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Development of a capillary electrophoretic method for the separation and determination of trace inorganic and organomercury species utilizing the formation of highly absorbing water soluble dithizone sulphonate complexes

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Abstract

This paper details results which show that rapid, good quality separations can be achieved for inorganic and organomercury species as their dithizone sulphonate complexes, using coated capillary columns. The complexes were pre-formed before injection and detection was by direct measurement of the absorbance of the complexes. Good linear calibration curves were obtained for all the mercury species studied and the exceptionally low background noise and straight base lines meant that detection limits in the low $\mu g\ l^{-1}$ range could be obtained.

Keywords: Organomercury compounds; Mercury compounds; Dithizone sulfonate complexes

1. Introduction

The environmental behaviour and toxicity of a metal are often related to the specific form or species in which it occurs [1]. It is generally recognised that the organometallic species of certain metals are far more toxic than the inorganic forms as a result of their greater biological compatibility [2]. The determination of total metal concentrations do not reflect this important information and this has led to the need to distinguish between the different species

There has been a continuing interest in the speciation of mercury for a number of years now owing in part to the high toxicity of methylmercury and the tendency towards bio-magnification as it passes through the food web [3]. This biomagnification is particularly large in marine ecosystems and leads directly to human exposure through the fish food chain. As expected, a great deal of effort has been put into developing methods to differentiate and determine organomercury species in biological and environmental samples. Analytical methods for the determination of mercury speciation up to 1994 have been critically reviewed by Puk and Weber [4]. In general, methods for the speciation of mercury can be divided into two major fields, selective reduction and chromatographic methods.

Selective reduction methods are based on the procedure proposed by Magos [5], which makes use of the differing reduction behaviours of inorganic and organic species in the presence of reducing agents. Hg(II) is reduced to Hg(0) by stannous

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chloride alone whereas alkylmercury compounds are only reduced to Hg(0) by stannous chloride in the presence of cadmium chloride. The mercury vapour generated is then detected by cold vapour atomic absorption spectrometry (CVAAS) [6] and cold vapour atomic fluorescence spectrometry (CVAFS) [7]. There are several variations to this method, mainly differing in the choice of reductants [5,7,8]. A major drawback to this method is that it only distinguishes between inorganic and organic mercury and does not differentiate the individual organic species.

The other common approach for the determination of methylmercury is based on the gas chromatographic (GC) method originally developed by Westoo [9], with detection of the organomercury halide by electron capture detection. Initial GC work utilised packed columns and suffered problems with low column efficiencies, decomposition and severe peak tailing leading to poor reproducibility [10]. These problems have been solved to some extent by the use of column conditioning [11] and capillary columns [12].

High-performance liquid chromatography (HPLC) methods have also been developed using a variety of detection systems including reductive electrochemical [13], inductively coupled plasma-mass spectrometry (ICP-MS) [14], UV–Vis [15] and CVAAS [16].

In recent years capillary electrophoresis (CE) has become available as a routine analytical technique. Although initially mainly used for the separation of biological macro-molecules, the technique is becoming increasingly applied to the separation of small species, including metal ions. One advantage of CE over conventional liquid chromatography methods is the lack of a substrate which could interact with the metal species. The authors considered that owing to this important aspect, CE should be investigated for the determination of mercury species, particularly in the light of the substrate interaction problems mentioned above.

The most common detection method is based on molecular absorption spectrophotometry, where achieving good sensitivity for trace analysis is a major problem. This is partially due to the very small volume of sample injected and the short detection path length, between 0.05 and 0.10 mm, as compared to 5 to 10 mm for standard HPLC detectors, although the sensitivity lost by the short path length is partly compensated by the very high efficiencies of CE separations. The most common detection method for metal ions is based on indirect measurement, where a migrating metal ion causes a vacancy in the absorbance of a chromophore which is added to the background electrolyte [17]. Although this unselective approach is capable of determining a large number of metal species, low detection limits are prevented by the relatively high background noise caused by the chromophore in the buffer.

