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Extraction of glyphosate by a supported liquid membrane technique

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Abstract

The possible application of the supported liquid membrane (SLM) technique for the extraction of glyphosate is presented. For the extraction of this compound the SLM system has been applied with utilisation of Aliquat 336 as a cationic carrier incorporated into the membrane phase. The extraction efficiency of glyphosate [*N*-(phosphonomethyl)glycine] is dependent on the donor phase pH, carrier concentration in the organic phase and NaCl concentration in the acceptor phase. The optimal extraction conditions are: donor phase pH>11, acceptor phase of 2 *M* NaCl solution and the organic phase composed of 20% (w/w) Aliquot 336 solution in di-hexyl ether. Counter-coupled transport of chloride anions from the acceptor phase to the donor phase is a driving force of the mass transfer in this system.

Keywords: Extraction methods; Membranes; Supported liquid membranes; Glyphosate; Pesticides

1. Introduction

The glyphosate, *N*-(phosphonomethyl)glycine (Fig. 1), a non-selective, post emergence herbicide is known for its extensive application to control herbaceous plants. Glyphosate is widely used all over the world not only due to its high herbicidal activity but also because of its low mammalian toxicity. Because of its prominence in agriculture, the determination and concentration measurements of glyphosate and its main metabolites (aminomethyl)phosphonic acid (AMPA) and sarcosine have been of great interest during the last few years. Several procedures based on application of such analytical methods as high-performance liquid chromatography (HPLC) [1–8], gas chromatography (GC) [9], GC–mass spectrometry (MS) [10], capillary electrophoresis (CE) [11,12] and recently liquid chromatography (LC)–MS [13,14] have been successfully used. All these methods can be characterised by good sensitivity, low limit of detection and reproducibility of obtained results. Conversely, the main problem in glyphosate and its metabolite analysis is their recovery from biological or field samples. These problems are caused by their polar nature and high water solubility, which make extraction difficult. Moreover, it might limit the options for various standard derivatization procedures. Usually, the clean-up requires utilisation of ligand-exchange and anion-exchange chromatography. The handling with this type of chromatography is very laborious and time consuming work. This is some drawback, which is not easy to avoid in glyphosate and its metabolites analysis. From this point of view, it could be interesting and highly desirable to find

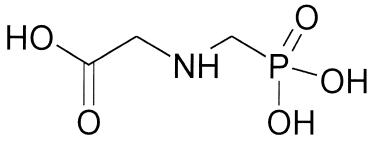

Fig. 1. Chemical structure of glyphosate.

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some alternative method for the clean-up of these compounds.

An opportunity to circumvent the inconveniences of glyphosate and its metabolites recovery could be an application of supported liquid membrane (SLM) extraction [15,16]. This versatile technique combines the benefits of liquid–liquid extraction (selectivity and flexibility) and classical membrane process (low operating cost, single-step operation). Thus, in comparison to ion-exchange chromatography or other classical preconcentration methods, it is a rather effective and easy to operate technique. The other important factor is the possibility of connecting SLMs on-line with an analytical system. Supported liquid membrane extraction has been successfully applied as a preconcentration technique for enrichment of various types of herbicides. Some of the examples are chlorophenoxyalkanoic acids [17], triazines [18,19] and chlorinated phenols [20]. There are two possibilities of operating with the SLM system depending on the charge of the extracted analyte. In the case of the acidic and basic compounds, the enrichment is achieved by suitable pH adjusting of two water phases [21]. In order to extract multicharged compounds such as free amino acids [22,23], it is necessary to use a carrier incorporated into the membrane organic phase. This carrier should bear a functional group with an opposite charge to the charge of transported molecule. Such a carrier facilitates the analyte passing over the liquid membrane by formation of a neutral, organic soluble ion-pair complex.

