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# Analytical Techniques for the *in situ* Measurement and Speciation of Trace Compounds in Natural Waters

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**Abstract.** A major research component of the analytical activities of the Analytical and Biophysical Environmental Chemistry group of the University of Geneva (CABE) is focused on the development of chemical sensors and mini or microanalytical systems for *in situ* measurements of trace compounds in aquatic environmental systems, including surface waters, sediments or water treatment plants. In this field, new concepts are required in order to determine not only the total concentration of environmental analytes but also the concentrations and physicochemical properties of their environmentally relevant chemical forms (chemical speciation). New selective analytical systems integrating reliable and rugged sensors with simple separation principles must be imagined to perform *in situ* (at depth), real-time, automatic measurements. Microtechnology is a key factor in such developments. New analytical methods must also be developed to characterize the nature and properties of the major natural, often colloidal or polymeric, complexing agents. In this context, the scientific approach of CABE is explained.

## Introduction

This paper reports the scientific approach and analytical activities of the Analytical and Biophysical Environmental Chemistry group of the University of Geneva (CABE). Research activities encompass several areas including:

- the development of new sensors and mini or microanalytical systems for *in situ* measurements in aquatic environments such as surface waters, sediments or water treatment plants;
- the analysis and characterization of natural macromolecules and colloids;
- the development of methods for speciation (see below) of trace elements in environmental medium.

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Developments in these areas are both socially relevant from a public policy point of view and scientifically challenging. The need of inexpensive, *in situ* probes for the continuous, real-time, automatic recording of pollutant concentrations and other environmentally relevant parameters is obvious. Decreasing cost and increasing the frequency of analytical measurements is an absolute requirement to enable the efficient monitoring of environmental conditions. These analytical developments are among the most challenging faced by analytical chemists in the last decades. Indeed, new concepts must be imagined to simplify the high tech laboratory techniques which are not usable *in situ*. Simultaneously, improved sensitivity and selectivity are required for environmental system analysis, since most important analytes are present at trace levels (typically  $10^{-12}$ – $10^{-7}$  M), and under a large variety of chemical forms which must be discriminated in order to correctly interpret their environmental impact. In addition, most trace compounds interact with complex natural colloids and biopolymers (e.g., clays, iron oxides, polysaccharides, humic substances), the properties of which must still be characterized and for which new analytical methods need to be developed. Finally, the development of *in situ*