Another, though less common approach to metal ion detection in CE, is to use direct absorbance measurement. Much lower background noise compared to indirect methods should produce better detection limits. The direct approach normally involves the formation of a strongly absorbing metal complex, usually with a chelating dyestuff, formed either pre-column or on-column. For example, 4-(2-pyridylazo resorcinol) and derivatives have been studied by a number of workers for the direct detection of metal ions [18–22]. However, a major drawback to this technique is the potential instability of metal complexes during electrophoretic migration. Thus, many complexes completely dissociate before they reach the detector [22,23]. It appears therefore that the complex must have a large stability constant preferably coupled with relatively slow kinetics of dissociation. Fortunately, mercury species in general form very stable complexes, particularly with sulphur containing ligands. Medina et al. [24] developed a CE method for the determination of organomercury species based on the direct measurement of the UV absorbance of cysteine complexes at 200 nm. This technique has been further investigated with respect to sample stacking [25] and validation [26] by Carro-Díaz et al. The authors considered that a similar approach, but involving highly absorbing mercury complexes in the visible region would be less prone to interference by organic impurities and improve sensitivity.

Dithizone is one of the most studied chelating dyestuffs and forms intensely coloured and very stable complexes with a wide range of metals including the heavy elements. A further important characteristic is dithizone's ability to form strong complexes with organometallic species, particularly

organomercury compounds [27]. Most dithizone complexes are uncharged and this together with their insolubility in water make them unsuitable for CE separation. Tanaka et al. [28] described the synthesis of a water soluble derivative of dithizone called dithizone sulphonate (DzS). The authors considered DzS would be worthy of study for application to the direct detection of metal species, with particular attention paid to mercury species because of the environmental concern discussed above. This paper details the development of an efficient CE separation system for selected inorganic and organomercury species after pre-column formation of their DzS complexes and describes the quantitative performance and detection limits using direct absorbance detection.

2. Experimental

2.1. Instrumentation and separation conditions

All experiments were carried out on a Dionex CES 1 capillary electrophoresis system (Dionex, Sunnyvale, CA, USA) equipped with a reversed polarity power supply. Sample injections were performed hydrostatically at the cathodic side from a height of 100 mm for a period of 30 s, (calculated sample volume 142 nl). A constant voltage of −25 kV was used throughout. Detection was carried out by on-column spectrophotometric measurements at 480 nm. Fused-silica capillaries of 100 μm internal diameter were supplied by Dionex (Camberley, UK). Data was recorded by a Dionex ACI computer interface, and processed using Dionex AI450 automated chromatography software, sampling at a rate of 50 Hz.

2.2. Samples and solutions

A 1000 μg ml^{-1} mercury (II) nitrate standard was obtained from BDH (British Drug Houses, Poole, UK). Methylmercury, ethylmercury and phenlymercury as chlorides were obtained from Shell UK. 1000 mg l^{-1} standard solutions of these were prepared by dissolving the appropriate amount in MilliQ water, with dissolution aided by sonification. All mercury standards were stored in the dark and fresh solutions prepared monthly. Sodium acetate and acetic acid were of Aristar Grade obtained from BDH. Acrylamide, potassium persulphate, *N,N,N′,N′*, tetramethylethylenediamine (TEMED) and γ-methacryloxypropyltrimethoxylsilane were obtained from Sigma (St. Louis, MO, USA). Dithizone sulphonate was manufactured in house, using a modified method based on that developed by Tanaka et al. [28]. All water used was 18 MΩ cm^{-1} obtained from a MilliQ high purity water system (Millipore, Bedford, MA, USA)

