



Steering protein and salt ad- and desorption by an electrical switch applied to polymer-coated electrodes

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ARTICLE INFO

Keywords:

Electrochemical protein separation
Capacitive deionization
Protein desorption
Polyelectrolyte
Carbon electrodes

ABSTRACT

Although solid-phase chromatography is a well-established method for protein separation, chemically intensive and often costly regeneration steps are needed to make reuse of the adsorbent possible. Here, we demonstrate the use of electrochemical principles as sustainable alternative. We make use of spontaneous adsorption of proteins to solid electrodes and reverse this process by applying an electric potential to regenerate the interface. This allows for adsorption of proteins to take place at 0 V difference between the electrodes, due to electrostatic interactions between the protein and the electrode surface. The desorption is then triggered by applying a potential difference (−1.2 V) between the electrodes.

It is demonstrated that the incorporation of negatively charged polystyrene sulfonate (PSS) or positively charged polydiallyldimethylammonium chloride (PDMAC) in or on top of the respective activated carbon electrodes increases the amount of exchanged protein from 1 to 10 mg g^{−1}, as compared to simple activated carbon electrodes. Interestingly, salt ad- and desorption occurs in opposite cycles compared to protein ad- and desorption, resulting in simultaneous concentration and desalting of the protein when 0 V is applied. On top of that, we also found that an enrichment in β-lactoglobulin could be achieved starting from whey protein isolate. These results clearly demonstrate that electrochemical technologies can be used not only for protein separation (including removal of salt), but also for protein fractionation, while not requiring solvent use.

1. Introduction

Proteins are an essential component in human nutrition and are also important in medicine and biotechnology [1–3]. Protein losses are high in the global food system, partly due to inefficient processing [4]. The utilization of byproducts and waste streams (biorefinery) can reduce their environmental impact [5–8], but only if this can be done in a sustainable, energy-efficient way.

Current protein extraction, separation and purification processes revolve mostly around filtration, (ultra)centrifugation, precipitation and chromatography, depending on the desired yield and purity of the product [9–12]. Filtration and centrifugation generally do not affect protein structure, although high selectivity between components is rarely possible, whereas precipitation aggregates proteins, which influences their functionality. In terms of purity, chromatography is the best option which also allows separation of individual proteins from mixtures. Commercially available resins for chromatographic

separation processes using ion exchange principles, most frequently contain sulfonic acid or quaternary ammonium groups as strong acid cation or strong base anion exchanger, respectively [13]. Thus, prominent options are sodium polystyrene sulfonate (PSS) or polydiallyldimethylammonium chloride (PDADMAC) [14–17]. Proteins adsorb to these polyelectrolytes due to electrostatic interactions, however other opposite-sign interactions such as hydration repulsion can oppose them and thus support reversible protein adsorption [18]. Nevertheless, desorption of the protein and regeneration of the column requires extreme conditions, and may lead to changes in protein functionality. Most desorption strategies target the solution conditions, e.g. by changing the pH [19] or the ionic strength [20] of the eluent and thus impact the charge of the proteins, or their screening length [21]. This leads to high eluent usage and consequently high environmental impact, especially in large-scale, preparative chromatography [22–26].

This research investigates the use of electrochemical stimuli to cycle surface properties from adsorptive to desorptive, without requiring

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<https://doi.org/10.1016/j.seppur.2020.117195>

Received 17 January 2020; Received in revised form 13 May 2020; Accepted 31 May 2020

Available online 03 June 2020

1383-5866/ © 2020 Published by Elsevier B.V.

external chemicals. By applying either a positive or negative potential, interfacial properties such as surface charge, double layer structure and oxidative state can be altered, which influences the ad- and desorption behavior of ions and other molecules [27,28]. This strategy has mostly been tested for small species (ions) in capacitive deionization (CDI) [29,30] and aromatic sulfonates or corticosteroids in electrochemically modulated liquid chromatography and related processes [31–35]. For proteins electro-responsive electrode coatings have been proposed based on backbiting self-assembled monolayers [36] or redox-active organometallics [37] and reversible protein ad- and desorption has been achieved. Nevertheless, both coatings are difficult and expensive to realize for large scale operations.

Here we propose for the first time an inverted capacitive protein separation process that allows for selective isolation of proteins via adsorption to and desorption from activated carbon electrodes that are coated with polyelectrolytes. Within this process, protein adsorption occurs at 0 V difference between the electrodes, merely due to electrostatic interactions with the electrode, while protein desorption is triggered by applying a potential difference between the electrodes. We found that incorporation of polyelectrolytes drastically increases reversibility of adsorption, and that salt ad- and desorption occurs in opposite sequences, which makes this process also highly interesting for desalination of protein rich streams.