Based on these considerations, it is evident that the only possibility to enrich glyphosate by supported liquid membranes is to use a proper carrier into the organic phase. Because these compounds resemble amino acids at their structure, it could be possible to apply either cationic or anionic carriers. However, the better choice might be the use of a cationic carrier as glyphosate is mostly negatively charged in water solution regarding nearly all the pH range. Therefore, in our work we have tried to apply the Aliquat 336 (quaternary ammonium salt) as a carrier of choice. Moreover, from our previous experiments [23], it is known that this compound served as a very efficient amino acids extractant. In the presented work, we have examined the influence of conditions, namely pH of the donor phase, concentration of counter-ion in the acceptor phase and carrier concentration in the membrane phase on the glyphosate transport through supported liquid membranes.

2. Experimental

2.1. Membrane equipment

The membrane unit is composed of two circular PTFE blocks (diameter 120 mm and thickness 8 mm) with grooves arranged as an Archimedes' spiral (depth 0.25 mm, width 1.5 mm and length 2.5 m, with total volume ca. 0.95 ml). To stabilise the whole construction aluminium blocks of 6 mm thickness were used on both sides of the PTFE blocks. A porous PTFE membrane with polyethylene backing (pore size 0.2 μm, total thickness 175 μm with 115 μm backing and porosity 0.70; Millipore FG, Millipore, Bedford, MA, USA) was impregnated with Aliquot 336 solution in hexyl ether for 30 min. In turn, the membrane was placed between two PTFE blocks and the whole construction was clamped tightly with eight screws. After installation of the membrane, excess of organic solution on the surface was eliminated by pumping ca. 20 ml of water through both channels. For a picture of the membrane unit, see Ref. [21].

The water and donor solutions used in experiments were pumped with a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Viliers-le-Bel, France) applying acid resistance tubing (Acid Mainfold Tubing, Elkay Products, Shrewsbury, MA, USA) connected to the membrane unit with Altex screw fittings.

2.2. Operation of enrichment system

The samples were the glyphosate–water solutions adjusted to the proper pH with HCl or NaOH. A 20-ml volume of sample solution was pumped through the donor channel at a flow-rate 0.2 ml/min. The acceptor was stagnant sodium chloride solution or water with volume of ca. 1 ml. After pumping of sample through donor side, the acceptor phase containing the extracted analyte was removed to a 2-ml volumetric flask with the acceptor phase solu-

tion. Subsequently both sides of the liquid membrane were refreshed with water prior to the next experiment.

2.3. Analysis

After every enrichment outlet the donor phase was analysed. The analysis was carried out by CE performed on P/ACE 5000 CE system (Beckman, Palo Alto, CA, USA) according to the procedure described elsewhere [12]. Capillary zone electrophoresis (CZE) was performed in a fused-silica capillary [57 cm (effective length 50 cm)×75 μm I.D.×375 μm O.D.] obtained from Polymicro Technologies (Phoenix, AZ, USA). The background electrolyte was 50 m*M* potassium phthalate with 0.5 m*M* tetradecyltrimethylammonium bromide used as an electroosmotic flow modifier adjusted to pH 7.5 with NaOH solution. The electrophoretic separation was performed at an applied voltage 20 kV (reverse polarity), the sample injection time was set at 6 s by applying pressurized nitrogen (80 p.s.i.; 1 p.s.i.= 6894.76 Pa). For detection on the capillary window a UV detector was used with a deuterium lamp operating at 254 nm.

2.4. Chemicals

Aliquat 336 – methyltrioctylammonium chloride was obtained from Jansson (Beerse, Belgium). Di-*n*-hexyl ether (DHE) (Sigma, St. Louis, MO, USA) was used as membrane liquid. *N*-(Phosphomethyl)glycine (NPG) was obtained from Sigma. CE were laboratory-prepared from potassium phthalate and tetradecyltrimethylammonium bromide (Sigma–Aldrich, St. Louis, MO, USA). All water used was purified with a Milli-Q-RO4 system (Millipore, Bedford, MA, USA).

