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High-throughput multiplexed capillary electrophoresis in drug discovery

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The demand for high-throughput analytical tools to support drug discovery applications has led to the development of multiplexed capillary electrophoresis and multichannel microfluidic devices to characterize libraries of compounds and alleviate backlogs in the discovery process. The capability to analyze multiple samples in parallel, and the diverse separation conditions that are permissible, facilitates rapid turnaround times. Examples of high-throughput applications of multiplexed electrophoresis in drug discovery include: physicochemical profiling, enzyme analysis, chiral separations and protein/metabolite analysis. Many single capillary electrophoresis methods can be potentially adapted to a multiplexed format, therefore, we anticipate the development of other high-throughput applications in the near future, which should facilitate decreases in sample analysis time and help improve laboratory efficiency.

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▼ Capillary electrophoresis (CE) [1–3] is an analytical technique that uses a high electric field of up to 1000 V cm^{-1} within a narrow glass capillary tube, in which the tubing is filled with conductive background electrolyte, to separate, identify and quantify component molecules in a mixture. Because of the high separation efficiency, fast separation time, low reagent consumption and small sample volume, high performance capillary electrophoresis (HPCE) has emerged as an important analytical tool for the separation of low MW (<600 Da) compounds of pharmaceutical interest, as well as large biological molecules (e.g. proteins and DNA). Various modes of CE can be used depending upon the characteristics of sample mixture to be separated. Capillary zone electrophoresis (CZE) [3] separates charged compounds based on their charge to mass ratio (electromobility), and micellar electrokinetic chromatography (MEKC) [4] can separate both charged and neutral compounds based on differential partitioning into a moving micelle pseudophase (hydrophobicity). Capillary isoelectric focusing (CIEF) [5] separates proteins and peptides based on their isoelectric points (pI values). Capillary gel electrophoresis [6,7] is often used for sized-based separations of protein and DNA. Many different types of detectors can be interfaced to CE [8]. Most organic molecules contain a chromophore that absorbs in the UV region from 195–254 nm, therefore, UV absorption detection is the most commonly used detection method. Laser induced fluorescence (LIF) is the most sensitive detection method but analytes must contain a fluorophore, or derivatization is required. Other detection modes used in conjunction with CE include MS or conductivity measurement [3].

Introduction

The increasing demand from the industry for higher throughput analytical techniques has led to the development of novel technologies, often derived from traditional, serial-based methods. In this regard, CE has a significant advantage in that it is more readily adapted to a highly parallel format than chromatographic techniques, which require precise fluid pumping and high pressures. The introduction of multiplexed CE nearly ten years ago significantly increased the analytical throughput for DNA sequencing and mutation analysis and had a key role in the completion of the Human Genome Project [9]. Such systems exclusively use LIF detection because of the ultra high sensitivity requirements for DNA sequencing. Most compounds of pharmaceutical interest or their byproducts do not fluoresce naturally, therefore, dye labeling is required for LIF detection. This limits the use of multiplexed CE-LIF for drug discovery and development because the modification of drug candidates is not desired. The recent introduction of 96-capillary multiplexed CE-UV absorption detection instrumentation [10]

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provides a more generalized approach for high-throughput drug discovery applications.

The majority of drug-like compounds possess MWs in the range of 250–600 Da with aromatic ring or conjugated double bond structures, therefore, they have strong UV absorption properties. The near universal detection capabilities of UV absorption thus enable the detection of underivatized molecules. Figure 1 shows a schematic of a multiplexed CE system using UV absorption detection. An array of 96 capillaries is packed side-by-side at the detection window where the capillary polyimide coating has been removed. At the sample injection end, the capillaries are arranged in an 8×12 format compatible to a standard 96-well plate. The outlet ends of the capillaries are connected to a common reservoir. A deuterium lamp is used as a UV light source. The transmitted light through the capillary array detection window is collected by a flat field lens, passed through an interference filter and imaged on a photodiode array detector. The separation buffer or sieving matrix can be filled into each capillary through the reservoir side with a pump for performing parallel separations of different samples. In addition, it is possible to introduce differing buffer solutions into different capillaries by vacuum to vary separation conditions for the same sample simultaneously, thereby speeding method development.

