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Liquid Emulsion Membranes and Their Applications in Biochemical Processing

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Abstract

The potential for liquid emulsion membrane (LEM) systems in biochemical applications and their advantages over conventional systems are discussed. Examples are cited where LEMs have been used to successfully separate organic acids, amino acids, and antibiotics. The use of LEMs to immobilize cells and enzymes to synthesize antibiotics and amino acids as well as decontaminate biological waste streams is described. Enzyme systems immobilized in LEMs are quantitatively evaluated via traditional engineering approaches. In light of the above analysis, the potential LEM process difficulties of membrane breakage, swell, and selectively are examined. New biological applications for LEMs are suggested.

One of the major challenges in biotechnology is the separation and subsequent concentration of fermentation products. The disadvantages of current techniques are many and varied. Some processes, like conventional ion exchange, are inherently batch operations that require pretreatment of the fermentation broth before separation and have high capital costs. Other techniques, such as whole cell broth extraction, while being used in a continuous mode, are often product and broth specific, require prederivitization of the solute, and will work for only a small number of solutes. Gel permeation chromatography (HPLC), while providing the capability for excellent separation, requires extensive pretreatment of the broth, is capital and labor intensive, and is not easily scaled. Those interested in the downstream processing of small bio-

chemicals seek the development of a separation scheme that avoids the pitfalls of currently used technologies. The properties of such an optimal system are listed in Table 1.

One system that demonstrates these attributes is the use of liquid emulsion membranes. Liquid emulsion membranes (LEMs) were first developed in 1967 by Li (*1*) and have been used for a variety of separations [see reviews by Frankenfeld and Li (*2*), Marr and Kopp (*3*), and Way et al. (*4*)]. Applications of LEMs to bioseparations were, until recently, largely limited to their use in biomedical applications such as prevention of drug overdose and drug delivery (*5–8*). In the last 5 years, several investigators have examined the use of LEMs for the downstream processing of biochemicals. While a brief review of this newer literature has been published recently (*9*), a more in-depth review of the available literature is necessary to establish the directions of needed research and to show the applicability of LEMs to downstream processing operations.

CONCEPT

Liquid emulsion membranes, when applied to biochemical separations, are three-phase systems consisting of a water-in-oil emulsion dispersed into an aqueous third phase (Fig. 1). The system is prepared by slowly adding an aqueous phase to a surfactant-laden oil phase under intense shear. The resulting kinetically stable water-in-oil emulsion is then dispersed using mild agitation into a continuous aqueous phase, resulting in a dispersion of emulsion globules in an aqueous solution. The encapsulated or "interior" phase never actually contacts the "exterior' aqueous phase; the oil acts as a liquid membrane between the two aqueous phases. It is typically assumed that the globules are noncoalescing and thus that they retain their integrity throughout the separation process. In addition, due to the presence of surfactant, the globules are

TABLE 1
Criteria for an Optimum Downstream Process

Selectivity
Ability to concentrate
Continuous
Minimal pretreatment
Inexpensive

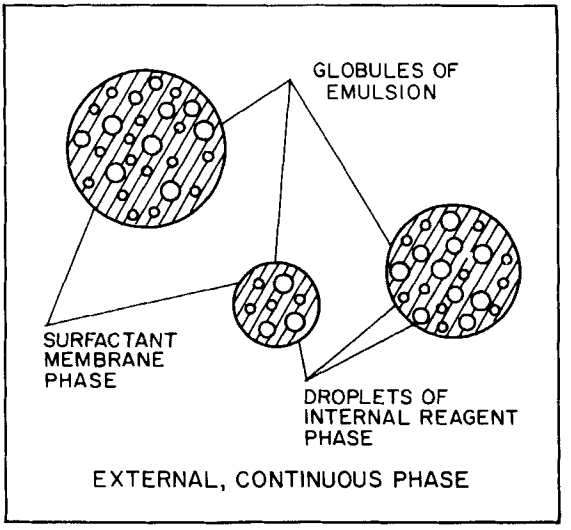


FIG. 1. Schematic of a liquid emulsion membrane system.

internally stagnant (*10*); there is no circulation of the aqueous droplets inside the globule.

MECHANISMS OF BIOCHEMICAL SEPARATIONS

Simple Diffusion Membranes

There are two mechanisms by which separation can be affected using LEMs. The first of these, the simple diffusion or Type I membrane (*11*), relies on the ability of the solute of interest to partition into the oil phase of the membrane. An example of this type of separation is shown in Fig. 2a using the separation of acetic acid as a model case. The nonionized form of acetic acid partitions into the organic phase of the LEM globule and diffuses through the membrane until the acid reaches an interior aqueous droplet. In membranes of this type, the interior phase is usually either a concentrated acid or base. In the case of acetic acid, the interior phase is often a concentrated sodium hydroxide (NaOH) solution. Upon reaching the droplet, the acid partitions into the aqueous phase where it is rapidly converted to acetate anion, CH_3COO^-. This ion is unable to partition back into the oil phase due to its charge. Since there is very little

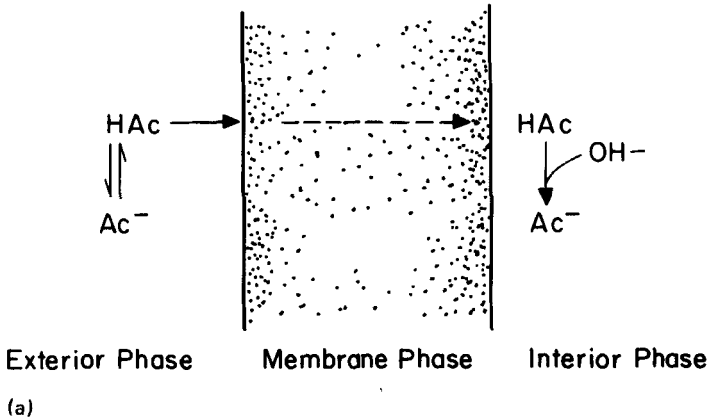


FIG. 2a. Mechanism of transport for a simple diffusion (Type I) LEM system.

nonionized acid in the given droplet, the driving force for separation and partitioning into the droplet is very high.

