NEW MICROCAPSULES USEFUL AS EXTRACTION MEANS IN PARTICULAR FOR EXTRACTING WATER OR SOIL CONTAMINANTS
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NEW MICROCAPSULES USEFUL AS EXTRACTION MEANS IN PARTICULAR FOR EXTRACTING WATER OR SOIL CONTAMINANTS

FIELD OF THE INVENTION

The invention refers to extraction techniques, more specifically to a novel extraction system suitable for contaminants like pesticides and herbicides removal.

BACKGROUND OF THE INVENTION

Presence of herbicides and pesticides in surface and ground water is a real problem. Because of physical processes such as erosion, ground water movements, pesticides used in agriculture and local gardens end up in local streams, making water hazardous for aquatic life and for human use. In traditional waste water treatment plants pesticides are not always detoxified, therefore these are sending back to the waterways. Atrazine is an example of pesticide most commonly found in surface and ground water because of its relatively high solubility in water and its extensive use in agriculture such as citrus, pineapples, Christmas trees plantations (Liu, Bennett et al. 1999). Organothiophosphorus such as parathions are other very common pesticides often found in drinking water. They are known to be at the origin of neurosis and carcinogenesis.

Different procedures exist to remove atrazine by chemical treatment such as ozonation of atrazine (Beltran, Gonzalez et al. 2000). Biotreatments using granular activated carbon associated to bacteria enables biodegradation of low concentrations of xenobiotics such as atrazine (Feakin, Blackburn et al. 1995; Jones, Owen et al. 1998). Photochemical processes also degrade atrazine since it is photolytic by ultraviolet irradiation. The main product obtained is hydroxyatrazine, and the ultimate product is cyanuric acid(Héquet, Gonzalez et al. 2001).

Nanofiltration is another way to remove pesticides simultaneously with nitrates from water(Wittmann, Côté et al. 1998; Van der Bruggen, Everaert et al. 2001).
Pesticides such as ethlyparathion or atrazine can also be reduced rapidly in the presence of iron powder (Ghauch, Rima et al. 1999).

Organothiophosphorus pesticides can be removed from contaminated water using a water-immiscible organic solvent immobilized on a supported liquid membrane. The phosphorus-based substance is extracted into the solvent by contacting the contaminated water on one side of the membrane, whereas on the other side there is solution containing a hydroxy-affording strong base that will react with the phosphorus-based substance to form a non toxic product (Vandergrift and Steindler 1989).

Another alternative for removal of organothiophosphorus pesticides is the use of supercritical CO₂ for their extraction, combined with their degradation by Fenton's reagent (Yu 2002).

Silver complexed chitosan microparticles were also shown to be adsorbents for pesticide removal such as methyl parathion (Yoshizuka, Lou et al. 2000).

SUMMARY OF THE INVENTION

The invention proposes a new and original extraction system composes of microcapsules which proved quite efficient for the removal of water or soil contaminants like herbicides or pesticides. These microcapsules contain an oil core surrounded by a hydrogel polymer membrane and have a diameter which can vary within a wide range, for example between about 0.800 and about 1.500 mm.

Therefore a first object of the invention is to provide microcapsules consisting of a lipophilic liquid core surrounded by a hydrogel polymer membrane, wherein:

- the lipophilic liquid core component is an oil of natural or chemical origin or a water immiscible organic solvent or a mixture of same;

- the hydrogel membrane is made of suitable polycations or polyanions associated with unsaturated monomers like acrylamide or its derivatives by electrolytic interactions or chemical cross-linking, preferably a cross-linked
polymer membrane made by copolymerization of alginate with monomers selected from acrylamide and acrylamide derivatives.

As further object of the invention there is a process for the preparation of said microcapsules which comprises

a) selecting an oil of natural or chemical origin or a water immiscible organic solvent or a mixture of same as lipophilic liquid core constituent;

b) selecting unsaturated monomers like e.g. acrylamide and/or acrylamide derivatives and suitable polycations or polyanions as constituents of the mixture subject to interaction;

c) initiating and then performing interaction of the selected constituents up to the desired polymerization level of the hydrogel membrane; and

d) subjecting then the core and the membrane components to a suitable microcapsulation technology, e.g. the laminar jet break-up co-extrusion technique.

