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A polydimethylsiloxane/glass capillary electrophoresis microchip for the analysis of biogenic amines using indirect fluorescence detection

A polydimethylsiloxane-glass capillary microchip is fabricated for the rapid analysis of a mixture of common biogenic amines using indirect fluorescence detection. Using a running buffer of phosphate and 2-propanol, and Rhodamine 110 as a background fluorophore, both co-ionic and counter-ionic systems are explored. Studies demonstrate the separation and analysis of cations using indirect fluorescence detection for the first time in a chip-based system. Resulting electrophoretic separations are achieved within a few tens of seconds with detection limits of approximately 6 μM. The reduced sample handling and rapid separations afforded by the coupling of indirect fluorescence detection with chip-based capillary electrophoresis provide a highly efficient method for the analysis and detection of molecules not possessing a chromophore or fluorophore. Furthermore, limits of detection are on a par with reported chip-based protocols that incorporate precolumn derivatisation with fluorescence detection. The current device circumvents lengthy sample preparation stages and therefore provides an attractive alternative technique for the analysis biogenic amines.

Keywords: Biogenic amines / Capillary electrophoresis / Indirect fluorescence / Microchip / Polydimethylsiloxane
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1 Introduction

Miniaturisation of conventional analytical instrumentation has been the focus of a great deal of attention during the last decade. Specifically, the development of the concept of the miniaturised total analysis system (μ-TAS) by Manz *et al.* [1, 2] has influenced many unique systems for genetic analysis, clinical diagnostics, chemical synthesis, environmental monitoring and drug screening [3, 4] to name but a few. The advantages inherent in miniaturising analytical systems lie in improved efficiency with respect to sample size, response time, cost, analytical throughput, automation, parallel analysis, and functionality. Many liquid phase separation techniques have been successfully transferred to microscale, and include capillary zone electrophoresis (CZE) [5–7], capillary gel electrophoresis [8], free-flow electrophoresis (FFE) [9, 10], open-channel electrochromatography [11, 12], open-channel liquid chromatography (LC) [13, 14], packed-bed chromatography [15], micellar electrokinetic capillary chromatography (MEKC) [16, 17] and synchronised cyclic capillary electrophoresis (SCCE) [18].

Due to the small sample volumes usually encountered in microfluidic environments (pL-nL) efficient detection is a key issue in the successful implementation of many μ-TAS. Detection techniques used in chip-based capillary electrophoresis (CE) analysis have included a diversity of optical and electrochemical methods [19, 20]. However, due to reduced channel dimensions of the microchip format, fluorescence has proven the most widespread optical detection technique. This is in part due to the fact that the reduced pathlengths obviate sensitive UV detection. Fluorescence methods are extremely sensitive, provide low limits of detection, and have consequently been the detection method of choice in most chip-based analytical systems. Although fluorescence techniques are inherently sensitive, associated instrumentation can often be costly and relatively few analytes exhibit intrinsic fluorescence. Consequently alternative optical detection methods are often required. Indirect absorption and fluorescence methods afford such an alternative, and are particularly useful for the analysis of species that do not possess an exploitable chromophore or fluorophore. Such techniques initially proposed by Hjertén *et al.* [21] and successfully applied to conventional CE by Kuhr and Yeung [22] and Foret [23], allow for the analysis of a variety of both anionic and cationic species through the use of a background fluorophore in

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Abbreviations: His, histamine; **IFD**, indirect flourescence detection; **PDMS**, polydimethylsiloxane; **Put**, putrescine; **Trp**, tryptamine

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the running buffer. More recently, indirect fluorescence detection (IFD) methods have been successfully transferred to chip-based formats. For example, Sirichai and de Mello [24, 25] demonstrated the use of IFD in conjunction with chip-based CE for the analysis of commercial photographic developing agents. At the same time, Wallenborg and Bailey [26] reported the use of IFD methods in the analysis of explosives, and Landers and co-workers [27] used IFD methods for the analysis of complex amino acid mixtures on chip.

In indirect fluorescence the mechanisms of detection are well established and understood. A fluorescing ion is used in the background electrolyte. When the analyte under scrutiny passes through the detection zone, an increase (positive peak) or decrease (negative peak) will be observed. The sign of the peak is determined by the relative charges of the analyte and background fluorophore. Although a large number of applications to-date have employed a co-ionic system (analyte and fluorophore of the same charge), there have been many examples where counter-ionic systems have been implemented. Collet and Gariel [28] have demonstrated that cations can be readily separated by conventional CE and detected using an anionic fluorophore. Desbene *et al.* [29] also demonstrated the use of fluorescein as an anionic fluorophore in the detection of metal cations by CE.

