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Review

Recent applications of affinity interactions in capillary electrophoresis

Systems biology depends on a comprehensive assignment and characterization of the interactions of proteins and polypeptides (functional proteomics) and of other classes of biomolecules in a given organism. High-capacity screening methods are in place for ligand capture and interaction screening, but a detailed dynamic characterization of molecular interactions under physiological conditions in efficiently separated mixtures with minimal sample consumption is presently provided only by electrophoretic interaction analysis in capillaries, affinity CE (ACE). This has been realized in different fields of biology and analytical chemistry, and the resulting advances and uses of ACE during the last 2.5 years are covered in this review. Dealing with anything from small divalent metal ions to large supramolecular assemblies, the applications of ACE span from low-affinity binding of broad specificity being exploited in optimizing selectivity, *e.g.*, in enantiomer analysis to miniaturized affinity technologies, *e.g.*, for fast processing immunoassay. Also, approaches that provide detailed quantitative characterization of analyte–ligand interaction for drug, immunoassay, and aptamer development are increasingly important, but various approaches to ACE are more and more generally applied in biological research. In addition, the present overview emphasizes that distinct challenges regarding sensitivity, parallel processing, information-rich detection, interfacing with MS, analyte recovery, and preparative capabilities remain. This will be addressed by future technological improvements that will ensure continuing new applications of ACE in the years to come.

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1 Introduction

Electrophoretic separation patterns are influenced by intermolecular and intramolecular interactions taking place during electrophoresis. This was realized already back in the early days of electrophoresis (for historical notes, see, *e.g.*, [1]). Thus, moving boundary electrophoresis was used to characterize antigen–antibody interactions more than 50 years ago [2]. Modern day electrophoresis also uses affinity interactions to manipulate and optimize selectivity, and the capability of affinity electrophoresis to extract quantitative information about interacting systems is of significant value. Recently, affinity CE (ACE) has even begun to be used as a preparative tool. The chronology of affinity electrophoresis development after the proof-of-principle in the era of moving boundary electrophoresis consists of two phases corresponding to gel-based electrophoresis (see overviews in, *e.g.*, [3, 4]) and then to the advent of CE [5–7].

Since the last review about the use of affinity interactions in CE [8], there has been a steady outpouring of theoretical and practical applications of noncovalent molecular interactions which we will try to review here. An emerging trend is that ACE is now more integrated in the experimental toolbox of many laboratories. It is now one of several tools available for studying binding interactions and for enhancing selectivity concurrently with other methods even though it is still considered a specialist approach by many. Real novelties within CE theory and practice have been limited during the period except maybe within the area of preparative ACE to develop, *e.g.*, aptamers (*cf.* Section 9).

New ACE applications within the last 2.5 years are the focus of this review. While the list is not guaranteed to be exhaustive, many new applications in this period are

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listed in the analyte-sorted Table 1. We include all applications where reversible interactions (in a wide affinity range) and electrophoresis occur simultaneously. Thus, we include interactions between analytes and free or immobilized ligands, and also both preequilibrated mixtures and dynamic ACE as well as cases with various combinations of these methods. Additionally, we include both applications where affinity interactions are used as tools, *e.g.*, to manipulate resolution or for preparative purposes, and applications where electrophoresis is used as a tool for characterizing interacting molecules. Below we try to spotlight, review, and discuss characteristic and important applications within the main application areas. In parallel with the new developments an avalanche of more or less straightforward acronyms and abbreviations has appeared. Many are explained in the Appendix to Table 1.

2 Reviews

A number of specialized and general ACE reviews have appeared in the period (see also the last part of Table 1). Gayton-Ely *et al.* [9] cover recent progress and innovation with respect to biomolecular interaction analysis in the 2003–2004 period and list close to 100 studies in that period. Other recent reviews have focused more specifically on the use of ACE techniques for the assessment of drug interactions with serum proteins and for the characterization of enantiomeric species [10, 11]. In addition, miniaturized electrophoretic systems used for enzyme and immunoassays were included in another recent review on microfluidic systems in biochemical analysis [12].

3 Immunochemistry

Antigen–antibody interactions form the basis of the most specific and sensitive quantitative assays for components in biofluids that are available, and antibodies are widely used as preparative tools in affinity chromatography. Thus, it is of great interest to characterize antigen–antibody reactions, and electrophoresis may be readily used to separate bound from free immunoreagents. The development of CE for this purpose appears to be following two different trajectories. One is the quest to miniaturize immunoassays (lab-on-a-chip) for simple, fast, and sensitive measurements in clinical settings. The other is the application of CE for the in-depth characterization of immunoreagents with respect to reactvity and binding strength, *e.g.*, so that antibodies for specific applications can be tailor-made. An important issue in the miniaturization of immunoassays is achieving suitable detection limits comparable to the performance of traditional ELISAs. In ELISA subpicomolar detection limits and parallel sample processing are standard [13].

Various CE immunoassay arrays that partially fulfil these requirements have been devised in recent work, *e.g.*, in [14, 15] where a 48-channel radial CE array was designed and shown to be able to assess the binding of the hapten trinitrotoluene to mAbs in a competitive, homogeneous (*i.e.*, involving no solid-phase interactions) preincubation assay format. Detection was achieved by means of LIF and reached a limit of about 1 ng/mL, corresponding to about 4 nM. In other work, chip-based CE for the analysis of human immunoglobulin using capacitively coupled contactless conductivity detection [16] was demonstrated in a model system where also immunochemical reactions were performed. Contactless conductivity detection appears to be relatively sensitive (LODs for purified immonoglobulin M (IgM) were around 15 pM in conventional capillaries and 3.4 nM on chips), but the approach also requires low-conductance running buffers. While the preliminary results showed detection of immunocomplexes and free antigen, it remains to be seen if the method is too restricted with respect to running buffer conditions to be widely applicable for immunoassays. In another report on chip-based immunoassays, a sample throughput capability of 10–12 *per* hour for the simultaneous analysis of several cytokines directly from cerebrospinal fluid, at 1 pg/mL levels of detection limits, was achieved [17]. Importantly, the quantitation correlated very well with independent ELISA-based measurements. The analysis time was down to about 2 min *per* sample for the electrophoresis step, while the incubation and labeling steps prior to that totaled about 15 min. Since the bound analytes were here quantitated by electrophoresis after being eluted from the immunocapture port that is integrated into the chip, the approach is, strictly speaking, not an affinity electrophoresis method because no interactions take place during electrophoresis. Similar methods may be used in the process of developing and characterizing antibodies. An application – also based on immobilized antibody–antigen reaction prior to electrophoresis – was recently published by our group (supplemental material in [18]). Here, the specificity of a mAb for structurally very similar antigens was ascertained by analyzing supernatants of antigen mixtures incubated with immobilized antibody. The high resolution of CE ensured separation of the protein species as well as the consumption of very little precious biological material. The use of immunoaffinity CE approaches for proteomics in general has recently been reviewed [19].

