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Enhanced pharmaceutical analysis by CE using dynamic surface coating system

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Abstract

The poor repeatability of migration times in capillary electrophoresis (CE) within an injection sequence and between capillaries can be a difficulty when implementing CE for routine pharmaceutical analysis. The use of a dynamic surface coating has been shown to improve the routine performance of CE. The surface coating generates an appreciable electro-osmotic flow at low pH, which reduces analysis times for basic drugs compared to the low pH buffers typically used in CE. The repeatability of migration times and repeatability of migration times between capillaries was improved. Peak tailing for basic drugs was also reduced which improved peak shapes and peak area integration precision. It is concluded that the dynamic coating system is a positive advance in the routine implementation of CE into pharmaceutical analysis.

Keywords: Pharmaceutical analysis; Capillary electrophoresis; Dynamic surface coating

1. Introduction

Capillary electrophoresis (CE) is becoming increasingly established as a routine technique in many industries. However, there remain some operational and technical areas of CE, which must be addressed before CE can become considered as a more frequent replacement and/or alternative for routine HPLC methods for assay, and related impurities determinations. These improvements are primarily improved method repeatability, sensitivity and injection precision. Particular technical operating problems can be solute adsorption and variable migration times both within injection sequence and between capillaries.

Solute adsorption is a significant problem for basic compounds as they are positively charged and are electrostatically attracted to the negatively charged capillary walls. Irreproducible adsorption can lead to increased peak area variability and lower precision. Peak tailing can also mask impurity peaks, which migrate closely after the main peak. Various approaches have been adopted [1] to reduce solute adsorption. These have included [1] use of high salt concentration, extremes of pH, internally coated capillaries and buffer additives. The use of high salt concentrations

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which shields the capillary wall and reduces adsorption. Operation at extremes of pH (e.g. pH 1 and 13 etc.) beneficially alters the charged nature of both the solutes and capillary wall and can reduce electrostatic interactions. Internal capillary surfaces can be chemically modified [2–4] to give a permanent neutral, positive or negative charge which may assist in the elimination of peak tailing and/or alter the speed/direction of electroosmotic flow (EOF). However, these internally coated capillaries have increased costs compared to uncoated capillaries and alteration must be paid to the repeatability, stability and durability of the coatings. Buffer additives such as triethylamine have been used to reduce peak tailing. Surface active additives such as cationic surfactants have been used to alter the speed/direction of the EOF. However, although the use of additives can be beneficial, they still do not necessarily give sufficient repeatability between injections, across different days, and between capillaries and instruments.

The migration time of a solute in CE is dependent upon both the charge/size of solute ion and the level of EOF generated in the capillary. The level of EOF is dependent on the pH of the buffer used as this controls the extent of the silanol dissociation. At high pH, the flow is substantial whilst at pH values below 3 it is significantly reduced but still present. Variations in pH therefore affect the flow rate and lead to changes in migration times and reduced method robustness. Differences in residual silanol contents between portions of capillaries from the same supplier and/or between capillaries from different suppliers allow leads to variable migration times and reduced method robustness. This variability between capillaries can be reduced somewhat by extensive rinsing of the capillaries with NaOH solution before initial use [5] but it remains an appreciable source of variability.

Recently, an approach to reproducibly dynamically coat the internal wall of the capillary has been developed, patented [6] and commercialised. The three-stage process involves initially flushing the capillary with NaOH solution to fully dissociate the surface silanols and generate a negatively charged surface. An initiator solution, which contains a polyamine substance, is then flushed through the capillary. The multiply charged polyamine coats the entire capillary wall making it strongly positively charged. The capillary is finally flushed (initiated) with a buffer solution at the required pH which contains a polyanion layer which gives (Fig. 1) a uniform coating resulting in the capillary being negatively charged. The negative charge of the polyanion layer is unaffected by pH variation as it contains the sulphonic acid groups remain ionised over the entire pH range. This approach of using the initiator and buffer systems is sold commercially as both CElixir or CEofix. There have been some reports of the use of this system to improve performance of CE in clinical [7,8], forensic [9,10] and bioanalytical [11] determinations.

