Capillary electrophoresis and small molecule drug discovery: a perfect match?

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Capillary electrophoresis (CE) is an analytical technique based on the separation of the analytes within a capillary owing to their different electrophoretic mobilities. It is widely used in pharmaceutical analyses owing to its versatility and high separation power. However, its penetration into the drug discovery scene has been relatively limited until recent years. Several factors have contributed to this low implementation, including the maturity of liquid chromatography, the scarcity of experienced CE practitioners, and certain limitations intrinsic to the technique. Recently, instrumental improvements and the growing demand for analytical information have lead to a continuously expanding range of routine electrophoretic applications throughout pharmaceutical discovery and development. In this article we review CE fundamentals, review well-established CE methodologies in drug discovery of small molecules and discuss trends that, in our opinion, might emerge in the coming years.

Drug discovery is an iterative, multistage process that covers diverse scientific disciplines, including biology, chemistry, toxicology and pharmacology [1,2]. Each step of the process produces a large volume of samples that demands fast turnaround time of analytical data with the highest information content. In this context, separation science becomes increasingly important because various decisions, at every step that can be considered in the drug discovery process, are taken on the basis of results generated by one or several separation techniques [3]. High-performance liquid chromatography (HPLC), together with advanced data-handling software, has become the walk-up tool for drug discovery compound identification, quantitation and purification, addressing needs for high throughput screening, structure–activity relationship development, and physicochemical property profiling [4,5]. Recent informatics tools advances are making the enhancement of the value of information derived from HPLC assays for lead compound series characterization possible. However, accessing complementary data from orthogonal separation techniques is of paramount interest for researchers supporting an increased demand for qualitative and quantitative data [6]. In the past few years, capillary electrophoresis (CE) has emerged as a useful orthogonal separation technique to HPLC in the drug discovery scene. The tremendous versatility, simplicity of use and cost-effectiveness of CE enables straightforward access to complementary information [7]. The background electrolyte (BGE) composition can be flexibly adjusted to match the analytical requirements and increase the chances of solving the particular problem. The high separation efficiency characteristic of electro-driven separations ensures a high enough peak number in the electropherogram to resolve extremely complex mixtures [8]. The relatively low number of experienced CE practitioners and the maturity of HPLC in the pharmaceutical industry certainly have had a significant impact on the limited utilization of CE in early drug discovery. However, the latest technological advances in the field of electro-driven separations in terms of performance, throughput and automation, lead to CE now becoming an affordable option in newer areas of drug discovery, including proteomics, metabolomics and biomarker technologies [8–11]. In this article, we present an overview of the diverse roles of CE in meeting the fundamental analytical requirements involved in early drug discovery of low-molecular weight drugs, with a special focus on strategies already applied at Lilly Research Laboratories (http://www.lilly.com/research/Pages/research.aspx) and other academic and industrial research groups (Fig. 1). We also discuss current trends of CE in drug discovery beyond pure separation, such as methodological advances in sample preparation and injection,

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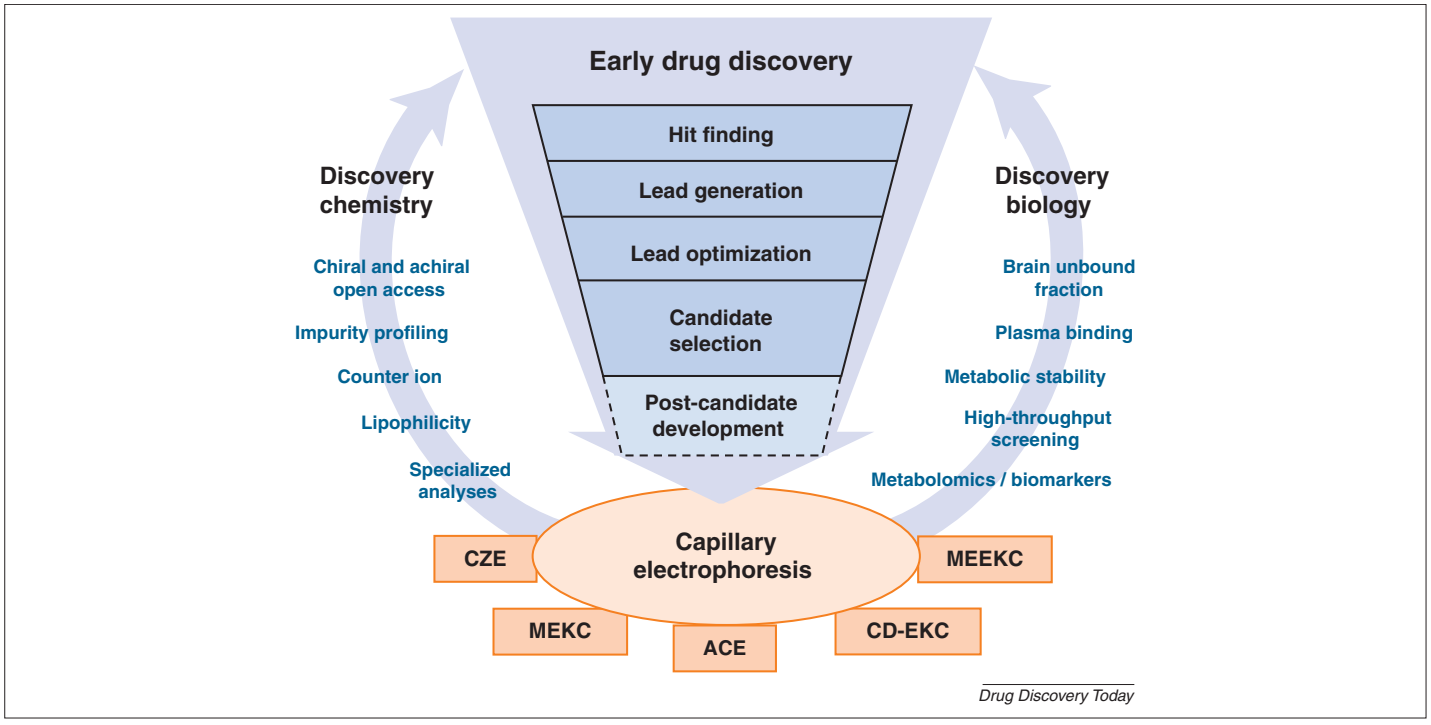

