

# Novel Electrospun Zwitterionic Nanofibers for Point-Of-Care Nucleic Acid Isolation Strategies Under Mild Conditions

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**Nucleic acid (NA) testing at the point-of-care requires efficient NA extraction followed by post-NA amplification to achieve necessary detection sensitivity. Nanofibers (NFs) are demonstrated to be an ideal solid surface in an NA extraction process but necessitate harsh conditions that interfere with the subsequent NA amplification process. It is demonstrated that novel, pH tunable, zwitterionic NFs composed of uncharged nylon doped with the weakly basic, cationic polyallylamine hydrochloride and the weakly acidic anionic polycarboxylic acid to address the issue. Unlike the other cationic polymers investigated, e.g. polybrene and polyaniline, these polymers allow efficient NA extraction in Tris-ethylenediamine tetra-acetic acid buffer under mild conditions (pH 4.5 containing 0.1% Tween 20 for adsorption, and pH 10 with 50 mM NaCl for elution). Adsorption and elution yields over 95% and 70%, respectively, are achieved. It also discovered a correlation between material morphologies and the NA extraction suggests that the combination of polymer chemistries and nanofiber morphologies facilitates efficient NA extraction at low concentrations (ng range) within a short time period (<10 min). Considering the simple protocols and instrument-free operation the as-developed NFs are highly attractive for use in sample-to-answer NA testing in point-of-care settings.**

detection procedures make them an ultimately relevant platform technology. Typically, within the NA test systems, the preceding extraction of the NAs is costly and tedious, and unless incorporated into a sophisticated cartridge, requires trained personnel and external equipment, hindering applicability for POC testing systems, especially in resource-limited areas.<sup>[2]</sup> Thus, an extraction of NAs that is readily integrated into the testing platform while easy to perform is essential.<sup>[3]</sup> Such an extraction system should also be fast, and suitable for small sample volumes, and importantly only involve non-hazardous chemicals to avoid problems in subsequent NA amplification.<sup>[4,5]</sup> Microfluidic chips, particularly as paper analytical devices, will be a viable platform that could fulfill the demands of this endeavor.

In general, a variety of NA extraction strategies are available, e.g., liquid-liquid or solid phase extraction (SPE) with various possible materials such as silica, ion exchange, or cellulose.<sup>[6]</sup> Many

commercial systems utilize silica materials in the form of beads or gel in spin columns due to the high binding affinity of NAs to silica under alkaline conditions.<sup>[6]</sup> To further POC applicability, researchers study other forms of silica-based materials, such as glass fibers or silica-coated magnetic nanoparticles.<sup>[4]</sup>

The glass fibers hold great promise for POC applications as they do not rely on equipment and can readily be implemented in paper-based microfluidics.<sup>[7]</sup> Nonetheless, the need for chaotropic salts to facilitate NA binding and ethanol for washing introduces some critical concerns,<sup>[1]</sup> specifically, in addition to posing the risk to untrained staff and the environment due to the hazardous nature of chaotropic salts, the latter step can also lead to co-existence of ethanol in the extracted NA and subsequent inhibition of NA amplification, especially when performed in microfluidic channels where complete evaporation of ethanol cannot be guaranteed.<sup>[4]</sup>

NA extraction-based ion exchange strategies offer a solution for these problems as mild conditions prevail and through changes in pH and salinity conditions adsorption and release of NAs can simply be realized.<sup>[8]</sup> However, it should also be noted that the current technologies often suffer from very high salinity in the extract which inevitably requires a dilution or desalting step prior to amplification.<sup>[6,9]</sup>

## 1. Introduction

Nucleic acid (NA) tests containing sample preparation, isothermal amplification strategies, and detection modules are of great interest as a viable tool for on-site detection of pathogens<sup>[1]</sup> with the hope of bridging the gap between lab-based systems, sophisticated cartridge approaches, and on-site point-of-care (POC) applicability. The superior sensitivity and specificity offered through NA detection combined with rapid and simple amplification and

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Nanofibers (NFs) are considered an attractive candidate to address these mentioned challenges due to their outstanding surface-to-volume ratio, high porosity, low cost, and tunable chemistries. There are many different fabrication methods available but electrospinning is most commonly used as fiber formation and morphology can be simply controlled by the spinning parameters and industrial up-scaling and production is possible.<sup>[10,11]</sup> Moreover, NFs can be made from a variety of materials and can even be further modified to tailor the chemical properties to the requirements of specific applications.<sup>[10,12]</sup> Consequently, NFs are widely used for SPE applications based on their physical and chemical properties.<sup>[13]</sup> In particular, due to their high specific surface area, formation of 3D frameworks, and low backpressure as a result of the immense porosity, NFs can be applied to all existing SPE setups such as classical columns or (membrane-)disks.<sup>[14]</sup> The chaotic structure of electrospun NFs can also promote efficient passive mixing within microfluidic systems,<sup>[15]</sup> thus no operator input is needed for this task, unlike using magnetic beads-based separation.

Despite their superior features only two NA extraction systems combining the advantages of ionic extraction of NAs and the advantages of NFs as sorbents in SPE have been reported in the literature. Brandão et al. used an in situ polymerization of polyaniline (PANI) on polystyrene (PS) NFs<sup>[16]</sup> and Xu et al. developed an anion exchange membrane based on NFs for NAs extraction.<sup>[17]</sup> Here, spin columns and harsh adsorption or elution conditions (pH and charged detergents) and the limited extraction ability only at high NA concentrations make them unsuitable for POC applications, in particular when combined with NA amplification where trace amounts of NAs are typically analyzed.

To overcome such problems, introducing pH-tunable zwitterionic systems into NFs is of great interest. The co-existence of cationic and anionic charge, especially from weak organic bases and weak organic acids, along the NFs can facilitate an efficient control of NA adsorption and elution under mild conditions and consequently favor the NA amplification process. Nevertheless, due to their intended applications as antifouling matrices or drug carriers, researchers usually strive for pH-stable zwitterionic properties to maintain certain functionality, hampering their extended utility in other areas. Moreover, a sophisticated synthesis method is usually required to couple the cationic and anionic functional groups to a single polymer chain,<sup>[18,19]</sup> making it difficult to manipulate or fine-tune their functionalities to serve for efficient NA extraction. Therefore, alternative and more flexible approaches in fabricating zwitterionic NFs need to be developed.

