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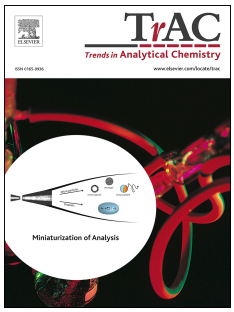

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Recent advances in column coatings for capillary electrophoresis of proteins

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Keywords: capillary coating, dynamic coating, permanent coating, nanomaterials, protein separation

Abbreviations:
EOF - electroosmotic flow; CE - capillary electrophoresis; LC - liquid chromatography; BFS - bare fused silica; BGE - background electrolyte; SDS - sodium dodecyl sulfate; CTAB - cetyltrimethylammonium bromide; PEO - polyethylene oxide; PVA - polyvinyl alcohol; MS - mass spectrometry; CZE - capillary zone electrophoresis; UV - ultraviolet; CIEF - capillary isoelectric focusing; CL - chemiluminescence; HMQC - hydrophobically modified quaternized cellulose; LIF - laser induced fluorescence, DAD - diode array detector; FD - fluorescence detector; CMC - carboxymethyl chitosan; rhEPO - recombinant human erythropoietin; AuNPs - gold nanoparticles; CEC - capillary electrochromatography; PEM - polyelectrolyte multilayer

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Abstract
Capillary coatings effectively improve the separation performance of proteins in capillary electrophoresis, mainly by reducing protein adsorption onto the inner capillary wall and by regulating the electroosmotic flow (EOF) to accommodate the separation problem in hand. In the first part of this review the newest trends in dynamic and permanent capillary coatings are summarized and discussed in detail. In the second part the application of nanomaterials as novel capillary coating materials is conversed. Nanomaterials have great potential in capillary coating preparations based on their advantageous properties such as large surface-to-volume ratios and a wide variety of chemistry options. Finally, some future prospective of capillary coatings in the emerging field of proteomics are given.

1. Introduction

Capillary electrophoresis (CE) is becoming a frequently used separation technique in protein analysis because of fast separation times, high efficiency, low reagent and minimal sample consumption compared to liquid chromatography (LC) [1]. Charged protein molecules are separated in CE by their differential electrophoretic mobilities in the background electrolyte at the appropriate pH, which should be defined based on their isoelectric points [2]. However, there are several issues that should be considered during protein analysis by CE. First of all, the most frequently used capillary tubing material of bare fused silica (BFS) becomes increasingly negatively charged at the separation pH>4 that induces a bulk electroosmotic flow (EOF) by the application of the electric field. This can be an advantage or a disadvantage during the separation, depending on the actual application. Protein adsorption onto the negatively charged silica surface [3] can also represent an issue. Capillary coatings can reduce protein adsorption and allow fine tuning the electroosmotic flow (EOF) in order to enhance minor differences in the electrophoretic mobilities of proteins and thus improve selectivity in CE [4].

Capillary wall coatings can be classified as dynamic or permanent types, based on the mode of attachment of the coating material [5, 6]. Dynamic coating approaches employ the addition of background electrolyte-soluble agents (e.g., amines or oligo-amines, anionic and cationic surfactants or neutral hydrophilic polymers, etc.) to the running buffer to cover the capillary wall during the separation [7] and maintain their surface concentration by sustaining the equilibrium between the liquid and solid-surface phases. Permanent coating materials are on the other hand, irreversibly attached to the inner surface of the fused silica capillary wall either by physical adsorption or by covalent bonding, therefore it is not necessary to add these materials to the BGE [8]. The use of nanomaterials as capillary coating agents opened up new possibilities to enhance separation efficiency and selectivity [9].

In this review we focus on the recent developments in column coating technologies applied in capillary electrophoresis of proteins. The dynamic and permanent coating approaches as well as the recently emerging nanomaterial coating layers are discussed in detail.