FIGURE 1

Capillary electrophoresis in early drug discovery. *Abbreviations:* ACE: affinity capillary electrophoresis; CZE: capillary zone electrophoresis; CD-EKC: cyclodextrin-mediated electrokinetic chromatography; MEEKC: microemulsion electrokinetic chromatography; MEKC: micellar electrokinetic chromatography.

instrumental improvements in the detection step, and multidisciplinary aspects of CE application in medicinal chemistry and bioanalysis.

CE fundamentals

Principles

CE is a powerful analytical technique with a unique separation mechanism, speed, efficiency and versatility. CE separation takes place in narrow-bore capillaries (10–75 μm internal diameter) filled with BGE and subjected to high electric fields (typically 5–30 kV). Analytes are separated on the basis of their differential mobilities in the running buffer, which can be a true or micellar solution or a microdispersed microemulsion. Accordingly, driving forces in CE are electrophoretic migration, the electroosmotic flow (EOF) and, in some modalities, the distribution of the analytes into pseudostationary phases (PSPs) [11,12]. The essential instrumentation of CE is simpler than in other techniques, consisting only of electrodes, a capillary, a power supply, a sample introduction system and the detector. The detection can be on-line [ultraviolet/diode-array (UV/DAD), spectrophotometric, spectrofluorimetric and electrochemical detectors] or off-line [mass spectrometer (MS)].

Modes of separation

The simplest CE mode is capillary zone electrophoresis (CZE), where a true solution fills the capillary and the analytes are separated based on their charge-to-mass ratios. Non aqueous capillary electrophoresis (NACE) is a complementary methodology to CZE and employs organic solvents instead of water in the BGE. This enhances hydrophobic analyte solubility and enables method selectivity to be tuned. CZE and NACE are well suited to the separation of charged analytes, but cannot be applied to uncharged molecules. Several electrokinetic chromatography modalities arise when one or more PSPs are added to the BGE (*i.e.* micelles, cyclodextrins and microemulsion). The chromatographic partition of analytes between the PSPs and the background electrolyte adds to the intrinsic electrophoretic migration of the charged free species, resulting in more general modalities. Micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) are general approaches that can separate neutral and charged species. The inherently chiral nature of cyclodextrins makes cyclodextrin-mediated electrokinetic chromatography (CD-EKC) extremely useful for chiral separations. Capillary electrochromatography (CEC) is another general modality that can be considered as a hybrid between CE and HPLC and is based on the employment of capillaries filled with stationary phases (retained with frits or covalently linked to the capillary). Affinity capillary electrophoresis (ACE) comprise several modalities that use the mobility shifts or peak areas of the injected analytes in running buffers containing different concentrations of the interacting molecules to determine their binding constant. Capillary isotachophoresis (CITP) is based on the presence of moving boundaries with different conductivities in a discontinuous buffering system and can be used not only as a separation method but also as a transient preconcentration step in certain injection modalities.

Other CE techniques have been developed and widely used to take advantage of traditional biomolecule methodologies in a capillary format. Among them, capillary isoelectric focusing (CIEF), based on the separation of molecules with different isoelectric points, and capillary gel electrophoresis (CGE), which resolves the analytes depending on their size and/or molecular

weight ratios, are well accepted for analysis of peptides, proteins, nucleic acids and antibodies.

Applications

Drug discovery chemistry

The potential of CE for pharmaceutical analysis has been successfully exploited since the 1990s [12,13]. Many of these early applications were dedicated to the analysis of well-characterized mixtures, most of them occurring at later stages of the drug discovery and development process. Analytical requirements in early drug discovery are significantly different, because most samples are generated from medicinal chemistry or parallel synthesis paths and the available chemical and/or analytical information is scarce. Furthermore, the lead or candidate is often present in a complex, crude reaction mixture containing multiple reagents in excess, unknown byproducts and catalysts. Consistently, there is a need for robust general methods to cover this wide chemical diversity. A second important scenario for CE corresponds to the interface between early drug discovery and drug development, where there are less synthesized molecules, the level of required characterization becomes more demanding and more specific analytical tasks need to be solved (Fig. 1).