2. Experimental section

2.1. Chemicals

The following chemicals and materials were purchased from Merck (The Netherlands): activated carbon (AC, particle size $\sim 10 \mu\text{m}$), poly(vinylidene fluoride) (PVDF, $534,000 \text{ g mol}^{-1}$), dimethylacetamide (DMAC), nitric acid (70%), ethylenediamine (99%), poly(sodium 4-styrenesulfonate) (PSS, $1,000 \text{ kg mol}^{-1}$), polydiallyldimethylammonium chloride (PDADMAC, $400\text{--}500 \text{ kg mol}^{-1}$), and sodium chloride ($> 99.5\%$). Furthermore styrene butadiene rubber (Zeon, Japan), whey protein isolate (Biopro, Davisco, USA) and a spacer mesh (Meshtec NBC, Japan) were used. Water in all experiments was deionized, and purified with a Merck Millipore system ($18.2 \text{ M}\Omega \text{ cm}$).

2.2. Electrode preparations

Various electrodes were prepared and combined in pairs, as described in the next sections and summarized in Table 1.

2.2.1. Activated carbon electrodes

To prepare activated carbon (AC) electrodes, activated carbon powder (2 g) was mixed with 10 wt% PVDF binder (2 g) in DMAC (5 mL) for 20 h and cast onto graphite foil using a rod coater with a $400 \mu\text{m}$ gap. Subsequently electrodes were dried over night at room temperature. All electrodes had circular shape with 5 cm diameter. [38]

2.2.2. Surface-modified activated carbon electrodes

To increase the negative charges on the surface of the AC powder, 10 g AC powder was oxidized by exposing it to concentrated nitric acid (100 mL, 70%) for 24 h (AC^-). Subsequently the AC^- powder was washed with around 1 L of water and dried. To obtain positive surface

charges on the activated carbon (AC^+), AC^- was suspended in nitrogen purged ethylenediamine (100 mL) at 120°C in an oil bath until all the liquid was evaporated [39,40]. Electrodes were made from these materials using PVDF binder as described for AC electrodes above.

2.2.3. Dip-coated activated carbon electrodes

To prepare dip-coated electrodes [41] (Dip^+ and Dip^-), AC electrodes, prepared as described above, were immersed for 15 min in 20 mM (based on Mw of monomer) PDADMAC or PSS solution, each containing 100 mM NaCl. Subsequently, the electrodes were immersed for 1 h in water and dried.

2.2.4. Composite polyelectrolyte-activated carbon electrodes

These electrodes (Comp^+ and Comp^-) were prepared by mixing 5 wt% of PDADMAC or PSS (0.11 g), respectively, with AC powder (2 g) and styrene butadiene rubber (0.28 g) as binder in water (5 mL). In this case PVDF could not be used as binder due to the immiscibility of the polyelectrolytes in DMAC. This slurry was then cast onto graphite foil. [42] Like the AC-based electrodes, these electrodes were circular with a diameter of 5 cm.

2.3. Characterization

X-ray photoelectron spectroscopy (XPS, JPS-9200, JOEL Ltd., Japan) was used to study the chemical composition of the electrodes. All samples were analyzed using a focused monochromated Al K X-ray source with a spot size of $300 \mu\text{m}$ under UHT conditions (base pressure 3×10^{-7} Torr). The radiation was set at 12 kV and 20 mA with an analyzer energy pass of 10 eV. In addition to wide scans, C1s, O1s, N1s and S2p narrow scans were also measured and the spectra were corrected using a Shirley background in CasaXPS. All spectra were referenced to the C1s peak at 285.0 eV attributed to C–C and C–H atoms. Thermogravimetric analysis (TGA) was conducted under a nitrogen atmosphere between 30 and 600°C . The weight loss between 30 and 100°C was related to residual water in the sample, while the weight loss between 101 and 600°C was related to the polymer content.

2.4. Electrochemical measurements

To determine the potential of zero charge (PZC) of all electrodes the procedure proposed by Gao et al. was followed [43]. Therefore, cyclic voltammetry measurements were conducted at 0.5 mV s^{-1} within a potential window of -1 to 1 V using an Ag/AgCl reference electrode and a compactstat (Ivium, The Netherlands). Subsequently, cathodic and anodic peaks were identified as indicated in Fig. S3 and the average was used as PZC value.

2.5. Capacitive separation measurements

The ad- and desorption of proteins and ions in response to capacitive current was measured using a flow-through capacitor system. Anode/cathode combinations as described in Table 1, separated by a spacer mesh (diameter: 6 cm, thickness: $124 \mu\text{m}$, mesh opening: $246 \mu\text{m}$), were assembled in a flow cell that was created by the technical workshop of our university, as depicted in Scheme 1 and described in previous articles [38,42].

Table 1
Electrode pairs and abbreviations.

Anode	Cathode	Abbreviation of combination
Activated carbon (AC)	Activated carbon (AC)	AC
Nitric acid-treated activated carbon (AC^-)	Ethylenediamine-treated activated carbon (AC^+)	$\text{AC}^{+/-}$
PSS mixed into the activated carbon slurry (Comp^-)	PDADMAC mixed into the activated carbon slurry (Comp^+)	$\text{Comp}^{+/-}$
AC electrode dip coated with PSS (Dip^-)	AC electrode dip coated with PDADMAC (Dip^+)	$\text{Dip}^{+/-}$



Scheme 1. Flow cell for capacitive separation experiments.