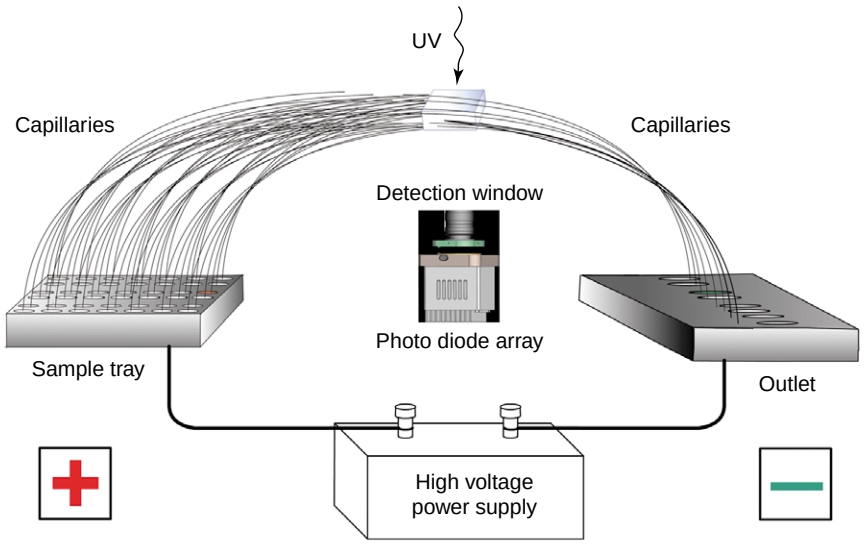

Figure 1. Schematic design of a multiplexed CE system with UV absorption detection. An array of 96 capillaries is packed side-by-side at the detection window where the capillary polyimide coating has been removed. The capillaries are arranged in an 8 x 12 format compatible to a standard 96-well plate for sample injection. The outlet ends of the capillaries are connected to a common reservoir. A deuterium lamp is used as a UV light source. The transmitted light through the capillary array detection window is collected by a flat field lens, passed through an interference filter and imaged on a photodiode array detector. UV absorption signals are monitored simultaneously for all 96 capillaries. High voltage is applied to the capillaries during electrophoresis separation.

Recent advances involving microfluidic devices provide an alternative separation tool. Microfluidic devices use networks of channels (<100 μm) fabricated in polymer, plastic, glass or fused silica substrates. Integration of sample manipulation, separation and even a detector has been achieved on a single device [11,12]. Although separation times are faster with microfluidic devices than with traditional HPCE, issues related to sample introduction, overall sample throughput and the need for LIF detection currently limit their use in drug discovery applications, although a bright future is anticipated.

This review focuses on recent developments relating to multiplexed capillary electrophoresis and multichannel microfluidic electrophoresis devices for performing high-throughput drug discovery and development applications. Applications using single capillary CE or single channel microfluidic devices are beyond the scope of this review and will not be discussed. Examples using multiplexed capillary or microfluidic electrophoresis systems for performing physicochemical profiling, chiral separations, enzyme and inhibitor activity screening, metabolomic analysis, immunoassay and protein analysis will be presented. Multiplexed CE-UV instruments and multichannel microfluidic devices were commercialized only recently, therefore, to date, only limited publications on their application in drug discovery and development have appeared. A summary of commercially available multiplexed CE systems is presented in Table 1. Most systems use LIF detection and are used for DNA sequencing and genotyping applications. However, recent developments indicate an exciting future for these newly available tools for an increasing range of drug discovery applications.