Analysis of metals and metal complexes

The analysis of metal and metal complexes containing pharmaceuticals by CE has recently received much interest owing to their prominent role in the treatment of diseases, such as anemia, arthritis, and in the chemotherapy of cancer. Foteeva and Timerbaev reviewed different CZE and MEKC approaches for analysis of charged metals and neutral complexes [14]. CZE-inductively coupled plasma (ICP)-MS and MEEKC-ICP-MS were used, respectively, to analyze therapeutic gallium nitrate formulations and to determine the anticancer drug candidate Tris(8-oxyquinolinato)-gallium(III) [15] and anticancer platinum complexes [16].

General analysis and physicochemical profiling

Lynen *et al*. recently published the evaluation of a multiplexed CE system for high-throughput pharmaceutical analysis in drug discovery by MEEKC [17]. Parallel CE has also been used for the MEEKC determination of log *P*, a key physicochemical property strongly correlated with the permeability of the compound, in high-throughput format [18]. More recently, Henchoz *et al*. focused on the utility of CZE for p*K*a determination and MEEKC for lipophilicity assessment in the context of physicochemical profiling of drug candidates to predict absorption and/or distribution [19,20].

Characterization of non-covalent intermolecular associations

Numerous ACE applications have been reported for the study of non-covalent intermolecular associations. Protein–drug, peptide–peptide, carbohydrate–drug and antigen–antibody binding are a few examples [21,22]. Preincubation ACE strategies were reviewed by Ostergaard and Heegaard, who discussed the different modalities and their potential and limitations [23]. Vuignier *et al*. reviewed the applicability of frontal analysis and zone elution CE to determine drug–protein binding, evaluating their advantages and limitations (the risk of protein adsorption on the capillary walls and the low detection limits of commonly used UV detectors) [24]. In addition to the conventional separation applications, CE possesses huge potential to perform functions far beyond these, as discussed by Nesbitt *et al*. [25]. Among these functions, low volume sample preparation and enrichment (known in general as stacking techniques [26]) and electrophoretically mediated reactions are particularly relevant. A recent example illustrates a protocol to screen L-glutamic dehydrogenase inhibitors with a gold nanoparticle-mediated enzyme bioreactor by capillary electrophoresis [27].

Achieving lower detection levels with alternative on-line detection techniques

Improvements in the detection step have been reported to reach lower detection limits. CE with electrochemical detection was used to determine 0.3 fmol of dopamine in optic lobes of drosophila [28] and 24 dopamine derivatives and biogenic amines [29] in drosophila brains. CE-laser induced fluorescence (LIF) enabled determination of epinephrine in human urine with a limit of detection (LOD) of 0.25 nM [30], and several anthracyclines (antibiotics that are regularly used in the treatment of cancers) with LODs of 10 ng/mL [31]. Aspartic and glutamic acid enantiomers [major excitatory neurotransmitters in mammalian central nervous systems (CNS)], were quantified by chiral CE-LIF with LODs below 0.04 ng/mL [32].

Exploiting the full potential of CE-MS

Advances in CE-MS hyphenated techniques are paving the way to the application of various EKC-MS modalities in drug discovery [33]. Two main trends have emerged to solve the main problem in EKC-MS hyphenation (low compatibility between conventional PSPs and MS): selection of alternative PSPs or employment of ionization modes more compatible with standard PSPs, such as atmospheric pressure photoionization (APPI) or inductively coupled plasma (ICP) MS. An example of the former was reported by Wan *et al*., who demonstrated the applicability of MEEKC-MS partitioning as a descriptor for biopartitioning of CNS drugs in brain tissue [34]. Recently introduced non-conventional PSPs include chiral polymeric surfactants [35], surfactant-coated single walled carbon nanotubes [36] and latex nanoparticles [37], suggesting that tailored PSPs might become prevalent in this research area in the coming years. All of them are stated to be highly compatible with MS detection using electrospray ionization (ESI) interfaces (widely applied in the industry), so we anticipate increased use of these novel PSPs in the pharmaceutical arena as they become more available to the general analytical community. Regarding APPI, in 2005 Mol *et al*. suggested the potential of this ionization technique for MEKC [38] and demonstrated the feasibility and usefulness of on-line MEKC-APPI-MS [39]. Soon after that, other authors also reported MEKC-APPI-MS for impurity profiling [40] and MEEKC-APPI-MS for drug analysis [41]. Concentrations up to 3% SDS can be used for MEEKC without significant ion suppression of the MS signals. LODs between 0.5 μg/mL and 5 μg/mL were achieved, demonstrating its technical feasibility adequate instruments. More recently, MEEKC-ICP-MS analytical features were shown to be comparable to MEEKC-UV in terms of sensitivity (with a lower resolution) and with the added value of its specificity [16], initiating another potential research field for successful EKC-MS hyphenation.

Discovery bioanalytical chemistry and in vitro *biology*

With the recent advances in genomics, combinatorial science and automation, scientists working in discovery bioanalytical

chemistry and *in vitro* biology are faced with the overwhelming task of developing, validating and running many new assays. These assays must be robust, rapid, sensitive and precise. In addition, there is a growing demand within analytical laboratories for more streamlined sample preparation techniques to reduced timeframes and increased overall throughput. CE is well suited as a micro-separation method for the identification and quantification of a great variety of substances from metabolic pathways, and thus it is a technique of choice for addressing questions related to neurobiology, oncology and metabolic disorders. With an adequate sample pretreatment, the high efficiency of CE can permit the rapid separation of closely related compounds and the analysis of drugs in plasma, blood, urine and tissues with relatively little development time and low interference from the matrix.

