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Effect of on-capillary large volume sample stacking on limits of detection in the capillary zone electrophoretic determination of selected drugs, dyes and metal chelates

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Abstract

On-capillary large volume sample stacking (LVSS) with hydrodynamic injections of up to 240 s can be used to significantly decrease the limits of detection (LODs) of selected cationic basic drugs, such as clenbuterol, narcotine, flurazepam, codeine and pethidine, by capillary zone electrophoresis (CZE) by up to two orders of magnitude. This technique for these cations requires the use of the electroosmotic flow (EOF) modifier, cetyltrimethylammonium bromide (CTAB), employed at a concentration of 0.002 mol l^{-1} in a run buffer of 0.05 mol l^{-1} disodium tetraborate adjusted to pH 2.2 with orthophosphoric acid. In addition, polarity switching has to be employed after stacking and sample solvent removal in order to effect migration to the UV detector set at 210 nm. Similarily, but using visible detection and a run buffer of 0.05 mol l^{-1} sodium acetate, 0.002 mol l^{-1} CTAB and $1.0 \cdot 10^{-7}$ mol l^{-1} of chelating agent 2-(5′-bromo-2-pyridylazo)-5-diethylamino phenol (5-Br-PADAP) at pH 8, cobalt may be determined as its cationic chelate by LVSS. An LOD of $5 \cdot 10^{-8}$ mol l^{-1} cobalt can be achieved. Anionic species, such as dyes containing SO_3^- groups, can also be subjected to LVSS using the EOF modifier, diethylenetriamine (DETA) at a concentration of 0.006 mol l^{-1} in a run electrolyte of 0.05 mol l^{-1} citric acid (pH 2.23). Using a 30 s hydrodynamic injection and reversed polarity CZE without post-stacking polarity switching, LODs can be decreased by up to 20 times. The anionic chelate of boron with the ligand Azomethine H can be subjected to LVSS using the same EOF modifier and run electrolyte. A decrease in LOD is again found, down to a value of $5 \cdot 10^{-8}$ mol l^{-1}. This method can be applied to the selective determination of boron at realistic concentrations in an artificial river water matrix containing some 20 trace metal ions.

Keywords: Sample stacking; Dyes; Metal chelates; Flurazepam; Narcotine; Clenbuterol; Codeine; Pethidine

1. Introduction

The popular UV–visible detector used in capillary zone electrophoresis (CZE) possesses relatively high limits of detection (LODs) of the order of 10^{-5}–10^{-6} mol l^{-1} due to the detector's short path length and also due to injection volumes which are limited to nl amounts, otherwise undesirable peak broadening takes place as dictated by Eq. (1).

$$V_{inject} = QV_{column}/N^{\frac{1}{2}} \quad (1)$$

where V_{inject} is the injection volume, V_{column} is the column volume, Q is the fraction of allowable peak broadening and N is the number of theoretical plates for the separation.

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Sample stacking is an on-capillary concentration technique for capillary zone electrophoresis (CZE) developed by Burgi et al. [1–3]. Commonly, a plug of water or low concentration buffer containing the analyte(s) is injected onto the capillary filled with relatively high ionic strength run buffer. On application of a voltage of 20–30 kV across the capillary, a high potential is developed across the sample plug which drives its ionic species towards the interfaces of the sample plug and the run buffer where they slow down and subsequently stack. In conventional polarity CZE, cations stack to the front of the sample plug and anions to the rear. Stacking carries on until the sample and buffer conductivities match and then separation based on the electrophoretic mobilities of the species and electroosmosis takes place. Small decreases in LOD values, of the order of a factor of 3, can be achieved by this technique which is limited to the size of the sample plug that can be injected.

A large volume sample can, however, be injected if the sample solvent is mostly removed using electroosmotic flow (EOF) modifier (cetyltrimethylammonium bromide, CTAB) [4] during the stacking process and prior to migration and detection of the analyte(s). Large volume sample stacking (LVSS) can be used with the EOF modifier CTAB [5] at concentrations >0.001 mol l^{-1} in the run buffer in conjunction with polarity switching, after stacking and sample solvent removal, to effect significant LOD improvement for the CZE determination of cationic basic drugs. McGrath and Smyth [5] have improved LOD values by approximately one order of magnitude by using 30 s hydrodynamic injections together with stacking and sample solvent removal. Oxspring et al. [6] have made a study of the CZE behaviour of metal chelates with the pyridylazodiethylaminophenol ligand and found an LOD of $5 \cdot 10^{-7}$ mol l^{-1} for cobalt using a single 30 s hydrodynamic injection and without CTAB in the run buffer.

