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# Ion chromatography on-chip

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**Abstract**

On-chip separation of inorganic anions by ion-exchange chromatography was realized. Micro separation channels were fabricated on a silicon wafer and sealed with a Pyrex cover plate using standard photolithography, wet and dry chemical etching, and anodic bonding techniques. Quaternary ammonium latex particles were employed for the first time to coat the separation channels on-chip. Owing to the narrow depths of the channels on the chip, 0.5–10 μm, there were more interactions of the analytes with the stationary phase on the chip than in a 50-μm I.D. capillary. With off-chip injection (20 nl) and UV detection, $NO_2^-$, $NO_3^-$, $I^-$, and thiourea were separated using 1 m*M* KCl as the eluent. The linear ranges for $NO_2^-$ and $NO_3^-$ are from 5 to 1000 μ*M* with the detection limits of 0.5 μ*M*. 

*Keywords:* Chip technology; Instrumentation; Latex nanoparticles; Microseparation; Inorganic anions; Thiourea

## 1. Introduction

There is a growing interest in the miniaturization of separation techniques on-chip because of the advantages of reduced reagent consumption, better analytical performance, shorter analysis time, and the applicability to process analysis and field analysis [1–3]. The majority of the published work is CE based, primarily due to the ease of implementation and the superior efficiency due to the flat flow profile that this method has over pressure driven methods. Even though traditional liquid chromatography techniques, such as high-performance liquid chromatography (HPLC) and ion chromatography (IC), have played an important role in routine analysis, there are very few research papers on miniaturized LC, particularly ion-exchange chromatography. One of the main difficulties is the necessity to introduce a stationary phase into the narrow channels on-chip. Several methods have been proposed, including packing [4], coating [5], in-situ polymerization of continuous bed [6], and in-situ micromachining of monolithic support structures and subsequent derivatization [7]. Among all these methods, coating is the easiest one to implement, but the phase ratio is usually not very high. Narrow channels and thicker stationary phase, such as a nanoparticle coating, could increase the opportunities for analytes to interact with a coated stationary phase, and hence for better separation.

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Latex nanoparticles have been widely used in coating capillaries in ion chromatography and capillary electrokinetic chromatography [8–10]. However, they have not been employed to coat microchannels on a chip. In this paper, a microseparation column for ion chromatography was fabricated on silicon substrate and covered with Pyrex glass. Latex nanoparticles were employed to coat the micromachined channel on-chip and on-chip separation of inorganic anions, nitrate, nitrite, and iodide, was tested.

## 2. Experimental

### 2.1. Materials and reagents

Quaternary ammonium latex particles (AS5A type) with an approximate size of 75 nm were supplied as an 11% (w/v) aqueous suspension from Dionex (Sunnyvale, CA, USA). Standards of 1 m*M* $NO_2^-$, $NO_3^-$ and $I^-$ were prepared from sodium or potassium salts of analytical grade. Polyether ether ketone (PEEK) tubing, PEEK sleeves, nuts, ferrules and zero dead volume fittings were obtained from Sigma–Aldrich (Dublin, Ireland). Fused silica capillary (50 μm I.D., type TSP050150; 40 μm I.D., type TSP040150) was purchased from Composite Metals (Hallow, Worchester, UK). Also, 18.2 MΩ Millipore water (Switzerland) was used in making up all standards.

### 2.2. Instrumentation

An Ultra Plus micro-pump system (Micro-Tech Scientific, USA), together with a 20-nl manual injector (Presearch, UK) was employed for off-chip pumping and injection. A Shimadzu SPD 10Avp detector (Shimadzu, Japan) fitted with a 35-nl flow cell (Presearch) was used for spectrophotometric detection at 214 nm. An HP3392A integrator (Hewlett-Packard, USA) was used to record the chromatograms. An LC-8A preparative liquid chromatographic pump was used for coating the capillaries and devices. Connections were realized using PEEK sleeves and standard LC zero dead volume fittings.