2.3. Capillary coating

The capillaries were coated using a procedure based on that developed by Hjertén [29]. Fused-silica capillaries with detection windows 5 cm from the end were washed with 1 *M* NaOH for 30 min, then rinsed with MilliQ water for 15 min. The capillaries were then filled with a 1% (v/v) γ-methacryloxypropyltrimethoxylsilane solution, adjusted to pH 3.5 with acetic acid. The silane solution was allowed to react at room temperature for 1 h, the capillaries were then rinsed with MilliQ water for 15 min. A 3% (w/v) solution of acrylamide was prepared by dissolving 0.3 g acrylamide in 10 ml water, degassed by sonification and helium. 10 μg of potassium persulphate and 10 ml of TEMED were added to the degassed acrylamide solution and thoroughly mixed. The capillaries were filled with the acrylamide solution, both ends sealed, and the capillaries placed in an oven at 40°C for 1 h. At the end of this period unbound polyacrylamide was removed from the capillaries by flushing with water, using an HPLC pump. The coated capillaries were then dried by allowing nitrogen to flow through them overnight.

3. Results and discussion

Dithizone is a well established colorimetric reagent which has been used in the past for the trace determination of many metal ions including organomercury species [27,30]. However, its aqueous insolubility and the even greater insolubility of the metal complexes formed, precludes its use in aqueous capillary electrophoresis. Water soluble derivatives or analogues of dithizone have been little studied because solvent extraction was an important

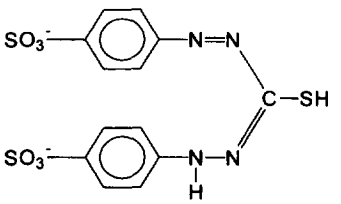

Fig. 1. Structure of dithizone sulphonate.

part of many classical methods to ensure the metal complex was separated from the excess reagent. Dithizone sulphonate (DzS), Fig. 1, is one of the few water soluble derivatives of dithizone described in the literature. DzS should be particularly useful for the determination of organic mercury species of general formula RHgX, by capillary electrophoresis since it forms complexes with a double negative charge of the type $[RHgDzS]^{2-}$ which should separate well from the triple negatively charged dye and its impurities. Inorganic mercury forms the negatively charged complex of the type $[HgDzS_2]^{4-}$, and so should also be well resolved from the organomercury complexes.

3.1. Optimisation of the CE separation

In all cases the DzS metal complexes were preformed prior to CE separation. On-column complexation was not feasible owing to the opposed mobilities of the dye anions and the metal cations. Four mercury cationic species were investigated, namely, inorganic mercury (Hg(II)), methyl mercury, ethyl mercury and phenyl mercury. All the mercury DzS complexes were found to be highly stable even in strong acid conditions. However, the dye itself was unstable below pH 4, though it did not degrade rapidly until below pH 3. This did not preclude the use of low pHs as the mercury species had plenty of time to react before the dye degraded.

Initial studies were carried out with uncoated silica capillaries. First attempts at separation were conducted using a sodium acetate buffer at pH 5, but at this pH the electrophoretic mobility of the complexes was found to be similar to, but opposed to that of the electroosmotic flow (EOF) and no peaks were detected within a reasonable time frame. A lower pH of 3 was then tried since the EOF is much reduced due to minimal ionisation of the surface silanol groups on the capillary wall. Under these conditions a good separation was achieved. This was quite pleasing as the separation of methyl and ethyl mercury was expected to be difficult owing to the small difference in molecular weight. The DzS was clearly not very pure and gave a number of small impurity peaks. These were well separated from the organomercury and did not cause any problems. However, when calibration curves were obtained, although linear, a

