these non-rubber substances and produce acids that neutralize the negatively charged stabilizing protein-lipid layer. Destabilization and coagulation of latex are the consequences [19]. Another model describes the hydrolysis of lipid substances in latex, which release fatty-acid anions. These anions possibly adsorb on the latex particle surface and displace adsorbed proteins [79]. In turn, it was reported that the adsorption of such soaps on the latex particle surface contributes significantly to the colloidal stabilization, while adsorbed proteins only have a minor impact [80]. However, general observations tend to support the second model describing the process of spontaneous coagulation. Accordingly, NRL cannot be prevented from spontaneous coagulation by setting the pH to at least approx. 7. At the same time, the pH of NRL must be lowered to below 5 in order to induce coagulation by acidification. In addition to spontaneous coagulation, the putrefaction of latex should also be mentioned, which sets in at a later point of storage and leads to bad odours. Both processes must be prevented by suitable preservation. A preservative of NRL must meet the following prerequisites. It must suppress the ac-

tivity and growth of microorganisms. It must enhance the colloidal stability of latex particles by increasing the surface charge density and the degree of surface hydration. In addition, the preservative must increase the ability of hydrophilic macromolecules to bind to the particle surface, which contributes to steric stabilization. Furthermore, multivalent metal ions must be removed. The latter are important for the well-being of microorganisms and reduce the colloidal stability of latex. Ammonia has been the standard preservative for NRL since the mid-19th century. It is added in an amount of 0.2% for short-term and 0.7% for long-term preservation of latex. The latter implies an ammonia concentration of almost 1 M in relation to the aqueous phase of latex with a rubber content of 60%. Ammonia is an effective bactericide in concentrations above 0.35%. Moreover, it can deactivate multivalent metal ions by formation of ammine ions through sequestration or by removal through precipitation as sparingly soluble compounds. Ammonia establishes alkalinity. The increased pH leads to an increased electric charge density and electrokinetic potential at the interface between latex particles and aqueous phase. In addition, ammonia accelerates the hydrolysis of non-rubber constituents. In particular, the hydrolysis of proteins produces polypeptides and amino acids, while the hydrolysis of phospholipids among glycerol, phosphate anions and organic bases produces long-chain carboxylate anions [79]. As already mentioned, it is assumed that latex stabilization is established by adsorption of these soaps on the particle surface [80]. Unfortunately, the formation of fatty-acid anions is observed during storage of ammonia-preserved latex. They associate cations, which in turn reduce colloidal stability in return. Air-free conditions favor the production of fatty acids and therefore reduce stability. In contrast, an initial increase in stability was reported for storage in excess air [79]. The use of ammonia as a preservative poses several problems. Ammonia is a toxic gas, potentially explosive and exhibits a bad odor. Safe and practical use for those involved in latex processing cannot be achieved. Other problems include corrosion of metals and steel from ammonia vapors and environmental pollution. Adjusting the dosage is also difficult due to its volatility [2]. Finally, ammonia leads to decreased turgor pressure due to cell necrosis and is therefore particularly unfavorable for the FE (see section 2.3.1.2) [6]. In accordance with the 12 principles of green chemistry, it is obvious to look for a substitute for ammonia in order to minimize the chemical hazards while maintaining the effectiveness [81]. A possible substitute for ammonia was found with ETA. It has already successfully replaced ammonia in many products such as hair dye applications [2]. With a pKB of 4.5 [82], ETA has a basicity comparable to that

of ammonia (pKB = 4.75). It contributes to a sufficiently deprotonated protein shell of the latex particles. In addition, safe and practical processing is guaranteed due to the very low vapor pressure and the absence of odors. ETA can be easily prepared by epoxidation of ethylene and subsequent ring opening. Furthermore, ETA showed a similar bactericidal activity as ammonia [2]. Concentrations of at least 1% (v/v) ETA could completely deactivate the bacterial populations within a few days and were as effective as 0.5% (v/v) ammonia [83]. ETA could successfully stabilize TKS latex. This was shown in a sufficiently low ZP that was comparable to values obtained using ammonia and KOH. ETA can therefore be regarded as a suitable preservative for TKS latex [2]. Just like the stabilization by naturally formed long-chain carboxylates after addition of ammonia, as described above, it is proposed to add carboxylates such as oleates as stabilizers [79, 6, 22]. Although it has also been reported that adding small amounts of carboxylate soaps accelerates spontaneous coagulation, adding larger amounts leads to a slower coagulation process. The effective concentration range of the soap addition depends on the presence and concentration of divalent metal ions [79]. It was also found that the adsorption of surfactant anions on the surface of the latex particles improves the mechanical stability of NRL. The extent of adsorption initially increased sharply with increasing surfactant concentration. At the critical micellar concentration (CMC) of laurate and SDS solutions examined, the adsorption reached a plateau. In addition, increasing adsorption of SDS on the latex particles was observed with decreasing pH. This showed a reduced interfacial activity of the present proteins and fatty acid soaps with decreasing pH [22]. In addition, BAC is a potential preservative [2] due to its cytotoxicity [84]. It is a mixture of alkyldimethylbenzylammonium chlorides with different alkyl chain lengths that influence the bactericidal activity. Accordingly, a longer chain increases will initially increase activity until a turndown point is reached. The latter depends on the respective microorganisms. It was found that the most effective component in the presence of organic compounds is C_{12}-BAC. The biological activity involves disorganization of the cell membrane and denaturation of proteins that are essential for metabolism and growth [85]. BAC is well known for its widespread use as a preservative for contact lenses [86]. Recently, it was added to TKS latex in an amount of 0.01% as an anti-bacterial deterioration agent. Finally, it was suggested to add NaSulfite to NRL as an antioxidant [2]. The chemical structures of the stabilizers important for this work are shown in Fig. 2.14.

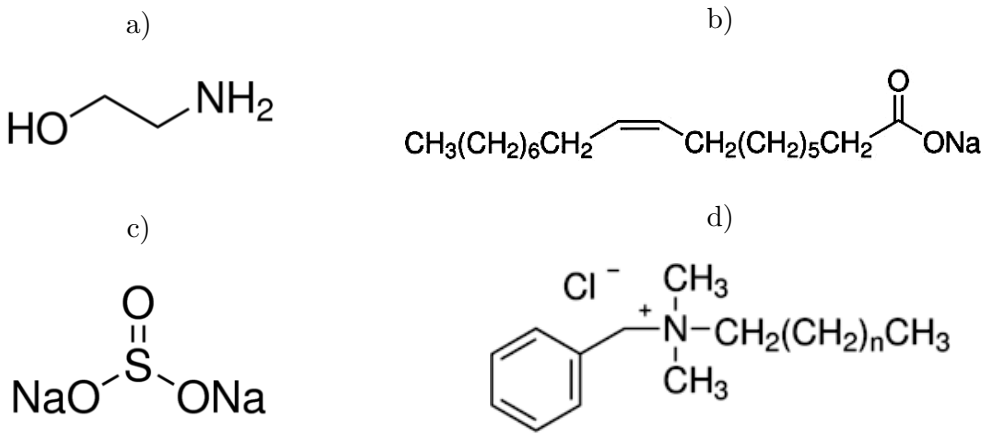


Figure 2.14 Chemical structures of ETA (a) [87], sodium oleate (b) [88], sodium sulfite (c) [89] and BAC (with n = 8 - 18) (d) [90].

In general, the storage time of latex affects the chemical composition of the latex and its properties. For example, it is known that the gel content of ammoniated HB latex increases with storage time. The detailed effects of stabilizers and storage times on the latex properties are still the subject of current investigations [80] and are examined further in section 4.3.2.

2.3.1.2 Extraction of Latex

As described in section 2.1.2, NRL is mainly obtained from the HB tree. The extraction technique commonly used is tapping. Due to the high osmotic pressure in the latex vessels, it is sufficient to cut them through incisions in the bark in order to subsequently achieve latex exudation [79]. Extraction by tapping is not practicable with TKS because of the relatively small size of the roots and the intrinsic viscosity of latex [3]. The cross-sectional structure of a typical TKS root is illustrated in Fig. 2.15. It shows that the latex-containing laticifers are aligned in a concentrically along the root [6]. The extraction of latex NR-bearing plants has been investigated since the 1930s. It was possible to separate the rubber fraction more or less completely from the other constituents which led to dispersions that could be treated in a similarly to common latex [91].

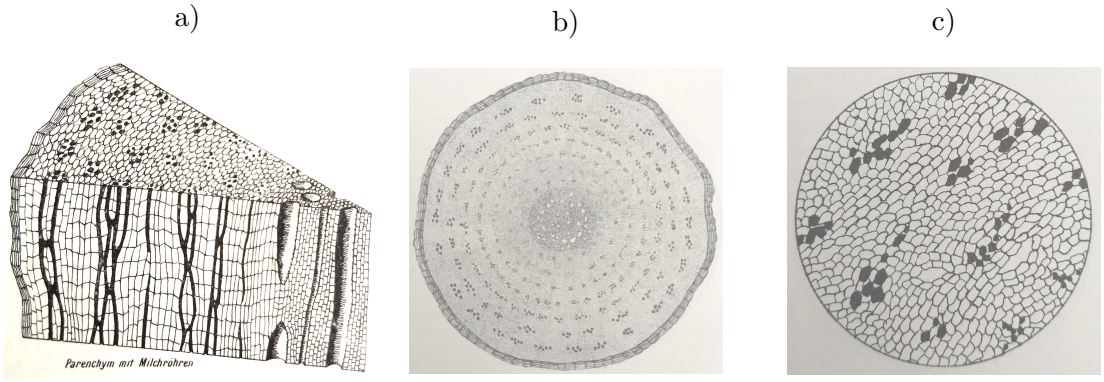


Figure 2.15 Illustrations of a TKS root showing the laticifers (black). Wedge cut (a), cross-section (b) and corresponding cut-out (c) [6].

The blender method is characterized by mechanical processing of the roots in the presence of a buffered stabilizing solution. After the bagasse is removed by filtration, the extracts can be further purified and concentrated. Remaining contaminants such as plant tissue are difficult to remove and reduce the extract quality. The latex yield in relation to the total rubber content of TKS was approx. 50%. It was shown that 96% of the extractable latex can be obtained after two 60 second grinds. [3]. A variation of the blender method is the extraction with a juicer (JE). In contrast to the former, the extraction does not take place immediately in the presence of stabilizing solution. Most of the bagasse is separated before the extracted sap drips into the stabilizing solution. For one, separating bagasse from the start may lead to easier subsequent purification. For another, the unprotected time between chopping and covering with stabilizing solution could possibly have a negative effect on the latex stability [2]. The FE is based on cutting the roots into circular sections. The latter are collected in a buffered stabilizing solution with periodic shaking to allow the latex to flow out [3, 6]. A detailed description of the FE parameters was published in the 1940s and is still relevant. The diameter of the laticifers, the appearance of the latex particles and their concentration influence the success of the procedure. It is also important to completely soak the roots in water prior to extraction. This increases the turgor pressure of the cells surrounding the laticifers, dilutes the viscous latex and facilitates the latex flow after cutting. Compared to extraction after soaking in water for 24 hours, soaking for 4 hours reduced the extraction yield by 25%, while no soaking reduced it by 31%. In order to ensure a sufficiently high turgor pressure, it is also necessary that not too many cells are destroyed during the subsequent cutting. A sharp cut is recommended for this purpose. Another crucial parameter

is the length of the cut root pieces, because latex can only flow out in vicinity of the cut. A size of about 0.5 cm is suitable for extraction, while smaller pieces would damage too many cells. Once the cut roots fall into the stabilizing solution, vigorous stirring is required. This is because the latex has a high rubber concentration and is therefore difficult to disperse. After the primary extraction, the pieces can be transferred to a second stabilizing solution to recover residual latex within the roots. The resulting extract can then be used to process fresh root pieces. This procedure can be repeated several times to obtain more concentrated extracts. Accordingly, the general idea behind the FE is to obtain latex extracts that are as pure and concentrated as possible. Overall yields of up to 49% were achieved with the FE in the lab-scale. It has been reported experiments on a larger scale gave poorer results [6]. Similar results have been reported recently. The latex yield in relation to the total rubber content of TKS was approx. 50% [3]. It was shown that 90% of the extractable latex can be obtained after 30 minutes of shaking. In general, the extracts are relatively clean and not contaminated with plant tissue, which makes the purification a lot easier. A filtration step is also not absolutely necessary. Since the root pieces are sufficiently thick, the extracts can be easily decanted [3]. In addition to these most common methods, approaches that combine various elements of the above have been proposed. One process involved chopping the roots, followed by suspending them in a stabilizing solution and shearing the slurry in an extruder. Extraction under high shear conditions in a continuously stirred tank reactor in the presence of stabilizing solution is also possible. It was assumed that the high shear and cold extraction solution is critical to detachment of latex from the crushed root laticifers. After that, plant fibers could be separated by filtration through a screen [92]. The extraction methods described each comprise differently strong forces that act on the latex. Therefore, it is ultimately important to consider the influence of mechanical agitation on latex stability. The systematic movement of lyophobic sols and Brownian motion itself can cause destabilization and induce flocculation. This is also important for the subsequent latex processing [5]. Beyond the mere extraction of latex, it is evident that the simultaneous extraction of other valuable root components such as solid rubber and inulin should also be taken into account. Such a process is shown in Fig. 2.16. [6].

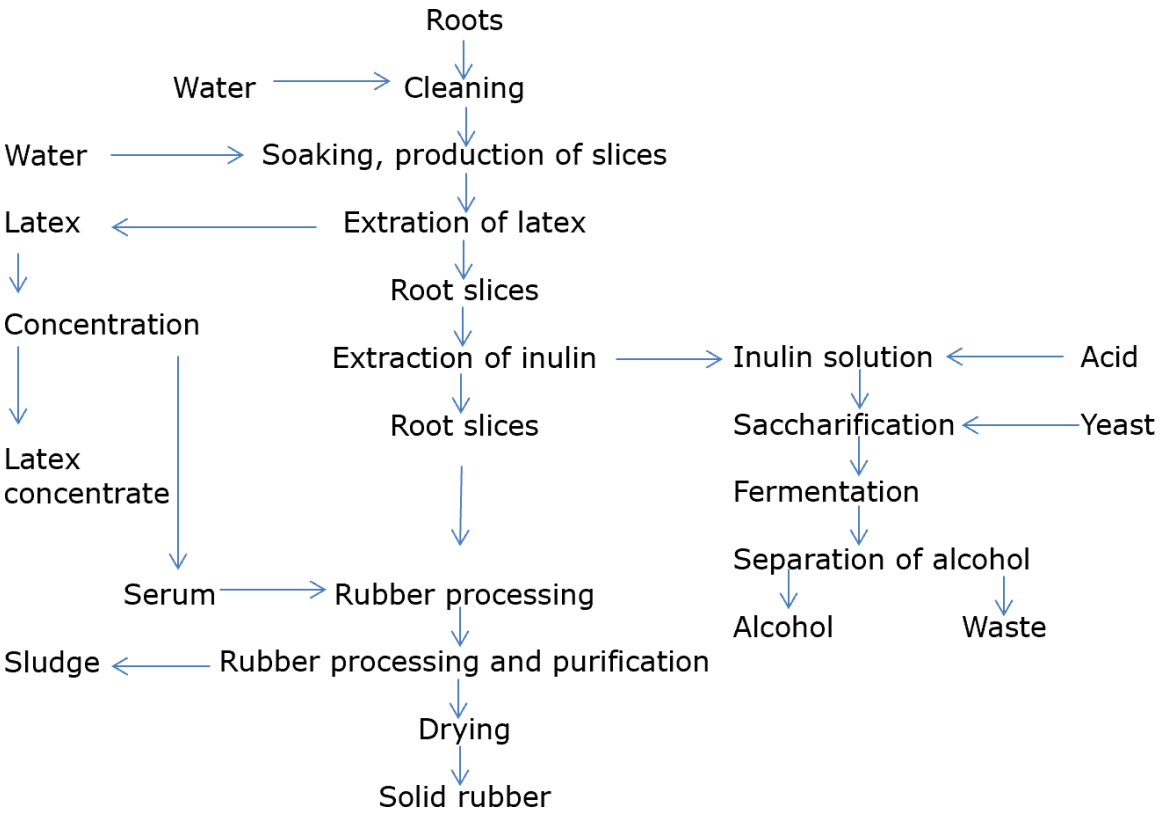


Figure 2.16 Scheme for TKS processing according to [6].

2.3.2 Concentration of Latex

It is necessary to concentrate NRL for several reasons. Unconcentrated latex contains a lot of water, which would be unreasonable to transport over long distances. In addition, some industrial processes, such as latex foam, require highly concentrated latices with rubber contents of more than 33%. Furthermore, the concentration process rules out quality differences between various field latices. Finally, the ratio of non-aqueous non-rubber substances to dry rubber can be reduced due to the preferential elimination of the former. It enhances the character of a colloidal dispersion of NR in water compared to common field latex, making it more suitable for certain industrial processes. Latex is usually concentrated to rubber contents of about 60% or more. To achieve this, various methods are used in industry. Evaporation simply removes water, which does not change the PSD. In contrast, creaming and centrifugation change the PSD, which is caused by the partial elimination of smaller particles

during concentration. Centrifugation is considered to be the most important method of concentrating NRL as 90% of NRL concentrates are obtained by this method. The remaining portions of concentrates are obtained either by creaming, evaporation, combining centrifugation and creaming, or combining multiple centrifugations with dilutions [79]. First, the relevant principles and practical aspects of latex concentration in general are presented. Then, particular approaches for the concentration of TKS latex are outlined.

2.3.2.1 Evaporation

When the dispersion medium is evaporated, the available volume of the dispersion medium for the particles is reduced. As a result, they must occupy positions with a reduced average interparticle distance. Destabilization of latex is observed, which leads to film formation. In general, the destabilizing effects can be predicted based on changes in the barrier height ΔG^*, as discussed in section 2.2.3. Accordingly, changes in the following factors with temperature are important: The average kinetic energy of the particles, collision frequency between the particles, thickness of the electrical double layer, permittivity of the dispersion medium and solvency of the dispersion medium for lyophilic stabilizing macromolecules [5]. Various ways of latex concentration by evaporation have been suggested. In a process by KDP Ltd, latex is heated in a double-walled drum by hot water circulating in the space between the walls. The continuous circulation of latex reduces skin formation and coagulation. In a process by Metallgesellschaft AG, latex is heated and sprayed into a partially evacuated chamber. Water evaporates as soon as the latex leaves the spray, with almost no foam formation [79].

2.3.2.2 Creaming

If colloidal particles have a different density than the dispersion medium and there is a gravitational force, movement of the particles occurs. If the density of the particles is lower than the density of the dispersion medium, they concentrate on the surface [79]. The main part of the rubber is then in this upper phase, which is referred to as the cream. Substances such as proteins, sugars and electrolytes are mostly in the lower phase, which is known as the skim and contains practically no suspended matter [93].

The opposite case of this creaming is sedimentation. It occurs when the density of the particles is higher than the density of the dispersion medium. Both processes are opposed by the Brownian motion of the particles. The variation of the equilibrium particle concentration with height is defined by the opposing tendencies of separation and dispersion and illustrated in Fig. 2.17. As a special case of the Boltzmann distribution, the ratio of the equilibrium particle concentrations at locations 1 and 2 can be written as follows [79]:

$$\frac{c_1}{c_2} = exp\frac{-W}{kT} \tag{2.24}$$

where W is the work for the reversible movement of a particle from location 1 to 2, T the temperature and k Boltzmann constant. In the case of a colloidal dispersion with spherical particles under the sole influence of gravity, W equals mgh with the effective mass m and the height h. With the particle diameter x, the particle density ρ and the density of the dispersion medium σ, m can be expressed by [79]:

$$m = \frac{\pi x^3(\rho - \sigma)}{6} \tag{2.25}$$

The equilibrium particle concentration c at height h can be thus be written as follows [79]:

$$c = c_0 exp - \frac{\pi x^3(\rho - \sigma)gh}{6kT} \tag{2.26}$$

with c_0 as the equilibrium particle concentration at zero height [79].

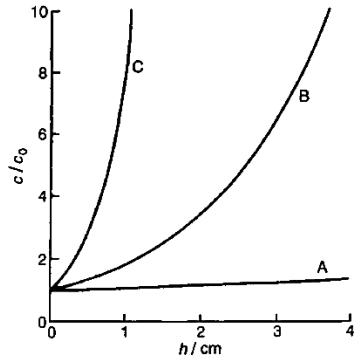


Figure 2.17 Predictions for variations of the ratio c/c_0 with h at 25 °C for dispersions of monodisperse spherical particles with diameters of 50 nm (curve A), 100 nm (curve B) and 150nm (curve C), with $\rho = 0.950$ Mgm^{-3} and $\sigma = 1000$ Mgm^{-3} [79].

For industrial processes the creaming rate is equally important. It can be derived from the equilibrium created by the forces acting on the particles. On the one hand,

there is the gravitational force, which for spherical particles with the diameter x reads [79]:

$$F_G = \frac{\pi x^3(\rho - \sigma)g}{6} \tag{2.27}$$

One the other hand, there is the force of the viscous drag, which is given by [79]:

$$F_V = 3\pi\eta x\frac{dh}{dt} \tag{2.28}$$

with the viscosity of the dispersion medium η and the particle speed $\frac{dh}{dt}$. In a steady state, this leads to the creaming rate [79]:

$$\frac{dh}{dt} = -\frac{(\rho - \sigma)g}{18\eta}x^2 \tag{2.29}$$

It becomes clear that $\frac{dh}{dt}$ is directly proportional to the density difference $\rho - \sigma$, to the square of the particle diameter and inversely proportional to the viscosity of the dispersion medium. So if $\rho < \sigma$, there is a positive creaming rate, which means that the particles are moving upwards. Large differences in density between particles and dispersion medium facilitate fast creaming. The same applies to large particle diameters and a low viscosity of the dispersion medium. These findings also mean that creaming time affects the PSD in both the cream and the skim. Eq. 2.30 defines x_h, which is the diameter of the largest particle that remains in a space with thickness δh at a level h after a creaming time h [79].

$$x_h = \sqrt{18\eta h}(\sigma - \rho)gt \tag{2.30}$$

The described particle diameter is directly proportional to $h^{1/2}$ and inversely proportional to $t^{1/2}$. The results is a cream fraction that is characterized by a broad PSD. In contrast, the skim fraction comprises a cut-off at a certain particle size depending on the creaming time. Substances that can facilitate the natural creaming process are called creaming agents (CA) [79]. These can be hydrocolloids such as alginates, gum, pectin or gelatin [93]. Water-soluble high-molecular alcohols such as polyvinyl alcohols, salts of polyacrylic acids, vegetable products such as cellulose, starch and dextrin [94] or carboxylate soaps can possibly be used as CA [79]. When CA is present in NRL, the skim flows out of the bulk phase due to interactions between latex particles. While the border between skim and cream moves upwards, the cream becomes more concentrate with decreasing volume. The skim shows no creaming after separa-

tion. At the same time, the creamy phase continues to concentrate while additional skim is expelled. Thus, the overall process can be described as an expulsion of skim from the latex. CA is added to latex as an aqueous solution in amounts of typically 0.1% on the aqueous phase [79]. After thorough stirring, it is left to stand for 16-40 hours and the skim then runs off [79, 95]. The specific parameters generally depend on the desired latex properties [95]. In addition, it has been reported that creaming can be accelerated at elevated temperatures [93, 94] due to the disappearance of the structural viscosity of the CA [93]. Fig. 2.18 shows how the concentration of ammonium alginate (AA) and locust bean gum affects the rubber concentration of the cream. Initially, the rubber concentration of the cream rises as the CA concentration increases. The curve passes a maximum and then decreases again. The rubber concentration of the skim decreases with increasing CA concentration and approaches lower values of $< 10\%$. It is also shown that a minimal CA concentration is required to achieve skim with very low rubber concentration [79].

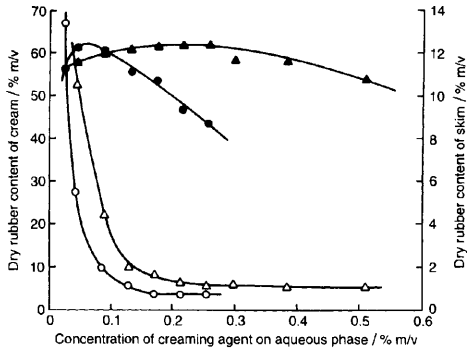


Figure 2.18 Dry rubber content of the cream and skim as a function of the concentration of CA. Investigated were ammonium alginate (● = cream, ○ = skim) and locust beam gum (▲= cream, △ = skim) [79].

The potential of the hydrocolloid to accelerate creaming is related to the increase in the viscosity of aqueous solutions. It is observed that separated skim has a lower viscosity than expected for solutions containing the initial concentration of hydrocolloid. An important aspect is the intensive stirring of the latex after adding CA. It ensures a proper homogenization of the mixture and induction of microflocculation of the particles. Latex obtained after creaming can be rediluted and subjected to further creaming processes. With certain CAs, a decrease in the creaming rate is observed each time until there is hardly any separation. This leads to the assumption of progressive interactions between CA and constituents of the aqueous phase of the

latex. Nevertheless, there are CAs where this finding could not be confirmed and the creaming can be repeated more often [79]. When using sodium alginate (SAL), latex can go through more than six creaming steps [93]. In general, it is believed that the enhancement of the creaming process is caused by an increased effective size of the latex particles. Their diameter enters the term for the buoyancy force of the particles with the third power. The viscous drag on the particles only increases with the first power of the diameter. In addition, the differences in equilibrium particle concentration become more pronounced with height. This is due to the fact that the work for the particle movement increases with the third power of the diameter. Apart from that, larger particle sizes are accompanied by a slower Brownian motion. This evidently also facilitates the creaming process [79]. The increased effective particle size in the presence of CA is explained by various theories. One of them suggests the adsorption of the hydrated hydrocolloids on the latex particles [79]. In this case, however, an unusually high degree of particle hydration would be required. Moreover, this extreme hydration would comprise the reduction of the density difference between particles and dispersion medium. This would be indeed detrimental to the creaming process [79]. Accordingly, it was confirmed that adsorption was not essential for the creaming process using SAL [93]. The CA concentration in the serum after creaming would necessarily become lower, the higher the initial rubber concentration of the latex before creaming. These results were not observed and weaken the theory of the adsorption of CA on the latex particle surface [96].

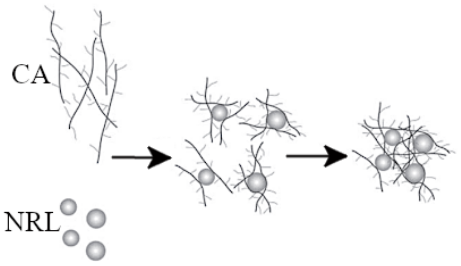


Figure 2.19 Mechanism for the action of CA on the creaming behavior of NRL. Interactions between CA and latex particles gradually lead to the formation of networks [97].

Another theory describes the formation of loose networks between CA adsorbed on the particle surface and CA that remains in the aqueous phase. The Brownian motion of latex particles is reduced due to their inclusion in these networks [79]. It has been suggested that creaming with hydroxymethylcellulose has a similar effect. The dispersed hydrophilic colloid was believed to interact with negatively charged

outer layers of the latex particles. As a result, the CA covered the surface of the latex particles, as shown in Fig. 2.19 [97]. In addition, there is a theory that explains the increased effective particle sizes with loose, reversible agglomeration of the latex particles induced by the CA [93]. This leads to a reduced electrical charge density on the surface of the latex particles [79]. Due to the high water-binding capacity of the hydrophilic CA, the adsorbed stabilizers are partially dehydrated [93]. This effect can be described as mutual desolvation caused by the coacervation of hydrophilic colloids of the same charge [96]. In return, the latex particles are destabilized and aggregate [93]. Therefore, there is a loose coherence of latex particles without coalescence. At the same time, it is assumed that the adsorbed layer of hydrocolloids prevents the coalescence of latex particles. However, for many anionic CAs such as alginates, no reduction in electrical charge density by adsorption has been reported. However, the same observations were made regarding increased creaming rates [79]. In addition to the creaming process by itself [79], the use of CA affects the particle size of latex particles. It has been found that a larger average particle size is obtained when lower CA concentrations are used [98]. General advantages of creaming are its simplicity and the relatively low energy consumption. Furthermore, only small amounts of rubber are lost in the skim [79]. Nevertheless, the process is slow, sensitive to differences in the original latex and problematic due to after-creaming during storage [79]. The presence of residual CA may also affect the colloidal and film-forming properties of the latex [79].

2.3.2.3 Centrifugation

Centrifugation is basically an accelerated creaming process. In contrast to the latter, the movement of latex particles within the aqueous phase is influenced by a centrifugal field and only to a minor extent by the gravitational field. The centrifugal acceleration depends on the particle location within the dispersion medium, which is not the case for the gravitational acceleration. For a particle path with the radius R, the centrifugal acceleration is $\omega^2 R$ with the angular speed of the particle ω. It is therefore directly proportional to the distance from the centrifuge axis. Nevertheless, the elementary requirements for concentration are the same. There must be a distinct difference in density between the particles and the dispersion medium. Centrifugation is also opposed by the Brownian motion of the particles. Again, the equilibrium

variation of the particle concentration can be predicted using the Boltzmann distribution. For the movement of a particle from a circular path with the radius R_0 to a concentric circular path with the radius $R < R_0$, the reversible work against the centrifugal force reads $\frac{1}{2}m\omega^2(R_0^2 - R^2)$. The effective mass m equals $\frac{\pi x^3(\rho-\sigma)}{6}$ for spherical particles with the diameter x, the density ρ and the density of the dispersion medium σ. The equilibrium variation of the particle concentration can then be written as follows [79]:

$$c = c_0 exp - \frac{\pi x^3(\rho - \sigma)\omega^2(R_0^2 - R^2)}{12kT} \tag{2.31}$$

Eq. 2.31 shows that the direction and degree of separation depend on the density difference $\rho - \sigma$. Therefore, NRL particles move towards the centrifuge axis, since $\rho < \sigma$. The degree of separation also correlates with ω^2 and $x^3(\rho - \sigma)$. The strong dependence on the particle diameter is illustrated in Fig. 2.20 [79].

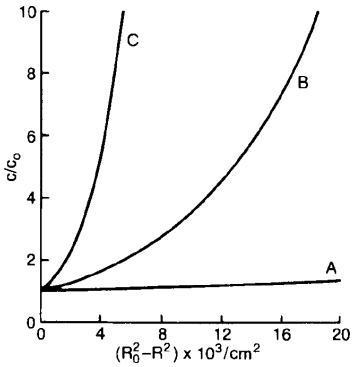


Figure 2.20 Predictions for variations of c/c_0 with $R^2 - R_0^2$ for a rotational frequency of 6000 min^{-1} at 25 °C for dispersions of monodisperse spherical particles with diameters of 50 nm (curve A), 100 nm (curve B) and 150 nm (curve C), with ρ = 0.950 Mgm^{-3} and σ = 1000 Mgm^{-3} [79].