2. Dynamic coating approaches

Dynamic capillary coating procedures are easy to perform under standard laboratory conditions and provide an effective way to alter EOF or diminish protein adsorption, since these coating materials are simply added to the BGE as depicted in Scheme 1a [7, 10]. Due to their equilibrium based nature, these coatings need to be continuously regenerated by refreshing the adsorbed layer on the capillary wall [1]. Dynamic coating materials reversibly adsorb onto the capillary surface with the advantage of easy removal and regeneration if necessary [11]. The most commonly used agents are either small ions, like amines or oligo-amines, anionic and cationic surfactants (sodium dodecyl sulfate - SDS, cetyltrimethylammonium bromide - CTAB, etc.) or neutral hydrophilic polymers, such as polyethylene oxide (PEO), polyvinyl alcohol (PVA), celluloses, dextrans and ionic liquids [7,

11-13]. One of the disadvantages of dynamic coating agents is their possible interference with the separation and/or detection systems, especially, mass spectrometry (MS). In this latter case, if the coating material enters the mass spectrometer it may cause problems such as analyte signal suppression and/or contamination of the ion source and ion optics [14]. The most frequently applied dynamic coating agents used in capillary electrophoresis analysis of proteins are summarized in Table 1.

Table 1. The most frequently used dynamic capillary coating types for capillary electrophoresis of proteins

Coating agent	**Separation method**	**Analyte types**	**References**
Hydroxypropylmethyl cellulose	CE-UV	H1 histones	[15]
1,4-diaminobutane (DAB) or spermine	CZE-UV	Carbohydrate-deficient transferrin (CDT) in human serum	[16, 17]
Zwitterionic sulfobetaines	CIEF-UV	Lentil lectins, myoglobin, carbonic anhydrase I, carbonic anhydrase II and trypsin inhibitor	[18]
Linear polyacrylamide, N-substituted acrylamide copolymers	CZE-UV	Bovine serum albumin, transferrin, α1-antitrypsin	[19]
1-butyl-3-methylimidazolium tetrafluoroborate	CE-UV	lysozyme, cytochrome C, ribonuclease A, albumin, and α-lactalbumin	[20]
Poly(1-vinyl-3-butylimidazolium) bromide	CE-UV	Lysozyme, cytochrome C, ribonuclease A and α-chymotrypsinogen A	[21]
Polyvinylpyrrolidone (PVP) combined with sodium dodecyl sulfate (SDS)	CE-CL	Hemeproteins	[22]
Quaternized celluloses or hydrophobically modified quaternized celluloses	CE-UV	Lysozyme, ribonuclease A, cytochrome C, bovine pancreatic trypsin inhibitor, and chymotrypsinogen	[23, 24]
N-methyl-2-pyrrolidonium methyl sulfonate	CE-UV	Lysozyme, cytochrome C, ribonuclease A, and α-chymotrypsinogen A	[25]
Poly(N-methyl-2-vinylpyridinium iodide-block-ethylene oxide	CE-UV	Basic proteins (cytochrome C, lysozyme and α-chymotrypsinogen A) and lipoproteins (VLDL, HDL_3, LDL)	[26]
Gemini pyrrolidinium surfactants and	CE-UV	Lysozyme, α-chymotrypsinogen A,	[27]

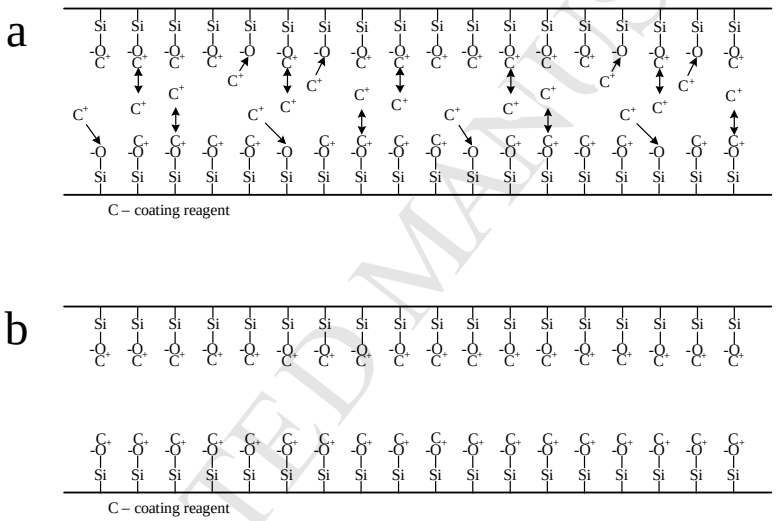
hexafluoroisopropanol		ribonuclease A, cytochrome C, hemoglobin, bovine serum albumin, trypsin inhibitor, and pepsin	
N-dodecyl-N,N-dimethyl-(1,2-propanediol) ammonium chloride	CE-UV	Lysozyme, ribonuclease A, cytochrome C, myoglobin	[28]