Omics-based and biomarkers approaches

There are numerous CE applications in the bioanalytical scene; quantitative bioanalysis, metabolite identification, biomarker analysis and metabolomics are just some of the common uses [42–45]. For example Jiang *et al.* have developed a simple and fast high-performance CE-UV method for the separation and quantification of modified nucleosides in urine samples [46]. Based on its separation efficiency and high reproducibility, this CE-based method meets the criteria for clinical diagnosis. The authors claimed the use of this approach for potential early cancer screening using nucleosides as biomarkers. Another successful case in the area of metabolomics is the development of a CE-MS/MS-based methodology to identify and characterize metabolites in single cells and subcellular structures. Lapainis *et al.* took advantage of the sensitivity of the ESI time of flight (TOF)-MS and ESI quadrupole time of flight (QqTOF-MS) systems in combination with CE to enable the detection of a large number of metabolites and the generation of high quality mass spectra from single-cell samples (>100 compounds yielded signals over 10^4 counts from the injection of only 0.1% of the total content from a single metacerebral cell). An additional advantage of such an assay is the low detection limits (key to success for managing sample preparation from femtoliter to nanoliter cell volumes), which are in the low nanomolar range for several cell-to-cell signaling molecules (*e.g.* acetylcholine, histamine, dopamine and serotonin) [47].

High-throughput screening and lab chip

Another potential application of CE in drug discovery is in *in vitro* biology, such as in HTS methods. The primary goal of the HTS stage is to identify chemical hits, against a therapeutic target of interest, from which lead candidates can be derived. Traditionally HTS-based methods rely on radiolabeling or fluorescent reagents and coupling assays with optical detection using multi-well microtiter plates and robotic liquid and/or sample handling enabling quantitation of enzymatic target inhibition. A limitation of optical detection is that typically fluorescence or radioisotope labels must be incorporated into the assay to generate detectable signal. This sometimes causes detrimental effects on the biological reaction because labeled substrates might not behave identically to native substrates, and there is significant risk of inaccurate results if test compounds affect the label or coupling reactions. High-throughput assays that can be performed without labels and coupling reactions are in need. Although HPLC/MS is a powerful label-free detection system for screening applications, CE-based methods have emerged as promising means of achieving high quality primary (hit finding) or secondary (confirmation post screen, determination of IC_{50} values [48], assessment of conformational stability) screening with suitable sample throughput. Thus, CE on microfabricated structures has achieved impressive throughput in enzyme assays by combining fast separation speed and parallel operations [49], and dynamic ligand exchange-ACE has been introduced as a convenient platform to determine the relative affinity of a holoprotein to various ligands integrating ligand exchange and protein unfolding in the capillary during electromigration [50]. Pei *et al.* have reported an electrophoretic microfabricated channel array for characterization and screening of enzymes using RGS-G protein interactions with fluorescence detection [51]. A 16-channel design demonstrated the potential of this technique to rapidly determine kinetics, enzyme concentration, and optimal enzymatic reaction conditions, by enabling serial electrophoretic assays from 16 different enzyme reaction mixtures at 20 s intervals in parallel. A second design, a chip with 36 channels, enabled performance of 36 electrophoretic assays in 30 s suggesting a potential throughput of up to 4320 assays/h with appropriate sample handling procedures. Another approach that is gaining widespread attention with screening groups throughout the pharmaceutical industry is a lab chip technology called microfluidic electrophoretic mobility shift assay (MEMSA) [52]. The principle of this technique is to measure the changes in mobility of molecules under electrophoretic conditions in a time-resolved fashion. Screening campaigns using MEMSA can be performed in two different modes, on-chip and off-chip. In on-chip mode, enzyme and substrate are sipped from reservoirs on the chip, whereas compounds are sipped from 384-well plates, and the reaction takes place in a nanochannel on the chip in 60 s, followed by an electrophoresis separation step and detection. In off-chip mode, the reaction occurs in the wells of the microplate, and after reaction termination a sample is sipped on the chip, that is used only for separation and detection. Perrin *et al.* described the development of a MEMSA for a kinase screen based on the electrophoretic separation of fluorescent products and substrates using a Caliper-based nanofluidic environment in on-chip incubation mode [53]. This technology permitted screening of a set of 32,000 compounds with a throughput of up to 12 × 384-well plates in 10 hours with excellent data quality. The flexibility of this technology enabled its application to a variety of targets, including protein kinases, protein phosphatases, histone deacetylases, sirtuins, among others [54–56]. The above examples confirmed that screening based on electrophoretic separation is an alternative that is not susceptible to the limitations imposed by radiolabel and coupling assays.