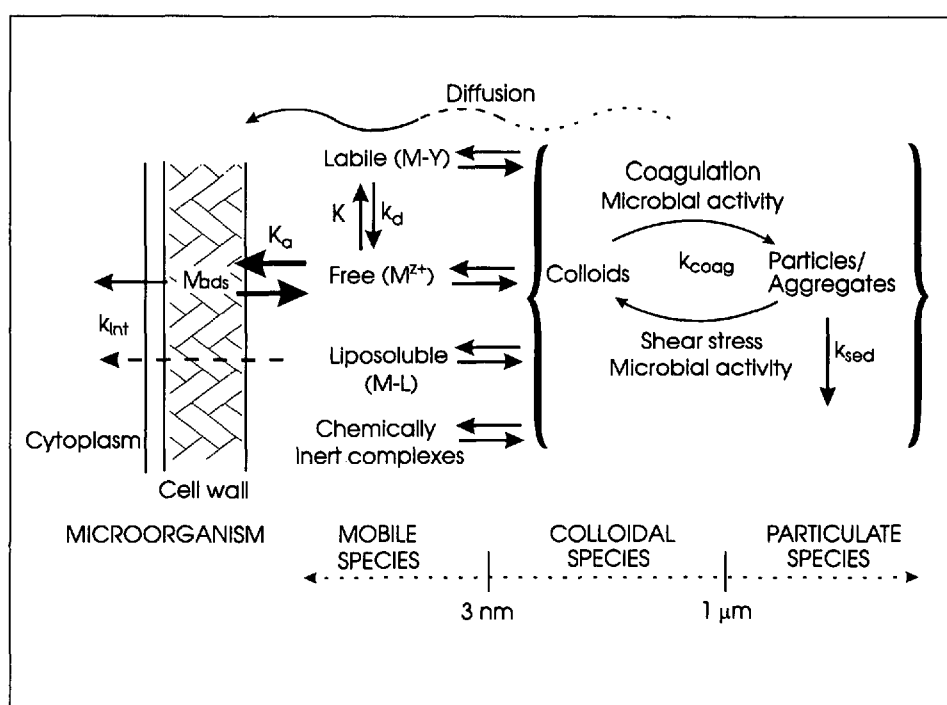


Fig. 1. Schematic discrimination among several groups of metal complexes, based on their behaviour in surface waters. Particulate bound metals ( $> 1 \mu\text{m}$ ) will sediment relatively quickly (residence time in water = hours to days) [5]. Colloid bound metals ( $1 \text{ nm} - 1 \mu\text{m}$ ) may either remain in suspension, be eliminated by horizontal transport or sediment following coagulation [1][3]. Mobile species ( $> 3 \text{ nm}$ ) include small labile complexes and the free metal ion which have been shown to be key species related to metal bioavailability [2][4]. Liposoluble complexes may also be bioavailable due to passive transport by dissolution/diffusion in the plasma membrane.

analytical probes which can function in *extreme* conditions (*e.g.*, at hundreds or thousands of meters in lakes or oceans) is a multidisciplinary task, since demanding criteria are imposed not only on the chemical properties of the sensing element, but also on factors such as the robustness of the material used, the long-term reliability of the devices, the capacity and rapidity of signal storage and the transmission and energy consumption of the probe.

### Speciation

Measurements of total metal concentrations alone are often inadequate for understanding the biological or physicochemical fate of vital or harmful metals in natural aquatic media and for undertaking possible remedial action, since the chemical combination of an element or compound with natural complexants will affect its bioavailability [1][2] and/or mobility [3][4] (*Fig. 1*). Examples of how such discrimination can be done *in situ* based on sound physicochemical principles are given in the subsequent section. Over the last few decades, the development of laboratory speciation techniques has been largely limited by the ill-defined properties of the natural complexing agents and the fact that not enough attention has been paid to the specific environmental processes of interest. Because these components may interfere strongly with the separation or detection principles, new developments of *in situ* probes must necessarily take into account the nature, physicochemical characteristics and properties of complexing ligands present in the test medium. This is often trivial for simple complexes, *e.g.*, with inorganic anions ( $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ ) or small organic complexants (*e.g.*, amino acids). However, most aquatic complexing agents are structurally heterogeneous macromolecules, colloids or particles or aggregates of those and can range in size from 1 nm to tens of micrometers. The characterization of natural colloidal complexing agents is itself an important analytical challenge, which includes not only measurements of chemical composition but also of conformation and aggregation as function of physicochemical (pH, ionic strength, hydration), and biochemical (enzymatic, microbial) parameters.

CABE is developing new techniques for such studies based in particular on

- transmission electron microscopy (in particular on site, non-perturbing specimen preparation) coupled to chemical probes;

Table 1. *Advantages of in situ Probes for Natural Water Monitoring*

- Elimination of many of the artefacts due to sample handling, *i.e.*, no or minimum sample transformation (see text)
- Minimization of the overall cost of data collection (in particular, due to a reduction of analysis time)
- Possibility of real-time analysis, allowing the rapid detection of pollutant inputs (*e.g.*, monitoring of industrial wastes or water quality in water treatment plants)
- Ability to accumulate detailed spatial and temporal data banks of complete ecosystems (lakes, aquifers ...)
- Possibility to perform measurements in locations which are difficult to access (boreholes, deep lakes or oceans)
- Concentration gradients and fluxes at environmental interfaces (sediment-water; air-water) may be measured directly

Table 2. *Criteria for the Design of in situ Probes for Natural Water Monitoring*

- Reliable, automatic measurements
- Simple, compact, low-cost apparatus
- No or minimum sample transformation during analysis (minimization of artifacts)
- Multielement (or -component) analysis
- High sensitivity ( $10^{-7}$ – $10^{-12}$  M or less)
- Speciation capabilities
- Use of microsensors/total analytical microsystems

- atomic force microscopy (study of conditions for non-perturbing observations of conformation, adsorption or aggregation processes);
- multidetector capillary electrophoresis (aggregation and conformation studies);
- correlation fluorescence spectroscopy (aggregation, conformation and adsorption studies).

### Advantages and Criteria of *in situ* Probes for Trace Metals

The major processes which may create artefacts during trace metal speciation in laboratory measurements include [1][4][5]: *a*) losses of trace metals or trace metal complexants on labware surfaces; *b*) contamination of metals or organics by desorption from the polymer/glassware, filters and analytical apparatus; *c*) gas reequilibration (*e.g.*,  $\text{CO}_2$ ,  $\text{O}_2$ ) of the sample with the atmosphere due to a pressure

or temperature change with concomitant pH or redox variations (and metal speciation); *d*) coagulation of colloidal matter, followed by sedimentation of the aggregates and the associated trace metals; *e*) physicochemical changes due to microbial activity. Most of these artefacts are avoided or largely minimized by using real-time analysis with adequate *in situ* probes. Other advantages of *in situ* measurements are given in *Table 1*.