Once the reagent in a given droplet is depleted in the process of converting nonionized acid to anion, the driving force for partitioning of the acid into the droplet quickly dissipates. The nonionized acid must then diffuse further into the globule to find a droplet with a sufficient amount of reagent. As it diffuses further into the droplet, separation time increases and the process becomes diffusion limited (*12*, *13*).

Carried-Mediated Processes

A second type of transport mechanism, that of facilitated transport via a "carrier" compound [Type II transport (*11*)], is shown in Fig. 2b using a phenylalanine anion as the solute and chloride as the counterion. This mechanism is used as the means of transport when the solute is a charged compound. In these systems, an oil-soluble, water-immiscible carrier of a charge that is complementary to the charge of the solute is dissolved in the oil phase. The carrier is usually an ionic surfactant consisting of a long hydrophobic tail section and a univalently charged hydrophilic "head group." The charge of the head group requires that the carrier always be complexed with a counterion to remain electrically neutral in the oil phase. Typically, the interior phase consists of a concentrated inorganic salt solution, which provides the driving force for transport.

Assuming that the carrier is initially complexed with the counterion in

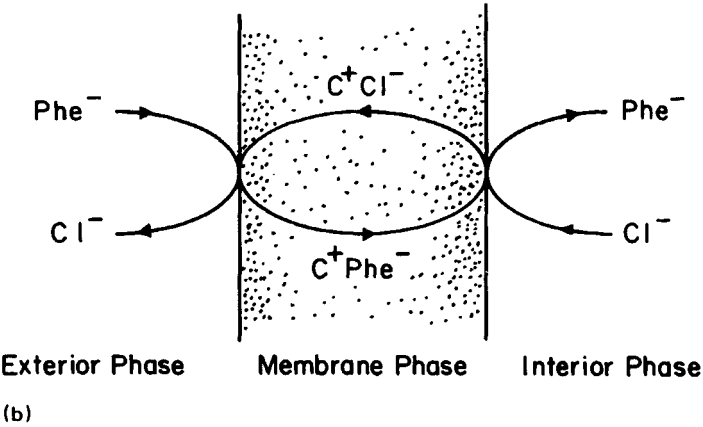


FIG. 2b. Mechanism of transport for a carrier-facilitated (Type II) LEM system.

the interior phase, separation occurs in the following fashion. The carrier/counterion complex adsorbs to the globule/exterior phase interface. An interfacial ion-exchange reaction occurs in which the phenylalanine is exchanged for the counterion. This reaction is primarily driven by the solute's affinity for the carrier and the oil phase. The carrier/solute complex diffuses across the membrane until it reaches an oil/interior droplet interface. At this interface, another interfacial ion-exchange reaction occurs. The solute is exchanged for the counterion in the interior phase, a reaction driven by "mass action." The carrier/counterion complex then diffuses back across the membrane and the process is repeated.

Advantages of LEM Bioseparation Systems

The advantages of the application of LEM systems are identical for the two mechanisms mentioned above and are best understood in terms of the properties of an optimal bioseparation system as described in Table 1. For example, the volume of exterior and interior phases can be varied independently and essentially at will, and thus one can easily make a system in which there is much more exterior phase than interior phase. With this condition, each time solute is transported from the exterior to interior phases, the solute is *simultaneously concentrated* by the ratio of the two phase volumes. In addition, if the reagent in the interior phase is concentrated enough, the solute can be concentrated against its gradient

(*14*). It is thus in two ways that separation and concentration can be achieved simultaneously with LEM systems.

In addition, LEM systems are scalable and continuous processes. The technology underlying LEM applications is that of liquid–liquid extraction. This type of technology is readily scaled up to commercial operation. LEM systems have been tested on the pilot scale for metal separation systems with good success (*2*, *15–17*), and an LEM process for the recovery of zinc from wastewater has been successfully scaled to a commercial process (*18*). These pilot studies, as well as the commercial unit, were based on continuous operation. In addition, Thien et al. examined the bench-scale continuous operation of an LEM system for the separation and concentration of L-phenylalanine with good success (*19*). Scheper et al. also worked with an enzymatic system for D,L-racemization of amino acids on a continuous basis (*20*). Modeling of continuous operation of LEM systems by Hatton and Wardius (*21*) indicates that continuous operations better utilize the reagent in the globules and should provide better separation. This improved efficiency has been verified in the work of Thien et al. using bench-scale multistage contactors (*19*). Experimental studies of LEM systems in continuous column extractors have been conducted (*22*, *23*) and have shown the feasibility of continuous column-based LEM systems. In particular, Scheper et al. (*24*) and Makryaleas et al. (*25*) used column extractors and complicated enzyme/LEM systems with success.

Unlike chromatographic separation systems, LEM systems have also been shown to require little or no pretreatment of the solute-containing exterior phase. Studies of LEM separation systems using solids-containing wastewater as a source of zinc for recovery (*22*) and uranium leach liquors containing significant quantities of "crud" (*17*) showed that particulates and flocs did not affect the mass transfer in these systems. It has also been shown that LEMs are not affected by the presence of either live bacterial cells (*26*) or enzymes (*27*), thus suggesting that fermentation broths could be handled with no pretreatment. This assertion is backed up by the studies of Thien et al. which showed no difference in separation between ultrafiltered cell-free broth and yeast whole cell broth (*19*). Scheper and coworkers (*24*) also worked with cell-free broth without difficulty. The inability of untreated broth to hamper separation will vary with the solute of interest. For example, Halwachs (*28*) showed that blood proteins can adsorb some solutes and thus decrease the driving force for transfer in the LEM system.

Pilot-plant studies, in addition to showing that LEM systems can be operated on a continuous basis, also showed that, for low-value products such as metal recovered from wastewaters or leachates, LEM systems can

be as economically advantageous, if not more so, than currently employed solvent extraction techniques (*2, 15–17*). To date, no pilot-plant scale separations of biochemicals using LEMs have been reported in the literature.

BIOCHEMICAL APPLICATIONS

Biomedical Applications

The first application of LEMs to bioseparations was for drug delivery and drug overdose prevention systems (*5–8*). The systems studied focused on the use of Type I diffusion membranes for the separation of a series of compounds of pharmaceutical interest. The system used was very similar to the example acetic acid Type I diffusion system discussed above; the exterior solution was at pH 2, the interior buffered at pH 10, and the drugs tested were all organic acids. With such systems, Rhodes and coworkers (*5, 6*) reported the successful separation of 12 benzoic and salicylic acid derivatives. Chiang et al. (*7*) examined the transport of seven barbiturates and similarly achieved quick separations. Since the interest of these authors was primarily in the areas of drug delivery and drug-overdose prevention, the authors were not interested in the recovery of the drugs from the emulsions and did not speculate on the ability of these systems to be used for bioproduct recovery.