The buffering agents using in this system are zwitterionic amino acids such as Tris, arginine and taurine. The buffers therefore have a low ionic strength and a good buffering capacity. The low ionic strength is of benefit as this reduces the operating current compared to standard inorganic buffers used in CE such as borate. The lower currents generated permit use of either wider bore capillaries for improved sensitivity or higher applied voltages. Migration times are also reduced for the basic drugs pH 2.5, as their electrophoretic migration is supplemented by an appreciable EOF. This combination of EOF and mobility reduces migration times by up to 50%.

Peak tailing occurs due to the variable reversible absorption of the solute onto the capillary wall. It would be anticipated that this tailing would be worse if the capillary wall were coated with negatively charged polyanion. However, the absorption effect is reproducible and constant as the

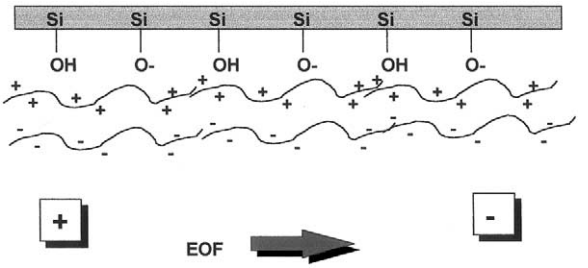

Fig. 1. Schematic of the dynamic coating process.

capillary wall is uniformly coated. The polyanion is also present in the running buffer and has interactions with the solute, which reduce tailing influences. This solute adsorption reduces resolution, and also reduces precision, as it is more difficult to reproducibly integrate tailing peaks.

The area of a peak in CE is related [8] to both the solute concentration and the migration time of the peak. Later eluting peaks move slowly through the detector compared to faster migrating solutes. The later migrating peaks therefore have wider peak widths and higher peak areas. Variability in the migration times throughout an injection sequence will lead to imprecision of the peak areas. The good migration time precision stability obtained with this buffer system reduces this source of peak area variability.

This dynamic coating system has recently been shown to offer significant operational advantages over uncoated capillaries in the area of forensics and clinical chemistry [7–10]. Particular advantages included improved migration time precision, and reduced sample losses that occur due to sample absorption onto the surface of uncoated capillaries. Peak shape was also shown to be improved [9,12] using this buffer system. It was anticipated that the use of this buffer system should improve the performance of CE methods in routine pharmaceutical analysis.

Basic drugs are commonly analysed used low pH phosphate buffers. These methods have been validated [13] and routinely implemented in several pharmaceutical companies. However, the migration time repeatability can be adversely affected by variable residual low level EOF that may be present. Highly basic drugs can interact with residual silanols, which result in peak tailing. Compounds with a high mass-to-charge ratio have low electrophoretic mobilities and their migration times can be extensive. This paper reports the evaluation of the commercial buffer system for the analysis of a range of basic drugs. It is concluded that the system offers significant advantages over traditional CE buffers. This improvement is primarily in terms of improved repeatability of migration times and between capillaries and within an injection sequence. Analysis times are also reduced due to the presence of an EOF at low pH, which augments the mobility of the basic drugs. Peak tailing is reduced which leads to an improvement in peak shape and peak area integration results. It is concluded that use of this buffer system is an advance in routine use of CE in pharmaceutical analysis by CE.

2. Experimental

A Beckman P/ACE 5000 instrument (Fullerton, CA) connected to a Hewlett Packard (Bracknell, Bucks, UK) HP1000 data system was used. Fused silica capillaries were obtained from Composite Metal Services (Hallow, Worcs, UK). Capillary detection windows were produced using an electrical filament device purchased from Electrokinetic Technologies (Capital HPLC, Broxburn, Edinburgh). Test compounds were either obtained from within GlaxoSmithKline or were obtained from Sigma (Poole, Dorset). The CElixir solutions were obtained from SRI (Gloucester, UK). The experimental multi-bore capillary was kindly donated by Mat/Sen division of UOP LLC, Des Plaines, IL.

3. Results and discussion

3.1. Use of dynamic coating system for efficient separation of basic compounds

Fig. 2a shows the analysis of six basic compounds. Two of them, triaminopyrimidine and diaminobenzoic acid are multiply charged and highly basic. Compounds of this nature strongly interact with the silanols on the capillary resulting in tailing peaks. The separation using the dynamically coated capillary shows Gaussian, non-tailing peaks. Fig. 2b shows the 10th injection in a sequence that shows the good reproducibility of migration times.