*Table 2* lists the key criteria for the development of *in situ* probes. In particular reliability is much more limiting for *in situ* measurements than for laboratory analysis. As discussed in [4], most traditional separation and detection techniques cannot be used for *in situ* speciation measurements. The development of microsensors and microanalytical systems [6] are key steps in the development of *in situ* probes. Not only are miniaturized systems easier to handle in the field but also sample contamination is greatly reduced. Above all, the development of sensors which

mimic natural processes is facilitated with microsystems in that they are of similar size (1–10  $\mu\text{m}$ ) and can be based on similar processes as those influencing the metabolism of plankton (bacteria, algae). Measurements can then be made which facilitate the comprehension of the reaction of biota to human impact. Despite the obvious environmental need for *chemical* probes [4], few exist, especially for depths > 2–3 m, largely due to the analytical difficulties which need to be overcome. Examples of recent developments are given below.

#### Example 1: Probes Based on Gel Integrated Voltammetric Microelectrodes

One possible approach for metal analysis is the development of voltammetric microelectrodes integrated in hydrophilic gels (Fig. 2). A microdisk (or a microdisk array) electrode can be covered with a gel layer much thicker than the radius of the disk, but thin enough to allow quick (minutes) equilibration with the water sample. After equilibration, voltammetric analysis is performed inside the gel. The gel is selected to allow equilibration of metal cations and small complexes, while pre-

venting the diffusion of potentially fouling colloids and macromolecules [7]. The analytical device thus integrates a fractionation by dialysis with voltammetric detection. This enables the microanalytical systems to be inserted directly inside sediments, in particular to measure concentration vs. depth profiles and, therefore, fluxes of metals at the sediment-water interface.

Either iridium or mercury plated iridium microelectrodes can be employed in the voltammetric probe depending on the required determination [8]. The iridium microelectrode can be prepared by electroetching an Ir wire to a diameter of a few microns and sealing it in a glass capillary (Fig. 2b) [8]. Alternatively, microelectrode arrays can be prepared by thin film technology on chips [9] and covered by the protective gel layer (Fig. 2c). Using microtechnology, microsystems of a few  $\text{mm}^2$ , including the gel covered microelectrode arrays, as well as the counter and reference electrodes can also be prepared. The sensitivity limit obtained with both single microelectrodes and microelectrode arrays is  $10^{-10}$ – $10^{-11}$  M.

*In situ* measurements combining gel separation with voltammetric microelectrodes have a number of advantages as discussed in [4][7]. A unique key characteristic of this technique is the specificity of the voltammetric response to the mobile metal species, *i.e.*, the free metal ion and complexes which diffuse fast enough to be reducible at the microelectrode during the time frame of the measurement. It can be shown [10] that the size limit between the mobile and larger colloidal metal species is a few nm. The specificity of voltammetric microelectrodes for such small species is important as these species are believed to form most of the bioavailable metal fraction in environmental systems.

A commercial portable *in situ* probe has been developed based on the gel integrated voltammetric microelectrode (Voltammetric *in situ* Profiling System), in cooperation between CABA and *Idronaut-Srl* (Via Monte Amiata 10, I-20047 Brughiero). It allows the measurement of Cu, Pb, Cd, Zn, Fe, Mn and S(-II) at variable depths down to 500 m. The probe contains all the hardware and firmware necessary for the voltammetric measurements and telemetric data (see [10] for detail).

