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Journal of Chromatography A, 834 (1999) 309–320

JOURNAL OF CHROMATOGRAPHY A

Construction and investigation of a post-capillary reactor for trace metal analysis by capillary electrophoresis

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Abstract

This paper describes the construction and investigation of a post-capillary reactor for the determination of trace metals by UV–Vis absorption after formation of intensely coloured complexes. A short review of the present published work on post-capillary systems is followed by a detailed description of the fabrication and performance of a post-capillary reactor. The main principle of operation is based on the infusion of the colorimetric reagent into a small 50 μm gap between the separation capillary and the reaction capillary. The gap is enclosed by a permeable membrane and the flow of reagent is aided by a slight overpressure in the post-capillary reactor cell. Careful choice of colorimetric reagent, electroosmotic flow and pH was required to prevent the post-capillary reagents migrating in the wrong direction. Two reagents were studied in detail, namely, xylenol orange and 4 (2-pyridylazo)resorcinol (PAR). The best separation and detection characteristics were obtained with PAR for Cu, Cd, Co, Ni, Zn and Mn. Good linear calibrations were obtained down to the 0.1 ppm level for all the metals except Cu.

Keywords: Post-capillary reactor; Instrumentation; Metal cations

1. Introduction

Post-column reaction systems have been routinely used as a means of detecting metal ions separated by liquid chromatography (LC) for many years now [1]. Those based on 4-(2-pyridylazo)resorcinol (PAR) have been the most popular combined with high efficiency ion chromatography columns. When capillary electrophoresis (CE) was first investigated for trace metal determinations, post-capillary reaction systems were little studied presumably because of the more difficult technical problems involved in their construction. Therefore, most of the work focused on the separation and detection of metal complexes formed either pre-column or on-column. Indirect detection techniques usually involving a UV–Vis absorbing buffer containing weak complexing acids gave good separations, but sensitivity can be limited by the relatively low molar absorptivities of visualising agents such as imidazole and creatinine. More importantly perhaps, the unselective nature of indirect methods could be a serious disadvantage if looking for traces of transition metals in the presence of large amounts of alkali and alkaline earth metals. The direct detection of highly absorbing metal complexes seemed a better way of obtaining improved selectivity and sensitivity and a number of studies have been published mainly involving highly coloured organic chelating agents. Although several of these produced good separation and detection characteristics using pre-column or on-

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0021-9673/99/$ – see front matter
PII: S0021-9673(98)00858-9

column chelation, there are several problems limiting this approach. Unless the complexes are kinetically or thermodynamically very stable, dissociation could occur, producing broad peaks or no peaks at all. Interaction with the capillary wall was also possible, again broadening peaks. The reader is directed to the recent comprehensive review by Macka and Haddad [2] detailing and comparing the published methods involving the CE determination of metal ions using indirect and direct detection.

The problems mentioned above, together with limitations on the choice of separation buffers that could be used, meant that post-capillary systems looked an increasingly attractive proposition if the technical problems could be overcome. However, the transfer of post-column technology from LC to CE is not straightforward. The smaller scale of CE makes the construction of a post-column reactor technically much more difficult and since the major attraction of CE is the high efficiency of the separations, the requirements in terms of peak broadening are far more critical than in LC.

A number of post-capillary reaction systems have been described in the literature [3–10]. All but one were designed for use with fluorescence detectors, with the majority of these being applied to the detection of *ortho*-phthalaldehyde (OPA) derivatized amino acids. The kinds of post-capillary reactors can be conveniently divided into two types, those where an electric field is present after the reaction right up until detection takes place (voltage-driven) and those where the electric field is absent after the reaction takes place (pressure-driven). In the former case electrophoretic migration or a combination of electroosmotic flow (EOF) and electrophoretic migration carry the reaction products to the detector. In the latter case, an overpressure is used to carry the products of the post-capillary reaction to the detector. However, with voltage-driven systems it should be noted that in addition, a slight overpressure is often used to reduce the time in the reaction zone.

1.1. Voltage-driven systems

Technically, the least complicated post-capillary reaction system for CE is the free solution approach adopted by Rose [3], in which a single separation capillary is terminated in the grounded reservoir of the derivatizing reagent. Zones migrating out of the capillary mix with the reagent and are detected using fluorescence just beyond the capillary outlet. Although simple in design, the system is restricted to very fast derivatisation reactions, since the zones become rapidly diluted on exiting the capillary. The system also requires a reaction cell capable of holding sufficient volume of reagent to prevent depletion during a run. Replenishment of the reagent at the capillary exit is solely due to turbulence caused by the flow exiting the capillary. Since the only source of mixing is in the reaction cell, several closely migrating zones may lead to localised depletion of the reagent at the capillary exit, resulting in a decline in response over time. The system also requires accurate focusing of the excitation source close to the capillary exit, which is difficult to achieve using standard fluorescence detectors. Although suitable for use with in-laboratory manufactured CE systems, the requirements of the reaction cell make this system difficult to apply to most commercial instruments, due to the focusing requirements of the excitation source and the need for both the inlet and outlet of the capillary to be at the same height if siphoning is to be avoided.