The EOF modifier diethylenetriamine (DETA) can be used at a concentration of 0.006 mol l^{-1} in the run electrolyte, 0.05 mol l^{-1} citric acid, without polarity switching for the reversed polarity CZE determination of anions, such as dyes containing SO_3^- groups. Oxspring et al. [7] have applied this technique to SO_3^- containing Remazol dyes and have improved LOD values by up to 20 times. The anionic chelate of boron with the ligand Azomethine H can be similarly subjected to LVSS using DETA as EOF modifier and this has resulted in significant LOD decrease, down to a value of $5 \cdot 10^{-8}$ mol l^{-1} [8].

This paper presents recent studies and results on the effect of on-capillary LVSS on LOD values in the CZE determination of selected cationic and anionic drugs, dyes and metal chelates. Particular attention is paid to the effect on both the LOD and the peak width of increasing the time of the hydrodynamic injection in the range 3–300 s and also the effect of multiple negative charges, located on relevant dye molecules, on the stacking process and the resulting LOD values.

2. Experimental

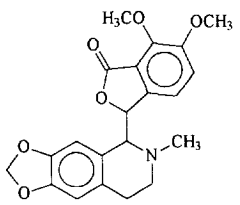
2.1. Reagents and analytes

All solvents were of HPLC grade. CTAB, DETA, $Na_2B_4O_7 \cdot 10H_2O$, orthophosphoric acid, citric acid, 2-(5′-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP), Azomethine H, ascorbic acid, acetic acid, ammonium acetate, disodium nitrilotriacetic acid (NTA) and ethylenedinitrilotetraacetic acid (EDTA) were purchased from Aldrich Chemicals (Gillingham, UK). Sodium acetate was obtained from BDH (Poole, UK) and cobaltous chloride from Hopkin and Williams (Essex, UK). Aqueous solutions were prepared in Milli-Q 18 MΩ water (Millipore).

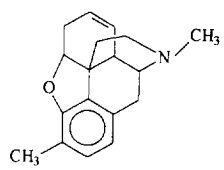
Benzodiazepine drug samples were obtained from Roche Products (Welwyn Garden City, UK) and other drug samples were obtained from the Northern Ireland Forensic Science Laboratory (Carrickfergus, UK). Drug structures are shown in Fig. 1. Remazol and Cibacron dyes were supplied by Fruit of the Loom, Buncrana, County Donegal, Ireland.

2.2. Preparation of analytical solutions

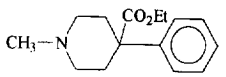
A 10^{-3} g ml^{-1} stock solution of each drug was prepared in methanol and dilutions (down to $1 \cdot 10^{-7}$ g ml^{-1}) were obtained from this solution for LOD studies. Serial dilutions were effected using 10% (v/v) run buffer in Milli-Q 18 MΩ water for the



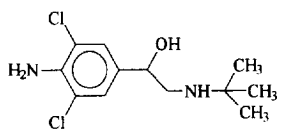
(I) NARCOTINE



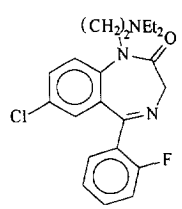
(IV) CODEINE



(V) PETHIDINE



(II) CLENBUTEROL



(III) FLURAZEPAM

Fig. 1. Structures of basic drugs I–V investigated by CZE.

LVSS studies. A $1.0 \cdot 10^{-3}$ mol l^{-1} solution of cobalt was prepared by dissolving 0.0288 g of $CoCl_2 \cdot 6H_2O$ in 100 ml Milli-Q water. A stock solution of $1.0 \cdot 10^{-3}$ mol l^{-1} 5-Br-PADAP was prepared by dissolving 0.0349 g of the ligand in approximately 50 ml of ethanol. The solution was then transferred to a 100 ml volumetric flask and made up to the mark with Milli-Q water.

The Remazol Black B and Remazol Red RB solutions were prepared from the solid dye, while the remaining 4 dyes were supplied in a 33% water–sulphuric acid solution [67% dye per 100 ml of water–18 mol l^{-1} H_2SO_4 (1:1, v/v)].

A 10^{-3} mol l^{-1} solution of boron was made up by dissolving 0.0364 g of $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml Milli-Q water. A stock solution of $1 \cdot 10^{-2}$ mol l^{-1} Azomethine H was made up by dissolving 0.44 g of Azomethine H and 1.0 g of ascorbic acid in 100 ml of Milli-Q water. A buffer masking agent was used to prevent other metal species from reacting with Azomethine H and this was made up with 100 ml Milli-Q water, 125 ml acetic acid, 250 g of ammonium acetate, 10 g of disodium NTA and 25 g of EDTA. This masking agent had a pH of 5.2.