A solution containing 1.5 mg g^{-1} whey protein isolate (WPI) and 0.3 mg g^{-1} sodium chloride was continuously flushed between the electrodes at 1 mL min^{-1} and the applied potential was switched between 0 and -1.2 V during 10 cycles. The time for each step was varied between 300 and 1800 s and the UV absorbance at a wavelength of 280 nm and the conductivity were measured continuously in the outlet. The gravimetric ad- and desorption capacity of salt (gSAC and gSDC, respectively), and proteins (gPAC and gPDC, respectively) were determined for cycles 3 to 10, based on the equations below,

$$gSAC = \frac{m_{s,ads}}{m_e} \quad (1)$$

$$gSDC = \frac{m_{s,des}}{m_e} \quad (2)$$

$$gPAC = \frac{m_{prot,ads}}{m_e} \quad (3)$$

$$gPDC = \frac{m_{prot,des}}{m_e} \quad (4)$$

where m is the mass of salt (s), or protein (prot) adsorbed (ads) or desorbed (des), respectively; m_e is the average mass of active material on the electrodes (0.186 g).

2.6. HPSEC measurements

When protein isolate consists of a number of proteins. The amounts of β -lactoglobulin, α -lactalbumin, and bovine serum albumin in the outflow of the electrochemical flow cell were determined using high-performance size-exclusion chromatography (HPSEC, Thermo Dionex Ultimate 3000 UHPLC, Thermo Fisher Scientific, USA) using two columns (TSKGel G3000SWXL $5 \mu\text{m}$ $300 \times 7.8 \text{ mm}$ and TSKGel G2000SWXL $5 \mu\text{m}$ $300 \times 7.8 \text{ mm}$). As eluent, 30% acetonitrile in ultrapure water with 0.1% trifluoroacetic acid was used at a flow rate of 1.5 mL min^{-1} and $30 \text{ }^\circ\text{C}$. The UV detection wavelength was set to 214 nm.

3. Results and discussion

In this section, the composition and characteristics of the electrodes are presented to which the components (protein and salt) adsorb. Then

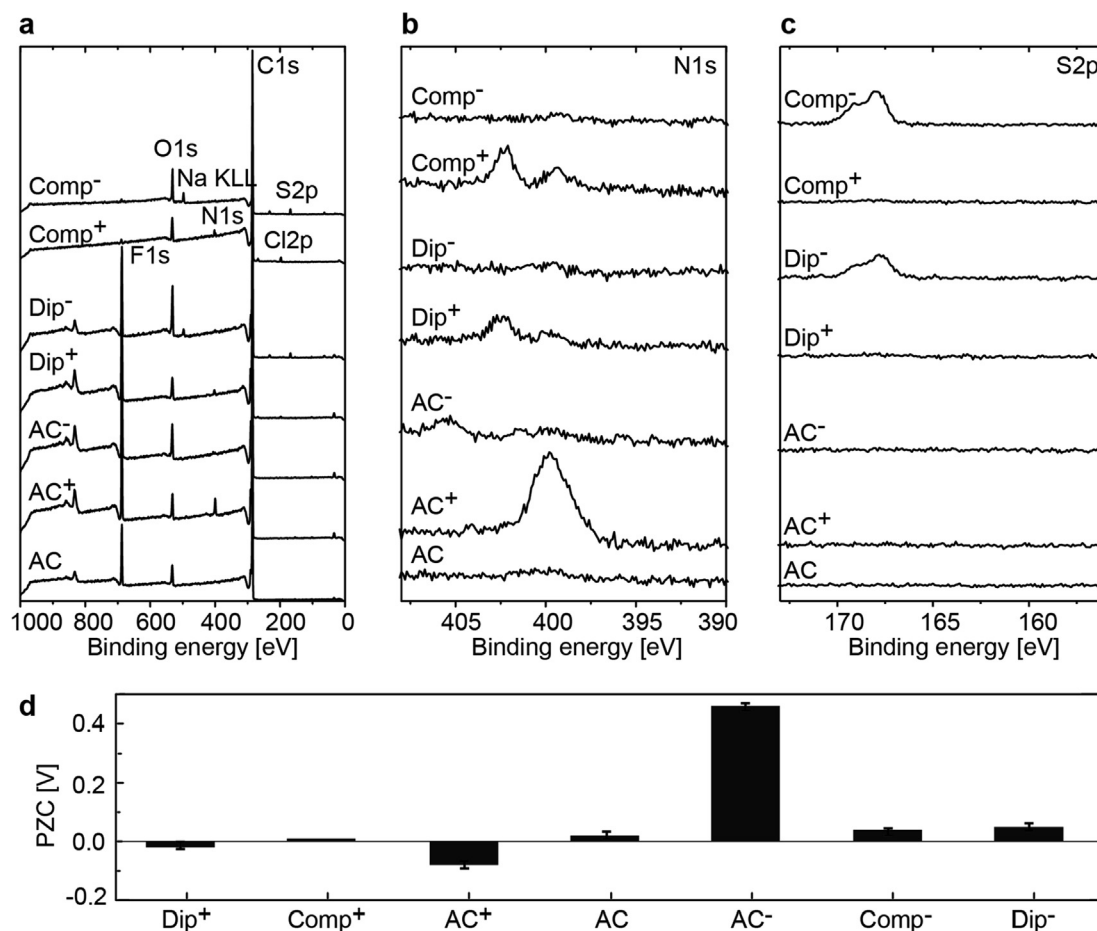


Fig. 1. X-ray photoelectron spectra of all electrodes as indicated in the plots. (a) Wide scan. High-resolution spectrum of (b) N1s and (c) S2p. (d) Potential of zero charge of all electrodes (CV scans are shown in Fig. S3).

