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Cationic and anionic polymeric additives for wall deactivation and selectivity control in the capillary electrophoretic separation of proteins in food samples

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Abstract

Both cationic and anionic polymeric additives were used for the capillary electrophoretic separation of proteins in food samples. The cationic polyelectrolyte polydiallyldimethylammonium chloride was more effective in minimizing protein–wall interactions at pH 3 than at pH 7, presumably due to greater repulsion between the adsorbed polymer and proteins. Improved resolution was observed in the presence of the co-additive sodium octanesulphonate, presumably due to ion-pairing interactions with protein sample components. The anionic polymer dextran sulfate produced relatively high efficiencies, 120 000–180 000 theoretical plates, for protein separation, presumably because the polymer adsorbed to the capillary wall, rendering the surface more hydrophilic. In addition to reduced protein–wall interactions, improved resolution was observed, presumably due to analyte–polymer ion-exchange/ion-pairing interactions. When poly(vinyl sulphonic acid) was used instead of dextran sulfate, broader profiles were obtained and fewer components were resolved, presumably due to reduced wall deactivation that is related to the lower hydrophilicity of poly(vinyl sulphonic acid).

Keywords: Coating; Food analysis; Proteins

1. Introduction

Protein separation, identification and quantitation is important in both science and commerce [1]. In particular, protein samples are useful in diagnosing the efficacy of meat storage and preservation conditions. Capillary electrophoresis (CE) is an interesting tool for the analysis of these complex samples. Due to the small diffusion coefficients of proteins, efficiencies in excess of 10^6 theoretical plates are expected, based on diffusional spreading as the major mechanism of peak broadening. Unfortunately such efficiencies have not been realized for proteins. The complex protein composition of foods leads to complex and poorly resolved electropherograms. Also, proteins interact with the fused-silica capillary, degrading separation efficiency. In order to prevent protein–wall interactions, buffer and capillary inner surface modifications have been developed. Buffer modifications include the addition of high concentration of potassium salts [2], ethylene glycol [3], neutral [4] or zwitterionic [5] surfactants. Although these approaches work for a number of separations, wall coatings often reduce electroosmotic flow (EOF) leading to slow separation [5].

Bonded phases have also been developed to minimize wall interactions [6–10]. Surface silanol groups have been derivatized with a variety of

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functional groups, with the goal to generate a surface of moderate or high hydrophilicity to shield the bare silanol sites. Although a number of these coatings allowed for efficiencies as high 300 000 theoretical plates, their routine use and development has been hampered by prolonged preparation times, limited pH stability, and relatively high expense.

Optimization of protein separations is challenging, particularly in complex biological samples [11]. Proteins partition poorly into conventional micellar aggregates and interact irreversibly with a number of buffer additives, leading to low efficiencies. Short-chain (C_6) sulphonate surfactants have been applied successfully to protein separation [12]. These surfactants ion-pair with positively charged amino groups, generating differential migration of protein species. Several disadvantages are associated with the use of surfactant additives. They can irreversibly interact with proteins, increasing their affinity for the capillary wall. Excessive Joule heating can occur at the high concentration of surfactant that is sometimes required to improve resolution.

Some investigators have employed neutral hydrophilic polymers both as capillary coatings and/or as dynamic additives for protein CE separations at pH< 7 [13–15]. Polymers used included poly(vinyl alcohol) (PVA), hydroxyethylcellulose (HEC) and poly(ethylene glycol) (PEG). These polymers are capable of hydrophobic and/or hydrogen bonding interactions with proteins and the surface silanols leading to selectivity control and wall deactivation, respectively. Although exceptionally high efficiencies were observed, polymer degradation (e.g., PVA) was observed at pH>5.

We report the use of water-soluble polydisperse polyelectrolytes as separation buffer additives. We expect that these polyelectrolytes will minimize protein–wall interactions because of polymer coating of the capillary wall. We also expect that these polyelectrolytes will modify selectivity and resolution by virtue of ion-pairing and/or ion-exchange interactions. We use these reagents for the analysis of proteins found in bovine and avian aqueous muscle extracts. The separation profiles were compared with the ones obtained with butane- or octanesulphonate in terms of resolution, peak shape and total analysis time.