Yagodin et al. (*29*) examined the application of LEMs for the removal of cholesterol from blood. The membrane was a Type I membrane containing cholesterol-specific digitonin in the interior phase. The digitonin acted to saponify the cholesterol, thus converting the cholesterol into a form which could not permeate back through the membrane once inside the membrane. With this system, Yagodin and coworkers, using only gentle agitation, were able to remove approximately 90% of the cholesterol from bovine whole blood within 2 h. In the same period, they saw essentially no drop in the concentration level of other blood components and no hemolysis. In reviewing the licensing of LEM-related technology for biomedical applications, Parkinson et al. (*8*) also noted that LEM systems were being applied to the oxygenation of blood and the treatment of chronic uremia.

Biochemical Separations

The first application of LEMs to the separation of commercially interesting biochemicals was that of Terry and coworkers (*30*). Using a

system very similar to the system of Rhodes and coworkers, Terry et al. focused on the separation of phenols and cresols from wastewater. In addition to being able to separate these contaminants from wastewater, they also found that Type I (simple diffusion) LEMs could also be used for the separation of acetic and propionic acids. Their study indicated that LEMs were particularly good at separating acetic acid from dilute solutions.

Larson et al. (*31*), studying the acetic acid system, noted that since LEMs were able to separate solutes from dilute streams, LEM systems should be well suited for separating dilute biochemicals from fermentation broth. The authors pointed out that separation times in Terry and coworker's system were large and that this was primarily due to the low partition coefficient of nonionized acetic and propionic acids in oil. In order to improve their permeability through the membrane, Larson and colleagues added a tertiary amine to the membrane to increase the partitioning of acid into the oil using the following chemistry:

$$R_3N + HAc \rightarrow R_3NH^+Ac^-$$

The addition of this tertiary amine made their system a carrier-facilitated, or Type II, system (Fig. 3). They compared the extraction versus time profiles for Type I and Type II acetic acid LEM systems and found the facilitated system to be much faster (see Fig. 4). In addition, they carried out a series of experiments designed to determine the controlling factors in the LEM-mediated separation of acetic acid. Not surprisingly, they

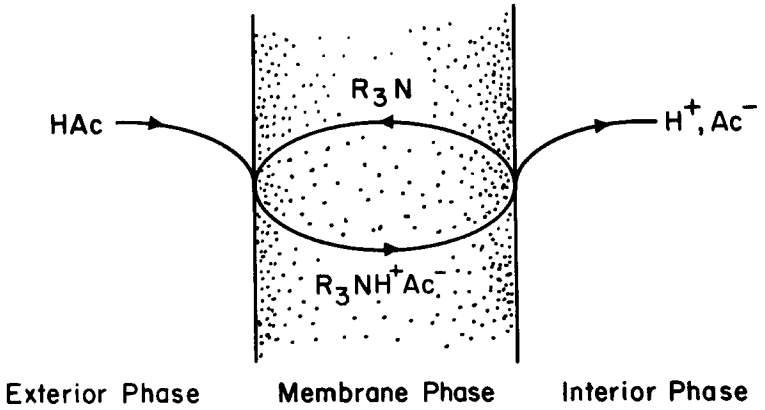


FIG. 3. Schematic of Type II LEM system for acetic acid separation.

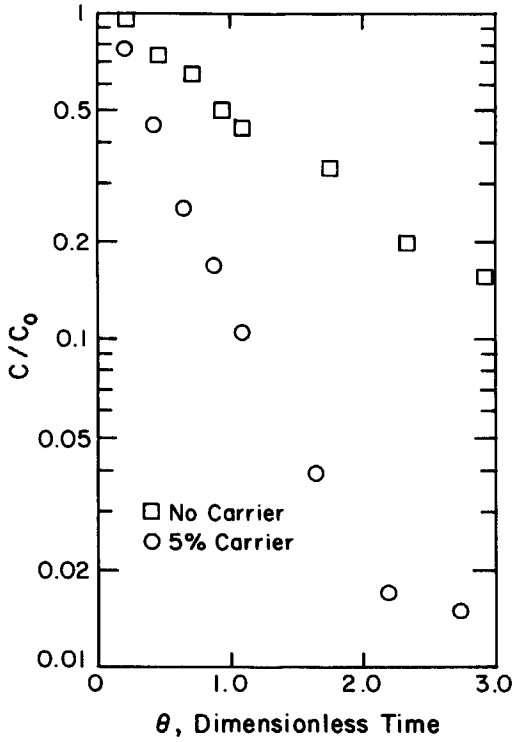


FIG. 4. A comparison of separation times for Type I and Type II systems for acetic acid separation (from Ref. *31*).

found that increases in carrier concentration, initial interior reagent concentration, and the amount of emulsion used to treat a given volume of acid solution, or a decrease in the initial concentration of acid, increased the extraction.

It should be noted that the above-mentioned systems can be used *in situ;* that is, as long as the conditions of the fermentation broth allow for some partitioning of the solute into the oil phase of the membrane (either by equilibrium partitioning of the solute itself or by complexation chemistry), these LEM systems can be used in the fermenter during a fermentation. This view is supported by the studies of Mohan and Li that show that the presence of LEMs does not adversely affect the activity of enzymes (*27*) or the growth of microorganisms (*26*). *In situ* LEM separations could be one means of relieving the build-up of toxic and feedback-inhibiting products in fermentations. Such "extractive fermen-

tations" would be extremely useful in the microbial production of organic acids such as acetate, acrylate, butyrate, citrate, and lactate. LEM systems for these fermentations have already been studied to some extent (*32*).