2.3. Apparatus and procedures

All experiments were performed on a Spectra Phoresis 1000 (Thermoseparation Products, Stone, UK) equipped with a UV–visible fast scanning detector. All equipment control and data handling were performed using Spectra Phoresis software. Separations were carried out in a 70 cm×75 μm uncoated fused silica capillary (Composite Metal Services, Hallow, UK), for which the length of the capillary, from injector to detector, was 63 cm. Before CE separations, capillary columns underwent a conditioning/equilibration procedure consisting of purging with 0.1 mol l^{-1} NaOH at 60°C for 5 min (to deprotonate the silanol groups) and with water at 60°C for 5 min. This was followed by equilibration with run buffer for 5 min and with a series of "no-injection" injections, whereby a voltage was applied for 5 min. The capillary was washed for 5 min with buffer before each injection. To prevent capillary blockage, buffers and sample solutions were filtered using 13 mm diameter discs with a pore size of 0.45 μm (Gelman Sciences, Ann Arbor, MI, USA) apart from the cobalt chelate samples, as the chelate was left behind on the disc when filtered. Samples were injected using the hydrodynamic mode via a vacuum controlled system with an injection time of 3–300 s. For injection times ≥30 s, as in LVSS studies of the drug substances, the sample solvent was removed by applying a positive voltage of +20 kV when the run buffer was 0.05 mol l^{-1} $Na_2B_4O_7 \cdot 10H_2O$, adjusted to pH 2.2 with orthophosphoric acid and including 0.002 mol l^{-1} CTAB for the purpose of reversing the EOF. For the stacking studies of the drug clenbuterol, the run buffer was diluted by a factor of 10 with Milli-Q water and used as a solvent for clenbuterol in order to minimise the time required to remove the sample solvent. On application of this +20 kV potential, the electric current was initially low and the resistance

was high due to the presence of the low ionic strength sample plug. As the sample solvent was removed, the current increased and when it had reached 95% of the pre-injection level, the polarity of the electrodes was switched to −(15–20) kV and the drug analytes were driven to the detector by the electroosmotic effect, with electrophoretic mobilities in the opposite direction towards the cathode.

LVSS of the Co chelate was performed using a run electrolyte of 0.05 mol l^{-1} sodium acetate, 0.002 mol l^{-1} CTAB and $1.0 \cdot 10^{-7}$ mol l^{-1} 5-Br-PADAP (pH 8). It was diluted ten times when used as the sample solvent. A similar technique of stacking was used as for the cationic drugs. A voltage of 25 kV was first applied to remove sample solvent from the sample plug. When the current reached 95% of its preinjection level, the polarity was switched and the cationic chelate driven to the detector by the EOF.

Stacking studies on the dyes were performed using a run electrolyte of 0.05 mol l^{-1} citric acid (pH 2.23) with 0.006 mol l^{-1} of the EOF modifier DETA. The instrument was operated throughout in the reverse polarity mode without polarity switching after stacking/sample solvent removal and using 30 s hydrodynamic injections.

Stacking studies on the anionic boron–Azomethine H chelate were carried out using a run electrolyte of 0.03 mol l^{-1} citric acid, 0.006 mol l^{-1} DETA with its pH being adjusted to 3.7 with the masking agent. Again, as with the dyes, reversed polarity CE was employed throughout with a potential of −25 kV and 30 s hydrodynamic injections were used.

3. Results and discussion

3.1. CZE determination of selected basic drugs

Table 1 shows the pK_a values and charge status of drugs I–VII at pH 2.2, the pH of the CZE run electrolyte. Fig. 1 shows the structures of I–V. Hydroxyethylflurazepam (VI) and desalkylflurazepam (VII) are similar in structure to flurazepam (III) with $-(CH_2)_2OH$ and –H groups replacing the $-(CH_2)_2NEt_2$ group, respectively. Four of the drugs have a charge status of +1, flurazepam (III) with an additional pK_a of 1.4 has a charge status slightly in excess of 1 (the degree of dissociation α for the protonated nitrogen of the diazepine group is 0.86) and the flurazepan metabolites (VI) and (VII) have a charge status of approx 0.5 (α value 0.46) and between 0.5 and 1 (α value 0.30), respectively. As positively charged drugs in pH 2.2 run electrolyte, they will stack at the forward interface of sample plug/run buffer while the sample solvent is removed using the EOF modifier CTAB. Even though at this pH there is a negligible number of dissociated silanol groups in the internal capillary wall for ion pairing to positively charged quaternary amine groups in CTAB, an association between Si–OH and N^+ still occurs, presumably involving the oxygen dipole in Si–O–H and N^+. At a certain critical concentration of surfactant, there is a reversal in the zeta potential of the double layer at the capillary internal surface due to association of the CTAB long hydrocarbon chains, giving rise to a hydrophobic bilayer and thus presenting an overall

