

## Perspectives

Guest Author  
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The form and bioavailable fractions of the metal ion are important factors that must be known in exposure risk assessment of any ecosystem. Supported liquid membrane extraction is capable of extracting both the form and bioavailable fraction of metal ions from environmental and biological media. Extraction is achieved by incorporating a carrier in the membrane that selectively extracts the bioavailable form of the metal ion. New miniaturized liquid-membrane extraction designs are especially suited for speciation studies of metal ions in small amounts of biological fluids.

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Sample Prep Perspectives Editor

# Speciation Studies of Metal Ions in Environmental and Biological Media Using Supported Liquid Membrane Extraction

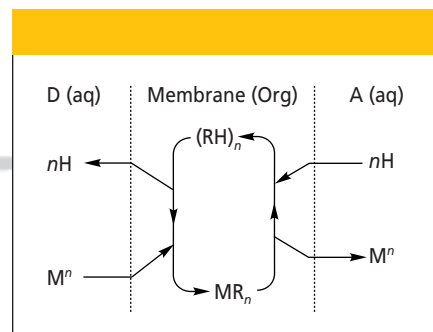
In ecological risk assessment for chemical pollutants, it is important to quantify the concentrations of freely dissolved substances in aqueous samples for approximate characterization of the bioavailable fraction. Such measurements are important because the concentration and toxicity of a chemical in an organism's body are linked to the bioavailable fraction and its chemical form within the surrounding environment. Metal ions, unlike organic pollutants, can be distributed in the surrounding environment in forms such as free-hydrated ions, dissolved inorganic and organic complexes, and metals associated with colloidal particles. Furthermore, some of these forms can exist in different oxidation states depending on the metal ion and environmental conditions. These include chromium(III) and chromium(VI), vanadium(IV) and vanadium(V), and arsenic(III) and arsenic(V). All of these forms play an important role in assessing the health hazards and toxicity of metal ions in ecosystems, and any analytical method must be based upon them. A good example is the contrast between chromium(III), a nutrient element used to control glucose and lipid metabolism in the cell membrane, and chromium(VI), which is carcinogenic (1).

However, many trace-metal analytical methods such as inductively coupled plasma spectrometry or atomic-absorption spectrometry have no speciation capabilities. These methods demand sample preparation techniques capable of isolating the chemical and bioavailable form of the metal ion from the matrix before its determination. Speciation studies are therefore challenging, especially at trace levels and in complicated samples such as biological fluids. Liquid membrane-based techniques have proved to be promising for speciation studies (2–4). Prominent among several types of liquid-membrane devices is the supported liquid membrane (SLM) technique (2,3).

## Speciation Studies Using SLM Extraction

**Extraction of the bioavailable fraction:** In an SLM configuration, an organic solvent is immobilized within the pores of an inert support material that separates the aqueous donor and the acceptor phases. The bioavailable analytes are partitioned from the aqueous sample stream into the organic membrane and then are reextracted into the aqueous acceptor phase. The driving force is the difference in analyte concentration between the donor and acceptor phases. The analyte also could be preconcentrated depending upon the ratio of acceptor and donor volumes. This approach has led to the development and application of numerous extraction methods for exposure risk assessment of various organic pollutants in both environmental and biological media. Such applications can be found in several review papers (5–8).

**Extraction of the bioavailable and chemical form:** In speciation studies using SLM extraction, a mobile carrier that is selective for the metal of interest is incorporated into an organic solvent held by capillary forces within the pores of a hydrophobic, microporous, and chemically inert membrane. The metal ions  $M^{n1}$  are transported toward the



**Figure 1:** Scheme of the metal-ion transport across the SLM extraction, where  $M^{n1}$  and  $(RH)_n$  represent metal species and acidic extractant, respectively.  $MR_n$  is the metal-extractant complex and  $nH^+$  is the hydrogen ion.

membrane by diffusion through the donor-diffusion layer and then through the hydrophobic membrane as a complex  $MR_n$ , formed with the organic carrier R present in the membrane.  $M^{n+}$  is finally released into the acceptor phase, where it is irreversibly trapped (Figure 1). Details of various transport mechanisms such as simple-carrier transport (with chemical reaction in the acceptor) (2,11), coupled cotransport (12,13), and coupled counter transport (4,10,13–16) have been discussed by Buffle and colleagues (9) and Djane and colleagues (10).