3. Results and discussion

Prior to further description of the obtained results, some more general remarks are required. As it is known from the previous works [15], in the case of the moving donor phase and the stagnant acceptor phase, the extraction efficiency *E*, is defined as the fraction of analyte extracted from the donor phase to the acceptor phase. It is a measure of mass transfer across the membrane that is stagnant at a specified extraction time, flow-rate, phase composition and ionic strength. There are two manners of calculating extraction efficiency described by the equations:

$$E = n_A / n_D \quad (1)$$

or

$$E = 1 - n_W / n_D \quad (2)$$

where n_A, n_D are the number of moles in the acceptor and donor phase, respectively and n_W is the number of moles found in the donor phase after extraction (in the stream leaving the donor channel). Eqs. (1) and (2) are the same provided that no analyte is accumulated in the membrane. Basically, the better way to calculate extraction efficiency is to use Eq. (1). However, in some cases, it is more convenient to use Eq. (2), especially when difficulties in the analyte concentration measurements might occur. For our purposes, we have chosen the second possibility, due to the high concentration of salt in the acceptor phase, which results in lack of a reasonable CE analysis. The high NaCl content in the sample influences the peak shape, and what is more important, it is not possible to create the proper calibration curve, in any concentration range for glyphosate (the regression coefficient was highly unacceptable). Probably, it is an effect of sample overloading that resulted in electromigration dispersion, a very undesirable phenomenon in CE with indirect detection.

3.1. The influence of carrier concentration on the glyphosate extraction

As was mentioned, Aliquat 336 was applied as a carrier of choice for the reasons given above. The results presented in Fig. 2 show the influence of the increasing carrier concentration in the organic phase on the glyphosate extraction efficiency. From this chart, it is evident that in glyphosate transport the participation of a carrier is necessary. The course of the plot in Fig. 2 is similar to that found for amino acid extraction [23] and is typical for such a type of facilitated transport mechanism. The extraction efficiency increases to the optimal carrier concentration value, where the further increase in the

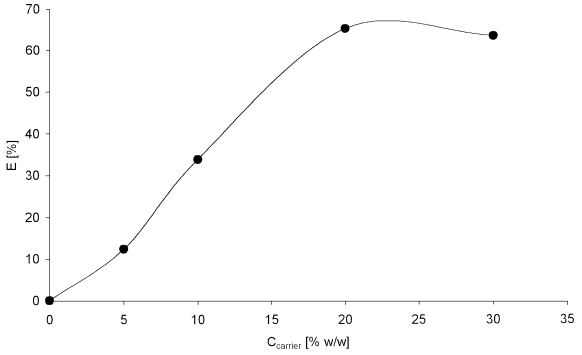

Fig. 2. The influence of the carrier concentration in the membrane phase on the extraction efficiency. Donor phase: 5 m*M* NPG, pH 11, flow-rate = 0.2 ml/min; acceptor phase: 2 *M* NaCl.

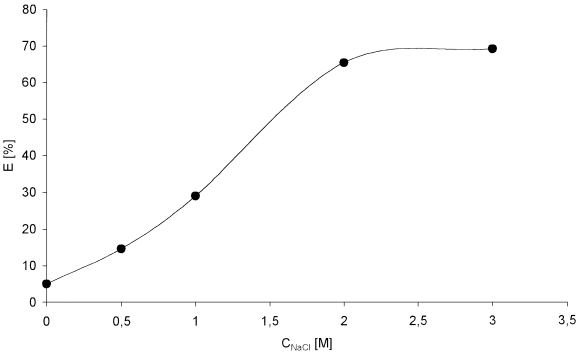

Fig. 3. The influence of the NaCl concentration in the acceptor phase on the extraction efficiency. Donor phase: 5 m*M* NPG, pH 11, flow-rate = 0.2 ml/min; membrane phase: 20% (w/w) Aliquat 336 in DHE.