Table 1
pK_a data and charge status at pH 2.2

Drug	$pK_{a,1}$	$pK_{a,2}$	Charge status (pH 2.2)
Clenbuterol (II)	0.7[b]	9–10[b]	+1
Narcotine (I)		9.8[b]	+1
Flurazepam (III)	1.4[c]	8–9[a]	>1
Codeine (IV)		8.2[b]	+1
Pethidine (V)		9.8[b]	+1
Hydroxyethyl flurazepam (VI)		2.26[c]	~0.5
Desalkylfluazepam (VII)		2.56[c]	Between 0.5 and 1

[a] Estimated using CZE [9].
[b] Estimated spectrophotometrically using Henderson Hasselbach equation [10].
[c] Reference [11].

positive charge to the run electrolyte in the capillary. Practically speaking, at concentrations of CTAB in excess of 0.001 mol l^{-1}, this results in reversal of the EOF allowing the sample solvent to be removed at the anodic end of the capillary in the LVSS studies. The use of surfactants, such as CTAB, to control surface charge and to reverse the EOF has been studied by other authors [4,12,13].

After stacking and sample solvent removal, the polarity is switched resulting in EOF reversal and this drives the drugs towards the detector, even though they have an electrophoretic tendency to migrate in the opposite direction to the cathode. Figs. 2 and 3 compare LVSS with a 30 s hydrodynamic injection with conventional CZE (3 s hydrodynamic injection) for drugs I–VII. The use of LVSS together with the EOF modifier CTAB to enable removal of sample solvent in all cases results in broadening of the peaks for these drug analytes. For example, the flurazepam (III) separation has an efficiency of 90 000 plates using conventional CZE (Fig. 2b) and

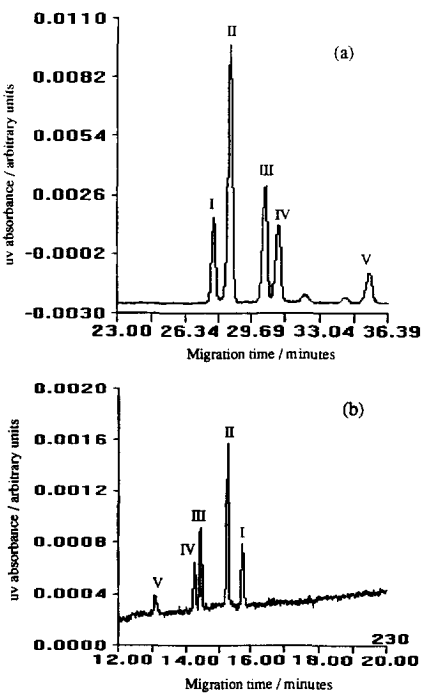


Fig. 2. (a) LVSS–CZE electropherogram of narcotine (I), clenbuterol (II), flurazepam (III), codeine (IV) and pethidine (V). (b) CZE electropherogram of the same mixture.

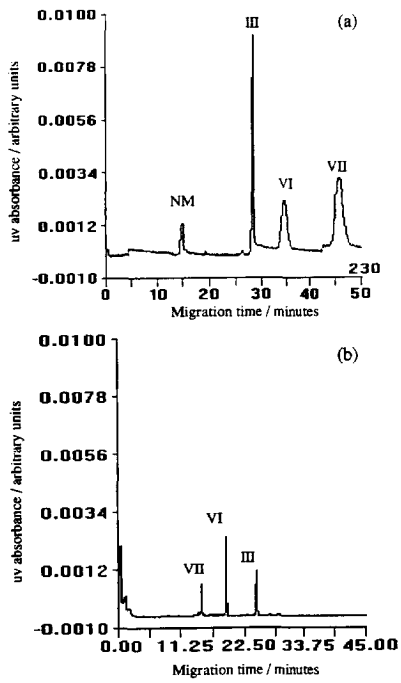


Fig. 3. (a) LVSS–CZE electropherogram of flurazepam (III) and its metabolites VI and VII. (b) CZE electropherogram of the same mixture.