A piece of research capillary tubing was obtained which contained nineteen 25-μm channels. The benefits of employing this capillary format would be that improved injection loadings and sensitivity could be obtained compared to a standard wider bore capillary. The current (and

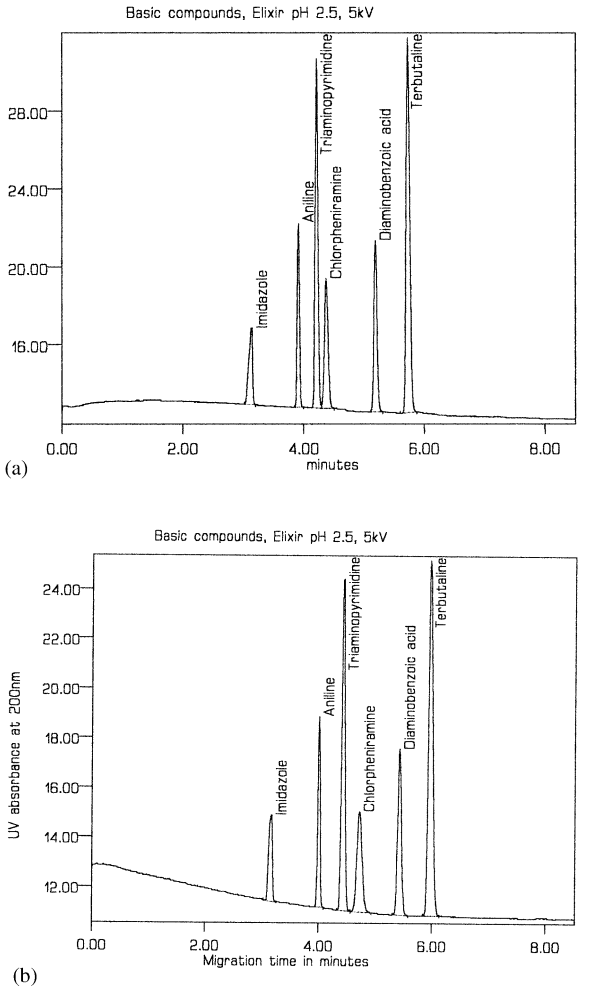

Fig. 2. Separation of pH 2.5 CElixir buffer, 27 cm × 50 μm, P/ACE 5000, +5 kV (about 17 μA), 30 °C, 1 s injection, 0.2 mg/ml in water, 200 nm.