Jorgensen and co-workers [4,5] applied a coaxial arrangement in which a smaller outer diameter (O.D.) capillary was inserted into a larger I.D. capillary, with a sheath flow of reagent driven by pressure running through the outer capillary. This arrangement, which is essentially an improvement of the free solution approach described above, utilises a reactor cell created from a section of larger I.D. capillary. This allows the use of on-column detection using standard CE detectors as well as providing a continually replenished supply of reagent. Zone broadening was minimised by closely matching the internal diameters of the separation and reaction capillaries. Close matching of the inner dimensions of the capillaries was achieved by etching the outside of the inner capillary with hydrofluoric acid to reduce the wall thickness. The end of the separation capillary was also tapered to reduce turbulence caused by the blunt end. With this system the grounding electrode is situated at the outlet of the reaction capillary, and the transport of solutes and electrolyte in the reaction capillary is a combination of electrophoretic processes and laminar flow. The

main problems associated with a co-axial system are difficulties in centring the end of the separation capillary inside the reaction capillary and the extreme fragility of the etched capillaries which makes assembly of the components very intricate.

Other approaches to the design of post-capillary reaction systems for CE also use a capillary as both reactor and detection cell. However, in these systems the separation capillary is coupled to a reaction capillary leaving a very small gap and the post-column reagent is introduced into the junction between the capillaries. The derivatized solutes are then detected using an on-column window. The main influences on zone dispersion with this type of post-capillary reaction system are dependent on the efficiency of mass transfer across the junction between the coupled capillaries and the kinetics of the derivatisation reaction. The grounded electrode is placed after the reaction capillary and the reagent can be introduced into the capillary by the electroosmotic process and if necessary the application of pressure. This pressure difference can be achieved either by elevation of the reagent vessel above the level of the capillary junction (height difference) or the application of a very slight gas pressure to the reagent vessel. Whichever method of reagent addition is used it increases the volume of the fluid in the reaction capillary. To compensate, the velocity of the flow in the reaction capillary must either be increased or a capillary with a greater internal diameter used, otherwise back pressure will be created in the separation capillary. The increased flow-rate in the reaction capillary can be created by the pressure used to introduce the reagent or by increasing the EOF. Although, the flat flow profile of EOF does not contribute much to zone dispersion, in a purely electroosmotically-driven system, some band broadening may be caused by laminar flow superimposed on the EOF or by the post-column reaction itself. Altering the size or charge of a solute will change its velocity. If the velocity of the derivatized solute is faster than that of the solute, zone dispersion will occur as the derivatized solute speeds up moving away from the remaining underivatized solute. On the other hand, if the velocity of the derivatized solute is slower than that of the solute, stacking may occur as the derivatized solute slows down allowing the underivatized solute to catch up. Although stacking is usually a desirable effect in CE, in this instance it may occur at the expense of peak resolution.

Pentoney et al. [6] designed a simple method for introducing the reagent into the capillary via a cross or tee junction. The junction was made by boring a hole through the capillary wall with a laser and bonding the reagent delivery capillary or capillaries at right angles to the separation capillary. Although the reagent flow was driven by pressure created by height difference, it was essentially an EOF controlled system with the transport of solutes in the reaction capillary due to the combined electrophoretic velocity of the derivatized solutes and electroosmotic flow, with a hydrodynamic pressure element superimposed over these. The major advantage of this system is the minimal loss of efficiency, which can be attributed to the low dead volume of the capillary junction and the perfect alignment of the separation and reaction capillaries. However, lasers capable of boring holes with this precision are highly specialist and not readily available.

Electroosmosis was used to introduce the reagent into the reaction capillary in the design of Albin et al. [7]. A 50 μm I.D. separation capillary and 75 μm I.D. reaction capillary were inserted into PTFE tubing sleeves to allow the use of a standard LC four-way connector to be used as the reactor. A small gap of between 10 and 50 μm was left between the capillaries. The cross channel of the reactor was connected to PTFE tubes which allowed the reactor to be flushed with reagent and electrolyte. The reagent was reported to enter the capillary by virtue of the greater volumetric flow-rate of the electroosmotic flow in the reaction capillary. However, introduction of reagent was probably aided by the dilution of the electrolyte resulting in an increase in field strength.

An alternative approach, providing a controllable means of reagent introduction, was developed by Cassidy et al. [8] in which a second independent voltage source is employed to create an ancillary potential across the reaction capillary. The EOF and hence the volume of reagent drawn into the reaction capillary could then be controlled by variation of the field strength in the reaction capillary. The major drawback of this system is that in order to allow the use of an increased field strength in the reaction

capillary, the field strength employed for the separation has to be less than optimal if Joule heating is to be avoided in the reaction capillary.

1.2. Pressure-driven systems

With a pressure-driven system the grounding electrode is placed at the junction between the separation and reaction capillaries and since there is no electric field in the reaction capillary, transport is achieved entirely by laminar flow. The total flow is made up of EOF exiting the separation capillary and pressure induced reagent flow. The pressure to introduce the reagent can be created by either height difference or the application of gas pressure to the reaction cell. A minimum pressure is required to prevent EOF from the separation capillary dispersing too quickly into the reaction cell. However, the pressure applied to introduce the reagent across the junction is transmitted to both capillaries and may result in a laminar flow element in the separation capillary, so care is needed to prevent the flow of post-column reagent into the separation capillary.