Zhang and coworkers applied zwitterionic sulfobetaine surfactants as dynamic coating agents for capillary isoelectric focusing (CIEF) of proteins. Separation of a mixture of seven protein standards was obtained with significantly improved resolution compared to bare fused silica capillary [18]. Applying ionic liquids (IL) as dynamic coating agents showed several advantages in capillary electrophoresis separations such as high solubility, heat stability, good electrical conductivity, adequate influence on EOF and efficient adsorption onto the capillary wall [29]. Wu et al. [20] employed 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid as dynamic coating material for the separation of basic and acidic proteins. As a matter of fact, ionic liquids assist better in the separation of proteins with reasonable background current compared to other common electrolytes. Separations of a large number of proteins can be performed in single run by association with ionic liquid cations under mild conditions without the need of any other selectivity enhancing modifiers and efficiencies up to 670,000 plates/m were obtained with such dynamic coatings. Guo et al. [25] used N-methyl-2-pyrrolidonium methyl sulfonate ionic liquid as dynamic coating agent and separated basic proteins within 15 min with high efficiency (theoretical plates 209,000 – 448,000 plates/m). The authors suggested the existence of hydrogen bonding between the ionic liquid and the capillary wall. Recent trends in capillary electrophoretic separation of basic proteins suggest the application of polymeric ionic liquids as even better dynamic coating agents. Polymeric ILs efficiently reduce protein adsorption due to their greater ability of capillary surface coverage compared to monomeric ionic liquids. Jiang and coworkers performed the separation of basic proteins with efficiencies ranging from 247,000 to 540,000 plates/m by using poly(1-vinyl-3-butilimidazolium) bromide as dynamic coating material [21]. Chen and coworkers applied polyvinylpyrrolidone (PVP) combined with sodium dodecyl sulfate (SDS) as dynamic modifier in capillary electrophoretic separation of hemeproteins with chemiluminescence (CL) detection [22]. They obtained good resolution, satisfactory reproducibility and symmetrical peak shapes. The proposed CE-CL method has a great potential for myoglobin detection in human urine samples. Zhao et al. [23] used cationic celluloses, namely quaternized celluloses (QCs) as dynamic coating agents. These coatings very effectively suppressed protein adsorption and reversed the EOF in a broad pH range, therefore basic proteins could be separated under physiological conditions and maintain their native conformation. The same research group developed new hydrophobically modified quaternized hexadecyl group containing celluloses (HMQCs) for dynamic capillary coatings [24]. HMQCs showed significant improvement in coating stability due to the additional hydrophobic interaction in addition to the electrostatic force and hydrogen bonding. HMQCs

generated stronger reversed EOF (approximately 10% increase) compared to QC coatings and resulted in better separation efficiency.

3. Permanent capillary coatings

Permanent coatings are either permanently adsorbed or covalently bonded layers to the capillary inner surface [8, 30]. Physically adsorbed permanent coatings offer some advantages compared to covalent coatings such as (i) simplicity of the coating procedure; (ii) option of easy coating regeneration; and (iii) minimal dependence on surface chemistry [12, 31]. In this case the coating materials are adsorbed onto the silica surface by means of physical forces like electrostatic, hydrophobic interactions or hydrogen bonding [32]. Important to note that permanent physical coatings, unlike dynamic coatings, do not require equilibrium sustaining agents in the background electrolyte to maintain surface coverage as depicted in Scheme 1b.



Scheme 1. Dynamic (a) and physically adsorbed permanent (b) capillary coatings.