Another application of LEMs to biochemical separations has been the separation of biochemical zwitterions from fermentation broth. A zwitterion is a compound possessing both a positive and a negative functional group at neutral pH. Typically, as in the case of amino acids, these functional groups are ionizable and can vary in charge as a function of pH:

$$HO_2C-\underset{\displaystyle R}{\underset{|}{CH}}-NH_3^+ \underset{H^+}{\leftrightarrow} {}^-O_2C-\underset{\displaystyle R}{\underset{|}{CH}}-NH_3^+ \underset{H^+}{\leftrightarrow} {}^-O_2C-\underset{\displaystyle R}{\underset{|}{CH}}-NH_2$$

Several important categories of biochemicals can be classified as zwitterions: phospholipids, amino acids, and β-lactam antibiotics. Due to the everpresent charge on these compounds, their solubility is greatly decreased in conventional organic solvents; traditional solvent extraction cannot be used to recover these small bioproducts from fermentation broth. As an alternative to the currently used techniques of derivitization followed by extraction or ion exchange, liquid emulsion membranes have been examined for the economical recovery of these compounds from fermentation broth.

While carrier-facilitated transport of amino acids across buoyant liquid membranes was first investigated in 1973 (*33*), the first detailed study of amino acid transport in LEMs was carried out by Thien et al. (*9*, *34*, *35*) who examined the separation and concentration of L-phenylalanine in an LEM system (see Fig. 2b).

Whereas those studying the use of LEMs for metal recovery as well as the studies of Larson et al. and Terry et al. focused on the *separation* of solute from dilute streams, Thien and coworkers pointed out that the economic use of LEMs as a biochemical downstream processing operation requires not only *separation* of the solute but *concentration* of the solute as well. To this end, the authors emphasized the need to examine the effects of process parameters on the final concentration of solute in the membrane via direct determination. In a comprehensive examination using realistic concentrations of L-phenylalanine in the exterior phase (10–15 g/L), Thien and coworkers delineated the effects of the following process parameters on separation and *concentration:* agitation speed (*9*), initial interior chloride concentration (*34*, *35*), carrier

and surfactant concentration (*34*), the presence of competing ions in the external phase (*34, 35*), the chemical nature of the counterion (*35*), and the chemical nature of the solute (*35*). The authors provided a rationale for manipulating the various parameters in LEM systems to obtain optimal performance. In addition, the authors provided a critical assessment of the shortcomings of LEMs when applied to bioseparations. These shortcomings will be discussed later.

Plucinski et al. (*36*), in a less detailed study, examined the transport of L-valine, glycyl-L-histidine, and L-(*N*-acylamino)ethane phosphate in LEM solutions for very dilute solutions (less than 1 g/L for valine). In contrast to previous studies however, Plucinski and coworkers had the interior phase be the source of solute and the exterior phase be the "receiving" phase. In addition, the exterior phase consisted of water only; there was no driving force other than the tendency to equalize the concentrations in each aqueous phase. The authors found that transport across the membrane was rapid for all three solutes and that equilibrium was reached within 20 min. Interestingly, the solute flux was not dependent on the carrier concentration or the carrier used to facilitate transport. Indeed, the solute transport rate was approximately the same for no carrier as it was for the various carriers tested. The authors attributed transport across the membrane in the absence of carrier to the formation of reversed micelles made from the emulsion-stabilizing surfactant, Rokwin 60.* While the formation of reversed micelles in emulsions has corroborating support in some types of emulsions (*37, 38*), experiments described by the authors do not include any light scattering or other techniques to prove the existence of reversed micelles. It is interesting to note that Thien and coworkers, in unpublished work, found no micelles present in the LEMs as determined by quasi-elastic light scattering. Nevertheless, the possibility that reversed micelles could be used as a mechanism for transport in LEM systems present intriguing possibilities that will be discussed later.

Yagodin et al. (*39*) examined the transport of L-lysine in its cationic form in LEM systems with the intent of using LEMs to recover lysine from fermentation broth. A schematic of the separation system is shown in Fig. 5. Since the solute is transported in the cationic form, a cation complexing agent, di(2-ethylhexyl)phosphoric acid (D2EHPA), was used as the carrier. This compound is a chelating agent typically used for the

*Rokwin 60 is described as a "mixture of esters of higher fatty acids and sorbitan." From this inadequate definition, it is difficult to make an authoritative statement as to whether or not the surfactant is likely to form reversed micelles.

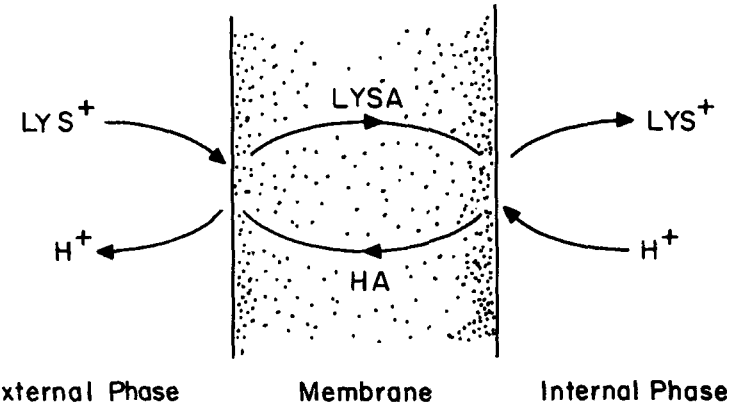


FIG. 5. Schematic of a Type II system for the separation of L-lysine as a cation.

separation of metal cations. While brief, Yagodin and colleagues described (but did not show) their studies of the effects of changes in emulsion phase volume, and carrier and surfactant concentration on membrane stability and amino acid separation. The authors pointed out, as do Thien et al., that to use LEMs as a downstream operation, the ability of the membrane to concentrate the solute in the interior phase is crucial. The authors only reported one figure as to the interior concentration of lysine. From this sketchy datum it is difficult to assess the effects of the various parameters on interior solute concentration for their system. The authors also mentioned the crystallization of lysine from the interior phase with a yield of 70%.

Enzyme and Cell Immobilization

The idea of using specific reagents as driving forces in the membrane has already been considered (*29*). Another method of supplying a specific driving force is to have a specific, catalyzed reaction in the interior phase. This reaction would continually convert solute to another chemical species as it reaches the membrane. If the reaction is fast enough, this conversion would allow for a maximum driving force at all times. One way of achieving this conversion would be through enzymatic catalysis.