In Fig. 2.20, the decreasing separation between the particles with increasing c was neglected and therefore also the contribution of the colloidal stability to W. This would in fact lead to a weaker increase of c with $R^2 - R_0^2$ for systems with high particle concentrations. The separation rate also needs to be discussed. Again, it can be derived from the equilibrium of forces that act on the latex particles. On the one hand there is the centrifugal force, which is given by [79]:

$$F_C = \frac{\pi(\rho - \sigma)\omega^2 R}{6} \tag{2.32}$$

where x is the diameter of a spherical particle at the distance R from the centrifuge

axis. The opposing force of the viscous drag is [79]:

$$F_V = 3\pi\eta x|\frac{dR}{dt}| \tag{2.33}$$

where η is the viscosity of the dispersion medium. Combining Eqs. 2.32 and 2.33, the steady state of $\frac{dR}{dt}$ can be written as follows [79]:

$$\frac{dR}{dt} = \frac{\pi(\rho - \sigma)\omega^2 R}{18\eta}x^2 \tag{2.34}$$

Eq. 2.34 shows the proportionality of the particle speed $\frac{dR}{dt}$ and the density difference $\rho - \sigma$. Again, it confirms a movement towards the centrifuge axis for NRL particles, where $\rho < \sigma$ and thus $\frac{dR}{dt} < 0$. Also $\frac{dR}{dt} < 0$ is directly proportional to x^2 and R, and inversely proportional to η. As a result, large particles, small distances from the centrifuge axis, large differences in density between the particles and the dispersion medium and a low viscosity of the dispersion medium altogether enhance the rate of centrifugation. The centrifugation time affects the PSD both within the cream and the skim. Considering a particle moving from a path with the radius R_0 to R, the diameter of the largest particle that remains in the skim after the time t is defined by x_R [79]:

$$x_R = \sqrt{\frac{18\eta}{(\sigma - \rho)\omega^2 t}ln\frac{R}{R_0}} \tag{2.35}$$

Fig. 2.21 shows predictions of x_R with respect to the elapsed time and the distance from the centrifuge axis. It also predicts the centrifugation time as a function of the distance from the centrifuge axis. Accordingly, particles smaller than x_R should be present in both cream and skim, while the skim does not contain any particles that are larger than x_R [79]. Both creaming and centrifugation generally result in NRL concentrates with bimodal PSDs. While creamed latex shows broader distributions, centrifuged equivalents show narrower distributions [97]. A comparison of the cumulative PSD of non-concentrated, creamed and centrifuged NRL is given in Fig. 2.22. It shows that the PSD of the concentrates is similar to the non-concentrated latex. The proportion of smaller particles in the concentrate is always lower than in the skim. At the same time, the skim does not contain any particles that are larger than specified in Eq. 2.35 [79].

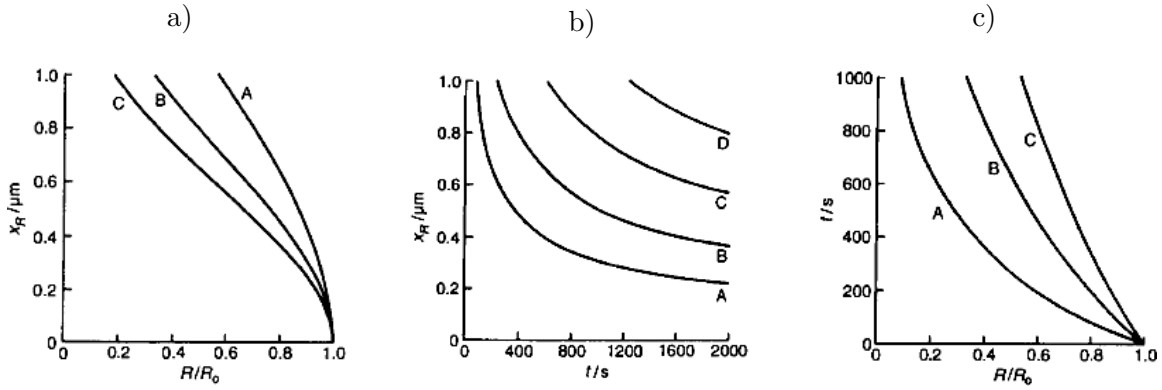


Figure 2.21 Predictions of x_R [79]. a) Variation of x_R with R/R_0 for times of centrifuging 500s (curve A), 1000s (curve B) and 1500s (curve C). b) Variation of x_R with time of centrifuging (t) for $R/R_0 = 0.90$ (curve A), $R/R_0 = 0.75$ (curve B), $R/R_0 = 0.50$ (curve C) and $R/R_0 = 0.25$ (curve D). c) Variation of x_R with R/R_0 of time of centrifuging (t) required for x_R to have values 1.5 µm (curve A), 10 µm (curve B) and 0.75 µm (curve C).

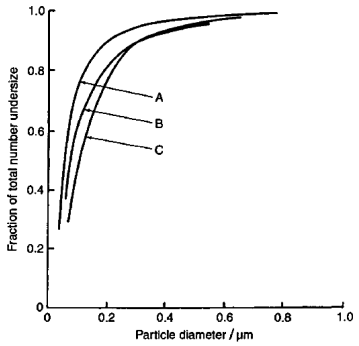


Figure 2.22 Influence of the concentration method on the cumulative PSD. The total particle fraction is plotted against the particle diameter of unconcentrated (curve A), creamed (curve B) and centrifuged NRL (curve C) [79].

In practical, latex is ammoniated prior to centrifugation in amounts from 0.25% to 0.8%, depending on the time since gathering [79]. The CAs discussed in section 2.3.2.2 can also be used for centrifugation [94]. The most popular type of centrifuge for concentrating NRL is the De Laval centrifuge. Latex is divided into proportions by several conical metal disks in a rotating centrifuge bowl, where it is separated into cream and skim. The distance that the particles travel from skim to cream is relatively small compared to the distance from the centrifuge axis. Thus, $\frac{R}{R_0}$ is almost unity and therefore x_R is very small, which results in a high separation efficiency.

2.3.2.4 Concentration of Latex From TKS and Guayule

The combination of the presented techniques has recently been used for the concentration of TKS latex [3, 92]. It was outlined that raw latex could be passed to a number of latex separators such as diffused air flotation tanks (DAF), continuous centrifugal decanters or quiescent settling tanks for purification and concentration. In addition, centrifugation could then be carried out. It has been suggested to add ammonium hydroxide and sodium carbonate for pH regulation, sodium sulfite or hindered phenols as antioxidants, as well as antimicrobial agents and antifoam reagents. Furthermore, AA could be used as a CA. It was stated that SCs of more than 40 or 50% could possibly be achieved. However, no concrete description of the results or latex properties was given [92]. Buranov et. al separated and purified raw TKS latex by centrifugation using agents such as polyethylene glycol, polyvinyl alcohol, agar, pectin or carboxymethyl cellulose. The upper latex layers were isolated with a pipette and a filler and collected in stabilizing solution. The centrifugation was carried out repeatedly at increasing speeds and time. This procedure made it possible to precipitate insoluble contaminants and tissue fibrils from the raw latex. A maximum rubber concentration of 7.5% was achieved using 3% polyvinylalcohol, while the lowest rubber concentration was 0.5% using carboxymethyl cellulose. Further flotation steps could be added to increase the latex concentration. A solution of 0.3% casein was used as a CA in a ratio of 1:5 (v/v). Purified and concentrated latex could be obtained after several phase separation steps, followed by draining the bottom layer and adding fresh CA. By gradually draining the bottom layer, the latex could be concentrated to a rubber concentration of up to approx. 35%, where no further separation was observed. The fluidity of the latex began to decrease when this particular concentration was reached [3]. In contrast, it was already shown in the 1930s that TKS latex can be concentrated by separators while maintaining normal viscosity up to an SC of 40-50% [6]. Finally, the discussed concepts were also used for concentrating latex from guayule. Repeated creaming and centrifugation sequences were carried out with the addition of glycerol or buffered AA solutions. SCs of approx. 50% were achieved. At this particular concentration, latex underwent a phase change and turned into a non-flowable cream [99]. Beyond these batch-type approaches, continuous concentration by centrifugal separators has also been described for the case of guayule extracts [99, 100]. Several problems arose due to the low rubber contents. First, large amounts of extract had to be fed into the separator until a sufficient differ-

ence in specific gravity was reached. This resulted in coalescence and coagulation of latex which interfered with the continuous discharge of latex cream. Afterwards the latex either overflowed the separator bowl without concentration or the serum was discharged from both the cream and the serum outlet. In contrast, milk can be easily concentrated this way, which was explained by the higher stability of milk particles compared to guayule latex particles. Therefore, it was believed that the concentration did not work properly due to instability under mechanical stress. Attempts to solve this problem resulted in modifications of the separator with a throttled serum outlet in order to generate a back pressure. This enabled a continuous displacement of latex cream and an up to tenfold concentration [100].

3 Experimental

3.1 Extraction of Latex

3.1.1 Preparation of TKS

3.1.1.1 Cultivation of TKS

The cultivation of TKS was managed by ESKUSA (Parkstetten, Germany) and carried out in cooperation with local farmers. TKS was grown mainly on alluvial and sandy grounds. The sowing was usually carried out in spring, the respective harvest in October/November. In addition, TKS was cultivated in greenhouses by ESKUSA and Hortilab (Telgte, Germany).

3.1.1.2 Preservation of TKS

If latex extraction was planned soon after harvest, TKS was stored outdoors on piles or in air-permeable containers under regular humidification. After up to two weeks,

the following storage methods were investigated to further increase the working window for successful latex extraction: TKS was stored in moist sand. In particular, perforated wooden boxes were provided with a grid, followed by a layer of straw and moist sand. By slightly raising the grid, fresh air could circulate. Then TKS was placed on top the sand before adding another layer of sand and so on. A damp cloth was placed on top of the last layer of sand. The boxes were stored inside at temperatures between −5 and 15 °C. Further TKS was placed in solutions of NaSulfite and BAC, ETA/ETA−HCl buffer (as specified in Tab. 3.1) or Agricol (Stähler Agrochemie, Germany), as well as by covering with Agricol or various waxes. The roots were then stored inside at temperatures between 5 and 15 °C. Finally, TKS was frozen by immersion in liquid nitrogen. They were then stored at −10 °C. In all methods investigated, the appearance of the roots was monitored regularly. After storage, the rosettes were cut off and the latex flow examined. Roots showing latex flow were collected and weighed. Further analysis was carried out using light microscopy, DLS and ZP measurements.

3.1.1.3 Separation and Cleaning of TKS

Batches of up to approx. 10 kg TKS were cleaned manually with a brush or with a conventional washing machine (Synthrox, Germany; Model: Chef Cleaner WM-400W-P). Larger batches were cleaned with a beet washing machine (Verfahrenstechnik Jaquet, Germany; Model: ESR 1.0-2.8), as shown in Fig. 4.2. For this purpose, TKS was placed in the water container of the beet washer. From there, the plants passed rotating brushes and were transported towards collecting containers on a shaking conveyor belt. This was repeated if coarser impurities were still visible. TKS was generally cleaned using tap water. In order to assess the influence of remaining soil contamination on the quality of the extracts, plants were washed manually to varying degrees. The addition of NaOleate to the wash water was also tested. The quality of the respective extracts was examined by light microscopy.

3.1.2 Extraction of Latex

Latex extraction was carried out by different methods depending on the amount of TKS available. Lab-scale extractions were performed at room temperature. Larger

scale extractions were performed in a hall at essentially ambient temperatures, which prevailed in November. Unless otherwise stated, the weight of TKS was related to the moist root weight after washing, draining and removing rosettes and leaves. Percentages of stabilizing solutions are defined as percent by weight. It should be emphasized that due to the design of the experiments on a larger scale it was neither sensible nor possible to keep all extraction parameters constant or to record them. In particular, the individual ratios of stabilizer per roots for each individual extraction step and the extraction time must be considered as guidelines. A strict differentiation of the individual fractions received was also not useful. However, the aim was to pursue an overall coherent practice.

3.1.2.1 Stabilizing Solutions

Stabilizing solutions were used to suspend the processed roots and to preserve the extracts. In addition, they were added to the raw latices in the course of concentration and purification. They were mainly based on an ETA/ETA-HCl buffer with NaSulfite, BAC and NaOleate. All ingredients used are given in Tab. 3.1, the exact compositions are given at the respective point.

Table 3.1 Chemicals used for stabilizing solutions. [1] = per analysis, [2] = not further specified by the producer.

Chemical	Producer	purity
Ammonia	Merck, Germany	25%, p. A.[1]
Ethanolamine	Merck, Germany	p. A.[1]
Ethanolamine	Acros organics, USA	99%
Sodium sulfite	Radiometer, Denmark	p. A.[1]
Sodium sulfite	Sigma-Aldrich, USA	≥98%
Potassium sulfite	Sigma-Aldrich, USA	90%
Ethanolamine hydrochloride	Sigma-Aldrich, USA	≥99%
Ethanolamine hydrochloride	Acros organics, USA	99+%
Benzalkonium chloride	Kao cooperation, Japan	n. sp.[2]
Benzalkonium chloride	Sigma-Aldrich, USA	≥95%
Sodium oleate	Sigma-Aldrich, USA	82% fatty acids
Potassium oleate	Synthomer, Germany	17%
Potassium laurate, CE 3111	Synthomer, Germany	n. sp. [2]
Potassium hydroxide	Merck, Germany	tech., n. sp. [2]

The solutions were prepared to achieve a pH in the range between 10.5 and 11. The pH was adjusted with pH paper or with a pH meter (VWR, USA; Model: PC 5000

L), which was calibrated with buffer solutions with pH 7 and pH 10. Stabilizing solutions used in the laboratory were prepared with deionized water. For larger scale extractions, tap water was mainly used for stabilizing solutions.

3.1.2.2 Juicer Extraction

The JE was carried out with a common juicer (Philips, Netherlands; Model: HR/1855). The roots were crushed and the outflowing juice was collected in the stabilizing solution, which was stirred with a standard laboratory magnetic stirrer. Between 0.1-1 L stabilizing solution (1 M ETA/ETA-HCl, 0.01% Na_2SO_3, 0.01% BAC, 0.05% NaOleate) were used per kg roots. Since juicer-type latex extractions have been well described on a lab-scale [3, 2], it was not investigated in more detail. It was mainly used as a simple and quick way to obtain latex to study its concentration potential as well as its colloidal characteristics. It was used for processing batches of up to 10 kg TKS.

3.1.2.3 Flow Extraction

Pretests of the FE were carried out with a slicing machine (Philips, Netherlands; Model: HR1388/80). The roots were cut into pieces of approx. 1-2 mm thick and fell into the stabilizing solution, which was stirred in a washing machine (Adler, Poland; Model: AD 8051). Approx. 1 L of stabilizing solution was used per kg roots. After the cutting was complete, the suspension was stirred for a further 30 min. The extract was drained and the remaining root pieces were squeezed with a potato press to recover the remaining absorbed extract. The resulting fractions were pooled and the pH was adjusted to 10.5 by adding ETA. The main part of the FE was carried out as follows. The roots were cut into pieces of 0.5-1 mm thick with a slicing machine (Kronen, Germany; Model: GS-10). They fell into 20 L stabilizing solution (0.5 M ETA/ETA-HCl 0.15% NaOleate, 0.05% Na_2SO_3, 0.005% BAC), which was stirred with an electric stirrer (Enotenica Pillan, Italy; type: Mill) in a container with a capacity of 90 L. Material that missed the container or accumulated in the machine housing was manually transferred to the stabilizing solution. The root pieces were fed into the stabilizing solution until the freshly cut roots could no longer be completely covered by liquid. The container was then removed from the slicer and

the suspension was stirred further for 30 min. The wet root pieces were removed from the container with an ordinary fishing net. After draining, the roots were transferred to another container with stabilizing solution, which was specifically used to absorb the root pieces from the primary extraction solutions. After another 30-60 min of stirring, the root pieces were removed with the fishing net and drained. Then they were transferred to a hydraulic press (Mannesmann, type: 08730, 20000 kg), where the root material was placed in press cloths that were separated by plastic plates. The hydraulic unit was placed on the top plate and a pressure of up to 20 t was applied. The outflowing extract was collected and pooled with the main extract. The resulting solution was then used for further uptake of roots. The number of repetitions of this sequence was based on the appearance of the extract. As soon as an increasingly brown color and viscosity was observed, fresh stabilizing solution was used for further extraction. A total of approx. 1.3 L of stabilizing solution were used per kg roots. A few changes were made in another implementation of the described FE. In particular, the compositions of the stabilizing solutions were changed to 0.5 M ETA/ETA-HCl, 0.3% KOleate, 0.13% K_2SO_3, and 0.01% BAC. The volume of stabilizing solution was also reduced, so that a total of Approx. 0.55 L of stabilizing solution was used per kg roots. In contrast to the first implementation, the root pieces were transferred directly to the hydraulic press after the primary extraction, without passing through another container with stabilizing solution. The final extract was filtered through a juice sieve (Rink, Germany) and mixed with ETA, resulting in a total addition of approx. 0.02 L per L of stabilizing solution used initially.

3.1.2.4 Mill Extraction

The ME was carried out with an electric mill (Helmut Rink, Germany; Model: Rätzmühle Edelstahl). The main elements that followed were essentially taken over by the FE. In particular, the roots were crushed and fed into the stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% KOleate, 0.1% K_2SO_3 0.01% BAC). The resulting suspension was periodically stirred with a rod. After processing approx. 9 kg roots per 40 L stabilizing solution, the container was removed from the mill and left to stand for up to approx. 30 min. Then, the process including pressing, pooling of the resulting fractions and repeated extraction was continued as described for the FE. A total of approx. 0.42 L of stabilizing solution was used per kg roots. Additional ETA

was regularly added to the RE during the course of the extraction to maintain latex stability. The final extract was filtered through a juice sieve (Rink, Germany) and mixed with ETA, resulting in a total addition of approx. 0.03 L ETA per L of the stabilizing solution used initially.

3.1.2.5 Chemical Digestion

For the purpose of latex extraction by chemical digestion, roots were placed in a solution of 2 M ETA/ETA-HCl, 1.2% KOleate and 1% KOH. Approx. 3.3 L of solution were used per kg roots. The KOH concentration was gradually increased to 7% over several days by adding solid KOH. Part of the suspension was heated with the aid of an oil bath.

3.2 Concentration of Latex

Unless otherwise stated, the latex was first processed within four weeks after the extraction. Percentages of solutions are defined as percent by weight. Creaming was carried out as follows. The CA was prepared as a 1% solution with deionized water and mixed with the latex in separatory funnels with a capacity of 0.1-2 L, containers with a capacity of 5 L or barrels with a capacity of 60-120 L. After thoroughly shaking, the mixture was allowed to stand until a distinct phase separation was observed. The resulting phases were then isolated by either draining the lower phase or removing the upper phase by suction using a water jet pump. Centrifugations were carried out with centrifuges from Sigma (Germany; Model: 3-18KS, swinging and fixed-angle rotor) Eppendorf (Germany; Model: 5415R, fixed-angle rotor), Heraeus (Germany, Model: Megafuge 1.0, swinging rotor) or Eppendorf (Germany; Model: 5415R) at room temperature. Latex was centrifuged in tubes with a capacity of 1-50 mL or buckets with a capacity of 400 mL. The resulting latex phases were isolated by suction with a water jet pump or with a spatula in the case of more concentrated latex. As with extraction, it is emphasized that the concentration process was a gradual process. This involved adaptations and variations of each method. In addition, emerging latex fractions of similar processing history have been pooled over time for an appropriate workflow. However, the individual approaches are summarized as best as possible in

the following and discussed in section 4.2.2.

3.2.1 Concentration by Evaporation

Evaporation tests were carried out using a rotary evaporator and an oil bath. The samples used were HB latex (Synthomer, Germany; 30% NR, 1% NH_3) that was diluted with water (millipore quality, ρ >18 MΩcm) or with a TKS serum. The latter was obtained by centrifuging a TKS extract and removing the latex and sediment phase. NaOleate was optionally added to the samples.

3.2.2 Concentration of JE Latex

Extracts obtained from the JE were processed using repeated centrifugation and dilution sequences. The centrifugations were carried out with the centrifuges from Sigma (swinging bucket-rotor) and Eppendorf (fixed-angle rotor). After centrifugation, the isolated latex was mixed with ammonia, NaOleate or stabilizing solutions. In addition, the sequence was tested using a centrifugable separatory funnel and the Heraeus centrifuge. For a more precise characterization, three types of latex were distinguished, which are described in Tab. 1. These included the RE, the intermediate obtained after the centrifugation and redilution sequence, and the resulting concentrate after the final centrifugation.

3.2.3 Concentration of FE Latex

Extracts obtained from the FE were processed as follows. Two weeks after extraction, NaOleate was added to the extent of 0.15%, whereby the total NaOleate concentration was increased to approx. 0.3%. The pH of the RE was increased to approx. 10 by adding ETA. Then three pretests were carried out using the Sigma centrifuge (swinging bucket-rotor). In Exp.1, the RE was centrifuged at 1000-4000 g for 1-10 min and the supernatant was isolated without the sediment. The supernatant was mixed with a 1% solution of SAL to the extent 1:5 (v/v) and left to stand. Then, the phase separation was monitored. In Exp.2, the RE was split into four samples denoted as A, B, C and D. A and B were mixed with additional NaOleate to the extent

of 0.3%, increasing the total NaOleate concentration to 0.6%. In contrast, C and D still had their initial NaOleate concentrations of 0.3%. All samples were centrifuged at 4000 g for 5 min and the supernatant was isolated without the sediments. Then the supernatants from A and C were mixed with a 1% solution of SAL to the extent of 1:5 (v/v) and left to stand. In contrast, the supernatants from B and D were left to stand without the addition of SAL. Then, the phase separation was monitored. In Exp.3, the RE was mixed with additional NaOleate to the extent of 0.15%, increasing the total NaOleate concentration to 0.45%. After centrifugation at 4000 g for 5 min, the upper phase (defined as solution U) and the serum (defined as solution L) were isolated separately without the sediments. Solutions U, L and the RE were mixed with a 1% solution of SAL and left to stand. Then, the phase separation was monitored. After these pretests, the main part of the RE was mixed in containers with a capacity of 60 L with a solution of 1% SAL to the extent between 1:20 and 1:10 (v/v). The mixture was shaken manually and left to stand until a distinct phase separation was observed. The latex was then isolated. The remaining extract was shaken, left to stand, and the resulting latex was isolated again. This creaming step without additional SAL was repeated once. The resulting latex fractions were pooled and transferred to containers with a capacity of 5 L and mixed with a solution of 1% SAL to the extent of 1:5 (v/v). The mixture was shaken and left to stand. The resulting latex was isolated and transferred to separatory funnels with a capacity of 1-2 L. Then stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% NaOleate and 0.1% Na_2SO_3) was added to the extent of up to 1:2 (v/v). The resulting latex was mixed with a solution of 1% SAL to the extent of 1:5 (v/v). The mixture was shaken, left to stand, and the latex was isolated. This purification step was repeated once. In addition to the main procedure described, minor variations regarding the amount of repetitions and additions of SAL and stabilizing solution were also investigated, which will be discussed in section 4.2.2. However, the underlying principle was always to first concentrate the latex as much as possible by creaming it with SAL and then to introduce redilutions with stabilizing solution for further purification at a later point in time. A part of latex obtained after approx. 5 creaming steps was left to stand to investigate the further phase separation over time. The resulting serum was removed periodically and the latex was isolated after approx. 3 weeks. Latex obtained after several creaming steps was mixed with 1:4 (v/v) of a 5% NaOleate solution and denoted as intermediate latex. Other parts of the intermediate stage were centrifuged with the Sigma centrifuge (swinging rotor). The phase separation was observed and

the resulting latex phases were isolated. Optionally, the latex was mixed with an additional 1% SAL solution before centrifugation. Finally, after examining the basic correlations, the final concentration was carried out. For this purpose, intermediate latex was mixed with deionized water or stabilizing solution to the extent of 1:1-1:3 (v/v). Then, the mixture was centrifuged at 4000-4248 g for up to 60 min and the latex phases were isolated and stored. Other parts of the intermediate latex were stored without further treatment and diluted and centrifuged in the same way at a later time. The exact respective parameters will be discussed in section 4.2.2. For a more precise characterization, three types of intermediate latices with increasing cleaning effort and the resulting concentrates were distinguished, which are described in Tab. 4.4.

3.2.4 Concentration of FE Latex (Variation)

Due to a natural occuring phase separation, the lower sludge phases of the RE were isolated approx. 4 weeks after extraction and examined separately from the main batch. The main batch was mixed in buckets with a capacity of 15 L with a solution of 1% AA to the extent of 1:4 (v/v). It was mixed with a rod and left to stand until a distinct phase separation was observed. The lower, latex containing phases were then isolated, pooled and left to stand for further separation. After removing the upper serum, the latex was mixed with a 1% solution of AA to the extent of up to 1:9 (v/v) and ETA to the extent of 3%. Since essentially no further phase separation was observed, then centrifugation of this intermediate latex was carried out using the Sigma centrifuge (swinging bucket-rotor) at 4000-4248 g for 5-60 min. Various additions and sequences were tested to quantitatively force the latex into the upper phase. For this purpose, the latex was diluted with water or solutions of KOleate or KLaurate before centrifugation. The removal of sediments by a centrifugation step prior to the latex-isolating centrifugation was also tested. Different geometries of the centrifugation tubes were tested. After determining the basic correlations, the intermediate latex was mixed with different amounts of KOleate, KLaurate and water and latex was isolated. This was repeated twice and the respective fractions were isolated. For a more precise characterization, five types of latex were distinguished, which are described in Tab. 4.5. These included the RE, the intermediate latex obtained after sedimentation and latex after each centrifugation step.

3.2.5 Continuous Concentration of Latex

The continuous concentration of latex was examined with a disc separator (Milky, model: FJ 90 PP). Pooled serum phases, which were obtained from the concentration sequence of the FE (variation) and not further characterized, were used for preliminary handling tests. The main investigation was carried out with an extract from the FE (variation), which had been stored for approx. 12 months and then preconcentrated to approx. 1% SC by isolating the naturally sedimented latex. Various influences on the phase separation during centrifugation were investigated. These were the position of the separator screw, the temperature of the extract, the addition of ETA and AA before centrifugation, the number of centrifugations and the amount of sediments removed. The approach of dilution and concentration was also tested. For this purpose, the centrifuged latex was diluted with deionized water to the extent of 1:1 (v/v) prior to centrifugation. Fractions obtained after centrifugation were left to stand and the phase separation was monitored. Latex was isolated and used for further concentration, which was carried out with the Sigma centrifuge (swinging rotor and fixed-angle rotor).

3.2.6 Concentration of ME Latex

Due to a natural occuring phase separation, the lower sludge phases of the RE were isolated approx. 6 weeks after extraction and examined separately from the main batch. The CA was prepared as a 1% solution of AA using tap water. The solution was mixed with ETA (approx. 25 mL per 10 L of AA solution) or stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% KOleate, 0.1% KSulfite, 0.01% BAC; approx. 500 mL per 10 L of AA solution) to increase the pH. Then, the mixture was added to the main RE to the extent of 1:9 (v/v). The influence of the repeated isolation of latex, addition of water and AA on the phase separation and the concentration efficiency was examined. Water was added to the extent of 1:2 to 1:1 (v/v) and CA solution was added to the extent of 1:19 to 1:4 (v/v). As soon as no further purification was observed, centrifugation of the resulting intermediate latex was investigated. The addition of water and KOleate before centrifugation was tested. The resulting latex fractions were isolated and stored.

3.3 Characterization of Latex

3.3.1 Determination of Rubber and Solids Content

The average rubber concentrations of the dry roots and extraction residues were determined by Lifespin (Germany) using NMR. The measurements were generally commissioned via ESKUSA. The SC was determined using a gravimetric method [2]. For this purpose, approx. 0.1-1 g of latex were centrifuged with the centrifuge from Eppendorf or Sigma (both fixed-angle rotor) at 16000 g for 10 min at room temperature. After adding of 0.1 mL glacial acetic acid (Merck, Germany), a further centrifugation was carried out at 16000 g for 10 min at room temperature. The resulting rubber pellet was isolated, washed with water and left to dry for several days. The analysis was carried out in duplicate. Although the method is widely used in ISO determinations [101], it appears that fractions of non-rubber substances are also present in the rubber pellet. Therefore, the values obtained from gravimetry have been referred to as solids content rather than rubber content, which was considered to be a more appropriate definition. The influence of non-rubber substances was reduced by thoroughly cleaning the rubber pellet with water. For the most part, washing with acetone could not remove any further substances from the pellet. An extraction of the residual resins by organic extraction, as described by Ulmann [6], was not used. Accordingly, the difference between solids and actual rubber concentrations should be emphasized and determined more precisely in future studies. However, in the framework of monitoring the various samples emerging during processing and due to the largely observed references to the SC (see section 2.3.2.4), the used determination was considered reasonable. Since extracts and serum fractions had a high proportion of foreign substances, their gravimetric analysis was only used to monitor the gradual concentration. The rubber pellets analyzed were heavily soiled and dispersed as tiny fractions, which led to flawed SC determinations. Final assessments on SC and latex yields were made using latex after at least a single concentrations step. For further investigation of latex constituents, ^{1}H-NMR-Spectroscopy was carried out. For this purpose, approx. 0.1-1 g of latex was dried at 45 °C until a constant weight was reached. After the addition of approx. 2.5 mg of 2,6-dimethoxyphenol (Fluka Chemika, Swiss, >97%) as an internal standard, approx. 1-1.5 g of toluene-d8 (Acros Organics, Belgium, 99.5 atom% deuterated, with 0.03% TMS) were added and the mixture was stirred for 1-2 days. The ^{1}H-NMR spectrum of a defined amount was

then recorded with a Bruker Avance 400 NMR spectrometer at 400 MHz. Since the quantitative analyis by means of NMR turned out to be inapplicable due to the high gel-content of latices examined, it was rather used for an assessment of the presence of non-rubber substances of organic origin.

3.3.2 Zeta-potential Measurements

The ZP of latex was measured with a Zetasizer Nano (Malvern Instruments Ltd, Malvern, UK) using the Doppler electrophoresis method [2]. The dilution medium was a 1 mM KCl solution, which was obtained by dissolving 74.6 mg KCl (Merck, Germany) in 1 L water (millipore quality, $\rho > 18$ MΩcm). The influence of additions to the dilution medium such as ETA (as specified in Tab. 3.1), Na_2SO_4 (Merck, Germany), NaCl (Merck, Germany), NaOleate (as specified in Tab. 3.1) and solutions of HCl (Merck, Germany) or NaOH (Merck, Germany) on the ZP were examined. Approx. 1 drop of native latex, 5 drops of RE, 3 drops of intermediate latex or a spatula tip of concentrated latex were diluted with 85 mL of dilution medium. A dilution series of the stock solution with a dilution factor (DF) of 1:2 in each dilution step was prepared and resulted in 9 dilutions, as summarized in Tab. 3.2. The ZPs were calculated with the Malvern Zetasizer software using the Helmholtz-Smoluchowski equation (Eq 2.19). Three measurements with 20 individual runs for each dilution were carried out at 25 ± 0.1 ℃.

3.3.3 Dynamic Light Scattering Measurements

Samples for DLS measurements were prepared analogously to the ZP measurements, using by a dilution series. The dilution medium was water (millipore quality, $\rho > 18$ MΩcm) which was adjusted to a pH of 10.5 by adding ETA (as specified in Tab. 3.1). It was filtered through a CA-filter (hydrophillic cellulose mixed ester, pore size 0.22 µm, Carl Roth, Germany). Approx. 1 drop of native latex, 5 drops of RE, 3 drops of intermediate latex or a spatula tip of concentrated latex were diluted with 3 mL of the dilution medium. A dilution series of the stock solution with a DF of 1:2 in each dilution step was prepared and resulted in 9 dilutions, as summarized in Tab. 3.2. An additional filtration through a PES-filter (hydrophilic

polyethersulfone, pore size 5 µm, Pall Corporation, USA) was carried out only with the stock solution. The dilutions were carried out directly in the measurement cells, which were dust-free cylindrical light scattering cells (10 mm outer diameter). The closed cells were placed in a tub of toluene of a CGS-3 goniometer system (ALV (Germany) with an ALV-7004/FAST Multiple Tau digital correlator and a vertical-polarized 22-mW HeNe laser (wavelength $\lambda = 632.8$ nm). The measurements were carried out at an angle of 90° for 300 s at 25 ± 0.01 °C. PSDs were calculated with the CONTIN algorithm using ALV correlator software (Version 3.0). Correlation functions were fitted with Table Curve 2D software (Version 5.01). Calculation of the hydrodynamic diameters was performed with the previously determined dynamic viscosity of the dilution medium and its refractive index. The values were obtained with an automatic micro-viscosimeter from Anton Paar (Austria, Model AMVn) and an Abbe refractometer from Krüss (Germany, Model AR4).