Covalent (chemical) capillary coatings generally involve the modification of the surface silanol groups on the fused silica capillary wall. The usual process starts with pretreatment of the capillary surface by NaOH to open up the Si-O-Si bonds and form Si-OH groups, followed by a silanization reaction with a bifunctional alkoxysilane reagent to form covalent bonds with the opportunity to attach the final or if necessary an intermediate coating material (e.g., linear polymer) [33, 34]. Covalent coatings feature long-term stability and they are compatible with MS detection [14]. Recently applied frequently used permanent coatings for capillary electrophoresis separation of proteins are summarized in Table 2.

Table 2. Most frequently used permanent capillary coatings for capillary electrophoresis of proteins

Coating type	**Coating agent**	**Separation method**	**Analytes**	**References**
Physically	N,N-	CE-UV	Lysozyme,	[35]

adsorbed	dimethylacrylamide-ethylpyrrolidine methacrylate (DMA-EPyM) copolymer		ribonuclease A, cytochrome C, α-chymotrypsinogen	
Covalent	Poly(ethylene glycol)-terminated alkoxysilanes	CE-LIF, CE-DAD	Bovine serum albumin, alcohol dehydrogenase, carbonic anhydrase and soybean trypsin inhibitor	[36]
Physically adsorbed	Carboxymethyl chitosan	CE-DAD	Recombinant human erythropoietin (rhEPO) glycoforms	[37]
Physically adsorbed	Polybrene and dextrane sulfate entrapment in polymerized silicate	CE-UV, CE-MS	Acidic proteins	[38]
Physically adsorbed	poly(N-ethyl morpholine methacrylamide-co-N,N-dimethylacrylamide), poly(N-ethyl pyrrolidine methacrylate-co-N,N-dimethylacrylamide), poly(N-ethyl morpholine methacrylate-co-N,N-dimethylacrylamide) and poly(N-ethyl pyrrolidine methacrylamide-co-N,N-dimethylacrylamide).	CE-UV	Ribonuclease A, bovine cytochrome C, horse cytochrome C	[39, 40]
Physically adsorbed	Poly(diallyldimethylammonium) chloride (PDADMAC) monolayer or PDADMAC/poly(sodium 4-styrenesulfonate)	CE-UV	Lysozyme, ribonuclease A, cytochrome C, myoglobin, α-lactalbumin	[41, 42]

	(PSS) multilayer			
Physically adsorbed	Poly(1-vinyl-3-butylimidazolium) bromide	CE-DAD	Lysozyme, ribonuclease A, cytochrome C	[43]
Physically adsorbed	Dioctadecyldimethylammonium bromide (DODAB) and poly-oxyethylene (POE) stearate	CE-UV	Histone type III-S proteins	[44]
Covalent	Poly(DMA-co-NAS-co-MAPS) or poly(DMA-co-GMA-co-MAPS)	CE-UV	Lysozyme, cytochrome C and ribonuclease A	[45]
Physically adsorbed	Poly(1,2-bis[10-(2',4'-hexadienoyloxy)decanoyl]-sn-glycero-2-phosphorylcholine)	CE-LIF, CE-FD (CCD)	histidine-tagged enhanced green fluorescent protein, R-phycoerythrin biotin conjugate, insulin chain A, trypsin inhibitor, bovine serum albumin, and α-chymotrypsinogen A	[46, 47]
Covalent	Trimethoxysilylpropyl(polyethyleneimine) and 1-(4-iodobutyl) 4-aza-1-azoniabicyclo[2,2,2] octane iodide	CESI-MS	Post-translationally modified Histones	[48]
Covalent	Diazoresin/poly(N-vinyl aminobutyric acid)	CE-UV	Lysozyme, ribonuclease A, cytochrome C, bovine serum albumin	[49]
Physically adsorbed	Poly(dopamine) and poly(acrylamide) mixture	CE-UV	Cytochrome C, lysozyme, ribonuclease A, α-chymotrypsinogen A, albumin, trypsin inhibitor	[50]
Covalent	Diazoresin/cyclodextrin-derived (CD) dendrimer	CE-UV	Lysozyme, ribonuclease A, bovine serum albumin, myoglobin	[51]
Covalent	Diazotized poly(ethylene glycol)	CE-UV	Lysozyme, cytochrome C, bovine serum	[52]

			albumin	
Covalent	Maltose-modified hyperbranched poly(ethylene imine)	CE-UV	Human serum proteins	[53]
Covalent	Linear poly acrylamide (covalently bonded on the silanized capillary surface)	CE-UV	Lysozyme, cytochrome C, RNase A	[54]