Table 1. Recent applications of affinity interactions in CE

Analytes	Interacting molecules	CE mode	References
Peptides and proteins			
	Thrombin–DNA (aptamer)	CE	[69]
	Homo-/di- and alanyl dipeptides–G-quartet DNA	OTCEC	[27]
	IgE–DNA	CE-SELEX	[66]
	Hyaluronan–HA binding proteins	ACE	[120]
	3C-like proteinase–octapeptide	ACE	[39]
	SH2-Bβ–phosphorylated peptide	FACE/APCE	[114]
	G proteins–BODIPY FL GTPγS	FACE	[113]
	Zn(II)–carbonic anhydrase II	CE	[34]
	Phosphatase–inhibitor	ACE	[37]
	HSA–Cu(II)	IMACE	[119]
	HSA–Pt(II)	CE-ICP-MS	[77]
	APP–heparin	ACE	[121]
	β_2-gp1–anionic ligands	ACE	[25]
	β-Lactoglobulin–pectin	CE-FA	[122]
	Mono-/diquaternarized diamines–protein/cell digest	CE	[22]
	GFP-CaM–Ca^{2+}	CE-LIF	[111]
Immuno ACE applications			
	Complement C4–scFv	ACE/SPR	[20]
	Cytokine–AB	Chip IACE	[17]
	Anti-PrPc–PrPc	ACE	[123]
	TNT–anti-TNT AB	MCP-CE	[15]
	IgM–anti-IgM	HVCCD-CEIA	[16]
	α_1-Antitrypsin–TMR-Fab-anti-α_1-antitrypsin	CE-LIF	[101]
Drugs, drug development, and small molecules			
	Neurotransmitter–sulfated β-CD	MC-ACE	[118]
	Oxprenolol enantiomers–HSA	AEC	[124]
	Enantiomers–CD	ACE	[109]
	Tramadol enantiomers–sulfated CD	ACE	[81]
	Bacitracin A1–divalent cations	ACE	[125]
	AGP–cellulase	ACE	[106]
	Glycopeptide antibiotics–dansyl compounds	ACE	[88]
	MPEG–β-CD	ACE	[126]
	Antalgic drugs–β-CD	CE	[127]
	Conjugated amino acids–β-CD	CE	[107]
	Basic drug–HSA	CE	[33]
	Drug–erythromycin	CE	[128]
	Hydrobenzoin–sulfated β-CD	CE	[84]
	Organic disulfates–QA-β-CD	CE	[129]
	Metadone–sulfated β-CD	CE	[86]
	Drug–CD	TGF-CE	[82]
	Aromatic 2-phenylglycine derivatives–crown ether	CD-CE	[130]
	N-Imidazole derivatives–neutral CD derivates	CD-CE	[87]
	Pantothenic acid–*s*-3-amino-1,2-propanediol	CE	[85]
	Basic enantiomers–heptakis CD	NACE	[108]
	Platinum anticancer–albumin	CE	[76]
	Flurbiprofen–CD	ACE	[131]
	p-Nitrophenol–CD	ACE	[100]
	Vancomycin–dipeptide	ACE-MS	[117]
	p-Nitrophenol–CD	ACE	[99]
	Cucurbit[*n*]uril (*n* = 6, 7)–amino compounds	CE	[132]
	Anti HIV-1 drug–TAR RNA	ACE	[64]
	HSA–drug	CE-FA	[71]
	Fmoc-peptide–glycopeptide antibiotics	ACE	[133]

Table 1. Continued

Analytes	Interacting molecules	CE mode	References
	β-Thujaplicin–goethite	CE	[134]
	Drug–adenosine receptor	CE	[135]
	Disopyramide–AGP	HPFA-CE	[83]
	Coumarin 153–apomyoglobin	CE	[110]
	Polyamines–organic phosphate	CE	[136]
	Clozapine–HSA	CE-FA	[72]
	Drug–HSA	HPFA-CE	[73]
	Alprostadil–α-CD	ACE	[137]
	Metal ions–MBP	tCITP-CE	[138]
	Drug–biopolymers	HPLC/CE	[10]
	Donepezil–HSA	CE-FA/CD	[74]
	Basic drug–HSA	CE-electrochemi-luminescence	[75]
	Tetraalkylammonium ion–inorganic anions	CE	[89]
	Mercapto group–alkaloids	CE	[139]
Molecular biology			
	Taxol–DNA	CE/FTIR	[59]
	Tau–ss/dsDNA	NECEEM	[30]
	SNP–ssDNA conjugates	CE	[56]
	Polyamines–DNA	ACE	[55]
	Ionic molecules–DNA/protein	CE	[51]
	Liposome–oligonucleotide	CE-LIF	[50]
	Divalent metal ions–DNA	CE	[52]
	Monovalent cations–DNA	CE	[140]
	Protein–DRD4 gene	CEMSA	[57]
	Carboxypeptidase G2–6S/R leucovorin	CE	[141]
	AZT–RNA	CE	[55]
	AZT–DNA	CE	[61]
	Fe(II)/Fe(III)–DNA	CE	[54]
	Nanogold-poly(ethylene oxide)–dsDNA	CE	[142]
	Chiral transition metal complexes–DNA	CE	[53]
	SSB–ssDNA	CE	[31]
Lipids and carbohydrates			
	Polysaccharide–iodine	ACE	[143]
	AGP–ConA	ACE	[144]
	Carbohydrate–lectin	ACE	[41]
	Disaccharide derivatives–serum proteins	ACE	[42]
	Apolipoprotein–lipid	VCE	[45]
	Drug–lipid membrane	CE	[145]
	LMW Heparin–G-CSF	CE	[146]
	LMW cationic drugs–dextran sulfate	CE-FA	[43]
	D/L-tryptophan–phospholipid-lysozyme	CE	[49]
	Drug–dextrin oligomers	CE-FA	[44]
Microbiology and cell biology			
	Antimicrobial compounds–YihA	ACE	[147]
	Human rhinovirus capsid–VLDLR repeat 3	CE	[110]
	Endothelin antagonist–receptor	ACE	[97]
	HRV2-RNA–RiboGreen	CE-LIF	[96]
	β-Carboline derivatives–TAR RNA	CE	[63]
	PAH–TAR RNA	QCM-CE	[148]
	Cationic peptides–membrane lipids	ACE	[46]
Reviews			
	Covalent drug–protein adducts	CE	[149]