32 000 plates using LVSS technique (Fig. 2a). Even allowing for peak broadening, molecules I–V are still baseline resolved with R_s values (at applied voltage of −15 kV and temperature 20°C) for I and II of 2.17 and for III and IV of 1.65, where an R_S value of ≥ 1.5 indicates baseline resolution. Fig. 3 again shows peak broadening on application of the LVSS procedure to flurazepam (III) and its two metabolites VI and VII. When the pK_a values of analytes are in the vicinity of the pH of the run electrolyte, the analyte with the lowest pK_a value emerges from the capillary first in LVSS, which is the reverse of migration order in conventional CZE when the polarity is not switched. This is found to be the case in Fig. 3a and b. For this interpretation of migration order, it is believed that the protonated aliphatic tertiary amine group in flurazepam (III) is ion-paired with $H_2PO_4^-$ anions from the run electrolyte. Such ion pairing of III has been postulated before in order to explain its solvent extraction behaviour [14].

The use of LVSS together with EOF modifier

CTAB does however significantly lower the LODs of analytes I–V by approximately one order of magnitude when compared to conventional CZE with a 3 s hydrodynamic injection (Table 2).

The decrease in LOD for VII and VIII is less marked due to significant peak broadening for these analytes when subjected to the LVSS procedure.

For the above mentioned LVSS procedures, software limitations restrict sample injections to 30 s. To obtain hydrodynamic injections from 30–240 s each run was stopped just before polarity switching until the appropriate number of 30 s injections had been made. A 240 s overall injection was the practical maximum in this case, since the sample plug caused a current cut-out beyond this value.

Using a −20 kV potential applied to the capillary, injection time was found to be linearly related to integrated peak area for a $3.2 \cdot 10^{-5}$ mol l^{-1} clenbuterol solution as would be expected. Over the range 3–120 s for $n=4$, a correlation coefficient of 0.9863 was achieved and it was also observed that the clenbuterol peak width increased with increase in injection time, being of the order of 1 min using a 120 s injection at a migration time of 17.5 min. This decrease in separation efficiency with increased analyte loading/sample solvent loading in the sample plug presumably occurs for the same sample volume remaining in each case just prior to polarity switching. This would suggest that the stacked cations within the sample plug are rendered less stacked the larger the volume of sample solvent that has to be removed from the capillary. It could be that this loss in separation efficiency is due to the mechanical effect of this latter process on the stacked cations.

Table 2
Comparison of LOD values of analytes I–VII using conventional CZE and LVSS with a 30 s hydrodynamic injection

Compound	Conventional CZE LOD (mol l^{-1}) ($\times 10^{-6}$)	LVSS LOD (mol l^{-1}) ($\times 10^{-7}$)
II	3.14	3.32
I	2.96	6.47
III	3.62	4.65
IV	3.45	4.04
V	5.26	4.05
VI	5.42	15.36
VII	4.51	28.47

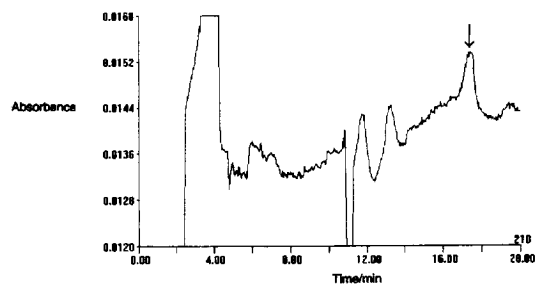


Fig. 4. LVSS–CZE of $3.2 \cdot 10^{-7}$ mol l^{-1} clenbuterol using a 120 s injection and a potential of −20 kV. Clenbuterol migration time is 17.5 min.

This peak broadening with increased injection time is illustrated in Fig. 4 for a $3.2 \cdot 10^{-7}$ mol l^{-1} solution of clenbuterol using a 120 s injection, the clenbuterol peak being seen at 17.5 min. The peak width in this figure of ca. 1 min can be compared with a peak width for clenbuterol of 0.67 min using a 30 s hydrodynamic injection (Fig. 2a, analyte II). Using a constant 120 s injection, a plot of clenbuterol concentration in the range $3.2 \cdot 10^{-4}$–$1.6 \cdot 10^{-7}$ mol l^{-1} was linearly related to peak area ($n=6$, $r=0.9987$). By increasing the injection time to 240 s, it was possible to estimate an LOD of $3.2 \cdot 10^{-8}$ mol l^{-1} where the signal intensity was equal to twice the peak to trough backround noise. This compares favourably with an LOD for clenbuterol of $2.3 \cdot 10^{-6}$ mol l^{-1} for conventional CZE using a 3 s hydrodynamic injection.