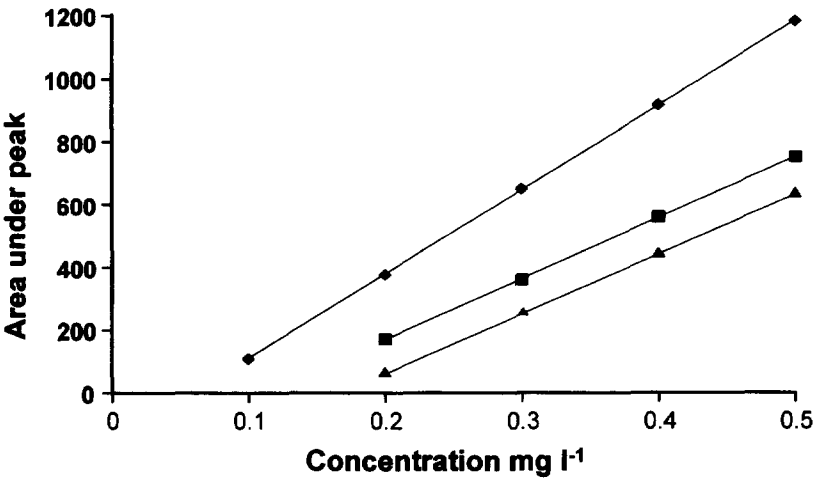

Fig. 2. Organomercury calibration. Electrophoresis conditions: buffer=10 m*M* sodium acetate, pH 3; voltage=−25 kV; detection=480 nm; hydrostatic injection for 30 sec. with a 100 mm height difference. Capillary=fused-silica, 65 cm L_t, 60 cm $L_d \times 100$ μm I.D. ♦=Phenyl mercury; ▲=Ethyl mercury; ■=Methyl mercury.

large negative intercept was found (Fig. 2). This was surprising, since any dissociation during electrophoretic migration should produce erratic or curved calibrations. One possibility was that trace metal impurities in the background buffer were exchanging with the DzS mercury complexes, thus producing a constant subtraction of a small amount of the mercury over the whole calibration range, resulting in the negative intercept. It was considered that the addition of small concentrations of DzS to the running buffer should reduce any exchange or dissociation reactions. When this was done the negative intercept was virtually eliminated while still maintaining linear calibrations. Nonetheless, adding DzS to the buffer gave rise to another problem connected with its instability at low pHs. Whereas instability was not a problem with pre-column formation of the complexes, having DzS in the buffer for long periods at pH 3 resulted in significant degradation in a few hours, reducing its effectiveness in eliminating the negative intercept. Increasing the pH to reduce the degradation of DzS would solve this, but of course the EOF would become significant again, destroying the separation. Using coated columns appeared to be the solution.

There are two methods of capillary coating routinely used in CE, dynamic coatings and bonded coatings. With dynamic coatings, suppression or reversal of electroosmotic flow is generally achieved by the addition of trimethylalkyl ammonium salts to the background electrolyte [31,32]. Unfortunately, in this instance, the use of these salts, for example, hexadecyltrimethylammonium bromide, proved unsuccessful, possibly due to an electrostatic interaction between the positively charged surfactant coating the capillary wall and the negatively charged complexes. It was therefore decided to investigate chemically coated capillary technology. Capillaries coated with neutral hydrophilic coatings have been developed in the field of protein separations [29,33–35]. These are designed to suppress EOF and also to eliminate surface interactions. A polyacrylamide coating was chosen for its neutral charge, hydrophilic properties and the relative ease of in-house manufacture.

A series of experiments were performed using the polyacrylamide coated capillaries with a sodium acetate buffer at pH 5 with the addition of 5 mg l^{-1} DzS. These capillaries were found to give very good separations (Fig. 3). A good feature of the separation was that the species of interest, the organomercury cations, were nicely separated from each other and well separated from the excess dye and its impurities. Nevertheless, the inorganic mercury although migrating in the middle of the impurity peaks was sufficiently resolved to obtain quantitative information.

3.2. Quantitative performance

All the mercury DzS complexes exhibited similar lambda (λ) max. values close to 480 nm. The absorbance spectrum of DzS and its impurities