Interaction of the selected polycations or polyanions with suitable monomers can imply electrolytic interaction and chemical cross-linking as well. Preferred hydrogel membrane components comprise alginate which is then cross linked to monomers selected from acrylamide or acrylamide derivatives.

Still a further object of the invention is a method for extracting lipophilic soil or water contaminants, which comprises adding the above mentioned microcapsules to contaminated waste water or the like in an amount sufficient to extract the said contaminants, keeping them in contact under stirring with the said contaminants for a time sufficient to achieve the desired extraction rate and eventually recovering the loaded microcapsules from the aqueous medium.
DESCRIPTION OF THE INVENTION

The process which is proposed within the frame of the invention is based on liquid-core microcapsules which combine long term stability with high extraction rates. Direct contact of the extracting medium and water is avoided, a high interfacial area is provided, very little agitation is required and the capsules may be simply removed by sedimentation or flotation. Due to the high stability it should be feasible to back extract the pesticide/ herbicide or carry out a chemical oxidation or other decomposition reaction directly, and recycle the capsules. Alternatively the capsules could be disposed of by incineration. Due to the high partition coefficient, the herbicide/pesticide can be accumulated to high concentrations within the capsules which further facilitates the determination of the quantity of herbicide/pesticide present in waste water.

The adequate solvent for the extraction of atrazine, methylparathion, ethylparathion and 2,4-dichloro-phenoxycetic acid is encapsulated in a porous hydrogel membrane, which is composed of alginate and polyacrylamide.

They offer the advantage of having a very large interfacial contact area thanks to their small radius, favorising a fast extraction. On the other side the presence of the membrane physically separates the organic phase from the water to be treated, leading to the use of less solvent compared to liquid-liquid extraction.

To show the feasability of extracting herbicides/pesticides from water with this type of microcapsules. Atrazine, 2,4-D, methylparathion and ethylparathion are chosen as examples.
Materials and Methods:

Chemicals:

2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (Atrazine), (O,O-Di-methyl) O-(p-nitrophenol) thiophosphate (Methylparathion or MP), (O,O-diethyl) O-(p-nitrophenol) thiophosphate (Ethylparathion or EP) and 2,4-dichloro-phenoxyacetic acid (2,4-D) were of analytical grade and obtained from Aldrich Fine Chemicals (Buchs, Switzerland).

Dibutylsebacate (dibutyl decanedioate or DBS, CAS 109-43-3) and oleic acid (cis-9-octadecenoic acid or OA, CAS 112-80-1) were of analytical grade and obtained from Fluka (Buchs, Switzerland). MIGLYOL® 812 (glycerol tricaprylate/caprate), glycerol trioctanoate/decanoate or M) was of analytical grade and obtained from Dynamit Nobel (Plüss Staufer AG, Basel, Switzerland).

Four stock solutions of the pesticides/ herbicides were prepared by dissolving atrazine, MP, EP and 2,4-D separately in ethanol (absolute) to the desired final concentration. (for example 12 mg MP in 10 mL ethanol). A defined volume of each stock solution (for example 0.2 mL of MP stock solution) was dissolved in water to obtain 10 mL of solution for use in extraction experiments.

Stock solutions of 40% (w/v) acrylamide(AA) and N, N'-methylene-bis-acrylamide(MBA) in a ratio of 19/1, N, N'-methylene-bis-acrylamide(MBA), Hydroxymethylacrylamide(NMAM) and Tert-butyl hydroperoxide (70% in water) were obtained from Sigma (Buchs, Switzerland). Acrylamide (purity >99.5%) and sodium pyrosulfite (Na₂S₂O₅) were obtained from Fluka (Fluka Chemie AG, Buchs, Switzerland). Alginate LVG (Batch n°005-281-03) was supplied by Pronova (Lysaker, Norway).

A stock solution of sodium alginate (7%) was prepared in Tris/HCl, pH 7 and filtered through a 0.2 μm filter under a pressure of 4-6 bar.
Determination of partition coefficients (LogP<sub>oct</sub>)

LogP<sub>oct</sub> is a criterion which describes the hydrophobicity of compounds with respect to an octanol/ water mixture (Vermüe and Tramper 1995). Values given in this study were obtained by calculation via the website of Syracuse Research Corporation (http://esc.syrres.com/interkow/kowdemo.htm) or determined experimentally. Experimentally determined values were measured by liquid- liquid extraction experiments in which each of the four herbicides/pesticides were added to 10 mL de-ionized water to the desired concentration. A quantity of each of the three organic phases (0.35 mL dibutyl sebacate, migyol or oleic acid) were added to the aqueous solutions followed by shaking for 5 min, on an orbital shaker set to 400 rpm. The emulsions were subsequently allowed to stand at room temperature for 18 hours to reach equilibrium, before measuring the concentration of pesticide/ herbicide in the aqueous phase.