### 2.3. Chip fabrication

The miniaturised liquid chromatography chips were fabricated using standard photolithography, wet and dry chemical etching, and bonding techniques described previously [11]. Chip layout was designed on a Sun Sparc workstation at the National Microelectronics Research Centre (Cork, Ireland). The separation channels were micromachined on 4 inches ⟨100⟩ orientation silicon wafers (1 inch=2.54 cm). These channels were isotropically etched to depths of 0.5–10 μm using the dry etchant $SF_6$. Using a second photolithography stage and anisotropic etching, V groove connection ports were micromachined on the same wafers. Finally, the patterned silicon wafer was anodically bonded to a Pyrex glass cover plate to seal the channels. After diamond sawing to reveal the V grooves, fused-silica capillaries were inserted into the grooves and held in place using a non-conductive epoxy. The fused-silica capillaries were then connected to external instrumentation using standard LC fittings.

### 2.4. Preparation of coated capillaries and devices

Coated capillaries were prepared by treating the capillary with 1 *M* NaOH for 30 min using a syringe placed in a modified ''glue gun'' attachment to provide a consistent and even pressure. The capillary was then rinsed with water before a 1:10 dilution of AS5A latex suspension was passed through the capillary for 30 min using the same syringe attachment. The suspension was left at rest in the capillary for a further 30 min before being conditioned with mobile phase for 30 min before use.

The miniaturised liquid chromatography chips were coated by flushing a dilute suspension of latex particles through the chip for 30 min using the HPLC pump. The suspension was left in the device for 30 min at rest before being flushed out with water to ensure that there were no blockages. Coated chips were equilibrated with mobile phase for 30 min before use.

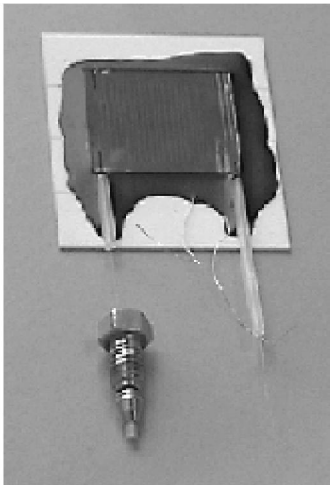

Fig. 1. Microfabricated column on silicon.

## 3. Results and discussion

### 3.1. Miniaturised LC on-chip system

The chips employed in this study are 20×20 mm in size (Fig. 1) and were manufactured according to details provided in Section 2. The micro-column dimensions are 23 cm×200 μm×3.6 μm having a volume of 115 nl and are ideally suited to perform open-tubular (OT) separations due to the smallest distance (1.8 μm) through which the analytes need to diffuse to reach the stationary phase. Off-chip injection and detection were employed so that both the chip and the connecting capillary can be regarded as the ''separation column''. Sections of fused-silica capillary (length between 4 and 15 cm) were used to connect the chip to the injector and the detector, which become coated with latex during the chip-coating procedure, and the significance of this was therefore also examined.

### 3.2. Comparison between OT-chip and OT-capillary columns

Cationic latex particles have been found to absorb onto fused-silica by Pyo et al. [10] who used particle coated capillaries for the separation of inorganic anions by high temperature OT-LC. The connecting tubing on the chip will therefore become coated during the chip-coating procedure and will provide some ion-exchange sites with which the analytes can interact. To examine the contribution from the fused-silica sections, a 3.5-m×50-μm I.D. section of capillary was coated with AS5A latex particles and the separation of selected inorganic anions examined in the OT-LC mode. This is shown in Fig. 2a where the separation of thiourea (neutral marker), $NO_3^-$ and $I^-$ was readily achieved using a 1.0-m*M* KCl eluent.

