

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231190650>

Scheme for classification of heavy metal species in natural waters. Reply to comments

ARTICLE *in* ANALYTICAL CHEMISTRY · OCTOBER 1980

Impact Factor: 5.64 · DOI: 10.1021/ac50062a043

CITATIONS

20

READS

8

2 AUTHORS, INCLUDING:



[Graeme E Batley](#)

Commonwealth Scientific and Industrial Res...

218 PUBLICATIONS **7,395** CITATIONS

SEE PROFILE

acid tends to adsorb onto the Hg electrode and causes shifts in the apparent concentrations of aquo and complexed forms of the metals (8, 9). The extents of such effects will clearly change between the preformed and the in situ modes of operation. The present experiments have been insufficient to delineate the significance of the Hg competition effect relative to the fulvic acid adsorption interference effect. Therefore, the above data may reflect both effects.

Although the value of the computational evaluations presented above depends largely on a lack of kinetic stability for the species present in natural water systems, they have been used as vehicles for demonstrating the potential influences of the recommended analysis conditions when the species are kinetically labile. Similarly, the limited experimental results presented are not necessarily representative of the attainment of equilibrium in each instance. They have been included primarily as illustrative examples of shifts in the estimation of electroactive ion concentrations which may be induced by various stages of the recommended sample treatment protocol. The computations and the example results indicate that the effects considered may, individually or collectively, play prominent roles in shifting the native equilibria extant in water samples into entirely nonrepresentative conditions.

Florence and Batley have emphasized that speciation by their procedures relies on behavioral differences and that the use of the term "labile metal" requires a statement of the exact experimental conditions used. They have also stressed that the fraction of metal contributing to the ASV stripping current may be comprised of electroactive complexes as well as aquo ions. The present authors strongly support these emphases; such speciation measurements must be qualified within the

context of the equilibrium shift possibilities which may be induced by the sample treatment and measurement steps. Ideally, they should also be referenced to the results of similar measurements on well-defined metal-ligand systems as validation models. Without such model evaluations, the actual analytical efficacies of speciation methods cannot be reliably assessed nor can the results obtained be realistically evaluated in terms of potential significance.

LITERATURE CITED

- (1) Batley, G. E.; Florence, T. M. *Anal. Lett.* **1976**, *9*, 379-388.
- (2) Batley, G. E.; Florence, T. M. *Mar. Chem.* **1976**, *4*, 347-352.
- (3) Batley, G. E.; Gardner, D. *Estuarine Coastal Mar. Sci.* **1978**, *7*, 59-66.
- (4) Florence, T. M.; Batley, G. E. *Estuarine Coastal Mar. Sci.* **1977**, *75*, 791-797.
- (5) Florence, T. M. *Water Res.* **1977**, *11*, 681-687.
- (6) Crow, D. R. "Polarography of Metal Complexes", Academic Press: London, 1969, pp 2-10.
- (7) Schnitzer, M.; Skinner, S. I. M. *Soil Sci.* **1966**, *102*, 361-365.
- (8) Greter, F. L.; Buffle, J.; Haerdi, W. J. *Electroanal. Chem.* **1979**, *101*, 211-229.
- (9) Buffle, J.; Greter, F. L. *J. Electroanal. Chem.* **1979**, 231-251.
- (10) Skoog, D. A.; West, D. M. "Fundamentals of Analytical Chemistry", 3rd ed.; Holt, Rinehart, and Winston: New York, 1976; p 786.
- (11) Reference 10, p 280.

¹ Present address: Department of Chemistry, State University of New York, Buffalo, NY 14214.

R. K. Skogerboe*
S. A. Wilson
J. G. Osteryoung¹

Department of Chemistry
Colorado State University
Fort Collins, Colorado 80523

RECEIVED for review December 21, 1979. Accepted June 26, 1980. Supported in part by EPA Grant No. R805-183-02.

Sir: Skogerboe et al. quite correctly point out that the trace metal speciation scheme described by Batley and Florence (1, 2) may not accurately measure the concentrations of the various species originally present in a water sample, because (a) the pH of the sample is changed before measurement, (b) acetate ion is added, and (c) mercury(II) nitrate is added for in situ mercury film formation. We were, in fact, very careful to emphasize in our applications (2-4) of this speciation scheme that the results obtained are *operationally defined* and do not necessarily reflect the true equilibrium concentrations of metal species. However, it should *not* be assumed that, by omitting acetate and Hg(II) and maintaining the pH at the natural value, the scheme will yield the original equilibrium species concentrations. Anodic stripping voltammetry (ASV) is a dynamic method of analysis, and all labile metal complexes will dissociate to some extent at the electrode surface (5), whatever the pH. Other perturbations from equilibrium may take place during passage of the solution through the chelating resin column and as a result of UV irradiation. Even the initial filtration of the sample through a membrane filter may affect the speciation by removing adsorbent in the form of particulate matter and by changing the concentrations of dissolved oxygen and carbon dioxide.