When a fluorescing anion is chosen as the buffer ion in CZE, a fluorescence signal is created at the detector at all times. If an analyte anion passes into the detector region, local charge neutrality requires that there is a displacement of the fluorescing background even though the analyte ion does not absorb or fluoresce, resulting in a negative peak. If an analyte cation passes into the detector region, an increase in the fluorescence is observed. This is due to the fact that more fluorophore molecules need to be present to preserve charge neutrality, resulting in a positive peak. Hence as long as the analyte in question is charged, some kind of response will be observed in the detection zone.

Bioactive amino compounds with molecular weights less than 500 are generally termed biogenic amines. Examples of biogenic amines include histamine (His), tryptamine (Trp) and putrescine (Put), which are illustrated in Fig. 1.

These amines are synthesised from amino acids by enzymatic decarboxylation and widely exist in nature. Biogenic or naturally occurring amines have been the subject of a great deal of research, especially in neuroscience. There is now a readily available body of evidence supporting the role of these amines as neurotransmitters [30]. They have also been implicated in a variety of psychiatric and neurological disorders [31]. For example, elevated urinary tryptamine has been reported in phenylketonuria [32], and in cases of carcinoid tumour [33], and thyrotoxicosis [34]. Many of these amines are also present in a number of agricultural products and analysis of such compounds in foodstuffs is an expanding area of interest [35, 36]. It has been known for some time that foods rich in *p*-tyramine can cause hypertensive crises in individuals suffering from asthma [37]. Therefore, it is conceivable that other structurally similar amines present in foods can also contribute to such effects. His and related compounds are also thought to be responsible for the unpleasant symptoms arising in scrombroid fish poisoning [38], or in spoiled foods, [39] especially those that are capable of inducing allergic reactions.

In general biogenic amines possess no chromophore or fluorophore, and the polyamines, such as Put, are inherently more difficult to derivatise completely than monoamines. Consequently, analysis of biogenic amines has usually been performed preferentially in the native form by CZE using indirect UV absorption. For example, Arce and co-workers [40] demonstrated the electromigrated separation of 21 amino compounds containing biogenic amines by CZE with indirect UV detection. Nevertheless, recently a more efficient separation of biogenic amines has been accomplished by chip-based MEKC (with analyte derivatisation) as reported by Li *et al.* [41]. They demonstrated that eight biogenic amines could be separated by microchannel MEKC using sodium dodecyl sulphate (SDS) as the surfactant. Detection limits were reported in the region of 3–6 μM (based on a signal-to-noise ratio of 2). However, the authors noted that the derivatisation step was time – consuming, negating many of the advantages associated with chip-based methods. Typically each amine sample took 5–12 h at room temperature to react with a fluorescein isothiocyanate-labelling moiety.

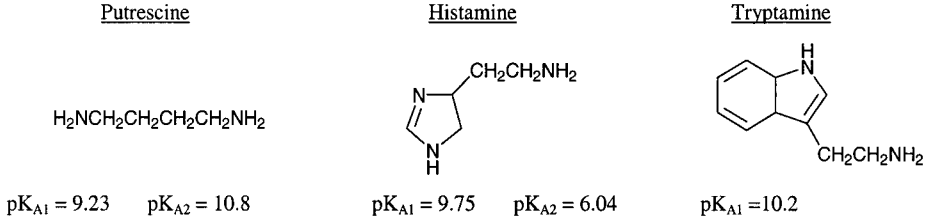

Figure 1. Chemical structures and pK_A values for the biogenic amines studied.

With this study in mind, we report herein the use of a chip-based CE system with indirect fluorescence detection for the separation and analysis of a synthetic mixture of biogenic amines in a rapid manner. Employing a microdevice constructed from polydimethylsiloxane (PDMS) and glass, both models of IFD are assessed (*i.e.*, co-ion and counter-ion detection) to optimise detection limits and component resolution.