2. Experimental

2.1. Instrumentation

The Quanta 4000 (Waters, Milford, MA, USA) CE instrument was equipped with a zinc pen-ray lamp (214 nm) detector cell assembly. Data acquisition was done via a Macintosh IIsi computer. A LabVIEW program was written to acquiring data. All pH measurements were done with a Fisher Scientific Accumet pH meter (Edmonton, Canada) equipped with a saturated calomel electrode.

2.2. Capillary preparation

Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75 μm I.D.×365 μm O.D. were used. Unless otherwise noted, capillary end-to-end length was 60 cm and end-to-detection window was 52 cm. Prior to the first run the capillary was conditioned with the separation medium for 15–30 min. Between runs, the capillary was rinsed with 100 mmol/l KOH for 2 min followed by distilled–deionized water for about 2 min using a vacuum (15 mmHg; 1 mmHg=133.322 Pa). When separation electrolytes were changed, the capillary was purged with deionized–distilled water, 100 mmol/l KOH for 5 min followed by a purge with deionized–distilled water. For studies where the polymers were used as rinsing agents (0.01–0.5% g/ml), purging of the capillary (15 mmHg) was carried out for 10 min followed by purging with the separation buffer for 5–10 min. Overnight conditioning of capillaries was done by rinsing with deionized–distilled water using gravity-flow. Sample injection was hydrostatic from a height of 10 cm for 5 s.

2.3. Reagents

Reagents, such as separation electrolytes, polymers were used as obtained from the manufacturer without further purification. The cationic polyelectrolyte was poly(diallyldimethylammonium chloride) (PDDACl, M_r 250 000 g/mol), available as 20% solids in water (Aldrich, Milwaukee, WI, USA). The anionic polyelectrolytes dextran sulphate (500 000 g/mol) was from Pharmacia (Uppsala, Sweden) and

poly(vinyl sulphonic acid) (PVS) was obtained from Aldrich. The ion-pairing reagents, 1-butanesulphonate and 1-octanesulphonate in the sodium form were bought from Aldrich. Dimethyl sulphoxide (0.1%, v/v in doubly distilled water) was used as the EOF marker and was purchased from BDH (Toronto, Canada). Phosphate buffer and phosphoric acid (100 m*M*, used for pH adjustments) were obtained from Waters.

2.4. Analyte

For the cationic polyelectrolyte studies, an aqueous extract of bovine muscle protein was used with lactate preservative. The sample was then stored at −4°C. For the anionic polyelectrolyte studies, an aqueous extract of thermally denatured avian muscle was employed. Frozen chicken or beef tissue was thawed for 1 h. A 30-g sample was homogenized in 30 ml of ice-cold water by grinding the sample at full speed for 15 s, followed by cooling on ice for 45 s. The sample was spun at 4°C for 30 min on an ultracentrifuge. Samples were aliquoted into 500-μl vials and frozen at −20°C. The tissue extract was thawed before use. The chicken sample was analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, which revealed a series of peaks at M_r 20 000, 32 000 and 44 000 corresponding to the proteins myosin (isoelectric point, p*I* 6.0), tropomyosin (p*I* 4.5) and actin (p*I* 4.8).

3. Results and discussion

3.1. Electroosmotic flow reproducibility studies

The control of EOF is essential for reproducible analyses, particularly when CE separations are performed in the presence of surface active agents. Cationic polymers have been used as rinsing agents, where the capillary is rinsed with polymer (typically 0.1%) dissolved in water for about 10 min initially, followed by a 2-min rinsing between runs [16–18]. In the absence of frequent replenishment of the polymer, progressive stripping of the coating leads to poor migration time reproducibility and unstable currents. Alternatively, polymer may be incorporated in the separation buffer, which can improve migration time reproducibility by replacement of stripped polymer with polymer from the separation medium.