#### Example 2: Supported Liquid Membrane Coupled to Voltammetry

The supported liquid membrane is a preconcentration technique based on the complexation of the metal ion of interest

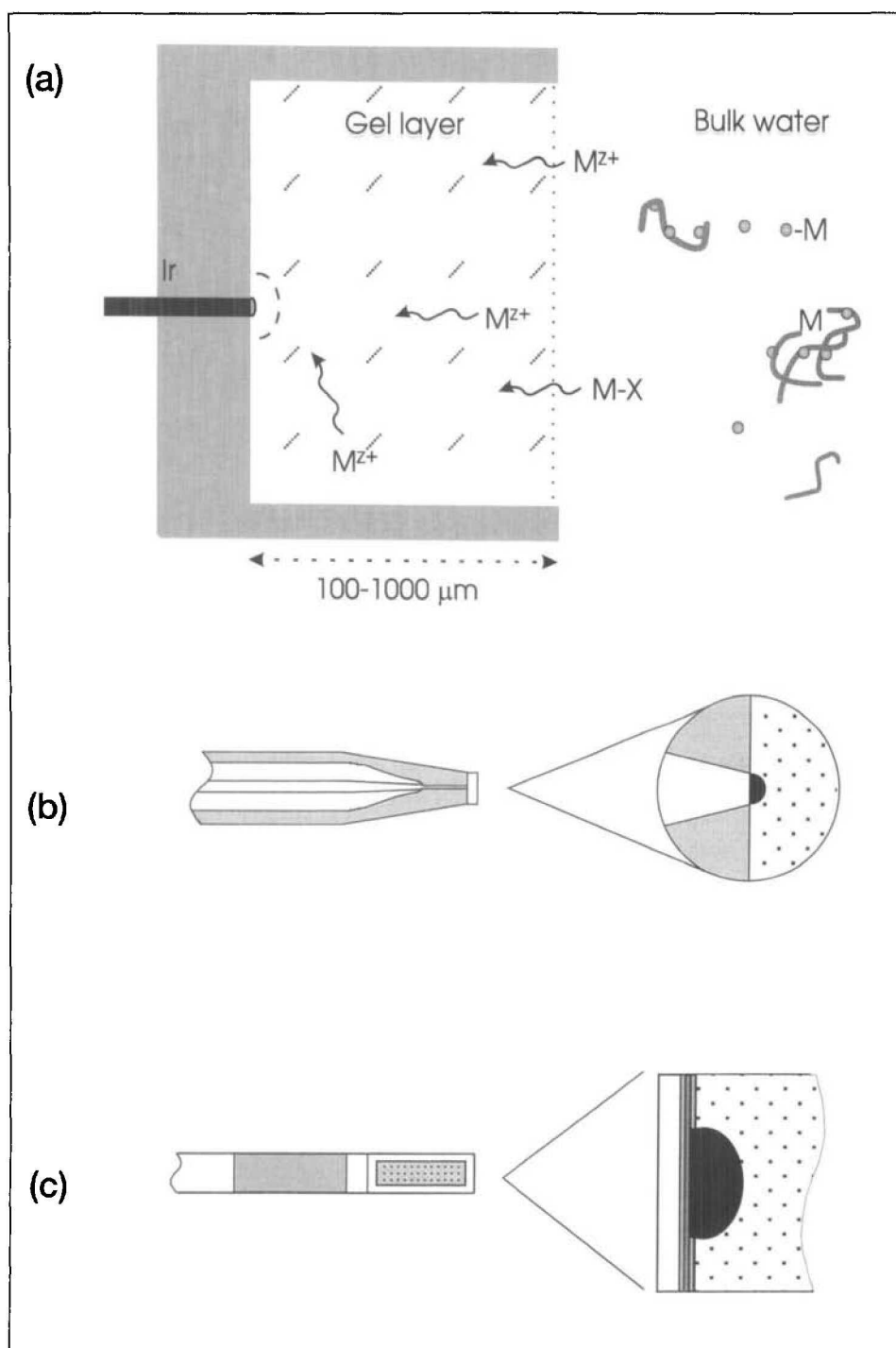


Fig. 2. Principle of gel integrated voltammetric microelectrodes (a) and schematic drawings of gel covered Ir based microelectrodes: single microelectrode sealed in capillary glass (b); microelectrode array (top) and Ir counter electrode (bottom) combined on the same support (c)