A synergy of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) is of interest, as the combination of a weak acid and base provides a pH depending surface charge.<sup>[20,21]</sup> As shown in previous studies, fibers generated from a PAH and PAA mixture can be used to adsorb small cationic ions or molecules such as heavy metals<sup>[20,22]</sup> or methylene blue,<sup>[22]</sup> in the latter case the fibers were tested as a candidate for drug release. They hence should hold promise also for adsorption and desorption of large biological polyanions such as NAs which are dramatically more challenging when considering their complex chemistries and morphological structures. Furthermore, even though PAH/PAA can be fabricated through electrospinning as demonstrated in the previous studies<sup>[20–22]</sup> an additional cross-linking step is required to enable water-stable NFs

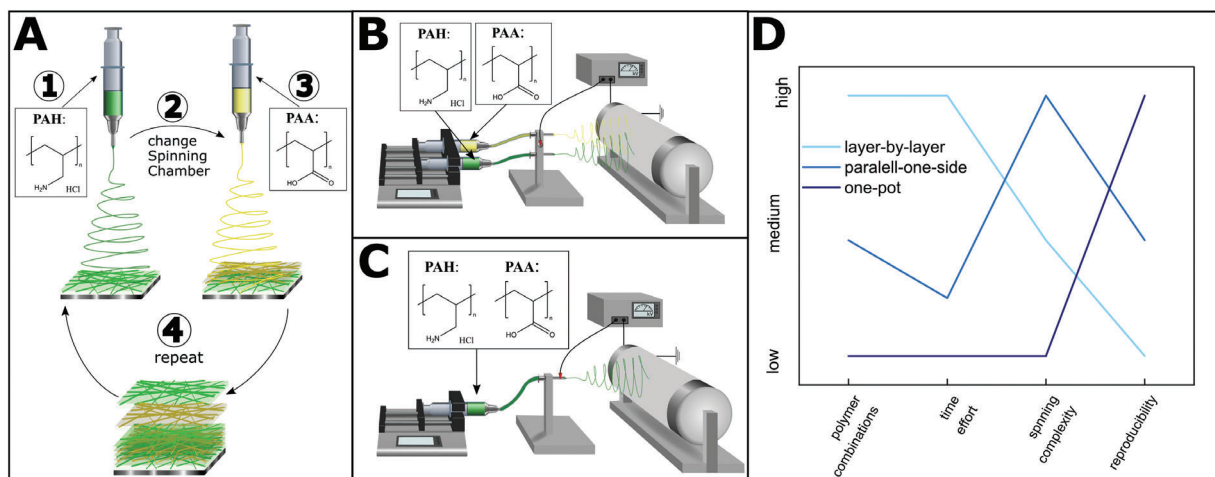
as needed for biological reactions. We hence hypothesized that co-electrospinning of PAH and/or PAA with a water-stable uncharged supporting polymer can overcome this issue. Here, Nylon serves as a promising candidate as nano-nets with ultra-fine fiber diameters (<20 nm) can be formed along with typical electrospun fibers (diameters in the range of a few hundred of nanometers)<sup>[23]</sup> and could offer NA extraction at very low concentrations.

The aim of this work is thus to create an NF-based anion exchange membrane readily and suitable for the extraction of NAs even in the POC application field. To fabricate such NF membranes, various polymer combinations, and strategies were explored in pursuit of characteristics favored for NA extraction and overall manufacturing. As part of this study, an in-depth material characterization was conducted to unravel the influence of the chemical and physical properties of NFs on NA extraction. Furthermore, NA extraction conditions were thoroughly investigated, ultimately rendering efficient pH-dependent extraction of NAs. High adsorption and elution efficiency within 10 min together with minimally required operator input make the as-developed NF membrane highly suitable for further integration to on-site devices applicable in POC and in-field settings.

## 2. Results and Discussion

### 2.1. Development of NFs

NFs suitable for NA extraction-based ionic exchange require a hydrophilic property and high stability with a pH-tunable surface charge. Thus, an initial polymer screening was performed to determine the optimal material (Table S2, Supporting Information). First, different supporting polymers were assessed, with nylon proving to be the most promising candidate due to its intrinsic hydrophilicity in contrast to PS and polylactic acid (Figure S1, Supporting Information) and its greater structural integrity compared to polyvinylpyrrolidone after exposure to aqueous buffers (Figure S2, Supporting Information). Zwitterionic moieties intrinsically introduced within polymer chains were not used due to their stable charge state over a wide pH range, which is unsuitable for pH-dependent adsorption and elution.<sup>[18]</sup> Instead, it was hypothesized that a mixture of individual cationic and anionic polymers can provide such functionality, especially since control over the ratio of positive and negative groups is easily feasible. PAH, as a weak base with a  $pK_a$  of 3.5,<sup>[19]</sup> was chosen as the cationic polymer because NAs could be efficiently adsorbed onto it at relatively mild acidic conditions, i.e. at a pH of 4.5. This is in contrast to PANI<sup>[16]</sup> where complete adsorption could only be achieved under harsher conditions under which NAs are more prone to degradation (Figure S3, Supporting Information). Studies with Polybrene (PB) demonstrated an adsorption yield highly comparable to that of PAH (data not shown). However, PB possesses a stronger base that does not offer protonatable groups and, in addition, the quaternary amino function is sterically more hindered than the primary one of PAH (Table S2, Supporting Information), hence elution of adsorbed NA is challenging. Polyacrylic acid (PAA) was chosen as the anionic component because of its lower  $pK_a$  compared to polystyrene sulfonate (PSS) ( $pK_a \approx 4.5$  and  $\approx 2$ , respectively)<sup>[19]</sup> which could allow the application of milder basic conditions in the elution step. More importantly, in



**Figure 1.** Comparison of different approaches for the generation of zwitterionic NFs. A) Layer-by-layer separate spinning; B) parallel-one-side spinning; C) one-pot spinning; D) comparison of the three methods in terms of flexibility, time effort, spinning methods, and reproducibility.

comparison to PSS, PAA provides greater miscibility with PAH and nylon in formic acid, which is vital for homogenous distribution of all components within the as-spun NFs.

