µTAS (micro total analysis systems) for the high-throughput measurement of nanomaterial solubility

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Abstract. There is a consensus in the nanoecotoxicology community that better analytical tools i.e. faster and more accurate ones, are needed for the physicochemical characterisation of nanomaterials in environmentally/biologically relevant media. In this study, we introduce the concept of µTAS (Micro Total Analysis Systems), which was a term coined to encapsulate the integration of laboratory processes on a single microchip. Our focus here is on the use of a capillary electrophoresis (CE) with conductivity detection microchip and how this may be used for the measurement of dissolution of metal oxide nanomaterials. Our preliminary results clearly show promise in that the device is able to: a) measure ionic zinc in various ecotox media with high selectivity b) track the dynamic dissolution events of zinc oxide (ZnO) nanomaterial when dispersed in fish medium.

Keywords: µTAS, nanoecotoxicology, dissolution, nanomaterial, zinc oxide

1. Introduction
Under the guidelines given by the Organisation for Economic Co-operation and Development (OECD) [1] and International Organization for Standardization (ISO) [2], solubility is an important physicochemical property to measure for nanomaterials. In particular there is a need to understand the extent of toxicity arising from the ions released as result of the dissolution process. In the past, it is the dissolution of metal oxides nanomaterial such as ZnO that has been of most concern and it is zinc in the ionic rather than the particulate form that has been blamed for potential toxicity [3]. Current protocols to measure solubility often involve a separate sample preparation step prior to analysis; this is often required to distinguish particulate zinc from ionic zinc. In the past this has been achieved either through the use of a dialysis membrane [4] or the extraction of a supernatant using a pipette after centrifugation [5]. In either case, the extracted sample can then be analysed using elemental analysis based techniques such as: Inductively coupled plasma mass spectrometry (ICP-MS) [5] or Inductively coupled plasma atomic emission spectroscopy (ICP-AES) [6]. The reliability of solubility data acquired from such methods hinges largely on the proper sample preparation step e.g. on the careful extraction of the supernatant without disturbing the resulting pellet after centrifugation, a highly operator-dependent step. In the case of the dialysis membrane, an assumption is made that any nanosize zinc particulates have smaller size than that of the pore size of the membrane, which may not be the case. In addition to the potential unreliability of the data, the methods proposed are laborious and not suitable for rapid routine analysis. What is needed is a highly selective device capable of measuring ionic zinc (as opposed to particulate zinc) that is potentially suitable for mass screening.
In the early 1990s, the concept of µTAS (Micro Total Analysis Systems) was put forward, which builds on the idea that a multifunctional chemical laboratory process can be integrated on to a single microfabricated device. The advantages of such devices in comparison to their “bulkier” counterparts are clear and have been well documented to include: faster analysis, lower cost, less sample consumption, and suitability for high-throughput analysis [7]. One such device is the CE(capillary electrophoresis)-conductivity microchip [8]. This is a µTAS device capable of performing: subsampling, sample separation and detection, all achieved by applying high voltages to microchannel networks. The microchip has a capillary electrophoretic column for the separation of ions and a contactless capacitive coupled conductivity detection (C4D) system at the end of the separation column to quantify the concentration of the separated ionic species. The theory of the CE-conductivity microchip has been well documented and was recently shown to be suitable for the quantification of ions in highly complex medium such as blood [9]. Its ability to quantify ion concentration in complex media suggests its potential to be used for the measurement of nanomaterial dissolution in nanocotoxicological applications.

In this study, the feasibility of the CE-conductivity microchip in nanocotoxicological applications is investigated. Our approach is to conduct a preliminary investigation that involves the use of ZnCl₂; this was chosen as our model for analyte dissolution due to the fact that ZnCl₂ is highly soluble in aqueous solutions. ZnCl₂ is dissolved in two separate ecotox media i.e. fish and Daphnia, in order to establish the performance of the microchip for analysis of ionic zinc. Results presented show, for the first time, the feasibility of the CE-conductivity microchip for the analysis of ZnO nanomaterial dissolution when dispersed in fish medium. Its usefulness in monitoring the dynamics of ZnO dissolution for a period of several weeks is assessed. As suggested by Tanyanyiwa et al., the choice of background electrolyte (BGE) buffer mixture is important as it contributes to both selectivity and efficiency of separation; for zinc the BGE of a hydroxyisobutyric acid (HIBA)/histidine (His) combination has been recommended [10].