various process conditions are illustrated, and the differences in ad- and desorption behavior, leading to suggestions for innovative separation design, are discussed.

3.1. Electrode characterization

Four electrode combinations are compared, all based on activated carbon as shown in Table 1: (1) two unmodified AC electrodes, (2) an anode with carboxylic groups (AC^-) and a cathode with ethylenediamine (AC^+) to create a net-negative or net-positive charge, respectively, when exposed to water at neutral pH [40]. Additional electrodes were made with charged polymers (PSS or PDADMAC) that were either (3) dip-coated onto unmodified AC electrodes or were cast as an integral part of the electrode slurry (4).

The XPS wide scan in Fig. 1a and the determined elemental peaks collected in Table S1 indicate that the AC^- electrodes were successfully modified with carboxylic groups, as evident from the larger oxygen content compared to the AC electrode, and the shift in the potential of zero charge (PZC) to more positive values (Fig. 1d). The N1s peak at 399 eV (Fig. 1b and Table S1) of the ethylenediamine treated electrodes (AC^+) proves the presence of amine groups, which shifted the PZC to negative values (Fig. 1d), as found by Gao et al [40]. The electrodes containing PSS obtained by dip coating or as integral part of the matrix, show prominent S2p peaks at 168 eV due to the sulfonate groups, whereas the PDADMAC containing electrodes have N1s peaks at 399 and 402 eV for C-NH₂ and C₄-N⁺, respectively [44] (Fig. 1b and Table S1). As described earlier for modified AC electrodes, also here a shift in PZC was observed; negatively charged polymers lead to more positive PZC, and positively charged polymers to more negative values (Fig. 1d). Similar PZC values were obtained in earlier work using impedance spectroscopy [42]. The shift in PZC is less pronounced compared to AC^+ and AC^- electrodes without polymers, since the majority of the incorporated polymer is not located directly at the interface of the activated carbon and thus influences the external electric double layer less strongly.

Thermogravimetric analysis indicates on average 7.7 wt% (Fig. S2 and Table 2) weight loss for $Comp^-$ and $Comp^+$, when heated from 100 to 600 °C. This is slightly less compared to the amount of polymer used to prepare the electrode slurry (5 wt% binder and 5 wt% polyelectrolyte). The weight loss of all AC electrodes is around 10.6 wt%, which is in line with the 10 wt% polymeric binder used for the electrode preparation. Dip^+ and Dip^- lose on average 12.4 wt% of their mass, which corresponds to 1.6 wt% polyelectrolyte content. Between 30 and 100 °C AC electrodes loose around 0.6 wt%, while electrodes containing polyelectrolytes loose on average 5.9 wt%, representative of the large amount of water captured in the polyelectrolytes.

3.2. Protein ad- and desorption

When testing the different electrode combinations in the flow cell, whey protein adsorption was detected in the passive phase during which 0 V was applied (minimum UV absorbance in the effluent). In contrast, during the active phase when a potential of -1.2 V was applied, an increase in UV signal indicated protein desorption (Fig. 2a). At

Table 2
Thermogravimetric weight loss after heating sample from 100 to 600 °C.

Material	Weight loss [wt%]	Polyelectrolyte content [wt%]
AC	10.6	0
AC^+	10.8	0
AC^-	10.5	0
Dip^+	12.4	1.8
Dip^-	12.1	1.5
$Comp^+$	7.8	3.9
$Comp^-$	7.5	3.8

the same time salt ad- and desorption, related to peaks visible in the conductivity readouts in the outlet, occurred vice versa: while proteins adsorbed, salt was released and while proteins desorbed, salt was stored in the electrodes as indicated in Fig. 2b and c. The underlying mechanisms are discussed in the following. The focus will first lay on protein separation and subsequently more insights about the salt interchange are discussed. Please note, during these measurements the mixture of whey proteins was analyzed as a whole and not individual proteins.

