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Enzymatic reaction in multiple w/o/w emulsions: effect of the nature of the lipophilic phase

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Structural characteristics of multiple water/oil/water type (W/O/W) emulsion systems offer a possibility to be used for physical immobilization of enzymes by incorporation into the internal aqueous phase [1–4]. These emulsion systems, acting as liquid membranes, are very attractive and interesting not only because they provide favorable transport rates through the membrane, but also because this mass transfer can be easily adjusted, designed and optimized. Therefore, by enzyme immobilization in multiple emulsions, two main goals could be achieved: to keep the enzyme intact and active and to optimize the enzymatic reaction by mass transfer modification [5, 6]. These facts are fundamental advantages from a biotechnological point of view [7].

In an enzyme-loaded W/O/W emulsion, substrate molecules, dissolved in the external continuous phase, should permeate through the middle layer toward the internal aqueous compartment, where the enzymatic reaction takes place. Since the permeant (substrate) is converted into a product, the concentration of permeant in the receiving phase is maintaining at zero or at a very low level (type 1 facilitation). Thus, the concentration gradient of the permeant across the membrane is maintained as high as possible [6].

The selectivity of transport through the lipophilic membrane layer and mass transfer limitations depend to a great extent on the liquid membrane properties [5, 8]. Therefore, the effect of membrane properties, defined by emulsion formulation, should be carefully evaluated. Alternatively, a modification of the substrate transport rate toward the internal aqueous compartment by changing the middle membrane phase properties nature, composition, surface and thickness could be considered as an approach for controlling the enzymatic reaction in a W/O/W emulsion system.

The aim of this study was to evaluate the effect of the nature of lipophilic component, used as an oil phase of the emulsion, on the enzymatic reaction in W/O/W emulsion system. Urea/urease was selected as a model substrate /enzyme system.

Enzyme urease was immobilized into the internal aqueous compartment (0.001% w/w in phosphate buffer pH 7) of W/O/W emulsion systems prepared by use of liquid paraffin, isopropylmiristate, olive oil, castor oil or almond oil as a lipophilic phase. Multiple emulsions were prepared with the emulsifier pair Span 83/Tween 80 = 7.5/1 (w/w) by a two-step emulsification procedure [8, 9]. First, a primary W/O emulsion was prepared by mixing the buffered solution of urease with oily phase containing lipophilic emulsifier Span 83, 20% w/w (propeller stirrer, 800 rpm, 5 min). In the second step, a W/O/W emulsion was obtained by reemulsification of W/O emulsion with aqueous phase containing the hydrophilic emulsifier Tween 80, 1.67% w/w (magnetic stirrer, 300 rpm, 5 min). Phase ratios φW/O and φW/O/W were 0.5. Emulsion samples were incubated at 25 °C. Enzymatic reaction was initiated by incorporation of urea (1.2% w/w) into the external aqueous phase of the prepared samples. The urea/urease reaction was followed by measuring the quantity of NH_3 (potentiometric titration, 0.05 M HCl), produced in the emulsion system in defined time intervals. Prior to product determination, $HgCl_2$ and NaCl have been added to the aliquots of the W/O/W emulsion. $HgCl_2$ induces cessation of the enzymatic reaction, while NaCl (2.8% w/w into an external aqueous phase) causes osmotically-induced conversion of W/O/W into a simple O/W emulsion system, as a prerequisite for detection of the total quantity of NH_3 in the aqueous phase. The obtained results for product amount vs. time were processed using the first-order kinetic model. The kinetic coefficient k, as well as $t_{1/2}$ have been calculated.

Experiments were performed on freshly prepared samples (n = 3), thus excluding the effect of the changes of the emulsion dispersion status during ageing, which may affect the membrane phase properties.