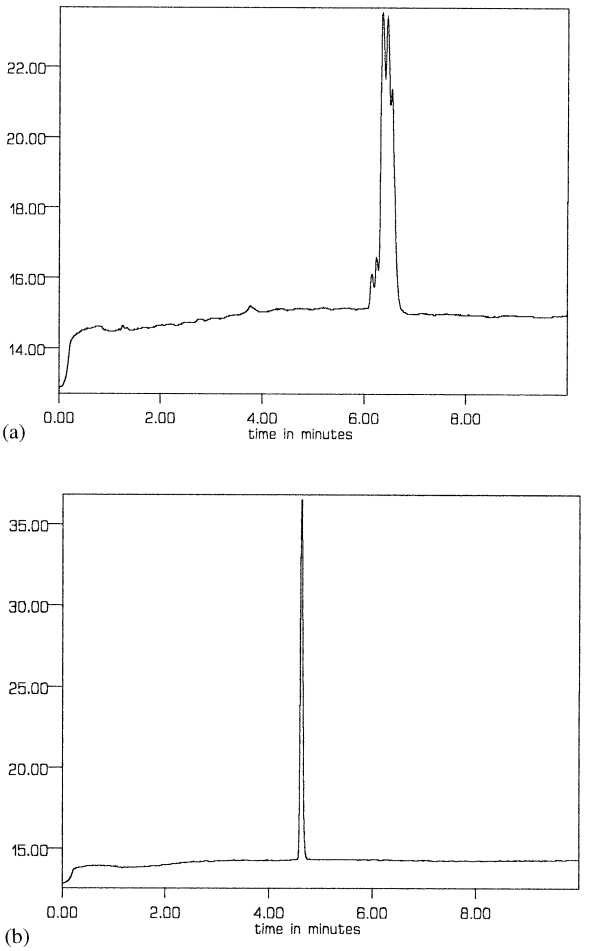

Fig. 3. Separations on a multi-bore capillary using phosphate buffer or CElixir buffer. (a) Separation using phosphate buffer: 50 mM phosphate 2.5, multi-bore capillary 19 × 25 μm channels, 27 cm long, 130 uA, +5 kV, 30 °C, detection at 200 nm, sample salbutamol 1 mg/ml, 1 s injection. (b) Separation using CElixir buffer: 50 mM phosphate 2.5, multi-bore capillary 19 × 25 μm channels, 27 cm long, 90 uA, +5 kV, 30 °C, detection at 200 nm, sample salbutamol 1 mg/ml, 1 s injection. Multi-bore capillary 19 × 25 μm channels, 27 cm long, +5 kV, 30 °C, Elixir buffer pH 2.5, 90 uA, 200 nm.

heat) generated in each 25-μm channel would be low as any heat generated would be effectively dissipated. However, Fig. 3a shows that use of the capillary with a phosphate pH 2.5 buffer produced a series of split peaks. This peak splitting was due to different levels of EOF being generated in each channel. Fig. 3b shows the analysis of the same sample solution on the multi-bore capillary using the pH 2.5 buffer to dynamically coat the surface. A single peak was generated which shows that the EOF is uniform across each of the channels. The peak time is reduced in Fig. 3b due to the EOF present. The current levels are high (90–130 μA) as the current is additive. The current levels and joule heating is however low in each of the 19 channels. This example especially exemplifies the improvement in repeatability using a dynamic coating system.

Reproducibility and control of EOF between capillaries is problematic as it relies on the silanol

density which changes between, and along capillaries. Extensive rinsing of the capillaries with NaOH to activate the silanols is recommended to improve reproducibility but variability still occurs which detracts from use in a routine environment. Fig. 4 shows separation of five basic compounds on three freshly prepared capillaries. All capillaries were rinsed for 10 min with 0.1 M NaOH followed by 1 min with the initiator solution and then 2 min with the pH 2.5 buffer. All capillaries gave an operating current of 16 or 17 μA. The separations shown in Fig. 4 were the first injection on each new capillary. The reproducibility of migration times for the three separate capillaries is better than the repeatability for three new HPLC columns or three normal untreated CE capillaries. The low operating current and high buffering strength also greatly assist in long-term injection repeatability as buffer depletion effects are minimised at low currents. The low operating current also reduces the internal temperature that reduces diffusion related broadening.

The injection precision in CE is related to the peak size and variability in injection volume. It is therefore recommended to use relatively high sample concentrations to minimise integration-