Analytical procedures

The concentrations of atrazine, MP, EP and 2,4-D in the aqueous phase were determined spectrophotometrically (Hewlett-Packard Model 845X) at a wavelength of 274, 278, 278 and 283 nm respectively. Standard curves were prepared by measurement of the absorbance of solutions of known concentration of each compound. The concentration of each compound in the extraction solutions was adjusted to fall within the linear response of the spectrophotometer (absorbance <1).

EXAMPLES

Preparation of liquid- core capsules

A stock solution of acrylamide (AA) /methylene-bis-acrylamide (MBA) (23.5% AA, 2.5% MBA) was prepared and filtered (Steritop 0.2µm, Millipore, corporation 80Ashby Road Bedford MA 01730-2271). The stock solutions of alginate and AA/MMBA were then combined, with agitation, in a ratio of 1:1 in order to prepare a solution with the desired final concentrations. To 10 ml of this polymer solution was
added 20μl Tert-butyl hydroperoxide. The polymer/monomer solution was then co-extruded with the organic phase (DBS, Miglyol or oleic acid), into the gelling bath composed of 8% CaCl₂, 20mM Tris/HCl, pH 7, 1% Tween 80 and 0.1-0.4% Na₂S₂O₅. The resulting capsules were incubated in the gelling bath for 45min, filtered and washed extensively with de-ionised water to remove any un-reacted reagents.

Liquid-core capsules of different sizes were prepared using the co-extrusion jet-break-up technique. The encapsulator (Inotech Encapsulator IEM) was fitted with a concentric nozzle with an internal diameter of 200μm and an external diameter of 300 μm or an internal diameter of 400μm and an external diameter of 500 μm. Two syringe pumps (200 series, kd Scientific, Boston, USA) were connected to the encapsulator to supply the organic phase through the central nozzle and polymer solution through the external nozzle. Spherical capsules were obtained by the application of a vibrational frequency with defined amplitude to the co-extruded jet and collected in a gelling bath placed 18 cm below the nozzle and agitated by a magnetic stirrer (length 4cm). Polymer flow rate, oil flow rate and vibration frequency were empirically determined for the different solutions and for the different nozzles used. Further details of the technique for the production of liquid-core capsules using the prilling technique have been described elsewhere (Peters et al., 2002).

The capsules can be described as having an external radius (rₜₑₓₜ) and an internal radius (organic phase radius, rₜᵢₙ). Three different size capsules were produced with the following characteristics: (1) small capsules, rₜₑₓₜ 0.398 mm, rₜᵢₙ 0.264 mm; (2) medium capsules, rₜₑₓₜ 0.496 mm, rₜᵢₙ 0.305 mm; (3) large capsules, rₜₑₓₜ 0.76 mm, rₜᵢₙ 0.407 mm. After extrusion and chemical cross-linking of the polymer membrane, the capsules were used immediately or incubated for 4 hours in 10 mL of tri-sodium citrate solution (20 g/L) to complex calcium ions and thereby release alginate into the solution (Peters et al. 2002).
Measurement of capsule size distribution

The size and size distribution of capsules was determined using a microscope (Zeiss Axiolab, Switzerland) fitted with a video camera (CCD-IRIS, Sony, Japan) interfaced to a PC operating with the Cyberview (Cervus International, Courtaboeuf, France) image analysis software. A sample of 60-200 capsules was examined and the mean standard deviation determined.