Applications

Physicochemical profiling

The widespread adoption of combinatorial methods in the synthesis of new chemical entities (NCEs) and the development of high-throughput activity screening methods has transformed the drug discovery process. Although tens of thousands of potential hits can be obtained for various targets from a screening library, many of these hits do not possess adequate ‘drug-like’ properties. It is desirable to

Table 1. Summary of commercial multiplexed CE systems and their applications

Manufacturer	System	No. of capillaries	Detection	Applications
Amersham Biosciences www.amershambiosciences.com	Megabase 500	48	LIF	DNA sequencing, genotyping
	Megabase 1000	96		
	Megabase 4000	384		
Applied Biosystems www.appliedbiosystems.com	ABI 3730	48,96	LIF	DNA sequencing, genotyping
	ABI 3730xl	96		
	ABI Prism 3100	4,16		
Beckman Coulter www.beckman.com	CEQ 8800	8	LIF	DNA sequencing, genotyping
	CEQ 8000			
CombiSep www.combisep.com	CePRO 9600	96	UV	pK_a, log P, chiral, purity, SDS-protein sizing, DNA sizing, peptide mapping, absorption amino acid analysis, CIEF, oligonucleotide QC
Genteon www.genteon.com	Capella 400	384	LIF	Genotyping, DNA sizing, oligonucleotide QC
Spectrumedix www.spectrumedix.com	SCE series	24,96,192,384	LIF	Genetic analysis, DNA sequencing, DNA/protein gel shift
	REVEAl series			Mutation discovery
	Ident series			Genotyping
	HTS series			Protein analysis
Caliper Technologies www.caliperls.com	Caliper 3000 HTS	4–12 channels Microfluidic device	LIF	Enzyme assay

Abbreviations: CIEF, Capillary isoelectric focusing; LIF, Laser induced fluorescence; QC, quality control.

identify hits with poor ADME properties at a relatively early stage of discovery, to reduce attrition rates later in the development process. It has been estimated that up to 30% of compounds that reach development are rejected as a result of poor ADME properties [13]. When studying the passive absorption of compounds across biological membranes, knowledge of compound acid dissociation constants (pK_a values), partitioning behavior (e.g. octanol–water partition coefficients or log P values), solubility and membrane permeability are of vital importance. To this end, multiplexed CE can provide a high-throughput, sample conserving approach that is relatively insensitive to impurities, for the screening of compound pK_a and log P values.

pK_a screening

In recent years, CE has emerged as a method of choice for the assessment of compound pK_a values. Historically, pK_a values have been determined by potentiometry or more recently by UV spectrophotometry [13]. Potentiometry requires milligram amounts of sample at relatively high concentrations (500 μM); spectrophotometry requires differences in spectral properties for the neutral and ionized form of a molecule. Both methods are susceptible to error from sample impurities or degradants. Advantages of CE include low sample requirements (μg range) and minute (ng) sample consumption; because CE is a separation technique, it offers the ability to handle impure samples; automated operation and the capability to analyze sparingly soluble (low μM range) compounds. In addition, only the migration time of the compound is needed for analysis, therefore, exact knowledge of sample concentration is not required. Detection is most often by UV absorption, therefore, a suitable chromophore is required, although detection by MS was recently explored [14]. The use of CE for the determination of pK_a values was recently reviewed [15]. Briefly, to determine the pK_a value of a compound by CE, the effective mobility (μ_{eff}) of the compound is measured as a function of pH, yielding a sigmoidally-shaped titration curve, the inflection point of which corresponds to the pK_a value. The effective mobility is calculated by Equation 1 [Eqn 1]:

$$\mu_{eff} = L_d L_t V \left(\frac{1}{t_a} - \frac{1}{t_m} \right) \qquad \text{[Eqn 1]}$$

where L_d is the capillary length to the detector, L_t is the total capillary length, V is the applied voltage, and t_a and t_m are the migration times of the analyte and a neutral

marker (usually DMSO), respectively. As equilibration between the charged and neutral forms of an ionizable compound at a specific pH are faster than the CE separation time, a single compound peak is observed and the μ_{eff} can be described as in Equation 2 (for a monobasic compound):

$$\mu_{eff} = \alpha\mu_b \quad \text{[Eqn 2]}$$

where α is the fraction present in the ionized form and μ_b is the effective mobility of the fully ionized species. Combination of Equation 2 with the familiar Henderson–Hasselbalch equation yields Equation 3 (again for a monobasic compound):

$$\mu_{eff} = \frac{\mu_b 10^{-pH}}{10^{-pK_a} + 10^{-pH}} \quad \text{[Eqn 3]}$$

Because the pH is known and μ_{eff} and μ_b can be determined experimentally, it is possible to use nonlinear regression analysis to determine the pK_a value. Equations describing the relationship between μ_{eff} and pK_a for up to three ionizable groups have been described previously [15].