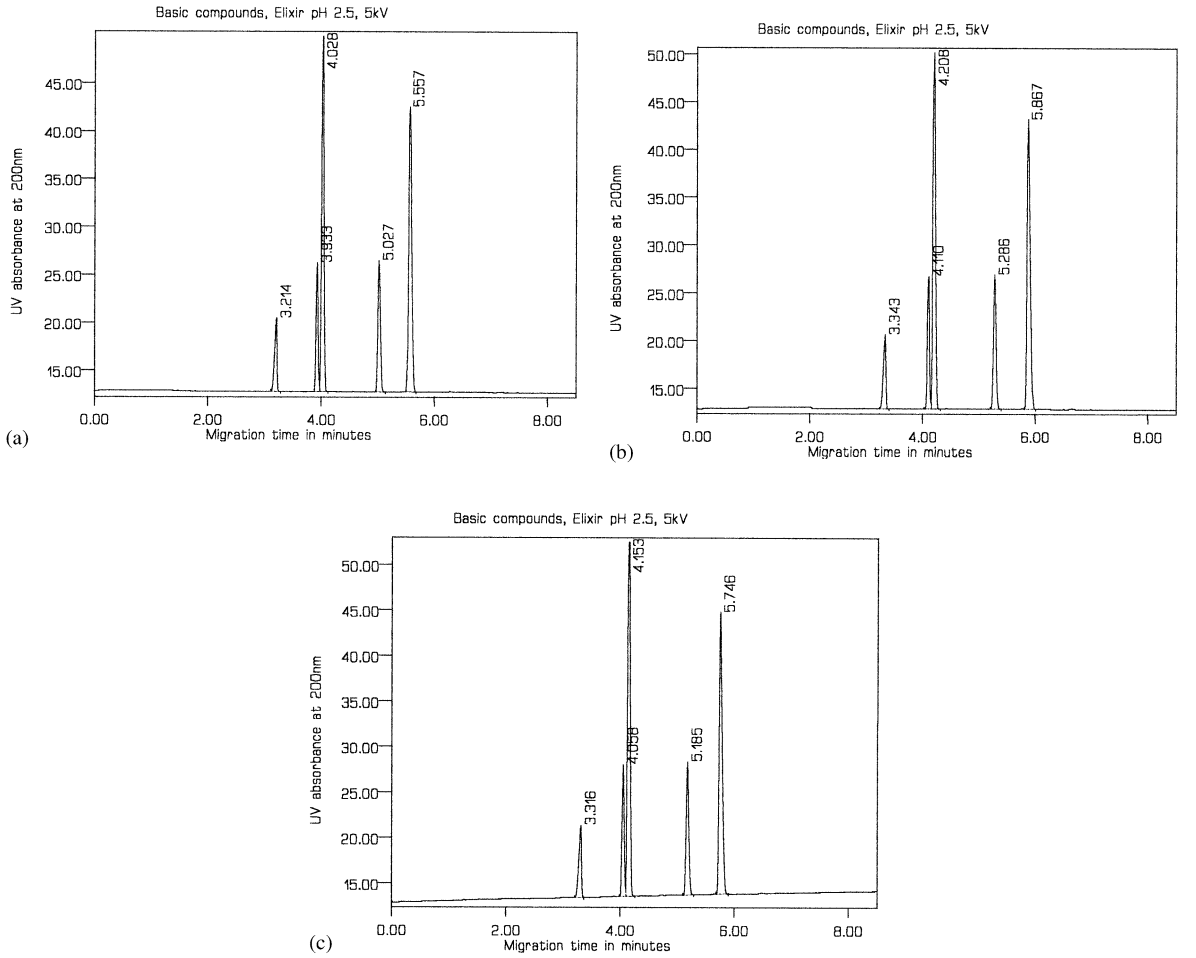

Fig. 4. Repeatability of separation of basic compounds on three freshly prepared capillaries: 10 min with 0.1 M NaOH followed by 1 min with reagent A and then 2 min with reagent B, 27 cm × 50 μm, +5 kV (about 17 μA), 30 °C, 1 s injection, 0.2 mg/ml in water, 200 nm. Peak identities in: (a) metformin, 3.2 min; aniline, 3.9 min; sumatriptan, 4.0 min; GW1 proprietary basic drugs, 5.0 min; labetalol, 5.6 min.

related errors. Internal standards are employed to eliminate effects of variable injection volumes. However, if tailing occurs for the internal standard or solute peak, then variable integration and areas will be obtained. The reduced tailing obtained with this these dynamically coated capillaries assists in precision measurements in routine application. In CE, the area of a peak is related to the migration time of the peak as this affects the residence time of the peak within the detector. Therefore, if the time of the peak is variable, then this will also reduce peak area precision. Reduced peak tailing improves the precision of peak area ratios as the integration of the peaks is more accurate and precise.

Table 1 shows data from studies measuring precision. Injection sequences were performed using a test-mixture containing three basic drugs and aniline, which is used as an internal standard. These measurements were conducted on different days and on three separate capillaries. It was found experimentally that it was necessary (as recommended by the manufacturers) to flush the capillary with NaOH, then initiator solution and then the buffer between each injection to ensure consistent migration times. The improved peak shape, reduced tailing and improved peak time repeatability made accurate, and more reproducible integration possible. The inherent injection volume variability in CE is shown in the 2–3% RSD values obtained for peak areas (Table 1). This injection volume variability is successfully counteracted by the use of the internal standard approach.