Table 1. Continued

Analytes	Interacting molecules	CE mode	References
	Peptide recognition	CE	[150]
	Small ions and proteins and DNA	CE	[51]
	Protein–protein	ACE	[151]
	Protein–DNA	CE-LIF	[112]
	Drug enantiomers–protein chiral selector	ACEC	[152]
	Molecularly imprinted polymers	MIP-CEC	[153]
	Macromolecule–ligand	CE-HD	[154]
	Drug–biopolymer	ACE	[10]
	CD interactions	CE	[80]
	Immunological interactions	CE	[155]
	Affinity interactions	ACE	[8]
	Peptides	CE methods	[156]
	Virus, bacteria, eucaryotic cells	CE	[93]
	ACE	ACE	[9]
	TNT–anti-TNT AB	Microchip-CE	[12]
	Drug–plasma proteins	ACE	[70]
	Biomolecular binding systems	CE/ACE/FACE/CEHD/CEVP	[157]
	Biomolecules	CE-MS	[158]

AB, antibody; **ACE**, affinity CE (reacting molecule added to the electrophoresis buffer); **ACEC**, affinity CEC; **AGP**, α_1-acid glycoprotein; **APCE**, affinity probe CE; **APP**, amyloid precursor protein; **AZT**, azidothymidine; **β_2-gp1**, β_2-glycoprotein 1; **BODIPY FL GTPγS**, 6-((4,4-difluoro-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-propionyl) amino) hexanoic acid guanosine 5'-*O*-[3-thiotriphosphate]; **CAE**, capillary affinity electrophoresis or capillary array electrophoresis; **CAGE**, capillary affinity gel electrophoresis; **CaM**, calmodulin; **CE-ECL**, CE-electrochemiluminescence; **CE-FA**, CE-frontal analysis; **CE-HD, CEHD**, CE-Hummel–Dreyer; **CE-ICP-MS**, CE-inductively coupled plasma-MS; **CEMSA**, capillary electrophoretic mobility shift assay; **CE-SELEX**, CE-systematic evolution of ligands by exponential enrichment; **CFTPFACE**, competitive flow-through partial-filling affinity CE; **tCITP-CE**, transient capillary ITP-CE; **CYP2C9**, drug metabolizing enzyme (cytochrome P450, family 2, subfamily C, polypeptide 9); **DE-ACE**, dynamic equilibrium affinity CE; **DCCE**, dynamic complexation CE; **DHPC**, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; **DMPC**, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; **DRD4**, dopamine D4 receptor; **FA**, frontal analysis; **Fab**, antigen-binding antibody fragment; **FACCE**, frontal analysis continuous CE; **FACE,** fluorescence anisotropy CE; **FTPFACE**, flow-through partial-filling affinity CE; **$G_{\alpha\alpha i1}$**, G protein $\alpha\alpha$i1; **G-CSF**, granulocyte-colony stimulating factor; **GFP**, green fluorescent protein; **HA**, hyaluronan; **HD**, Hummel–Dreyer; **HIV**, human immunodeficiency virus; **HPFA-CE**, high-performance FA-CE; **HRV2**, human rhinovirus serotype 2; **HVCCD-CEIA**, high-voltage contactless conductivity detector-capillary electrophoretic immunoassay; **IACE**, immunoaffinity CE; **ICP-MS**, inductively coupled plasma-MS; **IDA**, iminodiacetic acid; **LMW**, low-molecular-weight; **MBP**, metal-binding protein; **MC**, microchip; **MC-ACE**, microchip-affinity CE; **MCP-CE**, microchannel plate-CE; **MPEG**, methoxypoly(ethylene glycol); **IMACE**, immobilized metal chelate affinity CE; **MSLIPFACE**, multi-step ligand injection partial-filling affinity CE; **NACE**, nonaqueous CE; **NECEEM**, nonequilibrium CE of equilibrium mixtures; **OCLSPFACE**, on-column ligand synthesis partial-filling affinity CE; **OsCBK**, *Oryza sativa* Ca^{2+}/CaM binding protein kinase; **OTCEC**, open-tubular CEC; **PAH**, poly(allylamine hydrochloride); **PFACE**, partial-filling capillary affinity electrophoresis; **PrPc**, normal cellular prion protein; **PSA**, prostate-specific antigen; **PTM**, post-translational modification; **QA-β-CD**, quaternary ammonium β-CD; **QCM-CE**, quartz crystal microbalance-CE; **scFv**, single chain variable fragment; **SELEX**, systematic evolution of ligands by exponential enrichment; **SH2-Bβ**, Src homology 2 (525–670) domain; **SNP**, single nucleotide polymorphism; **SSB**, single-stranded DNA-binding protein; **TAR**, *trans*-activating response region; **TGF**, temperature gradient focusing; **TNT**, trinitrotoluene; **YihA** (essential GTP-binding protein from *E. coli*); **VACE**, vacancy affinity CE; **VCE**, vesicle affinity CE; **VLDLR**, very low-density lipoprotein receptor; **VP**, vacancy peak

New quantitative binding studies of immunoreagents have confirmed the validity of CE-derived binding constants by comparison with independent techniques such as surface plasmon resonance measurements [20]. In this study a singe-chain variable fragment antibody reacting with fluorescently labeled C4 complement protein was evaluated. It is one of the few CE-based studies of interactions between two proteins that are both >25 000 in molecular mass. A K_D of 15–30 nM was estimated by both the SPR and CE methods.

Summarizing the use of antigen–antibody interactions in CE there are–despite the advances in detector technology and chip designs–still plenty of obstacles to conquer

with regard to LODs and parallel processing. The idea of replacing solid-phase immunoassays with microelectrophoretic methods only becomes attractive in routine clinical chemistry after these issues have been solved. In contrast, CE has a lot to offer in the development and functional characterization of immunoreagents.