Since whey proteins have their isoelectric point at a pH of 4.5 [45] and the pH of the bulk solution is always above pH 6 (Fig. S14) the proteins used in this study have an overall negative charge, leading to storage at the cathode, carrying positive chemical charges. For $Comp^{+/-}$ and $Dip^{+/-}$ electrodes, the observed amount of ad- and desorbed whey proteins of about 10 mg g^{-1} , is much larger than that of, about 1 mg g^{-1} , for the AC and $AC^{+/-}$ electrodes (Fig. 3a), with stable values for cycles 3–10 (Fig. S4–S7). This can be related to the fact that the cathode carrying PDADMAC can store more proteins than the standard AC^+ electrodes due to the presence of the polycation, potentially also leading to multi-layer formation [18]. Furthermore, the interaction strength between the protein and polyelectrolyte is weaker than between protein and activated carbon, thus proteins detach more readily. This is due to the strong hydration effects in the polyelectrolyte opposing the electrostatic interactions with the protein [18]. The protein exchange also takes place at lower potential, but lower amounts can be separated (e.g. -0.4 V leads to 3 mg g^{-1} on average (Fig. S8)).

It is important to mention that for all electrodes protein desorption occurs faster than adsorption (Fig. 3c). For example, when adsorption and desorption periods are both set to 1800 s the adsorption peak of $Comp^{+/-}$ has a long tail and a total width of 1800 s (Fig. 2a), the desorption peak has however only a width of 880 s. This asymmetry in rates can be related to the interaction between the different ionic species, and the difference in driving forces during ad- and desorption. During protein adsorption at 0 V diffusion is the key driving force however, during protein desorption at -1.2 V migration assisted due to the applied potential is dominating, accelerating the process. Furthermore, at the cathode, the positive chemical charges of the PDADMAC are screened by the negative electronic charges. This increases electrostatic repulsion of negatively charged proteins, while adsorbing additional cations. The latter effect is also of relevance since the increase in ionic strength in the electrodes can further weaken electrostatic interactions between the proteins and the electrodes due to Debye screening.

This asymmetry is also known in ion-exchange processes in which the replacement of a slower diffusing molecule with a faster one is usually much faster than the opposite scenario. Ion exchange is, for example, three times faster if a cation exchange resin, loaded with Na^+ , is exposed to H^+ , than in the opposite case. This is due to the buildup of a potential gradient depleting the film around the resin if fast-moving H^+ ions are loaded onto the resin [46]. In our case, this rate-determining factor could be especially important for polyelectrolyte-containing electrodes. Since proteins diffuse much slower than chloride ions, protein adsorption is slow if the chemical charges in the polyelectrolyte are screened by Cl^- ions. In contrast, if the protein concentration in the polymeric layer is high, the ion concentration in its vicinity is high and thus facilitates protein- Cl^- exchange.

In general, the adsorbed amounts of protein are slightly smaller than the desorbed amounts, and the same trend is found when adsorption and desorption periods are both set to either 900 or 300 s (Fig. 4 and S11–S12). For these times, the amount of adsorbed protein is 5, and 3 mg g^{-1} , respectively. Only when a longer adsorption period (1800 s) is combined with a shorter desorption period of 300 s the amounts were found to be similar (Fig. 4 and S13). Interestingly, this particular combination seems to lead to higher protein adsorption overall compared to the process run at 1800 s for adsorption and desorption. This could be indicative of protein re-adsorption to the anode at extended