The potential for chip-based separations was ex-

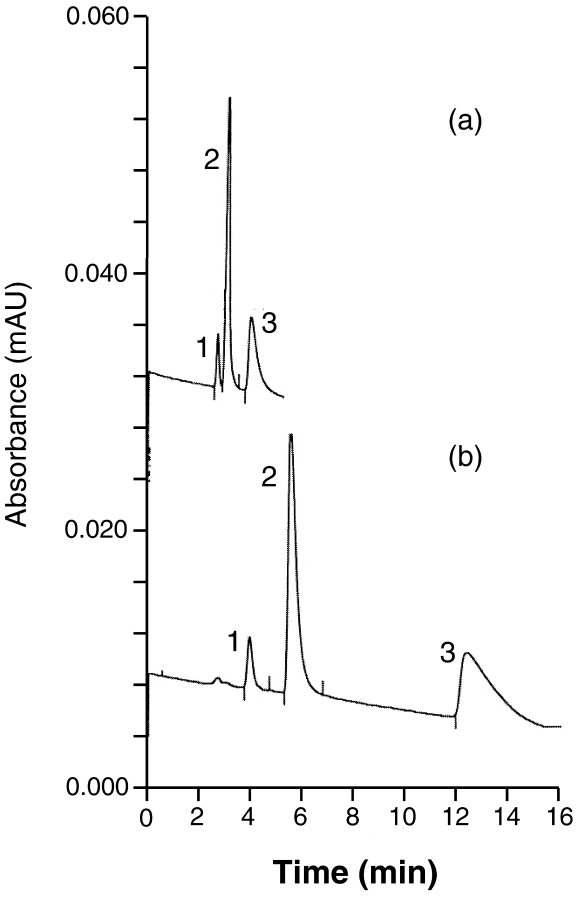


Fig. 2. (a) Separation of inorganic anions using a 3.5-m×50-μm I.D. capillary column coated with anion-exchange AS5A latex particles. Flow rate of 2.6 μl/min. (b) Separation of inorganic anions using a silicon chip (micro-channel dimensions 23 cm× 200 μm×3.6 μm) and 34 cm of coated connecting capillary. Flow rate of 150 nl/min. Eluent in all separations is 1 m*M* potassium chloride. Peaks: 1=thiourea, 2=$NO_3^-$, 3=$I^-$.

amined by coating a chip having a total of 34 cm of 50-μm I.D. connecting capillary. Initial attempts to coat the chip simply by flushing the device with latex suspension were successful and no further attempt to optimise the coating procedure was undertaken. Fig. 2b shows the separation of the same analytes in the same conditions as the capillary column, the only difference being the magnitude of the flow-rate. It can be seen, that there is significantly more retention of the analytes in the chip-based separation in comparison to the capillary column. To examine the change in retention, the capacity factor ($k'$), which is independent of flow-rate, can be calculated according to the equation:

$$k' = \frac{t_r}{t_r - t_0}$$

where $t_r$ is the retention time of the analyte, and $t_0$ is the retention time of an unretained solute, with thiourea being used for this purpose. Calculating the capacity factor for the analytes, then $NO_3^-$ increased from 0.12 and 0.40 on changing from capillary to chip-based column while, for $I^-$, it changed from 0.48 and 2.27 (shown in Table 1). This increase in capacity is not surprising given the narrow depth of the channels on the chip and as such there will be more interaction of the analytes with the stationary phase.

While the separations obtained in the chip were promising, the large length of capillary used to connect the chip to the off-chip devices was long (17 cm on each end). This provides further distance through which the analytes must travel to reach the detector, making the separation slow, and also provides the potential for interaction through the coated capillary. To rectify this, the connecting capillary was reduced to 8 cm (4 cm on each end), the smallest possible length that could safely be connected to the external devices. The influence of reducing the length of capillary can be seen in Table 1, where it can be seen that the void now occurs at 0.83 min in the chip with the shorter connecting capillary, in comparison to 3.97 min in the chip with the longer capillary. This therefore provides more rapid separation with $I^-$ now eluting in 2.75 min, almost 10 min quicker. Values for retention factors remained virtually unchanged as the capillary was shortened indicating that the majority of analyte retention originates on the coated microchannels on the chip and not from the connecting capillary.

### 3.3. Demonstration of ion-exchange behaviour

The separations obtained above suggest ion-exchange behavior of the analytes on adsorbed latex particles on the chip and typical approaches employed in IC to control retention should likewise be applicable. This is most readily achieved by changing the concentration of the competing ion where an increase in eluent anion concentration will result in a decrease in analyte retention. To examine this, separations of thiourea, $NO_2^-$, $NO_3^-$ and $I^-$ with eluent concentrations of 0.1, 0.5, and 1.0 m*M* $Cl^-$ were carried out. It can be seen from Fig. 3 that as the concentration of $Cl^-$ is decreased, analyte retention increases so that thiourea, $NO_2^-$ and $NO_3^-$ are easily separated at 0.1 m*M* $Cl^-$. This increase in retention should vary with the concentration of eluent anion ($E$) according to the equation:

$$\log k' = C - \frac{x}{y}\log[E]$$

where $C$ is a constant, $x$ is the analyte anion charge

Table 1
Comparison of retention times and retention factors of $NO_3^-$ and $I^-$ in chips with differing lengths of coated capillary used to connect chips to off chip devices

| Analyte | 350 cm capillary | | Chip + 34 cm capillary | | Chip + 8 cm capillary | |
|---|---|---|---|---|---|---|
| | $t_r$ | $k'$ | $t_r$ | $k'$ | $t_r$ | $k'$ |
| Void | 2.64 | – | 3.97 | – | 0.83 | – |
| $NO_3^-$ | 2.98 | 0.12 | 5.57 | 0.40 | 1.15 | 0.39 |
| $I^-$ | 3.93 | 0.48 | 13.0 | 2.27 | 2.75 | 2.31 |