Using a single capillary CE instrument and 12 evenly spaced pH buffers from pH 2–11, the typical throughput for the determination of pK_a values is 40–60 minutes per compound [15]. The use of predictive software to pre-select the pH range and measurement with six pH buffers can yield a throughput of ~4 compounds per hour [16]. However, when screening compounds with multiple pK_a values over a wide pH range, this approach is limited. The introduction of 96-capillary multiplexed CE-UV provides a high-throughput method for screening pK_a values.

The application of vacuum-assisted multiplexed CE (VAMCE) for the rapid estimation of pK_a values of compounds was recently described by Zhou and co-workers [17]. In this study, a 12-point pH buffer series from pH 2.2 –10.7 was used, and eight different compounds were loaded in replicates of 12 across a 96-well plate. The 12 different pH buffers were injected in an orthogonal pattern to the sample plate into the 96-capillary array. The use of vacuum-assisted CE permitted the completion of a single run in ~5 min. The optimized method was capable of performing the pK_a analysis of between 128 and 164 compounds over 12 pH values in an eight-hour period. A set of 96 different compounds, mostly monoacids and monobases, were estimated by the VAMCE method with an average absolute difference to literature values of ~0.22 pK_a units.

Recent (unpublished) work from the laboratory of Pang *et al.* has involved the development of a 24-point buffer system spanning a wide pH range (pH 1.8–11.2) to expand the determinable pK_a range and improve the pH resolution. Figure 2 shows 24 simultaneously collected electropherograms from a 96-capillary array for a sample of 100 ppm quinine (a dibase) in 0.1% DMSO (performed in the laboratory of Pang *et al.*). The first peak corresponds to the positively charged quinine, which migrates ahead of the neutral DMSO marker. A plot of μ_{eff} vs pH yields the curve in Figure 3, from which two inflection points at pH 4.34 and 8.51 correspond to the pK_a' values (I=50 mM). Over 75 acidic, basic, and zwitterionic compounds with single and multiple pK_a values have been determined with good agreement to literature values. A throughput of 12 compounds per hour (each over 24 pH points) can be achieved. For low solubility compounds, an approach employing methanol-containing buffers and extrapolation to 0% cosolvent is under evaluation.

log P screening

The octanol–water partition coefficient (log P value) of a compound is a common measure of lipophilicity and is used during drug design, development and formulation. The log P values of compounds are commonly used to estimate ADME properties and have often been employed for establishing quantitative structure–activity relationships (QSARs). Traditionally, log P has been determined by the shake-flask technique, which is time consuming and requires large amounts of solvent and pure samples. It is also possible to indirectly determine log P values using chromatographic or CE techniques, as recently reviewed [18]. A variation of CE, microemulsion electrokinetic chromatography (MEEKC) [19], has found increasing use for the indirect determination of compound log P values. Microemulsions are immiscible oil droplets consisting of heptane or octane in water stabilized by surfactants and cosurfactants. The separation mechanism in MEEKC is based upon differences in solute partitioning between the microemulsion and aqueous phases. The migration time of a solute relative to internal standards fully incorporated in the microemulsion (e.g. dodecylbenzene) and in the aqueous phase (DMSO) is used to calculate the retention factor *k*, which previously has been shown to correlate well to log P [18]. A universal calibration curve can be constructed by plotting experimentally determined log *k* values against literature log P values of standard solutes ranging from –1 to 6 to obtain a linear relationship. The log *k* value of an unknown sample can then be measured under the same MEEKC conditions and its corresponding log P value determined by interpolating from the calibration curve. Advantages of the MEEKC method for log P determination include small sample consumption (µg range), insensitivity to impurities, and automated operation. It is important to note that for a compound to be well correlated to log P with MEEKC, it must be present in its neutral form; therefore, weakly basic