3.2. CZE determination of cobalt as its cationic chelate with 5-Br-PADAP

The same technique of cation stacking and sample solvent removal using CTAB, as for the cationic drug species, can be applied for LVSS of this Co chelate. Three 30 s hydrodynamic injections were made into the capillary of a solution containing $1.0 \cdot 10^{-5}$ mol l^{-1} cobalt and $2.0 \cdot 10^{-5}$ mol l^{-1} 5-Br-PADAP, made up in water. In the high speed scanning mode between 450 and 650 nm, a peak was observed which had a migration time of 4.6 min, as shown in Fig. 5. A spectral display of absorbance vs. wavelength showed that the chelate possessed 2 peaks at 549 and 587 nm, which is indicative of the cobalt chelate [6] (Fig. 5). Using a sample solution

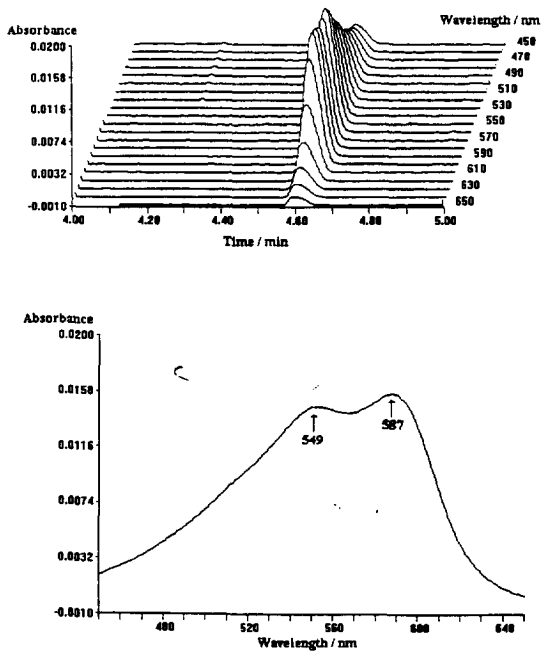


Fig. 5. (a) LVSS–CZE operating in the high speed scanning mode for the Co–PADAP chelate. Three 30 s hydrodynamic injections of a solution $1.0 \cdot 10^{-5}$ mol l^{-1} Co and $2.0 \cdot 10^{-5}$ mol l^{-1} PADAP are applied. (b) Visible spectral scan of the migrating peak at 4.60 min.

containing $1.0 \cdot 10^{-6}$ mol l^{-1} Co and $2.0 \cdot 10^{-6}$ mol l^{-1} 5-Br-PADAP, prepared in 10 times diluted run buffer, an increasing number of 30 s hydrodynamic injections were made, up to a maximum of 10 when the instrument cut-out occurred due to low conductivity. The results of multiple injections are illustrated in Fig. 6 where it was observed that peak area increased linearly with increasing number of injections ($r=0.9872$) with a tolerable degree of peak broadening. The migration times corresponding to Fig. 6a–d are 5.59, 5.32, 4.95 and 5.15 min, respectively. An LOD of $5.0 \cdot 10^{-8}$ mol l^{-1} was estimated using ten 30 s injections. This compares favourably with an LOD of $5.0 \cdot 10^{-7}$ mol l^{-1} estimated by Oxspring et al. [6] for a single 30 s hydrodynamic injection without CTAB in the run buffer.

3.3. CZE determination of dye anions

A stacking method that can be used for anions

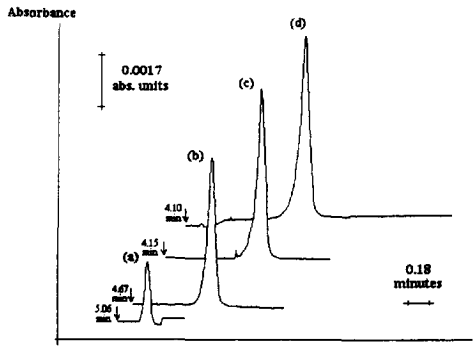


Fig. 6. Variation of the CZE signal with the number of 30 s hydrodynamic injections performed. Sample solution contained $1.0 \cdot 10^{-6}$ mol l^{-1} Co and $2.0 \cdot 10^{-6}$ mol l^{-1} PADAP prepared in ten times diluted run buffer. (a) 2, (b) 4, (c) 8, (d) 10 injections.

employs the EOF modifier DETA and does not require polarity switching after stacking and sample solvent removal [1]. This technique can be used for LVSS of SO_3^- containing Remazol and Cibacron textile dyes. When DETA is added to a 0.05 mol l^{-1} citric acid run electrolyte at a concentration of 0.006 mol l^{-1} with the capillary already in the reversed polarity mode, anionic dyes in a 30 s hydrodynamic injection will stack at the forward interface of the sample plug/run electrolyte.