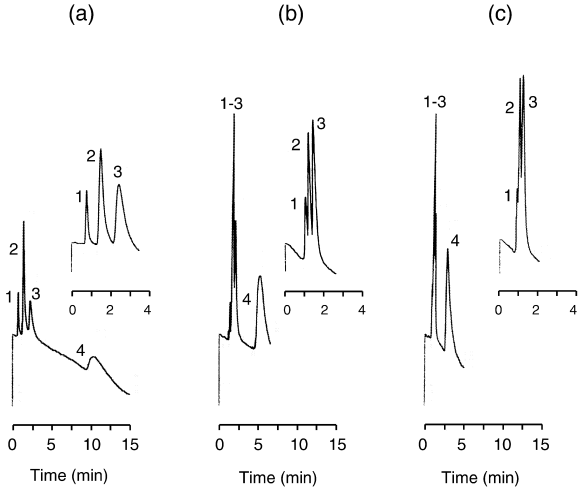


Fig. 3. Influence of varying the eluent concentration on the on-chip ion-exchange based separation of inorganic anions. (a) 0.1 m*M* KCl, (b) 0.5 m*M* KCl, and (c) 1.0 m*M* KCl. Peaks: 1= thiourea, 2=$NO_2^-$, 3=$NO_3^-$, 4=$I^-$.

and $y$ is the eluent anion charge. A plot of log $k'$ vs. log[$E$] for monovalent anions using a monovalent eluent anion should be linear with the slope equal to $-1$. The construction of a plot using retention factors of the three different analyte anions at the three different eluent concentrations was linear ($r^2>$ 0.99) with values of the slope ranging from $-0.77$ for $NO_2^-$ to $-0.82$ for $I^-$. This is lower than the theoretically expected value of $-1$ and can be explained by the dissolution of $CO_2$ from the air into the unbuffered eluent. This resulted in the formation of hydrogencarbonate ions, which increased the concentration of anions in the electrolyte, thus increasing the eluotropic strength of the eluent. Therefore retention times were lower than would be expected in a carbonate free eluent, leading to a deviation from the ideal value of $-1$ for the ratio $x/y$.

### *3.4. Linearity and detection limits*

Using an eluent concentration of 0.1 m*M* $Cl^-$, injections were made of solutions of thiourea, $NO_2^-$ and $NO_3^-$ of varying concentrations. A calibration graph was constructed by plotting the peak areas against the concentrations of the injections of nitrite and nitrate (Fig. 4). It was observed that linearity of

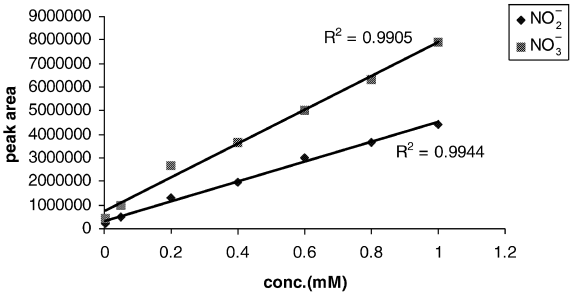


Fig. 4. Plot of peak area versus concentration over the range 5 $\mu M$–1 m*M* for nitrite and nitrate.

peak area and concentration in the range 5 $\mu M$–1 m*M* is obtained for nitrite and nitrate with coefficient constants, $r^2=0.9944$ and 0.9905 for nitrite and nitrate, respectively. The detection limits for nitrite and nitrate were determined at three times the standard deviation of the noise and found to be 0.5 $\mu M$.

## 4. Conclusions

Latex nanoparticles have been successfully employed to coat a microfabricated column on a silicon substrate and an on-chip separation of inorganic anions was realized. Due to the narrow depth of the micromachined column and the nanoparticle coating, it is easier for analytes to interact with the stationary phase on the chip than in a capillary, which leads to better separation. With on-chip injection and detection integrated on these chips, better separation can be expected in the near future.

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