The comparative study of the emulsion systems indicated that the composition of the middle oil layer affected the rate of the enzymatic process (Fig., Table). By changing the lipophilic component in the membrane layer, the transport rate through it has been changed significantly. For instance, when isopropylmiristate in the membrane layer of the emulsion was replaced with almond oil, the kinetic coefficient k (min^{-1}) was almost four times lower (Table). This could be a result not only of the different permeability of the layer (function of the oil nature, density, viscosity etc.), but also of the different surface and thickness of the liquid membrane layer i.e. the obtained dispersed status of the emulsion. Practically, the emulsion formulation controls the transport across the membrane. On the other hand, the comparison of the urea/urease reaction in emulsion media with that in the aqueous solution showed up to ten times lower values of k (min^{-1}) in the emulsions (Table). This suggests that the liquid membrane keeps the

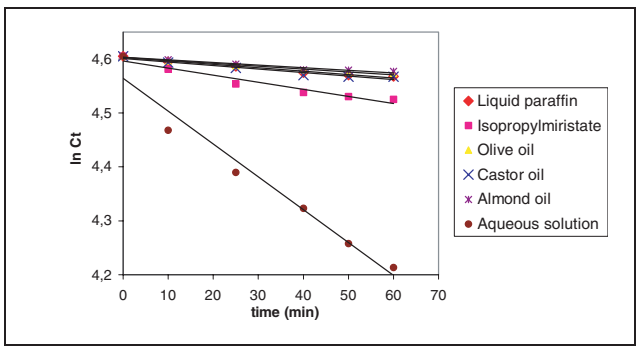

Fig.: Ct (mmol/kg), versus time plot for urea/urease reaction in W/O/W emulsion samples as a function of the nature of lipophilic phase (n = 3)

Table: 1st order kinetic parameters of urea/urease reaction in a multiple W/O/W emulsion system as a function of the nature of lipophilic phase (n = 3)

Lipophilic phase in W/O/W emulsion	Kinetic parameters (1st order)		
	k (min^{-1})	Correlation coefficient	$t_{1/2}$ (min)
Liquid paraffin	5×10^{-4}	0.984	1386
Isopropylmiristate	1.1×10^{-3}	0.984	630
Olive oil	5×10^{-4}	0.982	1386
Castor oil	6×10^{-4}	0.984	1155
Almond oil	3×10^{-4}	0.989	2310
Aqueous solution	6.4×10^{-3}	0.986	108

enzyme intact and acts as a rate-controlling factor of the mass transport toward the internal aqueous phase. Further investigations involving study of the effect of membrane surface and thickness on the effective kinetic coefficient are in process.

In conclusion, the emulsion system itself affects the kinetic parameters of an enzymatic process. Modification of the composition of the middle oil layer in a multiple W/O/W system, used as a medium for an enzymatic process, could be considered as an approach for controlling the rate of the enzymatic process.

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Three new butyl glycosides from *Inula crithmoides* L. growing in Egypt

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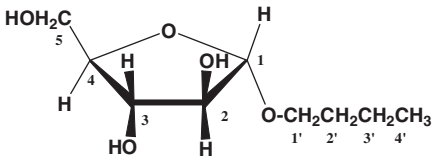
Three new glycosides have been isolated from *Inula crithmoides* L. Structure elucidation of the isolated compounds was established by the application of spectroscopic analyses including, 1 D and 2 D NMR spectroscopy and MS.

The genus *Inula* (Asteraceae) comprises 200 species [1]. The major secondary metabolites of the genus are sesquiterpene lactones mainly eudesmanolides [2], monoterpenes [3], diterpenes [4] and flavonoids of diverse chemical structures [5]. Several pharmacological activities are attributed to these secondary metabolites, including treatment of asthma, dysentery and inflammatory diseases [6, 7]. Inulin, a fructose polymer and fructo-oligosaccharides are of common occurrence in Asteraceae [8, 9]. Fructo-oligosaccharides have been shown to exhibit beneficial effects by stimulating the growth of Bifidobacteria in the human colon, by suppression of putrefactive pathogens, and by reduction of serum cholesterol concentration [10]. In addition, fructose is used as a food by diabetic patients and



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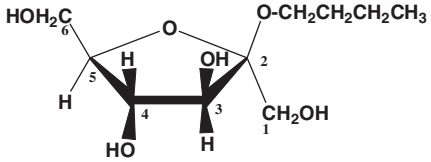

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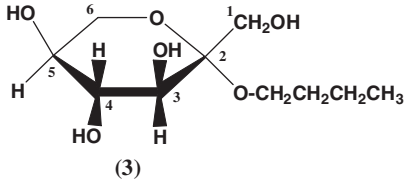

(3)

Isolated Compounds