MICROSCALE PHOTOLYTIC ELUENT GENERATION FOR
CHROMATOGRAPHY
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ABSTRACT
This paper describes a new method for the generation of gradients for microscale chromatography based on the photolytic degradation of photosensitive reagents. An inert dissolved photochemical is placed in the eluent and irradiated by variable intensity LEDs to proportionately degrade the photochemical into simple chemical components, such as HCl, which can be used to create pH gradients which can be used to perform gradient separations of antipsychotics.

KEYWORDS: chromatography, gradients, photochemistry

INTRODUCTION
One of the most significant challenges for the miniaturisation of chromatography is the problem of creating temporal eluent composition gradients in systems with flow rates in the order of one microlitre per minute and lower. Chromatographic gradients are typically created using programmable pumps that combine the liquids from two or more different solutions, and this has been implemented in microchips by Zhang and Roper [1] using a series of pneumatic valves and Lazar and Karger [2] using electroosmotic pumps. Brennan et al. [3] designed a microfluidic chip which can produce smooth gradients by carefully combining a sequence of flows of two solutions. The chip worked by splitting the flow into a series of 20 parallel channels of different lengths which recombined downstream.

All of the methods of creating eluent gradients discussed thus far have involved combining at least two different through adjective or diffusive processes. However, there is an entirely distinct approach based on dynamically changing the chemical composition of a single flowing liquid. In the past decade, Dionex Corporation developed electrolytic eluent generation for ion chromatography applications, which had been pioneered by Strong et al. in 1991 [4,5]. This method relies on electrolysis of water and migration of ions across a semi-permeable membrane to create eluents with variable concentrations of KOH, H₂CO₃ or methanesulfonic acid determined by the applied electric current.

We have previously demonstrated a new concept for for the generation of salt gradients for cation-exchange chromatography: photolytic eluent generation [6]. The photochemical reagent, desyl chloride, was degraded by exposure to UV light through the simultaneous use of 4 UV 250 – 290 nm LEDs to release HCl. The concentration of HCl released could be varied from 0.5 – 1.5 mM by simple variation of the LED current intensity. In this work, we examined a number of different photochemical reagents for the production of pH gradients.

EXPERIMENTAL
The chromatographic setup used was similar to that described previously [6] and is shown in Figure 1. Briefly, eluent (50/50 w/w ACN) with 1-15 mM photochemical reagent was passed through the reaction cell, a piece of 1 mm quartz tubing placed on an aluminum groove (Figure 2) with pieces of capillary glued into each end to interface to the capillary LC column (a Zorbax SB-C18 with 5 μm particles, measuring 150 x 0.5 mm, Agilent, Santa Clara, CA, USA). Eluent flow was controlled by an Agilent 1200 series capillary pump G1376A and detection was performed with an Agilent 6320 Series Ion Trap with an electrospray ionisation source.

2-chloro-1-(2,5-dimethylphenyl)ethanone (CDMEP) was used as the photosensitive eluent component and the absorbance spectra in 50% w/w acetonitrile-water were at a concentration of approximately 0.63 mmol/L using a cell width of 1 mm is shown in Figure 3.

Chlorpromazine hydrochloride (CHL), clozapine (CLO), haloperidol (HAL) and thioridazine hydrochloride (THI) were obtained from Sigma–Aldrich whilst amisulpride (AMI) and risperidone (RIS) were obtained from Sequoia (Oxford, UK). 2-chloro-1-(2,5-dimethylphenyl)ethanone 95% was purchased from ChemBridge (CA, USA).

Figure 1: Schematic of the photolytic eluent generation system for the generation of microscale pH gradients.
RESULTS AND DISCUSSION

Investigation of a number of photochemical reagents revealed that CDMEP was the most appropriate photochemical to work with due to its greater acid generation and reduced tendency for reaction cell fouling compared to desyl chloride which was used in our previous work [6].

Beginning with a solution consisting of 10 mM CDMEP in 50% acetonitrile-water, two separate buffers were formulated. The first buffer used basic compounds, whilst the other used acidic compounds. The cation exchange buffer system uses 1.0 mmol/L each of imidazole and piperazine as buffers. The effect of LED current on the pH of this eluent is shown in Figure 4, (hollow squares). Meanwhile, the anion exchange buffer system uses 0.87 mM each of ammonium acetate and ammonium bicarbonate. The effect of LED current on the pH of this eluent is shown by the solid triangles in Figure 4. The results demonstrate that it is possible to create a microscale photochemical eluent pH control system by the methods described. Both eluent systems were able to reach pH* values below 3 and higher than 8.

Figure 4 shows the separation of 6 basic antipsychotics using the photolytic eluent generation system. The pH* of the eluent should be 5.5 at 10 mA and approximately 2.7 at 22 mA. Considering the 10 mA separation (Figure 5.2, top), it can be seen that the drugs elute in an early group (consisting of AMI, RIS, CLO and HAL) and a late group (CHL and THI) after 30 minutes. The separation can be shortened to just 10 minutes by increasing the current to 22 mA to give a pH* of 2.7 (Figure 5.2, bottom). However, the selectivity between AMI, RIS and CLO is lost, possibly because they enter a doubly charged state at this low pH that reduces their hydrophobicity to very low levels. The gradient capability of the photochemical eluent pH control system was demonstrated by performing a gradient elution (Figure 5.2, middle) with ascending current (descending pH*) as described in the figure caption. The gradient elution program allowed the selectivity of AMI, RIS and CLO to be maintained whilst the peak shape of THI was improved and the elution time was reduced to 17 minutes.

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