Applications at Lilly

CE in an open access environment

A crucial factor in drug discovery is the turnaround time. At Lilly, we have deployed two orthogonal methodologies in an open access environment. The general applicability of MEEKC in achiral analyses [57,58] and the potential of electrokinetic chromatography (EKC) with highly sulfated cyclodextrins (HS-CDs) for chiral analyses [59] were used to reduce the lead time (from sample submission to a user receiving her and/or his result) to a minimum. Buffer replenishment, vial interchanging and short-end injections were used to control buffer depletion, a phenomenon unique to

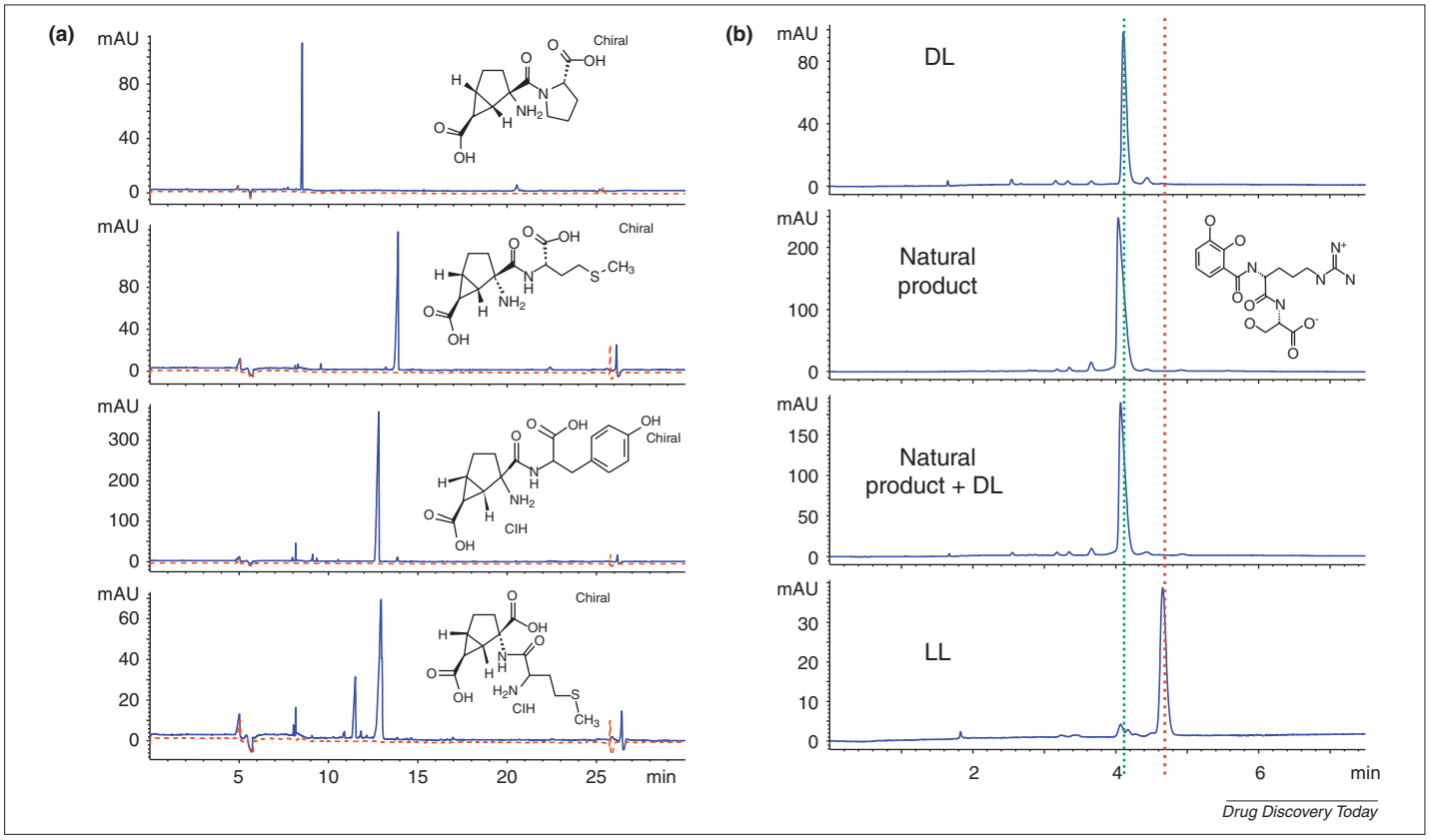

FIGURE 2

Analysis by MEEKC (left) and chiral EKC (right). **(a)** Representative electropherograms for purity assessment by achiral MEEKC of several Lilly proprietary polycarboxylic acids (blue) and overlayed blank injections (red). Capillary dimensions: 50 μm i.d., 363 μm o.d. fused-silica capillary, Ld: 50 cm, Lt: 58.5 cm, 200 mbar s injection, +20°C, +20 kV. Running buffer: 115 mM sodium dodecyl sulfate, 50 mM octane (0.81%, v/v), and 722 mM butanol (6.61%, v/v) in 40 mM sodium borate, adjusted to apparent pH 9.2 with 5 M NaOH. **(b)** Confirmation of the stereochemistry of a natural product with synthetic analogues by chiral EKC with negatively charged cyclodextrins. Capillary dimensions: 50 μm i.d., 363 μm o.d. fused-silica capillary, Ld: 8.5 cm, Lt: 38.5 cm, 60 mbar s injection (short-end injections), 20°C, +15 kV. Running buffer: 50 mM ammonium phosphate, pH 2.5:20% γ-highly sulfated cyclodextrin: milli-Q water (w:w:w 2:1:1). Experiments were run on an Agilent Technologies (http://www.home.agilent.com/agilent/home.jspx?cc=NL&lc=dut) ^{3D}CE equipped with a deuterium lamp and a photodiode array detector. *Abbreviations:* EKC: electrokinetic chromatography; DL: Dextrogyre Levogyre; MEEKC: microemulsion electrokinetic chromatography.