Table 3.2 DF of the prepared sample numbers (SN). Samples were prepared up to SN8 for ZP and up to SN9 for DLS measurements.

SN	0	1	2	3	4	5	6	7	8	9
DF	-	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512

3.3.4 Light Microscopy

For light microscopy of latex, a Nikon Eclipse E400 microscope was used. Microscopic images were taken with a Canon EOS 650 D camera along with Canon EOS utility software (Version 2.11). The latex examined was placed on a microscope slide without further preparation. Images were taken with 200x and 400x magnification.

3.3.5 Determination of Protein Concentration

Before the actual determination of the protein concentration, a sample pretreatment was carried out in order to minimize matrix effects. The removal of rubber from samples examined was already achieved in course of purification, as described in section 3.3.6. Other interfering substances were removed using the Pierce Compat-Able protein assay preparation reagent set (Thermo Fisher Scientific, USA). The

determination of the protein concentration was carried out using the BCA assay with the Pierce BCA protein assay kit (Thermo Fisher Scientific, USA). The protocols were taken from the respective user manuals [102, 103]. Accordingly, 0.1 mL of sample was mixed with 0.5 mL of Compat-Able solution 1. After 5-15 min, 0.5 mL of the Compat-Able solution 2 was added. After centrifugation with the Sigma centrifuge (solid angle rotor) at 10000-14000 g for 10-30 min at room temperature, liquids were removed and the tubes containing the protein pellet were left to drain. Samples that had undergone the Compat-Able procedure were mixed with 2.1 mL of the BCA working reagent. Samples that had not undergone the Compat-Able procedure were divided into 0.1 mL portions and mixed with 2 mL of the BCA working reagent. Samples for calibration curves were prepared according to Tab. 3.3. Finally, the mixtures were incubated either 1 hour at 37 °C using the standard protocol or 30 min at 60 °C using the enhanced protocol. Then, the absorption at 562 nm was measured with a spectrophotometer at 25 °C (Tecan, Switzerland; Model: infinite 200Pro).

Table 3.3 Dilution series of the standard test tube protocol with a working range of 20-2000 µg/mL (a-i) and the enhanced test tube protocol with a working range of 5-250 µg/mL (A-F).

Sample	**V(water) [µL]**	**V(BSA) [µL]**	**c(BSA) [µg/mL]**
a	0	300 of stock	2000
b	125	375 of stock	1500
c	325	325 of stock	1000
d	175	325 of b	750
e	325	175 of c	500
f	325	325 of e	250
g	325	325 of f	125
h	400	100 of g	25
i	400	0	0
A	700	100 of stock	250
B	400	400 of stock	125
C	300	300 of stock	50
D	400	400 of B	25
E	400	100 of C	5
F	400	0 of E	0

In a separate experiment, the allergenic potential of various serum fractions obtained after centrifugation of FE intermediate latex was investigated by Synthomer using the FITKit Hev b 1, Hev b 3, Hev b 5, Hev b 6.02 in accordance with ASTM D 7427:2014-09.

3.3.6 Reduction of Protein Concentration

In general, various solutions were added to the sample and mixed. After incubation for 1 hour at room temperature, the mixture was centrifuged with the Sigma centrifuge (swinging rotor and fixed angle rotor) at room temperature. Then the latex phase was isolated and analyzed according to section 3.3.5. Chemicals used for protein removal sequences were SDS (Merck, Germany), Triton X-100 (Sigma-Aldrich, Germany), sodium deoxycholate (DOC, Sigma-Aldrich, Germany) and urea (Merck, Germany). For dilutions and the preparation of additives, millipore water was used (ρ >18 MΩcm). Percentages of solutions are defined as percent by weight. Intermediate latex from the FE served as starting and reference point for the experiments. It originated from concentration by combined creaming, centrifugation and dilution as described in Tab. 4.4. To get an overview of the general protein reduction performance, latex was mixed with different solutions. The compounds used and the resulting mixtures are summarized in Tab. 3.4. After incubation the mixtures were centrifuged at 4700 cm^{-1}. The latex was isolated, transferred to a fresh tube with a spatula, and diluted with 9 parts of water. Another centrifugation was then performed at 4700 cm^{-1} for 20 min. The resulting serum was isolated using a syringe. The latter was then subjected to the Compat-Able pretreatment and the the protein concentration was measured with the BCA assay as described in section 3.3.5.

Table 3.4 Compositions of investigated mixtures to screen the protein removal performance of additives (added as 10% solutions).

V(latex) [mL]	**Additive**	**V(additive) [mL]**	**V(water) [mL]**
0.5	-	-	0.5
0.5	SDS	0.1	0.4
0.5	Triton X-100	0.1	0.4
0.5	SDS + Triton X-100	resp. 0.1	0.3
0.5	SDC	0.1	0.4
0.5	SDS + SDC	resp. 0.1	0.3

Then, purification sequences using urea, SDS and Triton X-100 was carried out analogously to the pretests. Different purification approaches were compared. The syringe approach (referred to as Syringe) was performed by repeatedly removing proteinaceous serum. For this purpose, a mixture of 5.0 mL latex, 1.0 mL of 10% SDS, 1.0 mL of 10% Triton X-100, 1.0 mL of 10% urea and 2.0 mL of water was incubated and centrifuged at 14000 cm^{-1} for 10 min. The serum was removed with a syringe and

subjected to the Compat-Able pretreatment and the protein concentration was measured with the BCA assay. The remaining latex was diluted to the original sample volume with a solution of 1% SDS and 1% Triton X-100, solubilized and carefully decanted into fresh tube without the sediments. 1.0 mL of the mixture was centrifuged at 14000 cm^{-1} for 10 min. The resulting serum was isolated with a syringe and subjected to the Compat-Able pretreatment and the protein concentration was measured with the BCA Assay. This procedure of serum isolation, dilution of remaining latex, solubilization and centrifugation was repeated several times to examine the concentration of removable proteins at each step. The spatula approach (referred to as Spatula 1) was performed by repeatedly transferring the latex to fresh tubes to minimize the carryover of contaminants. For this purpose, a mixture of 5.0 mL of latex, 1.0 mL of a 10% solution of SDS, 1.0 mL of 10% Triton X-100, 1.0 mL of 10% urea and 2.0 mL of water was incubated and centrifuged at 14000 cm^{-1} for 20 min. The latex was isolated, transferred to a fresh tube with a spatula, diluted with 9 parts of a solution of 1% SDS and 1% Triton X-100 and solubilized. It was centrifuged at 14000 cm^{-1} for 20 min. The resulting serum was isolated with a syringe and subjected to the Compat-Able pretreatment and the protein concentration was measured with the BCA Assay. The described procedure of latex transfer, dilution and solubilization was repeated several times to examine the concentration of removable proteins with each step. In a variation of the spatula approach (referred to as Spatula 2), a mixture of 7.5 mL of latex, 1.5 mL of 10% SDS, 1.5 mL of a 10% solution of Triton X-100, 1.5 mL of 10% urea and 3.0 mL of water were incubated. The mixture was centrifuged at 4700 cm^{-1} for 10 min. The latex phase was isolated, transferred into a fresh tube with a spatula and diluted with 9 parts of a solution containing 1% urea, 1% SDS and 1% Triton X-100. The mixture was centrifuged at 4700 cm^{-1} for 10 min. The resulting serum was isolated with a syringe and subjected to the compat-able pretreatment and the protein concentration was measured with the BCA Assay. Again, the described procedure of latex transfer, dilution and solubilization was repeated several times to examine the concentration of removable proteins with each step.

4 Results and Discussion

The following results are primarily presented to give a holistic picture of the isolation and characterization of TKS latex. In addition, more general and less quantifiable observations are discussed as they could be of great importance for future investigations. It should also be emphasized that selected and successful parameters such as centrifugation speeds and times, additions and repetitions of certain sequences are assumed to vary greatly between the extracts, although they were prepared similarly. This is clearly owed to the fact that the roots are a natural product with varying ingredients and soil contamination.

4.1 Extraction of Latex

4.1.1 Preparation of TKS

The preparation of TKS included the cultivation, harvest, storage and cleaning. In general, the preparation and handling of TKS prior to the actual extraction had a major impact on the success of the subsequent latex processing.

4.1.1.1 Cultivation of TKS

In the case of field cultivation, the plants were exposed to various types of stress. First, climatic influences evidently threatened optimal plant growth and even led to minor crop failures. Second, the use of common harvesting machines was associated with damage to the plants. In particular, severed root parts and cracks on the root surface were observed, which increased the tendency of the latex to coagulate. Third, the roots partially burst within a few days after harvest, as shown in Fig. 4.1. Most of the latex from burst roots was coagulated. Moreover, an increased degree of bursting was observed after moistening. It was assumed that this was caused by an increased turgor pressure of the plant cells [6]. Since moistening was just as important for root preservation (see section 2.3.1.1) as high turgor pressure for subsequent extraction (see section 2.3.1.2), its extent should be adjusted depending on the extraction method and time [6]. The implementation with greenhouses generally resulted in smaller roots than obtained from field cultivation, as shown in Fig. 4.1. The advantages were overall undamaged and cleaner roots and the ability to control climatic influences. In the framework of this work, however, field cultivation proved to be the most suitable for investigating the upscaling potential of latex isolation. A common finding was the relatively low stability of latex from roots harvested from May to August compared to fully mature roots from October to November. This was attributed to an incomplete formation of the protein shell of the latex particles [5, 6].

Figure 4.1 Field-grown TKS (a,b) and burst root (c). TKS grown in greenhouses using the substrates vermiculite (d) and perlite (e).

4.1.1.2 Storage of TKS

The main challenge in storing TKS was maintaining latex stability. 3-5 days after harvest and cleaning, 85% of the roots showed latex flow. The losses were attributed to the aforementioned bursts and injuries. With prolonged storage, greater exposure to fresh air reduced the amount of roots with latex flow. This was reflected in roots that had been stored outside in piles for approx. 5 weeks. Roots taken from the bulk were in good condition and maintained latex flow, while roots taken from outer layers showed much poorer results. Ulmann has already reported on the influence of layering and the associated differences in humidity on the success of storage [6]. Undamaged roots could be stored in moist sand. After 4 weeks, they were in good condition and showed no deterioration. Latex flow was observed for approx. 96% of the roots. For this purpose, it was necessary to keep the sand containers continuously moistened by regular pouring and covering. For larger amounts of TKS, this approach might be realized by humidity control devices such as those used in greenhouses or agriculture [99, 104]. The storage of TKS in liquid media such as water, ethanol, vegetable oil or solutions of 1% Na_2SO_3 and 0.1% BAC was not successful. After a few days, the roots showed maceration, a more or less severe fungal infestation and no latex flow. The lack of latex flow might be due to excessive dilution of the latex within the roots, which was made possible by burst laticifers. A change in the colloidal state in terms of coagulation, flocculation or gelation might also have played a role [5]. Covering the roots with the alginate-based Agricol, beeswax or wax, which is used for plant preservation, was also not successful. The seal was insufficient to prevent the roots from drying out. Storage of TKS by simple freezing or shock freezing was not successful either. Essentially, the aim of shock freezing was to inhibit the formation of larger ice crystals, which are known to damage cellular plant structures [77]. Apparently this was insufficient to stabilize latex. In fact, about 1 week after shock freezing and storing at -10 °C, liquid latex was observed upon thawing and cutting the roots. However, the inside of the root was mushy and showed no latex flow, which could be explained by the freeze-thaw destabilization [5]. The stability of the native latex before freezing might be increased according to Blackley [5] and should be investigated further, e.g. by soaking the roots in solutions of NaOleate. The latter has been shown to increase the latex stability, as described in Section 4.3.2.1. Overall, the storage of TKS in moist sand turned out to be the most suitable method to bridge the time between harvest and extraction.

4.1.1.3 Cleaning of TKS

The cleaning of TKS and separation of insignificant parts of the plants was important, since foreign substances would inevitable also be transferred into the extracts. Manual cleaning enabled the complete removal of impurities (see Fig. 4.2), but was tedious and only suitable for lab-scaled extractions of up to 10 kg TKS.

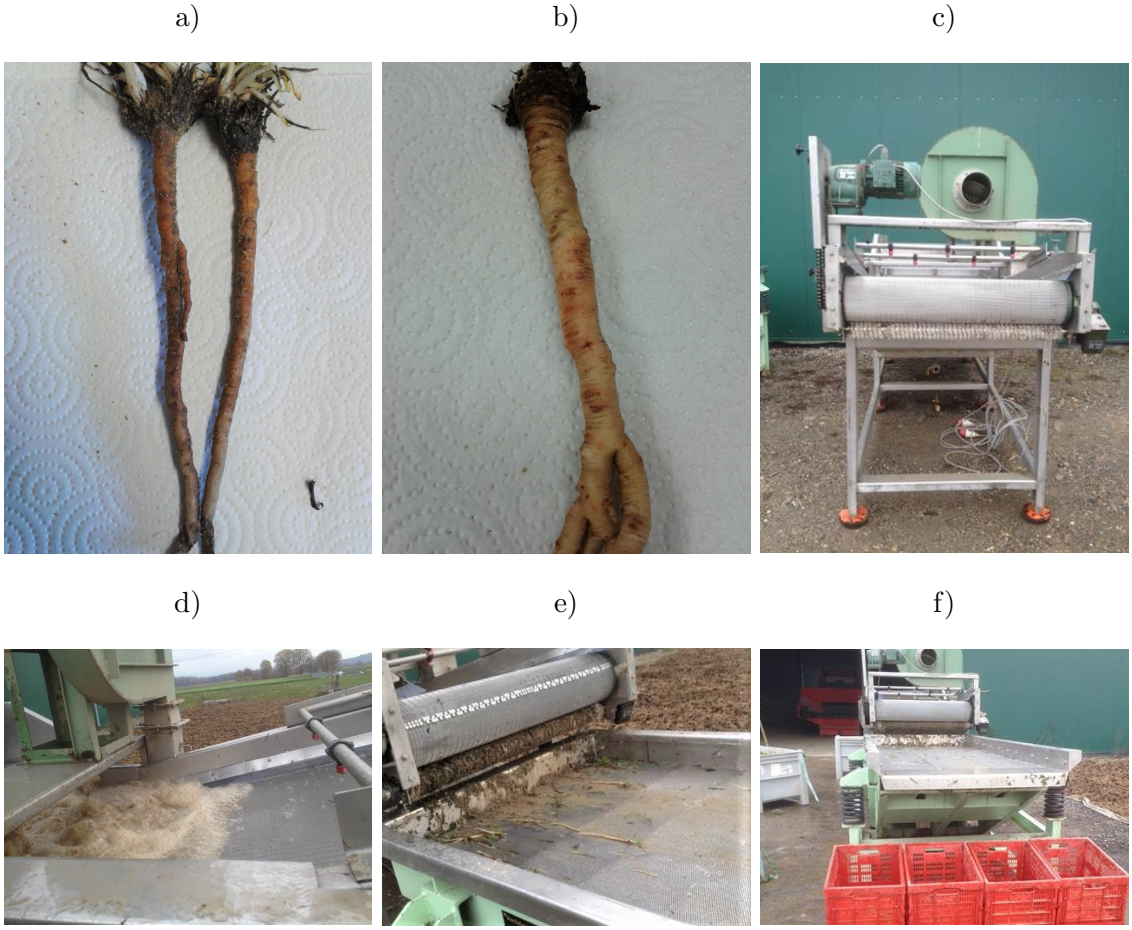


Figure 4.2 Native (a) and manually cleaned root (b). Beet washing machine (c) and corresponding cleaning procedure. After passing through a water container (d), the roots were pressed by rotating brushes (e) and finally transported further on a vibrating belt (f).

Using an ordinary washing machine facilitated the cleaning of TKS for lab-scaled extractions. The overall cleaning performance was acceptable, but stickier impurities on the root surface and loose root and leaf cords could not be completely removed. Coarser material accumulated in the machine and had to be removed regularly. A far more automatic cleaning of TKS was possible with the aid of a beet washer (see Fig. 4.2). With this, a single person could clean approx. 50-100 kg of TKS per

day. Adding NaOleate to the wash water did not significantly improve the cleaning performance as the dirt was generally rather loose. Though, it resulted in a more homogeneous distribution of latex particles, as revealed by light microscopy in Fig. 4.3. This additional stabilization might be utilized for extraction techniques that require an increased latex stability.

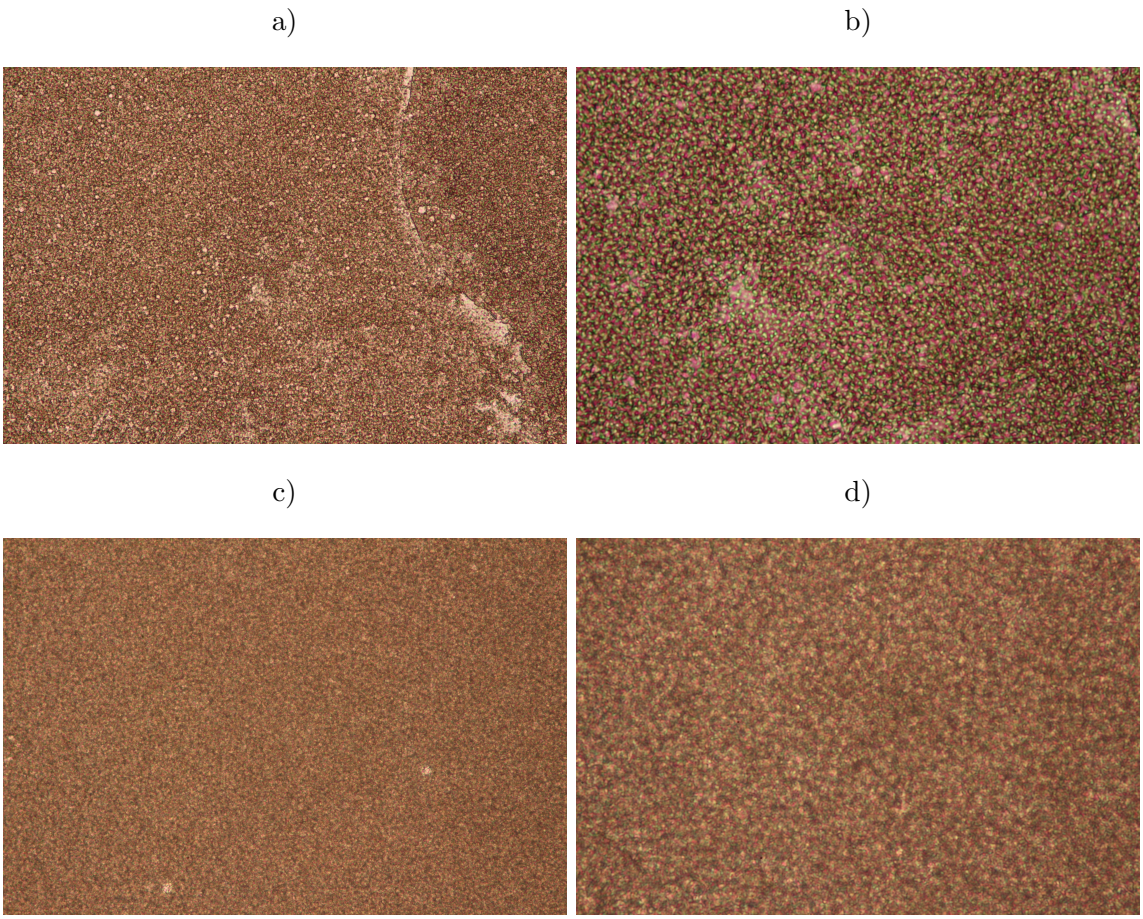


Figure 4.3 Light microscopic images of native latex from roots soaked in water (a,b) and 0.05% NaOleate solution (c, d) for 30 min. Images were taken at 200x (a,c) and 400x magnification (b,d).

In this context, the influence of root contamination on the latex stability of JE extracts was investigated. The use of both poorly and intensively cleaned roots resulted in extracts with hardly any particle aggregation directly after extraction (see Fig. 4.4). The formation of agglomerates began after 4-8 weeks in RE originating from poorly cleaned roots. This destabilizing effect could be explained by an increased activity of microorganisms in the presence of soil, which caused a reduced electrostatic repulsion of the latex particles (see sections 2.2.3 and 2.3.1.1). In addition, soil might

have served as a germ that induced the agglomeration of latex particles. In the case of intensively cleaned roots, no major agglomeration was observed during storage.

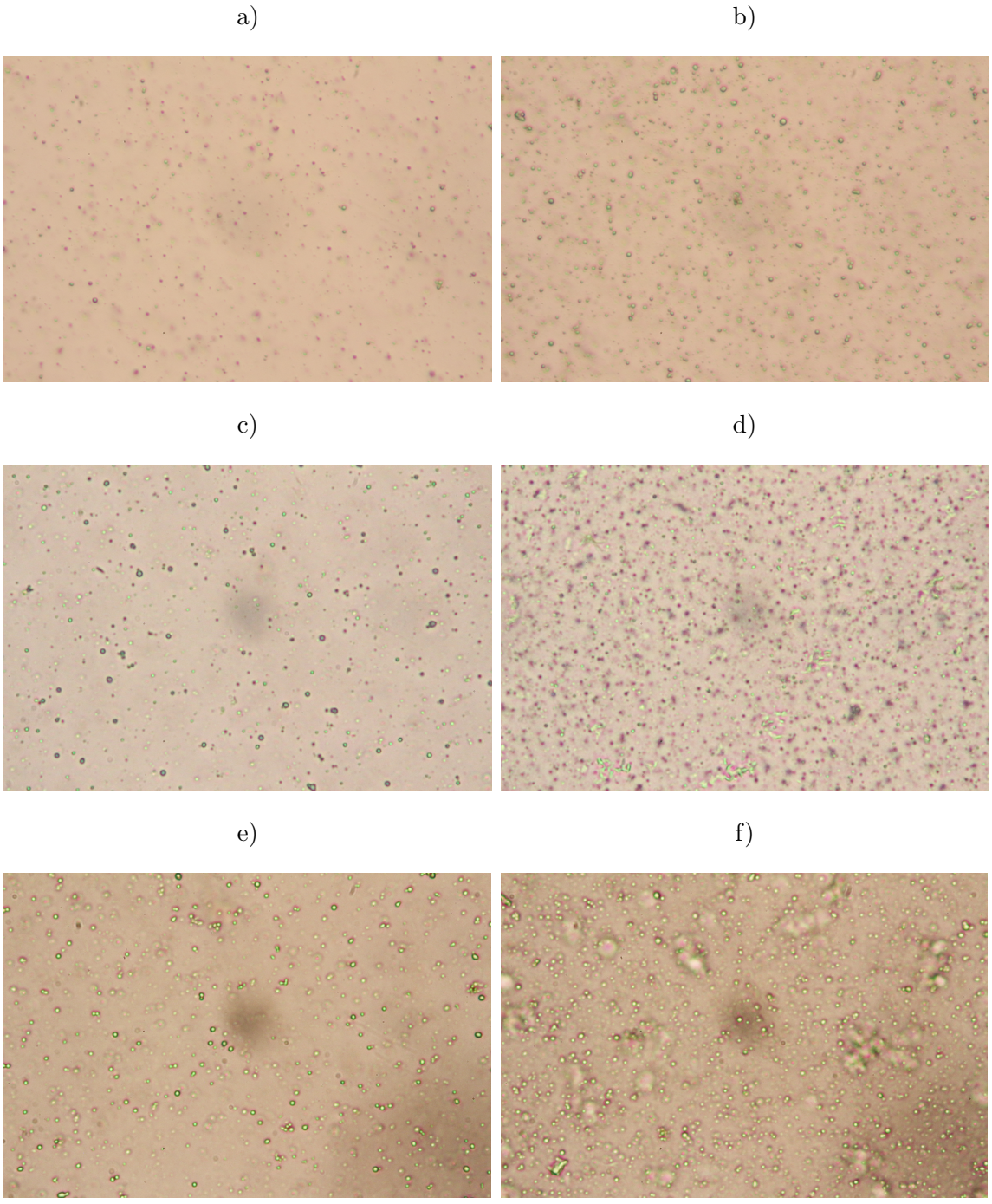


Figure 4.4 Light microscopic images (400x magnification) of RE from intensively cleaned roots (a, c, e) and from poorly cleaned roots (b, d, f), both stabilized with 1 M ETA/ETA-HCl buffer. Images were taken directly (a, b), 4 weeks (c, d) and 8 weeks (e, f) after extraction.

It should be noted, however, that the amount of foreign substances in extracts and their effects on latex quality depended to a large extent on the extraction method (see section 4.1.2). For the latex extraction, only the roots of TKS were required. The rosettes and leaves might be processed in other ways to recover other valuable constituents [105, 106, 107, 108], which was not further investigated. This raised the question of whether rosettes and leaves should be removed before or after cleaning. On the one hand, these generally carried a large part of the soil contamination, which meant additional cleaning efforts. On the other hand, removing rosettes and leaves caused latex losses during cleaning. In any case, rosette removal was best done as late as possible before the extraction step, as damaging the roots facilitated the coagulation process. In practice, it is recommended to decide depending on the appearance of the plant. Greenhouse plants grown on substrates and plants grown on sandy soil were already relatively clean and should therefore ideally be cut after cleaning. For latex extraction with the FE it was important to soak in water for at least 30 min in order to increase the turgor pressure [6], which has usually already been achieved through the cleaning process. Depending on the size of the roots, approx. 10-20 kg TKS could be cut by a single person. However, cutting was the bottleneck of the entire process chain. Efforts to use TKS as a whole for extractions resulted in rapid clogging of the extraction devices. Automation of this step, e.g. with the aid of cutting machines, was not investigated further. According implementations should be able to handle the very different root sizes and produce sections with minimal losses.

4.1.2 Extraction of Latex

After harvest, storage and cleaning, the roots were finally processed into extracts. The main challenges of latex extraction were the release and suspension of latex particles from the laticifers. At the same time, colloidal stability had to be guaranteed. In addition, the amount of suspended plant material had to be minimized. Accordingly, a compromise had to be found between the degree of root shredding and the purity of the extract. The latter greatly influenced the success of the subsequent latex processing. With a dry root weight of approx. 18-19% of the wet root weight, the average rubber concentration was reported to be 3.0% in 2016 (JE), 5.9% in 2017 (FE), 8.0% in 2018 (FE (variation); Extraction by decomposition) and 6.6% in 2019

(ME). Based on the specific root preparation, it was assumed that the actual roots used vary in rubber concentration. Accordingly, using the batch specially prepared for the extraction (2019), only an average rubber concentration of 2.5% was found. It is likely that the cleaning and rosette removal decreased the potentially extractable rubber content. However, these values served as foundation for the following discussions on extraction yields. In this context, the basis for rubber determination in section 3.3.1 should be noted.

4.1.2.1 Design of an Upscaled Flow Extraction

The focus of latex extraction was on the FE. Based on various studies, it was considered as the most promising in terms of up-scaling potential and latex quality. As outlined in section 2.3.1.2, the overall advantage was the high extract purity due to the immediate removal of defined pieces of biomass after the extraction [3, 6]. Preliminary tests, which were not published, indicated that some problems still had to be solved in order to implement the FE on a larger scale. A heavily stirred container capable of draining liquids was introduced to take up the cut root material and replaced the glass flask as the primary extraction container [3]. This allowed the biomass to be separated quickly after extraction and before processing fresh roots for further latex enrichment. The set-up could accommodate approx. 1 kg of roots per L of stabilizing solution. Overall, it served as a first step to increase TKS throughput. The cutting performance of the slicer was not optimal as smaller root strands were simply pushed through the openings. As a result, they could not be cut properly and could not release latex. Softer roots were more likely torn around the rotating knife housing than actually cut. The best cutting performance was observed with larger and stiffer roots. The direct contact of the cut root pieces with fresh air was kept to a minimum due to possible destabilization of the latex [6]. In fact, no such effects were observed, as the root pieces were immediately covered with stabilizing solution. If coagulation was observed, two options would be recommended. The cutting process might be realized directly in the stabilizing solution. Another implementation might include a stabilizing solution that continuously drips onto the cutting edge. These types of processing might lead to increased latex stability, but were found to be difficult to realize in practice. Another practical problem was the residual extract absorbed by the biomass, which was removed after extraction. This was first solved by subsequently

pressing out the separated biomass using a conventional potato press. The production of smaller root pieces facilitated the extract recovery, but also increased the proportion of plant material suspended in the extract. Performed in this way, the pressing process was obviously cumbersome, barely scaleable, and failed to achieve a full extract recovery. These preliminary tests resulted in the following requirements for the further development of the FE. First of all, the extraction device should constantly produce well defined root slices regardless of the appearance of the roots. It should process batches of > 100 kg TKS within one day. The root uptake should be designed so that the roots are straightened in order to produce uniform slices. Furthermore, the slices should be transferred directly to a container with a stirred stabilizing solution. In practice, this meant that the discharge height had to match the height of the container. A sharp material discharge width also had to be guaranteed. Finally, it was found that the industrial slicing machine GS-10 from Kronen AG (Germany) could meet most of these requirements. However, the implementation also encountered some problems. Since the root slices were rather moist, a small proportion stuck to the inside of the machine housing and had to be transferred manually into the stabilizing solution. In addition, the slices were spread over a relatively large area over the tub that contained the stabilizing solution. Installing a small funnel on the material outlet could solve this problem. In contrast to the initially used slicer, the GS-10 could handle any type of roots and produced well-defined slices. Stirring was achieved by an automatic mixing station from Eibenstock (Germany). In addition to the sufficiently vigorous stirring, it could be separated from the stabilizing solution quickly and easily. The latter was necessary for the removal of biomass after each extraction step. In this way, up to approx. 0.5 kg of cut roots per L of stabilization solution could be handled. Beyond this limit, stirred extract showed an increasingly severe imbalance. As the degree of enrichment increased, the effective capacity of roots decreased. Because the sequence of root processing and subsequent biomass removal had to be repeated several times, a more efficient workflow was required. To solve these problems, three interchangeable extraction stations were set up to allow for a continuous process. In particular, the respective tub could be removed from the material discharge after its capacity has been exhausted. Thereafter, stirring could be continued to complete the extraction. At the same time, a second stirred stabilizing solution was be placed under the material discharge, so that the root processing could be continued immediately. As soon as the root capacity of the second tub was exhausted, the procedure was repeated with a third stirred stabilizing solution. In

the meantime, the first extract has finished stirring and the root material could be removed. Finally, this extract could be used again for further enrichment.

Figure 4.5 Extraction sequence. Slicing machine (a), decimal balance for root weighing (b), placement of the roots on the transportation band (c), simultaneous extraction (d), extraction container (e), removal of biomass from extract (f), filling the press with moist biomass (g), pressing process with additional inserts for increased pressure (h) and extraction residue (i).

The biomass could be completely removed from the extract with the aid of a synthetic net. After reviewing various pressing methods with specialists from H. Rink (Germany), a hydraulic pack press was chosen. It was found to be the most effective method of recovering the absorbed extract in terms of the properties of the root material produced. In contrast to conventional hydraulic basket presses, there was the

advantage that the liquids had to travel shorter distances through the bulk material before they were recovered [109, 110]. The extraction sequence is illustrated in Fig. 4.5.