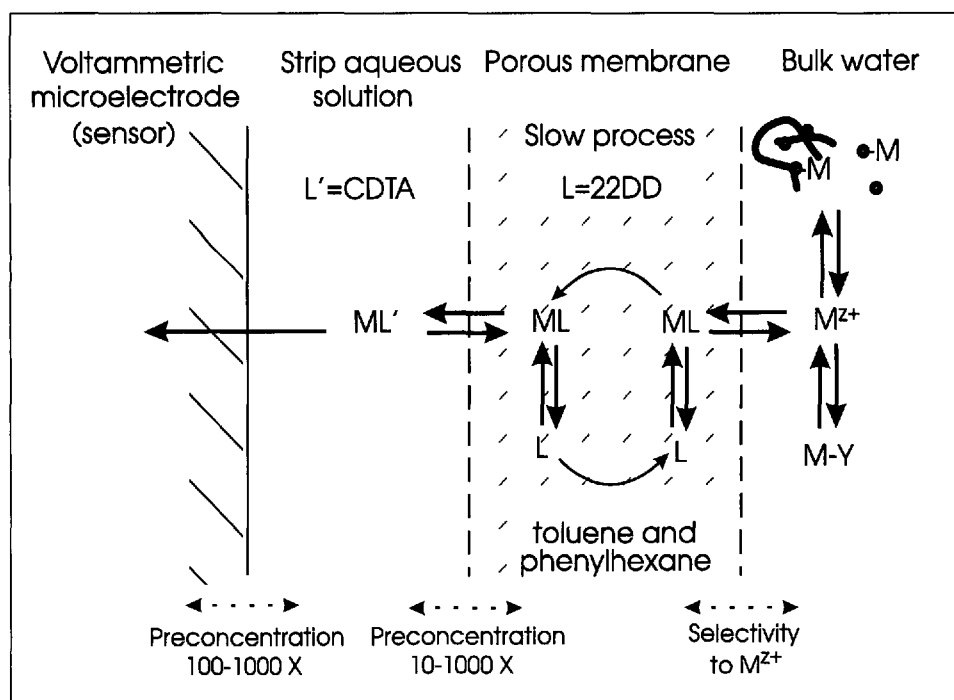


Fig. 3. Schematic representation of the Supported Liquid Membrane preconcentration technique coupled to voltammetric detection. Solve = Toluene + Phenylhexane. L = 22DD = 1,10-didecyl-1,10-diaza-18-crown-6; L' = CDTA = *trans*-cyclohexane-diamino-tetraacetic acid.

by a hydrophobic ligand dissolved in a non water-miscible solvent which is immobilized in a porous inert membrane, followed by a simultaneous back extraction into an aqueous solution (strip) (Fig. 3) [11]. The interest of this technique is that it mimics some of the processes which occur for the transport of metal ions through biological membranes. In particular, it is selective to the free metal ion concentration (Figs. 1 and 3) [4][11] which is essential to our understanding of biogeochemical cycles [12]. In addition, the pumping system which is created allows high preconcentration factors to be attained in the strip solution (see below). The coupling of this technique with highly sensitive detection techniques such as fluorescence and voltammetry should allow the determination of ultra trace free metal ion concentrations below  $10^{-12}$  M, *i.e.*, at the levels encountered in environmental systems. As mentioned above, the selectivity of the supported liquid membrane to free metal ion [11] is a key feature for environmental applications. In certain conditions, lipophilic complexes may also pass the membrane. However, since their determination is also important for the interpretation of environmental functioning (Fig. 1), this is a positive factor.

In order to get high preconcentration factors in a reasonable time, a high volume ratio of test to strip solution is required. For mass transport occurring only by molecular diffusion, the diffusion time increases with the square of the diffusive

cell dimensions. Thus, preconcentration should be done inside channels with radial dimensions not larger than hundreds of microns to get preconcentration times of 1–100 min (depending on the desired preconcentration factor). Preconcentration factors of up to 3000 have been obtained, using small internal diameter (200–600  $\mu\text{m}$ ) hollow fibers as the porous membrane [13]. Simultaneous preconcentration of several metals [11][13] is possible with preconcentration factors which depend on the stability of the metal complexes inside the membrane and in the strip solution. Because the use of small systems is a key requirement for such developments, microtechnology based devices coupling supported liquid membranes with the microelectrode arrays mentioned in the previous section (and other sensors) appears to be a promising area of future research.

### Conclusion

As shown by the above mentioned examples, the development of probes for the *in situ* analysis and speciation of trace compounds in aquatic systems is a real scientific challenge. To facilitate further advances:

- 1) Probe development should be based on modern microanalytical systems and microtechnologies.
- 2) New analytical approaches should be developed for *in situ* measurement of

environmentally relevant groups of specific metal species.

- 3) These new analytical approaches must take into account the properties of the natural complexing agents. Detailed physicochemical and analytical studies of these components are needed which may lead to fundamental discoveries of interesting new characteristics of these compounds.
- 4) The probes must be reliable. This implies that traditional academic developments of new concepts must be complemented by substantial technical developments in order to obtain routine apparatus which work *in situ*. Industry should be strongly encouraged to invest in this aspect of the research.

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