extraction efficiency is not observed. This interesting phenomenon is apparently caused by two competing factors: the concentration gradient of the glyphosate complex, and the viscosity of the organic phase in the liquid membrane. As it is known, the flux of the species through the membrane of the thickness (l) is related to the concentration gradient (Δc) through Fick's first law:

$$J = -D \cdot \mathrm{d}c/\mathrm{d}l \quad (3)$$

where D is diffusion coefficient. High fluxes can be obtained when a large chemical potential (concentration gradient) and diffusion coefficient are maintained. Nevertheless, the diffusion coefficient depends on the viscosity of solvent (η) and the radius (r) of the species through the Stokes–Einstein relation:

$$D = kT/6\pi\eta r \quad (4)$$

Generally, when raising carrier concentration, both the amount of glyphosate that could be extracted into the membrane and the viscosity of the organic phase increase. An increase of the Aliquat 336 concentration will cause the growth in the complex flux, but at a certain carrier concentration [in the case of the glyphosate transport, 20% (w/w) Aliquat 336] the viscosity of the solution increases. Therefore, it might lead to the decrease in mass transfer of the carrier–analyte complex over the membrane phase.

3.2. Effect of the salt concentration in the acceptor phase

The dependence of the NaCl concentration in the acceptor phase on the glyphosate extraction efficiency is shown in Fig. 3. In this case, the extraction efficiency increases with increasing salt concentration. The maximal extraction was reached at an NaCl concentration of around 2 *M*. Further increase in salt concentration did not result in the increase of the extraction efficiency. The observed NaCl concentration dependence suggests that in glyphosate transport through SLM counter-coupled transport takes place. The mechanism of transport with this type of cationic carrier is proposed in Fig. 4 and can be described as follows: glyphosate in anionic form is transported due to presence of a

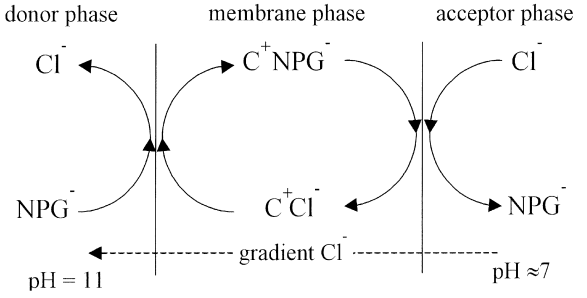

Fig. 4. Transport mechanism of glyphosate (NPG^-) through the SLM with Aliquat 336 as a carrier (C^+).

driving force, which is a gradient of counter Cl^- ion from acceptor to donor side of the liquid membrane. The function of the carrier is to facilitate the transfer of glyphosate from donor to acceptor phase and counter-ion in opposite direction. Basically, at the interface between organic and donor, glyphosate and carrier form an ion-pair complex, which becomes neutral. Then, the complex diffuses through the membrane and at the interface between organic and acceptor phases carrier exchange glyphosate to counter-ion. Glyphosate is released in the acceptor phase, the carrier–counter-ion complex diffuses back to the other side of the liquid membrane. A total effect of counter-coupled transport is diffusion of glyphosate from the donor to acceptor phase and Cl^- in the opposite direction. Therefore, when no driving force is present in the system the extraction of glyphosate reaches equilibrium. Increasing NaCl concentration increases the driving force and in turn higher extraction is achieved. However, as was mentioned, further increase in the salt presence in the acceptor phase does not bring about the rise in the extraction efficiency. It could be a result of the changes in the composition of the acceptor phase during the extraction. These changes could be attributed to the two interacting factors. Firstly, it might be assumed that during the extraction process the glyphosate concentration rises in the acceptor phase and the number of transportable (active) glyphosate molecules increases (the pH of acceptor phase is about 7, therefore some amount of glyphosate bears a negative charge). Hence, there is competition between chloride and solute anions in the active form in the transport from the acceptor to the donor side. The second factor is that during extraction the concentration of glyphosate reaches the limit of solubility and no more glyphosate could be transported to the acceptor side.