Tsuda et al. [9] developed a system which coupled CE to a conventional LC post-column set-up. The solutes were first separated in a fused-silica capillary which was co-axially coupled to a piece of 0.5 mm PTFE tubing via a four way connector. One of the free ports of the connector was used to ground the capillary and the other was used for the introduction of an alkaline buffer to the PTFE tubing. A fluorimetric reagent was subsequently added via a T-junction and the derivatized solutes detected using a standard HPLC fluorescence detector. The instrumentation was relatively complicated in this system, requiring the use of three pumps, one to deliver the alkaline buffer, a second for the fluorescent reagent and the third to provide a compensating pressure to the capillary inlet. The grounding electrode is immersed in the alkaline buffer, whereas the capillary was filled with a different buffer resulting in a discontinuous electrolyte. This is likely to result in a progressive change in the pH and conductivity of the separation electrolyte.

Zhu and Kok [10] developed a pressure-driven system in which the junction between the separation and reaction capillaries was made by inserting the capillaries into a porous PTFE tube. The coupled capillaries were fixed inside a grounded reagent vessel of approximately 1 ml capacity with finger-tight fittings. The porous PTFE tube was principally used to align the capillaries and was reported to offer little or no resistance to the flow of fluid, except when high flow-rates were used. The reagent was driven through the porous tube by air pressure applied to the reagent vessel. This air pressure was simultaneously applied to the reagent vessel and the capillary inlet to counteract Poisseuille flow in the separation capillary. Improved efficiencies were reported by coupling 75 μm I.D. separation capillaries to 50 μm I.D. reaction capillaries and enhanced sensitivity was obtained using ''bubble-cell'' capillaries. The only real drawbacks of this system were that the gap between the coupled capillaries could not be accurately controlled and therefore reproducibility from one assembly to another may be a problem and also the inability to flush the reagent vessel.

1.3. Choice of post-capillary reactor design for trace metal determinations

When considering the published designs outlined above it was thought the simplest and best option would be to use the system involving a short gap between the separation and reaction capillaries. The original idea was to introduce the reagent into the capillary by inserting short section of hollow fibre porous membrane between the two capillaries, and then apply a small amount of pressure to allow the reagent to migrate across the membrane into the capillary. The introduction of the reagent from virtually all around the junction should have the advantage of improved mixing when compared with a single point of introduction as in the system developed by Pentoney et al. [6]. To accomplish this, the internal diameter of the hollow fiber should be closely matched to that of the fused-silica capillaries, particularly with respect to zone broadening. However, these hollow fibers are manufactured for specific purposes such as ultra-filtration and dialysis. As they are supplied as complete units containing bundles of the fibers, their availability is severely limited. After an extensive search one company was able to supply the hollow fibers, but only in 0.5 mm I.D. Since the I.D. of the hollow fibre was so large, it

was decided to modify the original idea of using a short section of hollow fibre and instead use the membrane as a sleeve over a pre-fabricated capillary junction with a very short gap. The I.D. of the sleeve allowed the use of a small brace holding the capillaries together. The brace also served to increase the O.D. of the capillary assembly to nearly that of the I.D. of the sleeve, reducing the dead volume inside the capillary junction.

This paper details the construction, operation and performance of a trace metal post-capillary reactor detector based on the design outlined above. The post-capillary reactor performance was evaluated using xylenol orange (XO) and PAR colorimetric reagents.

2. Experimental

2.1. Instrument considerations and modifications

A modified Dionex CES 1 (Dionex, Sunnyvale, CA, USA) was used for all experiments as detailed in Fig. 1. The main modifications included the splitting of the helium line to allow the post-capillary reactor cell to be pressurised and an extra lead in the voltage supply so that the grounding point could be varied.

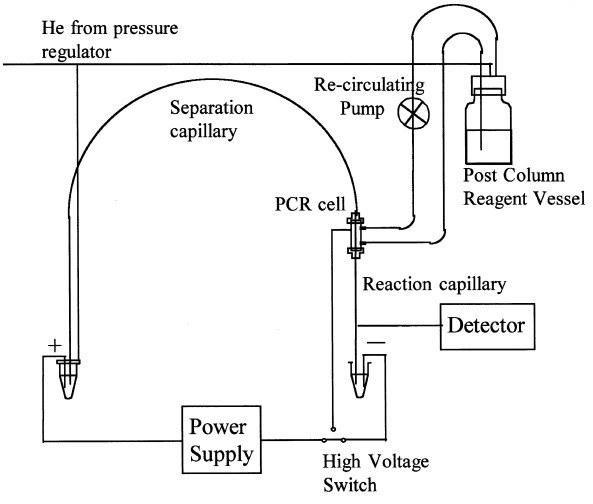

Fig. 1. Schematic diagram of the modified Dionex CES 1 instrument. PCR=Post-capillary reaction.

The CES 1 rinses the capillary by pressurizing the destination vial, forcing electrolyte through the capillary towards the source vial. This can result in colorimetric reagent being drawn into the separation capillary from the reaction cell, resulting in the possibility of on-column complexation during a run. To prevent this problem the capillary was rinsed from source to destination by performing 120 s pressure injections from a carousel vial containing electrolyte. Using this method, reagent may be drawn into the reaction capillary, but this was deemed to be of no consequence to the separation. The capillary rinse was performed after the destination vial had been rinsed and refilled, since the destination vial is emptied by the application of pressure to the vial.