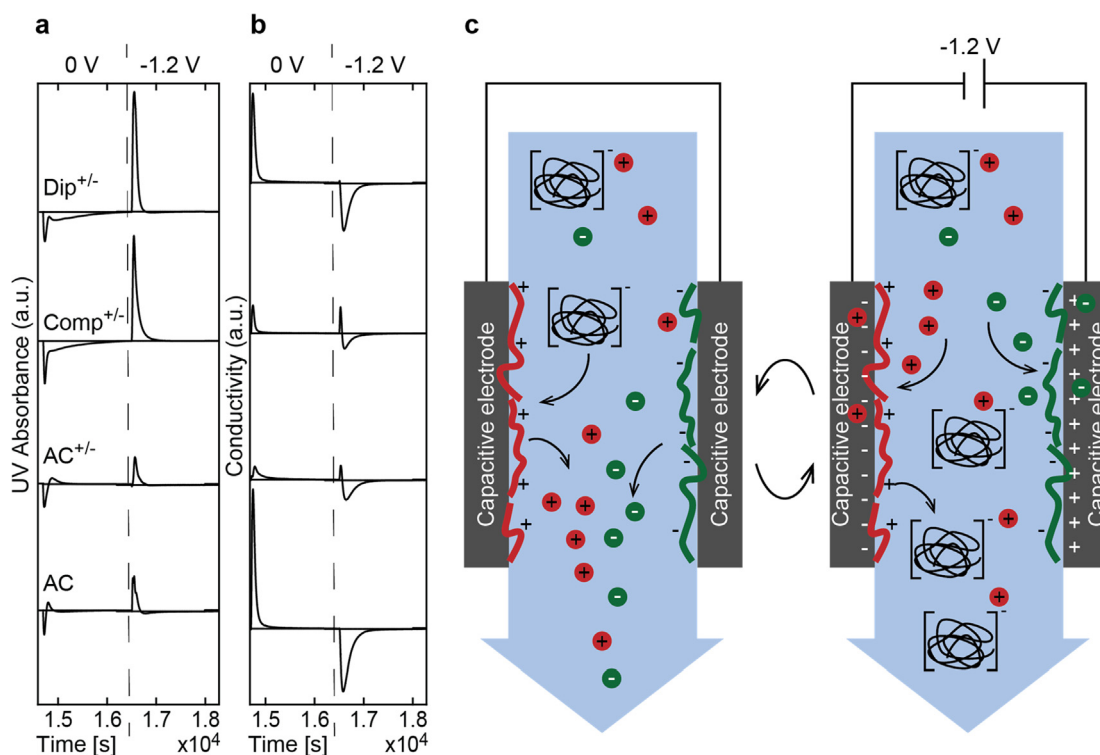


Fig. 2. Protein and salt separation using an electrical switch. (a) Change in UV signal of the eluent during cycle 5. (b) Change in conductivity of the eluent during cycle 5. (c) Schematic of salt (red and green dots) and protein (black ravels) ad- and desorption. At 0 V proteins adsorb and salt is released (c, left), while at a potential bias, proteins desorb and salt is stored in the electrodes (c, right). The red and green curved lines at the electrode interface represent the polyelectrolytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

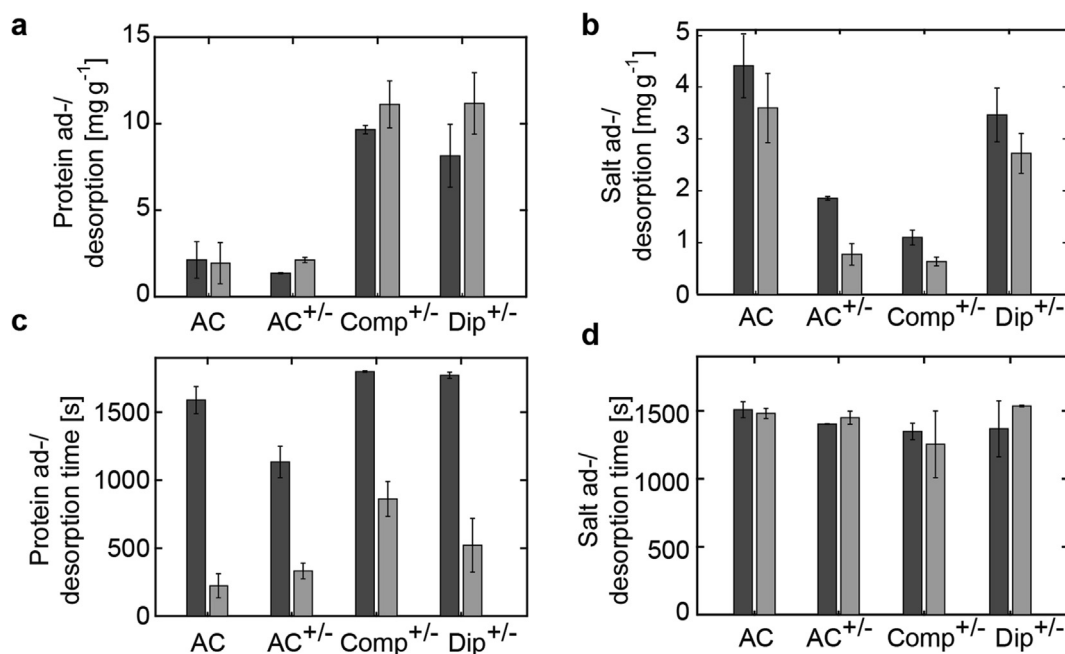


Fig. 3. Protein and salt exchange for all electrode combinations while keeping both the adsorption and desorption cycle at 1800 s. (a) Amount of protein ad- and desorption. (b) Amount of salt ad- and desorption. (c) Protein ad- and desorption times. (d) Salt ad- and desorption times. All values are averages over cycles 3–10 for all replicates. The dark grey bar represents values related to protein or salt adsorption and the light grey bar indicates desorption values.

periods during which -1.2 V is applied. This could be favored by pH decrease in the anode due to faradaic water splitting reactions, leading to a decrease in negative charges on the protein and thus reduced repulsion from the negative chemical charges of the PSS.

The Comp^{+/-} electrodes contain more polymer than the Dip^{+/-} electrodes (3.9 wt% versus 1.6 wt%, Table 2). This is especially relevant

for the anode, since the presence of the polymer reduces protein adsorption on the ‘wrong’ electrode (the anode) as depicted in Scheme 2. If, for example, two Comp⁺ electrodes are used (Fig. S9), protein desorption can be detected during the active as well as the passive phase while if two Comp⁻ electrodes are used (Fig. S10) desorption occurs only during the active phase, with small adsorption peaks during the