With 1.3 L stabilizing solution per kg of moist root mass, approx. 0.22 L of juice could be extracted. Since the sharply cut roots could be removed completely, the extracts did not contain any coarser plant material. As a result, the subsequent purification and concentration was generally the least difficult and cumbersome compared to extracts from other extraction approaches. However, the total amount of solution produced turned out to be impractical in terms of storage, resource consumption and processing. For this purpose, the ratio of stabilizing solution to processed roots was investigated. Using 1.3 L stabilizing solution (0.5 M ETA/ETA-HCl, 0.15% NaOleate, 0.05% Na_2SO_3, 0.005% BAC) per kg of moist root mass, the extracts had a light brown color and could be purified and concentrated best in comparison. Using only 0.55 L of stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% KOleate, 0.13% K_2SO_3, 0.01% BAC) per kg of moist root mass, referred to as FE (variation), the extracts were much darker in color and obviously contained higher amounts of plant material. Although no immediate negative effects on latex stability were observed, this led a more difficult purification and concentration. Accordingly, the ratio of stabilizing solution to processed roots must be adjusted depending on the respective further processing, as described in section 4.2.2. The latex yields, based on the extracted solids relative to the average rubber contents of native roots were calculated to be between 14 and 17%. Considering that the rubber content of roots analyzed immediately before the extraction step was much lower than that of native roots, extraction yields might be established relative to this. With such a correction factor based on the roots from 2019 (ME), it would theoretically increase to 37 to 45%. However, this approximation has not yet been adequately supported by experimental data and would definitely imply an enormous loss of latex from rosette removal. In any case, these results show that only a small fraction of the rubber could be extracted as latex. This discrepancy has already been reported by Ulmann and Buranov, where total yields of up to approx. 50% extractable latex were achieved with the FE on a laboratory scale [3, 6]. In larger-scale experiments, however, poorer results were also reported [6]. It was evident that the majority of the differences were due to the removed root material. Although a final assessment was not possible, this could be explained for several reasons. Parts of the latex were still trapped in the laticifers and removed along with the root material. However, it was assumed that given

the intense soaking of the root slices and the structure of the laticifers, this aspect is not critical. It is more likely that latex particles were actually released from the laticifers and thereafter remained in a dispersed state within the root material as part of the extract. It was suggested that interactions within this soaked alkaline biomass prevented the latex particles from being released into the bulk extract. In addition, some of the latex could have been in a coagulated state within the roots before the actual extraction. As a result, it was not possible to extract it in a dispersed state. In this case, after extraction, the latex was present as a solid rubber fraction that was adsorbed to the root material and then removed with it. In order to take some of these aspects into account, further extraction approaches were investigated and discussed in following sections. It should be noted that apart from the primary goal of isolating liquid latex, the solid rubber fraction might of course be recovered for other uses with additional effort as outlined in Fig. 2.16.

4.1.2.2 Mill Extraction

Given the results of section 4.1.2.1, the aim was to minimize the latex losses caused by the root material removed after extraction. By producing smaller root pieces that could be finely dispersed in the stabilizing solution, better release of latex particles was intended. This was investigated by replacing the previously used cutting machine with a mill. The roots were ground into a moist flour with more or less coarse particles, depending on the grid behind the knife housing. Smaller roots were not ground properly when using the 13mm mesh, and rapid clogging was observed when using the 5mm mesh. Using the 8mm grid allowed the best workflow. However, the ground material adhered strongly stuck to the inner walls of the knife casing and the grid due to its inherent moisture. Accordingly, the ground material was fed into the stabilizing solution in portions rather than as a constant stream. In addition, the knife housing had to be emptied regularly to prevent clogging. Contrary to the assumption that this prolonged exposure to air could cause latex coagulation [6, 16], no immediate destabilization was observed. It was suggested that the inherent plant juice in the ground material kept the latex stability until the stabilizing solution was reached. Hardly any coagulated latex was observed within the extract itself. Because of their white color, these fractions of solid rubber were most likely already in the roots before being ground. Considering the extracts, which were strongly interspersed with plant

material and impurities, this approach differed from the FE, which was based on a controlled cutting of roots to minimize the extraction of foreign substances. However, the overall essence of the FE including the removal of biomass after extraction and gradual enrichment was retained. An immediately observed benefit was the more efficient pressing, which was due to the small size of the root material produced, as shown in Fig. 4.6.

a) b) c) d)

Figure 4.6 Extraction setup with mill and stabilizing solution (a), processing of roots into stabilizing solution (b), root material during extraction (c) and separated root material before pressing (d).

Using 0.4 L stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% KOleate, 0.1% K_2SO_3, 0.01% BAC) per kg of moist root mass, approx. 1.2 L of juice could be extracted. As expected, this affirmed an increased suspension of root material compared to the FE. The latex yields were calculated to be about 5% and 14%, based on the extracted solids relative to the rubber content of native roots or roots that were analyzed immediately before the extraction. The dry mass of the remaining root material after pressing was 22% of its moist mass. A rubber content of approx. 2.8% was determined, which corresponds to 54% of the rubber content of the roots immediately before extraction. Apparently a large part of the rubber was again separated off together with the residue. Contrary to the previous considerations, the stronger mechanical processing of the roots could not significantly improve the latex recovery. In other words, these observations partially disprove the assumption that the loss of latex is mainly caused by too coarse a root material. The following explanations for the reduced latex yield by analogy with earlier considerations from section 4.1.2.1 are possible: Again, the latex fractions might have already been in a changed colloidal state within the roots, which made suspending as latex impossible.

It is most likely, however, that the latex particles interacted strongly with the ground material after the laticifers had been opened and therefore could not pass into the stabilizing solution. In any case, this fraction remained adsorbed on the root material removed after the extraction and could not be recovered as a latex dispersion. Finally, the remaining approx. 31% of the rubber was distributed in the sludge and serum phase after processing. This is evident in view of the problems with phase separation as described in Section 4.2.3. At the same time it shows that this latex fraction might still be purified and concentrated. According to the current state of the art, this proposal unfortunately turned out to be extremely difficult and lengthy due to the heavy pollution. Future investigations should focus on a thorough detachment of latex particles from the root material, since simply increasing degree of comminution did not lead to the desired effect. For this purpose, various surfactants might be added to the stabilizing solution. The controlled destabilization of latex, which then leads to microflocculation, should also be investigated. In this way a separation from the root material might be achieved, similar to the results from section 4.2.3. Different types of comminution might also lead to less strong interactions between latex particles and biomass. Finally, the influence of mechanical agitation on the weakening of these interactions should be examined [92].

4.1.2.3 Extraction of Latex by Decomposition

The previous attempts at latex extraction have been characterized by the removal of biomass immediately after the primary extraction step. This was of course beneficial in terms of extract purity, latex stability and concentration potential (see section 4.2.2). However, this separation resulted in a loss of latex, which was presumably caused by interactions with the biomass. Accordingly, quantitative extraction of latex was now attempted, in which the removal of biomass should be achieved at a later point of time, e.g. by other means such as creaming or centrifugation. Therefore, latex was extracted by completely suspending the roots, while particular attention was paid to latex stabilization. This approach was developed based on a method that is actually used to quantify rubber in TKS roots. It involved the release of latex particles by dissolving the outer root surface in the presence of a lye [6]. This would eventually suspend the entire root in the stabilizing solution. In addition, the advantage of chemical decomposition was the avoidance of the relatively high mechanical stress

that arised from the flow or mill extraction. The amount of processed roots per extraction run was limited by the given volume of stabilizing solution (2 M ETA/ETA-HCl, 1.2% KOleate), which was up to approx. 0.3 kg roots per L. After gradually increasing the KOH concentration from 1 to 7% over several days, the roots showed a consistence similar to mash. Coagulated latex was hardly observed and could already have been present in the roots used. In addition, the changed chemical environment could have induced spontaneous coagulation [6]. However, the extract showed an overall stable latex, which was mainly attributed to the strongly buffered stabilizing solution used. Since no root material was removed and latex stabilization could be maintained, an almost complete latex extraction could be achieved at first glance. Evidently, extraction by decomposition would be not practical in this way, because the ratio of processable root mass to solution was far too low. Much faster decomposition would be required to process additional roots. Contrary to what has been reported, a hydroxide concentration of 3-5% in combination with heating was not sufficient to rapidly decompose the roots [6]. As expected, the viscosity of the extract also increased sharply with increasing amount of processed roots, which hindered the addition of more roots. Unfortunately, the purification and concentration of the extracts was extremely difficult, which is discussed in section 4.2.3.

4.2 Concentration of Latex

4.2.1 Concentration of JE Latex

Studies on latex concentration were initiated using extracts from the JE due to their availability and well-known isolation [2]. The approaches discussed in section 2.3.2.4 were resumed and used to develop an efficient and easily scaleable process on a laboratory scale.

4.2.1.1 Preliminary Handling Tests

First, the behavior of extracts during centrifugation is discussed. In general, it was not possible to isolate pure and concentrated latex after a single centrifugation. The upper phase formed was not thick and concentrated enough for proper latex isolation without the carryover of contaminated serum. In addition, the upper phase was in itself full of impurities and of a brownish color. Centrifugation at higher speeds of 16000 g even led to coagulation of the latex. These observations were attributed to too low a SC and high levels of plant material and impurities. It was assumed that the latter hinder the formation of a thick and purified latex phase and have a destabilizing effect [5, 6]. Purification of the extracts by vacuum filtration before centrifugation was not successful. When using pore sizes of at least 8-12 µm, hardly any impurities could be removed. This led to the conclusion that centrifugation cannot be used when a direct, one-step concentration of RE to the final latex products is desired. For now, this should at least be the case for batch centrifuges. For this purpose, the continuous centrifugation of extracts is discussed in section 4.2.4. However, centrifugation should generally only serve as an initial concentration step or be combined with creaming according to the methods given for isolating and stabilizing latex with up to 25-35% SC from juicer or blender extracts [3, 2]. Preliminary tests showed that subjecting such a semi-concentrated latex intermediate to further centrifugation usually resulted in coagulation. To improve latex stability and purity, a sequence of adding water and stabilizers, centrifugation, removal of sediments, and repeated centrifugation could be used. Since the latex formed was white in color and showed no signs of destabilization (see Fig. 4.7), this proved to be the basic approach to enable the isolation of more concentrated latex.

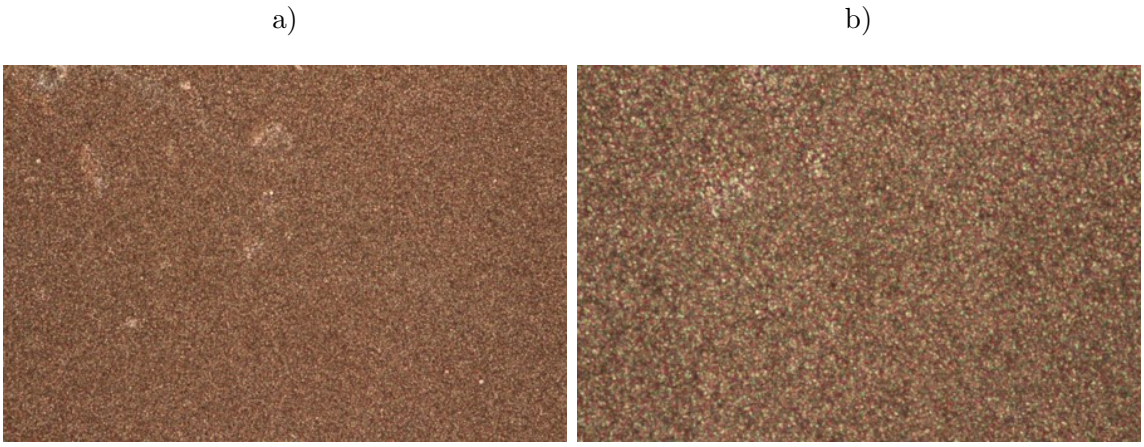


Figure 4.7 Light microscopic images of latex concentrate obtained by a purification sequence. Latex intermediate was prepared by centrifugation (4000 g, 30 min) and natural occuring creaming. Then this intermediate was mixed with 1:9 (v/v) of a 3% solution of NH_3 and NaOleate was added to the extent of 0.3%. After centrifugation (16000 g, 30 min), removal of sediments and repeated centrifugation, a stable and purified latex concentrate was obtained. Magnifications are 200x (a) and 400x (b).

Especially NaOleate proved to be beneficial for purification and concentration. By increasing its concentration from 0.05 to 0.3%, an optimum could be found at which the amount of removable sediments from latex serum could be doubled after centrifugation. This could be explained by the general cleaning effect of soap solutions [111]. The colloidal stability of the latex was further improved, which was already suggested by Ulmann [6]. In particular, the adsorption and accumulation of latex container surfaces during centrifugation and the subsequently increased tendency of coagulation could be reduced. Finally, a centrifugable separating funnel was introduced, which facilitated the repeated purification and concentration. It allowed the lower phase to be easily drained and stabilizing solution added without opening the container. As a result, the process could mainly be carried out in a single container. In contrast, the process above involved transferring latex by vacuum suction through a small nozzle, which easily induced coagulation.

4.2.1.2 Isolation of JE Latex Concentrate

Based on these preliminary tests, centrifugation and dilution were carried out repeatedly in order to achieve an even higher latex concentration than described above. After the extract was subjected to 3-4 processing steps, a purified intermediate latex

with a SC of approx. 3% could be isolated. A total of 3-4 times the amount of the particular dilution medium in relation to the initial volume of the extract was added. Fig. 4.8 shows the increasing phase separation efficiency and purity of the upper latex phase with each purification step. The improved separation performance could be explained by the decreasing viscosity of the aqueous phase, which in turn was due to the decreasing amount of foreign matter [79]. The intermediate latex was white, free of visible impurities and had a low level of particle aggregation as shown in Fig 4.9.

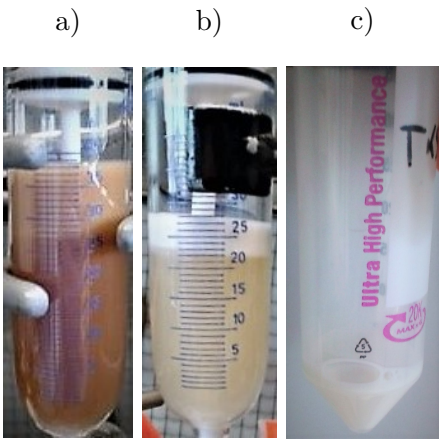


Figure 4.8 Progressing latex purification with centrifugable separatory funnel. Separation of raw latex (a), separation of intermediate latex (b), intermediate latex (c).

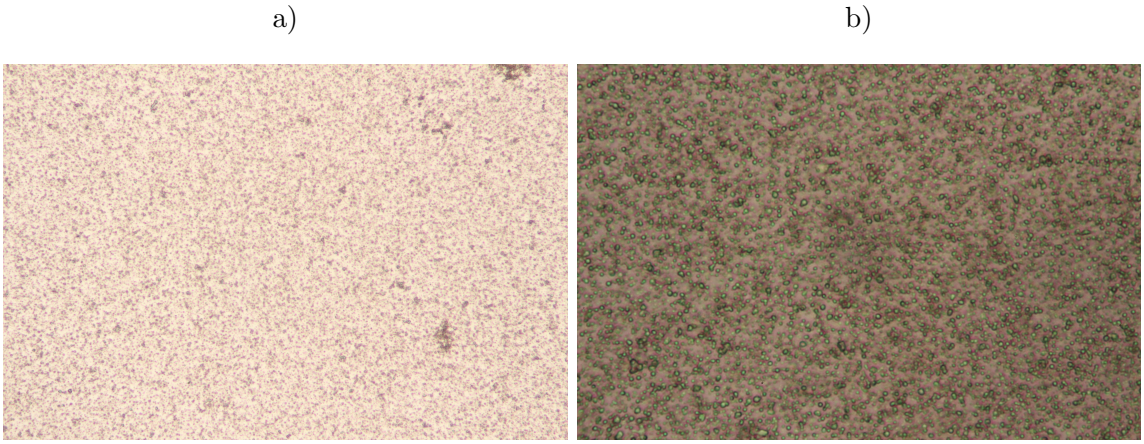


Figure 4.9 Lightmicroscopic images of intermediate latex, obtained by a purification sequence according to Tab. 4.1. Magnifications are 200x (a) and 400x (b).

By subjecting this intermediate to a final centrifugation, small amounts of latex concentrate could be isolated. It was creamy, extremely viscous but not coagulated

or flocculated. Tab. 4.1 summarizes chosen protocols for the concentration of latex using extracts from the JE.

Table 4.1 Successive protocols for the concentration of latex using extracts from the JE. Stabilizing solutions used contained 0.5 M ETA/ETA-HCl 0.3-0.5% NaOleate, 0.01% Na_2SO_3 and 0.01% BAC. The differences in parameters were mainly due to experimental reasons and resulted from the stepwise investigation. Volumes of more than 50 mL could only be handled by the Sigma centrifuge, the centrifugable separatory funnel could only be used with the Heraeus centrifuge, and speeds of more than 4000 g could only be achieved with suitable tubes with the Eppendorf centrifuge. Accordingly, these protocols do not claim to be exclusively valid. Protocol (top) led specifically to the latex characterized above, which was also used for DLS. Protocol (bottom) led to latex as used for ZP-measurements.

• Centrifugation (Sigma, 4000 g, 30 min), isolate cream by vaccuum suction, add 7 parts of stabilizing solution • Centrifugation (Heraeus, 3000 g, 15 min), drain 75% of extract, add 3 parts of stabilizing solution • Centrifugation (Heraeus, 3000 g, 15 min), drain from the bottom until phase separation line (led to intermediate as used for DLS) • Centrifugation (Eppendorf, 16000 g, 10 min), decant latex without sediments • Centrifugation (Eppendorf, 10000 g, 10 min), remove serum with syringe, isolate remaining latex cream (led to concentrate as used for DLS)

• Centrifugation (Sigma, 4000 g, 30 min), isolate cream by vaccuum suction, add 7 parts of stabilizing solution • Centrifugation (Heraeus, 3000 g, 15 min), drain 75% of extract, add 3 parts of stabilizing solution • Centrifugation (Heraeus, 3000 g, 15 min), drain from the bottom until phase separation line • Centrifugation (Sigma, 4000 g), remove serum with syringe, isolate remaining latex cream (led to concentrate as used for ZP-measurements)

Even if it was not yet possible to make a reliable statement due to the small amount available, the concentrates indicated a SC of over 41%. The latex particles were homogeneously distributed, as shown in Fig. 4.10. In contrast, the dilution of this sample with a solution of 0.3% NaOleate resulted in the aggregation of latex particles.

However, no coagulation or flocculation was observed upon dilution. This property was considered beneficial for applications where different SCs are required.

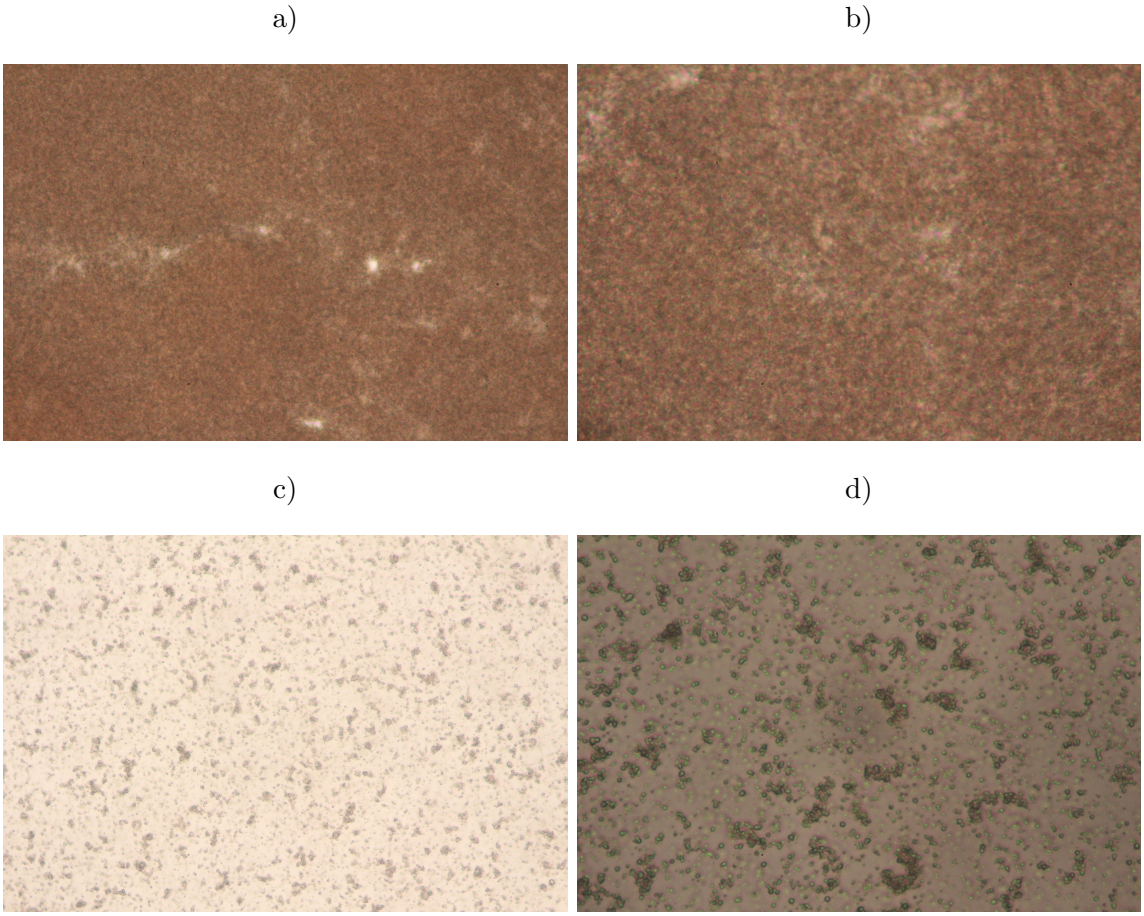


Figure 4.10 Lightmicroscopic images of latex concentrate (a,b) and the dilution two weeks after the final concentration using a solution of 0.3% NaOleate (c,d) (see Tab. 4.1). Magnifications are 200x (a,c) and 400x (b,d).

The overall success of the concentration process was attributed to the thorough removal of impurities by repeated centrifugation and dilution steps. It should be noted that in addition to the generally contaminating plant material, certain non-rubber components were also removed, which may have contributed to the stability of the latex [5, 19], as described in section 2.3.1.1. Since stabilizing solutions were used for the dilution steps, the reduced amount of natural stabilizers was apparently sufficiently compensated for. However, this approach had to be proven on a larger scale in order to reliably estimate the concentration potential.

4.2.2 Concentration of FE Latex

As shown in section 4.1.2.1, much larger amounts of FE latex were available compared to JE latex. This offered the opportunity to develop a concentration process on a larger scale. For this purpose, the concepts described in the previous sections were used. Nevertheless, preliminary creaming and centrifugation tests were required due to the different properties of the extract. In addition, the specific implementation had to be discussed.

4.2.2.1 Preliminary Handling Tests

A complete concentration sequence from extract to the final product exclusively by centrifugation according to Tab. 4.1 and [3, 2] turned out to be too tedious for larger amounts of extract. Therefore, creaming was included in the concentration sequence, as this was considered a simple method for handling larger amounts of latex [79]. In general, the separation efficiency was significantly reduced by suspended matter. Therefore, it was first investigated whether the separation can be improved by removing impurities beforehand by centrifugation. Centrifugation of extracts was carried out with gentle parameters (1000-4000 g, 1-10 min) due to the previously observed increased tendency to coagulate (see section 4.2.1.1). This could keep the shear stress on the latex at a lower level [5]. Two days after adding a 1% solution of SAL to the extent of 1:5 (v/v) to such partially purified extracts, latex concentrated as part of the lower phase. This indicated an increased effective density of the latex particles [79], which was explained by the still too high proportion of suspended matter in the extract. However, this would necessarily have also increased the density of the dispersion medium. Therefore, the relative increase in density of the latex particles must be explained by the occurrence of strong interactions with suspended matter (see section 2.2.3.2). These interactions ultimately led to the observed sedimentation instead of the generally expected creaming. Since NaOleate was found to improve not only latex stability but also the separation of suspended matter (see section 4.2.1.1), it was assumed that increasing its concentration might alter the outcome of the creaming experiment. Based on the interaction models between latex particles and CA (see section 4.2.1.1) discussed, this influence was also considered. Accordingly, different concentrations of NaOleate added before centrifugation and SAL added afterwards were investigated. The results are summarized in Tab. 4.2.

Table 4.2 Phase separation of extracts after removal of sediments by centrifugation. c(NaOleate) corresponds to the concentration of NaOleate in the extract before centrifugation. c(SAL) is the concentration of SAL added after centrifugation and removal of sediments.

c(NaOleate) [%]	**c(SAL)[%]**	**Phase separation**
0.6	0.17	Latex in upper phase
0.6	-	As above, but slower separation
0.3	0.17	Latex in lower phase
0.3	-	Hardly any phase separation

It could be shown that latex creaming is preferred to sedimentation with a higher NaOleate concentration. Obviously, the density of the latex particles could be effectively reduced below that of the dispersion medium. In contrast, the presence of SAL only increased the separation rate [79], but not the overall phase distribution. In addition, the simultaneous removal of sediments and isolation of the resulting upper, more concentrated phases and the lower, less concentrated phases were considered. The upper phase in particular should serve as a better starting point for the subsequent creaming. Accordingly, the separation of native extract and the resulting purified upper and lower phases after centrifugation was compared. The observations two days after the addition of SAL are summarized in Tab 4.3.

Table 4.3 Comparison of the phase separation of extracts after adding SAL to 0.17%. The samples examined were the native extract (0.45% NaOleate) and the resulting upper and lower fractions, which were obtained after removing sediments by centrifugation.

Sample	**Phase separation**
Extract	Latex in upper phase, lower phase or both
Upper fraction of centrifuged extract	Latex in upper phase
Lower fraction of centrifuged extract	Latex in upper phase

The extract formed two or three phases with latex in both the upper and lower phases. The extent to which latex could be isolated in the upper phase varied with respect to the batch of extract investigated, the geometry used and the size of the creaming experiment. In contrast to this, the purified latex fractions both showed a creaming behavior that is generally known for NRL [79]. The latex was accordingly concentrated in the upper phase. As discussed above, these observations could be explained by the changed density difference between the latex particles and the dispersion medium. The separation of the upper and lower phases facilitated the

subsequent latex isolation, but also meant a considerable amount of additional work. For extracts with lower SCs of <1% as in this case, it is recommended to skip this step and simply decant the supernatant from the sediments to quickly proceed with creaming. The preliminary concentration tests can be summarized as follows. Since creaming is based on the density difference between latex particles and dispersion medium [79], a corresponding relation was established. The stronger the interaction between the latex particles and suspended matter, the higher the effective density of the latex particles, which ultimately determined their tendency to sediment or cream. An adsorption of latex particles within networks of suspended matter was assumed, similar to a mode of action described for CAs [97]. The tendency for latex sedimentation decreased after purification, which involved the removing sediments after centrifugation. Apparently this step caused a relatively greater decrease in the density of the latex particles compared to the dispersion medium. Thus, two options for an initial concentration of extracts obtained from the FE could be proposed, as shown in Fig. 4.11.

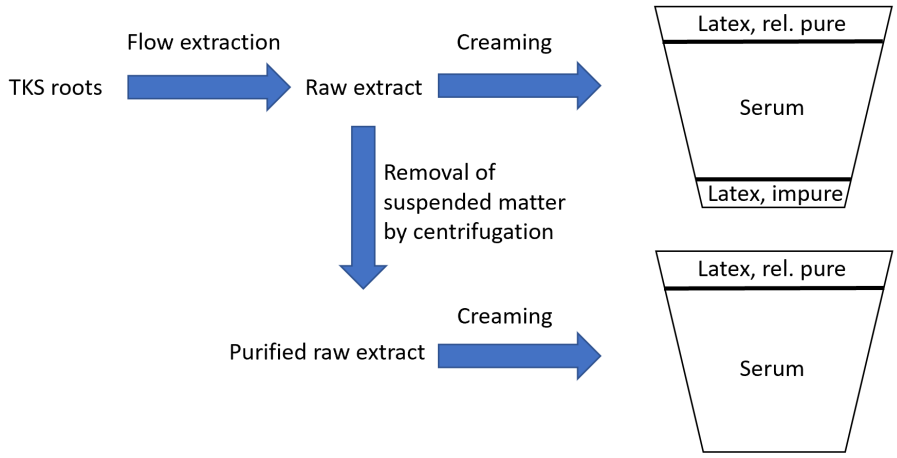


Figure 4.11 Scheme for extraction and pre-concentration of latex.

The concentration might be started directly with creaming after the NaOleate and SAL concentrations have been adjusted. In this case, the latex would have to be partially isolated in the lower phase. For one, this would save the initial purification step. For another, heavier impurities would then also accumulate in the lower phase and have a negative effect on the subsequent processing. The concentration might also be initiated with a removal of suspended matter by centrifugation, after the NaOleate concentration has been adjusted. This centrifugation of large amounts of extract

proved to be cumbersome and destabilized the latex. However, the purified extract would then show latex creaming rather than sedimentation after the addition of SAL. In this case, the latex could mainly be isolated in the upper phase, which would result in a higher purity than in the first approach. Based on these considerations, the specific implementation should be individually adapted with regard to the properties of the extract and the latex to be obtained. In particular, the main batch examined here showed a relatively low degree of latex sedimentation. Therefore, the approach of immediate creaming was chosen for the pre-concentration.

4.2.2.2 Isolation of Intermediate Latex

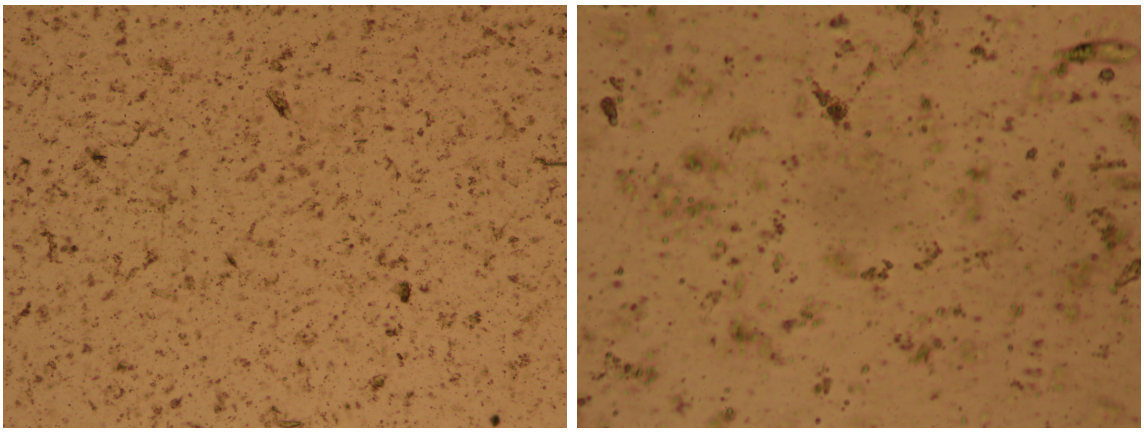
After the addition of SAL, the resulting upper phases could be successively isolated, pooled and subjected to further separation steps. This led to pre-concentrated latex after up to 4 weeks, depending on the phase separation progress of the individual batches. After 2-3 separation steps, the latex was still brownish in color, which indicated that the level of contamination was still high. However, it showed no coagulation or flocculation and confirmed sufficient stability. At this point, a purification sequence analogous to section 4.2.1.2 could be used to remove further impurities and then allow a stronger concentration. In particular, the latex was first diluted with stabilizing solution. The mixtures were then concentrated again by creaming. This sequence was carried out up to 3 times. Accordingly, the applied principle has always been to first reduce the volume and then introduce dilutions with stabilizing solution as late as possible. This enabled a reduced ratio of the required stabilizing solution per latex, an increased purification efficiency and, in general, easier handling. In particular, it was found that stabilizing solutions are best added to the extent of at least 1:2 (v/v) to achieve a substantial purification per separation step. At the same time, SAL solutions are best added to the extent of at least 1:5 (v/v), since smaller additions of SAL significantly increased the separation time. During these purification steps, latex again showed sedimentation to varying degrees. These fractions could be isolated and were pooled with the cream fractions. The effects of the applied sequences could be described with the following relations, similar to that established in section 4.2.1.2. First, the redilution with stabilizing solution served as a washing step to solubilize impurities that interacted with latex particles. Accordingly, the expelled serum fractions showed an increasingly light color, which confirmed the gradual la-

tex purification. Second, the purification was associated with a decreasing amount of suspended matter within the dispersion medium and thus reduced its viscosity [79]. Accordingly, the phase separation has become increasingly efficient. Third, the additional stabilizing solution contributed to latex stability throughout the process, as natural stabilizers may have been removed during creaming [79]. Accordingly, no coagulation or flocculation was observed.