Minor modifications were made to the instrument to allow automated operation with the post-column system installed. A low-pressure helium line, providing pressure to the reagent vessel, was created by detaching the helium line from one of the spare electrolyte bottles. A manually operated pressure reduction valve was placed in line to allow independent control of the pressure applied to the reagent vessel. A low-pressure line could also be connected to the capillary inlet via the pressure injection line. To facilitate automatic switching between the pressure injection and the inlet low-pressure system, a three-ported, electrically operated, low-pressure gas valve was inserted in the pressure injection line. The second inlet of the valve was connected to the low-pressure line. This valve was operated by the switch normally used to operate the capillary cooling valve on the instrument. During a run, the low-pressure system could be automatically switched to the capillary inlet and when the run was complete the capillary inlet was reconnected to the pressure injection line. The manually operated valve was designed to be used for pressures above 1.0 p.s.i. and operation at pressures below this, although usable, provided poor precision (1 p.s.i.=6894.76 Pa). A more precise, electrically operated pressure controller was obtained from Dionex and used to provide pressure to the capillary inlet. This device was able to control pressure in 0.1 p.s.i. steps from 0.1 p.s.i. upwards and was used to evaluate the effect of pressure on the separation efficiency.

The location of the instrument's power supply ground connection is inaccessible, being situated

below the optical bench. Therefore, to enable easy switching of the grounding electrode from the destination vial to the cell, a wire was connected to the power supply ground and soldered to the ''female'' part of a push-in connector. The connector was then insulated and fixed to the instrument in an accessible position. The grounding wire from the destination vial was disconnected from the power supply, lengthened and a ''male'' push-in type connector soldered to the end. A similar connector was also soldered to the wire from the cell electrode.

2.2. Post-capillary cell design

The major consideration in the design of the cell was to create a cell small enough to fit inside the light box of the instrument enabling the use of a reaction capillary with a relatively short effective length, defined as the distance from the junction to the detection window. A number of designs were investigated and they all involved enclosing the capillary gap with a reservoir containing the post-capillary reagent, pressurised externally with helium. Although they all worked reasonably well, the early cells had the disadvantage of being permanently glued together so that the capillary junction and polysulphone sleeve could not be renewed.

The final cell arrangement is shown in Fig. 2. The capillary junction was enclosed with a 50 mm×4 mm I.D. Dionex guard column. This design allowed the capillary assemblies to be installed and removed from the cell without damage, using finger-tight fittings. The minimum effective length of the reaction capillary in this design was 50 mm.

Care was needed when inserting the capillary assembly into the cell to prevent the capillaries becoming unaligned as a result of twisting when the finger-tight fittings were fastened. This was done by tightening the lower fitting first then gently gripping the PTFE sleeve of the upper fitting in a pair of pliers as it was gently tightened. The lower fitting was tightened more than the upper, since leakage from this fitting could be unseen, running directly into the detector, whereas leakage from the upper fitting was easily visible and of far less consequence. The cell also contained an electrode in case a pressure-driven system was required.

The reservoir of colorimetric reagent was stored in a 100-ml glass bottle which was connected by tube to the inlet of the cell. The outlet of the cell could be run to waste or crimped as need be. The reagent reservoir was also connected to a low pressure helium line controlled by a manually operated pressure reduction valve, allowing the bottle to be pressurized.

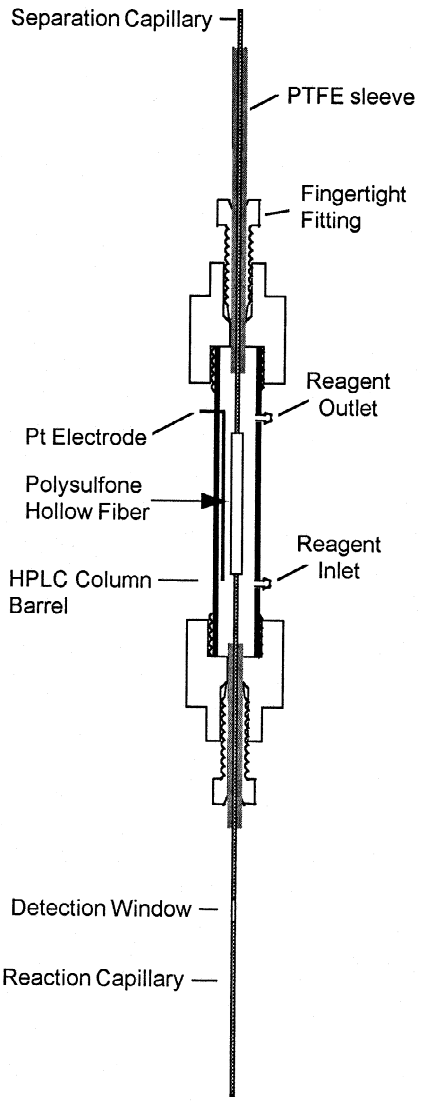

Fig. 2. Details of the post-capillary reactor cell.

2.3. Construction of the capillary junction

The adhesive used to construct the capillary

assemblies and reactor cells was Loctite, UV curing acrylic adhesive. The glue fully cured within a few seconds after exposure to an intense UV light source of about 285 nm. A schematic diagram of the capillary assembly is shown in Fig. 3.