We compared the EOF reproducibility under two experimental conditions. In the first case, 0.1% (v/v) of PDDPACl was used to rinse the capillary for 10 min prior to the first run. We obtained a 25% relative standard deviation (R.S.D.) in EOF over a 2.5 h period. In the second case, 0.01% of the polymer was incorporated in 10 mmol/l phosphate buffer at pH 7. We observed a 4% R.S.D. in EOF over a 2.5-h period. In the latter case, the more stable EOF is presumably due to replacement of stripped polymer by polymer in the separation medium. This observation is in agreement with results published by Towns and Regnier [4], who incorporated 0.005% Brij in a separation buffer, which acted to replace 0.1% Brij initially sorbed onto C_{18} bonded phases.

3.2. Selectivity studies with bovine muscle protein samples – cationic polyelectrolyte

Cationic polyelectrolytes have been used both as rinsing agents and as additives in the CE separation of small ions and proteins. In the case of proteins, efficiencies greater than 100 000 theoretical plates have been achieved at pH 3 [19]. Most proteins are below their isoelectric point at this pH and will be repelled from the positively charged capillary surface. The authors did not report changes in electrophoretic profiles with pH or in the presence of ion-pairing agents.

A study was conducted to examine electrophoretic profiles of a bovine muscle extract at pH ranging from 3 to 7 or in the presence of 5–20 mmol/l octanesulphonate at pH 3. A comparison of the electrophoretic patterns indicates a sharper profile at pH 3 (Fig. 1A) compared to pH 7 (Fig. 1B). Broad profiles were also observed in the presence of a higher concentration of PDDACl (0.1%, v/v) at pH 7. Slow protein–cationic polyelectrolyte complex formation may contribute to peak broadening [20,21].

Although the cationic polymer minimizes wall interactions for a number of proteins under acidic pH conditions, selectivity and resolution are not ideal. A

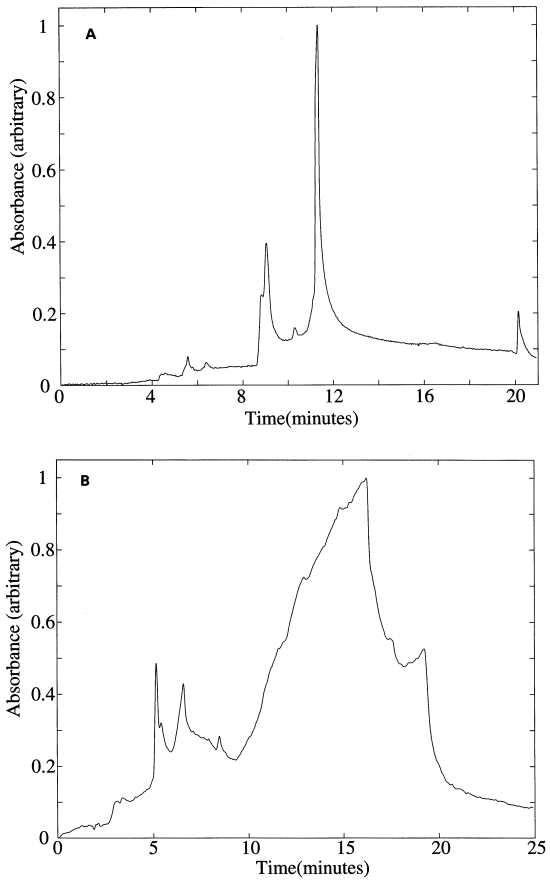

Fig. 1. Electrophoretic profile of a bovine muscle protein extract that has been thermally denatured, spiked with lactate, frozen, and thawed before extraction. Other conditions: hydrostatic injection from 10 cm for 15 s; electric field 500 V/cm; negative polarity (detector close to positive end). (A) Separation buffer: 5 mmol/l phosphate buffer–0.01% (v/v) PDDACl at pH 3.7. (B) Separation buffer: 5 mmol/l phosphate buffer–0.01% (v/v) PDDACl at pH 7.2.