Mohan and Li first showed that enzymes could be encapsulated in LEMs in 1974 (*27*). In their first study, the authors encapsulated cell homogenate from *Micrococcus denitrificans* in the interior phase of an

LEM. The authors took great care to demonstrate that the membrane stability was not compromised by the presence of homogenate. Further, the authors showed that the nitrogen reductase enzyme system used for the reduction of NO_3^- to NO_2^- and the reduction of NO_2^- to other nitrogen compounds still functioned (see Fig. 6). Figure 6 shows the profile of NO_2^- in the exterior phase. The system initially had only NO_3^- in the exterior phase. The graph indicates that some catalytic conversion must be occurring to change the form of the NO_3^-. The exterior phase had no catalytic activity after having been taken out of contact with the LEM phase. These results indicate that enzymes can function inside LEMs and that these enzymes can be used to carry out separations whose driving forces are as specific as the enzyme itself. It is curious to note that transport of NO_3^- occurred with no carrier in the membrane. The authors state that "apparently" the membrane was permeable to the charged compound NO_3^-. It is unlikely that NO_3^-, even as an ion pair, would have the high permeability that is reported. The authors give no mechanism for the transport across the membrane.

In a continuation of the above work, Mohan and Li (*26*) then immobilized whole, live cells in the interior phase of a liquid emulsion membrane. The cells, *M. denitrificans,* showed viability inside the LEMs for up to 5 days. In addition, the bacteria were able to reduce nitrates and nitrites, showing continued (though decreasing with time) enzyme

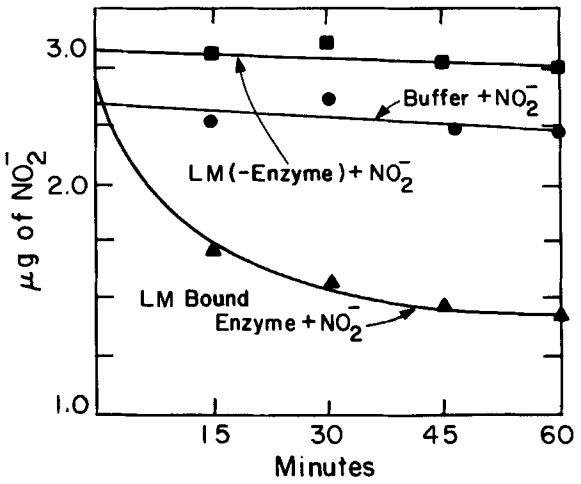


FIG. 6. Profile of exterior phase [NO_2^-] as a function of time for LEM-based and unbound reductase systems (from Ref. *27*).

activity. Unlike the cell homogenate system, in this case the authors added an anion carrier, a secondary amine, to the oil phase of the membrane. Much like the chemistry of tertiary amines, the chemistry by which the amine facilitates transport is

$$R_2NH + H^+ + NO_3^- \rightarrow R_2NH_2^+NO_3^-$$

The mechanism of transport and reaction for this LEM system is shown in Fig. 7. The authors found that the LEM could protect the enzyme from potential inhibitors as shown by putting $HgCl_2$ in the exterior phase. Normally, this powerful inhibitor of reductases would quickly and totally deactivate the enzyme. In the case of the enzyme protected by the liquid emulsion membrane, there was essentially no change in activity of the cells. The authors also showed the combined effects of transport and reaction by varying the pH of the outer phase* (Fig. 8). While the optimum pH for nitrate reduction of the unbound cells is around 7.5, the relative activity for the LEM-bound cells is higher at a lower pH (pH = 6.0). This increase in activity was most likely due to increased transport of the solute. As the pH was lowered, the solute was more likely to form the amine/solute complex, and thus more solute could be transported across the membrane for reduction. Since the transport of solute changes the activity of the bound cells, it is likely that the system is transport limited.

While the focus of the work of Mohan and Li was to demonstrate the applicability of LEMs to enzyme and cell immobilization for use in wastewater treatment, Halwachs et al. (*28*) considered the use of enzyme-impregnated LEMs for the phenol detoxification of hepatic blood. Their system consisted of phenol in bovine blood or plasma as the exterior phase, and UDPGA (urine diphosphoglucuronic acid) and UDPGT (urinediphosphoglucuronyltransferase) in the interior phase (Fig. 9). The system mimics the liver in that lipophilic phenol is linked to UDPGA via the catalytic action of UDPGT. The hydrophilic nature of UDPGA makes the new compound easy to excrete from the kidneys. In the LEM, the phenol (and only the phenol; the other blood products should not be affected) must diffuse through the membrane where it can be processed by the enzyme, while the product must diffuse back through the membrane and into the blood where it can be naturally processed.

The studies of Halwachs and coworkers addressed this system in

*The interior pH is held constant at the free cell optimum pH by a 0.1 *M* phosphate/Tris HCl buffer.

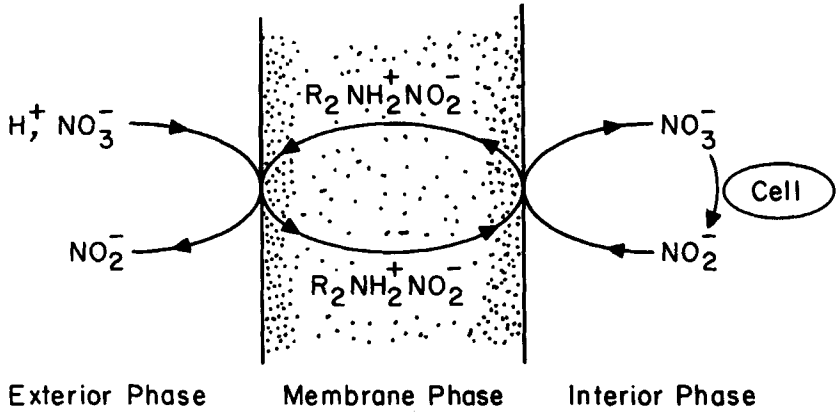


FIG. 7. Schematic of LEM-bound whole cell system for the reduction of NO_3^- to NO_2^-.