2 Materials and methods

2.1 Microchip fabrication

CE microchips were manufactured in-house and comprised an unstructured glass substrate and a PDMS-moulded layer containing the microchannel network. Fabrication of the PDMS layer involved a three-step process: (i) the creation and fabrication of a chromium mask, (ii) the generation of an SU-8 master, (iii) the moulding of the device in PDMS. Brief details of each step are now presented: (i) The channel pattern was created using AutoCAD 95 and transferred onto a glass wafer precoated with a positive photoresist layer and a chromium layer (Nanofilm, Westlake Village, CA, USA) using a direct-write photolithographic system (DWL2.0, Heidelberg Instruments, Heidelberg, Germany). The exposed photoresist was first removed using a 5:1 ratio of developing agent (Microposit, Coventry, UK) to water. This was followed by a chromium etching procedure using a Lodyne etch (Microchem Systems, Coventry, UK). This resulted in a transparent microchannel layout of the chip design. (ii) A plain glass wafer was spin-coated with a negative photoresist XP SU-8 10 (Microchem, Newton, MA, USA) at 2500 rpm. The spinning speed determined the thickness of the SU-8 coating and thus the channel depth, and for the current studies was approximately 10 μm +/− 1 μm. The transparent channel network from the chromium mask was then transferred onto the SU-8 substrate by placing them together and curing under a 10 cm diameter collimated UV beam from a 200 W mercury lamp (Goulding and Partners, Sussex, UK). The unexposed SU-8 was then removed using 1,2-propanediol monomethyl ether acetate (PMEA) (Lancaster, Morecambe, UK), leaving the SU-8 network fixed to the glass substrate. This was then hard baked for 24 h on a hotplate at 120°C. (iii) PDMS base and curing agent (Sylgard 184; Dow Corning, Wiesbaden, Germany) were mixed in a 10:1 w/w ratio, degassed and decanted onto the SU-8 master. The resulting structure was cured overnight in an oven at 40°C. Reservoirs were created by punching holes at the end of the channels of the PDMS device using a specially constructed bradawl, thus allowing fluidic access. The PDMS structure and a plain glass substrate were cleaned in 1 M sodium hydroxide and ethanol before being brought into contact, to form the complete microdevice. The layout of the CE microchip used in this work is shown schematically in Fig. 2a. A scanning electron micrograph of the injection port is also shown to demonstrate the quality of the PDMS channels throughout the fluidic network. The periodic structures apparent in the injection channel (B) are inherent to the functioning of the DWL system, in that the laser spot size does not completely overlap in the *x*-plane resulting in the observed 'ripple' effect in the injection channel. This periodic structure did not

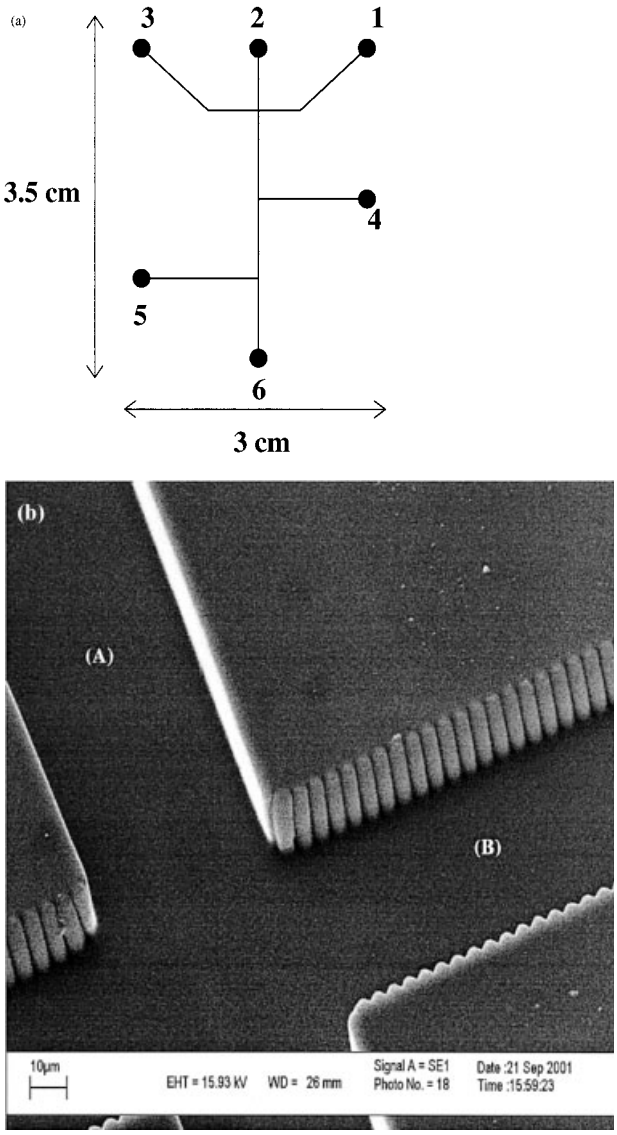

Figure 2. (a) Microchannel pattern of the CE device. Channels referred to in the text are identified by corresponding numbers. (b) Scanning electron micrograph of injection port: (A) separation channel, (B) injection channel.

affect the performance of the device during separation. The device includes an injection channel and a variable length separation channel. The distances from the injection intersection to the buffer inlet (2), buffer outlet (4, 5, 6), sample inlet (1) and sample outlet (3) reservoirs were 6.6, 26.0/35.5/27.2, 18.2 and 18.2 mm, respectively. The width and depth of the entire channel network were 40 and 10 μm, respectively.