Capsular extraction

All extraction experiments, both using liquid-liquid or capsules, were carried out with a constant volume of organic phase extractant (0.35 mL) and aqueous phase containing pesticide/herbicide (10 mL) such that results could be directly compared. In the case of capsules the constant volume of organic phase was achieved by measurement of the capsule size ($r_{int}$) and estimation of the volume of organic phase enclosed. Since three different size capsules were tested, the volume of the organic phase (0.35 mL) was maintained constant by varying the number of capsules. The estimated volume of each size capsule used in the extraction experiments are: (1) 1.7 mL small capsules ($r_{ext}$ 0.398 mm, $r_{int}$ 0.264 mm); (2) 2.0 mL medium capsules ($r_{ext}$, 0.496 mm, $r_{int}$, 0.305 mm); (3) 3.0 mL ($r_{ext}$, 0.76 mm; $r_{int}$, 0.407 mm). The volume of capsules required was determined using a graduated tube. The capsules were filtered through a porous mesh and placed in a conical flask followed by the addition of 10 mL of the aqueous solution containing pesticide/herbicide and agitated in a rotary shaker. A sample of the aqueous phase was removed immediately and at intervals throughout the extraction experiments for spectrophotometric determination of the pesticide/herbicide. The kinetics of extraction were estimated from the rate of uptake of the compounds from the aqueous phase.
Estimation of mass transfer

The main resistance to mass transfer of the herbicide/pesticide into the capsules was determined using a model described by (Stark 2001). In this model the capsule is considered to be a bead composed of an imaginary phase of alginate/polyacrylamide and organic phase.

The mass transfer can then be described by the simple mass transfer equation (eq. 1):

$$\frac{-dc_{aq}}{dt} = k \left( c_{aq} - \frac{c_{b}}{K} \right)$$  \hspace{1cm} (eq. 1)

where \( k \) is the overall mass transfer coefficient and \( K = \frac{c_{b}}{c_{aq}} \) (ratio of the concentration of the herbicide in the bead to the concentration in the aqueous phase at equilibrium).

The concentration of the herbicide inside the bead is obtained through a mass balance (eq. 2):

$$V_{aq}^{0}c_{aq}^{0} - V_{aq}c_{aq} = V_{b}(c_{b} - c_{b}^{0})$$  \hspace{1cm} (eq. 2)

where \( V_{aq} \) is the aqueous phase volume, and \( V_{b} \) is the capsule volume. A similar model has been applied to the extraction of 2-chlorophenol from aqueous solutions by liquid membranes (Lin, Pan et al. 2001).
Results

Choice of organic phase extractant

In order to choose the optimum organic phase for encapsulation and extraction of the four test pesticides/herbicides, three oils (dibutyl sebacate, oleic acid and MIGLYOL – fractionated coconut oil) were selected based on their high logP_{oct} values (6.2 and 7.7 respectively for DBS and OA, (Stark 2001)). The relatively high degree of hydrophobicity of these oils ensures that the equilibrium concentration of the oils in water, through exo-diffusion from capsules, is very low. In a first series of experiments capsules were incubated at room temperature in solutions of the 4 different pesticides/herbicides and the level of liquid-liquid extraction determined after a period of 18 h.

The UV absorption spectra (Figure 1) for methylparathion (MP) show a maximum at a wavelength of 278 nm. At this wavelength the partition coefficient K, defined as the ratio of the MP concentration in the organic phase to that in the aqueous phase after 18 h incubation, were estimated to have values of 116, 98 and 29 for DBS, MIGLYOL and oleic acid respectively. Similar results were observed for atrazine, EP and 2,4-D with the three different oils tested (results not shown). As a result DBS showed the highest level of extraction of MP (Figure 1). Since the hydrophobicity of MP, atrazine, EP and 2,4-D are very similar, with logP_{oct} values of 2.75, 2.82, 3.73 and 2.62 respectively, it would be expected that each would be efficiently extracted by DBS. As a result DBS was chosen for the preparation of liquid-core capsules.
Extraction using liquid-core capsules

Comparison of the extraction efficiency

In order to compare the efficiency of extraction of the four test compounds by capsular extraction with liquid-liquid extraction, capsules ($R_{\text{ext}}$, 0.496; $R_{\text{int}}$, 0.305) were prepared containing a core of DBS. Extraction efficiencies were compared after incubation of the test compounds with capsules at an agitation rate of 400 rpm. The EP, MP and atrazine concentrations in the aqueous phase rapidly declined to reach an equilibrium at which approximately 75% of the initial concentration remained after 10, 45 and 100 min respectively (Figure 2). The higher rate of EP extraction compared to the other two compounds, reflects the considerably higher hydrophobicity ($\log P_{\text{oct}}$, 3.73) of this compound. In the case of 2,4-D, an equilibrium was attained after only 15 min at 76% of the initial concentration (Figure 2) and probably reflects that this compound is the least hydrophobic of those tested.