Fig. 5 shows separation of low-level impurities in salbutamol drug substance, which confirms the utility of these buffer systems for impurity determinations. Use of low wavelengths such as 200 nm is possible as the buffer system has low background UV absorbance. The good peak shape and improved peak efficiency [11] allowed acceptable resolution to be obtained.

Table 1
Injection precision measurements (precision ($n = 10$))

	Time (min)	Time (%RSD)	RMT (%RSD)	Peak area (%RSD)	PAR (%RSD)
Capillary 1 day 1					
Metformin	1.511	0.38	0.34	3.34	0.32
Aniline	1.585	0.33	N/a	3.28	N/a
Sumatriptan	2.055	0.41	0.21	3.50	0.46
Labetalol	2.281	0.44	0.29	3.72	0.61
Capillary 1 day 2					
Metformin	1.534	0.93	0.30	3.49	1.03
Aniline	1.63	1.04	N/a	2.96	N/a
Sumatriptan	2.121	0.98	0.24	3.34	0.72
Labetalol	2.353	1.02	0.22	3.11	0.70
Capillary 2					
Metformin	1.598	0.26	0.32	1.11	0.37
Aniline	1.683	0.29	N/a	1.09	N/a
Sumatriptan	2.242	0.19	0.19	1.34	0.62
Labetalol	2.528	0.31	0.31	1.33	0.59
Capillary 3					
Metformin	1.542	0.60	0.33	3.42	0.35
Aniline	1.618	0.49	N/a	3.41	N/a
Sumatriptan	2.103	0.67	0.25	3.56	0.38
Labetalol	2.337	0.76	0.37	3.63	0.43

50 μm × 27 cm, 5 kV, 30 °C, 200 nm, CElixir pH 2.5, samples 0.2 mg/ml in water, rinse 1 min with 0.1 M NaOH then rinse 1 min initiator solution then 1 min with pH 2.5, injection time 1 s (and 1 s post-injection of buffer from separate vial).

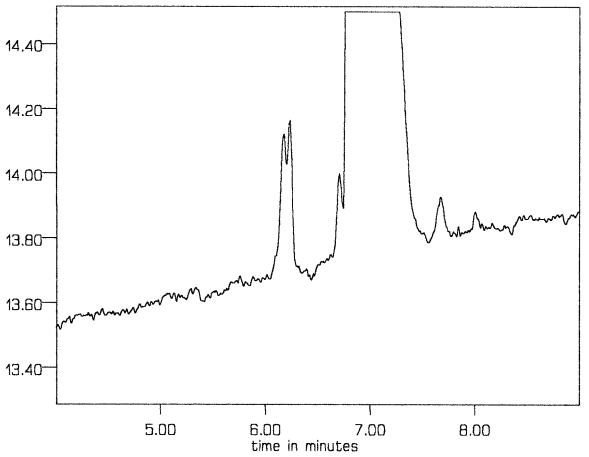

Fig. 5. Separation of salbutamol related impurities using dynamically coated capillary.

3.2. Disadvantages of the use of the dynamic coating system

A preliminary attempt to utilise a high pH (9.2) buffer for analysing a range of acidic drugs was partially unsuccessful. It was observed that the EOF generated was lower than for a traditional borate or phosphate high pH buffer. This lower flow allowed smaller or highly charged acids to successfully migrate against the flow resulting in them not being detected. These compounds would have been detected using a conventional CE buffer.

Micellar CE separations cannot be performed currently with these buffers as the SDS micelles interfere with the separation process. The buffers are more expensive than standard pre- or self-prepared buffers. The presence of the additives is prohibitive to use with MS detection.

4. Conclusions

The use of a dynamic surface coating has been shown to improve the routine performance of CE in pharmaceutical analysis. The buffer system was easy to use and significantly improved the repeatability of migration times and repeatability of migration times between capillaries. Peak tailing for basic drugs was also reduced which improved peak shapes and peak area integration precision. The presence of an EOF at low pH also reduced analysis times for basic drugs compared to the low pH buffers typically used in CE. It is concluded that the dynamic coating system is a positive advance in the routine implementation of CE into pharmaceutical quality control.

Acknowledgements

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