2. Experimental

2.1 Materials

Unless stated otherwise, all chemicals used in the study were of analytical grade or better, purchased from Sigma Aldrich UK and used as received. De-ionised water was used throughout and had a resistivity value of 18.2 MOhm/cm. Standard test solutions of: a) 1 mM LiCl, KNO₃ and Na₂SO₄ in deionised water and b) BGE of 0.5 M acetic acid, needed to qualify the CE-conductivity microchip, were purchased from eDAQ Europe; these were supplied with the associated certified weight reports provided by Absolute Standards, Inc. Nanomaterial Z-Cote Zinc Oxide was supplied by BASF SE through an OECD global initiative for nanomaterial testing [1]. The physicochemical property of this material has been reported elsewhere and it has been revealed by scanning electron microscopy that the powdered form has a primary particle size of 151.0 ± 55.6 nm [5]; the range reported here represented the broadness of the size distribution.

2.2 Sample Preparation

Stock solutions of ZnCl₂ (100 mM) were dissolved either in fish or Daphnia medium; the appropriate dilutions were made from this stock for subsequent studies. The fish and Daphnia media were prepared based on the recommendations of the OECD guidelines and the detailed protocol for this has been covered elsewhere [11]. The main components of the ecotox media and final pH are summarised in Table 1.
Table 1. Components of Daphnia and fish ecotox medium

| Chemical formula | Fish medium concentration (mM); final pH~7 | Daphnia medium concentration (mM); final pH~8 |
|------------------|------------------------------------------|------------------------------------------|
| Calcium Chloride | CaCl$_2$ 4.87 | 1.08 |
| Magnesium Sulphate | MgSO$_4$ 1.92 | 0.43 |
| Sodium Bicarbonate | NaHCO$_3$ 1.54 | 0.51 |
| Potassium chloride | KCl 0.15 | 0.05 |
| Sodium selenite | Na$_2$SeO$_3$ N/A | Trace i.e. 0.004 |

The protocol employed for the dispersion of the ZnO nanomaterial was based on the recommended protocol used for recent global OECD nanomaterial testing, as previously described [5]. In summary, this involved mixing the nanomaterial powder into a paste before adding more liquid and sonicating using a sonicating probe (Cole Palmer® 130 – Watt Ultrasonic Processors (50/60 Hz, VAC 220)) for 20 s. A final dispersion of 500 mg/L concentration (0.5 L total volume) was prepared and stored in a 1L storage media bottle. A corresponding “control” bottle containing just the fish medium i.e. no ZnO was also prepared. Throughout a 3 week period, 2 mL samples were aliquoted from each bottle; prior to extracting the sample each bottle was shaken gently by hand to aid homogeneity. Each 2 mL aliquot was passed through a 20 nm Anatop syringe filter syringe (Fisher Scientific UK) and the filtrate was collected for further analysis.

For the microchip experiments, a BGE solution of 5 mM His and 3 mM hydroxyisobutyric acid (HIBA) was prepared (the final pH adjusted to 4.5 with acetic acid). All solutions were filtered through a 20 nm syringe filter prior to introduction into the microchip to prevent potential blockages in the microchannels.

2.3 The CE-conductivity microchip

Borosilicate glass microchips were purchased from Micronit, Netherlands (model ET145-4). The fabricated microchip contained a manifold in a double-T geometry and integrated contactless conductivity detection, as schematically shown in Figure 1. The chip has a separation channel ~33 mm long, with a semi-circular cross section of ~100 µm width and ~10 µm depth. The arrangement of the detector consisted of two parallel strips of ~ 200 µm width, ~500 µm height and ~200 nm thick, with the electrodes separated by ~2.9 mm.

The CE-conductivity instrument was purchased from eDAQ Europe and consisted of the following units:

a) Two high voltage sequencers (HVS, Model ER230). Each HVS unit has a dual channel high voltage power supply especially designed for electroosmotic flow applications.

b) Capacitively Coupled Contactless Conductivity Detection (C4D) data system (ER225); this comprised of a conductivity detector and signal recording functions.
c) C4D Micronit chip electrophoresis platform (ET225). This unit has high voltage cables that allow the microchip to be connected to the HVS units; there are four reservoirs that connect the microchip to outside platinum electrodes. The unit includes a safety interlock, to switch off the HVS if the cover plate is lifted during the experiment. The platform also has a ground connector to minimise noise in the C4D signal.

