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Supported liquid membrane extraction of peptides★

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The application of supported liquid membrane (SLM) extraction for the enrichment of short peptides is presented. The extraction efficiency is dependent on the pH of donor phase and salt concentration in acceptor phase. Moreover, the extraction efficiency is also influenced by the peptide amino-acid sequence and hydrophobicity.

In the present time, more and more peptide-type compounds are introduced and applied in different areas of human activity, mainly as drugs or agrochemicals. Often, those compounds are in fairly low concentrations which sometimes renders it impossible to directly measure peptides as well as their metabolites content and fate in different matrixes. Those features obviously brought about a need for sensitive analytical methods for such purposes. Moreover, such methods have also to be selective enough to be able to differentiate between sometimes structurally similar peptides. However, commonly used analytical instruments often suffer from insufficient selectivity and sensitivity, therefore application pre-concentration and sample pre-treatment methods is necessary prior to further analysis. Among other methods, supported liquid membrane (SLM) extraction can be proposed. This technique has successfully been applied for the extraction and determination of a vast number of chemical compounds [1, 2].

Formerly, SLM has been successfully applied to amino acids [3, 4] and aminophosphonates extraction [5] and to peptide facilitated transport [6]. Those outcomes enable us

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Abbreviation: SLM, supported liquid membrane.

to look for a similar way of short chain peptides separation using a cationic carrier incorporated into the liquid membrane. In this short report, preliminary results of experiments concerning SLM extraction of peptides are presented. The influence of the acceptor phase composition, the type of counter-ion and the structure of peptide on the extraction efficiency were examined.

MATERIALS AND METHODS

The preparation of liquid membrane and the experimental set-up are similar to those described elsewhere [4]. Below, the general procedure of the experiments are described. Additional information can be found in the captions to figures or tables.

The samples were mixtures of peptides in various basic solutions. Twenty ml of sample solution was pumped through the donor channel at a flow rate 0.2 ml/min. The acceptor was about 1 ml stagnant sodium chloride solution or water. After pumping of sample through the donor side, the acceptor phase containing extracted peptide was removed to a 2 ml volumetric flask and filled up with the acceptor phase solution to the mark. Subsequently, both sides of the liquid membrane were rinsed with water prior to a next experiment. The sample was analysed with a UV-Vis spectrophotometr Beckman DU 640 B (U.S.A.).

RESULTS AND DISCUSSION

In Fig. 1 the dependence of Leu-Phe extraction efficiency on the pH of the donor (source) phase can be seen. The extraction efficiency increases with pH, with a rapid growth observed above pH = 8. The highest extraction efficiency was obtained when the donor phase pH was in the range 10–12. These results are not surprising if it is assumed that the transport mechanism through SLM is carrier facilitated counter-ion type. In this transport mode the peptide is transferred over the organic, liquid membrane as its ionic form (anion) enhanced by complexation with the cationic carrier (Aliquat 336 — quaternary ammonium salt) incorporated in the membrane phase. This indicates that to obtain maximal mass transfer it is necessary for the peptide to be

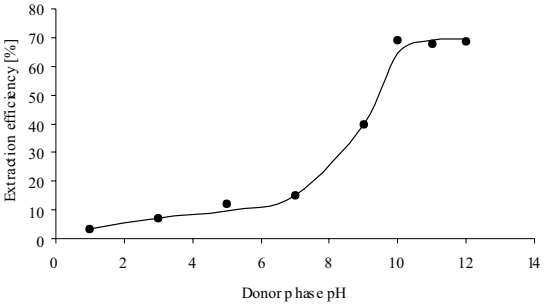

Figure 1. The influence of donor phase pH on the extraction efficiency of Leu-Phe.

Donor phase: 0.1 mM Leu-Phe, flow rate: 0.2 ml/min; membrane phase: 20% Aliquat 336 in dihexyl ether; acceptor phase: 2 M NaCl.

negatively charged. This can be achieved only if the high pH of the donor phase is kept which assures the presence of Leu-Phe in an extractable, anionic form. This is evident comparing Fig. 1 and Fig. 2 in which the distribution of Leu-Phe ($pK_{a\ -COOH} = 3.14$, $pK_{a\ -NH_2}$ = 8.41) ionic forms with pH is shown. There is a good correlation of the observed high extraction efficiency in the pH above 10 with the