Effect of agitation rate and capsule size on extraction rate

In order to determine whether the external (bulk liquid) mass transfer resistance is the main factor controlling the rate of extraction of the different test compounds by the capsules a series of experiments were conducted in which the bulk liquid was agitated at different rates and the extraction rate determined.

The rate and level of extraction using small capsules ($R_{\text{ext}}$, 0.398; $R_{\text{int}}$, 0.264 mm) for both EP and MP were independent of agitation rate above 200 rpm, with a higher rate for EP. Both compounds were extracted at a lower rate in the absence of agitation, as would be expected since mass transfer was limited by diffusion through the bulk alone (Figure 3 and 4). Repeating these experiments with medium-sized capsules ($R_{\text{ext}}$, 0.496; $R_{\text{int}}$, 0.305 mm) resulted in an approximate 10% decrease in the rate of extraction of MP, with equilibrium being attained after between 100-150 min compared with 60 min for smaller capsules (Figure 5). With large
capsules ($R_{\text{ext}}$, 0.760; $R_{\text{int}}$, 0.407 mm) the rate of extraction decreased considerably (>50%) compared with smaller capsules and equilibrium was not attained even after 150 min of incubation (Figure 6). Similar results were observed for the other compounds tested.

Effect of citrate on extraction rate

The capsule membranes are essentially composed of alginate together with crossed linked polyacrylamide of which the former is complexed with calcium ions to form a hydrogel while the latter is considerably more hydrophobic in nature. As a result it might be expected that the presence of calcium alginate in the membrane may create a resistance to mass transfer of the pesticides/ herbicides into the organic phase core In order to test this hypothesis capsules were prepared and incubated in the presence of sodium citrate which complexes calcium ions and results in the liquification of the alginate, which may then diffuse out of the capsular membrane. In these studies, the capsules which were not treated with citrate had an $r_{\text{ext}}$ of 0.388 mm and $r_{\text{int}}$ of 0.264 mm, while those treated with citrate had an $r_{\text{ext}}$ of 0.428 mm and $r_{\text{int}}$ of 0.26 mm. Thus citrate treatment resulted in a slight swelling of the capsules, indicating that alginate had indeed been lost from the capsular membrane.

The results (Figure 7) show that the rate of extraction of MP was over 50% faster for capsules treated with citrate compared with untreated ones, and equilibrium was attained within 40 min. When the same experiment was repeated with larger capsules ($r_{\text{ext}}$ 0.496 mm) a similar swelling was observed ($r_{\text{ext}}$ 0.530 mm) and an even more pronounced increase in the rate of extraction observed (Figure 8).

Overall mass transfer coefficients

The overall mass transfer ($k_c$) coefficients for capsules of different sizes were determined using equations 1 and 2 and experimentally measuring the extraction of Methylparathion. (Figures 4, 5 and 6) as a function of time and agitation rate.
Discussion

As with any liquid-liquid extraction or extraction system, the efficiency of the system is dictated by the partition coefficient of the solvent with respect to the products to be recovered. A high partition coefficient of the herbicides/pesticides between the organic and the aqueous phase enables a rapid transfer of the compounds to the organic phase, accumulation of high concentrations within the organic solvent as well as allowing the use of small volumes of the solvent.

With liquid-liquid extraction, since there is direct contact between the organic and the aqueous phases, a solvent is usually chosen which has a lower density than water, in order to allow good separation of the two phases, and should not form stable emulsions. The solvent should also be poorly soluble in water (high logP), have a melting point below room temperature as well as a low viscosity in order to facilitate the handling of the solvent. These properties mean that the concentration of residual solvent in the aqueous phase is very low thereby avoiding phase and/or molecular toxicity of organisms coming into contact with it (Bar 1987; Osborne, Leaver et al. 1990; Vermüe and Tramper 1995). For these reasons the choice of extractant is limited to solvents having a logP_{oct} higher than 5, where logP_{oct} is defined as the logarithm of the partition coefficient of the solvent in a standard two-phase system of 1-octanol and water. For these reasons three organic solvents (oils) were chosen and the rate and efficiency of extraction determined using a liquid-liquid extraction system. Of the three solvents studied dibutyl sebacate showed the highest partition coefficient with respect to methylparathion and the other pesticides/herbicides.