Different spinning methods for the preparation of zwitterionic NFs were investigated, including the layering of cationic and anionic layers, parallel-one-side spinning from two separate needles, and a simple one-pot approach (Figure 1). The former two strategies allow electrospinning of incompatible anionic and cationic polymers. As for the layer-by-layer spinning two spinning chambers are needed, where the two polymer solutions are spun individually this technique permits effective control over individual fibers, i.e. each polymer is subjected to its optimum spinning conditions as shown in exemplary case of PS and nylon (Figure S4, Supporting Information). Yet, such a technique is time-consuming and needs a lot of input from the operator. Additionally, the static collector makes fiber distribution on the collector inhomogeneous, leading to poor reproducibility within the NF mat (Figure S5A,B, Supporting Information). Using a rotating drum collector may be a viable option to overcome such issues, but neither for this nor for the parallel-one-side spinning approach a uniform distribution of individual fibers was achievable, where the position of spinnerets had a great impact on the collected location, thus affecting overall NA extraction efficiency as illustrated in Figure S6 (Supporting Information). This necessitated a switching of the spinnerets up and down from time to time to allow homogeneous distribution of both polymers. While this somewhat cumbersome approach results in desirable mats, the one-pot spinning approach, if possible, is highly desirable. Here, a homogenous mixture of the spinning solution was feasible and consisted of PAH, PAA, and nylon in formic acid. This one-pot spinning strategy not only requires minimal personal input but also provides satisfied reproducibility within and between NF batches (Figures S6 and S7, Supporting Information).

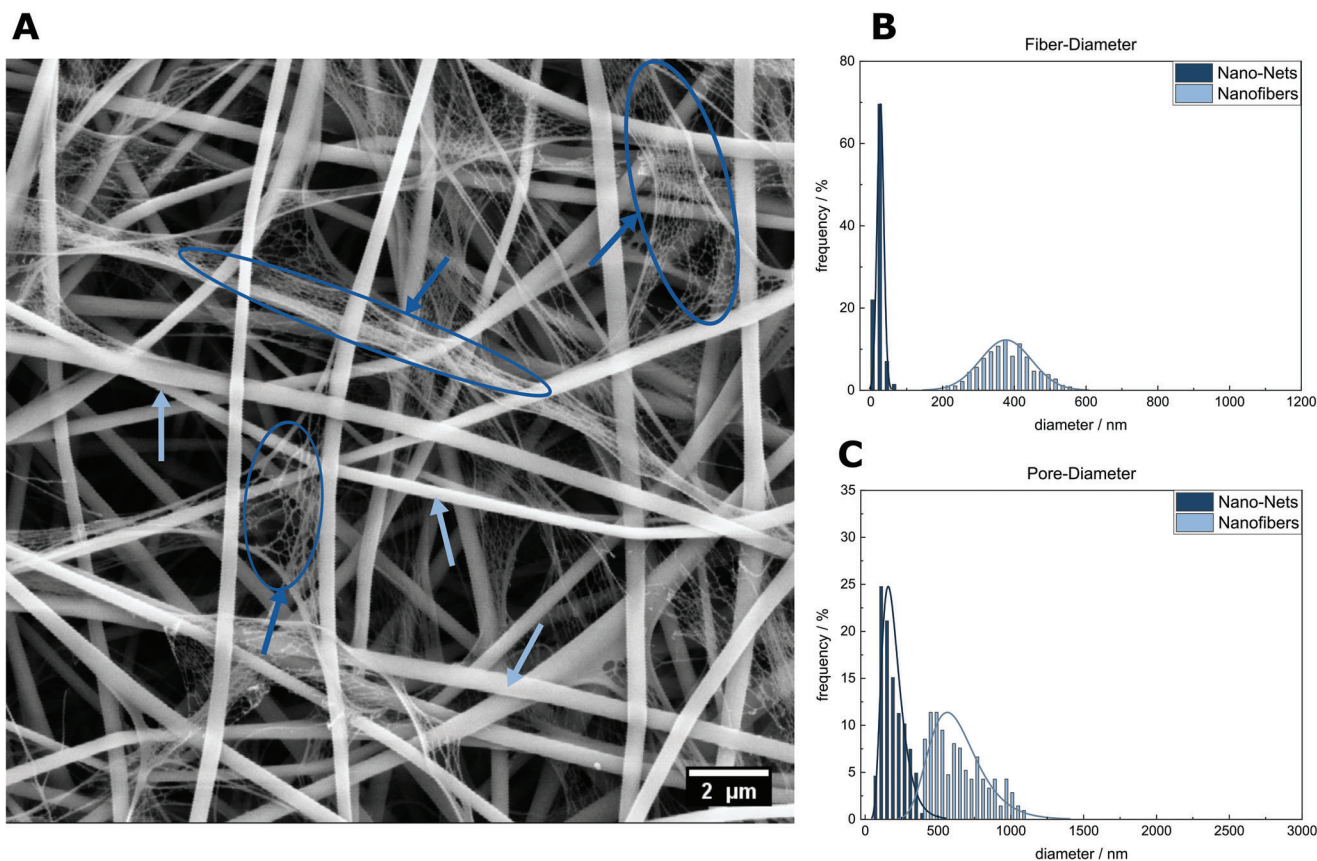
## 2.2. Fiber Morphology

Previous studies have shown that under specific conditions nanonets can be formed within the as-spun nylon NFs, which

is considered an attractive feature for boosting NA extraction efficiency.<sup>[23,24]</sup> The presence of nano nets can be efficiently controlled through the polymer concentration in the spinning solution, type of solvent, and spinning parameters such as an applied voltage. In this study, 20 wt.% total polymers consisting of nylon, PAH, and PAA in formic acid were used as a spinning solution, which successfully allowed the simultaneous formation of NFs (diameter of  $380 \pm 70$  nm) and nanonets (diameter of  $25 \pm 9$  nm) with a narrow size distribution (Figure 2), showing the same structure as pure nylon-NFs (Figure S8, Supporting Information). It should be noted that electrospinning of PAA, PAH, and PAA/PAH without supporting polymer did not result in the generation of nano nets as reported in other studies<sup>[25]</sup> Therefore, this material offers not only an increased surface-to-volume ratio but also very small pore sizes (NFs  $600 \pm 200$  nm; nets  $200 \pm 80$  nm), facilitating efficient interaction between NA and NFs. To unravel the beneficial features of as-spun NF-contained nano nets we first attempted to study the parameters that control the formation of nano nets in which an increase voltage plays great role in promoting the formation of nanonets as shown in Figures S9 and S10 (Supporting Information) and previously reported in literature.<sup>[23]</sup> We also investigated the effect of solution viscosity attributed to the presence of HCl in PAH used and stirring time (Figure S11, Supporting Information). Interestingly, no effect of PAH was detected in terms of fiber morphology or extraction efficiency, confirming the compatibility of PAH with nylon for electrospinning.