CE that can dramatically deteriorate its performance and therefore must be carefully controlled [60]. The MEEKC methodology employs the achiral surfactant sodium dodecyl sulfate (SDS), and is in general analogous for neutral compounds to reversed phase (RP)-HPLC methods on C18 columns [58]. As illustrated in Fig. 2a, MEEKC was also successfully applied to analyze polar compounds (low molecular weight polycarboxylic dipeptides [61]) thanks to the pure migration of the charged compounds and their distribution in the PSP, outperforming C18 RP-HPLC in this case. This technique empowers the medicinal chemist to monitor reaction progress and check final purity of these samples, significantly decreasing the lead time. In chiral EKC, one or more chiral selectors are typically added to the running buffer. In the platform we applied, three HS-CDs to increase the chances of obtaining an acceptable resolution for a wide diversity of chiral chemical entities [59]. Even without exhaustive method development (a constant concentration of selector and a single set of operating conditions have been used), this approach has been useful for selection of the optimum combination of solvent and counter ion in enantioselective crystallization and for the determination of enantiomeric excess in enantioselective synthetic paths. Another example involving the identification of one naturally occurring chiral compound *versus* its enantiomeric form is shown in Fig. 2b. The comparison of its migration time with the two existing synthetic analogous species enabled assignment of its stereochemistry [62]. In all these cases, sample treatment is extremely simple (dissolution of the samples in 1:1 acetonitrile: 50 mM triethylammonium phosphate buffer (pH 2.5)), without requiring conversion of the compounds to their respective free bases (as needed with conventional chiral HPLC). Analysis times of less than 10 min can be achieved. The fact that the pHs of the running buffers for MEEKC and chiral EKC can be different (pH 7.4, 9.2 or 10 in MEEKC *vs.* pH 2.5 in EKC), poses no problem because both modalities are run in the same bare silica capillary, and its equilibration is achieved automatically by setting blank injections or using programmed preconditioning steps. The platform developed for purity assessment by MEEKC-UV was also used for log *P* estimation. A calibration curve was built from standards with known log *P* values, including dimethylsulfoxide and n-dodecylbenzene as electroosmotic flow and microemulsion markers. The running buffer composition was 25 mM boric acid pH 10:butanol:octane:-sodium dodecyl sulfate (w:w:w:w 89:8:0.6:2.37), so that most basic analytes were analyzed as free bases. The capillary dimensions were 50 μm i.d., 363 μm o.d., Ld: 40 cm, Lt: 48.5 cm and the injection was 60 mbar. The method was applicable to compounds with log *P* ranging from 0 to 4.5, covering the range of interest in early drug