2.4 Electrophoretic analysis on a microchip

The capillary electrophoresis measurement was performed using a BGE that is optimal for the said analyte. The instrumental setting is governed by the type of BGE employed and this was established by following the manufacturer’s instructions [12]. The instrument settings for the test standard BGE (0.5 M acetic acid) were: frequency 800 kHz, amplitude 100%, high gain off, 5 Hz low pass. The instrument settings for the BGE employed for zinc analysis i.e. 5 mM His, 3 mM HIBA, pH 4.5 were: frequency 1150 kHz, amplitude 20%, high gain on, 5 Hz low pass).

For the electrophoretic analysis, the microchip was pre-conditioned prior to use (on a day-to-day basis). This was achieved by flushing the microchannels with the following solutions under vacuum: 0.5 M NaOH for 2 minutes, DI water for 10 minutes and finally BGE for 5 minutes. The microchip was mounted on the C4D Micronit chip electrophoresis platform and electrophoresis was carried out by applying the appropriate voltages to the four reservoirs (see Figure 1b). The voltage programme was set so as to achieve: sample plug formation, sample injection, separation and detection [12]. After use at the end of the day, the microchannels were flushed with deionised water, placed on a thermal hotplate (100ºC) for a few minutes and stored dry in a petri dish. PowerChrom ® software purchased from eDAQ Europe (ES280) was used to collect, display and analyse data.

3. Results and Interpretation

Figure 1c. shows a typical electropherogram for the standard test sample containing cations of lithium, potassium and sodium. The three peaks are attributed to the elution of the three cations, with retention times between 1 and 1.4 minutes. Taking into account that sample injection occurred after 47 s, the net elution time of the cations are: K$^+$ 22s, Na$^+$ 29s and Li$^+$ 38s; these results coincided with findings previously reported [12]. Results show that the peaks are negative, which has been previously associated with the fact that the cations analysed are less conductive than that of the H$^+$ cation that is being displacing in the acetic acid BGE [12, 13]. The expected retention time of the ions is correlated to their corresponding electrophoretic mobility, which in turn is a function of its charge and size that is modulated by the structure and dynamics of the hydration shells and the solvent [14]. Hence, high mobility cations are expected to be eluted first i.e. K$^+$ in this case. The lower electrophoretic mobility of Li$^+$, compared to the other two cations, is attributed to the large solvation shell around Li$^+$ [14].
Figure 1. CE-conductivity microchip analysis. The Figure illustrates: a) a schematic of the microchip set up detailing the equivalent circuit model of the C4D b) a schematic of sample flow depicting plug formation and subsequent injection at the double cross T-junction; the Figure also details the corresponding voltage programme applied on the four reservoirs c) a typical electropherogram of the cations eluted as a result of analysing the test solution at 100 microMolar concentration (with an acetic acid buffer BGE).

Figure 2 shows the electropherograms of ZnCl₂ (at concentrations of 0, 1 and 5 mM) when dissolved in Daphnia and fish medium. The electropherograms clearly show a peak on top of a flat baseline that is attributed to zinc cations. At 0 mM, both electropherograms show a small peak eluted at 1.3 minutes; this peak is due to cations present in the ecotox medium and shall be referred to as the blank peak for the remainder of the paper. It is likely that the broad peak in the blank is attributed to a combination of: Na⁺, Mg²⁺ and Ca²⁺ that exists at the few mM concentrations levels found in the blank ecotox medium. However, in order to verify this, there is a need to spike the blank sample with the appropriate cations to see what their corresponding elution times. It is important to note that the elutions times for the cations observed in the electrophoregram Figure 1c. (this is the reference test samples (containing only Li+, Na+ and K+)) cannot be employed to elucidate the elution times in Figure 2, simply because different BGEs are being employed in each case. The choice of BGE for the analysis of a particular ion is important as they affect separation selectivity.

In the presence of ZnCl₂, another peak elutes straight after the blank peak (at 1.4 minutes), and the size of the peak increases with increasing ZnCl₂ concentration. It is clear that this peak, which will be referred to as the zinc peak, is attributable to the concentration of the ionic zinc present in the media.
Interestingly, there are distinct differences in the profile of the zinc peak between the two media, in how much the blank peak interferes with the zinc peak. When in Daphnia medium, the blank peak is present in all concentrations and at 5 mM the blank peak exists as a shoulder peak on the left of the zinc peak. In the corresponding fish medium, the blank peak does not interfere with the zinc peak and at 1 mM concentration the blank peak disappears from the electropherogram. Another apparent difference between the two media is the size of the zinc peak, with this being much larger in the case of the fish medium compared to the corresponding Daphnia. At 5 mM, the peak height for the fish is ~40 mV whereas this is ~16 mV in the case of Daphnia. The much higher signal in the fish medium can be attributed to the actual concentration of free zinc at the different pH in the two different media. It is well known that zinc dissolution is not straightforward and the zinc can exists in various forms depending on factors such as temperature, the presence of complexing ions, and pH [15, 16, 17, 18]. According to Reichle and co-workers [15], the lower the pH the more likely it is that the zinc will exist as a free ion. In this case, the lower pH of fish medium at pH ~ 7 will mean that more of the zinc will exist as the free ion compared to the Daphnia medium at pH ~ 8, thus explaining the larger signal associated with the fish medium. As suggested by past workers [16], at the higher pH of 8 the following reaction can occur, in which the ion forms a protective, water insoluble zinc hydroxide (Zn(OH)$_2$):