study was conducted to examine the effects of 5–20 mmol/l octanesulphonate on the electrophoretic profiles in the presence of 0.01% (v/v) PDDACl. A number of components are resolved and a more detailed pattern is revealed, Fig. 2. This enhancement of selectivity and resolution could be due to ion-pairing interactions between the proteins and octanesulphonate. In other cases, octanesulphonate may coat some proteins via a combination of electrostatic and hydrophobic forces, imparting differential

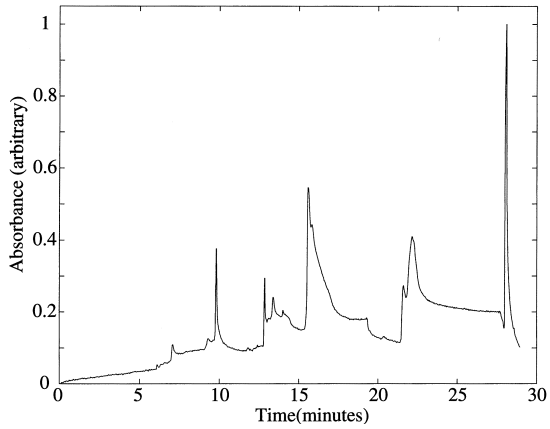

Fig. 2. Electrophoretic profile in the presence of 10 mmol/l sodium octanesulphonate of a bovine muscle extract sample that has been cooked, spiked with lactate, and frozen. Separation buffer: 5 mmol/l phosphate buffer–0.01% (v/v) PDDACl–10 mmol/l sodium octanesulphonate at pH 3.7. Other conditions: hydrostatic injection from 10 cm for 15 s; electric field 333 V/cm; negative polarity (detector close to positive electrode).

charge-to-mass ratios. This process may also be taking place between octanesulphonate and the polycation, reducing the effectiveness of the surfactant additive as well as making the modified capillary surface more hydrophobic.

3.3. Selectivity studies with avian muscle protein samples – anionic polyelectrolyte

Because of the electrostatic attraction between proteins and surface silanol groups at pH<~8, alkaline buffers are often used so that the negatively charged proteins will be repelled by the ionized silanol groups. However, in addition to electrostatic forces, hydrophobic effects are often responsible for protein–wall interactions [17]. These two trends suggest that a hydrophilic, negatively charged coating may be useful in protein separations. In addition, a negatively charged coating will generate large EOF [22], which may lead to rapid separations; in contrast, neutral coatings reduce EOF, resulting in slow separations.

A study was carried out to examine the use of two anionic polymers, dextran sulfate (DSA) (0.001–0.1% g/ml) or PVS (0.001–1%, v/v). These polymers were chosen because of their moderate (PVS)

to high (DSA) hydrophilicity and their different functional groups. An electrophoretic profile of a thermally denatured avian muscle sample is shown in Fig. 3A. The separation buffer was 25 mmol/l phosphate buffer–0.05% g/ml DSA at pH 7.2. The presence of 0.05% DSA results in a richer profile (12 peaks) and a more rapid (25 min), higher efficiency separation (120 000–180 000 theoretical plates) compared to buffers with 10 mmol/l octanesulphonate, Fig. 3B. An electropherogram generated with 10 mmol/l butanesulphonate at pH 7 was similar to that obtained with octanesulphonate.

The reduction in the background envelope for the DSA electropherogram indicates that protein–wall interactions are reduced, which is presumably due to DSA adsorption onto the capillary wall to render the inner surface highly hydrophilic. The high efficiencies and symmetric peaks indicate that protein–polyanion interactions are non-mass transfer limiting. Similar results were observed when DSA was used in the concentration range 0.001–0.01% in terms of resolution; the analysis time was optimum at the latter DSA concentration. Use of higher DSA concentration (0.1%) resulted in peak tailing, presumably due to slow polymer–analyte interactions.