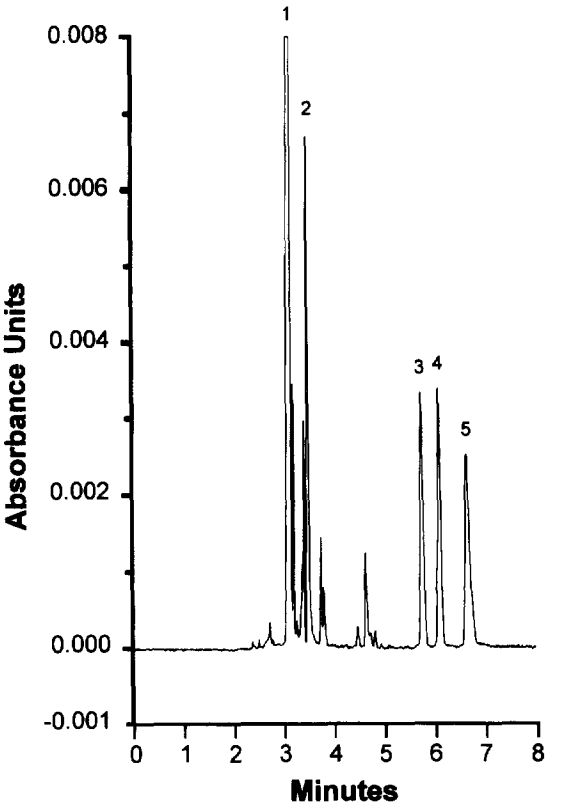

Fig. 3. Separation of inorganic and organomercury species. Separation conditions: buffer=10 m*M* sodium acetate, pH 5, containing 5 mg l^{-1} DzS. Voltage=−25 kV; detection=480 nm. Hydrostatic injection for 30 s with a 100 mm height difference. Capillary, fused-silica, internally coated with polyacrylamide, 60 cm L_t, 55 cm L_d×100 μm I.D. 1=DzS; 2=Hg, 1 mg l^{-1}; 3=MeHg, 1 mg l^{-1}; 4=EtHg 1 mg l^{-1} and 5=PhHg 1 mg l^{-1}.

seriously overlapped those of the mercury complexes, but this was not a problem as long as they were all separated from each other during electrophoresis. Calibration curves were found to have excellent linearity for all four species. A reproducibility study of the methyl mercury peak at the 1 mg l^{-1} level, over 10 consecutive runs, returned a relative standard deviation of 1.6% using peak height and 3.4% using area under the peak. Peak areas have been used for quantitative data calculations throughout. Although peak height generally gives greater precision, it has been observed that at concentrations above 1 mg l^{-1} peak height has been found to produce a non-linear response to concentration [36]. For the lowest calibration ranges a slight negative intercept was found showing the DzS in the buffer did not solve the problem entirely. Nevertheless, this did not prevent determinations down to low μg l^{-1} levels as can be seen in Fig. 4 for methylmercury giving a negative intercept of 5 μg l^{-1}.

In all studies it was found that the background noise was exceptionally low emphasising the potential advantage of direct absorbance methods over indirect methods. This is clearly seen in Fig. 3 with organomercury concentrations at the 1 mg l^{-1} level and also in Fig. 5 showing mercury and methyl mercury at the 0.01 mg l^{-1} levels, close to the limit of detection. The noise levels were found to be ±0.00002 absorbance units coupled with very flat baselines. These are the lowest absorbance noise levels ever seen by the authors in liquid separation detection. Because of the very low noise, the main disadvantage of short path lengths in CE detection has been almost completely off-set and detection limits are similar if not better than ion chromatography with post-column reaction detection. In fact the limit of detection in this case was controlled by the extent of the negative intercept rather than the noise. Thus, the detection limit found for methyl mercury was less than 0.01 mg l^{-1}.

A brief study was carried out involving the CE of other metals as their DzS complexes, which might produce potential interference in the determination of mercury species. Ag, Au, Bi, Cd, Co, Cu, In, Ni, Pb, Pd, Mn, Tl and Zn were investigated. Most were found not to show any peaks at all even at the 10 ppm level, indicating that significant dissociation had taken place before the detector. Some of the metals, Ag, Au, Cd, Co and Pd, gave distorted peaks close to inorganic mercury but well away from the organomercury peaks which are the main ones of interest for this work. These results emphasise the fact that very stable metal complexes are required to survive the electrophoretic migration.