3.1 Physical adsorption

Wu and coworkers applied hydrophilic carboxymethyl chitosan (CMC) as a physically adsorbed permanent capillary coating material for the analysis of recombinant human erythropoietin (rhEPO) [37]. Under optimized separation conditions (concentration and pH of the separation buffer, temperature, applied voltage) rhEPO glycoforms were separated by a CMC-coated capillary within 8 min with high efficiency. Different type of cationic copolymers were also applied as physically adsorbed coatings and their effectivity was investigated by the separation of model protein mixtures [35, 39, 40]. Cationic coatings significantly improved separation efficiency and resolution, compared to the use of bare fused silica capillary and also decreased analysis time. The net charge of the coating depends on the nature and composition of the copolymer as well as on the working pH, therefore by modifying these parameters the EOF can be altered to accommodate the CE separation problem [40]. By applying polymeric ionic liquids as physically adsorbed coatings, separation of basic proteins was performed with high efficiencies and good repeatability. The efficiencies for these protein separations were between 110,000 and 160,000 plate/m and the migration time %RSD was in the range of 0.45-1.6% (n=5) [43]. Nehmé et al. developed stable multiple ionic-polymer layers using highly charged polyelectrolytes (e.g., poly(diallyldimethylammonium) chloride (PDADMAC) / poly(sodium 4-styrenesulfonate - PSS) multilayer) as physically adsorbed capillary coating for protein analysis [41, 42]. These multilayer coatings were very stable at alkaline conditions, therefore enabled excellent separation performance even at pH=9.3 with high efficiency (700,000 plate/m) [42]. To improve the stability of physically adsorbed coatings, Ishihama and coworkers [38] applied a positively charged polymer entrapped in a polymerized silicate matrix as capillary wall coating. This coating was simple to prepare like any other physically adsorbed coatings, but offered the stability of covalent coatings. The polymerized silicate coated capillaries were reportedly very stable over a wide pH range and showed good performance in the analysis of acidic proteins at physiological pH.

3.2 Covalent (chemical) coatings

Chiari and coworkers [45] presented a novel covalent coating approach where chemically reactive groups such as N-acryloyloxysuccinimide (NAS) and glycidyl methacrylate (GMA) and [3-(methacryloyl-oxy)propyl]trimethoxysilane (MAPS) were part of the dimethyl acrylamide (DMA) polymer chain. The copolymers with poly(DMA-co-NAS-co-MAPS) or

poly(DMA-co-GMA-co-MAPS) were attached to the capillary wall. The reactive groups enhanced the stability of the film by reacting with surface silanols beside the alkoxysilane moiety. NAS is highly reactive towards primary amines, therefore it should be blocked after the coating procedure, e.g., with ethanolamine rinsing. Excellent separations were obtained for acidic and basic proteins with these hydrophilic coatings. Another type of covalent capillary coating was based on the use of a photosensitive diazo polymer [51, 52]. Firstly, the diazo polymer was electrostatically adsorbed to the negatively charged (pH > 3) [55] bare fused silica capillary surface. The physically adsorbed layer was then exposed to UV light forming the covalently cross-linked diazo coating. This coating was chemically stable and showed good performance in CE separation of proteins [52]. Guttman and coworkers [54] developed a fully automated covalent linear polyacrylamide (LPA) coating and regeneration procedure for CE of proteins, based on the original Hjertén approach [33]. The technique was implemented in a commercial CE instrument, where the capillary was not removed from the system during the entire coating and/or regeneration process. The resulting coated capillary columns were tested with a protein mixture of lysozyme, cytochrome C, and ribonuclease A and showed excellent stability up to at least a 100 runs with good reproducibility (migration time %RDS<0.67).