**With supported liquid membrane extraction, it is possible to extract the uncharged bioavailable fraction of the sample from the matrix, which also could interfere with the measurements.**

In simple carrier transport, the carrier in the membrane forms a complex with the analyte in the donor ( $MR_n$ ) that diffuses to the acceptor phase, where the analyte portion (M) is converted to a nonextractable form. This is accomplished through the analyte's interaction with the strong complexation reagent found in this phase. In most commonly used carriers, a coupled-transport mechanism (Figure 1) transports the metal ion ( $M^{n+}$ ) from the donor to the acceptor phase. In such a case, once  $M^{n+}$  reaches the acceptor phase, it is exchanged for a proton ( $H^+$ ) and converted to a nonextractable form. The proton gradient across the membrane is the driving force (14). Anionic di-(2-ethylhexyl) phosphoric acid (DEHPA) (4,14,17), cationic methyltricaprylammonium chloride (Aliquat 336) (16), and neutral crown ethers (18) have been used as carriers for this purpose.

The carrier in the membrane is capable of extracting the free-hydrated metal ions and the labile complexes. The labile complexes are defined as those that can dissociate and form many times during their transport by diffusion through the donor-diffusion layer (19). These complexes, as well as free metal ions, play an important role in bio-uptake by microorganisms in

ecosystems. In almost all speciation studies, the total concentration of both the free metal ions and the labile fraction selectively taken by the carrier in the donor has been quantified in the acceptor phase. This is quite important because it is this concentration that will give resulting toxicity when exposed to an organism. However, recent studies have shown that by controlling the donor diffusion-layer thickness, the time it takes for the metals to cross the diffusion layer can be varied (2). Controlling this variation could allow the labile fraction plus the free species or only the free species to be transported and preconcentrated into the acceptor phase. It also is possible to study the metal-binding capabilities of various matrices in the sample using the SLM extraction incorporated with a carrier. For natural and surface waters, humic substances are the most widespread matrices where metal ions bind. The complexing of metal ions by humic substances plays an important role in determining the metal toxicity, mobility, and bioavailability in these environmental compartments. Jönsson and colleagues (17) used the SLM extraction, incorporated with di-(2-ethylhexyl) phosphate as carrier, to investigate the fractions of copper(II) ions that are bound to humic acids and that are bioavailable in the water sample.

#### **Recent directions in SLM technology:**

Recently, there has been a shift toward designing simple and easily miniaturized SLM extraction modules. For the most part, the miniaturized SLM extraction designs have an acceptor-phase volume of less than 1 mL down to a few microliters. These new designs are very much suited for speciation studies. Many of the reported miniaturized SLM extractions to this point have been used to extract the bioavailable fraction of organic compounds in water or biological fluids. Jönsson and Mathiasson (20,21) recently have reviewed many of these new configurations. Some of these miniaturized SLM extraction designs include solvent microextraction and back-extraction (SME–BE), which preconcentrates analytes into a single drop (22), and liquid-liquid-liquid–microextraction (LLL–ME) (23). The LLL–ME uses a miniaturized disposable polypropylene hollow fiber in the form of a U-tube. The inside of the hollow fiber serves as the acceptor solution. An XT-tube extractor that uses a hollow fiber also has been reported (24). This device is so named because a cross (X) connector, a tee (T) connector, and PTFE tubing were used to