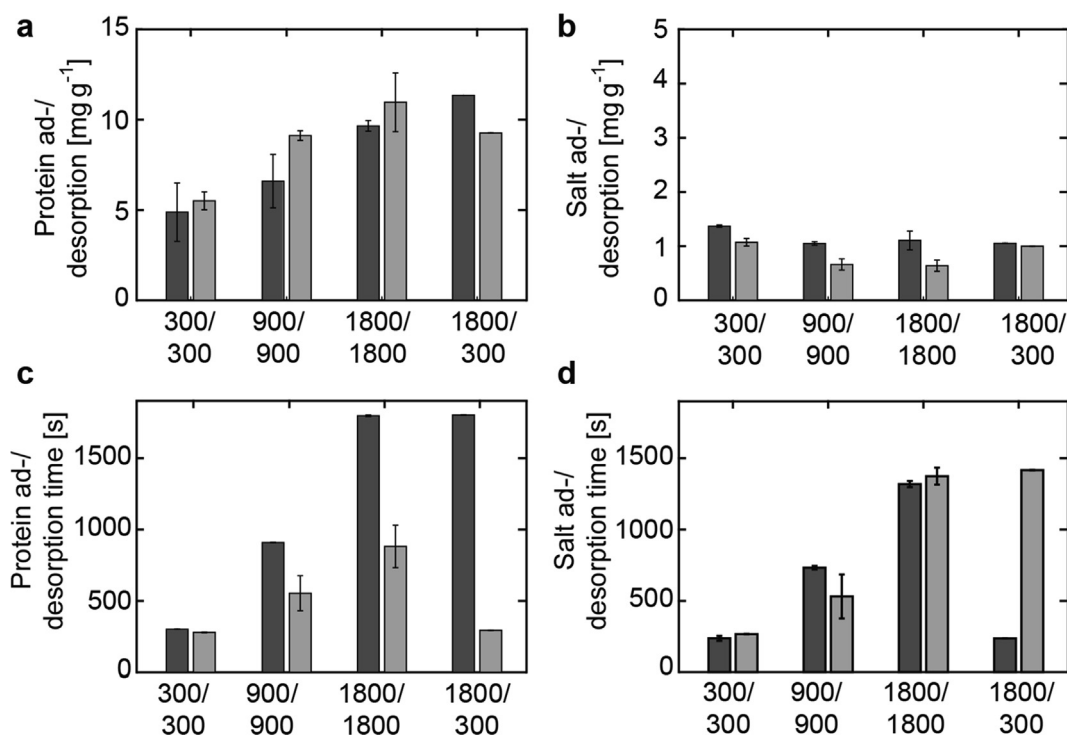
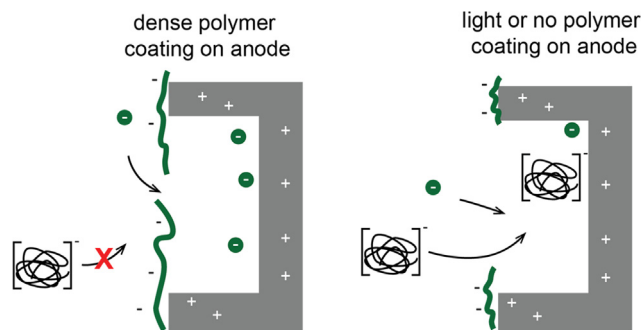


Fig. 4. (a) Protein and (b) salt ad- and desorption recorded using Comp^{+/-} electrodes at different ad- and desorption times indicated on the x-axis (adsorption time/desorption time). (c) Protein ad- and desorption times. (d) Salt ad- and desorption times. The dark grey bar represents values related to protein or salt adsorption and the light grey bar indicates desorption values.



Scheme 2. Protein adsorption due to capacitive current on anode (active phase) with dense polyelectrolyte coating (left) and anode with less or no polyelectrolyte coating (right). The grey box represents a pore in the carbon electrode and the white plus symbol indicate electron holes, the green curved lines at the electrode interface represent the polyelectrolytes. The green dots are the anions, while the black ovals represent the proteins.

passive phase. The migration of proteins into the anode also occurs for AC and AC^{+/-} electrode combinations. This explains the second desorption peak right after the adsorption peak in the passive phase (Fig. 2a).

By analyzing fractions of the effluent using HPLC the overall WPI protein concentration could be related to that of its three major constituents, β -lactoglobulin, α -lactalbumin, and bovine serum albumin [47]. As indicated in Fig. 5, β -lactoglobulin is the most prevalent, and its response to the electrical switch is the strongest. This indicates an enrichment in β -lactoglobulin in the effluent during the active phase.

3.3. Salt ad- and desorption

In addition to proteins, the low-molecular weight ions (Na^+ and Cl^-) need to be considered. As discussed above, some ions are stored due to an ion-exchange mechanism, especially when considering the

electrodes containing polyelectrolytes, leading to salt adsorption when proteins are released and desorption while proteins are adsorbed. The largest driving force the ions experience is, however, the applied potential difference of -1.2 V, leading to ion storage in the electric double layer of the electrodes as would be the case in capacitive deionization (Fig. 2b and c). However, the amount of stored ions is strongly dependent on the properties of the electrodes (Fig. 3c). In more detail, AC^{+/-} or Comp^{+/-} electrode pairs that have chemical charges directly at the surface or distributed within the electrode matrix, show low salt ad- and desorption values (0.5 mg g^{-1}). This could be explained by less efficient capacitive ion storage, e.g. due to a negative electric charge of the cathode. In the cases of the AC^{+/-} and Comp^{+/-} electrode pairs, the cathode also carries positive chemical charges which partially repel the cations. This was not the case for Dip^{+/-} electrode pairs that allow ions to penetrate through the polymer layer, thus not impacting electric double layer formation due to capacitive current. As a result, ad- and desorption is high at around 3 mg g^{-1} , which is comparable to the values obtained for AC electrodes.