Two sections of 375 μm O.D.×100 μm I.D. capillary of approximately 60 cm and 15 cm in length were cut using a ruby cutter. One end of each capillary was polished, initially using the face of a ceramic capillary cutter, then finished with an ultra fine abrasive film. A small hand held chuck was used to ensure the polished faces of the capillaries remained as perpendicular to the longitudinal plane as possible. Holding the capillaries by hand tended to produce slightly rounded faces due to flexing of the capillary. The capillaries were checked under a microscope to ensure the ends were as smooth and perpendicular as possible. The capillaries were aligned by inserting a piece of 0.0038 in. (96 μm) diameter tungsten wire into the unpolished end of the 15 cm capillary until a short length protruded, the wire was then threaded into the polished end of the 60 cm capillary. The cut end of the tungsten wire was first smoothed with the abrasive film to prevent scratching of the inner surface of the capillaries. The gap between the capillaries was fixed by inserting a short length of tungsten wire of the appropriate diameter between the two sections of capillary. The capillaries were then joined together by gluing a short section of 100 μm O.D. fused-silica capillary between them. A sleeve of 0.5 mm I.D. polysulfone hollow fibre was slipped over this assembly and bonded in place. Prior to use, the capillaries were cut to the desired lengths then conditioned by flushing with 0.01 *M* HCl for 30 min, followed by 0.5 *M* NaOH for 60 min, then rinsing with deionised water. The on-column window was made by removing 2–3 mm of the polyimide coat with a razor blade. This method of making the on-column window is preferable to burning the capillary, which appears to make the capillary brittle and produces windows that are larger than necessary.

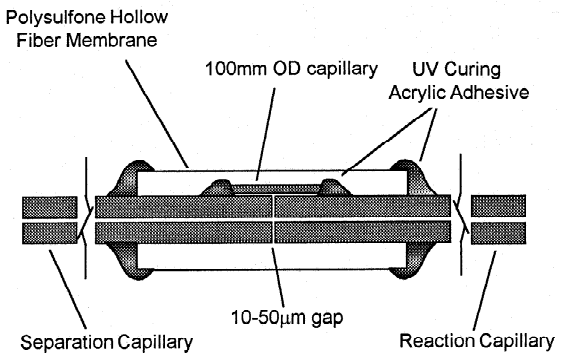

Fig. 3. Details of the inter-capillary junction.

2.4. Materials and reagents

Fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

Polysulfone hollow fiber membranes with internal diameters of 0.5 and 0.25 mm and a nominal molecular-mass cut-off of 10 000 were obtained from A & G Technology (Needham, MA, USA).

All chemicals were of analytical-grade purity. Sodium oxalate, citric acid, pyridine-2,6-dicarboxylic acid (PDCA) and XO [*o*-cresolsulfonphthalein-3′-3″-bis(methyliminodiacetic acid)] were obtained from Fluka (Buchs, Switzerland). Glycine and 2-(*N*-morpholino)ethanesulphonic acid (MES) were obtained from Sigma (St. Louis, MO, USA). Sodium hydroxide, sodium tetraborate decahydrate, boric acid, tartaric acid, disodium hydrogenphosphate, ammonium dihydrogenphosphate and ammonium hydroxide solution were obtained from BDH (British Drug House, Poole, Dorset, UK). PAR was obtained from Dionex.

3. Results and discussion

3.1. Choice of post-capillary reagent

Although there is a very wide range of colorimetric reagents to choose from, in practise there are only a few which meet the requirements of post-capillary reactors. The reagent needs to be water soluble, form intensely coloured metal complexes and react with a large number of metal ions. Furthermore, for use in CE, the presence of an EOF puts a further restriction on the number of suitable chelating agents. This is because the best arrangement for the separation of metal cations in CE is to inject into a buffer containing a weak complexing acid in the presence of a cathodal EOF, where the bulk capillary flow is

towards the destination vial. However, many water soluble colorimetric reagents and their complexes are negatively charged so when entering the reaction capillary they will tend to migrate backwards against the EOF. It is important therefore that the EOF is quite strong and the reagent in particular is not too negatively charged or mobile. Otherwise, any colorimetric reagent entering the separation capillary will cause on-column chelation and adversely change the separation of the metal ions before they reach the reaction capillary. Fortunately, there are a number of colorimetric reagents which form negatively charged metal complexes at high pH where the EOF will be high or close to maximum. Taking the above factors into account two reagents seemed worthy of initial investigations, namely, PAR and XO.

3.2. PAR post-capillary reactor

PAR is perhaps the most obvious reagent to investigate because its characteristics are well known from extensive use in classical spectrophotometric procedures and as a post-column reagent in ion chromatography. The reagent itself becomes negatively charged above pH 5 and remains singly negatively charged until about pH 11. Therefore its tendency to migrate against the EOF will not be very strong. In the presence of excess reagent, metals will form complexes of general formula ML_2^{x-} though the pH will have to be relatively high to ensure the weaker chelating metals such as Cd and Mn will form the complex close to completion. Therefore 2+ metal ions will produce PAR complexes with a double negative charge. However, they will not be that mobile in the electric field because of the large size of the complex ions. Concerning the separation of the metal ions in the main capillary, this is best carried out using a buffer containing a relatively weak complexing acid such as tartrate, oxalate, citrate etc. Choice of pH is important to achieve the desired charges on the complexes to maximise separation. However, it should be borne in mind that unlike ion chromatography–post-column reaction systems, where the separation of the metal ions and the reaction can be performed under conditions of different pH and ionic strength, with a CE–post-capillary reaction system these factors need to be kept similar to avoid excessive joule heating and loss of efficiency. Thus, if the pH of the post-capillary reaction is high as it is with PAR, then the separation pH needs to be similar resulting in a very strong EOF.