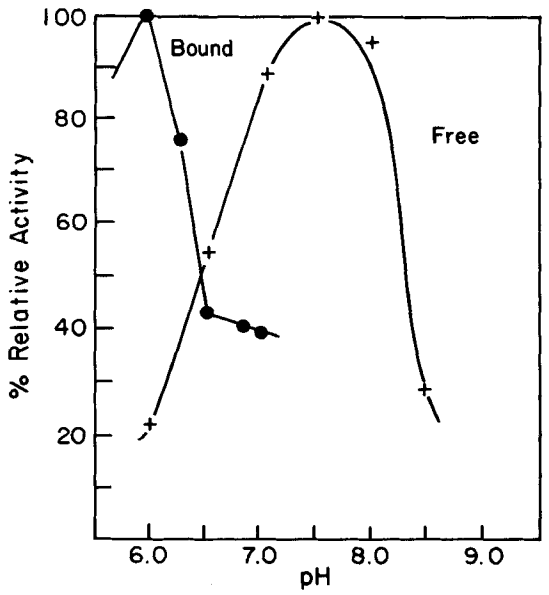


FIG. 8. Percent relative activity versus pH for LEM-bound and free whole cells (*26*).

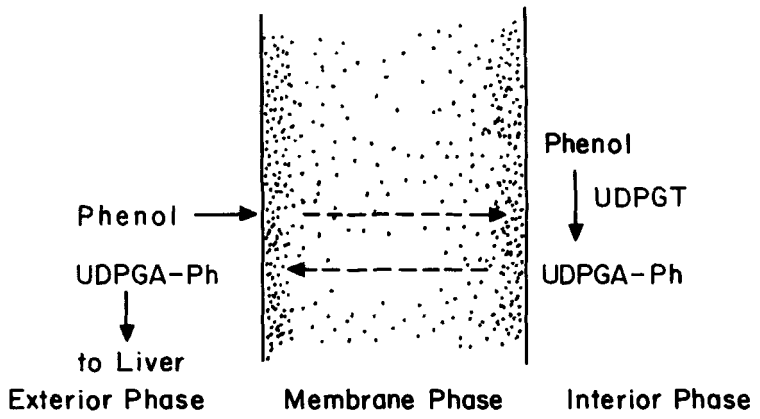


FIG. 9. Schematic of LEM-based system for the phenol detoxification of hepatic blood.

several different ways. The authors formulated the emulsion for membrane stability (so as not to recontaminate the blood). Loss of enzyme activity was found to be a first-order process in internal enzyme concentration as measured by enzyme activity. Unlike Mohan and Li, Halwachs et al. carefully considered the rate-limiting step of their system. In a clever comparison, the authors compared a mass transfer limited separation (where the interior reagent is base, thus having essentially *instantaneous* conversion of phenol to phenolate) to that of a kinetic limitation (plain water in the interior phase; essentially infinitely long reaction time). They found that their fluxes lay somewhere in between the two regimes. As they increased the enzyme concentration in the interior phase, their system began to approach the mass transfer limited regime. The authors then use a crude resistance theory to estimate the quantity of enzyme required to reach diffusional limitation. The theory that is developed greatly oversimplifies the geometry of the problem, especially in light of more sophisticated and mechanistic models that have been developed for Type I diffusion systems (*12*, *13*). Unfortunately, the authors do not provide enough data to perform a Thiele modulus calculation. Regardless of the theoretical development, Halwachs and colleagues justly emphasize the need to determine the rate-limiting step; if the process is mass-transfer controlled, there is no need to add more expensive enzyme in the hopes of increasing solute flux rates.*

*The data of Mohan and Li (*27*) demonstrate this point. Data from their system indicate that when the concentration of cells increases beyond a certain point, the specific activity of the interior phase decreases (the total activity remains unchanged). This is clearly indicative

In perhaps the most commercially applicable LEM/enzyme system studied, Scheper and coworkers (*40*) examined the stereospecific production of L-phenylalanine from a racemic mixture of D,L-methylphenylalanyl ester (Fig. 10). In this system the unprotonated form of the D,L-ester diffuses across the membrane to the interior phase. In the interior phase, α-chymotrypsin selectively hydrolyzes the L-ester into the L-amino acid and methanol. The D-ester is recycled. The L-amino acid, being present as a zwitterion in the interior phase, cannot partition into the oil phase due to its charge. An anion carrier, a quaternary ammonium salt, acts as a facilitative carrier for the amino acid.* The methanol, being uncharged, can diffuse across the membrane without facilitation. The exterior phase (and thus the interior phase via the nonspecific carrier) was maintained at a pH of 6. The conversion was measured by monitoring the ester concentration in the exterior phase.

The authors, in addition to showing excellent conversions, also attempted an analytical model of the system using enzyme kinetics developed earlier (*41*). The kinetics developed are those for the concentration of L-ester in a CSTR with enzyme reaction kinetics:

$$\frac{dC}{dt} = \frac{F_V}{V_R}(C^0 - C) - \frac{\upsilon_{max} C}{C + K_S}$$

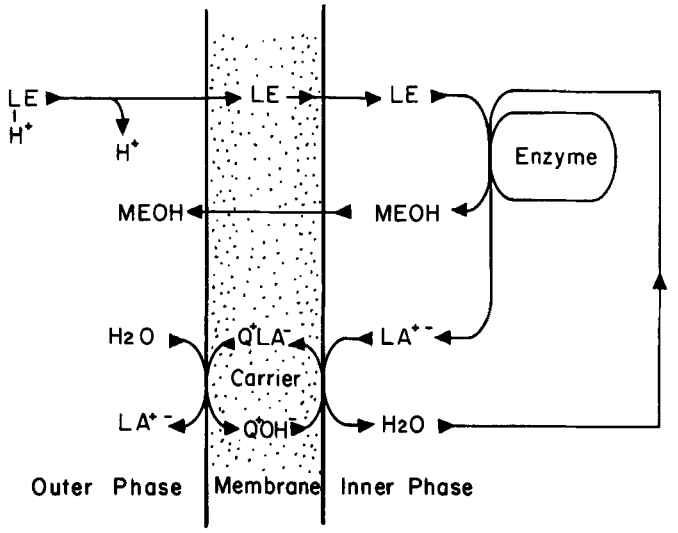
This equation inherently assumes that the system is reaction controlled. Unlike the earlier work by Halwachs (*28*), there is enough data to perform a simple Thiele modulus calculation for this system. For diffusion and enzyme reaction in a sphere, the Thiele modulus is given as

$$\phi^2 = \frac{R^2 \upsilon_{max}}{D_{eff} K_S}$$

For the system under consideration, assuming initially 400 m*M* of D,L-ester, we have $\upsilon_{max} = 0.33$ m*M*/s, $K_S = 100$ m*M* (*41*), $D_{eff} = 10^{-5}$ cm^2/s (*42*,

of mass-transfer limitation. In addition, for increases in carrier concentration at constant enzyme concentration, the rate initially increases and then stabilizes. This is indicative of reaction control.