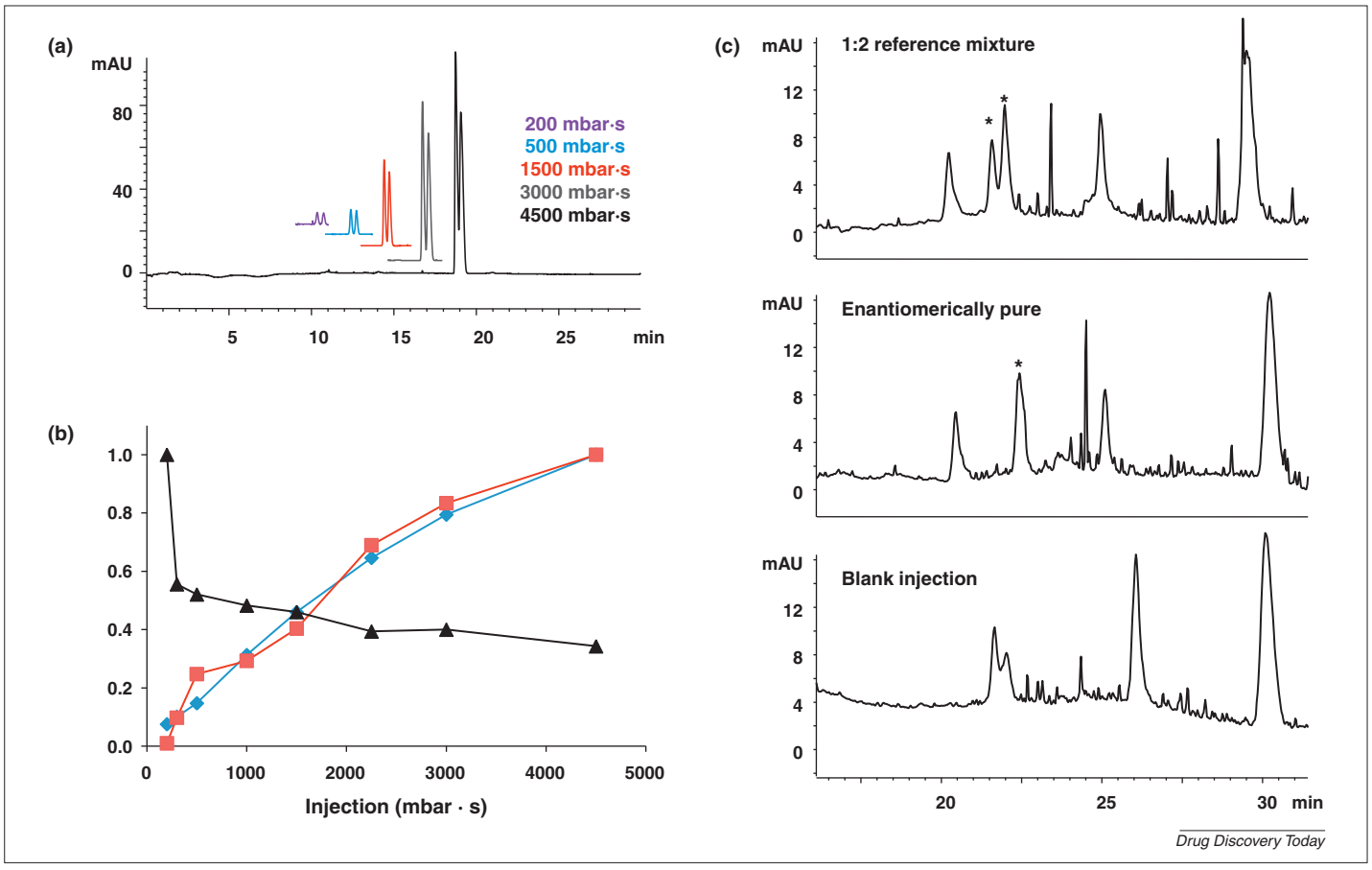

FIGURE 3

Use of sample stacking in chiral electrokinetic chromatography with highly sulphated cyclodextrins to access biologically relevant concentrations. **(a)** Overlayed electropherograms of 50 μg/mL solution of a racemic Lilly research mixture prepared in milli-Q water (the electropherograms have been shifted for clarity reasons). **(b)** Normalized optimization functions used in the stacking studies (milli-Q water): (◆) peak height, (■) S/N, (▲) resolution. **(c)** Electropherograms from rat plasma samples spiked with 5 μg/mL of a 1:2 reference mixture of a Lilly research compound and its epimer (upper), enantiomerically pure research compound at 5 μg/mL incubated with the plasma for one hour at room *T* (middle), and a blank rat plasma injection (lower) (5000 mbar s). Compounds of interest are denoted with asterisks. Capillary dimensions: 50 μm i.d., 363 μm o.d. fused-silica capillary, Ld: 30 cm, Lt: 38.5 cm, 20°C, −15 kV. Running buffer: 50 mM ammonium phosphate, pH 2.5:20% γ-highly sulfated cyclodextrin:milli-Q water (w:w:w 2:1:1). Experiments were run on an Agilent Technologies ^{3D}CE equipped with a deuterium lamp and a photodiode array detector.

discovery. At this stage, the compounds need to undergo structural optimization and therefore chemical series with lower log *P* values are needed to avoid the well-known issues of highly apolar candidates.

Specialized analyses

As mentioned previously, CE is particularly suited for charged species. A typical application is the quantitation of counter ions, employed to prepare the compound of interest (originally a free base) as a salt, to confirm its stoichiometry. The availability of commercial kits for rapid set up of the methods, the fact that robust results are easily obtained, and the inexpensiveness of the components required make this option a cost-effective approach for metal and organic ion determinations. Typically a charged absorbing species is added to the running buffer to have a background ultraviolet signal. The ions present in the sample separate along the capillary provoking a local decrease in the concentration of the charged additive (the analyte ions and the additive must have like charge) that results in negative peaks in the UV detector. CE-ESI-MS has also been successfully used in Lilly, operating the MS detectors at low m/z to determine small metal and inorganic ions. For quantitation purposes, calibration plots with reference compounds were chosen to quantify the ions. We employed ACE in the determination of the binding constants of drugs to sulfated cyclodextrins (Captisol®, among others), which are used in formulation groups to solubilize and administer drugs for *in vivo* studies (M. Molina-Martin, *et al.*, unpublished results). The feasibility of this approach has been demonstrated in early drug development at two independent laboratories, and the results are consistent with the literature values [63].

Sample stacking is a popular CE alternative in bioanalysis to increase sensitivity and selectivity owing to its flexibility and easiness of use [26]. The presence of a vast diversity of potential interfering substances in biological matrices, such as urine, plasma or cerebrospinal fluid presents a challenge with standard (not electrodriven) preconcentration steps. The unique separation mechanism of CE enables the optimization of the injection protocol in combination with the sample treatment and the analyte properties to overcome this difficulty. Figure 3 shows an application developed to evaluate the stability of a chiral carboxylic acid in rat plasma samples by chiral EKC with HS-CDs. Deionized water was used as low conductivity matrix model, achieving up to 80-fold improvement in sensitivity with