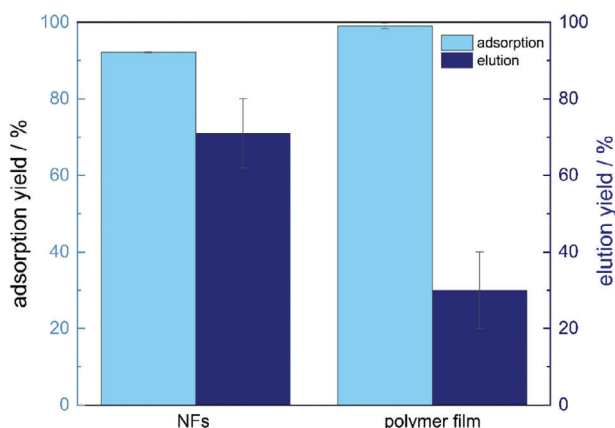
We further elucidated the impact of the nanostructures in governing NA adsorption and elution behavior. Here, nylon-PAH-PAA in the form of a knife-coated porous polymer film and nanofibrous membranes of similar thickness were compared. The adsorption of NA was not enhanced by having nanostructures as seen from the highly comparable adsorption yields obtained from the NFs and the polymer film (Figure 3). This suggests that the nanostructures are needed to allow a repulsion of NAs during the elution process. We assume that the closer proximity of the cationic and anionic functional groups in the nanofiber mat, especially in the very small nano-pores,





**Figure 2.** A) Scanning electron microscopy (SEM) picture of nylon-PAH-PAA fibers with a magnification of 1000  $\times$ . NFs are marked with light blue arrows, nano-nets with dark blue; size distribution of the NFs and nano-nets from the fibers B) and the pore diameter C);  $n > 200$ .

is needed to ensure that not only cationic but also anionic functional groups are surrounding the adsorbed NAs. Therefore, the use of nanomaterials does not only provides a high surface-to-volume ratios but also provides functionality in the elution process.

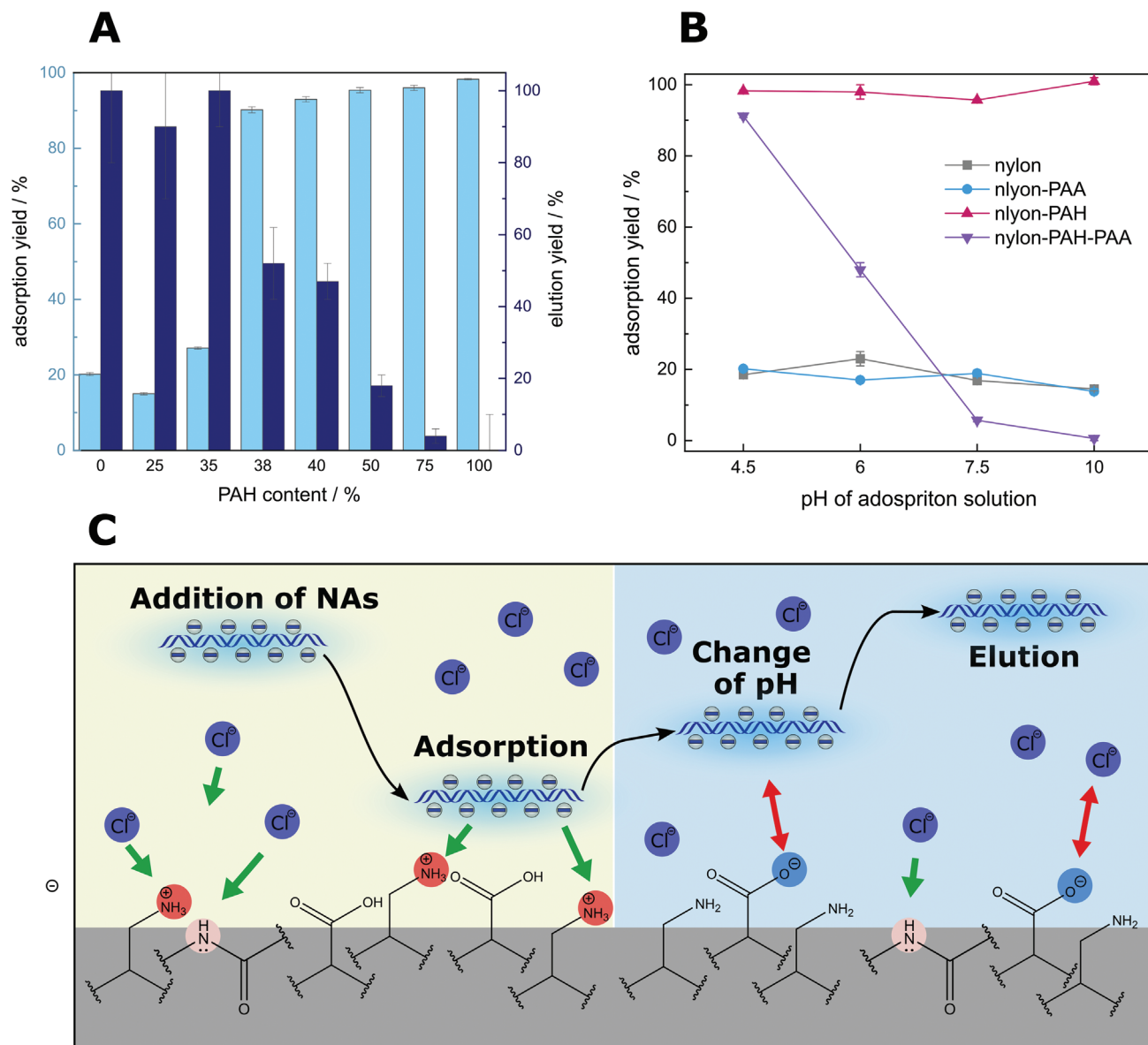


**Figure 3.** Comparison of the extraction efficiency of nylon-PAH-PAA NFs and a polymer film with a similar thickness. The polymer film and NFs were cut into circles with a diameter of 6 mm using the standard protocol as for all NFs samples. Afterward,  $7.83 \pm 0.01$  ng NAs were added to each sample for the adsorption.