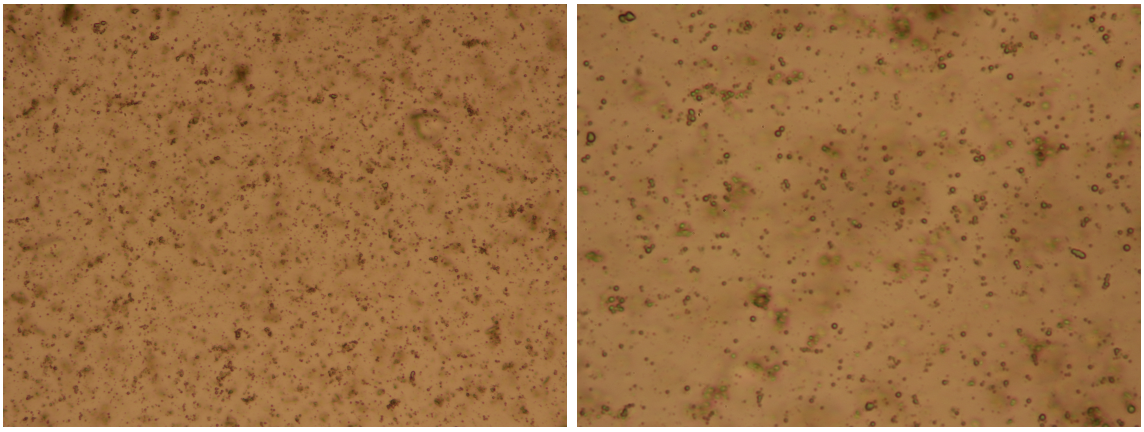
Figure 4.12 Samples of a purification series by creaming with SAL and dilution steps with stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% NaOleate). Up to the 3rd separation step a 1% SAL solution was added to the extent of 1:5 (v/v). For the 4th and 5th separation step, stabilizing solution was added to the extent of up to 1:2 (v/v), depending on the degree of pollution. Then a 1% SAL solution was added to the mixture to extents of 1:20-1:5 (v/v). Corresponding samples are the native extract (left) and the successively obtained latices after the 1st to 5th separation step (right).

After approx. 5 separation steps the color was considerably lighter, as shown in Fig. 4.12, and the SC increased to approx. 6%. Additional serum was expelled only very slowly. Apparently equilibrium was still not reached after a few weeks. This also meant that the viscosity of the dispersion medium was still relatively high, thereby limiting further concentration within a reasonable period of time. Another limiting factor was thought to be the presence of SAL, which has been reported to affect the final latex concentration [79]. An increasing formation of SAL induced networks of latex particles could be confirmed by light microscopy, as described in section 2.3.2.2 and shown in Fig. 4.13 [97].

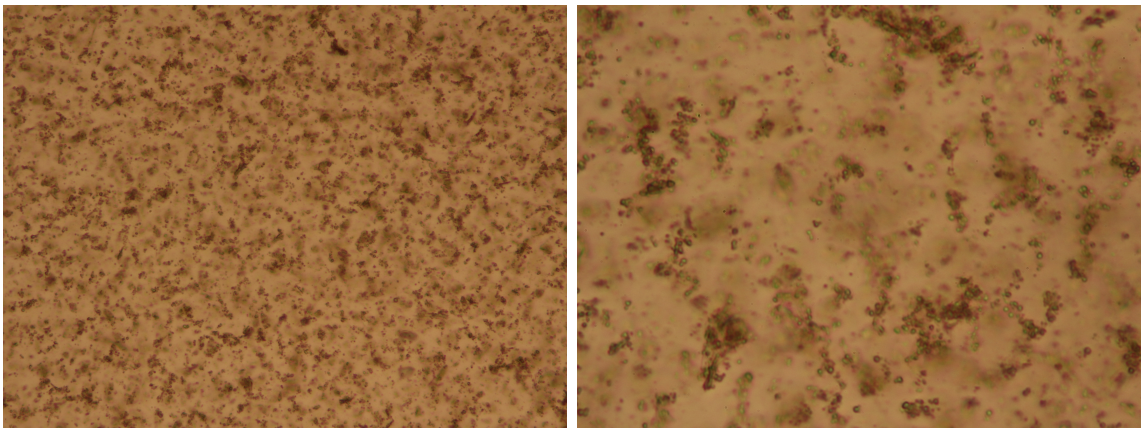
a) b)



c) d)



e) f)



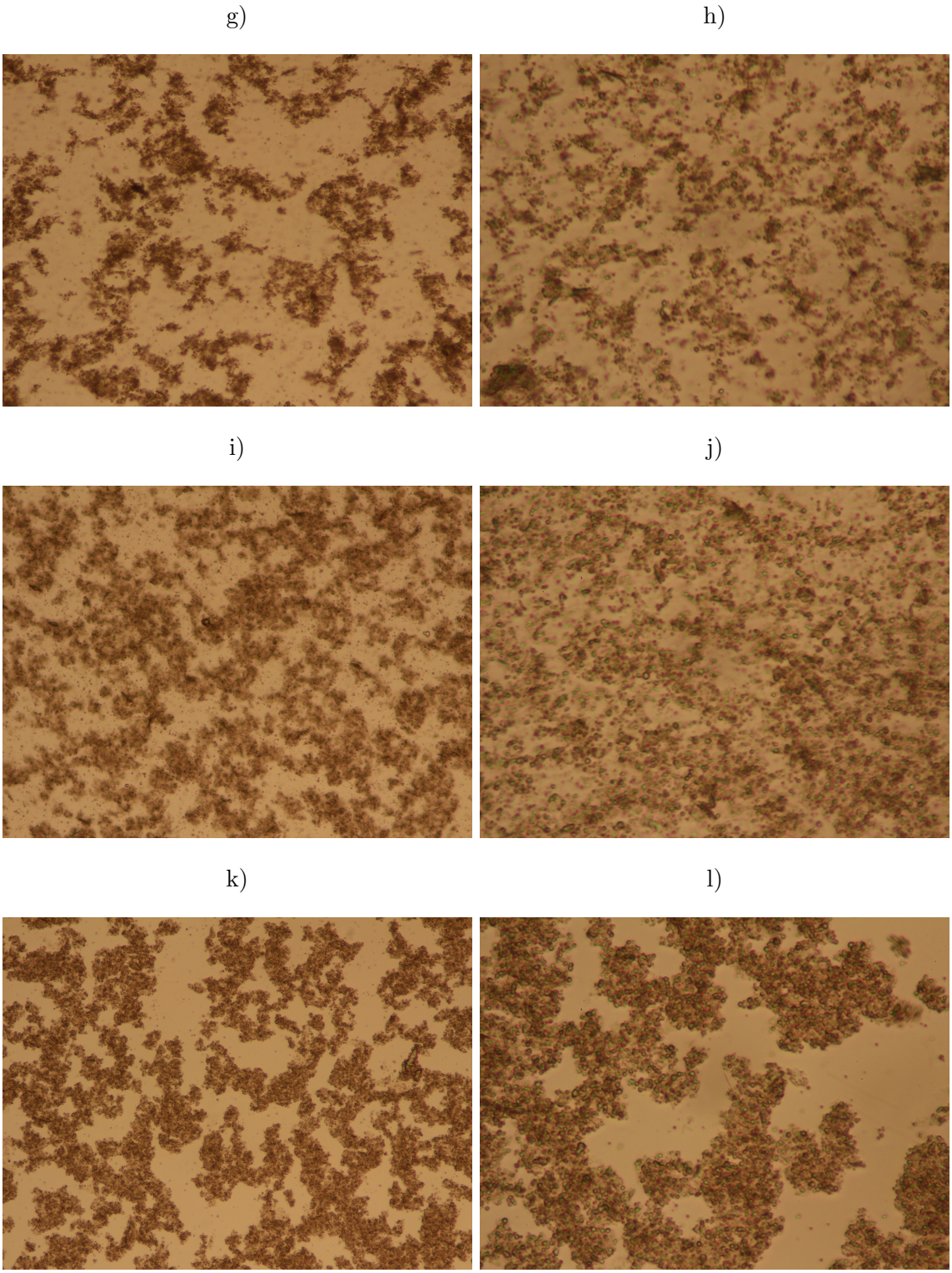


Figure 4.13 Light microscopic images of a concentration series by creaming with SAL and dilution steps with stabilizing solution (0.5 M ETA/ETA-HCl and 0.3% NaOleate). They correspond to the samples shown in Fig. 4.12. Magnifications are 200x on the left hand side and 400x on the right hand side.

However, at least a single addition of SAL was essential for an efficient creaming process, as already shown in the preliminary tests (see section 4.2.2.1). In this context it was observed that the amount of further SAL additions in each purification step had little influence on the success of further processing. In summary, a total of approx. 5 separation steps, which included dilution and creaming sequences, resulted in stable raw latex with increased purity and SC. In view of the reduced volume and the relationships of the Eqs. 2.26 and 2.31, it was assumed that a more efficient and stronger latex concentration might now be achieved by centrifugation. First, the latex phase formed after centrifugation was isolated by vacuum suction. A major disadvantage here was that the concentrated and viscous latex had to be transported through narrow nozzles and tubes before it reached the bulk concentrate. Due to the enlarged surface, the latex was heavily exposed to air, which finally led to the formation of latex films [5] and coagulation. Another problem was the formation of latex rims on the inner surfaces of the tubes after the cream was removed. These fractions could be recovered by immediate suspension in the remaining serum. Otherwise latex coagulated within a few hours due to film formation [5]. Occasionally, flocculated latex was observed after centrifugation, which could be prevented by adding NaOleate. Accordingly, latex was mixed with a 5% NaOleate solution to the extent of 1:5 (v/v) for stabilization prior to centrifugation. In general, the phase separation was insufficient for reasonable latex isolation. Latex was contained in substantial amounts in the resulting serum or as a layer on top of the bottom sediments. The addition of further 1% SAL solution to extents of 1:10-1:5 (v/v) only slightly improved the phase separation. These aspects outline that further concentration of the latex by centrifugation was tedious and difficult. Nevertheless, a SC of approx. 30% could be achieved by repeating sequences of centrifugation and removal of the serum, as summarized in Tab. 4.4. This latex was used in dipping tests for the production of gloves, which were carried out on behalf of Synthomer. It was reported that the preparation of the dipping compound could be achieved without any incompatibilities. However, the sample showed a behavior that is known from pre-crosslinked latices. This was also considered to be the cause of cracking during drying and vulcanization. These findings must be investigated in future dipping tests, using TKS latices with different processing histories (see Tab. 4.4).

4.2.2.3 Isolation and Processing of High Solids Latex

As outlined in the previous sections, latex could not be substantially be concentrated beyond a certain point using creaming and centrifugation. This was mainly attributed to an increased viscosity of the dispersion medium, which controls the separation rate for creaming and centrifugation (see sections 2.3.2.2 and 2.3.2.3). Based on the observations of section 4.2.2.2, it was assumed that redilution before further centrifugation could result in a sufficiently low viscosity of the dispersion medium for a more efficient concentration. In addition, a relationship between the viscosity of the original latex and the rubber concentration of the resulting creamed latex was reported. Accordingly, the lower the viscosity of the original latex, the higher the final rubber concentration [98]. Since the ultimate goal has always been the isolation of latex with a high SC, the principle of rediluting already tediously concentrated latex was evidently considered as unusual. However, it could be successfully applied. In particular, various preconcentrated intermediates with an SC of 6-30% were mixed with water to the extent of 1:1-1:3 (v/v). After centrifugation, highly concentrated latex could be isolated. It was a white paste, similar to heavily whipped cream and depicted in Fig. 4.14.

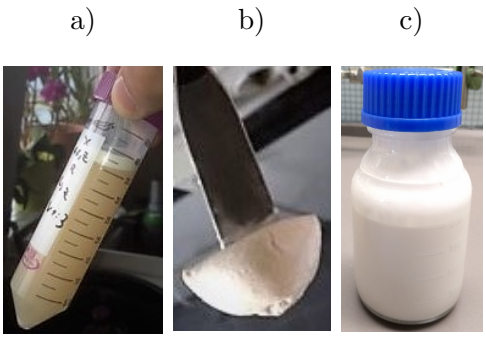


Figure 4.14 Defined phase separation and efficient concentration after redilution of intermediate latex and centrifugation (a) Isolation of high solids latex concentrate (b). Latex as used for producing latex foam (c).

Latex concentrates showed SCs of 54-76% with an average of 67 (± 9), the protocols for stepwise concentration is summarized in Tab. 4.4 and illustrated in Fig. 4.15. It was found that the respective processing history is not decisive for a successful concentration as long as the intermediate latex was diluted to an SC between approx. 2 and 10%.

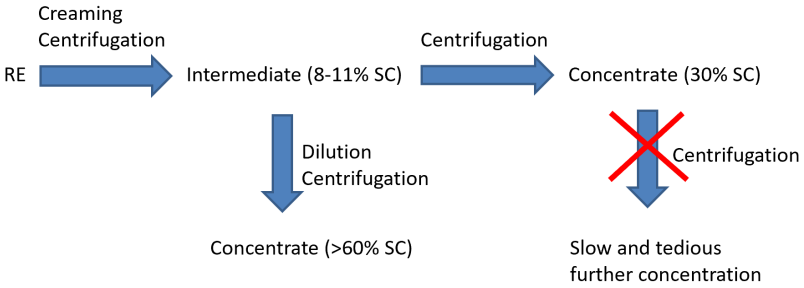


Figure 4.15 Illustration of concentration sequence of FE latex from RE to concentrate.

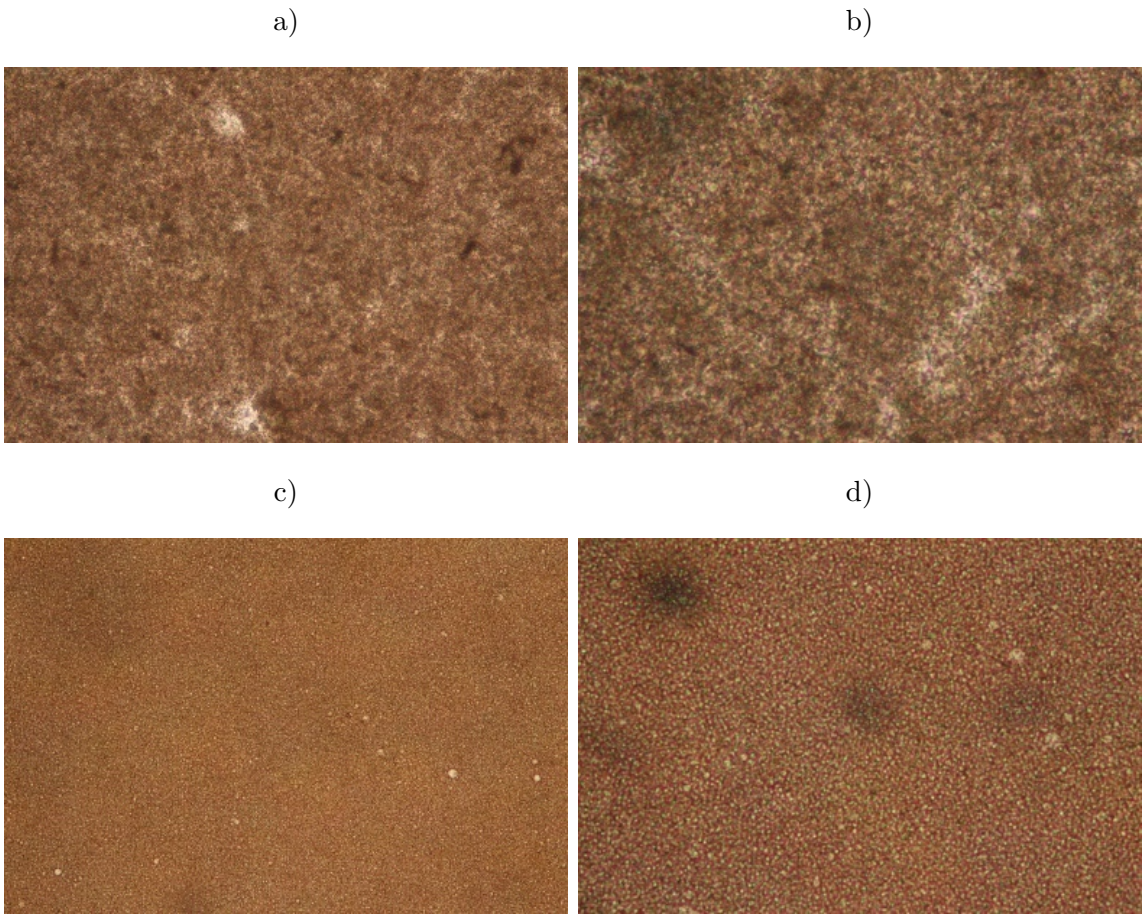


Figure 4.16 Light microscopic images of latex of stored intermediate and high solids latex intermediate approx. 10 months after concentration. The intermediate was prepared by 5 processing steps and additionally stabilized with NaOleate to 0.5% (a,b).The resulting concentrate was prepared by redilution and centrifugation of the intermediate (c,d). Magnifications are 200x (a,c) and 400x (b,d).

After 10 months of storage, the latex particles of both the intermediate and the concentrated latex were still homogeneously distributed without major aggregations,

as shown in Fig 4.16. More detailed discussions about the stability and PSD of latex in relation to storage or redilution are covered in section 4.3.

Table 4.4 Successive protocols of concentration and purification of RE obtained by FE. The actual repetitions depended on the purification progress of the particular batch. Optionally, the second sequence was omitted completely. Accordingly, high solids latex was always produced by intermediate latex, which varied in the extent of applied creaming or centrifugation steps. The respective additions were made up to 0.2% for SAL, up to 1:1 (v/v) for stabilizing solutions, up to 1:4 (v/v) for NaOleate (5% solution) and between 1:1 and 1:3 (v/v) for water. Centrifugations were performed at up 2000-4248 g for 5-60 min at 20-25 °C.

Concentration and purification of RE by creaming with a 1% solution of SAL and dilutions with stabilizing solution (0.5 M ETA/ETA-HCl, 0.3% NaOleate)
• Latex after one separation step was referred to as A intermediate (5% SC).
• Up to the 3rd separation step a 1% SAL solution was added to the extent of 1:5 (v/v). For the 4th and 5th separation step, at first stabilizing solution was added to the extent of up to 1:2 (v/v), depending on the degree of pollution, followed by the addition of a 1% SAL solution to extents of 1:20-1:5 (v/v) on the mixture.
• Latex after approx. 5 separation steps was mixed with a 5% NaOleate solution to the extent of 1:5 (v/v), which was then referred to as B intermediate (6% SC).
Further concentration and purification of latex obtained after 3-5 separation steps
• Centrifugation (4248 g, 15 min) of such a latex, subsequent removal of expelled serum, naturally ocurring creaming and the addition of a 1% solution of SAL to 1:5 (v/v) led to latex referred to as C intermediate (6-11% SC).
• Further centrifugation (4248 g, 10-30 min) and naturally ocurring creaming led to latex with 22% SC. Further addition of a 1% solution of SAL to 1:10 (v/v), centrifugation (4248 g, 180 min) of this latex and removal of expelled serum led to latex of approx. 30% SC.
Isolation of high solids latex
• Dilution of intermediates with water and centrifugation • Accordingly, latex as shown in Fig. 4.14b showed SCs of 76-83%, measured directly after concentration.
• Latex prepared from various intermediates showed SCs of 54-76% with an average of 67 (±9), measured 3-5 months after the final centrifugation and after-creaming.

Because TKS latex concentrate was processed in a similar way to HB latex, its macroscopic properties were of great importance. As discussed in section 2.2.5, it is known that the viscosity of NRL increases along with the SC [5]. In addition to this correlation, a relatively higher viscosity of TKS latex compared to HB latex was observed. Especially latex with a SC of >30% showed an exceptionally high viscosity. Since the high viscosity and whipped cream-like properties were already reported for guayule latex, it was suggested that the underlying principles may also apply to TKS. Accordingly, the unusual flow properties could possibly be explained by a changed PSD [23]. In view of the differences between the calculation methods on the one hand (see section 2.2.4) and the ranges given for HB latex on the other hand (see section 2.2.2.2), an explanation on the basis of the particle sizes proved to be difficult. However, comparing CONTIN results of FE latex and HB latex, it could be argued that the smaller particle sizes compared to HB latex led to smaller distances between the particles. In addition, the smaller mode was only weakly represented in the bimodal latex. Thus, the proportion of smaller particles that were accommodated in the spaces between larger latex particles was low, resulting in an increased effective volume fraction. These aspects might have contributed to the increased viscosity [5]. In addition, various components of the latex particle surface such as proteins and lipids of the biomembrane possibly caused the increased viscosity [23]. Furthermore, an influence of non-rubber components was suspected. These were possibly residual impurities from the extraction or naturally occurring non-rubber compounds from TKS latex. In this context, entrapment of the dispersion medium in SAL-induced particle networks might have increased the effective volume fraction of the disperse phase and thus the overall viscosity [5, 97]. For dipping tests in which latex with a SC of approx. 30% was used, the increased viscosity was still acceptable. In contrast, latex, which was to be used in foam production, required both a sufficiently high SC and a certain level of flowability. For this purpose, high solids latex was diluted approx. 11% to a SC of 56% with NaOleate solution to roughly meet processing requirements. Since this value was already the lower limit for the production of latex foam, TKS latex was also mixed with HB latex for more applicable flowability. Accordingly, by adding the synthetic latex, the viscosity of the mixture could be adjusted so that processing was possible. This blend was a typical industrial formulation for processing NRL in combination with synthetic latex. As a result, a first latex foam sample could be produced by Synthomer. It was reported that the production and overall foam structure of this latex foam was comparable to common HB latex foams. Differences from

the latter were found to be the softer appearance and the characteristic dandelion odor. Since the viscosity of latex is strongly controlled by the viscosity of the dispersion medium [5], the additions to intermediate latices before the final concentration steps (see Tab. 4.4) were considered important. It was found that larger NaOleate additions generally resulted in higher viscosity in relation to the SC. This applied to additions before the final and penultimate centrifugation. Since it was known that the respective potassium compounds are more soluble in water [112], the replacement of sodium compounds by the corresponding potassium equivalent was also taken into account. Latex concentrates with an overall lower viscosity could be isolated from sodium-free extracts. However, the relationships discussed still have to be examined quantitatively in order to control the viscosity of TKS latex concentrates.

4.2.2.4 Concentration of FE Latex (Variation)

As described in section 4.1.2.1, the extraction of latex was also investigated using an increased ratio of roots per stabilizing solution. It was intended to reduce the effort of concentration and the consumption of resources by reducing the extract volume. The application of the approach in section 4.2.2.2 to these extracts is discussed below. Obviously, a higher level of impurities was observed compared to previous extracts. Moreover, the addition of CA led to a complete sedimentation of latex (see Fig. 4.17).

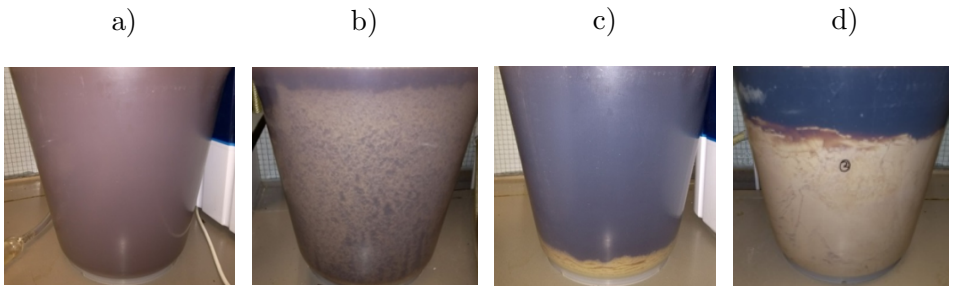


Figure 4.17 Sedimentation of FE latex. After 5-10 days, a distinct phase separation was observed. Extract after addition of a 1% AA solution to the extent of 1:4 (v/v) (a), after 1 day (b), after 5 days (c), after pooling of lower phases and further phase separation (d). After two separation steps no significant further compaction by expelling of serum was observed. Also increasing the extent of AA by up to 0.1% did not lead to further concentration.

Accordingly, the lower phase consisted of latex mixed with non-rubber substances. This result apparently represented an extreme case of increased effective latex par-

ticle density due to interactions with impurities, as previously described (see section 4.2.2.1). It would have been best to pre-purify the extract and to force the latex particles into the upper phase from the start. However, this could not properly be implemented in practice for the reasons mentioned in section 4.2.2.1. In this context, the continuous centrifugation is discussed in section 4.2.4. Nevertheless, the isolation of the lower phase as a whole was considered to be the best way to concentrate latex to a SC comparable to intermediates from section 4.2.2.2. Again, a point was reached at which no significant further concentration was observed, which was due to the high viscosity of the dispersion medium. Since impurities were carried along in the lower phase, this particular concentration of approx. 2% SC was lower than described in section 4.2.2.2. Subsequent centrifugation led to a similar, unfavorable phase separation with the lower sludge phase consisting of latex and non-rubber substances. This was expected because the same principles apply to centrifugation as to creaming [5]. Thus, it has not been possible to produce high solids latex by centrifugation using this sedimented intermediate. Since pure, stable and concentrated latex could only reasonably be isolated as part of the upper phase, the effective density of the latex particles had to be reduced. Due to the reduced volume compared to the extract it made sense to use the approaches from section 4.2.2.1 in order to force the latex particles into the upper phase. The latter could achieved by adding KOleate and water before centrifugation, which confirmed that the effective density of the particles was sufficiently reduced. In general, adding more KOleate resulted in a higher proportion of latex in the upper phase, while adding more water resulted in a much faster phase separation but also less concentrated latex. In both cases, a purer latex was observed when the amount added was increased. This means that the latex was either too polluted or too dilute to form a highly concentrated upper phase. As a result, it was found that latex could be best isolated by repeating a sequence of adding KOleate at 1-2% and water at 1:1 (v/v) and centrifugation, as summarized in Tab. 4.5. The latex concentrates obtained are characterized in detail in section 4.3. With the modified, sodium-free stabilizing solutions, the samples had a relatively lower viscosity. Since the sodium of the CA was exchanged for ammonium for this approach, the claim for ammonia-free latex isolation must be temporarily excluded here. Based on the results from section 4.2.2.3, the latex was tested by Synthomer for its suitability for foam production without mixing with synthetic latex. Experiments on handling during compounding, gelling foaming confirmed a behavior known from HB latex. However, the foam structure was rather large-pored with the formation of holes in

the foam, which was caused by the SC of the latex. As a result, it becomes clear that the SC will have to be increased further, while the viscosity must be kept at a reasonable level, in order to produce latex foam from pure TKS latex with properties comparable to HB latex.

Table 4.5 Successive protocols of concentration and purification of RE obtained by FE (variation).

Concentration of RE by sedimentation with a 1% solution of AA
• Addition of a 1% AA solution to the extent of 1:4 (v/v) to the RE.
• Latex after successive removal of expelled upper serum phases was referred to as 1st intermediate (approx. 2% SC).
Further concentration and purification of latex obtained after sedimentation
• Latex after the addition of a 1% KOleate solution to the extent of 1:1 (v/v) and centrifugation (4248 g, 15 min) was referred to as 2nd intermediate
• Latex after the addition of a 1% KOleate solution to the extent of 1:1 (v/v) and centrifugation (4248 g, 15 min) was referred to as 3rd intermediate
Isolation of high solids latex
• Addition of a 1% KOleate solution to the extent of 1:1 (v/v) to the 3rd intermediate and centrifugation (4248 g, 15 min) led to latex referred to as concentrate (approx. 55% SC)

4.2.3 Concentration of Latex from ME and Decomposition

Due to the overall greater size reduction of the roots, mill extracts (ME) were more permeated with suspended matter than FE extracts. It was assumed that this caused the comparatively more difficult subsequent concentration and purification. When AA was added, the latex sedimented in the same way as in section 4.2.2. Purification of this phase by further sedimentation steps after adding water and AA was hardly possible. After the subsequent centrifugation, a compact lower sludge phase was formed again as a mixture of latex with and non-rubber substances. Unfortunately, the previously successful approach of adding KOleate and water could not force the

latex into the upper phase after centrifugation. Since it was assumed that the obviously increased latex particle density was due to strong interactions with suspended matter, an attempt was made to reduce these interactions by intensive manual shaking after the respective additions of KOleate and water. Primarily, this action did not facilitate the stripping of impurities. Instead, the latex particles showed excessive flocculation, which was induced by the dilution step in combination with shear stress. This apparently represented an extreme case of the microflocculation, which was reported to be necessary for a successful phase separation when using CA [79]. The subsequent centrifugation resulted in the formation of an extremely viscous upper latex-containing phase. It was found that adding KOleate to 7% and water by 1:1 (v/v) before shaking provided the most efficient latex isolation. For one, smaller additions of KOleate reduced the latex purity. For another, larger KOleate additions resulted in a lower level of microflocculation and, in turn, reduced the proportion of latex in the upper phase. Unfortunately, the latex obtained only had a SC of approx. 15%. Attempts to further purify latex in order to subsequently achieve a higher SC, as developed in section 4.2.2.3, were not successful. As a result, it has been suggested that the interactions of latex particles with suspended matter are too strong to be efficiently separated by conventional creaming or centrifugation. This assessment also applied to the concentration of extracts obtained by decomposition. After adding AA and isolating the sedimented latex, further purification and concentration could hardly be achieved. Accordingly, the discussed advantage of complete inclusion of the root in the stabilizing solution in relation to the latex yield turned out to be a limiting factor for the isolation of latex concentrate.