PVS, at a concentration of 0.005% (v/v), was less effective in reducing the broad envelope, Fig. 4, although as many as eight components in the sample could be resolved. The lower hydrophilicity of PVS, compared to DSA, probably did not reduce the protein–wall interactions. The presence of the sulphonate groups may allow for protein–PVS ion-pairing interactions with a concomitant improvement in resolution. When higher concentrations of PVS

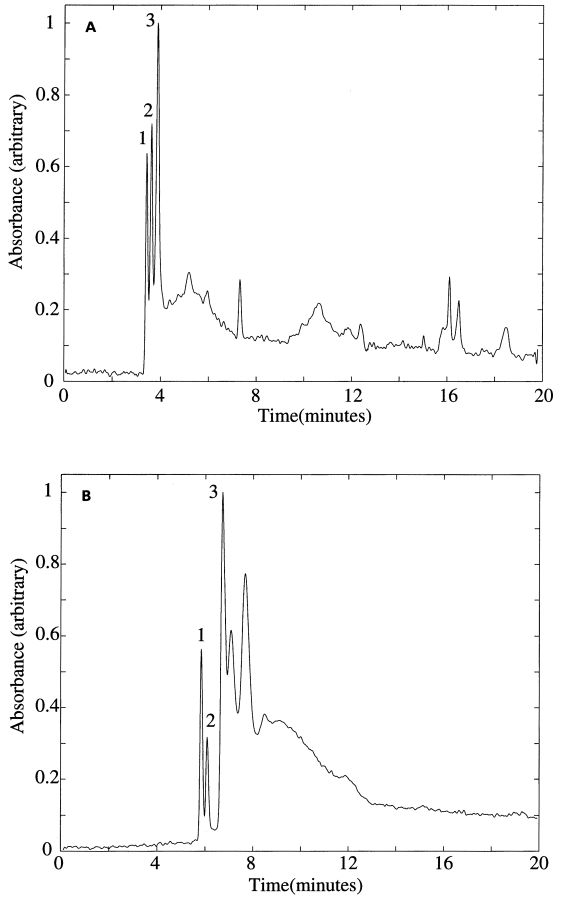

Fig. 3. Electrophoretic profile of a thermally denatured avian muscle tissue sample. In each case, hydrostatic injection was from 10 cm for 5 s; separation was at a 250 V/cm electric field; positive polarity (detector close to negative electrode). Peaks 1–3 were identified as actin, tropomyosin and myosin, respectively, based on co-migration with standards. (A) Separation buffer: 25 mmol/l phosphate buffer–0.05% (g/ml) DSA at pH 7.2. (B) Separation buffer: 25 mmol/l phosphate buffer–10 mmol/l sodium octanesulphonate at pH 7.2.

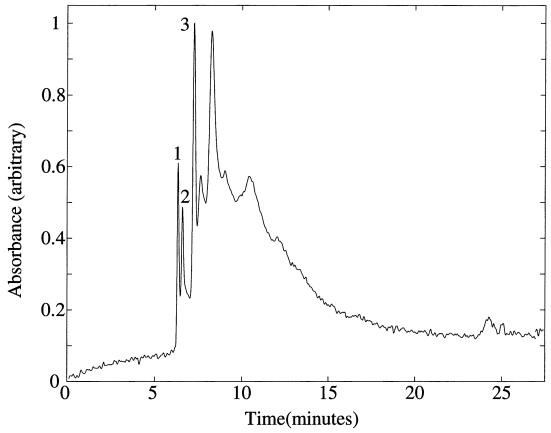

Fig. 4. Electrophoretic profile in the presence of 0.005% (v/v) PVS of a thermally denatured avian muscle tissue sample. Separation buffer: 25 mmol/l phosphate buffer–10 mmol/l sodium butanesulphonate at pH 7.2. Other conditions: hydrostatic injection from 10 cm for 5 s; electric field 250 V/cm; positive polarity (detector close to negative end). Peak identification as in Fig. 3.

were employed (0.01–1%, v/v), broad profiles were observed and a smaller number of components could be resolved.

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