DETA, $H_2N-(CH_2)_2-NH-(CH_2)_2-NH_2$, initially dissolves from the internal capillary walls into the aqueous sample plug [1]. It will exist as a tripositive ion at the pH of the run electrolyte and will thus remove sample solvent by its electrophoretic migration, complete with associated hydration shells, to the cathode. In contrast to the mechanism of sample solvent removal in the case of CTAB which involves electroosmotic flow generated from the internal walls of the capillary, the mechanism in the case of DETA is believed to be one that operates in the bulk of the run electrolyte with some electroosmotic contribution from the internal capillary walls where the Si–OH groups are essentially unionised at pH 2.23 and where any adsorbed DETA will have its N^+ sites associated with the oxygen dipole in Si–OH.

The use of LVSS in conjunction with DETA for sample solvent removal, results in LOD decreases for six Remazol and Cibacron dyes when compared with conventional CZE using 1 s hydrodynamic injections [7] (Table 3).

Remazol Black B (VIII) undergoes a 20 fold

Table 3
LOD values for CZE determination of Cibacron and Remazol dyes

Dye	LOD (mol l^{-1})		
	Conventional CZE	LVSS with DETA	LVSS with DETA for hydrolysed dye
Cibacron Red C-2G	$1\cdot10^{-6}$	$5\cdot10^{-7}$	$5\cdot10^{-7}$
Remazol Golden Yellow RNL	$1\cdot10^{-6}$	$5\cdot10^{-7}$	$1\cdot10^{-6}$
Cibacron Orange CG	$1\cdot10^{-6}$	$2.5\cdot10^{-7}$	$2.5\cdot10^{-7}$
Remazol Navy Blue GG	$1\cdot10^{-6}$	$1\cdot10^{-7}$	$1\cdot10^{-6}$
Remazol Red RB	$1\cdot10^{-5}$	$1\cdot10^{-6}$	$2.5\cdot10^{-6}$
Remazol Black B	$1\cdot10^{-5}$	$5\cdot10^{-7}$	$2.5\cdot10^{-6}$

improvement in its LOD by reduction of the LOD to $5\cdot10^{-7}$ mol l^{-1}. This dye has four SO_3^- groups in its structure and, on hydrolysis, the vinyl sulphone groups are transformed to vinylhydroxyl groups (Fig. 7). This is a competing side reaction in a dye bath in the textile industry. The overall Remazol Black B

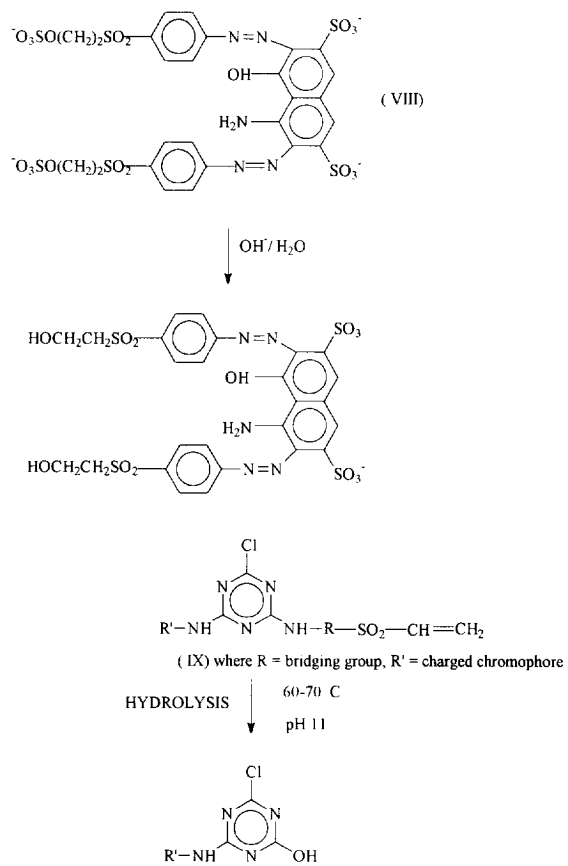


Fig. 7. Hydrolysis of Remazol Black B (VIII) and a Cibacron dye (IX).

then possesses two SO_3^- groups, and it is noticeable that the LOD of the hydrolysed dye increases on application of the LVSS/DETA procedure. This would suggest that the greater the negative charge on the dye molecule, the better the stacking process at the concentration boundary and hence the lower the LOD value. This observation is valid for all the hydrolysed Remazol dyes (Table 3) and it is also worth observing that the LODs of the Cibacron dyes, such as IX, do not alter on hydrolysis (Table 3) since such a chemical reaction does not alter the overall charge on the dye molecule [7], i.e. hydrolysis makes the following transformation as shown in Fig. 7.