2.2 Instrumentation

Electrophoretic separations were observed *via* fluorescence and indirect fluorescence detection methods. Using an inverted microscope (DMIL; Leica, Milton Keynes, UK) and a filter cube comprising of an excitation filter (BP 450–490 nm), a dichroic mirror (RKP 510), and a suppression filter (BP 515–560 nm). Excitation light (488 nm) from a 50 mW tuneable air-cooled argon-ion laser (Melles-Griot; Cambridge, UK) was passed through an excitation filter, reflected by the dichroic mirror and focused onto the microchip. Fluorescence emission was collected by a microscope objective (10×, 0.42 NA) (Newport, Irvine, CA, USA), passed through the dichroic mirror, a suppression filter, and finally through an adjustable detection window set at 20 μm × 60 μm. A photomultiplier tube (MEA153; Seefelder Messtechnik, Germany) functioning in current mode was employed to detect fluorescence photons. Data were acquired at a frequency of 20 Hz, stored using a PC data acquisition program (Picolog; Pico Technology, Hardwick, Cambridge, UK) and processed in Origin 6.0 (Microcal Software, Northampton, USA). A specially constructed 8-channel power supply was used to supply the drive voltages for electrophoresis and operated between 0 and +3000 V relative to ground. Voltage control was effected using a programme written under the LabView 5.0 graphical programming environment (National Instruments, Austin, TX, USA). Prior to experimentation, the microchip was charged with ethanol by capillary action. This process was crucial to efficient operation, and allowed subsequent aqueous solutions to be introduced to the somewhat hydrophobic PDMS channels. The microchip was operated in one of two modes, 'sample loading' or 'separation' mode. Electrical contact between the power supply and fluidic reservoirs was made using platinum electrodes. In all experiments, sample in the sample inlet reservoir (1) was injected *via* the 'transept' of the microchip through the sample outlet reservoir (3) by applying voltages. The size of the sample plug to be injected was controlled by the use of 'pinching' voltages applied to buffer inlet (2) and outlet (4) reservoirs. The pinching voltages were optimised at 83% for reservoir (2) and 93% for reservoir (4) of the sample loading voltage applied. The sample plug formed at the intersection was then injected into the separation channel by applying voltages across the buffer inlet (2) and outlet (4) reservoirs. During the separation mode, the sample inlet (1) and outlet (3) are maintained at 36% (optimised) of the potential applied to the buffer reservoir. This prevents leakage of the sample into the separation channel from the sample inlet (1) and sample outlet (3) channels during separation. All experiments were carried out using freshly prepared solutions, to reduce the experimental errors inherent in the analysis.

2.3 Chemicals

The biogenic amine standards Put, His and Trp were all 96–99% pure (Sigma-Aldrich, Gillingham, Dorset, UK). Buffer solutions were made from *ortho*-phosphoric acid, sodium phosphate, disodium phosphate and trisodium phosphate (Sigma-Aldrich). The pH of the buffer system was altered using sodium 0.1 M hydroxide and *ortho*-phosphoric acid. Rhodamine 110 chloride (Fluka Chemicals, Gillingham, Dorset, UK) was used as received. Fluorescein (Fluka Chemicals) was used in its disodium salt form, and along with 5-carboxyfluorescein (Molecular Probes, Leider, Netherlands) were used as received. To aid with the dissolution of the biogenic amines, each were dissolved in a small amount of ethanol and/or methanol before dissolving in the running buffer. Furthermore, to prevent the amines solubilising in the microchannel during separations, a small percentage of organic solvent (~3%), in this case 2-propanol, was used. This procedure has been well documented as an efficient way of encouraging hydrophobic compounds to stay dissolved in an aqueous environment [42].

3 Results and discussion

The microchip employed in this work was first characterised by separating two fluorescent dyes, fluorescein and 5-carboxyfluorescein. As expected, the two dyes could be resolved and separated within a few seconds using moderate electric field strengths. In the presence of electroosmotic flow, the efficiency of an electrophoretic separation can be defined in the terms of the number of theoretical plates (*N*) as defined in Eq. (1).

$$N = \frac{(\mu_{ep} + \mu_{eo})V}{2D} \quad (1)$$

$$N = 5.54\left(\frac{t}{w^{0.5}}\right)^2 \quad (2)$$

Table 1. Efficiencies, reproducibilities and detection limits obtained for Put, His and Trp