### 2.3. Electrostatic Properties of the NFs

The pH dependency of the NFs is easily realized by a mixture of cationic PAH and anionic PAA. Their ratio was optimized, aiming for leveraging a full adsorption capacity and a maximum elution yield. This study revealed a significant impact of PAH content (Figure 4A; Table S3, Supporting Information). Shifting from 100% to 0%, it was found that pure PAH NFs achieve full NA adsorption but lack elution capability. Approaching the optimal ratio of 38% PAH yields a substantial increase in elution while maintaining a satisfied adsorption yield. However, a turning point is observed at 35% PAH, leading to more anionic fibers with minimal adsorption yield. Standard deviations of the elution signals are very high as the amount of eluted NAs is close to the limit of detection and therefore the quantification is not precise anymore.

An in-depth analysis of the fibers' pH dependency demonstrates that the nylon and nylon-PAA fibers show a pH-independent and unspecific adsorption of just  $\approx 20\%$  while the nylon PAH adsorb the NAs completely over the whole pH range investigated. (Figure 4B) A strong pH dependency could only be observed for the zwitterionic nylon-PAH-PAA NFs with a strong NA adsorption at pH 4.5 then decreasing for higher pH values. The lower adsorption at a basic pH may either be a result of the mixture of PAH and PAA or an experimental mistake. Overall, this pH dependency does fit with the  $pK_a$  values reported in



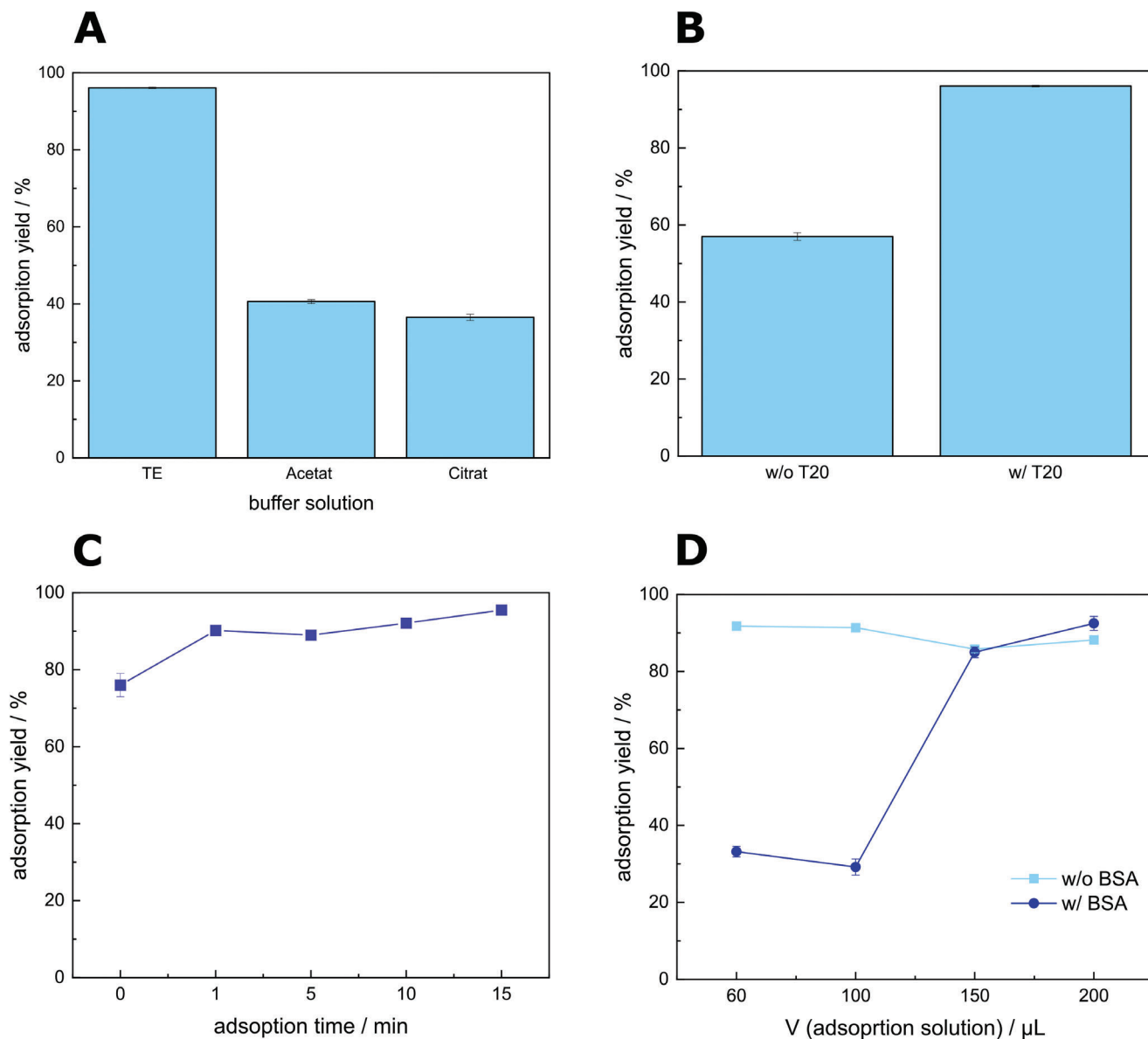
**Figure 4.** pH dependency of nylon-PAH-PAA NF,  $n \geq 3$ . A) Adsorption and elution yield of nylon-PAH-PAA NFs with varying ratios of PAH and PAA while the absolute mass of added charged polymer remained constant at 16.7 wt.%. Adsorption in Tris-ethylenediamine tetra-acetic acid (TE) solution pH 4.5 + 0.1% T20, elution in TE pH 12 + 100 mM NaCl; B) adsorption yield dependency on pH for different non-charged, cationic, or anionic and zwitterionic NFs C) schematic illustration of the pH-dependent NA extraction process from zwitterionic nylon-PAH-PAA NFs.

literature, as at pH 4.5 and pH 10 mainly the amino and the carboxylic acid groups are presented, respectively.<sup>[19]</sup> In conclusion, the mixture of PAH and PAA can combine the properties of both polymers, where the fibers are capable of adsorbing NAs at a low pH and eluting NAs due to electrostatic repulsion at a high pH (Figure 4C), resulting in zwitterionic NFs with a pH-tunable surface charge.