4 Proteins and peptides

The analysis of proteins by CE has always been troubled by adsorption problems, and this is also evident in CE-based binding studies with proteins. Much effort is spent on working out conditions for reproducible protein analysis before embarking on the binding studies themselves. A typical problem is that the best conditions for CE-based protein separations (*e.g.*, acidic pH) are rarely the most suitable conditions for studying physiologically relevant binding interactions. In some cases low-affinity interactions can be utilized to make peptides and proteins analyzable under suitable buffer conditions. The way such buffer additives are used is different from wall-shielding dynamic coating additives [21], where interactions with the analytes are highly undesired. The new types of additives are meant to complex with the analytes and thereby shield them from wall binding. An example of both mechanisms described in one paper [22] is the use of mono- and diquaternized diamines for peptide and protein separations. This approach mainly improved peptide recovery, while the analysis of larger peptides/whole proteins still was questionable. In the case of a specific protein analyte, the glycosaminoglycan-binding plasma protein β_2-glycoprotein I, the so-called pH-hysteresis effect of uncoated fused-silica capillaries [23, 24] was exploited. In this way reproducible analyte recovery was achieved at neutral pH and binding studies could be carried out [25]. The pH-hysteresis effect refers to the slow deprotonization of silanol groups when brought to neutral pH from the acidic side compared with the faster equilibration when coming from the alkaline side. It is achieved, *e.g.*, simply by flushing the capillary with 0.1 M HCl prior to running the CE analysis. This gives a slower electroendosmotic flow and a decreased tendency for positively charged analytes to stick to the capillary walls under the very same running buffer conditions that are incompatible with analyte recovery when preceding the run with an alkaline flush.

Peptide–peptide interactions have been studied in recent work dealing with amyloid β-peptides [26]. The fate of the incubated peptides was followed over time by repeated CE analyses. It could be demonstrated that the peptides aggregated and that the aggregation pattern of the 40-amino acid variant was vastly different from that of the more amyloidogenic 42-amino acid variant.

Mechanisms underlying the selectivity of binding interactions between proteins or peptides and so-called quadruplex (G4) DNA were explored in a study of 14 different homodipeptides and nine different Ala-X dipeptides separated in capillaries containing immobilized quadruplex DNA [27]. Using the regular quartet sequence, it was possible to resolve the peptides better than in a bare capillary under otherwise identical conditions. It could be shown that some amino acid combinations in the dipeptides had a higher binding than others. Thus, these amino acid motifs were possibly involved in the separation of proteins achievable by open-tubular CEC using quadruplex DNA as a stationary phase [28]. There is an affinity continuum from these selectivity enhancing oligonucleotide structures that has no affinity for specific proteins to the very specific, high-affinity artificial DNA and RNA sequences called aptamers. Aptamers are defined as DNA/RNA molecules that have been selected from random pools based on their ability to bind ligands. They are combinatorially developed to bind specific targets and often contain G-quartet motifs, as is, *e.g.*, the case for the thrombin-binding aptamer originally described more than 10 years ago [29]. The use of aptamers in ACE is reviewed in more detail in Section 9.

In an interesting application of combinations of different ACE modes, the dsDNA- and ssDNA-binding capability of the microtubule-associated tau-proteins was examined [30]. The authors discovered that tau actually may dissociate dsDNA to ssDNA and is bound to ssDNA contrary to earlier beliefs. Peak profiles in experiments separating preincubated mixtures in empty electrophoresis buffer (an approach named NECEEM, nonequilibrium CE of equilibrium mixtures) and in analyses including a displacer of unbound material in the running buffer (here the ssDNA-binding protein from *Escherichia coli*) [31], made it possible to obtain quantitative measurements of the DNA-tau binding.

5 Protein conformation

A special case of interaction studies is when CE is used to unravel and characterize intramolecular interactions such as those involved in protein folding. CE has been of considerable value in this field, because different conformations may be resolved provided that their lifetimes are sufficiently long and the folding variations impose sufficient changes in the shape/size, charge distribution, or exposure of interacting domains to change electrophoretic velocities. This makes con-

formers otherwise not readily resolvable by other techniques amenable to characterization by CE as we have shown for variants of the amyloidogenic protein β_2-microglobulin [32]. In these studies the functional consequences, *e.g.*, binding of Congo red and other ligands to the individual conformers that were separated by CE could be evaluated. Thus, in these studies two selectivity factors conferred by intramolecular as well as intermolecular interactions simultaneously contributed to the separation patterns. The advantage of using solution techniques under near-native conditions for studying protein conformation is obvious.

Also the differential interactions of conformers of proteins with chiral drugs can be exploited in CE to characterize folding patterns as shown, *e.g.*, in studies of HSA [33]. In this application the focus was not to quantitate drug enantiomers, but to characterize protein conformers indirectly from the changes in the enantiomeric selectivity associated with different conformations. In other studies, refolding of proteins partly denatured by chaotropes or anionic detergents was followed by CE. CE allowed evaluation of the effects of the addition of various cofactors, for example divalent metal ions, on the refolding process at the same time as it was possible, as, *e.g.*, in the case of refolding enzymes, to probe for activity [34].

6 Enzymes

Enzyme-substrate reactions are also noncovalent molecular interactions, and have been analyzed by microelectrophoretic methods for some time [35]. Substrate formation and inhibitor action may be screened and characterized quantitatively online by electrophoretically mixing and separating zones of reactants and products in a setup that has been named electrophoretically mediated microanalysis (EMMA). The field was recently reviewed [36]. An example of the use of this approach is the screening of microbial culture extracts for protein tyrosine phosphatase inhibitors and extraction of substrate conversion kinetics data entirely based on CE analysis [37]. In another study the substrate specificity of an acid phosphatase from greater duckweed (*Spirodela oligorrhiza*) toward a wide range of organophosphate insecticides was readily evaluated by an MEKC method [38]. A binding constant determination at different temperatures for the interaction between a proteinase from the severe acute respiratory syndrome (SARS) coronavirus and an octapeptide inhibitor was published [39], and CE in this way continues to be a valuable tool for studies of enzyme-substrate specificity, rate constants, and thermodynamics.

7 Glycoconjugates

Carbohydrates are important for many biological reactions. Lectins constitute a group of proteins with specific carbohydrate-binding capabilities. Lectins continue to be used as tools in CE for identification and characterization of carbohydrate moieties [40]. Conversely, lectins may themselves be characterized with respect to binding specificity using panels of well-defined carbohydrates in CE [41]. Bergström and coworkers used Con A as a partial-filling affinity ligand in ACE to separate microheterogeneous variants of purified α_1-acid glycoprotein (AGP), and further used the system to characterize disease-related variations in the glycosylation of serum APG from rheumatoid arthritis patients.

Nakajima *et al.* [41] used the reverse strategy to search for specific carbohydrate binders in crude extracts of tulip bulbs. Using a library of oligosaccharides added to the electrophoresis buffer, the tulip lectins were probed for binding activity by looking for migration shifts. Subsequently, purified lectins were used as analytes to precisely delineate carbohydrate-binding specificities.