Biogenic amine	Concentration detection limit (μM)	Theoretical plates per channel (*N*/2 cm)	Theoretical plates (*N*/m)	*H* per channel (μm)	% RDS for migration times	
					intraday	interday
Put	6	1026	51 317	19.49	2.68	6.80
His	7.5	3168	158 445	6.31	3.21	6.58
Trp	6.5	4291	214 590	4.66	5.80	7.44

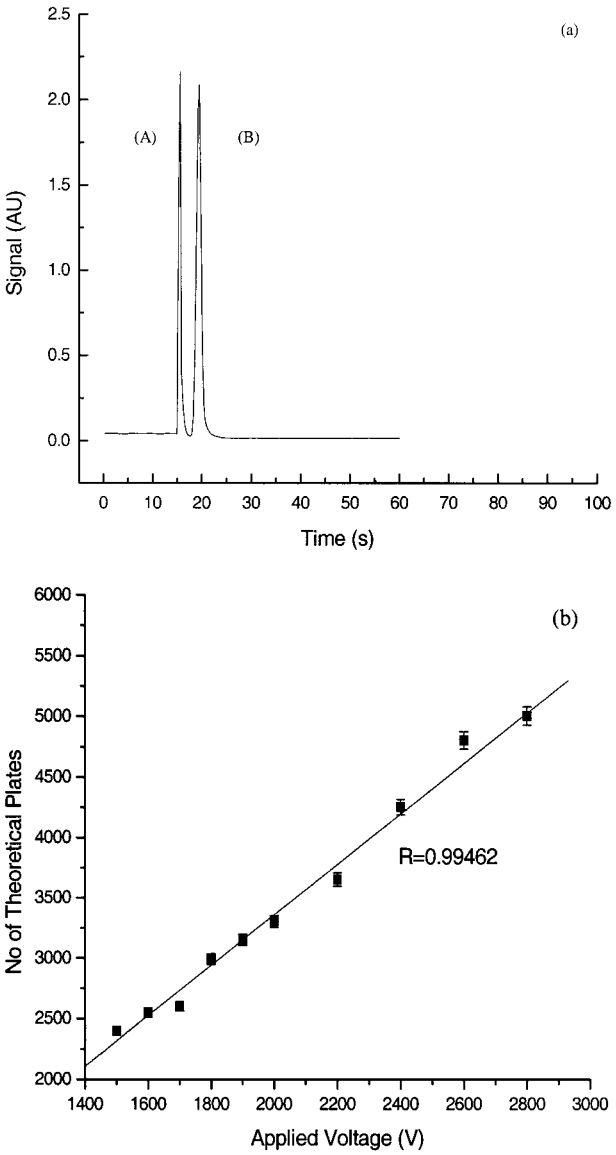

Figure 3. (a) Electrophoretic separation of two fluorescent dyes; peak identities: (A) fluorescein (100 μM), (B) 5-carboxyfluorescein (100 μM); buffer, 30 mM phosphate at pH 9.4. (b) Variation of number of theoretical plates (*N*) with applied voltage for injections of 100 μM fluorescein; buffer: 30 mM phosphate at pH 9.4. For all experiments, separation channel, 40 μm × 10 μm × 26 mm.

Here μ_{ep} and μ_{eo} are the electrophoretic mobility ($cm^2V^{-1}s^{-1}$) and electroosmotic flow ($cm^2V^{-1}s^{-1}$), respectively, *V* is the total applied voltage (V), and *D* is the specific molecular diffusion coefficient (cm^2s^{-1}). For calculations described in Table 1, *N* is calculated directly from electropherograms using Eq. (2), where *t* is the migration time (s) and $w^{0.5}$ is the width of the peak at half its height (s). Figure 3a illustrates a typical electrophoretic separation of fluorescein and 5-carboxyfluorescein, thus demonstrating the quality of the separations achievable within the fabricated microchannel network. The electropherogram shows very little peak tailing and good resolution. Figure 3b shows the variation of the number of theoretical plates as a function of the applied voltage. The plot demonstrates that the relationship between *N* and *V* is a linear one as predicted by Eq. (1). As well as agreeing with theory, this also indicates that there is no significant Joule heating within the range of electric field strengths used. In conclusion, Fig. 3 demonstrates that the fabricated microchannels are free from artefacts or deformities, and that the separations obey the theoretical relationships.