#### 2.4. Optimization of the Analytical Process

The extraction efficiency of the zwitterionic NFs was further enhanced through the optimization of the extraction conditions.

Specifically, adsorption solutions and incubation times were investigated (Figure 5), where the TE buffer outperformed the others. The addition of Tween 20 (T20) further improved the adsorption yield due to a shortened wetting time. Compared to other studies where complete adsorption was achieved after 9<sup>[16]</sup> or 20 min,<sup>[17]</sup> in this work over 70% of NAs were adsorbed immediately and complete adsorption is achieved within 5 min, making this system suitable for flow-through extraction. Furthermore, while the other studies used high NA amounts ( $\mu\text{g}$  range), our study focused on low NA amounts, i.e. in the ng range, as this is deemed significantly more relevant for POC application, for example, NAs concentration in serum is  $\approx 1.8\text{--}35 \text{ ng mL}^{-1}$ , in whole blood  $\approx 30 \mu\text{g mL}^{-1}$  and only a few copies of pathogen NAs

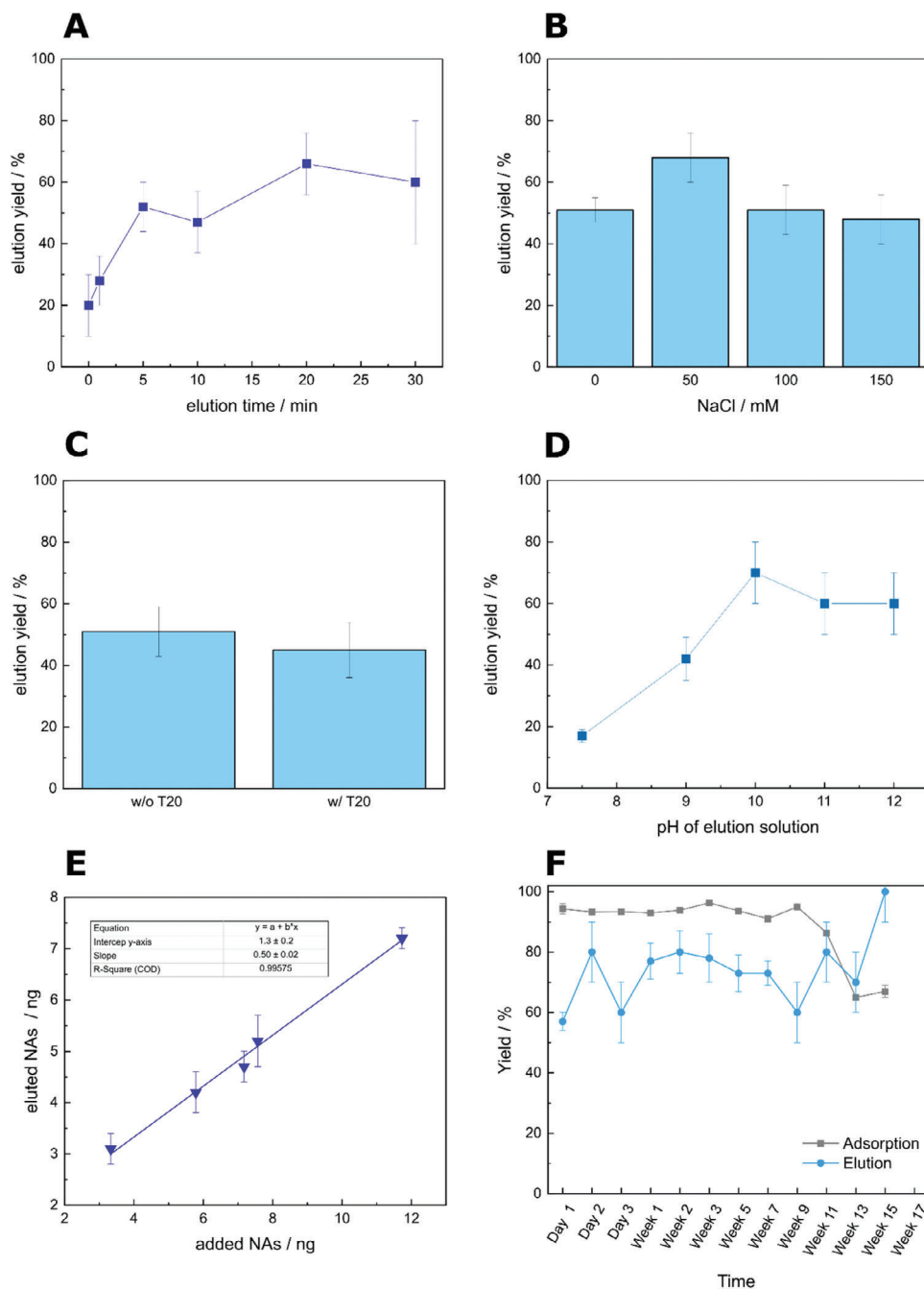


**Figure 5.** Influence on the adsorption yield on nylon-PAH-PAA NF of A) buffer system while the molarity was kept constant at 11 mM and a pH of 4.5 containing 0.1% T20, B) the addition of 0.1% T20, C) adsorption time and D) the dilution in the presence of BSA. Here, a constant amount of 100 mg BSA and 6 ng NAs were diluted in 60–200  $\mu\text{L}$  and the responding adsorption yield was determined;  $n \geq 3$ .

per sample are expected,<sup>[8,26]</sup> emphasizing the beneficial feature of nanonets within the as-developed zwitterionic NFs. An interference study with bovine serum albumin (BSA) showed no difference in adsorption performance at only 3-fold dilution, even though  $\approx 100 \mu\text{g}$  of BSA and only  $\approx 5 \text{ ng}$  of NAs were added, which correlates to  $\approx 2\text{--}3 \mu\text{L}$  of human serum.<sup>[27]</sup> Nevertheless, human serum does contain more interfering species such as lipids or salts. Due to the low specificity of the electrostatic extraction process, more in-depth research is needed here to study the influence of different sample types. However, this simple and promising dilution approach showed that NAs have a high attraction to the NFs due to their negatively charged backbone. Consequently, future studies will investigate the effect of individual

sample matrices, such as serum, saliva, or urine, and different types of pathogen NAs, such as DNA or RNA, in more detail.