4.2.4 Further Approaches on Latex Concentration

In addition to the established methods for concentrating latex (see section 2.3.2), less discussed approaches were examined. It turned out that they are not readily applicable to TKS latex, however the basic experiences obtained are described below. First, continuous centrifugation was used to automate the concentration approaches, which were based on the principle of repeated dilution and concentration sequences. In general, the problems reported for centrifugation of guayule extracts [100, 99] were also found with TKS latex. A direct concentration of raw latex with a SC of up to 1% could not be achieved with the disc separator. The volume ratio between

the heavy and light phase hardly changed when the position of the separator screw was varied. Accordingly, neither the heavy nor the light phase could be significantly concentrated. Heating the latex to 40 °C before centrifugation did not change the result either. These observations could be explained by a too low SC and an insufficient difference in density between latex particles and dispersion medium [100]. In addition, latex coagulation was often observed between the separator discs. This could be attributed to insufficient latex stability for the high mechanical stress that occurred during centrifugation [100]. Although this could be compensated to a certain extent by the further addition of ETA, the stability could not be maintained with higher concentrated samples. Further preparation of the separator itself was necessary to completely accommodate the observed problems with phase separation, latex destabilization and concentration factor. Unfortunately, proposed modifications of the outlet for the light phase, as described for guayule latex [100] could not be examined yet. Second, latex concentration by evaporation of water is mentioned. The investigations were discontinued after the preliminary tests using mixtures of HB latex and TKS serum. Evaporation of water was generally associated with considerable coagulation of latex. This destabilization could be attributed to a reduced barrier height ΔG^*. The particles faced a reduced available volume of dispersion medium and had to occupy positions with a reduced average distance between them [5]. In addition, the resulting latex was heavily polluted because non-rubber substances were also concentrated in addition to rubber. As a result, the evaporation concentration was found to be adequate, if any, when the latex samples were of high purity and well stabilized. However, this method should be considered if the ratio of non-rubber to rubber substances has to remain unchanged [5]. This could potentially apply to already concentrated latex with an established PSD that needs to be further concentrated.

4.2.5 Summary and Discussion of Concentration Concepts

As a result of the previous sections, general concepts for the concentration of TKS latex could be developed. They had to be adapted with regard to the colloidal properties of the extracts. The main factors determining the success of the concentration were the degree of root comminution and the amount of suspended matter in the extracts. On the one hand, these factors were related to the viscosity of the disper-

sion medium and limited the concentration rates during creaming and centrifugation. The concentration rates could be increased either by removing suspended matter by centrifugation or by dilution prior to further concentration. On the other hand, interactions between latex particles and suspended matter presumably caused an increased latex particle density, which in turn led to latex sedimentation rather than creaming. Extracts obtained from more comminuted roots or using lower volumes of stabilizing solution per root mass generally showed stronger interactions. This occurrence made concentration and purification more difficult. However, latex particles could be forced into the upper phase with the aid of oleates. It was assumed that the latter contributed to the release of impurities and thus to a reduction in the density of the latex particles. Extracts obtained from the FE were found to be most suitable for the preparation of latex concentrates. In addition to colloidal aspects, the amount of extract to be processed had to be taken into account. While lab-scale processes have been well described, reasonable implementations for larger-scale extractions were found to be challenging from a practical point of view. In the case of FE extracts, the concentration to latex with 6% SC was achieved by repeated sequences that included creaming and redilution. From there, latex with up to 25-30% SC could be prepared by centrifugation. The preparation of latex with >54% SC was achieved by rediluting latex with 6-30% SC as obtained above and a final centrifugation. High solids latex was a pasty cream with a relatively high viscosity compared to HB latex. This was attributed to differences in natural compounds of TKS latex, interactions with non-rubber compounds, the presence of NaOleate and an altered PSD. Such latex could be rediluted to prepare lower SC latex without coagulation. With regard to the concentration process itself, some general results could be summarized. First, a pre-concentration of extracts was necessary because a single-step concentration to final products was not possible. For a reasonable latex isolation after centrifugation of such a preconcentrated intermediate, the upper phase had to be purified, thick and compact enough. This could only be achieved if the intermediate latex had at least an SC of 2%. In contrast, an SC of >11% resulted in a thin, highly concentrated upper layer and a broad, less concentrated phase below. All in all, the following qualitative relationship might be proposed for FE latex:

$$P(C) \underset{2\% \lessapprox SC \lessapprox 11\%}{\approx} \frac{1}{f(SC) * f(I)} \tag{4.1}$$

In Eq. 4.1, $P(C)$ defines the probability of forming a highly concentrated latex phase at a fixed centrifugation speed and period, and I the amount of suspended matter in the dispersion medium. The amount of latex lost during creaming and centrifugation was generally negligible. Before the main batches were subjected to the respective process, the latex stability was examined with handling tests. Therefore, coagulation could be reduced to a minimum. Smaller amounts were intentionally left behind during latex isolation to avoid the transfer of contaminated adjacent serum. In other words, at each isolation step there was a tradeoff between recovery, purity and concentration of latex. In this context, the isolation of latex became more efficient, as the phase boundary between concentrated latex and serum became increasingly sharp in the course of the purification. Obviously, residual latex in the serum might be enriched and recovered by further creaming. It was also demonstrated that latex remaining as part of the sediment phases could be recovered by resuspending with oleate-containing stabilizing solutions and centrifuging. Although these attempts turned out to be tedious, it was found that basically complete processing of extracted latex into concentrates is possible. This is in contrast to the extraction processes themselves, where significant losses were observed.

4.3 Characterization of Latex

In view of the numerous properties of NRL (see section 2.2) on the one hand and the variables of the latex isolation (section 2.3) on the other hand, it is clear that only selected correlations were investigated within the scope of this work. While the general processability was already discussed during latex isolation, the focus is now on latex properties such as PSD and stability as well as reducing the protein concentration. As latex isolation was gradually developed, differently extracted and concentrated fractions occurred. Apart from native root latex, this also included the necessary simplification to neglect the influence of the age of isolated latex at the time of investigation. These circumstances also mean that their colloidal properties could not be easily compared. It is therefore intended to provide an overview of latex properties from different isolation approaches rather than a direct comparison. An immediate significance for the latex isolation was not necessarily given for all of the determined characteristics. Nevertheless, the basic knowledge of these relationships should be regarded as valuable for future investigations and for comparisons with conventionally produced HB latex. The latices examined were generally native latex, extracts, intermediates, concentrates and their diluted equivalents.The results of DLS and ZP measurements are presented and discussed in relation to the respective processing history.

4.3.1 Particle Size Distribution

4.3.1.1 Development of Hydrodynamic Diameters by DLS

Before analyzing the PSD, the calculation of hydrodynamic diameters (HD) had to be developed, which was achieved using various methods according to section 2.2.4. The method of cumulants is based on the assumption of a monodisperse system of spherical particles without interactions and utilized a single exponential function. The logarithm of the field correlation function $lng^{(1)}(t)$ is then a decreasing straight with the slope $-\Gamma$. This function would differ if interactions or different modes occurred. The difference can be described by a Taylor series $K_n(\Gamma)$ of $lng^{(1)}(t)$ after potencies of $-t$. This leads to series coefficients, denoted as cumulants, which can be

written as follows [40]:

$$\ln g^{(1)}(\tau) = \sum_{n=1}^{\infty} K_n \frac{(-t)^n}{n!} \tag{4.2}$$

The cumulants are connected with the momenta of the distribution function, so that the cumulant K_1 describe the mean value, K_2 the width and K_3 the tilt of the distribution. In practice, the ideal value $\ln g^{(1)} = 0$ is not achieved due to experimental circumstances. Overall, this leads to the following relationship [40]:

$$\ln \sqrt{g^{(2)}(t-1)} = \ln \sqrt{\Xi} - K_{(1)}t + K_{(2)}\frac{t^(2)}{2!} - K_{(3)}\frac{t^(3)}{3!} + ... \tag{4.3}$$

where Ξ is a quality criterion for the autocorrelation function that can be derived from the y-intersept. The normalized second cumulant $\frac{K_{(1)}}{K_{(1)}^{(2)}}$ is known as the polydispersity index (PDI) and quality factor [40]. Assuming a monomodal or a bimodal distribution, mono-exponential (ME) and double-exponential (DE) fit models can be used to describe the measured correlation functions $g^2(t)$, which reads [2]:

$$g^2 t = C + \beta(|g^{(1)}(t)|)^2 \tag{4.4}$$

In Eq. 4.3, $\ln g^1(t)$ is the normalized autocorrelation function of the intensity, t the decay time and C and β fit parameters. In the case of a monomodal distribution, the following fit function of the field autocorrelation was applied [2]:

$$g^1 t = a_1 \exp(-a_2 t) \tag{4.5}$$

In the case of a bimodal distribution, the fit function can be written as follows [2]:

$$g^{(1)}(t) = a_1 exp(-a_2 t) + a_3 exp(-a_4 t) \tag{4.6}$$

The obtained decay rates a_2 and a_4 can be used to calculate the diffusion coefficients D_1 and D_2 of the two modes 1 and 2 [2]:

$$a_{(2/4)} = D_{(1/2)} q^2 \tag{4.7}$$

In Eq. 4.7, q is the scattering vector and reads $[(4\pi\eta)/\lambda] sin(\frac{\theta}{2})$, with the wavelength of the incident radiation λ, the refractive index η and the scattering angle θ. The

HD d_{H1} and d_{H2} are calculated using the Stokes-Einstein relationship [2]:

$$d_{(H)} = \frac{kT}{3\pi\eta D} \tag{4.8}$$

In Eq. 4.8, k denotes Boltzmann's constant, T the temperature and η the dynamic viscosity [2]. In addition to cumulant analysis and exponential fitting, the distribution functions were also calculated by the CONTIN algorithm using the ALV correlator software [47].

For both exponential fitting and CONTIN, the dynamic viscosity of the dispersion medium (ETA (aq), pH = 10.5) and the refractive index had to be known and were determined to be 0.946 mPas and 1.334, respectively [2]. Furthermore, the scatter concentration had to be sufficiently low that only single scattering occurred. Thus the samples were diluted until they were optically clear [49], which generally applied to samples with SN $\geq$ 4. Accordingly, more concentrated samples were not used for analysis. In addition, normalized correlation functions with values of > 1 or calculated HD of > 2000 nm were not taken into account (see Appendix) [2]. In the case of more dilute samples samples with SN $\geq$ 7. in particular, an additional decay was observed at higher decay times. Therefore, it was necessary to apply a data cut-off at certain decay times. The respective decay time was either 100 or 500 ms depending on the resulting fit quality. Since the PDI was mostly > 0.1, the PSD could be determined with a DE fit and CONTIN. Overall much lower HD were calculated by CONTIN compared to exponential fitting. This trend was also reported previously [2]. Certain modes could be separated by filtration to allow a more accurate analysis [46]. In this case, an ME fit was appropriate.

4.3.1.2 PSD of Native Latex

In view of the controversy surrounding the PSD of native TKS latex and the influence of plant age [5, 6, 24], as well as the possible use of roots from storage or early harvest (see section 4.1.1), corresponding latex fractions are analyzed below. The PSD of native TKS latex was recently identified by our group [2] as bimodal with the smaller mode at 130-190 nm and the larger mode at 380-580 nm. The analysis of latex from roots that had been stored in sand for approx. 3 months is shown in Fig. 4.18.

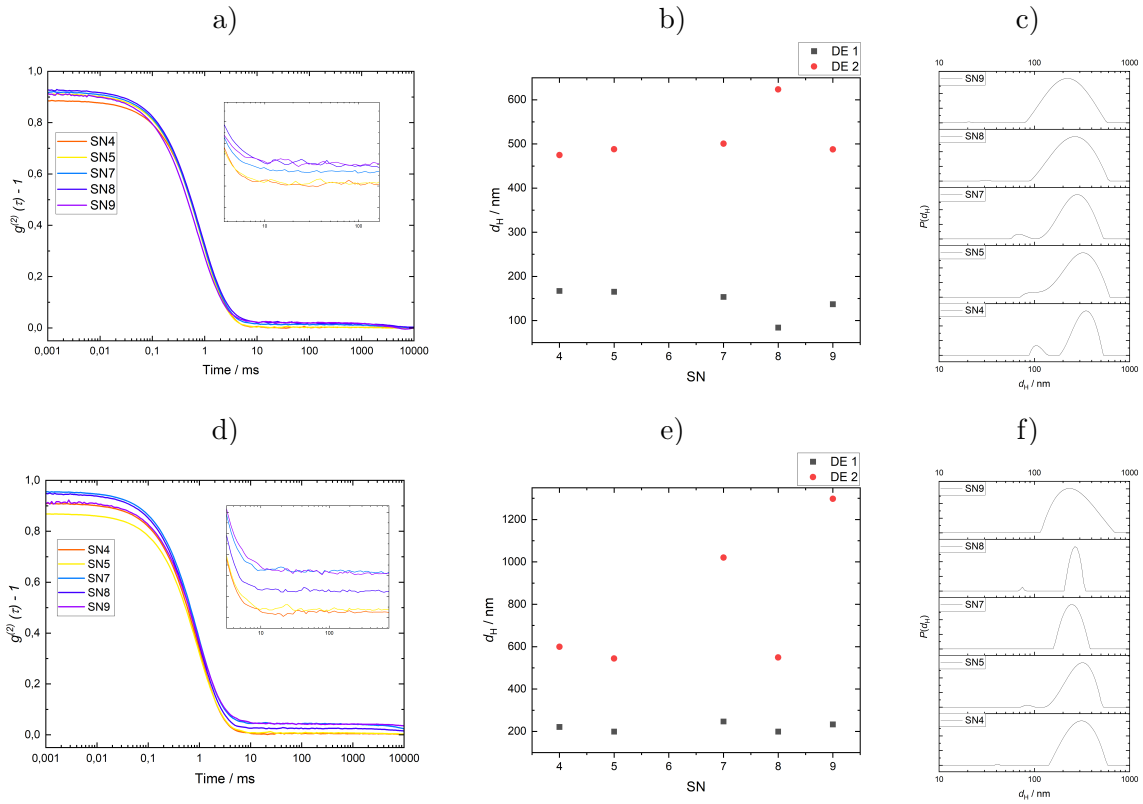


Figure 4.18 Normalized intensity correlation functions $g^2(\tau) - 1$ for the dilution series of native latex from roots stored in sand for approx. 3 months (a) and from roots harvested approx. 3 months early (d). Particle diameters d_H in nm of latex from roots stored in sand for approx. 3 months, calculated with DE fitting (b) and from roots harvested approx. 3 months early (e). Intensity weighted particle size distribution $P(d_H)$ of native latex from roots stored in sand for approx. 3 months, calculated with CONTIN algorithm. (c) and from roots harvested approx. 3 months early (f).

DE fitting gave a smaller mode at 84-167 nm with an average of 141 (±34) nm and a larger mode at 488-624 nm with an average of 515 (±61) nm. With CONTIN a bimodal PSD was found for SN4, but the smaller mode disappeared for higher SN. However, the smaller mode could be isolated and detected by filtration of SN 0 through a 0.2 µm filter. This confirmed that the initial bimodal character was essentially retained after storage. Cumulant analysis of the isolated smaller mode gave HD of 111, 106 and 106 nm for first, second and third order cumulants and a PDI of 0.13. The ME fit gave an HD of 110 nm. The less dominant smaller mode could have been caused by the aggregation of smaller particles during storage [5]. Nevertheless, the overall bimodal character and range of particle sizes agreed with the previous values for native latex [2].

In addition, the influence of the harvest time on the PSD was examined, which was of relevance if fresh TKS were required before the usual harvest. Fig. 4.18 shows the analysis of latex taken from roots harvested approx. 3 months prior to the usual harvest. DE fitting gave a smaller mode at 199-247 nm with an average of 220 (±21) nm. The size of the larger mode strongly varied between 544-1297 nm. Apparently the expected mode at 380-580 nm was not yet fully developed. CONTIN also confirmed the presence of a mode at 247-299 nm with an average of 273 (±22) nm. A larger mode could not be identified, but SN5 and SN7 showed an even smaller mode with an average of 80 nm. This was possibly a fragment of advancing rubber biosynthesis. Overall, it was shown that the formation of modes was not yet complete, which must be taken into account when targeting latex with special features.

4.3.1.3 PSD of JE Latex

The PSD of latex is affected by the extraction and concentration [2]. Specifically, the PSD of JE latex concentrated to approx. 25% SC was recently investigated by our group. The initial bimodal character of the native latex disappeared and a monomodal distribution with HD of 1040-1260 nm was found [2]. Given the purification sequence and protocols developed to isolate high solids latex in section 4.2.1.2, it was important to examine and compare the various stages of processing, namely RE, intermediate and concentrate (see Tab. 4.6). The corresponding analysis is shown in Fig. 4.19.

Table 4.6 Summarized processing history of investigated JE latex. Protocols are discussed in detail in Tab. 4.1.

Investigated latex	**Processing history**
RE	-
Intermediate	Repeated centrifugation and redilution of RE
Concentrate	Centrifugation of intermediate

In the case of the RE, DE fitting gave a smaller mode at 89-126 nm with an average of 112 (±14) nm and a larger mode at 337-462 nm with an average of 419 (±49) nm. This is consistent with the previous results for native latex and suggests that the extraction step itself has little impact on the PSD. For the intermediate, DE fitting gave a smaller mode at 139-197 nm with an average of 168 (±28) nm and a larger mode at 766-842 nm with an average of 807 (±40) nm.

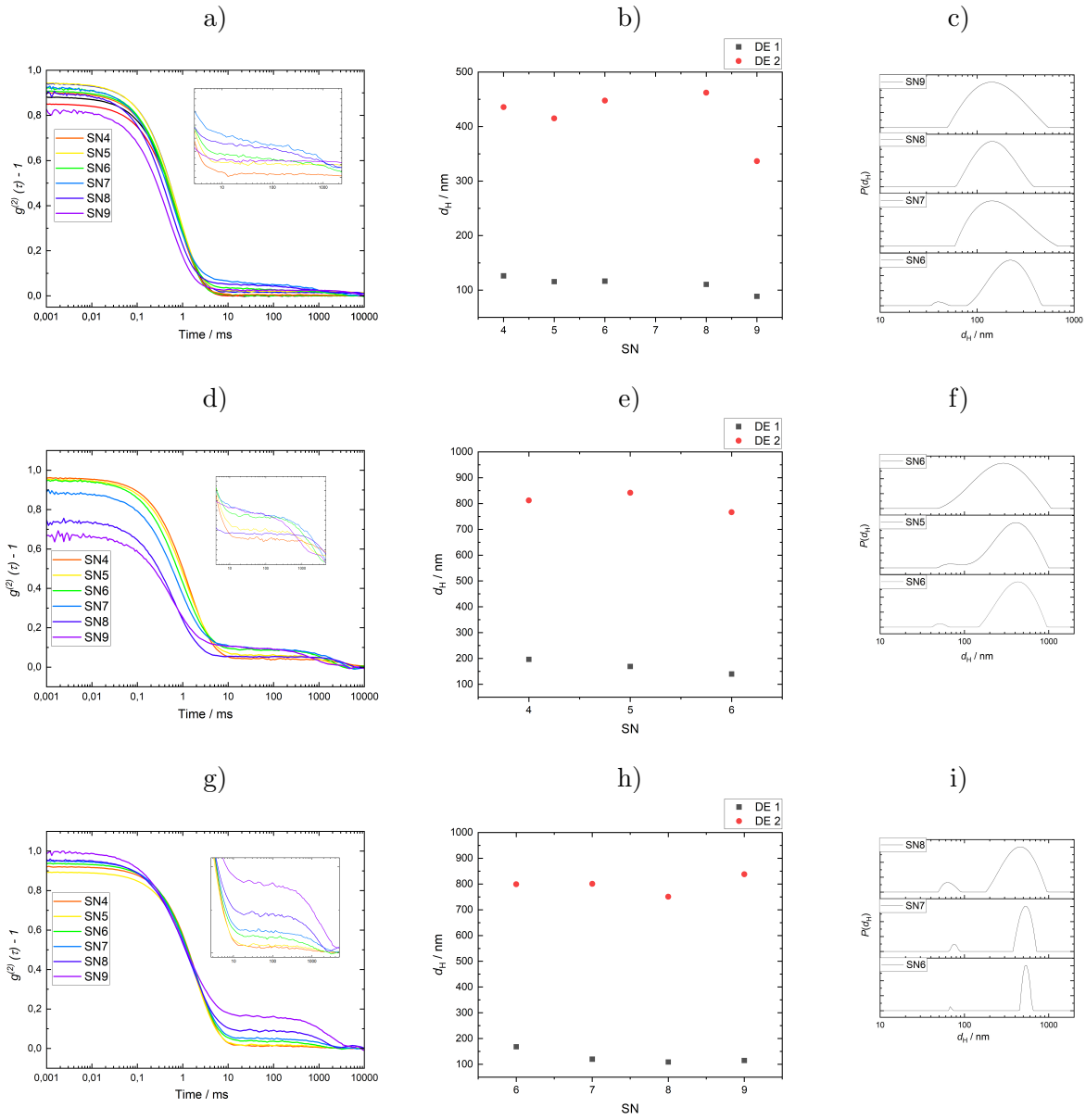


Figure 4.19 Normalized intensity correlation functions $g^2(\tau) - 1$ for the dilution series of JE RE(a), intermediate (d) and concentrate (g). Particle diameters d_H in nm of JE RE (b), intermediate (e) and concentrate (h), calculated with DE fitting. Intensity weighted particle size distribution $P(d_h)$ of JE RE (c), intermediate (f) and concentrate (i), calculated with CONTIN.

Compared to the RE, the second mode became significantly larger during centrifugation, which was probably caused by agglomeration of smaller particles [79]. For the concentrate, DE fitting gave a smaller mode at 109-168 nm with an average of 127 (±27) nm and a larger mode at 750-838 nm with an average of 797 (±36) nm. While

the mean particle sizes were apparently not affected by the significant final concentration, their distribution became much narrower, which was expected [97, 79].

4.3.1.4 PSD of FE Latex

Since the main latex isolation was achieved through the FE, the corresponding PSD was examined more closely. Three selected latex intermediates, hereinafter referred to as A, B and C, were analyzed in particular. They were characterized by an overall increasing purification effort of the initial RE. The resulting concentrates, which were obtained by subsequent centrifugation of the respective intermediates were also examined, as summarized in Tab. 4.7.

Table 4.7 Summarized processing history of investigated FE latex for DLS. The respective protocols are given in detail in Tab. 4.4.

Investigated latex	**Processing history**
A intermediate	Creaming of RE
A concentrate	Dilution of A intermediate, centrifugation
B intermediate	Repeated creaming of RE
B concentrate	Dilution of B intermediate, centrifugation
C intermediate	Repeated creaming of RE, centrifugation
C concentrate	Dilution of C intermediate, centrifugation

For the A intermediate (see Fig. 4.20), DE fitting led to a smaller mode at 140-148 nm with an average of 144 (±4) nm and a larger mode at 775-810 nm with an average of 798 nm. CONTIN gave a smaller mode at 88-132 nm with an average of 103 (±25) nm and a larger mode at 535-681 nm with an average of 596 (±76) nm. In the case of the resulting A concentrate, the DE fit was only suitable for SN6 and gave a smaller mode at 139 nm and a larger mode at 667 nm. CONTIN gave a smaller mode at 59-87 nm with an average of 75 (±14) and a larger mode at 374-650 nm with an average of 557 (±158) nm. For the B intermediate no suitable fit could be applied, even with a data cut-off at 100 ms. In addition, CONTIN gave extremely broad signals that made it difficult to determine the particle size. These aspects were likely caused by residual SAL, given the processing history, that included repeated creaming steps. For the B concentrate (see Fig. 4.21), DE fitting resulted in a smaller mode at 211-249 nm with an average of 231 (±19) nm and a larger mode at 791-892 nm with an average of 830 (±55) nm. CONTIN gave a smaller mode at 95-118 nm with an average of 104 (±12) and a larger mode at 481-545 nm with an average of 522 (±36) nm.

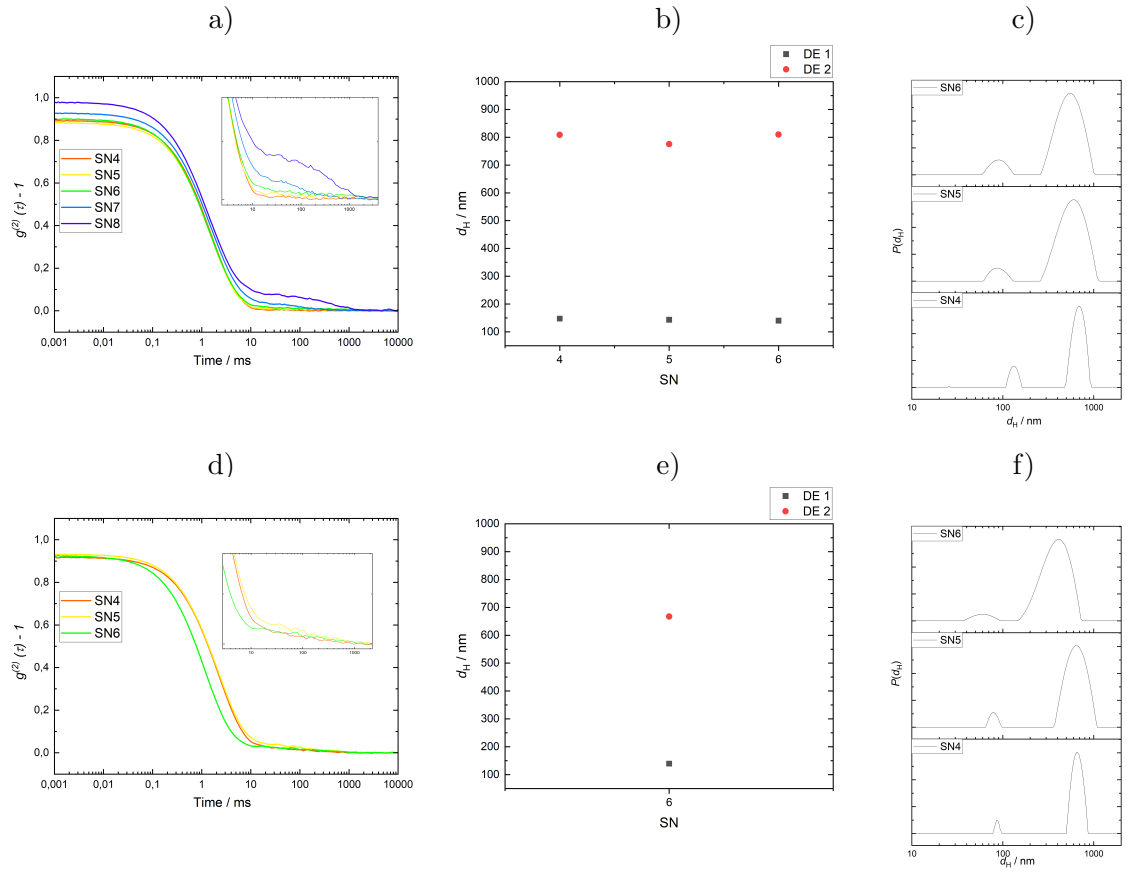


Figure 4.20 Normalized intensity correlation functions $g^2(\tau) - 1$ for the dilution series of A intermediate(a) and concentrate (d). Particle diameters d_H in nm for A intermediate (b) and concentrate (e), calculated with DE fitting. Intensity weighted particle size distribution $P(d_H)$ of A intermediate (c) and concentrate (f), calculated with CONTIN.

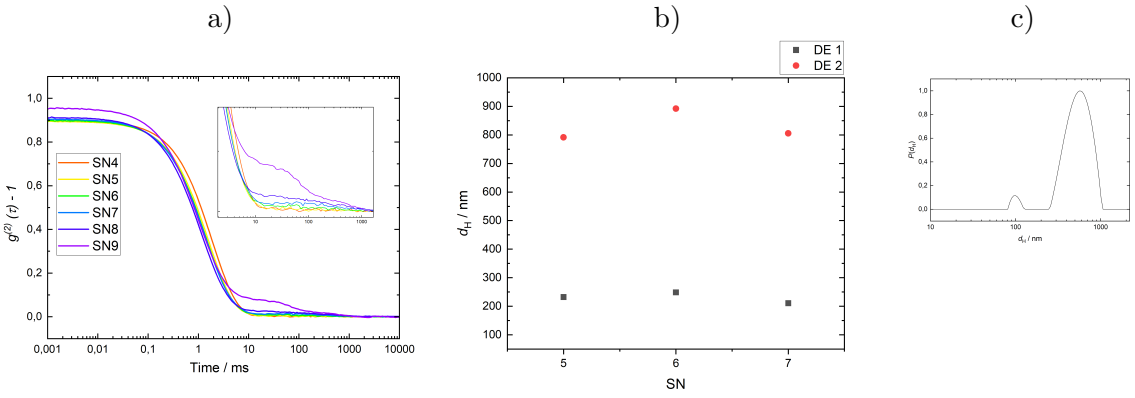


Figure 4.21 Normalized intensity correlation functions $g^2(\tau) - 1$ for the dilution series of B concentrate (a). Particle diameters d_H in nm for B concentrate (b), calculated with DE fitting. Intensity weighted particle size distribution $P(d_H)$ for B concentrate (c), calculated with CONTIN. Correlation functions of B intermediate did not provide consistent results.

For the C intermediate (see Fig. 4.22), DE fitting gave a smaller mode at 146-164 nm with an average of 155 (±9) nm and a larger mode at 731-785 nm with an average of 760 (±27) nm. CONTIN resulted in a smaller mode at 100-118 nm with an average of 109 (±9) nm and a larger mode at 448-520 nm with an average of 493 (±39) nm. In the case of the resulting C concentrate, a DE fit was only suitable for SN5 and gave a smaller mode at 289 nm and a larger mode at 1096 nm. CONTIN resulted in a smaller mode at 57-118 nm with an average of 92 (±31) and a larger mode at 707-814 nm with an average of 745 (±60) nm. In the case of C latex in particular, the smaller mode was less strongly represented in the concentrated form and larger size of the second mode was observed. This could be ascribed to the selective separation of particles below a minimum size and the agglomeration of smaller particles [79].

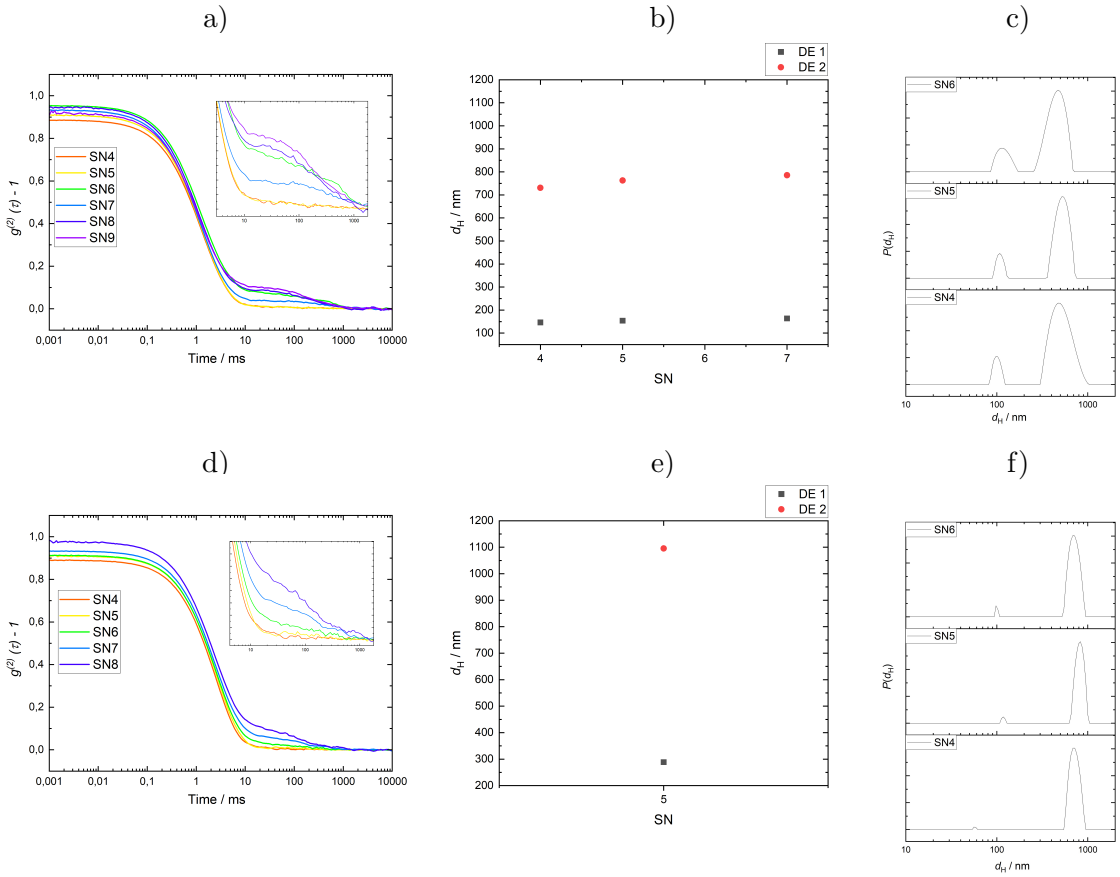


Figure 4.22 Normalized intensity correlation functions $g^2(\tau) - 1$ for the dilution series of C intermediate(a) and concentrate (d). Particle diameters d_H in nm for C intermediate (b) and concentrate (e), calculated with DE fitting. Intensity weighted particle size distribution $P(d_H)$ for C intermediate (c) and C concentrate (f), calculated with CONTIN.