The measurement of ASV-labile metal at the natural pH of the water, and in the absence of buffer, poses some practical problems. The slopes of the ASV peak height-metal concentration curves for Cu, Pb, Cd, and Zn are sensitive to pH and decrease rapidly at pH values higher than 6 (6). Each water sample would have to be carefully calibrated by standard addition of metal, a procedure difficult to perform without altering the pH of unbuffered waters of pH around 7. Deaeration of unbuffered waters also alters their pH, and electrolysis of unbuffered, near-neutral solutions results in the pH at the electrode surface varying considerably from that of the bulk solution. With many fresh waters, an inert elec-

trolyte would have to be added to provide sufficient electrical conductivity, particularly if differential pulse ASV is used. ASV measurements on unbuffered waters would therefore be poorly characterized by comparison with measurements made in a buffered solution of constant pH.

ASV at a thin mercury film electrode (TMFE), formed in situ by simultaneous deposition of mercury and trace metals, is now widely recognized as the most convenient and sensitive ASV technique, particularly when the differential pulse mode is used (7-10). When we first applied the in situ mercury deposition method to trace metal speciation, we carried out experiments on seawater and freshwater in which results with a TMFE were compared when the electrode was both preformed and in situ formed. No significant differences were found. This result can be explained by assuming that if a complex, ML, is sufficiently labile to exchange with 10^{-5} M Hg(II) after about 15 min at 25 °C, then it will also exhibit considerable ASV lability, and the concentration of M measured in the absence and presence of Hg(II) may not be greatly different. A similar argument could be used to predict that low concentrations of acetate would not seriously affect labile metal measurements. The very large differences found by Skogerboe et al. between preformed and in situ deposited films are surprising, and we wonder if they were the result of calibration problems caused by working with unbuffered solutions. Also, high concentrations of fulvic acid lead to electrode fouling, and calibration by standard addition under these conditions is difficult because the calibration curves are nonlinear.

It is quite possible, however, that in some samples the addition of Hg(II) and acetate could affect speciation results. This is not a problem as long as it is understood that the operationally defined scheme includes the addition of fixed concentrations of Hg(II) and acetate to the sample. Since it is impossible to implement a speciation scheme involving ASV

lability measurements without altering the sample in some way, it is better to have a buffered, well-defined system so that results on different waters can be more readily compared.

There are some deficiencies in our speciation scheme which were not mentioned by Skogerboe et al. It does not cope very well with mixed organic/inorganic colloids such as humate-coated iron oxide, which may be the predominant type of colloid in many natural waters (11). Also, for metal toxicity studies, the Chelex-100 resin, which complexes metals via oxygen and nitrogen bonds, does not simulate metal-binding proteins in biological systems. Passage of heavy metals across biological membranes probably occurs principally by carrier-mediated transport with metallothionein-type proteins to which the metal is complexed by sulfur (thiol) bonds (12). We are at present developing an alternative speciation scheme by using a thiol complexing resin which is equilibrated with the sample at the original pH. ASV is used only for total, not labile, metal measurements. Results from this new scheme will be compared with those obtained by our published procedure.

LITERATURE CITED

- (1) Batley, G. E.; Florence, T. M. *Anal. Lett.* **1976**, *9*, 379-388.
- (2) Batley, G. E.; Florence, T. M. *Mar. Chem.* **1976**, *4*, 347-363.
- (3) Batley, G. E.; Gardner, D. *Estuarine Coastal Mar. Sci.* **1978**, *7*, 59-70.
- (4) Florence, T. M. *Water Res.* **1977**, *11*, 681-687.
- (5) Davison, W. J. *Electroanal. Chem.* **1978**, *87*, 395-404.
- (6) Florence, T. M.; Batley, G. E. *Talanta* **1976**, *23*, 179-186.
- (7) Florence, T. M. *J. Electroanal. Chem.* **1970**, *27*, 273-281.
- (8) Lund, W.; Onshus, D. *Anal. Chim. Acta* **1976**, *86*, 109-122.
- (9) Valenta, P.; Mart, L.; Rutzel, H. J. *Electroanal. Chem.* **1977**, *82*, 327-343.
- (10) Florence, T. M. *Anal. Chim. Acta*, in press.
- (11) Senesi, N.; Griffith, S. M.; Schnitzer, M.; Townsend, M. G. *Geochim. Cosmochim. Acta* **1977**, *41*, 969-976.
- (12) Lerch, K. *Nature (London)* **1980**, *284*, 368-370.