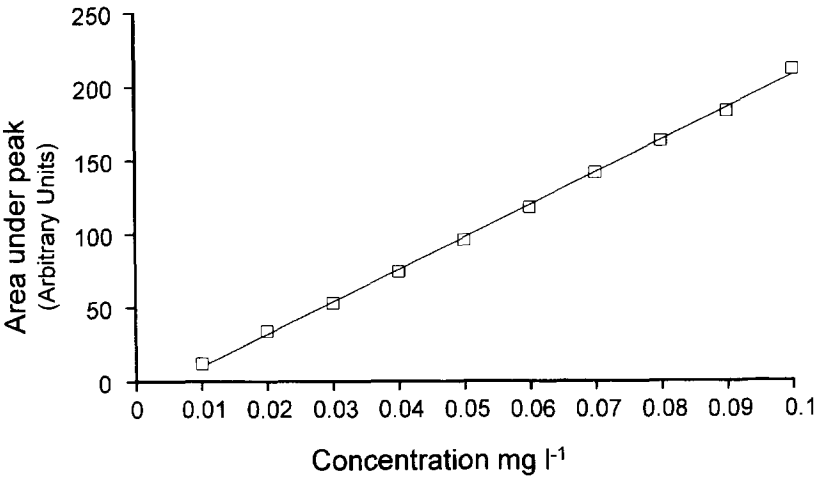

Fig. 4. Methyl mercury calibration from 0.01–0.10 mg l^{-1}. Separation conditions are the same as in Fig. 3. Correlation coefficient r=0.9995.

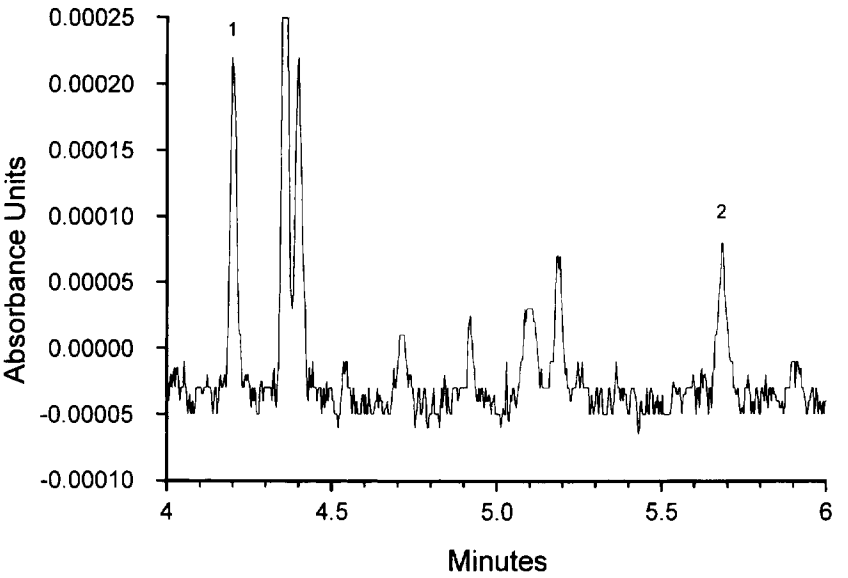

Fig. 5. Electropherogram of inorganic mercury and methyl mercury near the limit of detection. Separation conditions are the same as in Fig. 3 except capillary length is 65 cm L_t, 60 cm L_d. 1=Hg, 0.01 mg l^{-1}; 2=MeHg, 0.01 mg l^{-1}.

4. Conclusion

The results show that good CE separations are relatively easily obtained, particularly for the organomercury complexes, proving that the very stable DzS complexes degrade very little if at all during electrophoretic migration. Neutral coated capillaries were found to be essential, as a significant EOF would ruin the separation. DzS was required in the running buffer to suppress large negative intercepts in calibration curves, but did not appear to have any effect on background noise. In fact, exceptionally low noise levels were found, emphasising the major advantage of direct over indirect absorbance detection. Detection limits were in the low $\mu g\ l^{-1}$ range and should be more than adequate for the next stage of this study which will be the determination of organomercury species in fish flesh, where reported values range from 0.01 to 2.0 $\mu g\ g^{-1}$ fresh weight.

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