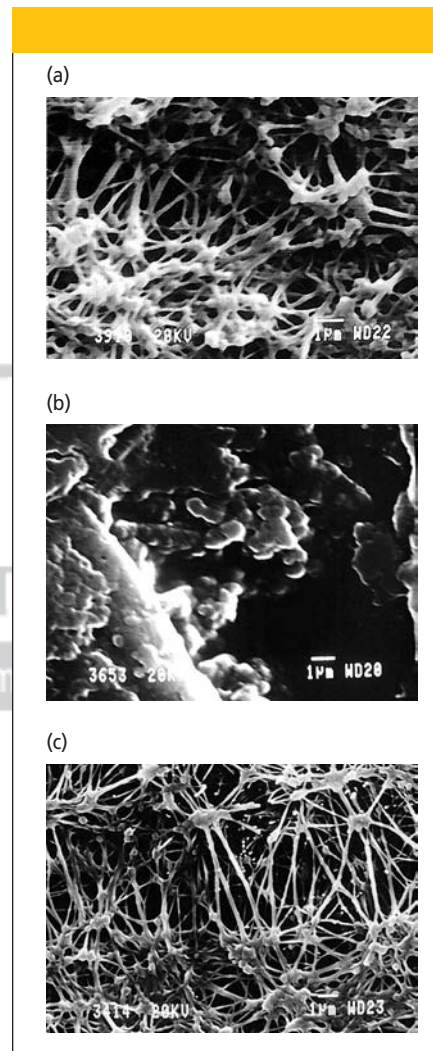
mount a hollow fiber membrane in the flow system. Buffle and his research group have designed a mini-SLM that allows one to record in real time the complete time evolution of metal in the acceptor phase during speciation studies (2,19). This approach has been used in speciation studies of copper (2), cadmium, and lead (19) in natural water. The design consists of a thin, flat sheet-SLM called minipermeation liquid membrane (minPLM). This membrane is incorporated within the acceptor phase, using voltammetric and reference microelectrodes, making it possible to perform real-time speciation studies. An off-line, miniaturized SLM extraction probe, which was used for speciation studies of manganese(II) in blood serum and milk with voltammetry for final detection, has been reported (4). This is similar to the one reported by Mullins (25), except that in the latter case, silicone rubber was used as a membrane instead of a liquid membrane. The SLM probe consisted of a miniaturized polypropylene tube. One end of the tube was closed, with the porous hydrophobic membrane sealed with PTFE tape and soaked in appropriate solvent containing a carrier. The inside of this probe served as an acceptor solution with a volume of 1 mL or less. This design proved to be simple but very suitable for speciation studies of metal ions.

### Applications of SLM Extraction in Speciation Studies

**Speciation studies in environmental samples:** To this point, many of the speciation studies have been performed with a variety of heavy-metal ions in natural and surface-water environmental samples. Most of this

work has been done by Buffle's research group (2,9) and Department of Analytical Chemistry's Research Group, Lund University (Lund, Sweden), (3,10,26–30). SLMs incorporated with either crown ethers, di(2-ethylhexyl) phosphate, or 8-hydroxyl quinoline as carriers have been used successfully to extract copper(II) (2,9,10), lead(II) (9,10), manganese(II) (29), cadmium(II) (9,10), and chromium(III) (3) from natural waters. In most of these speciation studies, metal ions have been extracted simultaneously (3,9,10,29,31) because the carrier is able to extract more than one metal ion of related charge. For anion metal ions such as chromium(VI), methyltricaprylammonium chloride (Aliquat) has been used as the carrier (3,30). In one study, it was possible to extract both chromium(VI) and chromium(III) in the same sample using two serially connected membranes with two different carriers specific to each chemical form. Success was achieved with Aliquat and DEHPA as extractants, respectively (3). The selectivity of a carrier toward a metal ion of interest in the sample was studied recently in the SLM extraction of uranium(VI) from natural water (11). The carrier was tributyl phosphate with sodium sulfate as the acceptor phase. Metal ions present in the natural-water sample before SLM extraction and in the extracts after extraction were scanned by inductively coupled plasma–optical emission spectrometry. Selectivity of the carrier toward the uranyl ion was demonstrated clearly. Despite occurring in high concentrations in the sample, most of the alkaline-earth metal ions were not extracted (11).