When comparing the concentrated latices A, B and C, the correlation of larger particle sizes as a result of an increased degree of purification could be suggested. All in all, the latices examined showed a bimodal character across all processing stages. This was affirmed by both exponential fitting and CONTIN.

4.3.1.5 PSD of FE Latex (Variation)

As discussed in section 4.2.2.4, certain extraction techniques led to latex sedimentation rather than creaming. Therefore, the influence of this altered phase separation on the PSD of the resulting intermediates and concentrates was of interest and is illustrated in Fig. 4.23. The latices examined are summarized in Tab. 4.8.

Table 4.8 Summarized processing history of investigated latex samples, which originated from FE (variation) RE prepared as described in section 3.1.2.3. The respective protocols are discussed in detail in Tab. 4.5.

Investigated latex	**Processing history**
Intermediate	Sedimentation of RE
Concentrate	Repeated dilution and centrifugation of intermediate

For the intermediate, DE fitting led to a smaller mode at 112-135 nm with an average of 127 (±13) nm and a larger mode at 412-672 nm with an average of 543 (±130) nm. Calculation with CONTIN showed that the smaller mode was only represented by a weak signal. The larger mode was found to be a broad signal resulting in particle sizes of 203-254 nm with an average of 230 (±23) nm. For the resulting concentrate, DE fitting gave a smaller mode at 382-493 nm with an average of 423 (±60) nm and a larger mode at 1195-1439 nm with an average of 1280 (±138) nm. Again CONTIN confirmed a smaller mode with weak intensities. The larger mode had sizes of 788-957 nm with an average of 896 (±94) nm. The concentrate showed much larger particle sizes than the intermediate and a narrower PSD, which was attributed to centrifugation in general [97, 79]. In addition, the particle sizes were largest compared to the previously analyzed FE concentrates, which could be explained by the comparatively high degree of centrifugation [79].

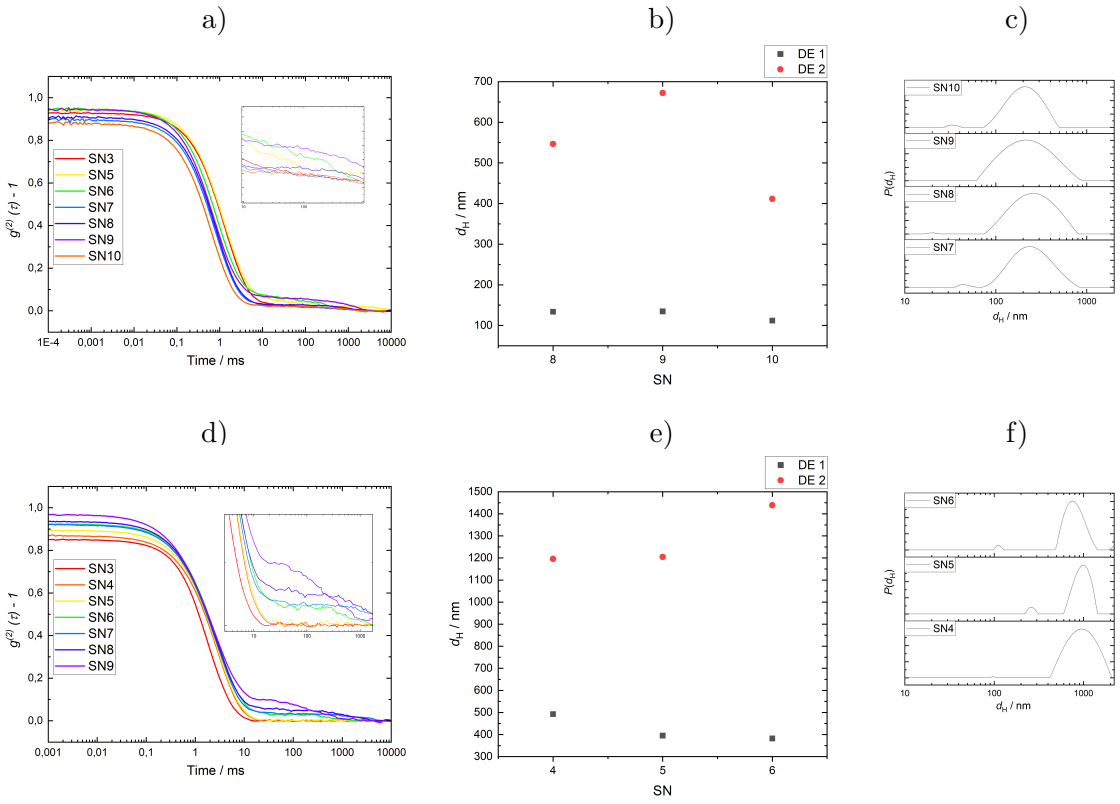


Figure 4.23 Normalized intensity correlation functions $g^2(\tau)-1$ for the dilution series of FE (variation) intermediate (a) and concentrate (d). Particle diameters d_H in nm for FE (variation) intermediate (b) and concentrate (e), calculated with DE fitting. Intensity weighted particle size distribution $P(d_H)$ for FE (variation) intermediate (c) and concentrate (f), calculated with CONTIN.

4.3.1.6 Summary of DLS Measurements

Tab. 4.9 summarizes the calculated particle sizes for DE fitting and CONTIN. The general character of the PSD was found to be bimodal for all latices examined. This is in agreement with results from HB latex [2]. Accordingly, a monomodal PSD could not be confirmed [24], but it must be noted that the smaller mode was usually only represented very weakly and the range of the particle sizes found was essentially similar (see Tab. 2.4). Taking into account the disappeared smaller mode after concentration, as for TKS latex with a lower solids content of about 25% [2], this result shows that TKS latex with high solids content can be isolated while maintaining the original bimodal PSD. The degree of purification, and in particular centrifugation,

was associated with an increase in particle size. Even if no quantitative statement was sought here, it should be possible to control the particle sizes in a controlled manner by varying the purification parameters described. Associated properties such as viscosity may be further adapted to individual needs.

Table 4.9 Average particle diameters d_H in nm and corresponding standard deviations of all samples investigated as a function of the DF of the dilution series of latex with the dilution medium ETA (aq) at 25 ± 0.1 C, calculated with DE fitting and with CONTIN algorithm (interm. = intermediate, conc. = concentrate).

Investigated latex	**DE**				**CONTIN**			
	d_{H1} nm	Δd_{H1} nm	d_{H2} nm	Δd_{H2} nm	d_{H1} nm	Δd_{H1} nm	d_{H2} nm	Δd_{H2} nm
Native latex, early harvest	220	21						
Native latex, stored roots	141	34	515	61	80		273	22
JE RE	112	14	419	49				
JE interm.	168	28	807		40			
JE conc.	127	27	797	36				
FE A interm.	144	4	798		103	25	596	76
FE A conc.	139		667		75	14	557	158
FE B interm.								
FE B conc.	231	19	830	55	104	12	522	36
FE C interm.	155	9	760	27	109	9	493	39
FE C conc.	289		1096		92	31	745	60
FE (variation) interm.	127	13	543	130	230	23		
FE (variation) conc.	423	60	1280	138			896	94

4.3.2 Colloidal Stability

The immediate importance of latex stability was manifested in yield-lowering coagulation processes during latex isolation. In addition, sufficient stability was required for dipping or for producing latex foam. In general, the discussed influence of the type of extraction, concentration and preservation on the latex stability was confirmed [2, 79]. While light microscopy has already been used to illustrate the gradual latex isolation, ZP measurements provided further assessment of latex stability. A relationship between dispersion stability and ZP was proposed by Riddick (see Tab. 4.10) and served to discuss the results presented below [113].

Table 4.10 Proposed dispersion stability with respect to ZP [113].

Dispersion stability	**ZP [mV]**
Maximal agglomeration and precipitation	0 ... +3
Range of strong agglomeration and precipitation	+5 ... −5
Threshold of agglomeration	−10 ... −15
Threshold of delicate dispersion	−16 ... −30
Moderate stability	−31 ... −40
Fairly good stability	−41 ... −60
Very good stability	−61 ... −80
Extremely good stability	−81 ... −100

By using a dilution series as for DLS, influences such as multiple scattering could be reduced [2]. The most consistent values were obtained in the range of SN4-7 and used to calculate the mean ZP. The ZP of native TKS latex was recently investigated by our group and showed extremely good stability with approx. −84 mV in the presence of ETA and was slightly less stable than HB latex with approx. −93 mV [2].

4.3.2.1 ZP of JE latex

A ZP of approx. −61 mV was already measured for concentrated JE latex with 25% SC [2]. In view of the slightly different approach that enabled the isolation of more concentrated latex according to Tab. 4.1, and the correlations discussed in section 4.2.1.2, an approximate range of stability in relation to pH was of interest and is shown in Fig. 4.24.

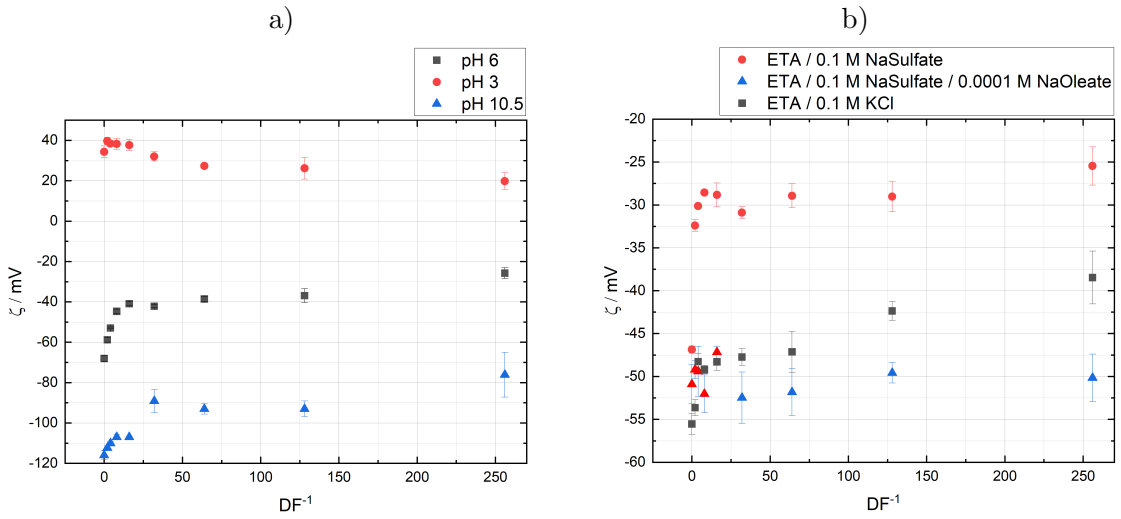


Figure 4.24 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the inverse dilution factor DF^{-1} of dilution series with latex concentrate of the JE, as specified in Tab. 4.1. In (a), the dilution series were prepared with the dilution media ETA (aq, pH 10.5), deionized water (pH 6) or HCl (aq, pH 3). In (b), the dilution series were prepared with the dilution media ETA (aq, pH 10.5) with additional 0.1 M NaSulfate, 0.1 M KCl or both 0.1 M NaSulfate and 10^{-4} M NaOleate. The dilution media of (b) finally resulted in a pH of 11.

In the presence of ETA at pH 10.5, the standard environment during latex isolation, extremely good stability was found with a ZP of −88 (±8) mV. It primarily confirmed that latex could be successfully stabilized during isolation. This was not evident due to the potentially destabilizing effects such as the initially strong dilution during extraction (see section 2.3.1.1), the mechanical stress caused by centrifugation (see section 2.3.2.4) and the increasing interactions due to the decreasing particle distance during concentration (see section 2.2.3). It was assumed that the high stability could be achieved through factors such as the slowed acidification due to the removal of impurities and obviously through the use of the respective stabilizing solutions. With regard to the pH stability, the measured ZP could be traced back to the relatively high pH value, which resulted in a negatively charged particle shell. At pH 6, the stability was reduced to a ZP of −36 (±7) mV. This could be explained by a reduced electrostatic repulsion between the latex particles due to the protonation of latex particle proteins and carboxylic acids (see sections 2.2.3.3 and 2.3.1.1). Although the stability could still be considered moderate, it confirmed the importance of a sufficiently high pH. At pH 3 the stability decreased significantly and showed a positive ZP of 26 (±5) mV, which means that the polarity of the protein shell was reversed.

In addition, the fundamental influence of salting out and stabilizing additives on the stability was of interest. The ZPs after the addition of KCl or NaSulfate and both NaSulfate and NaOleate in the presence of ETA at pH 11 are shown in Fig 4.24. With 0.1 M KCl, a fairly good stability with a ZP of −44 (±4) mV could be measured. With 0.1 M NaSulfate, a lower stability of −29 (±2) mV was found, which represented the threshold of a delicate dispersion. This was apparently caused by the screening of stabilizing charges on the surface of the latex particles (see section 2.2.3.3.). The addition of 10^{-4} M NaOleate to the dispersion medium containing NaSulfate was able to restore the stability and led to a ZP of −51 (±1) mV. This observation demonstrated the general ability to control the stability of TKS latex when salting out compounds reduce the electrostatic repulsion of latex particles. It might be used in protein-reducing treatments, in which the protein is denatured by salting out (see section 2.2.6.3).

4.3.2.2 ZP of FE Latex

Table 4.11 Summarized processing history of investigated FE latex for ZP measurements. The respective protocols are given in detail in Tab. 4.4.

Sample	**Processing history**
A intermediate	Single creaming of RE
A concentrate	Dilution of A intermediate, centrifugation
A dilution	Dilution of A concentrate
A concentrate (stored intermediate)	Dilution of A intermediate, centrifugation
B intermediate	Repeated creaming of RE
B concentrate	Dilution of B intermediate, centrifugation
B dilution	Dilution of B concentrate
B concentrate (stored intermediate)	Dilution of B intermediate, centrifugation
C intermediate	Repeated creaming, centrifugation
C concentrate	Dilution of C intermediate, centrifugation
C dilution	Dilution of C concentrate
C concentrate (stored intermediate)	Dilution of C intermediate, centrifugation

The ZP of latex obtained from FE is discussed below. As used for DLS, three latex intermediates A, B and C and their resulting concentrates, characterized by increasing purification effort were analyzed approx. 10 months after isolation. Immediately before the measurement, further samples were prepared in order to investigate the effects of the storage form on the latex stability. On the one hand, latices were prepared by diluting stored concentrates with water by 1:1 (v/v). On the other hand, concentrates were prepared from stored intermediates by centrifugation according to

Tab. 4.4. The samples examined and their processing history are summarized in Tab. 4.11. In Fig. 4.25, the ZPs of the stored intermediate, the concentrate and the concentrate produced from the stored intermediate for each sample group A, B and C are compared.

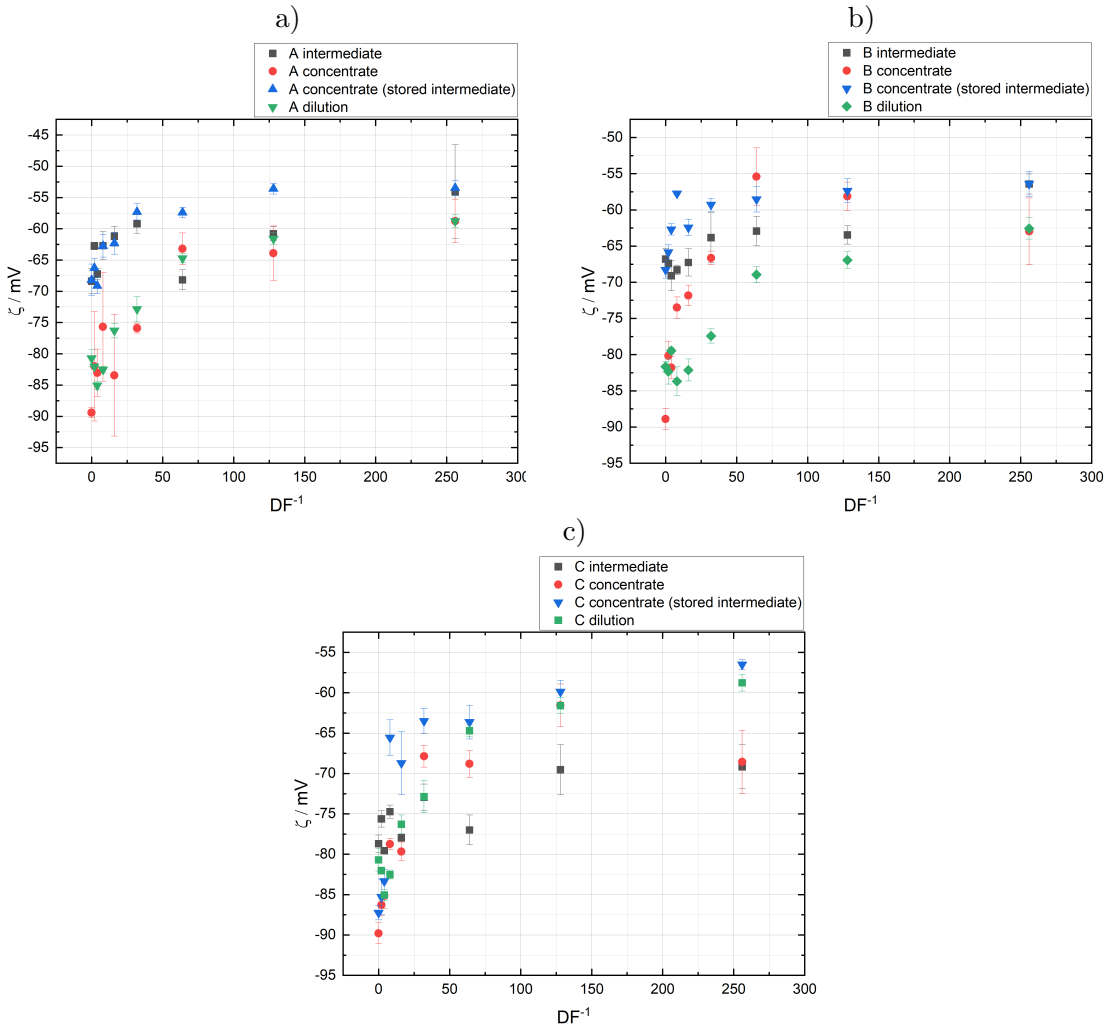


Figure 4.25 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the inverse dilution factor DF^{-1} of dilution series. The latter were prepared with samples of the FE, as specified in Tab. 4.4, and the dilution medium ETA (aq, pH 10.5).

The A intermediate showed a ZP of −61 (±6), the A concentrate was measured to be −65 (±7), the A dilution had −64 (±6) mV and the A concentrate, prepared from the stored intermediate gave −55 (±2). For the B intermediate, a ZP of −62 (±4) was measured, the B concentrate had −61 (±5), the B dilution gave −69 (±6) mV

and B concentrate, prepared from the stored intermediate had −58 (± 1). The C intermediate showed a ZP of −72 (±4) mV, the C concentrate had −67 (±3) mV, the C dilution was measured to be −65 (±4) mV and C concentrate, prepared from the stored intermediate had −61 (±3) mV. It gets clear that all latices examined showed fairly good to very good stability. Moreover, the differences between intermediates, concentrates, diluted concentrates and concentrates prepared from stored intermediate could be considered as small. However, some conclusions might be drawn. Although no trend could be derived for a lower stability of stored intermediates compared to stored concentrates, the intermediates apparently had a reduced stability against centrifugation as a result of the storage. This was shown by the lower stabilities of concentrates from stored intermediates compared to stored concentrates. Since other factors had to be taken into account in addition to colloidal stability, this finding did not allow a decision for a better form of storage. In particular, the stored concentrate showed after-creaming with oxidations and film formation on the progressively concentrating latex surface. To prevent this, the proportion of NaSulfite might be increased, taking into account the strong sensitivity of the ZP to changes in the electrolyte concentration (see below). In addition, the latex should be stirred regularly to avoid the consequences of after-creaming. Because of the lower SC, this was less of a problem for the stored intermediate. However, the up to 15 times higher volume of the intermediate compared to the concentrate was a disadvantage in terms of storage. The ZPs of rediluted concentrates confirmed that a controlled production of latex with a desired SC and viscosity can be achieved from stored concentrates without destabilization. Even if direct comparisons between A, B, and C should be treated with caution, the higher stability of the stored C intermediate and concentrate could be observed. This might be due to a more efficient separation of impurities as a result of the additional centrifugation step.

4.3.2.3 ZP of FE Latex (Variation)

Due to the changed phase separation discussed in section 4.2.2.4, the ZP of latex from the FE (variation) was of interest. The latices examined are summarized in Tab. 4.12.

Table 4.12 Summarized processing history of investigated latex samples, which originated from FE (variation) RE prepared as described in section 3.1.2.3. The respective protocols are discussed in detail in Tab. 4.5.

Sample	**Processing history**
RE	-
1st intermediate	Sedimentation of RE
2nd intermediate	Dilution of 1st intermediate, centrifugation
3rd intermediate	Dilution of 2nd intermediate
Concentrate	Dilution of 3rd intermediate, centrifugation

The isolation of latex within the lower phase was accompanied with the accumulation of non-rubber substances. It is known that some of these serve as natural stabilizers, but it has also been suggested that higher levels of contamination would reduce latex stability in the long term by causing microbiological deterioration (see section 2.3.1.1).

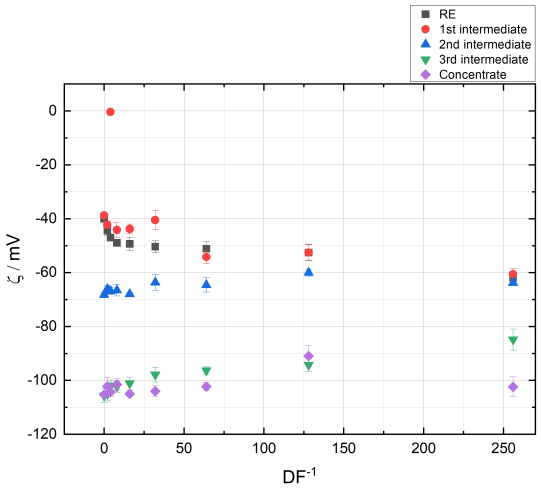


Figure 4.26 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the inverse dilution factor DF^{-1} of dilution series. The latter were prepared with samples of the concentration sequence of FE latex (variation), as specified in Tab. 4.5, and the dilution medium ETA (aq, pH 10.5).

Overall, the latex was able to withstand this effect and showed no significant decrease in stability after sedimentation (see Fig. 4.26). Both RE and 1st intermediate had fairly good stabilities with ZPs of −54 (±5) and −52 (±8) mV, respectively. The 2nd intermediate, which was obtained by centrifugation of the 1st intermediate, showed very good stability with a ZP of −63 (±2) mV. This slightly increased stability was mainly attributed to the addition of KOleate before centrifugation. For one, it was assumed that carboxylates concentrated on the surface of the latex particles to improve overall electrosteric stability (see section 2.2.3.2). For another, possibly

destabilizing impurities were preferably removed with the lower phase. Accordingly, both the 3rd intermediate as well as the final concentrate showed an even better, extremely good stability with ZPs of −93 (±6) and −100 (±6) mV, respectively. These observations confirmed that despite the initial sedimentation step, a highly stable latex concentrate could be produced after the complete concentration sequence.

With regard to the concentration sequence and further processing of the latex as well as for general comparability, the resilience of the latex stability to electrolytes was examined. Accordingly, the stabilities of RE, 1st intermediate and concentrate in the presence of an increasing NaCl concentration are shown in Fig. 4.27. The expected tendency of decreasing stability with increasing electrolyte concentration was confirmed (see section 2.2.3.3). This could be explained by insufficient repulsion between latex particles due to the screening of electrical charges. For RE, which was initially fairly well stabilized, the presence of 0.01 M NaCl led to a lower, moderate stability with a ZP of −41 (±2) mV. Increasing the NaCl concentration to 0.1 and 0.5 M led to a further decrease in stability with ZPs of −21 (±3) and −14 (±3) mV, respectively. The 1st intermediate showed the same trend as the RE with a stronger decrease in stability for a given increase in electrolyte concentration. In the presence of 0.01 M NaCl, a ZP of −36 (±3) mV was measured, which meant that the stability was still moderate. Increasing the NaCl concentration to 0.1 M and 0.5 M led to ZPs of −26 (±3) and −15 (±1) mV, respectively, which corresponded to the agglomeration threshold for the latter. The concentrate showed an even stronger decrease in stability when the electrolyte concentration was increased. A ZP of −41 (±2) mV was measured for 0.01 M NaCl, which could still be regarded as fairly stable. A further increase to 0.1 M and 0.5 M NaCl led to a similar destabilization as for the intermediate, with ZPs of −21 (±3) and −14 (±3) mV, respectively. As a result, more concentrated latex was shown to be more sensitive to electrolyte concentration. The ZPs showed the same trend as reported for HB latex. The TKS latex concentrate in the presence of 0.001 M NaCl was more stable than HB latex with −66 mV. For 0.01 M NaCl and 0.1 M, the TKS latex concentrate had a stability similar to that of HB latex with approx. −40 mV and −16 mV, respectively [26]. Finally, this basic knowledge on destabilization might be used in latex processing for controlled destabilization [33].

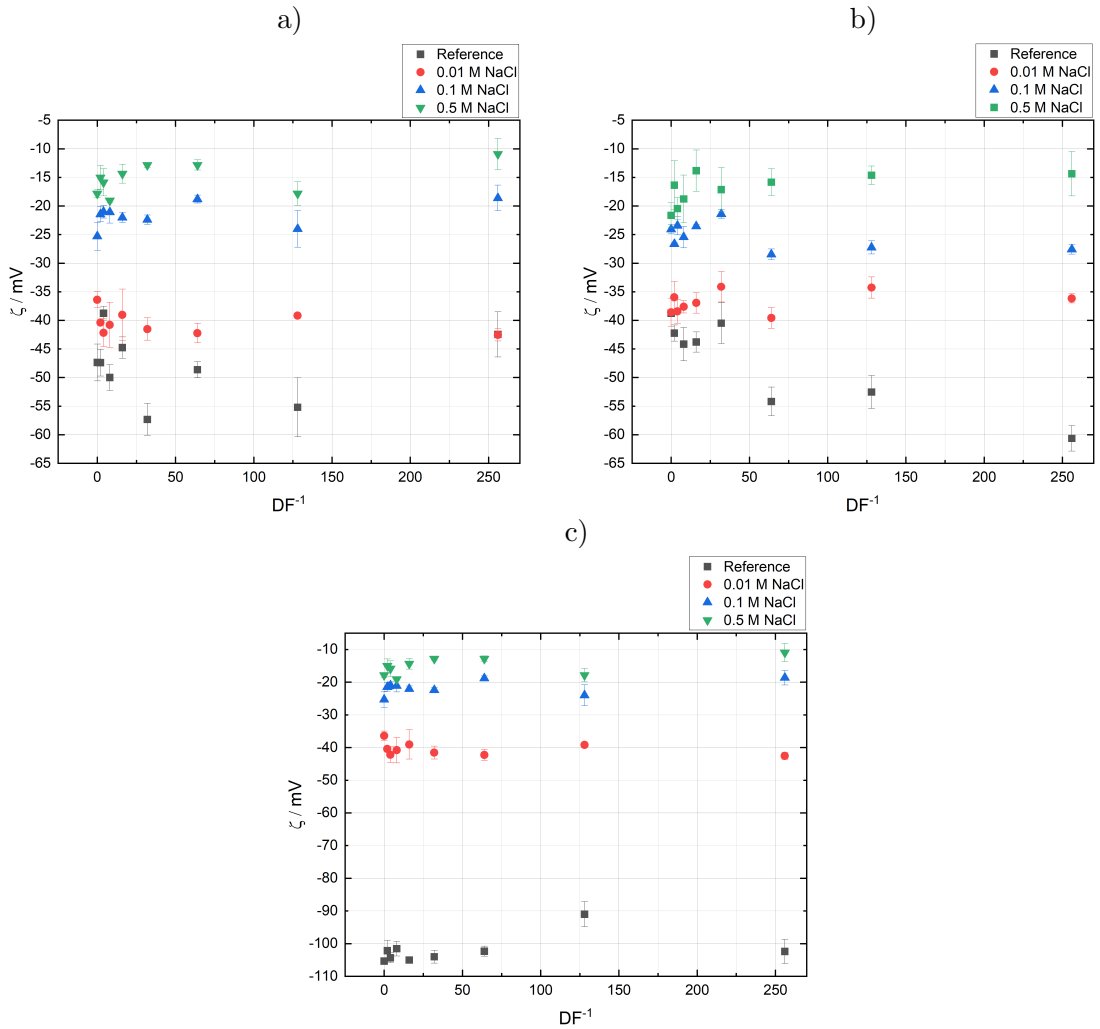


Figure 4.27 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the inverse dilution factor DF^{-1} of dilution series. The latter were prepared with RE (a), 1st intermediate (b) and concentrate (c), as specified in Tab. 4.5, and the dilution medium ETA (aq, pH 10.5) with electrolyte concentrations c(NaCl) = 0.01 M, c(NaCl) = 0.1 M and c(NaCl) = 0.5 M. It is compared to the samples obtained with c(KCl) = 0.001 M (reference).

4.3.2.4 Summary of ZP Measurements

All in all, it can be seen that after the isolation sequences, at least a fairly good stability was achieved. The ZPs of all latices examined are summarized in Tab. 4.13.

Table 4.13 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV for all samples investigated (NaSu = NaSulfate, NaOl = NaOleate).