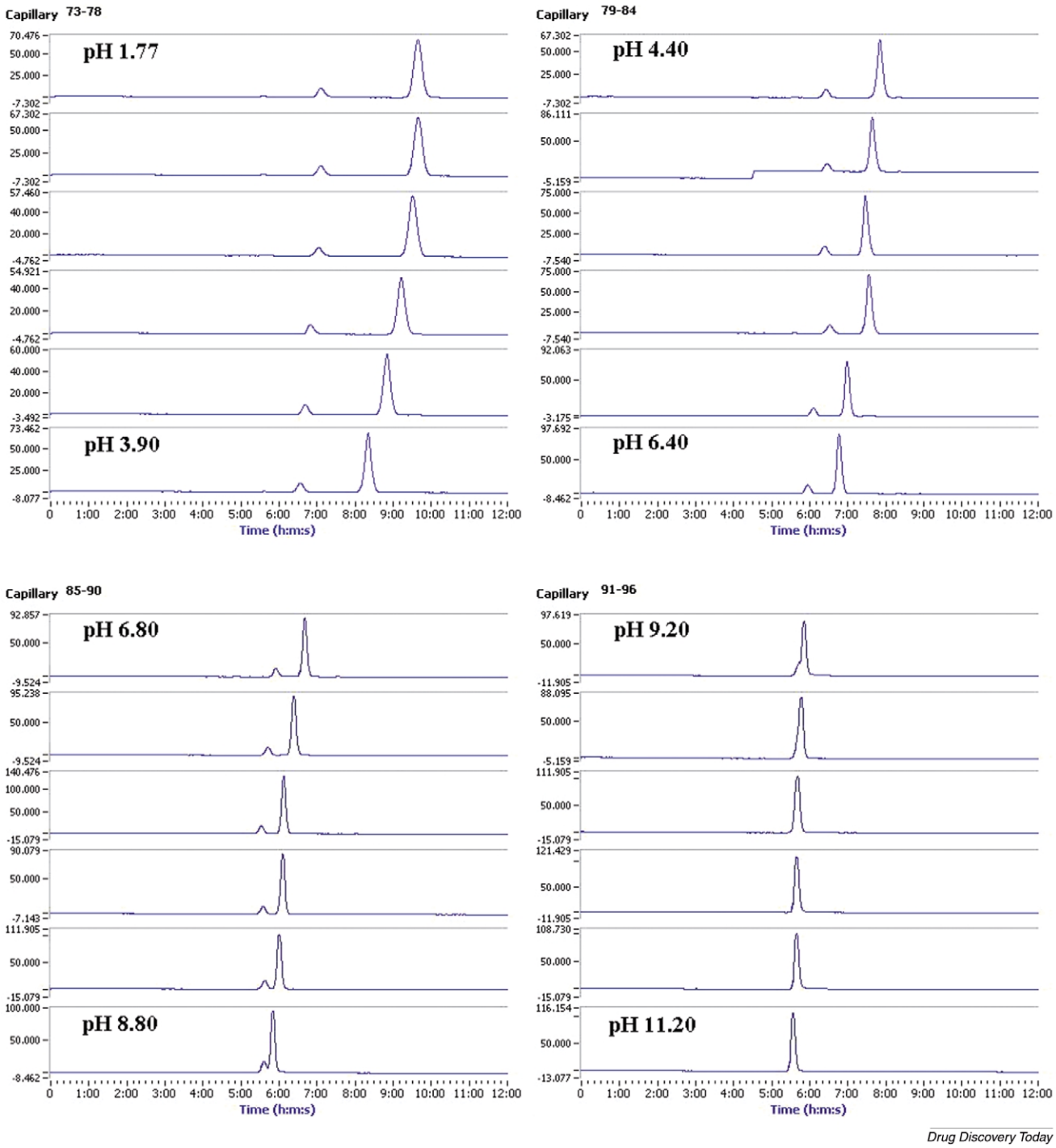

Figure 2. Simultaneously collected electropherograms at 24 different pH values for a sample of 100 ppm quinine (peak 1) with 0.1% DMSO (peak 2). A series of phosphate, formate and borate buffers of equal ionic strength (I=50 mM) were used to collect the CE data.

compounds must be analyzed at high pH values and weakly acidic compounds at low pH values.