T. M. Florence*
G. E. Batley

Analytical Chemistry Section
AAEC Research Establishment
Lucas Heights, NSW, 2232
Australia

RECEIVED for review June 10, 1980. Accepted June 26, 1980.

Liquid Chromatography of Alkaloids, Drugs, and Nitrogen Heterocycles on a Porous Polymer

Sir: Macroporous resins are coming into use as sorbents for liquid chromatography. They are stable over the entire aqueous pH range, and their behavior toward organic acids, bases, and ampholytes has been studied (1-3). The Amberlite XAD resins (Rohm and Haas Co.) have been widely used. As sold, they are not suitable for high-performance chromatography because their particles are too large; ground to sizes of 10 μ M, however, they have given plate heights of 0.05 mm and less (4).

Recently, porous polymers have been produced in Japan expressly for liquid chromatography. They have small, uniform, spherical particles and have given good chromatography of drug mixtures (5) and urine (6). Both styrene and acrylic polymers are available. Since they are stable at high pH, it is natural to use them for organic bases, such as alkaloids.

Alkaloid mixtures have been analyzed by liquid chromatography using a silica-bonded cation exchanger (7) and reversed-phase, hydrocarbonaceous bonded silica (8-11); paired-ion chromatography has been used (12-14) as has normal-phase chromatography on silica (15). In several cases eluents of high pH were used, and degradation of the silica columns must have been a problem. Wu and Wittick (10) made a careful analysis of the alkaloids of gum opium, using a reversed-phase column with phosphate buffers in aqueous acetonitrile.

We have found that alkaloids of various classes, as well as other nitrogen bases, can be separated on a column of porous styrene-divinylbenzene copolymer, using alkaline acetonitrile-water eluents.

EXPERIMENTAL SECTION

Resin, Column, Equipment. Hitachi Gel 3010, a macroporous styrene-divinylbenzene copolymer, was kindly supplied by S. Ganno and K. Fujita of Hitachi Corp., Tokyo, Japan. It was a special grade having particles 10 μ m in diameter. A stainless-steel column, 4.6 mm i.d. by 22 cm long, was packed under pressure with a slurry of the resin in 50% acetonitrile. It was fitted with

a water jacket and run at 50-60 °C. At 1 mL/min the back-pressure was 500 psi (35 atm). The pumps were Waters Model 45 or 6000. Injections were made with a Rheodyne valve, Model 7125, having a 20- μ L sample loop. Detection was by UV absorbance at 254 nm, using an Altex Model 152 detector.

Solvents, Chemicals. Acetonitrile of chromatographic grade came from J. T. Baker Co. and Burdick and Jackson Laboratories; alkaloids, including U.S.P. opium powder, were kindly provided by the University of Colorado School of Pharmacy, with Merck and Co. labels; Aldrich Chemical Co. supplied other materials. Solvents were degassed and then measured and mixed by volume; "45% acetonitrile" means that 45 parts of acetonitrile by volume was mixed with 55 parts of water. After the solvents were mixed, a small volume of concentrated ammonia or tetrabutylammonium hydroxide was added, and the base concentration was found by titration with standard acid. In some experiments, where successive amounts of water were added to the solvent in the reservoir in order to study the effect of solvent composition on retention, the density of the mixed solvent was measured by pipetting 5.00 mL into a weighed flask at ambient temperature (22 °C) and then weighing the flask and contents. The volume percent of acetonitrile was found to within 1% from a calibration curve.

Sample Preparation. To test the chromatographic method, we ran chromatograms of brown U.S.P. opium powder. Weighed quantities were extracted overnight with water; the extracts were centrifuged and made to known volume. The clear aqueous extracts were mixed with equal volumes of acetonitriles before injecting. No other pretreatment or "cleanup" was performed.

RESULTS AND DISCUSSION

Figure 1 shows chromatograms of six alkaloids. At 1 mL/min the plate number for papaverine was 1600; at 2 mL/min it was 1050. Figure 2 shows the separation of three opium alkaloids plus ethylmorphine, and Figures 3 and 4 show chromatograms of opium extracts at different solvent compositions, along with a standard mixture. The peak identifications in Figure 3 were made by comparing the retention volumes of known compounds. Figure 5 shows the dependence of retention on solvent composition and indicates that better resolution is possible at lower acetonitrile concentrations.