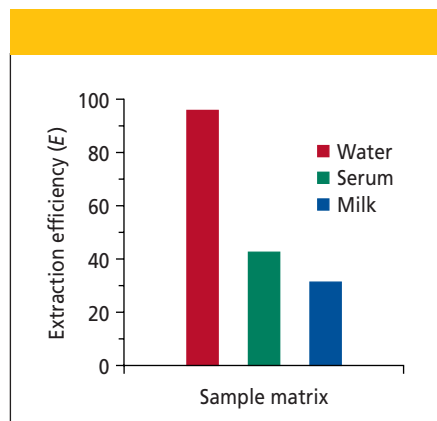
**Speciation studies in biological samples:** Thus far, most of the speciation studies reported have been done in natural-water environmental compartments, with few in biological samples (27,28). In exposure risk assessment of ecosystems for possible contaminants, measuring the analyte concentration in the organism itself complements that of measuring the surrounding environment. Taking into account the fact that once a chemical species enters the organism it can be changed easily to other forms, it is important to determine the bioavailable form within the organism itself, too. Urine, blood, and hair are known to be indicators of metal toxicity in humans (32). Therefore, it is important to perform speciation studies in these biological samples. The normal level of chromium in human fluids such as urine, blood serum, and plasma is less than 1



**Figure 3:** Scanning-electron-microscope pictures of membrane filters after extraction of manganese(II) in (a) water, (b) milk, and (c) blood serum.

ng/g (33). Speciation studies at such low concentrations is therefore a huge challenge. It also requires a very sensitive detection system that can handle a few milliliters down to microliters of biological samples. A combination of SLM extraction and graphite-furnace atomic absorption spectrometry (27) or voltammetry (4,16,28) for final detection is promising. Such combinations have been applied to speciation studies in urine (27,28) or blood serum (4) with detection limits below 1 mg/L.

Although SLM extraction, especially with miniaturized designs, is promising in speciation studies of biological fluids, there are still some challenges that must be overcome. These challenges can be highlighted easily in a few attempts made so far (4,16,27,28). Figure 2 shows a comparison of the extraction efficiency obtained in spe-



**Figure 2:** Influence of sample matrix on manganese(II) extraction efficiency using an SLM probe. Stirring time: 60 min. RSD ( $n = 3$ ): water 5%, milk 5%, and serum 5%.

ciation studies of manganese(II) from water, blood serum, and milk using a miniaturized SLM probe (4). Clearly, it is seen that speciation studies in water samples are quite possible with SLM extraction. However, in biological fluids, the extraction efficiency is lower compared to water because of the matrix effect. Milk is more complicated than blood serum, so the extraction efficiency is lowest. These lower extraction efficiencies are due to the metal ions being complexed to sample matrix components.

There is another factor in dealing with more complex matrices. Scanning-electron-microscopy studies of the used membranes (Figure 3) showed that the matrix itself tended to block the pores of the membrane, especially when analyzing milk samples. However, for blood serum and urine samples (data not shown), the scanned membranes were very much similar to membranes that came into contact with reagent water. This demonstrates that to a large extent, membranes are not fouled easily by the matrix components found in urine and blood serum.

The extent to which the matrix effects influence the extraction efficiency could also depend upon the size of the metal ion. In SLM speciation studies of lead in urine using di(2-ethylhexyl) phosphate, extraction efficiencies close to 100% were obtained (27). However, speciation studies of chromium(VI) in urine in a similar setup but with Aliquat as the carrier resulted in very low extraction efficiency; close to 3% (16). In water, the corresponding extraction efficiency was about 40%. This difference in extraction efficiency could be due to the fact that chromium(VI) is a much bigger atom than lead(II) and tends to be complexed more to organic macromolecules such as proteins found in biological fluids.

**Comparison of SLM extraction with other techniques in speciation studies:** Liquid-liquid extraction is a traditional technique that has been used to extract metal ions from aqueous solutions (34,35). Extraction is based upon the partitioning of the dissolved metal ions between the organic phase (extraction liquid) and the aqueous solution (sample solution). However, unless the metal ions are present already as neutral or nonpolar, the first step is to convert them into such forms. Adding the organic carrier in the organic phase similar to SLM extraction does this for the most part. Carriers such as dithiocarbamates (34,35), 8-hydroxyl quinoline

(36,37), and organophosphorus (38) have been used. The technique is less attractive because it is tedious and time consuming, is not easy to automate, and is environmentally unfriendly as a result of the large volumes of organic solvents used. It also forms emulsions, which makes it difficult to separate the two phases, especially for biological fluids. SLM extraction is very easy to automate because a flow system is used, and it also eliminates the problems of emulsion formation. Only a few microliters of organic solvent are used to fill the pores of the membrane support.