Preliminary studies of the post-capillary system were carried out with PDCA as the complexing buffer in the separation capillary. Metal PDCA complexes are very stable and 2+ metal ions will form complexes with an overall charge of 2−. It was considered this high charge would counteract the strong EOF. Injections of Cu(II), Zn(II), Mn(II), Co(II) and Ni(II) ions were made and the concentration of PAR was optimised to obtain the highest sensitivity consistent with sharp peaks. A PAR concentration of 5 m*M* in pH 8.6 buffer produced good results. Lower PAR concentrations gave slightly better sensitivity, but broader peaks. A higher pH would have resulted in a more complete reaction for some of the metals with PAR, but as mentioned earlier, it is not advisable to have too large a difference between the separation and post-capillary reaction pH values.

Using the PDCA buffer, peaks were obtained for all the above mentioned metal ions, but there was very little difference in migration time, so no separations were achieved. It was assumed that the PDCA complexes were too strong and produced complexes with similar size and charge. A number of weaker chelating carboxylic acids were investigated for use in the separation buffer. Oxalic acid was found to be the most promising. Interestingly other acids such as citric and tartaric produced no peaks at all. Fig. 4 shows a separation of five metal ions with oxalic acid in an ammonium phosphate buffer at pH 8.6. The migration order was consistent with the relative stabilities of the oxalate complexes. Zinc is also well separated (not shown) coming out last as a very broad peak. The extreme broadness of the zinc peak indicated possible wall interactions or rapid dissociation in the reaction capillary. It was not clear why zinc behaved like this as the magnitude of its oxalate stability constant indicated it should come out before copper as a sharp peak. When the quantitative performance of the post capillary detector was investigated, copper showed anomalous behaviour in that the signal decreased non-linearly with decrease in concentration. Thus, a strong signal at 1 ppm, essentially disappeared at 0.5 ppm. The other

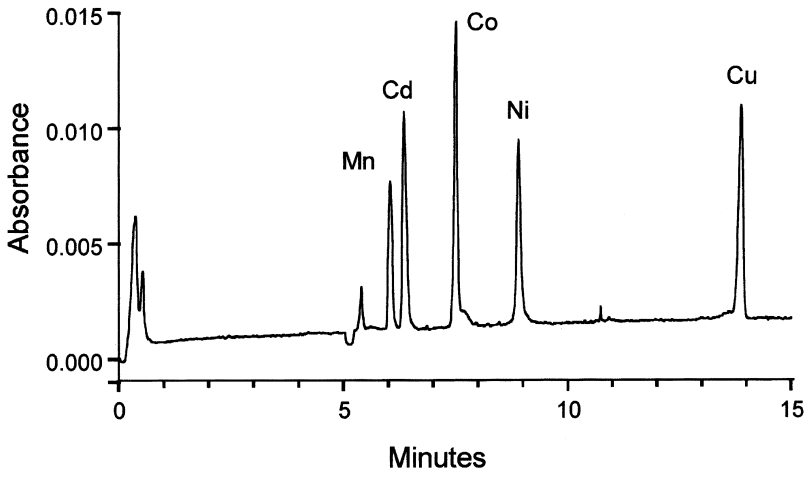

Fig. 4. Electropherogram of five metal ions using a PAR post-capillary reactor. Separation conditions: buffer, 5 m*M* oxalic acid and 10 m*M* ammonium phosphate adjusted to pH 8.6; injection, 100 mm hydrostatic for 15 s; voltage, 18 kV. Post-capillary reaction; 10 m*M* ammonium phosphate and 5 m*M* PAR adjusted to pH 8.6. Metal concentrations: cadmium cobalt and copper (2 ppm); manganese and nickel (1 ppm).

Table 1
Reproducibility of 1 ppm metal injections using the PAR post-capillart reaction

Metal	R.S.D. (%)[a]	Efficiency (N, m^{-1})
Mn	5.0	25 000
Cd	8.6	47 000
Co	3.5	39 000
Ni	3.3	81 000
Cu	4.1	200 000

[a] For six repeat injections.

four ions, Ni, Co, Cd and Mn gave good linear calibrations with correlation coefficients of 0.9991, 0.9966, 0.9974 and 0.9884, respectively. Reproducibility at the 1 ppm level was also good as shown in Table 1 along with information on peak efficiency. Fig. 5 shows an electropherogram of four metal ions close to the detection limit illustrating typical baseline noise and drift. The detection limits found (defined as twice the average peak to peak back-