The typical throughput of MEEKC for indirect log P determination is 2–4 compounds per hour [18]. To increase the throughput of log P determinations by MEEKC, Wehmeyer and co-workers introduced the use of 96-capillary multiplexed-MEEKC with UV detection at 214 nm [20]. The log P values for 42 different weakly basic and neutral compounds were determined to within 0.5 log units of literature values, with an average deviation of ±0.23. The

method was shown to be highly reproducible between different analysts and different days, with the capability to perform hundreds of analyses per day.

Wong and co-workers [21] further evaluated the operating parameters and long-term performance of multiplexed-MEEKC for indirect log P determinations. Good reproducibility (<0.1 log *k*) and precision (<0.1 log P unit) of log P values for the majority of 36 tested compounds were obtained over a period of 8 months using more than 10 different batches of microemulsion buffer. The same capillary array was used throughout the entire study, thus demonstrating the durability of the method. A throughput of 46 samples per hour was demonstrated. Importantly, a linear free energy relationship analysis performed on the data using solvation parameters validated that the multiplexed MEEKC method could serve as a good model for log P determination.

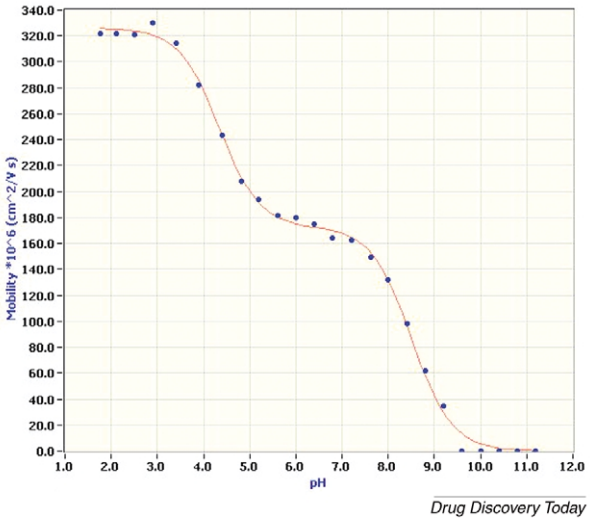

Figure 3. Plot of μ_{eff} vs pH for quinine, indicating the presence of two different pK_a values.

Enzyme activity screening

Determination of enzyme or inhibitor activity, potency and selectivity is important for clinical studies of disease mechanisms or the development of pharmaceutical products. The existence of large numbers of enzymes and inhibitors as potential drug targets requires high-throughput analytical techniques for their characterization. When using CE, substrates and products can be separated and detected individually and any influence of impurities on enzyme activity can be reduced. Multiplexed capillary electrophoresis and multichannel microfluidic electrophoresis devices can provide the means for high-throughput investigation of enzyme activity.

He and Yeung presented a method for kinase inhibitors HTS using a 48-capillary electrophoresis system [22]. The use of UV absorption detection enabled the direct measurement of peptide substrates without any modification or labeling, preserving the biochemical and electrophoretic properties of the native substrates. The approach provided a general platform for high-throughput analysis of native peptides and their phosphorylated counterparts. The specificity and reactivity of the protein kinases could then be determined by comparing the ratio of the native and phosphorylated peptides. The same principle could be used to determine the potency of kinase inhibitors following the introduction of the inhibitor into the mixture of kinase and peptide substrates. The IC_{50} value was estimated by using eight different concentrations for each kinase inhibitor. The use of a 48-capillary array permitted the study of six inhibitors simultaneously.