**Generally, it is more difficult to perform speciation studies in biological samples such as milk, whole blood, blood serum, and plasma because heavy-metal ions occur at trace levels and most of them are complexed to various macro-organic molecules.**

Solid-phase extraction (SPE) using ion exchange and chelating polymers has been used for speciation studies of metal ions (39). In this case, polymer beads are packed in a mini-column (typically 50 mm  $\times$  4.0 mm or smaller) that provides sufficient capacity. Chelex 100 (iminodiacetate resin) has been the most widely used polymer and has a strong preference for heavy, alkaline, and alkaline-earth metals (40,41). Its major drawback is swelling and contracting of the resin, which limits its application in a flow system (41). Other extractants that do not swell have been used following immobilization on suitable support, especially polyvinyl-divinyl benzene (42,43) and 8-quinolinol (44,45). However, almost all speciation studies have been applied to environmental samples, which is not surprising. In most cases, it would be difficult to extract a biological fluid such as milk through a solid-phase minicolumn because matrices will block it easily. On the other hand, SLM extraction has been proven to tolerate harsh conditions, and any aqueous sample can be extracted as long it contains no suspended solids. SLM

can tolerate dissolved organic matrices such as milk (4), urine (16), and humic substances (17) that can block the SPE column easily, especially with large sample volumes. Membrane fouling can occur in SLM extraction of samples like milk, but this does not completely stop the extraction of the metal ion of interest by the carrier (4).

## Conclusion

SLM extraction is capable of complementing conventional techniques (liquid-liquid and solid-phase extractions) in environmental and biomedical speciation studies of metal ions. The attractiveness of SLM extraction in speciation studies, especially for biological samples, is based on its capability for miniaturized configurations that can suit small sample volumes. Also, SLM extraction can tolerate the harsh conditions found in both environmental and biological samples.

## References

- (1) A.R. Anderson and A.S. Kozlovsky, *Am. J. Clin. Nutr.* **41**, 1117 (1985).
- (2) L. Tomaszewski, J. Buffle, and J. Galceran, *Anal. Chem.* **75**, 893 (2003).
- (3) N. Djane, K. Ndung'u, C. Johnsson, H. Sartz, T. Törnström, and L. Mathiasson, *Talanta* **48**, 1121 (1999).
- (4) L. Soko, L. Chimuka, E. Cukrowska, and S. Pole, *Anal. Chim. Acta* **485**, 25 (2003).
- (5) J.Å. Jönsson and L. Mathiasson, *LCGC* **21**, 424 (2003).
- (6) J.Å. Jönsson, *Chromatographia* **57**, S-317 (2003).
- (7) J.Å. Jönsson and L. Mathiasson, *J. Sep. Sci.* **24**, 495 (2001).
- (8) J.Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.* **18**, 325 (1999).
- (9) N. Parthasarathy, M. Pelletier, and J. Buffle, *Anal. Chim. Acta* **350**, 183 (1997).
- (10) N. Djane, K. Ndung'u, F. Malmus, G. Johansson, and L. Mathiasson, *Fresenius J. Anal. Chem.* **358**, 822 (1997).
- (11) L. Chimuka, E. Cukrowska, L. Soko, and K. Nalke, *J. Sep. Sci.* **26**, 601 (2003).
- (12) M. Mulder, *Basic Principles of Membrane Technology* (Kluwer Academic Publishers, Norwell, Massachusetts, 1996).
- (13) B.D. Smith and S.J. Gardiner, in *Advances in Supramolecular Chemistry: Facilitated Transport of Small Hydrophilic Biomolecules through Artificial Membranes*, G.W. Gokel, Ed., (1997), p. 1, vol. 5.
- (14) P. Wiczorek, J.Å. Jönsson, and L. Mathiasson, *Anal. Chim. Acta* **346**, 191 (1997).
- (15) P. Dzygiel, P. Wiczorek, L. Mathiasson, and J.Å. Jönsson, *Anal. Lett.* **31**, 1261 (1998).
- (16) L. Soko, E. Cukrowska, and L. Chimuka, *Anal. Chim. Acta* **474**, 59 (2002).
- (17) T. Trtic-Petrovic and J.Å. Jönsson, *Desalination* **148**, 247 (2002).
- (18) F. Guyon, N. Parthasarathy, and J. Buffle, *Anal. Chem.* **71**, 819 (1999).
- (19) P. Salaun and J. Buffle, *Anal. Chem.* **76**(1), 31-39 (2004).
- (20) J.Å. Jönsson and L. Mathiasson, *J. Sep. Sci.* **24**, 495 (2001).