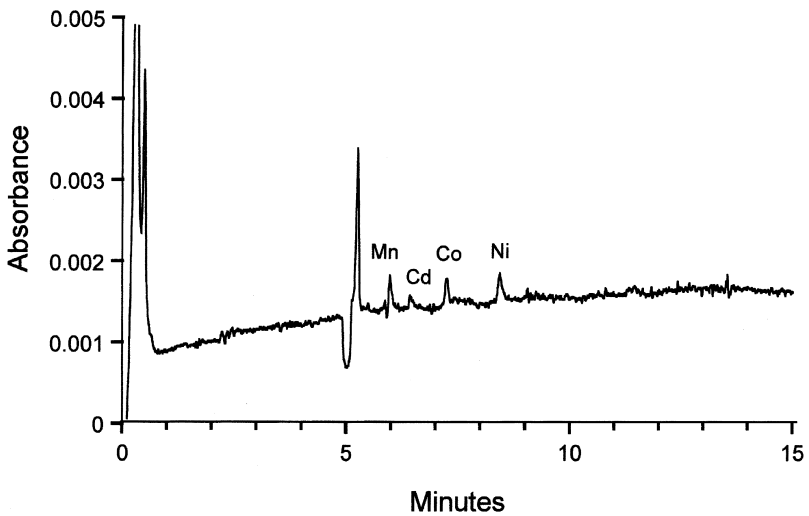

Fig. 5. Electropherogram of four metal ions near the detection limit using a PAR post-capillary reactor. Separation and post-capillary reaction conditions as in Fig. 4. Metal concentrations all 0.1 ppm.

ground noise level) were 0.02, 0.1, 0.03 and 0.03 ppm for Mn, Cd, Co and Ni, respectively. When comparing these detection limits with present published methods involving indirect or direct detection, as detailed in the review by Macka and Haddad [2], care should be taken to equate only with those methods using similar injection techniques i.e. hydrostatic or pressure injection. Electrokinetic injection invariably gives rise to stacking leading to preconcentration of the analytes. Bearing this in mind, the detection limits using PAR in this work are similar to or slightly better than those found using indirect detection. For example, Beck and Englehardt [11] quote detection limits between 0.05 and 0.1 ppm for a range of metals using imidazole as the visualising agent. However, considering the high sensitivity normally associated with the detection of coloured metal complexes, the post-capillary detection limits are not quite as good as present direct methods using pre-column or on-column approaches. Typically, direct detection using pre-column or on-column formation of strongly absorbing metal complexes, give detection limits around 0.01 ppm [12,13]. One of the reasons why detection limits were not as good as anticipated is because of the lower than normal peak efficiencies usually associated with CE separations (see Table 1). Efficiency is almost certainly lost due both to disturbance at the capillary gap and the time spent in the reactor capillary. It is expected that further improvements in design of the capillary gap and reaction conditions should produce better efficiencies and therefore better detection limits.

Another factor affecting efficiency is the presence of a high conductivity buffer. One of the main problems with some of the carboxylic acids operated at high pH, is that they have such a high conductivity, necessitating the use of low field strengths. Since efficiencies generally improve with increase in field strength it was decided to look at a complexing buffer with a lower conductivity. The one chosen was glycine which has a pK_a of 9.78. Thus for the pH range under study, the glycine will be a mixture of L^- and the zwitterionic form. The mobility of the fully ionised form is $-37.4 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (cf. oxalic acid, $-74.6 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}). This lower mobility combined with lower ionic strength of glycine should allow the use of higher field strengths. Fig. 6 shows the separation of Cu, Co, Ni, Zn and Mn in a glycine buffer at pH 10. Although reasonable peak efficiencies were obtained for Cu, Co and Ni, the resolution was not good. Furthermore, the peak shapes for Zn and Mn were very poor. It was considered that the higher pH was responsible for this, possibly due to metal ion hydrolysis and/or wall interactions, so this approach was not studied further.

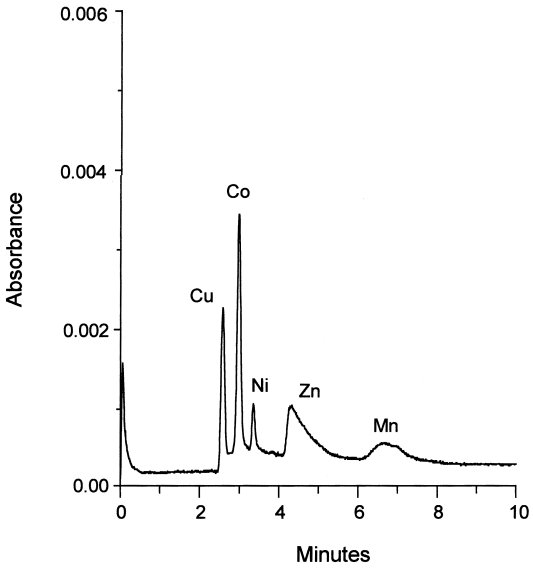

Fig. 6. Electropherogram of five metal ions using a PAR post-capillary reactor with glycine in the separation buffer. Separation conditions: buffer, 10 m*M* glycine adjusted to pH 10 with sodium hydroxide; injection, 100 mm hydrostatic for 20 s; voltage, 30 kV. Post-capillary reaction; 0.5 m*M* PAR and 10 m*M* glycine adjusted to pH 10 with sodium hydroxide. Metal concentrations; all 2 ppm.

A pressure-driven set-up was also investigated with the voltage grounded in the post-capillary reactor cell. The peaks obtained were very broad and the system was unstable, with multiple spikes appearing after a short interval. It was suspected that small gas bubbles were forming in the membrane. With the voltage-driven system the main effect causing reagent to flow across the membrane was the EOF aided by slight overpressure. With the pressure-driven system the reagent is forced through the membrane solely by pressure difference, which could be sufficient to cause bubble formation. No further work was done at this stage as it was considered a radical redesign of the capillary junction and reactor

cell was required before stable results could be obtained.