*While providing convincing data that the ester was taken up by the membrane, the authors provided no data concerning the fate of the interior amino acid. The authors note that a pH of 6 was maintained both in the interior and exterior phases. At this pH, the amino acid is predominantly present in the zwitterionic form, a form that cannot be transported across the membrane by a cationic carrier.



LE
H+ = Protonated L-Ester
LE = Neutral Ester
LA+- = Zwitterionic L-Amino Acid
Q+ = Adogen 454

FIG. 10. Schematic of LEM-based system for the production of L-amino acids from racemic ester mixtures.

43), and $R = 0.05$ cm. With this, $\phi = 0.9$; the system can be considered to be under both types of control. This analysis indicates that the assumption of reaction control is not reasonable. The above analysis is confirmed by the data of Halwachs et al.. The authors gave a data set for the continuous operation of an enzyme/LEM system in a CSTR for two different residence times. When comparing the authors' model prediction of steady-state values to the experimental steady-state values, the model overpredicts the conversion. This overprediction can be interpreted (and is so by the authors) as the manifestation of the failure to include transport effects.

In a later work, Scheper and coworkers (*24*) examined three additional enzyme/LEM systems of potential commercial value. The first of these systems is designed for the removal and subsequent derivatization of

penicillin G (Pen G) from cell-free fermentation broth. The system investigated is shown in Fig. 11. In this system, Pen G forms a complex with the carrier, a asecondary amine, and diffuses across the membrane. In the interior phase it is hydrolyzed by the enzyme penicillin acylase, forming the products 6-amino-penicillinic acid (6-APA) and phenylacetic acid (PAA). The PAA is transported via carrier across the membrane and is delivered to the exterior phase. Batch tests of simulated broth and cell-free broth were conducted with good separation and conversion. Further tests were conducted in a 10.5-L Kuhni extractor column followed by breaking of the emulsion and reaction of the interior phase with D-phenylglycyl methyl ester to form ampicillin.

In the same work Scheper et al. also discussed two other systems: a system for the production of L-leucine from α-ketoisocaproate and the stereoselective conversion of racemic 4-acetoxycyclopent-2-en-1-one (4-AOCP). The former will be discussed below. In the racemic system of 4-AOCP, the 4-AOCP diffuses through the membrane where it is stereospecifically acted upon by pig-liver esterase. The products, rates, and yields are shown in Fig. 12. The data indicate that the stereoselectivity increases when the emulsion is immobilized. No rationale is given for this enhancement.

Much more complex enzyme/LEM systems than those discussed

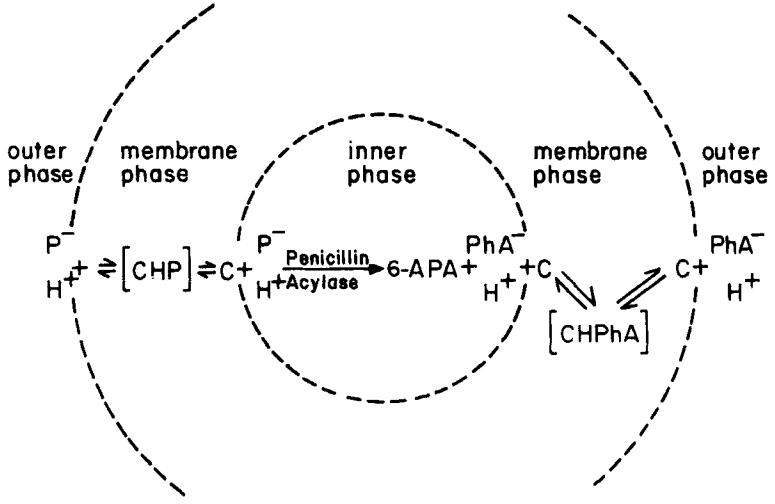
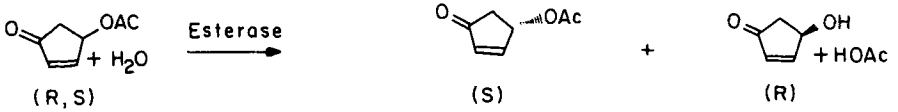


FIG. 11. Schematic of mechanism for LEM-based production of 6-APA from penicillin G (adapted from Ref. *24*).



	Ester Yield (R-form)	Rate (mM/hr)
Native Enzyme	65%	8.25
LEM-Bound Enzyme	75%	3.57

FIG. 12. Products and yields for stereo-specific reaction of 4-AOCP using free and LEM-bound pig-liver esterase.

above can be developed. In related work by Makryaleas et al. (*25*), the authors immobilized a complex enzyme/coenzyme network in LEMs for the production of L-leucine from α-ketoisocaproate (Fig. 13). In this system the ketoisocaproate forms a complex with the carrier (a quaternary ammonium salt) and diffuses across the membrane. Formate (via carrier) and ammonia also diffuse across the membrane from th exterior phase. Under the action of leucine dehydrogenase, LEUDH, the ammonium ions and α-ketoisocaproate are converted to L-leucine. This leucine is then transported back across the membrane to the exterior phase via carrier. The enzymatic reaction for the production of L-leucine requires the conversion of NADH to NAD^+. The cofactor is originally placed in the interior phase and is regenerated by the presence of a second enzyme in the interior phase: formate dehydrogenase. The formate that reaches the interior phase is oxidized to CO_2 during the reduction of NAD^+ to NADH.

Makryaleas and coworkers showed that activity of this complex enzyme system decreases about 70% after 7 h time. This decrease is after optimizing the membrane formulation to minimize breakage and protein inactivation. In addition, the work of these authors indicates that the transport of NH_3 and formate control the rate of leucine production.