- (21) J.Å. Jönsson and L. Mathiasson, in *Advances in Chromatography*, P. Brown and E. Grushka, Eds. (Marcel Dekker, New York, 2000), vol. 41, p. 53.
- (22) M. Ma and F.F. Cantwell, *Anal. Chem.* **71**, 1879 (1999).
- (23) S.P-Bjergaard and K.E. Rasmussen, *Anal. Chem.* **71**, 2650 (1999).
- (24) O.B. Jonsson, U. Nordlöf, and U.L. Nilsson, *Anal. Chem.* **75**, 3506 (2003).
- (25) F. Mullins, *J. Sep. Sci.* **24**, 593 (2001).
- (26) N.-K. Djane, Ph.D. dissertation, Lund University, Lund, Sweden (1997).
- (27) N.K. Djane, I.A. Bergdahl, K. Ndung'u, A. Schütz, G. Johansson, and L. Mathiasson, *Analyst* **122**, 1073 (1997).
- (28) N.K. Djane, S. Armalis, K. Ndung'u, G. Johansson, and L. Mathiasson, *Analyst* **123**, 393 (1998).
- (29) K. Ndung'u, N.K. Djane, and L. Mathiasson, *J. Chromatogr. A* **826**, 103 (1998).
- (30) K. Ndung'u, N.-K. Djane, F. Malcus, and L. Mathiasson, *Analyst* **124**, 1367 (1999).
- (31) M. Papantoni, N.K. Djane, K. Ndung'u, J. Å. Jönsson, and L. Mathiasson, *Analyst* **120**, 1471 (1995).
- (32) R.A. Goyer, "Toxic Effects of Metal Ions," in *Casarett and Doull's Toxicology*, C. Klassen, Ed., (McGraw-Hill, New York, 1996), vol. 5.
- (33) C. Veillion and K.Y. Patterson, *Anal. Chem.* **66**, 856 (1994).
- (34) K.W. Bruland, R.P. Franks, G.A. Knauer, and J.H. Martin, *Anal. Chim. Acta* **105**, 233 (1979).
- (35) G.R. Bradford and D. Bakhtar, *Environ. Sci. Technol.* **25**, 1704 (1991).
- (36) R.E. Sturgeon, S.S. Berman, A. Desaulniers, and D.S. Russell, *Talanta* **27**, 85 (1980).
- (37) D.C. McAvoy, R.C. Santore, J.D. Shosa, and C.T. Driscoll, *Soil Sci. Soc. Am. J.* **56**, 449 (1992).
- (38) M.B. Shabani, T. Akagi, H. Shimuzu, and A. Masuda, *Anal. Chem.* **62**, 2709 (1990).
- (39) B.S. Garg, R.K. Sharma, N. Bhojak, and S. Mittal, *Microchem. J.* **61**, 94 (1999).
- (40) C. Haraldsson, B. Lyven, M. Pollak, and A. Skoog, *Anal. Chim. Acta* **284**, 327 (1993).
- (41) G. Valcarcel, in *Flow Injection Atomic Spectroscopy*, J.L. Burguera, Ed., (Marcel Dekker, New York, 1989) p. 189.
- (42) A. Siriraks, H.M. Kingston, and J.M. Riviello, *Anal. Chem.* **62**, 1185 (1990).
- (43) S. Motellier and H. Pitsch, *J. Chromatogr. A* **739**, 119 (1996).
- (44) O. Abollino, E. Mentasti, V. Porta, and C. Sazanini, *Anal. Chem.* **62**, 21 (1990).
- (45) N.-K. Djane, F. Malcus, E. Martins, G. Sawula, and G. Johansson, *Anal. Chim. Acta* **316**, 305 (1995).

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