Investigated latex	**Dilution medium**	ζ **[mV]**	$\Delta\zeta$ **[mV]**
JE conc.	ETA (pH 10.5)	−88	8
JE conc.	Water (pH 6)	−36	7
JE conc.	HCl (pH 3)	26	5
JE conc.	ETA, 0.1 M KCl (pH 11)	−44	4
JE conc.	ETA, 0.1 M NaSu (pH 11)	−29	2
JE conc.	ETA, 0.1 M NaSu, 10^{-4} M NaOl (pH 11)	−51	1
FE A interm.	ETA (pH 10.5)	−61	6
FE A conc.	ETA (pH 10.5)	−65	7
FE B interm.	ETA (pH 10.5)	−62	4
FE B conc.	ETA (pH 10.5)	−61	5
FE C interm.	ETA (pH 10.5)	−72	4
FE C conc.	ETA (pH 10.5)	−67	4
FE A dilution	ETA (pH 10.5)	−64	6
FE A conc. (stored interm.)	ETA (pH 10.5)	−55	2
FE B dilution	ETA (pH 10.5)	−69	6
FE B conc. (stored interm.)	ETA (pH 10.5)	−58	1
FE C dilution	ETA (pH 10.5)	−65	4
FE C conc. (stored interm.)	ETA (pH 10.5)	−61	3
FE (variation) RE	ETA (pH 10.5)	−54	5
FE (variation) 1st interm.	ETA (pH 10.5)	−52	8
FE (variation) 2nd interm.	ETA (pH 10.5)	−63	2
FE (variation) 3rd interm.	ETA (pH 10.5)	−93	6
FE (variation) conc.	ETA (pH 10.5)	−100	6
FE (variation) RE	ETA (pH 10.5), 0.01 M NaCl	−41	2
FE (variation) RE	ETA (pH 10.5), 0.1 M NaCl	−21	3
FE (variation) RE	ETA (pH 10.5), 0.5 M NaCl	−14	3
FE (variation) 1st interm.	ETA (pH 10.5), 0.01 M NaCl	−36	3
FE (variation) 1st interm.	ETA (pH 10.5), 0.1 M NaCl	−26	3
FE (variation) 1st interm.	ETA (pH 10.5), 0.5 M NaCl	−15	1
FE (variation) conc.	ETA (pH 10.5), 0.01 M NaCl	−41	2
FE (variation) conc.	ETA (pH 10.5), 0.1 M NaCl	−21	3
FE (variation) conc.	ETA (pH 10.5), 0.5 M NaCl	−14	3

On the one hand, various factors were assumed to reduce latex stability. At first the extraction itself represented a drastic change in the chemical environment (see section 2.3.1.1). The latex was then subjected to mechanical stress by centrifugation (see section 2.3.2.4). In the course of concentration, the distance between the particles was reduced, which led to stronger interactions between the latex particles (see section 2.2.3). On the other hand, there were aspects that maintained latex stability. It was assumed that the removal of impurities in the course of concentration slowed down acidification processes and thereby increased long-term stability. Presumably, maintenance of the stability could be explained primarily by the stabilizing solutions

used. While the FE concentrates generally showed lower stabilities compared to native TKS latex (−84 mV [2]), they were similar to JE latex with a lower SC of 25% (−61 mV [2]). FE concentrates from sedimented intermediate products were also successfully stabilized. In addition, their stability was at least as high and resistant to electrolytes as HB latex.

4.3.3 Reduction of Protein Concentration

As shown in section 2.2.6, latex proteins can cause allergies. For this reason, a reduced protein concentration was aimed for in the course of latex isolation. The main challenge was to maintain the colloidal stability, since proteins bound to the latex particle shell contribute to a large extent to the stabilizing, repulsive electrostatic force, as described in section 2.2.3. B intermediate latex was used as the starting and reference point for the respective tests, as given in Tab. 4.4. It should be noted that the measured protein concentration corresponded to the serum phase after the respective protein removal step. It was assumed that this concentration correlated with the protein concentration of the original latex, according to sec. 2.2.6.

4.3.3.1 Determination of Protein Concentration

As discussed in section 2.2.6.2, ISO determination methods were generally limited to the protein concentration of solid NR, while few were applicable to NR in its dispersed latex state. Unfortunately, the Lowry method commonly used was not suitable for protein determination because of its poor compatibility with the surfactants used for latex stabilization [68]. Since the Kiehldahl method measures the nitrogen content and the main stabilizer was ETA, this method could not be used either [69]. Finally, the BCA assay proved to be suitable for the system under study [67, 75]. However, a direct measurement of the latex samples was not successful, since clear solutions without other interfering substances were required for the UV/Vis spectroscopy of protein-metal complexes. For this reason, the latex particles were removed first, and thus inevitably also particle-bound proteins. At the same time, complete removal of latex particles was not possible for experimental reasons. Therefore, this step was considered critical to the final evaluation of the results. Second, non-rubber substances were removed by adding a solution containing TCA. The reagent induced the precipitation of proteins as well as residual NR. After centrifugation, a protein pellet was formed which could be separated from the remaining solution. Since the pellet was barely visible and could easily be lost as the solution drained, particular caution was required in this step. The UV/Vis absorption measurements were generally not reproducible when samples were compared to a separately measured BSA standard curve. This was attributed to insufficient protein recovery after the precipitation step and could be solved by using higher centrifugation speeds and times than

recommended [102]. In addition, it had to be taken into account that the method was limited to protein solubilization by TCA. As shown in section 2.2.6.2, there may be protein fractions that are only soluble in other agents, which should be tested in further experiments. Overall, the outlined pretreatment in combination followed by the BCA assay was considered to be a reasonable approach for monitoring the protein concentration of TKS latex. The respective data for calibration are given in Tab. 6.12. Furthermore, a standard addition approach for protein quantification was considered [92]. This enabled the matrix effects of the latex serum to be minimized by adding the BSA standards directly to the latex without further treatments such as protein precipitation or NR removal. However, no linear correlation between protein concentration and absorption signal was observed. Fitting the absorption maxima to a polynomial term only allowed a rough estimate of the protein concentration. After appropriate adjustments, this approach might be used for direct protein quantification in TKS latex and plant extracts in general, but was not used for the following analysis.

4.3.3.2 Solubilization of Proteins

In order to reduce the protein concentration, a sequence of protein denaturation and solubilization followed by repeated purification steps was used, implementing concepts of Fig. 2.12 and Tab. 4.1. Before that, a screening of the solubilizers proposed for HB latex provided an assessment of their suitability for TKS latex. As described in section 2.2.6.3, Triton X-100 is used for accurate protein determinations because of its ability to solubilize particle-bound proteins. In that embodiment, resulting serum phases were used for protein analysis while latex was removed as a coagulated residue [75]. Therefore, the combination of adding Triton X-100 followed by centrifugation was seen as promising. If the remaining latex was properly stabilized and isolated in a dispersed instead rather than a coagulated state, this process would ideally result in low-protein latex. Other surfactants were screened accordingly. In fact, the samples examined did not coagulate after centrifugation and protein reduction was possible in this way. With Triton X-100, 96 µg/mL could be removed from the latex phase. The greatest protein reduction was achieved in the presence of the anionic surfactant SDS with 145 µg/mL. This higher efficiency of anionic surfactants than of nonionic surfactants in removing oligopeptides in the latex stage has been reported

[114]. Using the combination of SDS and Triton X-100, the value fell slightly to 138 µg/mL. Similarly, the nonionic surfactant sodium deoxycholate alone on its own could remove 118 µg/mL, while the combination with SDS resulted in a lower value of 77 µg/mL. These differences might be due to interactions between the surfactants that affected the overall solubilization efficiency. Using water, 81 µg/mL could be removed, which was attributed to C-serum proteins. In addition, it could be suggested that the differences to the samples with additives were due to the solubilization of particle-bound proteins. The corresponding protein concentrations in the serum after centrifugation are shown in Tab. 4.28.

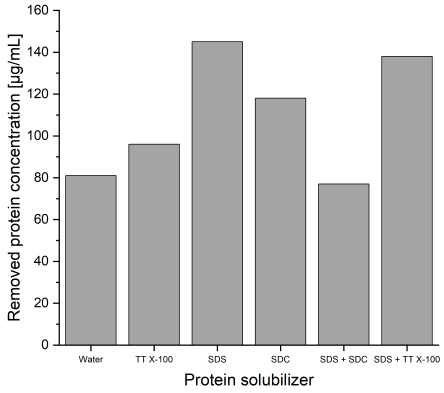


Figure 4.28 Removed protein concentrations after addition of various solubilizers and centrifugation. The additions were made using TT X-100, SDS and SDC, respectively to the extent of 1%. Detailed compositions are given in Tab. 3.4.

4.3.3.3 Purification by Denaturation and Solubilization of Proteins

Preliminary tests on the purification sequence indicated the importance of a suitable SC of the initial latex prior to the centrifugation that separated the proteinaceous serum and the purified latex (see section 4.2.5). That is, a compact concentrate was not formed if the SC was too low. In turn, this resulted in contamination of the isolated latex with the serum that carried the solubilized proteins. If the SC was too high, a part of the latex was still dispersed as a dilute phase between the concentrate and the proteinaceous serum. In view of this, two approaches of latex isolation were used to address these circumstances. In the spatula protocol, purification was achieved by resuspending the latex in each new container, whereas in the syringe protocol, purification was generally achieved in a single container by resuspension after removal of the serum. In addition, the SC of the original la-

tex and obtained samples differed due to resuspension during the purification steps. Therefore, the initially measured protein concentrations were corrected by the respective dilution to $c(Protein, dilution-corrected)$ and related to the respective SC using a correction formula that provided the final removable protein concentration $c(Protein, removable)$, which was given by:

$$\text{c(Protein, removable)}[\mu g/mg] = \frac{\text{c(Protein, dilution-corrected)}[\mu g/mL]}{\text{SC}[mg/mL]} \tag{4.9}$$

After incubation with the solution of urea (1%)/SDS (1%)/Triton X-100 (1%) for one hour and centrifugation, protein concentrations of approx. 1000 µg/mL latex were measured in the serum, which corresponds to a removable protein concentration of approx. 11 µg/mg solids. At the same time, this could be viewed as the protein concentration of the initial latex. Given the tendencies of the solubilization test and the approaches discussed in section 2.2.6.3, SDS (1%)/Triton X-100 (1%) was chosen as wash solution for the following purification sequences. As a result, the protein concentration could be reduced significantly, as shown in Fig. 4.29. With the syringe protocol the removable protein concentration could be reduced to 1.08 µg/mg solids. There was less tendency to latex coagulation and contamination by proteinaceous serum during the transfer step. The serum could be removed thoroughly without disturbing the concentrated latex phase. It was assumed that the syringe protocol would result in poorer protein removal due to the carry-over of the B-serum proteins formed at the bottom of the tubes. Although samples were decanted into new tubes after resuspension, minor fractions of bottom sediments which contained B-serum proteins might have been carried along. In contrast, the spatula protocol had the advantage of not dragging B-serum proteins, but required a clean transfer of the concentrated latex, which was not evident. After 5 purification steps, the removable protein concentration decreased to 0.59 µg/mg solids, which was slightly less than for the syringe protocol. However, the differences between the methods were small. This could be explained by an already greatly reduced amount of B-serum proteins as a result of the purification history of the intermediate latex used. Since a prolonged urea incubation time slightly improved protein removal, incomplete denaturation was assumed after one hour in contrast to what has been reported for HB latex [114]. For that purpose, the purification steps of the spatula protocol were also carried out using the urea (1%)/SDS (1%)/Triton X-100 (1%) solution, which resulted in improved protein removal. After 2 purification steps, the removable protein concentration was

measured to be 0.12 µg/mg solids.

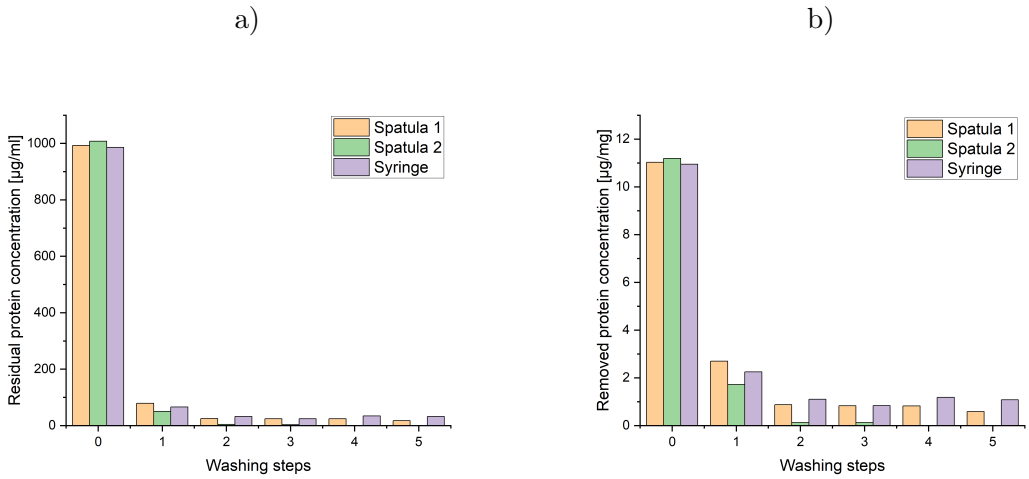


Figure 4.29 Dilution corrected, removable protein concentration $c(Protein, dilution - corrected)$ in $[\mu g/mL]$ (a), and removable protein concentration related to the respective SC $c(Protein, removable)$ in $[\mu g/mL]$ (b), after purification sequences for different protocols. Spatula protocols were based on a direct latex isolation from the upper layers. The syringe protocol comprised the removal of protein containing serum while leaving the latex mostly within one container. All variations were intitiated with an treatment with urea (1%)/SDS (1%)/Triton X-100 (1%) solution, which represented the purification step 0. While the syringe protocol and spatula 1 protocol involved a purification sequence using SDS (1%)/Triton X-100 (1%) solution, the spatula 2 protocol involved a purification sequence using urea (1%)/SDS (1%)/Triton X-100 (1%) wash solution.

However, the proteins could not be completely removed within these tests. This could be attributed to the equilibrium of proteins between the aqueous phase of the concentrated latex phase and the serum. In other words, some proteins will always remain in the latex unless all of the dispersion medium is expelled, which would obviously lead to coagulation. That is, even if particle-bound proteins were initially successfully denatured and solubilized, they could remain in the dispersion medium of the isolated latex. Nevertheless, the described approach proved to reduce the removable protein concentration up to approx. 99% with respect to the initial intermediate latex. At this point, it seems obvious to consider the general allergenic potential of proteins in TKS latex. While the purified samples obtained above could not yet be analyzed, the allergenic potential of similar serum fractions obtained after centrifugation of FE intermediate latex was investigated by Synthomer. It was found that none of the samples examined contain allergens known from HB latex. However, another study demonstrated that both soluble and particle-bound proteins

in TKS latex show cross-reactivity with anti-HNRL antibodies [61]. In view of the different origins and processing history of the latices, the remaining 1% proteins after the purification described above must still be suspected of being allergenic for Type I latex-sensitized humans. Eventually, it confirmed that previously discussed methods (see section 2.2.6.3) for producing low-protein HB latex can be applied to TKS extracts with a similar extent of protein reduction. The protein concentration might be further reduced by additional purification steps or by using different solubilizers. Although PEG was not successful [73], the approach to use polymers, in particular to replace the particle-bound proteins, holds great promise. For this purpose, polymers with an even higher affinity for the positively charged inner phospholipid layer must be investigated. More importantly, this should be further explored along with an appropriate upscaling of this fundamentally designed protein reduction test. Due to the basically same concept as described in section 4.2.2, it should best be implemented directly with the latex isolation.

5 Conclusion and Outlook

Within this thesis, NRL from roots of TKS could be isolated and characterized. By adapting various concepts for colloidal systems, plant extraction and common latex production, a process from the root to the characterized latex was described that made important advances in the field of TKS latex. Initially, testing different concepts for handling TKS roots prior to extraction provided basic information, especially for a larger scale application. The significance of appropriate harvesting technique, storage and cleaning for successful latex extraction was confirmed. This included an intact root surface that could be improved by more controlled cultivation in greenhouses. While storage in moist sand has allowed roots to be used for latex extractions for up to several weeks, research is needed to further extend the working window. Better root cleaning resulted in higher long-term stability of the subsequently extracted latex. Rosette and leaf removal methods need to be explored to improve workflow and scalability. The comparison of different approaches to latex extraction itself showed that a weaker root crushing yields extracts that are of better quality and easier to process. In turn, greater comminution did not make it possible to isolate more latex. Significant latex losses during root processing need to be further unraveled and addressed. The flow extraction provided the best extract in terms

of purity and processing properties. By adapting suggested approaches, an upscaled extraction sequence could be designed and applied. Concentration and purification of the extracts were achieved by a step-by-step process that involved repeated sequences of creaming, centrifugation and redilution. It was possible to set up protocols for the production of latex with a SC of over 60%. The non-rubber substances contained therein and their effects on processability compared to HB latex must be investigated in the future. Detailed correlations between SC, contamination, stability and their effects on latex processing could be determined. For each type of extract or raw latex, concepts for the best possible processing could be outlined. Latex could be stabilized during extraction, purification, concentration and storage with ammonia-free stabilizing solutions based on ETA/ETA-HCl, oleates, sulfites and BAC, which represented a further step for a safe and green latex isolation. In future, a compromise between the amount of stabilizing solution used and the type of root shredding should be found, taking into account the purity of the latex and the workload. If necessary, other ways of latex purification must be investigated. Even if some of the sequences described were able to concentrate the latex sufficiently, a more automatic way of removing contaminants must be developed. In particular, the selective reduction of the interactions between contaminants with latex particles should be further examined. In addition, a more automatic concentration concept has to be developed, since continuous concentration could not be achieved due to problems with phase separation and latex stabilization. In application tests by Synthomer, mixtures of TKS latex with HB latex could be processed into rubber foam. Unfortunately, the overall amount of latex produced was not yet sufficient for industrial applications. Even so, the protocols discussed provide a better understanding and a starting point for a future resumption of latex isolation. Representative latex fractions could be characterized with regard to colloidal properties that could be related to latex from other NR-bearing plants, the respective isolation approach and finally the resulting handling and processing properties. DLS measurements showed a bimodal PSD with a generally weakly pronounced smaller mode and particle sizes increasing with the extent of the purification. ZP measurements confirmed the stability of differently obtained latices after storage, in the presence of electrolytes and when rediluted for the purpose of adapting the SC. In addition, the protein concentration of extracted latex could be determined and significantly reduced. In combination with the established latex isolation protocols, this demonstrated the possibility of producing low protein TKS latex.

For an assessment of future applications of TKS latex, the individual processing steps must be evaluated from a holistic economic and practical point of view. First, this includes the demand for fields under suitable growing conditions where established regional crops must be taken into account. Apart from the relatively low overall yields achieved with the protocols described, only a few liters of latex per hectare might currently be produced, even assuming ideal processing conditions. In addition to the cultivation itself, the corresponding costs for root preparation and latex production are estimated to be in the order of several thousand euros. In view of the discussed prices for common latex, this illustrates the general framework and limits of latex production from TKS. However, an increasing added value is expected for hypoallergenic TKS latex, considering the demand for products with the valued properties of NRL combined with hypoallergeneity. A step towards such latex could be shown in this work. In addition, the consumption of resources during processing must be taken into account. The main part of the concentration and purification could be achieved by creaming, which is advantageous due to the low energy consumption. Another aspect is the use of water and chemicals. Aside from root cleansing, most of the water and chemicals used are attributed to the cleansing and stabilization of the generally heavily polluted extracts. However, the main problem for further application is not seen primarily in the consumption of chemicals, but rather in the challenging logistical aspect. Obviously, the factor of the volume of stabilizing solution used per isolated latex is extremely high. The provision, handling, storage and transport of such high amounts of solution are certainly not optimal in an overall view, even if they were found to be necessary for the production of high-quality latex.

6 Supplementary Data

6.1 DLS Measurements

Samples		Native latex, early harvest				Native latex, stored roots			
		DE		CONTIN		DE		CONTIN	
SN	DF	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm
4	1:16	221	599		299	167	475	108	332
5	1:32	199	544	85	293	165	488		280
6	1:64								
7	1:128	247	1020		247	153	501	69	288
8	1:254	199	549	75	266	84	624	31	251
9	1:508	233	1297		260	137	488	21	219

Table 6.1 Particle diameters d_H in nm of latex from roots harvested approx. 3 months early and from roots stored in sand for approx. 3 months as a function of the DF of the dilution series of latex with the dilution medium ETA (aq) calculated with DE fitting and with CONTIN algorithm.

Table 6.2 Particle diameters d_H in nm of JE RE, JE intermediate and JE concentrate as a function of the DF of the dilution series of latex with the dilution medium ETA (aq), calculated with DE fitting and with CONTIN algorithm.

JE		RE				Interm.				Conc.			
		DE		CONTIN		DE		CONTIN		DE		CONTIN	
SN	DF	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm
4	1:16	126	436	46	266	197	812	52	405	303	902	75	619
5	1:32	116	415	28	237	169	842		346	270	865	67	583
6	1:64	116	448	41	208	140	766		266	168	799	69	538
7	1:128									120	801	76	525
8	1:254	111	462		147					109	750	65	438
9	1:508	89	336		153					115	838		301

Table 6.3 Particle diameters d_H in nm of FE A, B and C intermediates and concentrates as a function of the DF of the dilution series of latex with the dilution medium ETA (aq), calculated with DE fitting and with CONTIN algorithm.

FE		A interm.				B interm.				C interm.			
		DE		CONTIN		DE		CONTIN		DE		CONTIN	
SN	DF	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm
4	1:16	148	809	132	681	105	660			146	731	100	510
5	1:32	144	775	88	571					154	763	108	520
6	1:64	140	810	89	535							118	448
7	1:128									164	785		

FE		A conc.				B conc.				C conc.			
		DE		CONTIN		DE		CONTIN		DE		CONTIN	
SN	DF	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm
4	1:16			87	658							57	715
5	1:32			79	637	232	791	100	545	289	1096	118	814
6	1:64	139	667	59	374	249	892	95	540			100	707
7	1:128					211	806	118	48				

Table 6.4 Particle diameters d_H in nm of FE (variation) 1st intermediate and concentrate as a function of the DF of the dilution series of latex with the dilution medium ETA (aq), calculated with DE fitting and with CONTIN algorithm.

FE (variation)		1st intermediate				Conc.			
		DE		CONTIN		DE		CONTIN	
SN	DF	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm	d_{H1} nm	d_{H2} nm
4	1:16					493	1196	97	943
5	1:32					395	1205	261	957
6	1:64					382	1439	112	788
7	1:128					184	1048	75	739
8	1:254	134	546	20	254	162	1028	61	680
9	1:508	135	672		220				
10	1:1016	112	412	34	203				

6.2 Zeta-Potential Measurements

Table 6.5 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the DF of dilution series of JE concentrate for different pH, additions of KCl, NaSulfate and NaSulfate/NaOleate.

JE conc.		pH 3		pH 6		pH 10.5		KCl		NaSu		NaSu/NaOl	
SN	DF	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	34	2,9	−68	1,3	−116	2,2	−56	1,2	−47	0,4	−51	2,3
1	1:2	40	1,9	−59	1,2	−112	0,9	−54	0,9	−32	0,7	−49	1,1
2	1:4	38	1,5	−53	0,4	−110	0,8	−48	0,9	−30	0,1	−49	2,9
3	1:8	38	2,7	−45	1,1	−107	1,4	−49	0,5	−29	0,3	−52	2,2
4	1:16	38	2,8	−41	0,9	−107	0	−48	1,0	−29	1,4	−47	0,7
5	1:32	32	2,3	−42	0,8	−89	5,7	−48	1,0	−31	0,7	−52	3,0
6	1:64	27	1,2	−39	1,2	−93	2,6	−47	2,4	−29	1,4	−52	2,7
7	1:128	26	5,3	−37	3,4	−93	3,8	−42	1,1	−29	1,8	−50	1,2
8	1:256	20	4,1	−26	2,7	−76	11	−38	3,1	−25	2,2	−50	2,8

Table 6.6 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the DF of dilution series of FE A, B, C intermediates and concentrates, concentrates (prepared from stored intermediate) and concentrates (rediluted after storage) with the dilution medium ETA (aq, pH 10.5).

FE		A interm.		B interm.		C interm.		A conc.		B conc.		C conc.	
SN	DF	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	−68	2,1	−67	1,5	−79	1,1	−89	0,8	−89	1,5	−90	1,3
1	1:2	−63	0,6	−67	1,9	−76	1,0	−82	8,8	−80	2,0	−86	1,3
2	1:4	−67	0,9	−69	2,1	−80	0,3	−83	3,8	−82	1,5	−85	1,6
3	1:8	−63	2,6	−68	0,7	−75	0,8	−76	8,7	−74	1,5	−79	0,7
4	1:16	−61	1,6	−67	1,9	−78	1,5	−83	9,7	−72	1,4	−80	1,2
5	1:32	−59	1,5	−64	3,4	−73	1,7	−76	0,8	−67	0,9	−68	1,4
6	1:64	−68	1,6	−63	2,0	−77	1,9	−63	2,5	−55	4,0	−69	1,7
7	1:128	−61	1,1	−63	1,3	−70	3,1	−64	4,3	−58	2,0	−62	2,7
8	1:256	−54	7,5	−56	1,4	−69	2,7	−59	3,5	−63	4,6	−69	3,9

FE		A conc. (st. inter.)		B conc. (st. inter.)		C conc. (st. inter.)		A dil.		B dil.		C dil.	
SN	DF	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	−68	2,5	−68	1,1	−87	0,9	−81	1,4	−82	0,7	−75	5,5
1	1:2	−66	1,6	−66	1,0	−85	2,2	−82	0,3	−82	1,7	−81	1,2
2	1:4	−69	1,3	−63	0,8	−83	1,4	−85	0,7	−79	0,5	−82	0,6
3	1:8	−63	1,8	−58	0,2	−66	2,2	−83	0,6	−84	2,0	−84	1,4
4	1:16	−62	1,8	−62	1,1	−69	3,9	−76	1,2	−82	1,5	−79	0,7
5	1:32	−57	1,3	−59	0,9	−64	1,6	−73	2,0	−77	1,0	−70	1,7
6	1:64	−57	0,9	−59	1,8	−64	2,1	−65	0,7	−69	1,1	−67	1,2
7	1:128	−54	0,9	−57	1,7	−60	1,4	−62	1,0	−67	1,2	−62	1,1
8	1:254	−53	1,2	−56	1,7	−57	0,6	−59	1,0	−63	1,5	−61	0,6

Table 6.7 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the DF of dilution series of FE (variation) RE, intermediates and concentrate, with the dilution medium ETA (aq, pH 10.5).

FE (variation)		**RE**		**1st interm.**		**2nd interm.**		**3rd interm.**		**Conc.**	
SN	**DF**	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	−40	1,7	−39	0,5	−68	0,9	−106	2,5	−105	0,6
1	1:2	−44	1,7	−42	1,4	−66	1,2	−105	2,6	−102	3,2
2	1:4	−47	1,2	0	0,3	−67	1,3	−102	2,1	−104	1,5
3	1:8	−49	1,1	−44	2,9	−67	2,1	−102	2,1	−102	2,2
4	1:16	−49	2,4	−44	1,8	−68	0,7	−101	2,4	−105	1
5	1:32	−50	2,2	−41	3,6	−64	2,9	−98	2,7	−104	2
6	1:64	−51	2,5	−54	2,5	−65	2,6	−96	1,4	−102	1,5
7	1:128	−53	3,1	−53	2,9	−60	1,3	−94	2,4	−91	3,8
8	1:254	−62	1,4	−61	2,2	−64	1,1	−85	3,9	−102	3,7

Table 6.8 Zeta-potentials ζ and the corresponding standard deviations $\delta\bar{\zeta}$ in mV as a function of the DF of dilution series of FE (variation) RE, intermediates and concentrate, for additions of NaCl.

FE (var.)		**RE 0.01 M NaCl**		**RE 0.1 M NaCl**		**RE 0.5 M NaCl**		**1st interm. 0.01 M NaCl**		**1st interm. 0.1 M NaCl**	
SN	**DF**	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	−36	1,4	−25	2,4	−18	0,7	−39	2,5	−24	0,8
1	1:2	−40	0,3	−21	1,3	−15	2,1	−36	2,7	−27	0,2
2	1:4	−42	2,4	−21	1,1	−16	2,4	−38	2,1	−23	1,6
3	1:8	−41	3,9	−21	1,9	−19	0,3	−38	1,1	−25	1,9
4	1:16	−39	4,5	−22	0,9	−14	1,7	−37	1,8	−24	0,2
5	1:32	−42	2,0	−22	0,8	−13	0,4	−34	2,7	−21	0,8
6	1:64	−42	1,7	−19	0,7	−13	0,9	−40	1,8	−28	0,9
7	1:128	−39	0,3	−24	3,2	−18	2,1	−34	1,9	−27	1,2
8	1:256	−43	1,1	−19	2,3	−11	2,8	−36	0,8	−28	0,9

FE (var.)		**1st interm. 0.5 M NaCl**		**Conc. 0.01 M NaCl**		**Conc. 0.1 M NaCl**		**Conc. 0.5 M NaCl**	
SN	**DF**	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV	ζ mV	$\Delta\zeta$ mV
0	-	−22	2,2	−36	1,4	−25	2,4	−18	0,7
1	1:2	−16	4,2	−40	0,3	−21	1,3	−15	2,1
2	1:4	−20	1,9	−42	2,4	−21	1,1	−16	2,4
3	1:8	−19	4,2	−41	3,9	−21	1,9	−19	0,3
4	1:16	−14	3,6	−39	4,5	−22	0,9	−14	1,7
5	1:32	−17	3,8	−42	2,0	−22	0,8	−13	0,4
6	1:64	−16	2,3	−42	1,7	−19	0,7	−13	0,9
7	1:128	−15	1,6	−39	0,3	−24	3,2	−18	2,1
8	1:256	−14	3,9	−43	1,1	−19	2,3	−11	2,8

6.3 Protein Concentration Measurements

The protein concentrations could be calculated from the calibration curves generated with the BSA using the standard test tube protocol [103]:

$$\text{Concentration } [\mu g/mL] = \frac{\text{Blank-corrected absorbance} - 0.00883}{0.0012} \tag{6.1}$$

Using the extended test tube protocol, the protein concentration could be calculated by:

$$\text{Concentration } [\mu g/mL] = \frac{\text{Blank-corrected absorbance} - 0.00204}{0.00307} \tag{6.2}$$

Table 6.9 Calibration with BSA and standard test tube protocol with working range = 20-2000 µg/mL (a-i) and enhanced test tube protocol (working range = 5-250 µ/mL (A-F) with absorbances at 562 nm.

Vial	BSA concentration [µg/mL]	Absorbance	Standard deviation
a	2000	2.25	0.05
b	1500	1.73	0.05
c	1000	1.25	0.03
d	750	0.96	0.06
e	500	0.69	0.03
f	250	0.36	0.02
g	125	0.17	0.05
h	25	0.04	0.03
i	0	0.00	0.01
A	250	0.76	0.11
B	125	0.40	0.11
C	50	0.18	0.08
D	25	0.07	0.04
E	5	0.04	0.06
F	0	0.00	0.03

Table 6.10 Absorbances at 562 nm and removable protein concentrations after addition of various solubilizers and centrifugation.

Sample	Absorbance	c(Protein, removable) [µg/mL]
Water	0.252	81
Triton X-100 (1%)	0.2965	96
SDS (1%)	0.446	145
SDC (1%)	0.363	118
SDS (1%) + SDC (1%)	0.239	77
SDS (1%) + Triton X-100 (1%)	0.425	138

Table 6.11 Absorbances at 562 nm, c(Protein, dilution-corrected) and c(Protein, removable) after further purification steps for the syringe, spatula 1 and spatula 2 procedures.

Purification step	Absorbance	c(Protein, dil.-corrected) [µg/mL]	c(Protein, removable) [µg/mg]
Syringe			
0	0.600	986	10.95
1	0.204	66	2.25
2	0.101	32	1.10
3	0.077	24	0.84
4	0.108	34	1.18
5	0.099	32	1.08
Spatula 1			
0	0.605	993	11.03
1	0.244	79	2.70
2	0.080	25	0.87
3	0.077	24	0.83
4	0.076	24	0.82
5	0.055	17	0.59
Spatula 2			
0	0.614	1008	11.19
1	0.156	50	1.72
2	0.013	4	0.12
3	0.013	3	0.12

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Berngau, den 17. November 2021

(Johannes Ramsauer)