Free carbohydrates and polysaccharides also are important in many biological processes and in industry. In one application, dialyzed serum was screened for specific proteins interacting with a panel of negatively labeled disaccharides [42]. Even though no specific identification of the protein(s) involved was achieved, the electropherograms indicated the presence of strong binders of several of the disaccharides. The authors then reversed the system and analyzed the disaccharides with serum proteins and identified some of the serum binders as being γ-, β-, and α-globulins, and AGP. They identified good binding of the globulins to gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucopyranose) and low binding to lactose (galactose-β(1–4)-glucose).

Carbohydrate oligomers have also been of interest in the analysis of pharmaceuticals [43, 44]. An interaction between soluble low-molecular-weight (LMW) ligands and oligomers where at least one is charged is well suited for CE-frontal analysis (CE-FA) in coated capillaries. Østergaard *et al.* [43] studied the effect of dextran sulfate polymers on the analysis of LMW drugs with respect to ionic strength of the electrolyte and polymer concentration. Complex formation was found to be independent on the dextran sulfate concentration at low ionic strength, and differences in charge of the LMW drugs resulted in different binding strength to the dextran polymer. Furthermore, the ionic strength of the buffer strongly influenced the LMW drugs–dextran sulfate interaction. During the analysis the applied voltage had to be adjusted with increases in ionic strength due to uncontrollable temperature

increase in the capillary. For one of the LMW drugs the authors found good agreement between binding data obtained with CE-FA and with equilibrium dialysis.

Hoffmann *et al.* [44] used preequilibration analysis in negative CE mode to analyze the affinity between labeled dextrin and a group of negatively charged model drugs. They obtained binding parameters and quantitative measurements that fit well with independently obtained NMR spectroscopy measurements.

8 Lipids

The analysis of lipids by CE is problematic because of the insolubility of many lipids in aqueous buffers and their lack of chromophores. In the case of lipoconjugates such as lipoproteins and glycolipids, however, enough hydrophilicity may exist to make ordinary analysis strategies work. Otherwise, pure lipids must be analyzed as micellar preparations, as vesicles/liposomes, in the presence of detergents (enabling the formation of mixed micelles), or by using nonaqueous CE. The lipid binding of apolipoproteins was studied using an ACE approach with negatively charged phospholipid vesicles added to the physiological electrophoresis buffer [45]. Unilamellar vesicles resembling cell membranes were found more suitable for binding studies than multilamellar vesicles and liposomes. A quantitative binding theory taking into account the possibility of many protein molecules binding *per* vesicle (vesicle surface area may be 1000-fold larger than the surface area of the protein analyte) was worked out. It was shown that the extracted binding constant values and values for the number of protein molecules bound *per* vesicle correlated quite well with measurements using independent techniques. The approach was called vesicle CE, and this important study should form the basis for studies of the binding of many other biologically relevant lipid-interacting molecules.

Lipophilicity and screening for membrane affinity was the focus of another study measuring the interaction of various cationic peptides with micelles and bicelles, *i.e.*, bilayered micelles. As in all biphasic systems, the analyte migration is a function of charge-to-size ratio, EOF, and residence time in the various micelles [46]. The approach is highly suited for assessing solution lipophilicity and membrane affinity of peptides exhibiting secondary structure, and is versatile with respect to the different micellar compositions that may be applied. In addition to the lipophases mentioned above, microemulsions may also be used to evaluate drug–membrane interactions and membrane permeability of drugs. A thorough study compared liposomal, micellar, and microemulsion (combinations of detergents with organic solvents) electrophoresis buffers for CE. The method was used for the estimation of membrane permeability of drugs (22 different drugs and 5 neutral alkylphenones) and found best correlation of liposome-based CE methods with independent measurements [47]. Information regarding cell membrane permeability characteristics is pivotal for estimating bioavailability in drug development projects. Such projects that often involve a high number of drug candidates should potentially benefit greatly from early and fast screening methods such as CE.

A composite method for chiral separations in CE involving phospholipid lysozyme stably immobilized on the capillary wall through a derivative of quaternarized piperazine called M1C4 [48] successfully separated D- and L-tryptophan, and may be useful as a general approach for chiral separations based on phospholipid-protein wall coatings [49].

Finally, a simple approach to evaluate the first step in the efficacy of liposomes for oligonucleotide delivery is to measure the binding of fluoresceinylated oligonucleotide to specific liposome preparations by ACE. This was shown in a study of two commercially available cationic liposome formulations that were used as electrophoresis buffer additives [50]. The method was complicated by the fact that the cationic liposomes above certain concentrations reversed the EOF. However, based on the migration time shifts of the labeled oligonucleotide as a function of liposome concentration binding constant estimates could be obtained.

9 RNA, DNA, and aptamers

Molecular biology relies routinely on CE for sequencing of DNA, but also the number of binding studies involving DNA or RNA as analytes is steadily growing. Emerging subfields are the characterization of small molecular ion–DNA interactions [51], and the characterization of drug–DNA/RNA interactions. M-DNA (DNA complexes with divalent metal ions) was characterized by CE in an attempt to understand the poor performance of CE when samples containing DNA and metal ions are analyzed [52]. The chiral characteristics of transition metal complexes were shown to be analyzable by CE, because the enantiomeric affinities toward calf thymus DNA is differential [53]. CE was also used to measure the 10^4–10^5/M binding constants of Fe^{2+} and Fe^{3+}-ions interacting with duplex DNA [54]. This was performed in conjunction with Fourier transform infrared difference spectroscopy to evaluate conformational changes. In another study by the same group, DNA interactions with polyamines and cobalt(III)hexamine were characterized. Information on the mechanisms of polyamine-induced DNA condensa-

tion and duplex stabilization *in vivo* including different degree of protection afforded by different types of polyamines was obtained [55].

In the use of DNA for diagnostic purposes the introduction of affinity interactions may also be advantageous. One example is the increased discrimination of single nucleotide polymorphisms afforded by the use of an oligo-DNA-polyacrylamide pseudo-immobilized affinity ligand [56]. In this study, the method was used to separate point mutated from normal CYP2C9 DNA. SNP analysis is one area where microarray analyses may encounter problems because single-base mismatches do not typically prevent dsDNA formation. This creates a need for CE-based, discriminatory analyses, and the study utilized the ability of the weakly interacting polyacrylamide-based affinity matrix to achieve differential migration without binding any of the target DNA too strongly. In addition, it was demonstrated that suitable immobilized ligand sequence designs may be inferred from analysis of melting temperature values, and by appropriate choices of oligonucleotide length, Mg^{2+}-concentration, and operating temperature.