As stated previously, in IFD fluorescing ions are located in the background electrolyte, and a change in the fluorescent intensity occurs due to displacement (negative peaks) or electrostatic attraction (positive peaks) mechanisms. In this work, both situations have been explored, since Rhodamine 110 is a zwitterionic ampholyte (with a calculated pK_A of 6.96 +/− 0.02). In an acidic environment the fluorophore is cationic and hence a negative peak from the amines is expected due to the need to retain local charge neutrality. This effect can be seen for an electrophoretic separation of Put, His and Trp (Fig. 4a). Under these experimental conditions it is observed that the His and Trp peaks could not be resolved. This is due to the fact that in an acidic environment the amines all carry the same charge, and the pH of the system is below that of the second pK_A of all the analytes. As a result the amines are now only separable by differences in their mass. This would require a separation length unattainable on our microchip (hundreds mm's) or electric fields $>$ 5000 V/cm. Consequently, instead of changing the physical geometry of the microchip the separation

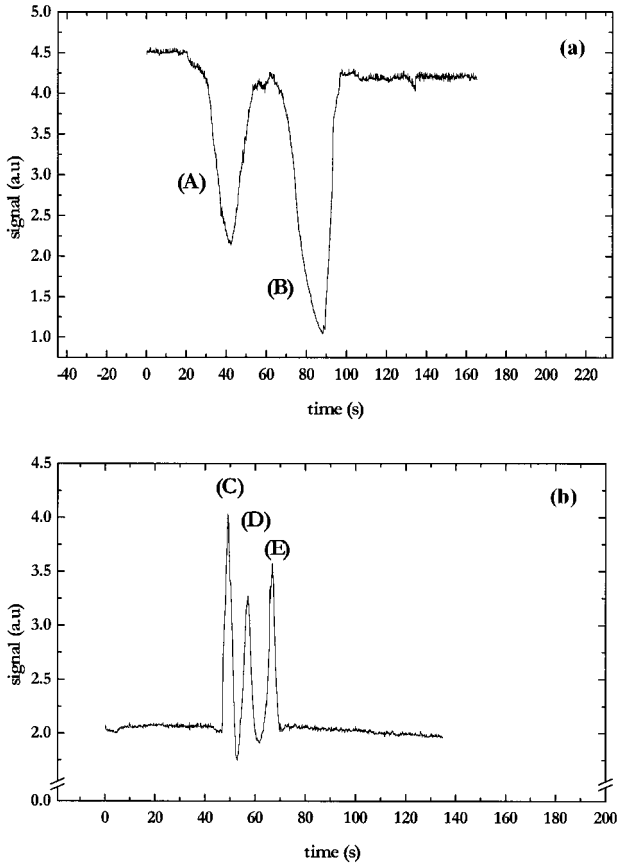

Figure 4. (a) Electrophoretic separation of biogenic amines using a co-ionic IFD system. Buffer, 30 mM phosphate, 3% 2-propanol and 500 μM Rhodamine 110 at pH 2.2. Peak identities: (A) Put, (B) His and Trp. (b) Electrophoretic separation of biogenic amines using counterionic IFD system. Buffer, 30 mM phosphate, 3% 2-propanol, 500 μM Rhodamine 110 at pH 9.4. Peak identities: (C) Put, (D) His, (E) Trp. For both systems, separation channel, 40 μm × 10 μm × 26 mm, distance to detection window, 20 mm; injection voltage, 1000 V between reservoirs 1 and 3 for 40 s; separation voltage, 2000 V between reservoirs 2 and 4.