$$\text{Zn}^{2+} + 2\text{OH}^{-} \rightarrow \text{Zn(OH)}_2(s)$$

Interestingly, this interpretation can also explain findings related with the previous OECD study, in which the solubility measurements of ZnO dispersion showed that measured zinc concentrations in fish medium was always higher in comparison to the corresponding medium. The reader should refer to the relevant NPL’s open access report for further details [5]. Another interesting observation from Figure 2 is the shape of the peaks, which is not Gaussian in shape. This can be attributed to the effects of electromigration dispersion, in which diffusional and dispersive forces can arise inside the channels resulting in wedge shaped peaks; these often appear when the sample concentrations are too high [13].

Figure 3 shows the performance of the microchip in the measurement of free zinc in fish medium at various concentrations of ZnCl$_2$, as assessed through its retention time and peak area; the plots show the mean value of 3 replicates with +/-1SD. Figure 3a shows that the eluted zinc peak at $t_R$ of ~ 1.4 minutes (with a 60s sample injection time) is consistent and as expected not affected with change in ZnCl$_2$ concentration. The RSD associated with the retention time is good, being less than 4% in all cases.
Figure 3. The effect upon increasing [ZnCl₂] when in fish medium as assessed by the analysis of eluted zinc peak: (a) retention time, tᵣ, and (b) peak area. Electrophoretic separation and detection were acquired as in Figure 2b. The plot shows mean of 3 replicates with +/-1SD.

Figure 3b shows the corresponding zinc peak area w.r.t ZnCl₂ (when in fish medium) concentration, indicating a strong linear relationship. Repeatability for peak area is less than 10%; this
is true apart from concentrations of 0.05 and 2.5 mM, with RSD of 37 and 34%, respectively. The high RSD associated with the low concentration of 0.05 mM can be attributed to the fact that the measurements are close to limit of quantification. The poor RSD associated with 2.5 mM, however, is puzzling, with no real concrete explanation put forward at this stage. Future repeatability study will need to be conducted in order to establish if this is a real effect and if there is a need to further develop the method further to ensure better repeatability.

Figure 4 shows results from the ZnO dissolution study. Figures 4a and 4b show the electropherograms of the blank i.e. fish medium only, and the 500 mg/L ZnO dispersion in fish medium, respectively; both were taken at the end of the 3 week study. It is clear from Figure 4b that there is a second peak eluted after the blank peak; the blank peak is apparent in both Figures. The second peak can be attributed to dissolved zinc from the ZnO nanomaterial dispersion; this result is similar to before (Figure 2b), in that the zinc peak elutes soon after the blank peak. The size of the zinc peak observed here, however, is very small with peak height < 5 mV. This zinc peak was monitored throughout the 3 weeks study and it first emerged at the end of the first week. After this, the zinc peak seems to appear and disappear within the 3 weeks timeframe. Even though the zinc peak is not always present after week 1, this peak was never present in the corresponding blank samples throughout the study.

Figure 4. Electropherograms from the ZnO dissolution study of: a) blank b) 500 mg/L ZnO dispersion at the end of the 3 weeks study. Electrophoretic separation and detection were acquired under the same experimental conditions as in Figure 2b.

The appearance/disappearance of the zinc peak in the case of the ZnO dispersion can only be explained by the fact that, as stated before, zinc dissolution from nanomaterial is not straightforward and there is a possibility that free zinc re-precipitates in the solid form. As indicated by past workers, several factors can affect the kinetics associated with this re-precipitation event including: pH, concentration of other dissolved ions/organic compounds in the liquid medium. [16, 17,18].