The Dip^{+/-} electrodes are also the most stable ones, with less fouling over repeated operation, since over the course of 10 cycles the gSAC of Dip^{+/-} electrodes decreases by 0.3 mg g^{-1} while AC electrodes lose 0.7 mg g^{-1} (Fig. S4 & S6). This proves that the incorporation of polymers either in or on top of AC electrodes results in a capacitive protein separation process, that on top of that also affects the salt concentration in the effluent depending on the position of the polymers, which makes this process also interesting for concurrent desalting of the proteins. The Dip^{+/-} electrode pair is most efficient per amount of polymer used, probably due to the density of the polymeric layers.

4. Conclusion

A novel capacitive, selective protein separation process based on electrically switched ion exchange is presented. As it is an exchange process, the protein and salt ad- and desorption were coupled. Via surface modification with charged polymers the protein ad- and

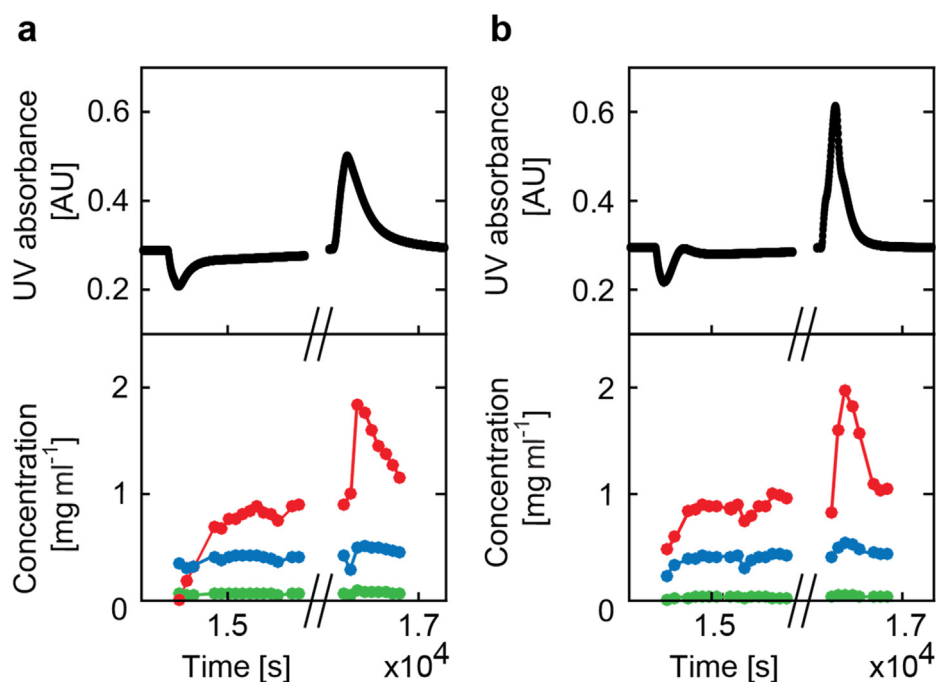


Fig. 5. Changes in β -lactoglobulin (red), α -lactalbumin (blue) and bovine serum albumin (green) concentration during active and passive phases (bottom) with respect to total UV absorbance change (top). (a) Using Comp^{+/-} electrodes. (b) Using Dip^{+/-} electrodes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

desorption could be increased to 10 mg g⁻¹, compared to 1–2 mg g⁻¹ for the unmodified electrodes, and even achieved an enrichment of β -lactoglobulin over other whey proteins. The stability of the modified electrodes remained high over 10 cycles. Since protein and salt ad- and desorbed in opposite half cycles this process is promising for concentration and desalting of protein streams in one step, which is relevant to industries such as food and biotechnology.

CRediT authorship contribution statement

Pina A. Fritz: Conceptualization, Methodology, Investigation, Data curation, Software, Writing - original draft. **Penghui Zhang:** Methodology, Writing - review & editing. **Tom Bruschinski:** Investigation, Data curation. **Sevil Sahin:** Methodology, Writing - review & editing. **Louis C.P.M. de Smet:** Methodology, Writing - review & editing. **Mary B. Chan-Park:** Conceptualization, Supervision, Writing - review & editing. **Remko M. Boom:** Conceptualization, Supervision, Writing - review & editing. **Catharina G.P.H. Schroën:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the institute for sustainable process technology (ISPT) for funding our project. Furthermore, we would like to thank Barend van Lagen (Laboratory of Organic Chemistry, Wageningen University) and Maurice Strubel (Laboratory of Food Process Engineering) for scientific support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seppur.2020.117195>.

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