Although liquid-liquid extraction has been successfully employed for the removal of pesticides/herbicides from water (Hernandez, Beltran et al. 1993; Yrieix, Gonzalez et al. 1996; Mahara, Borossay et al. 1998; Liu, Howell et al. 2001), the problem still remains that relatively large quantities of solvent are required, and a system has to be used which involves vigorous agitation, in order to provide a high surface contact between the two phases and thus achieve a high mass transfer. The system must
also allow for efficient separation of the two phases after the extraction process. As a result an alternative system has been developed here involving capsular extraction. In the latter system liquid- core microcapsules have been developed in which the organic solvent is surrounded by a hydrophilic, polymeric membrane which provides for a high mass transfer area while avoiding direct contact between the two phases and the formation of any stable gels. Using this capsular extraction system, containing dibutyl sebacate, four different pesticides/ herbicides (ethylparathion, methylparathion, atrazine and 2,4-D) could be efficiently recovered from aqueous solutions (Figure 2) with the extraction efficiency increasing with increasing hydrophobicity of the compound. With the exception of 2,4-D, over 80% of all compounds (320 g) tested could be recovered in a quantity of capsules containing 0.35 ml dibutyl sebacate (Figure 2). Higher levels of pesticide/ herbicide extraction could be achieved by simply increasing the number of capsules added to the aqueous solution.

The rate at which the pesticides/ herbicides are extracted by the capsules is a function of the different mass transfer resistances: (1) the aqueous diffusion layer around the capsule; (2) the resistance within the polymer membrane and (3) the resistance in the organic phase core. In order to define which of these resistances was responsible for controlling the rate of extraction a series of experiments was undertaken with capsules of the same size but with different agitation speeds and with capsules of different sizes with the same agitation speed.

It is observed that when the solution was agitated (Figures 3-6; Table 1) the rate of extraction increased to between 0.42- 1.3 x 10^6 m/s and was independent of the agitation rate (200- 400 rpm) for capsules with a r_{ext} of 0.398 mm. These results show that, providing that the bulk solution is mixed at a sufficiently high rate (turbulent conditions), there are no concentration gradients within the bulk aqueous solution and the liquid film on the capsule surface has a constant resistance. This indicates that the external resistance to mass transfer is very low. For larger capsules (r_{ext} > 0.398 mm) an external mass transfer resistance was observed for agitation rates below 300 rpm (Table 1). Since capsules composed of cross-linked polyacrylamide
and alginate have a density higher than that of water, sedimentation of the capsules can occur. Consequently lower agitation rates are required to maintain smaller capsules in a turbulent state compared with larger capsules.

The rate of extraction of methylparathion is clearly related to capsule size (Figures 4-6), with the smallest capsules showing the fastest rates. This is clearly the result of the interfacial mass transfer area per unit volume (ratio of the total interfacial area of the capsules to the total volume) increasing with decreasing capsule size, thereby leading to a more rapid mass transfer. These results indicate that it is the mass transfer resistance in the capsule membrane which controls the extraction of compounds such as methylparathion.

The physical properties of capsules, composed of a dibutyl sebacate core surrounded by a cross-linked polyacrylamide/alginate membrane, may be altered following treatment with chelating agents such as tri-sodium citrate. After such treatments, the capsules become more elastic, although the mechanical resistance (burst force) is unaffected over the pH range 4 to 9, and the membrane thickness increases. In addition the rate of extraction of pesticides/herbicides increases by a factor of 3 to 5-fold compared with similar capsules, which have not been treated with citrate (Figures 7 and 8; Table 1). Since the membrane thickness of capsules treated with citrate is larger than that for non-treated capsules, it might be expected that the mass transfer resistance within the membrane is higher and that the rate of extraction would decrease. That this is not the case is due to a change in structure of the capsule membrane. In the absence of citrate the capsule membrane is probably composed of a dense mixture of polyacrylamide and calcium alginate. When capsules are treated with citrate, calcium ions are removed from the calcium alginate complex resulting in a more loose membrane structure composed essentially of polyacrylamide interpenetrated by sodium alginate. As a result the membrane becomes more hydrophobic and less dense, thereby facilitating diffusion of the hydrophobic herbicides and pesticides.