4. Conclusion
In this study, a µTAS device was shown to be useful in the measurement of dissolution of metal oxide nanomaterial. In particular, we had shown the feasibility of the CE-conductivity microchip to investigate the solubility ZnO nanomaterial in fish medium. The device was used to highlight the complexity the dissolution events and that the re-precipitation of ionic zinc (and its kinetics) must be considered and further investigated in order to elucidate the mechanism of nanomaterial toxicity.
References

[1] OECD Series on the Safety of Manufactured Nanomaterials, ENV/JM/MONO(2010)46, No. 27 - 46, 2010 List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials: revision. http://www.oecd.org/document/53/0,3746,en_2649_37015404_37760309_1_1_1_1,00.html

[2] ISO, ISO/TR 13014 2012 Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment.

[3] Gilbert B, Fakra S C, Xia T, Pokhrel S, Mädler L, Nel A E, 2012 The fate of ZnO nanoparticles administered to human bronchial epithelial cells ACS Nano. 6(6):4921-30.

[4] Fabrega J, Tantra R, Amer A, Stolpe B, Tomkins J, Fry T, Lead J R, Tyler C R and Galloway, T S 2012 Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod corophium volutator Environ. Sci. Technol 46(2) 1128-35.

[5] Tantra R, Boyd R, Cackett A, Fry A T, Gohil D D, Goldberg S, Lee J L S, Minelli C, Peck R, Quincey P, Smith S, Snowden J, Spencer S, Tompkins J, Wang J, Yang L 2012 NPL report: final report on the physico-chemical characterisation of PROSPECT engineered nanomaterials: http://publications.npl.co.uk/dbtw-wpd/exec/dbtwpub.dll?&QB0=AND&QF0=ID&QI0=%206281%20&TN=NPLPUBS&RF=WFullRecordDetails&DL=0&RL=0&NP=4&AC=QBE_QUERY.

[6] Pasqui D, Golini L, Della Giovampaola C, Atrei A and Barbucci R 2011 Chemical and biological properties of polysaccharide-coated titania nanoparticles: the key role of proteins. Biomacromolecules 12(4) 1243-49.

[7] Weigl B H, Bardell R L and Cabrera C R 2003 Lab-on-a-chip for drug development Adv Drug Deliver Rev 55(3) 349-377.

[8] Elbashir A A and Aboul-Enein H Y 2012 Recent advances in applications of capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C4D): an update Biomed. Chromatogr. 26(8) 990-1000.

[9] Vrouwe E X, Luttge R, Vermes I and van den Berg A 2007 A Microchip capillary electrophoresis for point-of-care analysis of lithium Clin. Chem. 53(1) 117-123.

[10] Tanyanyiwa J and Hauser P C 2002 High-voltage capacitively coupled contactless conductivity detection for microchip capillary electrophoresis Anal. Chem. 74(24) 6378- 82.

[11] Tantra R, Jing S and Gohil D 2010 Technical issues surrounding the preparation, characterisation and testing of nanoparticles for ecotoxicological studies, V.B. Popov (Editor) WIT Press, Environmental Toxicology 3 165-176.

[12] eDAQ. Application Note C4D016. Procedure for microchip electrophoresis with C4D. http://www.edaq.com/index.php.

[13] Brito-Neto J G A, da Silva J A F, Blanes L and do Lago C L 2005 Understanding capacitively coupled contactless conductivity detection in capillary and microchip electrophoresis. part 1. fundamentals Electroanal. 17 (13) 1198-1206.

[14] Koneshan S, Rasaiah J C, Lynden-Bell R M and Lee S H 1998 Solvent structure, dynamics, and ion mobility in aqueous solutions at 25 degrees C J. Phys. Chem. B 102(21) 4193-4204.

[15] Reichle R A, McCurdy K G and Hepler L G 1975 Zinc hydroxide: solubility product and hydroxy-complex stability constants from 12.5 - 75 degrees C Can. J. Chem. 53 3841- 45.

[16] Degen A and Kosec M 2000 Effect of pH and impurities on the surface charge of zinc oxide in aqueous solution J Eur Ceram Soc 20(6) 667-673.

[17] Lv J, Zhang S, Luo L, Han W, Zhang J, Yang K and Christie P 2012 Dissolution and microstructural transformation of ZnO nanoparticles under the influence of phosphate Environ Sci. Technol. 46(13) 7215- 21.
Mudunkotuwa I A, Rupasinghe T, Wu C M and Grassian V H 2012 Dissolution of ZnO nanoparticles at circumneutral pH: a study of size effects in the presence and absence of citric acid *Langmuir* 28(1) 396-403.

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