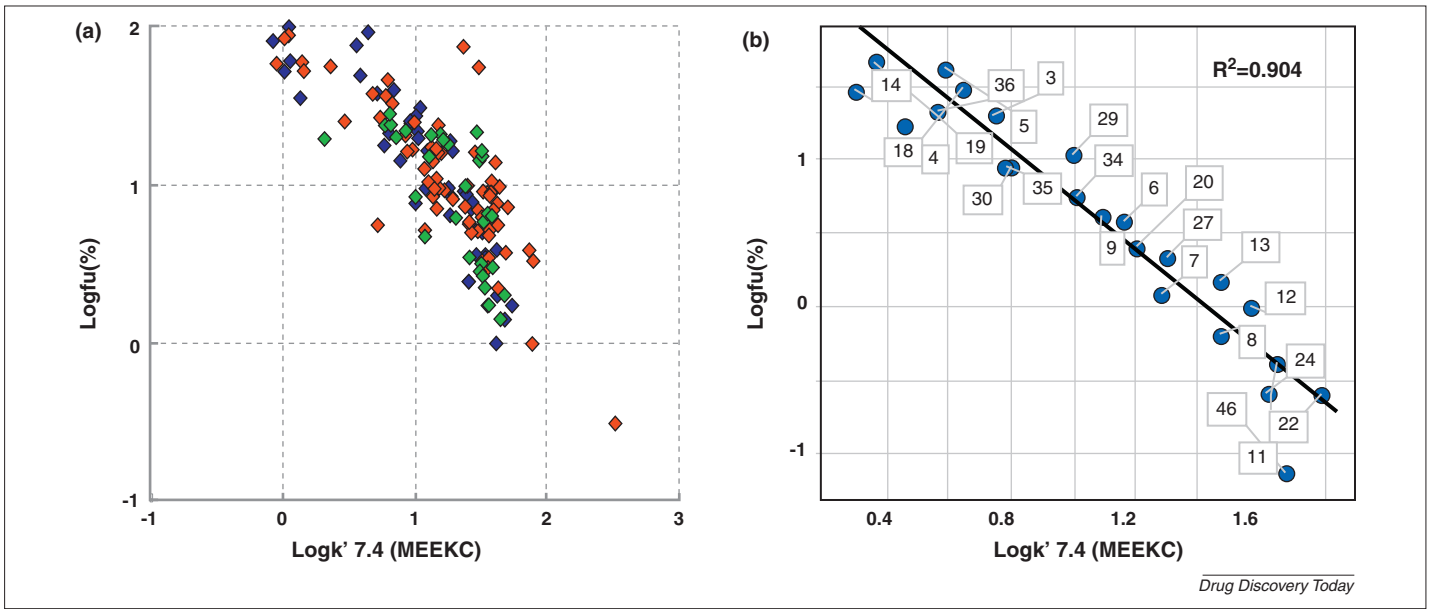

FIGURE 4

Correlation between brain unbound fraction (determined by conventional microdialysis technique) and MEEKC. Apparent distribution factors include: **(a)** analytes: two validation sets from Lilly collection (in blue and red, containing 45 and 70 compounds, respectively) and diverse molecules from neuroscience projects (in green, 31 compounds) (R50.808 for the 146 compounds). MEEKC running buffer: 82 mM sodium dodecyl sulfate, 37 mM octane (0.6%, v/v), and 874 mM butanol (8%, v/v) in 9 mM NaH_2PO_4, apparent pH 7.4. Capillary dimensions: 50 μm i.d., 363 μm o.d. fused-silica capillary, Ld: 8.5 cm, Lt: 38.5 cm, 60 mbar s injection, +10 mbar, +20 kV, $T = 20^{\circ}C$. Experiments were run on an Agilent Technologies ^{3D}CE equipped with a deuterium lamp and a photodiode array detector. (b) Reprinted and modified with permission from [34]. Copyright 2009, American Chemical Society. Analytes: 23 CNS drugs. MEEKC running buffer: 80 mM LA, 37 mM octane (0.6%, v/v), and 874 mM butanol (8%, v/v) in 85 mM methyl ammonium, apparent pH 7.4. Capillary dimensions: 50 μm i.d., Lt: 50–60 cm, 250 mbar s injection. +25 mbar (applied voltage not specified), $T = 25^{\circ}C$. Experiments were run on an Agilent Technologies ^{3D}CE ion trap. *Abbreviation:* MEEKC: microemulsion electrokinetic chromatography.

hydrodynamic injection at the expense of an acceptable loss in the resolution. LODs were below 200 ng/mL and the quantitation limit was 0.5 μg/mL (Fig. 3a,b). Interestingly, an extremely simple work-up (precipitation of 200 μL of sample with 200 μL of acetonitrile and centrifugation at 1000 rpm for 5 min) was sufficient to reproduce results obtained for deionized water solutions. We could confirm that the analytes did not epimerize in plasma after one hour at room temperature (Fig. 3c).

MEEKC as brain unbound fraction surrogate

We have developed a conventional MEEKC-UV system based on SDS to yield similar results to those reported by Wan *et al.* [34] opening up this application to more simple and inexpensive instruments without MS, although with a lower detection power. Figure 4 illustrates the good correlation between the brain unbound fraction determined by the gold standard methodology and the apparent MEEKC distribution factors in these systems, and the consistency of the results provided by SDS and lauric acid microemulsions. A pH of 7.4 was selected to mimic physiological pH in biological fluids, and the platform was validated against two sets of compounds selected independently using chemical structural diversity as criterion (polar surface area, lipophilicity and pKa were considered among other factors). The good overlap of these two sets demonstrates the robustness of the methodology.

Concluding remarks

CE has matured to become an integral part of pharmaceutical research owing to its tremendous versatility, simplicity of use, data quality and cost-effectiveness. This review suggests that the flexibility of CE and related techniques provide extremely powerful tools to obtain quantitative and qualitative information in all the stages of the small molecule drug discovery endeavor. Recent technological advances in the field of electro-driven separations (*e.g.* micro and/or nanofluidic, hyphenation and multiplexing) in terms of performance, throughput and automation, illustrate how CE is now becoming affordable in areas of drug discovery where HPLC is already well established, including HTS, proteomics, metabolomics and biomarker technologies.

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