The $k_c$ values for methylparathion (Table 1) clearly show that the main resistance to mass transfer is located in the membrane. For relatively hydrophilic low molecular
weight proteins and compounds, such as glucose, it has been shown that they can diffuse freely through hydrogel membranes composed of alginate (Tanaka, Matsumura et al. 1984). This situation is comparable to the membranes composed of cross-linked alginate/ polyacrylamide which show a \( k_L \) of \( 1.3 \times 10^{-6} \) m/s for methylparathion (Table 1). However, in the case of relatively hydrophobic compounds, such as the pesticides and herbicides tested in this study, \( k_L \) values are approximately three-fold higher (\( 3.6 \times 10^{-6} \) m/s) after treatment with citrate. These \( k_L \) values are of a similar order of magnitude to those reported by Stark (Stark 2001) for the extraction of phenylethanol from a bioconversion process using capsules composed of an alginate membrane. The results also agree with those reported for the liquid membrane extraction of benzene (\( \log P = 2 \)) where the main resistance to mass transfer was found to be in the aqueous membrane phase, with an overall mass transfer coefficient of \( 0.289 \times 10^{-6} \) m/s for a membrane thickness of 30\( \mu \)m (Gupta, Goswami et al. 1990). The latter \( k_L \) value is of the same order of magnitude as the largest, non-citrate treated capsules (\( r_{\text{ext}} = 0.76 \) mm, \( r_{\text{int}} = 0.407 \) mm) used in the present study and confirms the importance of the membrane resistance.

In addition to the mass transfer resistances in the membrane and the external aqueous phase (which can be overcome by the application of suitable mixing), the internal resistance, due to the absence of agitation within the capsules and the relatively high viscosity of dibutyl sebacate (9mPa s), cannot be eliminated. Diffusion within the liquid core is thus almost entirely through convection and can only be improved by reducing the size of the capsules. However the results have shown that sufficiently high extraction rates can be obtained due to the very large interfacial contact area of the capsules with the aqueous phase, that the internal resistance may be neglected. The rates of extraction achieved with liquid-core capsules, the facility of recovery and lack of formation of stable emulsions offer many advantages over classical liquid-liquid extraction methods. In addition, for biological applications, phase toxicity is reduced by the presence of a membrane that physically separates the oil phase from the aqueous phase. Molecular toxicity is also reduced since the
solvents used have very high partition coefficients, resulting in low concentrations of solvent in the aqueous phase at equilibrium.

Bibliography


Legends to Table

Table 1. Determination of overall mass transfer coefficients \( k_L \) (m/s), using methylparathion, for capsules of different sizes at different agitation rates. Data from Figures 4, 5 and 6 were applied in equations 1 and 2.

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<th>Size of capsules, ( R_{ext} ) (mm)</th>
<th>Mass transfer coefficient, ( k_L ) (m/s)</th>
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Figure legends

Figure 1. UV spectra of aqueous phase concentrations of methylparathion (MP) during liquid-liquid extraction using dibutyl sebacate (DBS), MIGLYOL and oleic acid over a period of 18 h. Symbols: Initial concentration (solid line) and concentration of MP in aqueous phase after 18 h (dashed line) in presence of DBS; Initial concentration (solid line with squares) and concentration of MP in aqueous phase after 18 h (dashed line with squares) in presence of Miglyol; Initial concentration (solid line with crosses) and concentration of MP in aqueous phase after 18 h (dashed line with crosses) in presence of oleic acid

Figure 2. Extraction kinetics for Atrazine, Methylparathion, Ethylparathion and 2,4-D using liquid-core capsules containing DBS with an external radius \( R_{ext} \) of 0.496mm
at an agitation rate of 400rpm. Symbols: c(t)/c0 ratio of concentration at time t to initial concentration; Ethylparathion (diamonds), methylparathion (squares), atrazine (triangles) and 2,4-D (crosses).

Figure 3. Effect of agitation on the extractions kinetics for Ethylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.398 mm. Symbols: No agitation (diamonds), 200 rpm (squares) and 400 rpm (triangles).

Figure 4. Effect of agitation on the extractions kinetics for Methylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.398 mm. Symbols: No agitation (diamonds), 200 rpm (squares) and 400 rpm (triangles). c(t)/c0 ratio of concentration at time t to initial concentration.