4. Nanomaterial layers

In capillary and microchip electrophoresis, different type of nanomaterials (e.g., gold nanoparticles, metal-oxide nanoparticles, polymer nanoparticles, carbon nanomaterials) have been employed to coat the surface of the separation channels [9, 56-58]. Nanoparticles can enhance separation efficiency, selectivity and reproducibility due to their advantageous properties such as large surface-to-volume ratios and a wide variety of surface chemistry options (e.g., organofunctional groups such as alkyl, amine and carboxyl groups) that may provide additional interaction sites [56]. Because of their size range, nanoparticles are exceptionally suitable for CE applications and can be used either as dynamic or permanent coatings if they meet the following criteria: 1) stable suspensions in a variety of CE background electrolytes; 2) desired selectivity in interaction with the analyte molecules; 3) charged but with different mobility that of the electroosmotic flow mobility; 4) matched mobility to the co-ions of the background electrolyte to alleviate peak broadening [59]; 5) small mass-transfer resistance; 6) no effect on detection efficiency; and 7) high surface area coverage [60]. The main disadvantage of the use of nanoparticles in CE applications is their possible interference with the detection systems, such as light-scattering problems in UV-Vis detection and effect on the ionization process in mass spectrometric detection. Therefore, nanoparticles applied mainly as permanent coating material in CE separation of proteins [9]. Recently used nanomaterial coatings for capillary electrophoresis separation of proteins are summarized in Table 3.

Table 3. Nanomaterial based coatings for capillary electrophoresis of proteins

Coating type	Nanoparticle	Separation method	Analytes	References
Dynamic	Didodecyldimethyl	CE-UV	Saliva, serum and	[61]

coating (and pseudostationary phase)	ammonium bromide (DDAB) - capped gold nanoparticles (AuNPs)		red blood cell lysate proteins	
Permanent coating (physically adsorbed)	PEG stabilized TiO_2 nanoparticles	CEC-UV	Chicken egg-white proteins (lysozyme, ovalbumin, conalbumin) and apo-transferrin	[62]
Permanent coating (covalent)	Fullerenol or latex diol (covalently bonded on the silanized capillary surface)	CE-DAD, CE-MALDI-MS	Five proteins (myoglobin, α-casein s1,s2, bovine serum albumin, ovalbumin and lysozyme) tryptic digest	[63]
Permanent coating (physically adsorbed)	Gold nanoparticles (multilayer structure; positively charged poly(diallydimethylammonium chloride), negatively charged poly(sodium-4-styrenesulfonate), and positively charged AuNPs.)	CEC-UV	Ovomucoid, lysozyme, avidin, ovotransferrin, ovalbumin	[64]
Permanent coating (covalent)	Citrate stabilized gold nanoparticles (covalently bonded on the silanized capillary surface)	CEC-UV	Human serum albumin tryptic digest	[65]
Permanent coating (covalent)	Graphene, graphene-oxide sheets (covalently bonded on the silanized capillary surface)	CEC-UV	Avidin, lysozyme, ovotransferrin, ovalbumin, ovomucoid, ovoflavoprotein	[66]
Permanent coating (physically adsorbed)	Carboxyl modified magnetic nanoparticles (physically adsorbed on a poly(diallyldimethylammonium) layer)	CEC-UV	Conalbumin, ovalbumin, α-lactalbumin, β-lactoglobulin	[67]
Permanent coating	Graphene oxide-SiO_2 hybrid	CEC-DAD	Chicken egg white proteins	[68]

(covalent)	nanoparticles (covalently bonded on the silanized capillary surface)			
Permanent coating (covalent)	Carboxyl fullerenes (covalently bonded through a photosensitive diazoresin)	CE-UV	Cytochrome C, lysozyme, bovine serum albumin, myoglobin	[69]