DNA–protein interactions also benefit from being characterized by CE. Upstream protein binding in the dopamine D4 receptor gene could be demonstrated by ACE [57]. The thermodynamics of DNA–protein interactions may also be studied in detail as described in recent work using *Taq* polymerase-aptamer and SSB protein from *E. coli* binding to a 20-base ssDNA as model systems [58]. Both rate constants and entropy and enthalpy parameters could be derived from a temperature-controlled analysis series.

In the field of DNA and RNA binding of drugs, CE has been useful together with spectroscopic and modeling methods for quantitating binding strengths and delineating binding sites such as in a study of the binding of taxol to DNA [59], and of the binding of azidothymidine (AZT) to DNA and RNA [60, 61]. Also, CE has been used as an adjunct technique for assessing binding in the development of new chemicals targeted at human immunodeficiency virus-1 (HIV-1). Thus, a drug development scheme for the inhibition of transcriptional activation caused by the Tat protein binding to the *trans*-activating response region (TAR) of HIV-1 mRNA was based on CE [62–64]. While the actual development of drugs based on studies like these may be a distant goal, it is clear that CE-based methods are often very suitable for screening purposes in situations where well-defined binding systems exist.

The field of aptamers, DNA or RNA based *in vitro* selected ligands from combinatorial libraries, is also an important and interesting area of development for ACE and for preparative ACE. Thus, several studies have proved the feasibility of using CE for selecting and isolating high-affinity aptamers for specific targets such as immunoglobulin E (IgE), neuropeptide Y [65, 66], and protein farnesyltransferase [67]. These publications utilize CE as both a preparative and an analytical method. The utility of even low-affinity aptamers for protein detection, in this case thrombin, has also been demonstrated [68], while high-affinity aptamer-thrombin complexation was studied in detail by others [69].

10 Drugs, protein–drug binding, enantiomers, and small molecules

CE-FA has long been a method of choice when analyzing drug–protein interactions [70]. Much literature focuses on analyses of HSA that acts as a molecular sponge for various drugs administered in humans [71–75]. CE-FA is used to determine protein binding and thus the bioavailability of possible drug candidates. Martinez-Pla *et al.* [71] have modified the traditional CE-FA set up to decrease the relatively long analysis times. They analyzed the interaction of 13 anionic, neutral, and cationic drugs with HSA at near physiological conditions. Short-end CE-FA was employed resulting in short injection plugs (15 s), and this was compared to CE-FA with normal long injection plugs (25–60 s). Five times shorter analysis time and overall similar results to traditional CE-FA were achieved.

Traditionally, UV absorption is the detection principle even though other detectors, *e.g.*, based on electrochemiluminescence [75] have been employed. In drug development, FA may require drug concentrations of 10 μM or lower. This approaches the limit of the normal UV detectors. Wan *et al.* [73] instead utilized MS for detection in drug-HSA/plasma interaction measurements. Where normal CE-FA methods require repetitive analyses of drug standards in order to obtain a calibration curve, the authors combined the standard run with the drug–protein analysis in a single run by first injecting a standard drug immediately followed by the drug–protein mixture. The mass spectrometer gave better sensitivity for protein binding at low drug concentrations, but required volatile buffers in order not to be affected by ionic suppression from usual CE running buffers. Thus, the interfacing CE with MS is still not routine. The authors assumed a one-to-one model with first-order dissociation for quantifying the obtained binding data. They found good agreement with plasma binding measured by UV and MS regardless of drug concentration and buffer type, although the reproducibility of the UV traces were more consistent due to smoother peak plateaus. MS as a sen-

sitive detector seems promising in CE-FA analyses although problems with time-dependent losses of low- and medium-binding drugs at low concentrations in the system also need to be addressed.

Another example of the analysis of drug interaction is the use of ACE for determining the binding behavior of platinum-based anticancer drugs toward HSA [76]. Timerbaev *et al.* [77] utilized a protein-independent metal-specific detector, an inductively coupled plasma-MS (ICP-MS), that allowed measuring the binding kinetics and stoichiometry of cisplatin and analogs to HSA and led the authors to propose a two-step mechanism of binding.

Chiral separation has always been a speciality area for low-affinity ACE and is characterized by a multitude of different chiral additives that may be used to effect separation and thereby quantitation of enantiomeric species. A number of examples are included in Table 1, and recent exhaustive reviews on this subfield including new insights into the separation mechanisms may also be consulted [78–80]. In Section 12 some new developments in chiral affinity separation theory are reviewed. Regarding applications, an interesting new contribution deals with titrating concentrations of highly charged chiral selectors (sulfated CDs) to a point where two drug enantiomers migrate in opposite directions without the requirement of counter pressure [81]. This, in principle, provides for infinite resolution capabilities and makes the use of ultrashort partial-filling approaches (zone lengths in the order of 5 mm in a 64.5 cm long capillary), and thus CE-MS methods, feasible. In another study, good resolution and impressive detection limit enhancements were achieved by using chiral temperature gradient focusing. Here, temperature-dependent ionic strength buffers were employed to move analytes to zero-velocity points in the separation path exposed to the temperature gradient. By this procedure, analytes were separated and concentrated efficiently. When combined with chiral selectors it was possible to temperature-focus highly concentrated enantiomeric species at different positions in very short separation channels (lengths on the order of mm) albeit at low peak capacity. The approach would seem very well suited for chip-based technologies [82].

An unusual application of enantioselective CE was a study of the binding of the racemic drug disopyramide to AGP for the purpose of separating genetic variants of this protein [83]. Separation conditions were worked out where the point-mutated AGP variants bound enantioselectively to the disopyramide enantiomers. Binding constants were estimated using FA, and it was envisioned that the approach will be useful for the functional analysis of genetic variants of AGP.

The influence of electrophoresis buffer selection for CD-mediated enantiomeric separations was studied in a report on hydrobenzoin and related compounds. It was found that lower concentrations of sulfated β-CD were necessary for effective enantioseparations at pH 9 in phosphate buffer than the concentrations needed in borate buffers [84]. This is presumably due to the well-known diol-complexing abilities of borate, *e.g.*, with carbohydrates. This has also been utilized in another study presenting enantiomer separations of pantothenic acid where a borate buffer in combination with (*S*)-3-amino-1,2-propanediol was found to be indispensable for achieving a chiral separation [85].