3.3. Influence of donor phase pH

Because glyphosate is a multicharged compound and it bears some functional groups either positively (secondary amino group) or negatively (phosphonic and carboxylic group) charged in water solution, the influence of the glyphosate solution pH is very important factor influencing the glyphosate extraction efficiency. In Fig. 5 the dependence of the extraction efficiency on pH is shown. It can be seen that together with the pH increase in the donor phase the extraction efficiency rises. This could be a result of the increasing in the amount of negatively charged molecules of glyphosate in the solution. The more transportable (active) form of glyphosate is present in the solution the more molecules can interact with carrier molecule at the donor–membrane interface. The overall effect is the higher extraction efficiency when the high donor phase pH is kept.

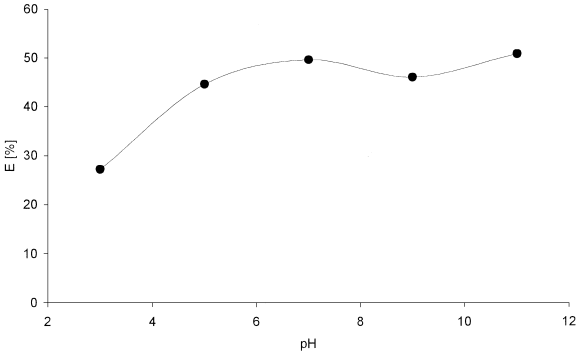

Fig. 5. The influence of the donor phase pH on the extraction efficiency. Membrane phase: 20% (w/w) Aliquat 336 in DHE; acceptor phase: 2 *M* NaCl; donor phase: 5 m*M* glyphosate at different pH of water; flow-rate = 0.2 ml/min.

3.4. Influence of the glyphosate concentration on the extraction efficiency

The effect of glyphosate concentration on its enrichment was investigated. The observed values are listed in Table 1. From the results it might be concluded that in the examined range of glyphosate concentration the extraction efficiency alters insignificantly. However, certain increase in the enrich-

Table 1
Effect of the glyphosate concentration on the extraction efficiency[a]

NPG concentration (m*M*)	*E* (%)
5	65.3
1	70.2
0.5	73.5

[a] Donor phase: NPG solution, pH 11, flow-rate = 0.2 ml/min; membrane phase: 20% (w/w) Aliquat 336 in DHE; acceptor phase: 2 *M* NaCl.

ment in the applied concentration range can be observed when the glyphosate amount in donor phase is smaller (5 m*M* in comparison to 0.5 m*M*). Probably, this effect derives from the same factors as it is in case of changes in salt content in acceptor phase. The lower glyphosate concentration the smaller amount of active molecules is accumulated in acceptor phase. As a result, the extraction of glyphosate is higher due to the competition between chloride and glyphosate anions is not as significant as when the higher glyphosate concentration is applied.

4. Conclusions

In this study we outline the possibility of using a SLM system with application of cationic carrier Aliquat 336 for extraction of glyphosate. This work is a part of larger programme dealing with the search for the possible utilisation of the SLM technique for amino acids and their phosphono derivatives. Hence, no application to ''real'' sample was attempted, as the intention was to find suitable extraction schemes. Presented results show that the transport and in turn, the extraction efficiency is governed by such factors as: carrier concentration, composition and pH of donor and acceptor phases. All these parameters are crucial for obtaining the optimal enrichment. A suitable donor phase pH should be alkaline to keep the glyphosate in active, transportable form. Moreover, it is important to point out that the transport of glyphosate is based on the counter-coupled transport mechanism and the presence of salt in the acceptor phase is necessary. However, some difficulties with the presence of salt in acceptor occurred when CE with indirect detection was used as the measurement method. Moreover, the limit of the detection was not satisfactory. Therefore, some other analytical methods should be considered or other types of detection might be used based on derivatization of glyphosate or electrochemical methods.

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