3.3. Xylenol orange post-capillary reactor

Although PAR shows potential as a sensitive spectrophotometric reagent in a post-capillary reactor, it was considered that it would be interesting to investigate a system where the pH was lower and the EOF not so strong. The possibility for hydrolysis of metal ions and wall interactions should therefore be less at the lower pH. XO is another well studied and used classical spectrophotometric reagent. It also reacts with a wide spectrum of metals, but at a lower pH than PAR, though the molar absorptivities of the XO metal complexes are significantly lower.

The optimum pH for the reaction of XO with 2+ metal ions is between 5.5 and 6. The buffer chosen for both the post-capillary and separation solutions was MES. The pK_a of MES is 6.1 which is ideal for use in the XO system. The fully ionised form also has a relatively low mobility of $-26.8 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, which allows the use of high field strengths. As used for the PAR system, a complexing acid is required in the separation capillary buffer to effect separation of the metal ions. Preliminary experiments showed that unlike the PAR system, weaker complexing acids such as tartaric acid in the separation buffer produced a response. However, the separation efficiency and detection sensitivity was poor. This can be seen in Fig. 7a where cobalt and nickel could not be resolved, though lead was well separated. Oxalic acid produced a better separation (Fig. 7b), but the peaks were even broader with a consequent further loss in sensitivity. The only advantage over the PAR reagent was the response to lead, though the sensitivity was not very good. It is interesting to note the relationship between the migration times of the peaks and the EOF marker, which is indicated by the dip in the baseline. For tartaric acid, all four peaks come out before the EOF marker, denoting that the tartrate complexes are positively charged, so travel ahead of the EOF. When using oxalic acid, Cd, Ni and Co come out before the EOF marker and Pb after it. This shows that at the oxalic acid concentration used, only the lead oxalate complex had an overall negative charge. Increasing the concentration of oxalic acid was not investigated in any detail as both the peak shape and sensitivity worsened considerably. This also happened to a lesser extent with increase in concentration of tartaric acid. Clearly, the XO reactor is much more affected

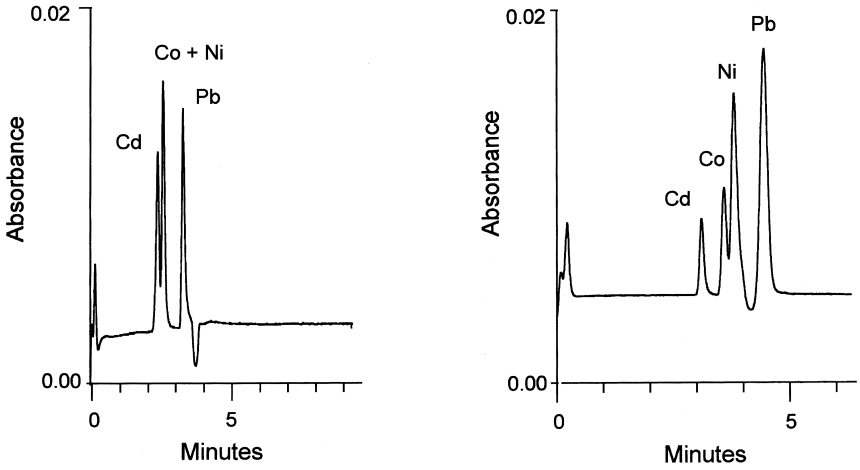

Fig. 7. Electropherogram of four metal ions using a XO post-capillary reactor. (A) Separation conditions: buffer, 10 m*M* MES and 1 m*M* tartaric acid adjusted to pH 5.8; injection, 100 mm hydrostatic for 15 s; voltage, 30 kV. Post-capillary reaction; 10 m*M* MES and 0.1 m*M* XO adjusted to pH 5.8. Metal concentrations: cadmium, copper and nickel (2 ppm); lead (20 ppm). (B) Separation conditions: buffer, 10 m*M* MES and 0.5 m*M* oxalic acid adjusted to pH 5.8; injection, 100 mm hydrostatic for 15 s. Voltage and post-capillary reaction as for (A). Metal concentrations: cadmium, cobalt and nickel (5 ppm); lead (50 ppm).

by these carboxylic acids than the PAR reactor, presumably because the XO metal stability constants are much lower than those of PAR.

4. Conclusions

The principle of using a small inter-capillary gap surrounded by a permeable membrane to introduce the colorimetric reagent worked successfully, though a slight overpressure was required to ensure a reproducible flow into the reaction capillary. The fabrication of a demountable cell was particularly convenient as the individual capillaries and polysulphone sleeve could be changed when necessary, though care was needed in resetting the gap. Grounding the electrode at the end of the reaction capillary rather than in the reaction cell gave the best results showing that an EOF right through to the detector window was required to keep peak broadening to a minimum. As anticipated, PAR gave the most sensitive reaction for most of the metal ions studied. However, some results were unexpected, such as the broadness of the zinc peak and non-linear calibration for copper. The poorer results for XO could be due to the lower stability of the metal complexes resulting in more dissociation, i.e., more peak dispersion in the reaction capillary, before reaching the detection window. More studies are underway to ascertain the effect of changes in the dimensions of the capillary gap and reaction capillary on sensitivity and to investigate other spectrophotometric and fluorimetric reagents.

Acknowledgements

We wish to thank Dionex (UK) and Dionex Corporation, Sunnyvale, CA, USA, for financial support and work experience during the research studentship for Simon Hardy.

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