In the final step, the elution process was optimized, focusing again on solution and time parameters. An optimum elution time of 20 min was determined. However, compared to an elution time of 5 min, only a marginal increase was found for longer incubation periods, making this system again also suitable for a flow-through setup (Figure 6A). Regarding the solution characteristics, the elution efficiency is primarily affected by the pH due to the NF's pH dependence. A significant increase was observed within the pH range of 7 to 10, with the optimum at pH 10. A higher pH could not increase the elution efficiency and is not recommended to ensure the NAs' stability (Figure 6D). In



**Figure 6.** Investigation of the elution from nylon-PAH-PAA NFs: Influence of elution parameters on the elution yield with A) the elution time, B) the addition of NaCl, C) T20, and D) the pH. Possibility for (semi-) quantitative detection due to a linear correlation of the added and eluted mass of NAs E). Aging study on nylon-PAH-PAA NFs. NFs over the storage time and adsorption and elution were measured under optimized conditions F);  $n \geq 3$ .

contrast to other NA extraction-based ion exchange,<sup>[6]</sup> a relatively mild concentration of salt is needed to allow the satisfied elution yields where 50 mM NaCl was optimal (Figure 6B). Thus, desalting or dilution is not needed prior to further NA amplification. The required low salt concentration may be attributed to the introduction of anionic moieties, which probably prevent strong binding between the NA and NFs. Unlike NA adsorption shown in Figure 5B, the addition of T20 in the elution solution had no

effect due to the already pre-wetted NFs occupied by adsorbed NA (Figure 6C).

When comparing this system to other NF extraction systems reported so far, our zwitterionic NFs show a comparable extraction time of in total 10 min without the need for centrifugation or tedious mixing step. Furthermore, due to the zwitterionic properties, this system does not require extreme pH conditions, high salt concentrations, or charged detergents to elute the



**Table 1.** Comparison of different nanofiber-based ionic NA extraction systems.

Membrane	Mass of NA	Adsorption time [min]	Adsorption pH	Elution time [min]	Elution pH	Elution Additives	Equipment demand	Refs.
PS-PANI	μg	9	2.8	10	11.7	SDS	High	[18]
Chitosan aerogel	μg	20–30	3–4	20	5	1.5 M NaCl	High	[19]
Nylon-PAH-PAA	ng	5	4.5	5	10	0.05 M NaCl	Low	This work

NAs, which are preferable for downstream amplification strategies (Table 1). Therefore, zwitterionic NFs are able to overcome the current problems of the ionic NA extraction system.

The zwitterionic NFs also offer the possibility of semi-quantitative readout when coupled with an amplification and detection system as a result of the linear correlation between the amount of NAs added and eluted (Figure 6E). Finally, the aging of the NFs should be investigated when stored under ambient conditions. The data was generated from a single NF mat the NFs showed consistent adsorption and elution for at least 9 weeks (Figure 6F). Future studies will study long-term stability also under protective conditions. The aging process itself started with a decrease in adsorption, but not in elution yield, suggesting that primarily PAH are degraded, while PAA and nylon properties might remain intact.

### 3. Conclusion

In this study, novel zwitterionic nylon PAH-PAA NFs fabricated by electrospinning were proposed to enable specific binding and elution of NAs under mild conditions, highly promising for the isolation of NAs for POC applications. The one-pot approach, i.e. electrospinning of a spinning solution containing nylon as carrier polymer together with the cationic PAH and the anionic PAA in formic acid, allows facile and reliable fabrication of the NFs. The adsorption and elution efficiency of NAs can be simply tuned via adjusting the polymer ratio and assay conditions. The high surface area, the inherent hydrophilic property, and the presence of nano-nets of the NFs undoubtedly allow the isolation of extremely low amounts of NAs achievable in a short period of time (within 10 min). The nylon PAH-PAA NFs are thus highly suitable for the extraction of NAs in flow-through systems without the need for additional equipment, making them a promising solution in the POC field, especially when they are integrated within microfluidic channels, μPADs, and origami-based assays. Furthermore, with precisely controlled material properties, these fibers offer adaptability for various applications and allow easy customization to specific challenges and needs, such as sample types, while ensuring predictable behavior of the NFs. In conclusion, with their demonstrated efficiency, these zwitterionic NFs can serve as an important tool to overcome current sample preparation challenges.

### 4. Experimental Section

**Electrospinning of the NFs:** Nylon 6,6 NFs were obtained by electrospinning of a 20 wt.% polymer solution in concentrated formic acid. All polymers were purchased from Sigma Aldrich, and formic acid was supplied by Carl Roth. The doping ratio of the charged polymers was kept constant at 20 wt.% of total polymer content. NFs were electrospun at 22 kV (unless stated otherwise) with a flow rate of 2 μl min<sup>-1</sup> using a 20-G

needle at 22 °C and 40% relative humidity and a distance of 20 cm with a rotary drum (Aluminum thin wall, diameter of 80 mm and length of S120 mm) system (Starter Kit-Aligned 40 kV, Linari Engineering srl, Pisa, Italy) where the rotation speed of 150 rpm was used. The fibers were collected for 5 h on grade 1 chromatography paper from Sigma Aldrich. Spinning conditions for all other polymers are summarized in the Supplementary Information in Table S1 (Supporting Information).

For parallel-one-side spinning, an in-house needle holder was used with a spacing of 2 cm between needles. For layer spinning, a vertical spinning system consisting of two individual spinning chambers was used. One chamber contained nylon-PAH and the other nylon-PAA setup. In this procedure, the collector was exchanged between chambers after 1 min of collection time, for a total spinning time of 30 min. Until use, the NFs were stored in a vacuum chamber.