Wehmeyer *et al.* reported the use of multiplexed capillary electrophoresis with LIF detection for the measurement of endogenous extracellular signal-regulated protein kinase (ERK) levels in cell extracts *in vitro* [23]. The separation of substrate, product and internal standard was performed in 50 mM borate buffer at pH 9.3. The levels of ERK enzyme in cell extracts was determined by monitoring the conversion of a fluorescently labeled peptide substrate to a phosphorylated fluorescently labeled peptide product. Accuracy (103–120%) and precision (3.5–22% RSD) of quantitation of ERK levels using both direct or immunoprecipitation assay formats were determined. It was demonstrated that 40 samples, in duplicate sets, could be analyzed in less than 2h using a direct assay. The authors did indicate that the introduction of a fluorescent group into the substrate peptide sequence could change the K_{max} and reaction kinetics relative to the unlabeled substrate. The same group also used a multiplexed CE–LIF system for enzyme inhibitor screening and endogenous enzyme level measurements [24]. It was demonstrated that multiplexed CE–LIF could be used to screen a large number of inhibitors at one concentration in a 96-well plate or construct dose–response curves for a smaller number of compounds.

Lin [25] and co-workers have developed a high-throughput application assay for lipid-modifying enzymes using micellar electrokinetic chromatography (MEKC, a variant of CE) separations of fluorescently labeled phospholipids in a microfluidic chip device. Phospholipase A2, D, C, and PtdIns-specific phospholipase C were studied with various inhibitors. The substrates and products were separated by MEKC in the microfluidic device in under 40s. Throughput of 80 min per 384-well plate could be achieved with the use of a four-channel microfluidic device to simultaneously analyze four samples.

Chiral discovery and screening

Many drug compounds contain at least one chiral center, therefore, the study of enantiomers is important in pharmaceutical development and production because, although one enantiomer might possess the desired pharmacological effect, the other could be inactive or even toxic. HPLC has traditionally been employed for chiral separations, along with gas chromatography (GC) and, more recently, supercritical flow chromatography (SFC). However, the cost of the specialized columns and reagents, limited resolution, and low sample throughput hinder the rapid development of enantiomer separations. Unlike HPLC, in which different columns are often required to vary enantioselectivity, CE methods entail the addition of chiral selectors, such as cyclodextrins (CDs) or crown ethers, directly in the running buffer to perform chiral separations [26]. Advantages of CE for chiral analysis include small sample and reagent consumption, the use of a universal capillary, low operating costs and high separation efficiency and resolution. Multiplexed CE can provide a significant increase in sample throughput for reducing chiral method development times and performing enantiomeric excess measurements.

Reetz *et al.* [27] have reported the use of a 96-capillary electrophoresis system with LIF for enantioselective catalyst screening. Chiral amines were derivatized with fluorescein isothiocyanate for LIF detection. Enantiomeric excess measurements (% ee) were performed in parallel with good agreement to conventional GC results. The authors speculated further method optimization to shorten the analysis time could yield a throughput of 15,000–30,000 ee determinations per day.

A 96-capillary array electrophoresis system, with UV detection for combinatorial chiral separations, has been applied by Zhong and Yeung [28]. Numerous potential buffers were evaluated, from which four systems under acidic conditions – one with sulfated-β-CD, and three dual CD buffers containing a mixture of a charged and neutral CD – were used to effectively separate the enantiomers of 49 out of the 54 test compounds. The system could evaluate 12 different separation conditions for eight compounds in the same run, dramatically decreasing the method optimization time for screening unknown compounds and enhancing the efficiency of the methods development.

Kenseth and Bastin [29] also demonstrated the use of a 96-capillary array electrophoresis system with UV detection for high-throughput chiral separations. The use of 50 μm internal diameter capillaries and enhanced capillary cooling to minimize CE run currents permitted the use of highly sulfated (HS) CD selectors in a parallel manner. Previous studies have demonstrated the applicability of HSCDs of various cavity sizes (α, β, γ) for achieving a broad range of enantioselectivity and high separation resolution.

Metabolomics

Metabolomics studies the physiological processes in relation to genes, the development of environmental interventions and the toxicity of drugs. CE offers a unique, high resolution, separation platform for metabolomic investigations. However, LIF is not a desirable detection format because of the difficulty of labeling unknown species, even though it provides high sensitivity. Although UV absorption is capable of measuring all components of a mixture, its detection limits are compromized due to the short absorption path length and small sample injection volume. Therefore, sample pre-concentration before separation becomes necessary. Using single column CE, several sample pre-concentration methods [30–31] have been developed to enhance the detection sensitivity from 20 to 1,000,000 times.