Charged chromophore–NH–triazine → charged chromophore–NH–hydroxytriazine.

3.4. CZE determination of boron as its anionic chelate with Azomethine H

The same technique of anion stacking and sample solvent removal using DETA as used for the SO_3^- containing dyes in Section 3.3 can be used for the anionic chelate of boron with Azomethine H [8]. A linear calibration plot over the range $1\cdot10^{-4}$–$1\cdot10^{-7}$ mol l^{-1} boron was achieved with correlation coefficient 0.991 ($n=5$), and an LOD of $5\cdot10^{-8}$ mol l^{-1} boron was found for Milli-Q water solutions spiked with boron. The method was then applied to an artificial river water matrix of $KHCO_3/Na_3PO_4/MgCl_2/CaCl_2$, which included some 20 trace metal ions at concentrations that occur in river water samples. Great selectivity was shown towards the boron chelate when masking agent and DETA were added to the run electrolyte (0.03 mol l^{-1} citric acid), bringing its pH to 3.7. Fig. 8 shows the electropherogram of a sample which contained 2.3·

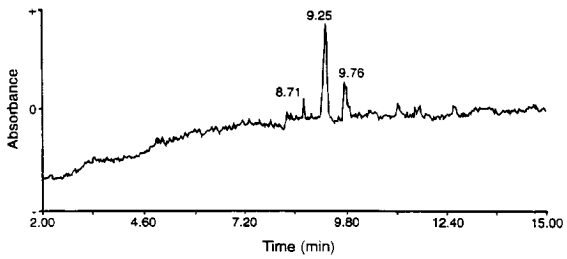


Fig. 8. Reversed-polarity CZE of $2.3 \cdot 10^{-6}$ mol l^{-1} boron in an artificial water matrix with masking agent and EOF modifier DETA added to the run buffer (0.03 mol l^{-1} citric acid), bringing its pH to 3.7. Boron chelate migration time 9.25 min.

10^{-6} mol l^{-1} boron in the artificial river water matrix with the migration time of the chelate being 9.25 min. The identity of this peak was confirmed as the boron chelate by scanning its spectrum over the wavelength range 350–450 nm. A linear relationship between peak area due to the boron chelate and concentration of boron in the artificial river water matrix was found in the concentration range $1.9 \cdot 10^{-6}$–$1.9 \cdot 10^{-5}$ mol l^{-1} and the LOD was estimated at $1.4 \cdot 10^{-6}$ mol l^{-1}. The recovery of the signal of a typical level of boron in a river water sample, e.g., $0.95 \cdot 10^{-5}$ mol l^{-1}, was 100% when a simple Milli-Q water matrix was compared with the artificial river water matrix.

4. Conclusions

On-capillary LVSS with hydrodynamic injections of 30 s can be used to significantly decrease the CZE LODs of selected cationic basic drugs clenbuterol (II), narcotine (I), flurazepam (III), codeine (IV) and pethidine (V) down to values of 3.32, 6.47, 4.65, 4.04 and 4.05 (all times 10^{-7}) mol l^{-1}, respectively. This technique requires the use of the EOF modifier, CTAB, in the tetraborate run electrolyte and polarity switching after stacking and sample solvent removal. By increasing the injection time to 240 s, the LOD of clenbuterol (II) can be lowered to $3.2 \cdot 10^{-8}$ mol l^{-1}. Other cations, such as the cobalt chelate with 5-Br-PADAP, can be similarily determined down to an LOD of $5 \cdot 10^{-8}$ mol l^{-1} using ten 30 s hydrodynamic injections.

Using a 30 s hydrodynamic injection with reverse polarity CZE and no post-stacking polarity switching, anionic species such as SO_3^- containing Remazol and Cibacron textile dyes can have their LODs lowered by up to 20 times using LVSS with the EOF modifier DETA in a citric acid run electrolyte. The anionic chelate of boron with the ligand Azomethine H can be similarily subjected to LVSS using citric acid/DETA. This has resulted in a method to selectively determine realistic levels of boron in an artificial river water matrix also containing some 20 trace metal ions down to an LOD of $1.4 \cdot 10^{-6}$ mol l^{-1}.

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