Chiral separations may also be of use for monitoring therapeutic drug concentrations of methadone (where the *R*-enantiomer dominates with respect to therapeutic effect) [86], and for monitoring new imidazole compounds that are under investigation for aromatase-inhibitor activities [87]. Another interesting study showed that it may be possible to decouple enantioselectivity from the effect of dimerization for given separation patterns. This study used the glycopeptide antibiotics vancomycin and balhimycin as chiral selectors in the separation of dansylated α-amino acids [88]. Finally, in the field of interactions between very small molecules, a study of the weak interplay (mM-range association constants) between tetraalkylammonium ions (used as electrophoresis buffer additive) and inorganic ions [89] underscore the broad applicability of ACE for binding studies based on its unique precision and reproducibility in mobility determinations.

11 Supramolecular assemblies

Particles, organelles, microorganisms, and eukaryotic cells may be analyzed by CE [90–93], and thus also interactions of such species can be examined electrophoretically. The main issue is the inhomogeneous nature of the analytes. No degree of purification, *e.g.*, of an organelle will result in a preparation as homogeneous as the molecular entities that are usually analyzed by CE. One exception may be virus particles that are very homogeneous. CE has been used successfully recently for studies of interactions between a domain (V3) of the very low-density lipoprotein receptor and human rhinovirus HRV2 [94]. The study revealed the stoichiometry of a V3-pentamer binding to virions by extrapolating migration shifts as a function of V3 concentration to saturation. This led to the proposal of a structural model for HRV2-receptor complexes. Working with the same virus, the same group also used CE to measure both the binding of fluorescent dye to viral RNA inside the intact virus and the fluorescent labeling of four capsid proteins [95, 96].

Formaldehyde-fixed eukaryotic cells were used as a stationary phase in an interesting study of endothelin antagonists. By coating the capillary with transfected Chinese hamster ovary cells overexpressing endothelin A-receptors, compound libraries could be easily screened for endothelin-like binding under physiological conditions as a first step in the search for new endothelin receptor antagonists. In this particular study, eight different peptide and nonpeptide antagonists were examined and ranked by the CE method in an order of affinity corresponding completely to the order determined by independent measurements [97]. The formaldehyde-fixed cells were simply immobilized by being washed through a poly-L-lysine-coated capillary with subsequent blocking of additional binding sites in the capillary by fetal calf serum. Binding strength was indirectly assessed by analyte migration times where longer times indicated higher affinity.

12 Theoretical innovation

An excellent review of the theory of CE-based binding experiments may be found in [98]. New work in this field deals with better and more efficient simulations, with the extraction of thermodynamic data, and with situations that do not conform to the standard assumptions in ACE. The latter include experiments involving more than one class of binding interactions, nonequilibrium conditions in dynamic ACE, ligand binding with no analyte mobility shifts, the presence of cooperative binding, and ligand added at a concentration that is not much higher than the analyte concentration. Simulation of ACE may use different methods for modeling the electrophoretic migration, and usually requires the application of a large number of differential equations. However, a recent study [99] showed that by assuming constant local field strength and by considering only the analyte and additive ions in the modeling, the computing time and memory requirements can be reduced significantly. A new algorithm reduced computing time by more than 90% and was able to simulate both equilibrium and nonequilibrium (preincubation) ACE. The method requires prior knowledge of binding and rate constants for simulations, but may be used to predict peak appearance times and peak shapes of real ACE experiments. In further work by the same group [100], simulation procedures were brought even closer to practical use. An enumeration algorithm that extracts combinations of binding constant and complex mobility from 3-D surfaces into 2-D plots under different experimental conditions was used to estimate accurate binding constants and complex mobilities from as few as two or three sets of ACE experiments. The approach had the added advantage that it works also for other than 1:1 binding stoichiometries.

A theoretical and practical study used mobility moment analysis to extract quantitative binding data from fluorescent antibody–antigen and fluorescent lectin-succinyl–glycoprotein interactions [101]. The idea is to replot traditional electropherogram data (signal *vs.* time) as signal *versus* mobility differences, *i.e.*, the plot depicts the data of a binding experiment relative to a reference experiment. The mobility moment (the average mobility of the detected analyte (reflecting the relative amounts of the free and complexed form of the analyte)) is plotted against antigen concentration and yields a binding constant value corresponding well with the outcome of plots of complex peak height against antigen concentration. This type of analysis cancels out contributions from differences in total amount of analyte from the analysis and also is not affected by fluorescence quenching or enhancement upon complexation. Additionally, it does not require assignment of peak species and this means, in cases where detection limits are critical, that large analyte plugs may be injected. The applicability of the approach for systems characterized by fast on and off rates, however, has not yet been experimentally verified.

Theory and practical approaches for intermediate kinetics interactions have been the topic of several recent important studies which, in addition, provide straightforward methods for the use of ACE as preparative methods for generation of affinity ligands with predestined affinity characteristics (*cf.* also Section 9) [58, 68, 102, 103]. The basic notion is that dissociating complexes from low-stability preequilibrated samples are observed as peak tailing between the complex peak and the peak representing free species. The tailing directly represents the unimolecular exponential decrease of dissociated complex, and the dissociation rate constant can therefore be calculated by analysis of the tailing curve. The approach has been named NECEEM [104]. The main prerequisite for this approach to work is that there is a quick and good separation between complexed and free species. In contrast to almost all other quantitative CE-based binding studies the method only requires a single experiment to yield dissociation rate and equilibrium dissociation binding constants using, *e.g.*, simple Excel programs for the computing. This makes it simple to carry out series of experiments as, *e.g.*, temperature experiments for studying the interplay between binding rates and temperature to extract thermodynamic parameters [58]. Also, the approach has been used as a chemical thermometer that noninvasively documented the temperature inside capillaries during CE in different instruments. It could be demonstrated that passive heat exchange (*e.g.*, air cooling) were less well suited for temperature-sensitive analyses than active heat-dissipation systems [105].

The theory of enantiomeric separations has also evolved during the past few years, and has been put to practical uses (see also Section 10). Thus, for binding constant estimates, a study using AGP and cellulase as chiral selectors and various drugs as analytes developed and showed a simple method for K_d estimates in partial-filling CE. This was based on migration time data, amount of protein loaded, and capillary radius. The study emphasized the relative importance of the absolute amounts of selector present in the system over plug length or selector mobility, and the authors recommended the use of low selector concentrations in long plugs [106]. It is theoretically possible to achieve an enantioseparation by CE even when the binding affinity of the selector for both enantiomers is equal. This may be possible if complex mobilities are different because of slight differences in ionization values, shape, size, lipophilicity, polarity, and solubility as summarized and tabulated recently [107]. Rational development and optimization of chiral separation conditions benefit from multivariate/multifactorial analyses as shown in several recent studies [108, 109]. This would seem to be true for the optimization of any ACE project.