Terabe *et al.* have used multiplexed capillary electrophoresis for metabolomic studies [32], thus enabling the detection of a large number of drugs and their metabolites. On-line sample pre-concentration using a dynamic pH junction was used to improve the sensitivity by over 50-fold for purine derivatives. The sample was dissolved in a phosphate buffer at pH 6.0 and a borate running buffer at pH 9.5 was used. As much as 13% of the capillary effective length was filled with sample compared to less than 1% of the capillary with normal CE injections. Because of the induced pH mismatch between the sample and the separation buffer, purine and its derivatives were concentrated into a sharp band at the inlet of the capillary.

Immunoassay

Immunoassay is a method to identify and quantify target molecules using the high selectivity of antibodies. Multiplexed electrophoresis devices can provide an alternative means for immunoassay analysis. Potential advantages over traditional immunoassay methods include high speed, high sensitivity, reductions in non-specific binding and the use of extremely small sample and reagent volumes.

A multichannel microfluidic device employing affinity capillary electrophoresis for immunoassay has been reported by Cheng *et al.* [33]. This microfluidic device contained six independent channels for simultaneous mixing, reaction and separation with LIF detection: immunoassays were demonstrated with ovalbumin and anti-estradiol, and both anti-ovalbumin and estradiol were labeled with a fluorescent dye. The device could be used to perform either six independent assays on one sample, or one assay on six samples. Although the entire process could be performed within 60 s, long flushing times (30 min) were

required to condition each channel to obtain reproducible migration times.

Bromberg and Mathies [34] have reported using a 48-channel micro-fabricated capillary array electrophoresis chip for high-throughput homogeneous immunoassays. Unlike the previously described microfluidic device, mixing and reactions were performed off-chip. Samples consisting of equilibrated mixtures of anti-trinitrotoluene (TNT) antibody, fluorescein-labeled TNT, and various concentrations for unlabeled TNT were electrokinetically injected into 48 channels. High speed and high-resolution separations were obtained. Although this demonstration was performed with explosives, the same principle could be used for clinical assays.

SDS-protein sizing

SDS–PAGE [35] is widely used for protein separations and MW determinations. However, the gel preparation and detection in SDS–PAGE is time consuming and labor intensive. SDS capillary gel electrophoresis (SDS-CGE) has been established as an alternative means for protein separation and sizing. Although separation by SDS-CGE is relatively fast (30 min) and can be automated, the lack of overall sample throughput has limited its use for protein separations. Luo [36] recently demonstrated the use of multiplexed SDS-CGE for high-throughput protein separation and MW determination. The method was capable of analyzing 96 protein samples in parallel within 30 min over a range of 14–116 kDa. The sizing accuracy and repeatability was comparable with conventional SDS–PAGE, while providing a significant increase in throughput and automation.

Future applications

The ability to adapt existing single capillary electrophoresis methods to a multiplexed format provides a host of potential future applications of multiplexed CE in drug discovery and development. Some examples include high-throughput purity profiling of combinatorial libraries, reaction monitoring of pharmaceuticals produced by fermentation or other natural synthetic processes, or quality control of drug manufacturing and stability. Protein related applications include protein expression studies or the examination of protein binding interactions. The growth of peptide and oligonucleotide therapeutics also presents future opportunities for multiplexed CE to have an impact.

Conclusion

In the past 15 years, capillary electrophoresis has found widespread acceptance as a complementary or alternative separation method in drug discovery. The evolution of highly multiplexed CE instrumentation provides a significant improvement in sample throughput relative to traditional, serial-based separation methods. The ability to analyze multiple samples or vary experimental conditions in parallel offers a unique and powerful tool for drug discovery. Multiplexed CE has a broad range of high throughput applications spanning the pharmaceutical, fine chemical, agrochemical and biotechnology industries. The multiplexed CE format provides the flexibility to simultaneously vary separation conditions to speed development processes. Many current rate-limited applications are scaleable to a multiplexed format; therefore, we anticipate a bright future for multiplexed CE to emerge as a routine analytical technique in drug development.

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