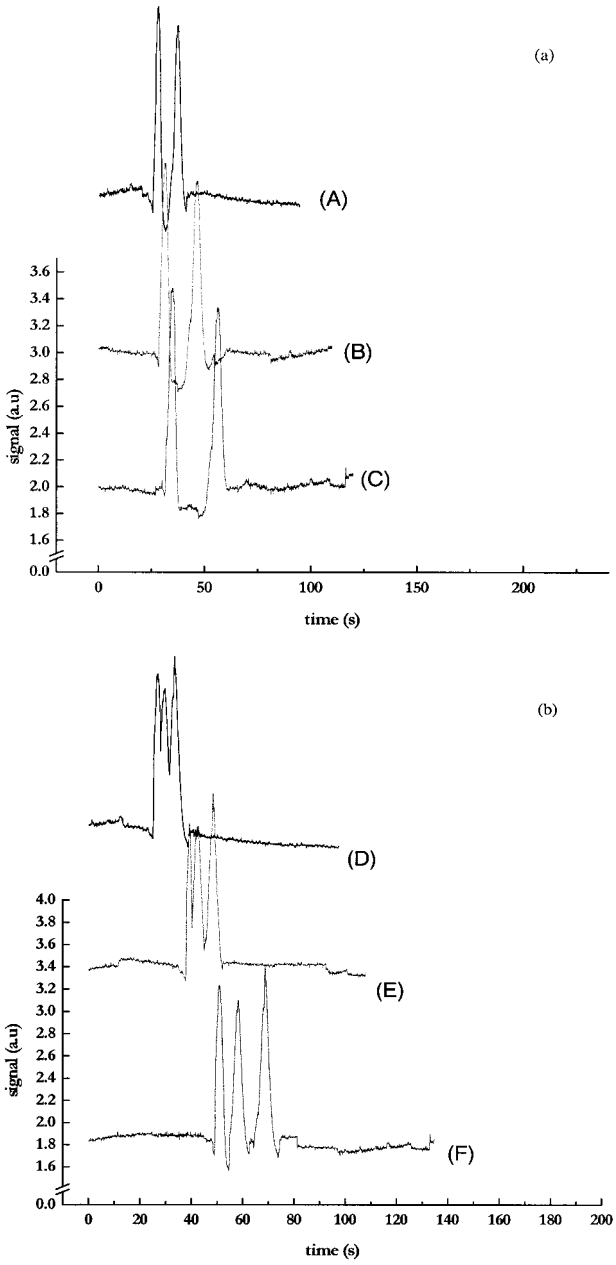

Figure 5. (a) Electrophoretic separation of Put and Trp as a function of separation length. Buffer, 30 mM phosphate, 3% 2-propanol, 500 μM Rhodamine 110 at pH 9.4; (A) 10 mm, (B) 15 mm, (C) 20 mm effective separation length. (b) Electrophoretic separation of Put, His and Trpt showing the effect of applied voltage on the separation. Buffer, 30 mM phosphate, 3% 2-propanol, 500 μM Rhodamine 110 at pH 9.4. (D) 3000 V, (E) 2500 V, (F) 2000 V. For all experiments, separation channel, 40 μm × 10 μm x 26 mm.

conditions were varied. Since buffer pH is an influential factor in defining component resolution a pH closer to the pK_A values of the biogenic amines (pH 9.4) was chosen to afford superior separation conditions. Studies of pK_A and its effect on the electrophoretic separation of various analytes have been discussed in detail by Zhang *et al.* [43–45].

Figure 4b demonstrates that by using a pH closer to the pK_A's of the biogenic amines the three-component synthetic mixture can be fully resolved. It should be noted that the peaks are now positive, since the Rhodamine 110 is now in its anionic form. Consequently, cations moving through the detection volume generate a positive peak due to electrostatic attraction. The efficiency of the separation was assessed by varying the applied separation length and electric field, as shown in Figs. 5a and b

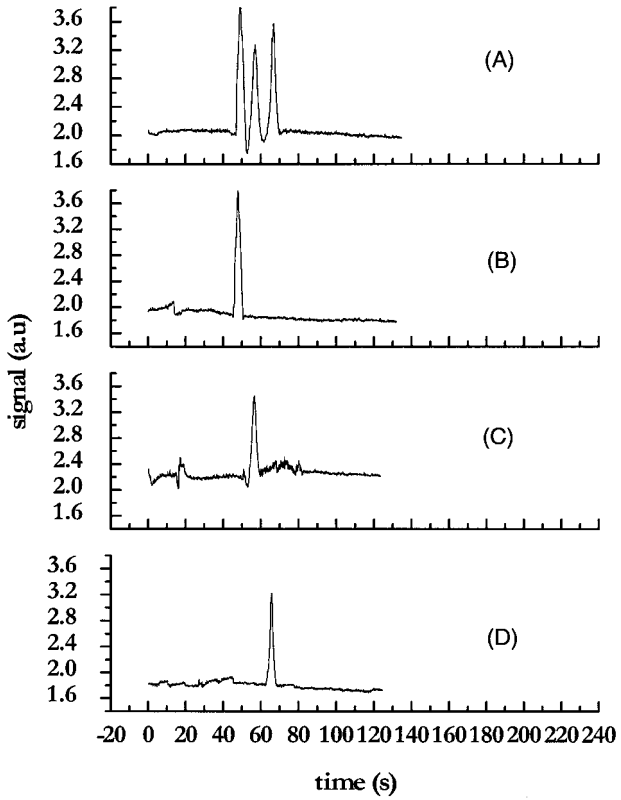

Figure 6. Electropherograms of a 3-component mixture and single component injections illustrating identity of each biogenic amine. Buffer, 30 mM phosphate, 3% 2-propanol, 500 μM Rhodamine 110 at pH 9.4. (A) 3-component mixture of Put, His and Trp, (B) single injection of Put, (C) of His, (D) of Trp. For all experiments: separation channel, 40 μm × 10 μm × 26 mm; distance to detection window, 20 mm; injection voltage, 1000 V between reservoirs 1 and 3 for 40 s; separation voltage, 2000 V between reservoirs 2 and 4.

respectively. Figure 5a demonstrates that by increasing the effective separation length to 20 mm putrescine and tryptamine peaks can be fully resolved. Furthermore, an increase in the applied electric field can reduce total separation times below 30 s (Fig. 5b). To identify the biogenic amines within the separation mixture, each biogenic amine was injected individually and subsequently classified according to its migration time. These results are illustrated in Figure 6. It can be seen that the amines elute in the order according to their mass-to-charge ratio, in which Put elutes first followed by His and finally Trp. Good correlation in migration time between single injections and the mixture analysis confirms peak identities.