**Characterization of the NFs:** Two 6 mm circles of NFs from two different spots of the NFs mat were placed on an SEM holder. Afterward, the samples were sputtered with a gold-palladium mixture. The NFs were characterized by SEM images obtained with a Zeiss/LEO 1530, Germany with 5–50 kV with 5 kV. SEM images from at least 8 different spots were taken. For the characterization of the NF diameter images with a magnification of 2500 kX (Image Pixel Size 10.78 nm) and for the nano-nets images with a magnification of 5000 kX (Image Pixel Size 5.391 nm). Thickness of the NF mat and knife-coated polymer foil was determined with SEM images from side cuts of the materials. Subsequently, nanofiber and nanonet diameter as well as pore sizes were determined using ImageJ. The pore size was determined with the ferret diameter from the automated particle counting function of ImageJ. Afterward, the number of pores below 400 nm was divided through the total number of pores to determine the nano-pore ratio.

For contact angle measurements, a drop of 5 μL water was placed on a fiber mat and an image was taken with a CCD camera on a Dataphysics contact angles system OCA 15EC and analyzed with ImageJ.

Further characterization was performed by determining the dynamic viscosity with a CVO 120 from Bohlin with a cone of 4° and a diameter of 40 mm. Linear extrapolation was used to determine the zero-shear rate from the data set.

**Buffers Preparation:** All TE solutions had a TrizimaBase (Sigma Aldrich) concentration of 10 mM and an EDTA (Carl Roth) concentration of 1 mM. The pH was adjusted with NaOH (CarlRoth) or HCl (Sigma Aldrich) with a pH electrode from SCHOTT instruments GmbH Mainz, Germany. The adsorption solution had a pH of 4.5 and contained 0.1% T20 (TE pH 4.5 + T20), and the elution solution contained 50 mM sodium chloride with a pH of 10. Sodium chloride and EDTA were delivered from Carl Roth and Tween 20 from Sigma Aldrich. Buffers from Citrate and Acetate, both bought from Sigma Aldrich, were made with the same recipe. Buffers were stored at 4 °C until use.

**NA Extraction Protocol:** NAs from *E. coli* bacteria were used as a model analyte and were extracted with a GenElute Bacterial Genomic DNA Kit from Sigma Aldrich following the protocol and afterward stored at –20 °C according to the manufactured clean, genomic dsDNA should be isolated in the extraction solution.

Before use, NFs were punched into circles of 6 mm diameter using a toggle press (Berg & Schmid GmbH, Germany) and carefully peeled from the filter paper to create free-standing NFs. The resulting fiber mats were placed on the bottom of a 96-well plate. For adsorption, 60 μL of a pre-diluted DNA solution in TE pH 4.5 + T20 was added to the NFs. After the incubation time of 5 min (without agitation), 40 μL of the supernatant was carefully transferred to a new well, and the pH was adjusted to pH 7 with 10 μL diluted NaOH. Here, it was not possible to determine the fluores-



cence from the NFs directly or within the same well because of the strong backscattering of the porous NFs. Therefore, measurement of the NA remained in the supernatant in a fresh well was chosen, even though this can lead to higher standard deviations. Before the elution, the remaining adsorption solution was completely removed without damaging the fibers before adding 60  $\mu\text{L}$  of elution solution. After incubation for 20 min, 40  $\mu\text{L}$  of the supernatant was again transferred to a fresh well and neutralized with 10  $\mu\text{L}$  HCl. Neutralization prior to the addition of the dye is mandatory to ensure a reliable and stable fluorescent signal since the dye is sensitive to pH. Finally, the NAs concentration was determined with ThermoFisher's OliGreen dye, since this dye is suitable for both double-stranded and single-stranded NAs. According to the protocol, the dye was freshly pre diluted 200-fold in TE pH 7.5 and then 50  $\mu\text{L}$  of dye solution was added to each well containing the neutralized NAs solution. After an incubation of 5 min at RT, the fluorescence was measured using a BMG Fluostar MTP (Ex:  $485 \pm 10$  nm, Em: 520 nm, Gain: 1636).

For each measurement, four samples with NAs and four samples without NAs were measured as blanks. Outliers were determined using a Q-test with a confidence level of 95%. To be able to determine the extraction efficiency the NA concentration of the adsorption solution was determined as well.

To process the data, the mean value of the obtained fluorescent signals was determined and corrected by the mean of the related blank measurement. To allow for a quantitative detection, a calibration curve for each solution was measured. Therefore, the concentration of the known NA solution was plotted against the fluorescence signal, and a linear calibration curve was plotted. From this, the mass per well of NAs in the adsorption solution,  $m_{\text{added}}$ , the mass NAs remaining in solution after adsorption,  $m_{\text{ad-sol}}$ , and the eluted mass,  $m_{\text{el}}$ , was calculated.

Afterwards, the mass of adsorbed NA  $m_{\text{ad}}$  was calculated as shown below:

$$m_{\text{ad}} = m_{\text{added}} - m_{\text{ad-sol}} \quad (1)$$

From this, the adsorption yield  $Y_{\text{Ad}}$  in % was determined:

$$Y_{\text{Ad}} = \frac{m_{\text{ad}}}{m_{\text{added}}} \quad (2)$$

Using the mass of eluted NAs, the elution yield  $Y_{\text{El}}$  in % was calculated:

$$Y_{\text{El}} = \frac{m_{\text{eluted}}}{m_{\text{added}}} \quad (3)$$

Errors were calculated using the standard deviation and Gaussian error propagation.

For the interference measurements, BSA was added to the same solution as the NAs. Here, a constant mass of 90  $\mu\text{g}$  per well BSA was added, obtained from Sigma Aldrich. For the measurements similar to the NAs protocol, 5  $\mu\text{L}$  of adsorption or elution solution was transferred to a new well before discarding the remaining supernatant. BSA concentration was determined using ThermoFisher's dye Pierce™ Detergent Compatible Bradford Assay Reagent according to the manufacturer's instructions. Here, 200  $\mu\text{L}$  of the dye solution was directly added to the protein solution and incubated for 10 min at RT. The absorbance was measured at 595 nm using BioTek (Agilent, USA). Adsorption and elution yields were then calculated according to the protocol for NAs.

For the aging study, one mat of NFs was stored under ambient conditions and tested over a time period of 16 weeks. Therefore, on each measurement day, NFs were freshly cut and transferred to a microtiter plate with the normal protocol. Adsorption and elution were tested under optimal conditions while the amount of added NAs was kept constant.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Keywords

anion-exchange, nanofibers, nucleic acids, pH-tunable surface charge, point-of-care, solid-phase extraction, zwitterionic

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