Figure 5. Effect of agitation on the extractions kinetics for Methylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.496 mm. Symbols: No agitation (diamonds), 200 rpm (squares), 300 rpm (light triangles) and 400 rpm (dark triangles). c(t)/c0 ratio of concentration at time t to initial concentration.

Figure 6 Effect of agitation on the extractions kinetics for Methylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.760 mm. Symbols: No agitation (diamonds), 200 rpm (squares), and 400 rpm (dark triangles). c(t)/c0 ratio of concentration at time t to initial concentration.

Figure 7. Extraction kinetics for Methylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.398 mm at an agitation rate of 400 rpm. Symbols: c(t)/c0 ratio of concentration at time t to initial concentration; Capsules not treated with citrate (diamonds), capsules treated with citrate (squares).

Figure 8. Extraction kinetics for Methylparathion using liquid-core capsules containing DBS with an external radius ($R_{ext}$) of 0.496 mm at an agitation rate of 400 rpm. Symbols: c(t)/c0 ratio of concentration at time t to initial concentration; Capsules not treated with citrate (diamonds), capsules treated with citrate (squares).
CLAIMS

1. - Microcapsules consisting of a lipophilic liquid core surrounded by a hydrogel polymer membrane, wherein:

   - the lipophilic liquid core component is an oil of natural or chemical origin or a water immiscible organic solvent or a mixture of same;
   - the hydrogel membrane is made of suitable polycations or polyanions associated with unsaturated monomers like acrylamide or its derivatives by electrolytic interactions or chemical cross-linking.

2. - Microcapsules according to claim 1, wherein the hydrogel membrane is a cross linked polymer membrane made by copolymerization of alginate with monomers selected from acrylamide and acrylamide derivatives.

3. - Microcapsules according to claim 1 wherein the lipophilic core component is selected from dibutyl sebacate, oleic acid, fractionated coconut oil, aliphatic or aromatic hydrocarbons like e.g. hexadecane or toluene, middle or high molecular weight alcohols, aldehydes, ketones or esters.

4. - Microcapsules according to claim 1 wherein the monomer used for preparing the polymer membrane is selected from acrylamide, methylene-bis-acryl, N-(hydroxymethyl)-acrylamide and methylolacrylamide.

5. - Microcapsules according to claim 1 which exhibit a low dispersion of capsule radius, preferably microcapsules having a diameter comprised between 0.800 and 1.500 mm.

6. - Microcapsules according to claim 1 useful as extraction means for extracting lipophilic soil or water contaminants from waste water or the like.

7. - Microcapsules according to claim 6, wherein soil or water contaminants are herbicides, pesticides or the like.
8.- Method for extracting lipophilic soil or water contaminants, which comprises adding microcapsules according to claim 1 to contaminated waste water or the like in an amount sufficient to extract the said contaminants, keeping them in contact under stirring with the said contaminants for a time sufficient to achieve the desired extraction rate and eventually recovering the loaded microcapsules from the aqueous medium.

9. - Method for the preparation of microcapsules according to claim 1, which comprises

   a) selecting an oil of natural or chemical origin or a water immiscible organic solvent or a mixture of same as lipophilic liquid core constituent;

   b) selecting unsaturated monomers like e.g. acrylamide and/or acrylamide derivatives and suitable polycations or polyanions as constituents of the mixture subject to interaction;

   c) initiating and then performing interaction of the selected constituents up to the desired polymerization level of the hydrogel membrane; and

   d) subjecting then the core and the membrane components to a suitable microcapsulation technology, e.g. the laminar jet break-up co-extrusion technique.

10. - Method according to claim 9, which comprises performing cross linking of alginate with monomers selected from acrylamide and acrylamide derivatives.
Figure 1
Figure 2
Figure 5
Figure 6
Figure 7
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 B01J13/14 B01J13/04 C02F1/28

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01J C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>US 2002/050659 A1 (STROHSCEIN RUDOLPH ET AL) 2 May 2002 (2002-05-02) paragraphs ‘0035!, ‘0036!; claims 1,2,13; figure 1</td>
<td>2,5-7,10</td>
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Date of the actual completion of the international search

12 May 2004

Date of mailing of the international search report

26/05/2004

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<tr>
<td>A</td>
<td>US 2001/002656 A1 (HOLZBRECHER MICHAEL ET AL) 7 June 2001 (2001-06-07) paragraphs '0025!- '0027!, '0044!; claims 1-11</td>
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