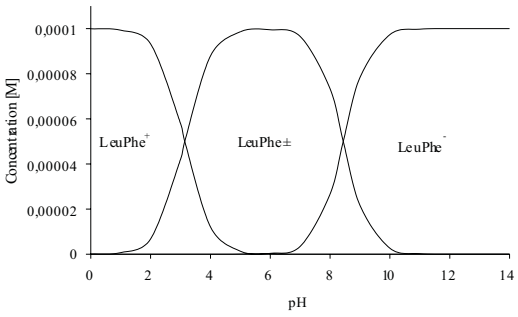

Figure 2. The distribution of Leu-Phe ionic forms in the pH range 0–14.

presence of anionic form of the peptide in the same pH region.

If the assumed transport mechanism is the major phenomenon responsible for the mass transfer of peptide over the liquid membrane, the driving force of the process is the difference in the concentration of the so-called counter-ion (in this case Cl^-) in the opposite direction. In Fig. 3 the dependence of the extraction efficiency on NaCl concentration in the acceptor phase is shown. It can be seen that higher chloride anion concentration results in a higher extraction efficiency but this is valid only for salt content in the acceptor phase below 1.5 M. Above this NaCl concentration the extraction efficiency reaches a plateau. For low salt content extraction of Leu-Phe is insignificant. These data clearly show that to obtain high mass transfer the presence of a chloride anion gradient from the acceptor to the donor phase is essential. The observed flattening of the extraction efficiency for high salt content is probably a result of reaching the limit of solubility by Leu-Phe in acceptor phase and the backward transport of the peptide.

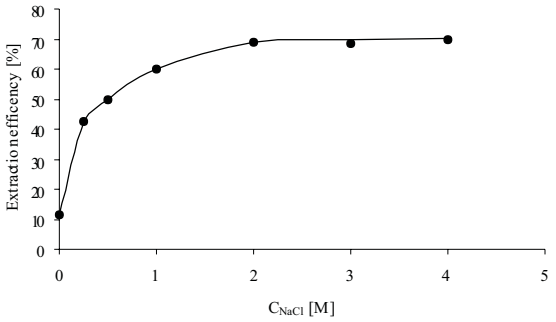

Figure 3. The influence of NaCl concentration on the extraction efficiency of Leu-Phe.

Donor phase: 0.1 mM Leu-Phe at pH 11, flow rate: 0.2 ml/min; membrane phase: 20% Aliquat 336 in dihexyl ether; acceptor phase: different NaCl solution.

In Table 1 the extraction efficiency of four different short peptides at two different initial concentrations in the donor phase is shown. Firstly, it can be seen that for the concentration of 0.05 mM the extraction is higher than for 0.1 mM. This is an effect previously observed for amines [7], amino acids [3] and aminophosphonates [5], a result of "incomplete trapping" that is caused by either reaching the limit of solubility in the acceptor phase and/or transport of the peptide back from the acceptor to the donor phase. Therefore, for lower initial concentrations more peptide can be accumulated in the acceptor phase and the extraction efficiency is higher. This is a very important implication from the analytical point of view, as in many cases the concentration of the analyte is far from the limit of detection of the available analytical instrument and efficient enrichment is needed to obtain the required concentration.

The second observation from Table 1 is that structurally different peptides are extracted with different efficiencies. This is probably a result of different hydrophobicities of the peptides examined. This is valid, however, with one noticeable exception – Phe-Phe (extraction efficiency around 30%), but it might be a result of low solubility of this highly nonpolar peptide in the acceptor phase.

Table 1. The extraction efficiency (%) of short peptides.

Donor phase: peptide solution at pH 11, flow rate: 0.2 ml/min; membrane phase: 20% Aliquat 336 in dihexyl ether; acceptor phase: 2 M NaCl.

Peptide	Concentration in donor phase [mM]	
	0.05	0.1
Met-Leu-Phe	100	84
Leu-Phe	100	69
Phe-Phe	53	33
Tyr-Glu	31	*

*Experiment not performed

Based on the presented preliminary results, as a conclusion it is important to notice that SLM extraction can be applied to the enrichment of short peptides using a transport based on carrier mediated coupled transport with cationic carrier dissolved in the mem-

brane phase. The obtained results paves the way for the already started experiments concerning SLM extraction of other peptides and their derivatives and the combination on-line of SLM peptide extraction with analytical instruments such as HPLC or CE for peptide determination.

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