Although nanoparticles applied mainly as permanent coating materials, gold nanoparticles in protein analysis by capillary electrophoresis have also been used both for permanent and dynamic coatings [70]. Yu et al. used didodecyldimethylammonium bromide (DDAB) capped gold nanoparticles (AuNPs) to form a stable dynamic capillary wall coating [61] that generated 75% greater reversed EOF compared to DDAB. To improve separation efficiency (by further reducing protein adsorption), the DDAB-capped AuNPs were modified with poly(ethylene oxide) (PEO). Using the 0.05% PEO modified DDAB-capped AuNPs dynamically coated capillary at pH 3.5, the separation of acidic and basic proteins featured high peak efficiencies and good reproducibility. Hu and coworkers applied a permanent coating approach, in which the gold nanoparticles were deposited on the silica surface via ionic adsorption to a polyelectrolyte multilayer (PEM) [64]. The first coating was constructed layer by layer with positively charged poly(diallydimethylammonium chloride), negatively charged poly(sodium-4-styrenesulfonate), and positively charged AuNPs. The second one was constructed layer by layer with positively charged poly(diallydimethylammonium chloride), negatively charged poly(sodium-4-styrenesulfonate), positively charged poly(diallydimethylammonium chloride) and negativelly charged AuNPs. After that, the multilayer coatings were derivatized by passing n-octadecanethiol solution through the capillary to stabilize the upper AuNPs layer. The resulted capillary coatings supported effective separation of acidic and basic proteins with good reproducibility in open tubular capillary electrochromatography (OT-CEC) mode and were reportedly stable over 800 runs. Gold nanoparticles can also be directly attached to the inner capillary surface by covalent bond. Hamer et al. reported a new method of immobilization of citrate stabilized gold nanoparticles (AuNPs) through covalent binding onto an APTES ((3-aminopropyl)triethoxysilane) functionalized fused silica capillary surface [65].

Carbon nanomaterials are mainly immobilized onto the capillary surface via covalent bonding [63, 66, 69]. Bonn and coworkers prepared a chemical capillary coating with fullerenol (derivatized fullerene) covalently bonded to silanized capillary surface [63]. The coated capillary showed great stability in the pH range of 2.0 to 10.0 and high separation performance were obtained in the analysis of a tryptic digest of five proteins with good reproducibility. Qu et al. applied a covalently coated capillary with graphene oxide and obtained good separation of five chicken egg white proteins [66]. Chi and coworkers used photosensitive diazoresin (DR) as coupling agent to covalently link carboxyl-fullerene to the inner capillary surface [69]. By replacing the toxic and highly moisture sensitive silane coupling agent with DR, the authors presented a promising, environmental friendly and

simple way to prepare the covalently coated capillaries for CE separation of proteins. This coating type featured good chemical stability and excellent separation efficiency with migration time RSD<2.5% for the test proteins in the course of 100 runs.

5. Conclusion and future prospective

In capillary electrophoresis of proteins, appropriate column coatings are important to improve separation performance mainly for the two reasons: 1) protein adsorption can be minimized onto the inner capillary wall, and 2) the electroosmotic flow (EOF) can be adjusted to enhance minor differences in the electrophoretic mobilities. The most important criteria of a good capillary coating is high stability over a wide pH range and no or minimal interference with the separation and/or detection system. Permanent coatings, especially covalent ones meet these requirements in most instances. Dynamic capillary coatings are on the other hand easier to apply, but have some disadvantages, such as possibly interfering with the detection systems (e.g., MS). Recent developments in nanoparticle based capillary coatings reportedly enhanced separation efficiency, selectivity and reproducibility. Due to their large surface-to-volume ratio and the wide range of surface chemistry options, nanomaterial layers hold great potential in future developments, especially in capillary electrochromatography applications. Because of their environmentally friendly nature, covalent coupling reagents are becoming more and more popular. In addition, fully automated capillary coating approaches for protein analysis are gaining recent interest as the process can be easily implemented in commercial CE instruments.

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Highlights

- Dynamic coatings for capillary electrophoresis of proteins are reviewed.
- Advances in permanent capillary coatings for protein CE analysis are discussed.
- The advent of nanomaterials as novel capillary coating option is introduced.