An optimised buffer concentration of 30 mM was used for all separations discussed. It was found that by increasing the concentration of the buffer a slight increase in resolution occurred, as has been demonstrated previously [25]. However, an increase in the conductivity is also observed as the buffer concentration is raised. This leads to increased Joule heating within the microchannel, and consequently the minimum buffer concentration that provided sufficient resolution for the biogenic amines was used for all analyses. The concentration of the background fluorophore Rhodamine 110 was also varied but little gains in sensitivity were observed. This is in agreement with previous studies in which marginally better stability and sensitivity with a lower concentration of background fluorophore has been reported. Furthermore, it should be noted that fluorescein was also tested as a background fluorophore. The background stability, however, was poor when compared with Rhodamine 110. This effect has previously been observed by Kennedy *et al.* [46] who reported Rhodamine B to be a much more stable fluorophore than fluorescein when detecting explosives by IFD. This is most likely due to the fact that the fluorescence quantum efficiency of fluorescein is highly dependent on local pH whereas Rhodamine 110 shows little variation in its fluorescence quantum efficiency over a wide pH range [47]. It has also been reported by Garner and Yeung [48] that the baseline of a fluorescein fluorescence signal becomes erratic when the pH of the buffer system is increased above 7.

Table 1 shows calculated efficiency, reproducibility and sensitivity measurements for each biogenic amine studied. Efficiencies were calculated using Eq. (2), and the resultant plate height is given in μm. The increase in plate number, when compared to previous chip-based analyses [41], is largely due to the control of the plug size with pinching voltages at the injection cross. This allows injection of a highly reproducible sample volume and prevents any leakage from the injection channel into the separation channel during the electrophoretic separation. The results presented here demonstrate, however, that the efficient separation of native biogenic amines can be achieved by manipulating the conditions of the separating media and exploiting the gains inherent in the microchip format. These include the ability to use high electric fields due to effective heat dissipation, and the ability to produce very small injection volumes.

The reproducibility of the system is quoted in terms of percentage relative standard deviation (%RSD) of the migration times, details of which can be found in Table 1. From this data it is concluded that the system has adequate reproducibility both intra- and interday. A slightly higher %RSD for interday measurements is to be expected, due to experimental differences in solution make-up. The detection limits for each of the biogenic amines were calculated using serial dilution of the stock solutions, and the results in Table 1 are based on a mini-

mum signal-to-noise ratio of three. Results indicate detectable concentrations in the range of 6–7.5 μM, which falls well within the range required for most applications within the food analysis [41] and dental industries (Wilkes, personal communication).

4 Concluding remarks

The results presented in this study demonstrate the use of microchip-based CE with indirect fluorescent detection for the separation and analysis of biogenic amines. By focusing more on the separation conditions, and less on the background electrolyte, satisfactory resolution and efficiencies are attainable. In particular, by adjusting the separation conditions to take into account the pK_A of the analytes, the greatest difference in charge and mass is realised, thus allowing separation to occur over much shorter separation lengths than on conventional capillaries. Plate numbers up to 214 590 (*N*/m) have been obtained for Trp with an associated plate height of 4.66 μm, and detection limits of 6.5 μM. These are surprisingly highly comparable to previous results incorporating precolumn derivatisation, in which detection limits (using fluorescence methods) on chip were in the low μM region (based on a minimum signal-to-noise ratio of 2) [41].

One of the primary advantages of the indirect fluorescence detection method presented herein is the omission of tedious pre- or postcolumn derivatisation of the analytes. As stated earlier, Li and co-workers [41] quoted a reaction time of 5–12 h between FITC and biogenic amines for labelling purposes [41]. However, it is noted that due to the nonselective nature of the indirect detection mode, and the complex matrices associated with most 'real' samples, possible interference in detection need to be addressed, in particular the stability of the background fluorophore and other irregularities common with indirect detection methods. Improvements in our IFD methods and investigations into incorporating labelling, separation and detection of biogenic amines on a single chip are currently underway. The incorporation of a labelling step with a rapid reaction time, and in-line separation, will afford improvements in both sensitivity and selectivity for 'real' samples.

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