13 Technical innovation

The limited sensitivity of detection and lack of analyte information offered by the traditional UV detection systems still constitute a major obstacle for many CE-based binding studies. Fluorescence detection improves detection limits, and connecting the CE instrument with novel systems providing more information-rich detection remains important for broadening the applicability of ACE.

Chowdhury *et al.* [110] used FA with LIF detection in a study of the apomyoglobin-complexing fluorophore Coumarin 153 to probe the overall solvation dynamics of the protein in polar solvents. The dissociation constants estimated by this method correlated well with other methods and confirmed a fairly strong binding of the fluorophore to apomyoglobin. CE with LIF detection was also exploited in a study of the interaction of Ca^{2+} with calcium-binding proteins in complex biological systems [111]. Here the authors studied the binding of Ca^{2+} to an *E. coli* expressed construct of green fluorescent protein (GFP) spliced to calmodulin, as well as the ensuing interaction with OsCBK, a high-affinity CaM-binding protein kinase. GFP was found to be a superior probe compared to chemical labels because it is stable and does not alter the Ca^{2+}-binding characteristics of CaM or the CaM–OsCBK complex. Also, it works well under physiological pH and ionic strength conditions.

An interesting approach for exploiting the higher sensitivity of fluorescence detection is fluorescence ansiotropy/polarization detection [112] which may also be used for quantitative analysis of noncovalent interactions [113, 114]. The detection principle is based on small fluorescent molecules with low anisotropy added to the electrophoresis buffer to probe for analyte binding. When bound in a complex with larger molecules, the probe molecules tumble less than when they rotate as unbound molecules, and this difference can be detected (as an anisotropy shift) by a polarized fluorescence detector. Thus, the method does not rely on mobility shifts for detection of binding. Whelan *et al*. [113] used the principle for studies of the binding of G protein isoforms using BODIPY FL GTPγS as the small fluorescent ligand probe. A high sensitivity at low probe concentrations (LOD 3 nM for $G_{\alpha i1}$) was achieved. In applying the approach a balance exists between choosing as low a probe concentration as possible (for achieving high sensitivity) and high probe concentrations that provide a higher dynamic range for detection of binding. In [114] fluorescence anisotropy CE and affinity-probe CE with LIF detection were compared for characterizing the rapid kinetics of the binding of the *Src*-homology 2 domain of the SH2-Bβ protein to a fluorescently labeled phosphorylated peptide (corresponding to the binding site of JAK2). Both approaches achieved relatively good LODs (100 nM (12 amol) and 300 nM (7.5 fmol)), respectively). The separation times were as short as 4 s which was suitable for the very rapid dissociation kinetics in the affinity probe CE experiments that were used to estimate the binding kinetics. Comparing the two methods, the resolution of multiple protein species seemed to be better in the fluorescence anisotropy CE experiments which, however, were used only for qualitative estimates of binding interactions in this study. The advances in contactless conductivity detection must also be considered an important technical development and have been described in Section 3.

Interfacing CE with information-rich detection systems is a continuing important issue for the applicability of ACE. Advances have recently been reviewed [115, 116]. The challenge of CE-MS remains the development of suitable interfaces that allow the separation buffer conditions of CE to be compatible with the ionization process of MS. Machour *et al.* [117] employed IT-MS as a parallel detector to UV in studying mobility shifts induced by the interaction of vancomycin derivatives with dipeptide substrates. It was concluded that CE-MS is a valuable tool by combining affinity separation with accurate mass determination, but it was found that binding constants could only be reliably calculated in this system based on the CE-UV data. The use of inductively coupled plasma MS as mentioned in Section 10 is a promising approach for

metal-specific detection in studies of metal-containing analytes. However, in general CE-MS is still technically challenging and only the invention of practical and simple interfacing technologies will release the significant potential of ACE-MS.

Miniaturization is also a continued focus for technological innovations within ACE (*cf.* also Section 3 on immunochemistry). The development of a fast analysis, *i.e.*, high-throughput screening together with binding data is attractive but still in the prototype phase. Stettler *et al.* [118] compared chip-based systems with UV and electrochemical detection with traditional CE-UV using the interaction between the neurotransmitters epinephrine and norepinephrine and CD in solution as a model application. The affinity microchip-CE system showed proof-of-principle ACE in qualitative terms, especially at lower CD concentrations, but was hampered by peak broadening. A number of immobilization strategies that originate from developments in affinity chromatography have been transferred to electrophoretic separation capillaries in preliminary applications. One example is Tsukagoshi *et al.* [119] who immobilized metal chelate affinity functional groups in silica capillaries. The inner wall was derivatized with polymerized *N*-(vinylbenzylimino)diacetic acid, and two modified surfaces were prepared: iminodiacetic acid (IDA) and Cu(II)-IDA. The modification did not eliminate the EOF. Immobilized metal ACE (IMACE) in the Cu^{2+}-capillary was applied to the analysis of purified HSA and gammaglobulin that were found to interact differentially with the fixed metal groups on the wall. Since these two analytes display very different p*I*s and thus can easily be separated, future work using more similar analytes, *e.*g., protein isoforms, is anticipated. More novel developments within the fields of immobilized affinity groups and new coatings are expected to originate from the interplay between the areas of LC, CEC, and CE.

14 Conclusions and future prospects

While the applicability of ACE is still challenged by technical limitations, it is now used quite broadly in analytical chemistry and the biological sciences as is apparent from above. The limitations chiefly concern analyte recovery, peak identification, limits in parallel processing, and in understanding the interplay between binding rates and separation parameters. Perhaps the most limiting factor for using the technique outside specialist laboratories is the fact that ACE is not one, but a suite of different techniques united by a capillary electrophoretic separation step. Therefore, its particular embodiment in specific applications depends on the questions and interacting systems to be examined. This is amply illustrated by the numerous modes of ACE used in the publications cited in this review. Despite the obstacles to being a universal systems biology tool, affinity interactions and CE will be increasingly integrated in many functional biology studies. CE will also find continued use as an analytical and preparative tool during development of specific binding reagents including immunoreagents such as mAbs, *in vitro* affinity-maturated single-chain Fv-fragments, and phage displayed variable regions as well as aptamers and chemical libraries. Also the functional and structural assessment of drugs and novel drug candidates will entail a growing use of ACE. Additionally, the specific area of protein conformational studies, which is important for, *e.g.*, biological drug development and for the understanding of disease, will continue to benefit from CE which is complementary to NMR- and MS-based methods in this field. Finally, technical innovations will continue in the areas outlined above, and to the extent that these are simple and easily accessible, the usefulness of combining affinity interactions with CE analyses will be increasingly obvious.

15 References

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