PROCESS CONSIDERATIONS

The work discussed above demonstrates the versatility of liquid emulsion membranes. Like any technology, LEMs are not without

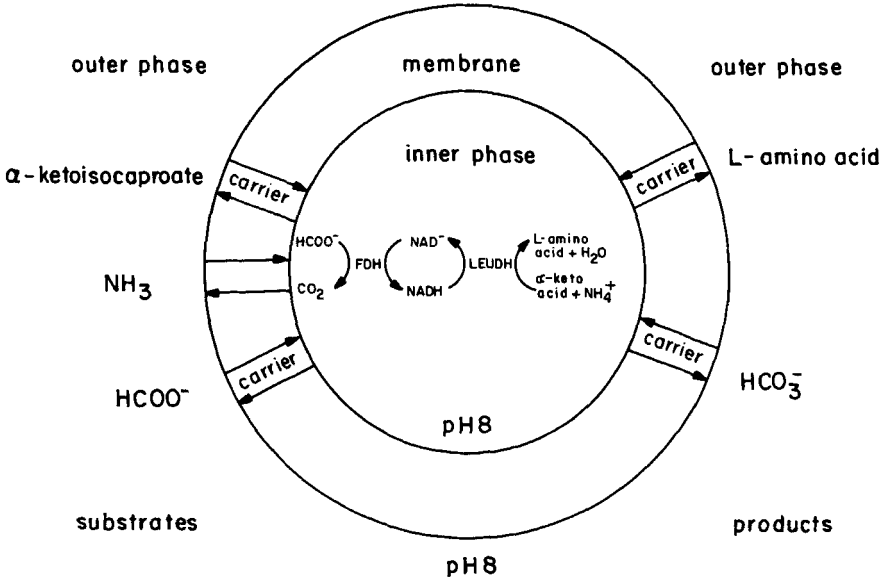


FIG. 13. Schematic of LEM-based enzyme/coenzyme system for the production of L-leucine (adapted from Ref. *25*).

potential problems. In the course of any application of LEMs, certain potential difficulties are repeatedly encountered. Because of their effects on LEM system applications, these potential process difficulties and their causes merit consideration.

Membrane Breakage

One of the most often mentioned difficulties in LEM systems is that of membrane breakage. The membrane of an LEM may break due to a variety of reasons: agitation-produced shear (*44–48*), excessive internal droplet size (*15, 44, 47, 49–51*), and poor membrane formulation (*15, 30, 44, 49, 50*). The detrimental effects of membrane breakage are primarily associated with the release of the interior contents of the membrane. When a membrane ruptures (for whatever reason), some of the internal contents are released into the exterior phase. This release returns separated solute to the exterior phase where it must be separated again by the remaining unruptured LEM. This need to separate the solute again seriously decreases the efficiency of the separation process. In addition,

interior phases usually contain some concentrated reagent. If this reagent is released into the exterior phase, the reagent may change the exterior phase conditions such that further separation cannot take place (*30*). Breakage can also cause contamination of the exterior phase as in blood treatment (*28, 29*) and drug-overdose prevention (*5–7*) applications.

While breakage can be a serious problem, it can, for the most part, be avoided by proper formulation of the membrane. Changes in formulation, such as increasing the viscosity of the membrane (*30*), increasing the surfactant concentration (*9, 34, 35, 39*), changing the surfactant type (*34*), and changing the phase ratio of the emulsion (*39*) can all contribute to membrane stability. Table 2 summarizes the incidence of membrane breakage in the studies discussed above. In almost all cases in which breakage appeared to be significant, the authors did not indicate that they had tried different formulations to avoid breakage. In all cases where the authors had tried different formulations to forestall membrane breakage, breakage was kept to a minimum.

Membrane Swell

Another potential problem associated with the use of LEMs is membrane swell. Membrane swell is a process by which water is transferred into the interior aqueous phase. This swell is driven by the large osmotic gradients that are typically found in LEM separation systems (*53*). It is important to consider the effects of swell in LEM systems, especially when the LEM system is used to both separate and concentrate a solute (*9, 34, 35, 39*). The water transferred acts to dilute the

TABLE 2
Incidence of Membrane Breakage

References	Significant breakage	Formulate to minimize breakage
30	Yes	No
5	Yes	No
39	Slight	Yes
9, 34, 35	No	Yes
28	No	Yes
24	No	Yes
26, 27	No	Yes

solute that has been concentrated in the interior phase as well as to dilute the driving force. This is illustrated by the data shown in Fig. 14.

Figure 14, adapted from Thien et al. (*34*, *35*) for the system shown in Fig. 2b, shows what happens to the interior phase concentration of L-phenylalanine at 40 min contact time as the initial interior salt concentration is increased. For small increases in salt concentration, the solute driving force, as well as the osmotic gradient, is increased. The concentration of solute in the interior phase increases. But as the salt concentration continues to increase, the swell becomes much more dramatic and the product in the interior phase is diluted. Besides diluting the product, excessive swell can lead to emulsion instability (*37*, *38*).

There are two possible mechanisms for swell in LEMs. The first, proposed by Colinart et al., suggests that swell occurs via hydrated surfactant (*53*). A schematic of this mechanism (Fig. 15a) indicates that the hydrophilic portion of the surfactant hydrates at the interface between the oil and the phase with the lowest salt concentration (highest activity of water, a_w; this is usually the exterior phase). The hydrated

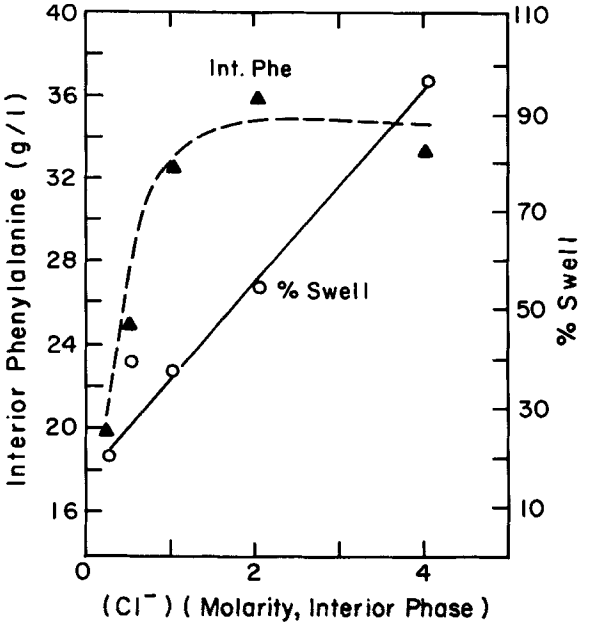


FIG. 14. Percent swell and interior phase phenylalanine concentration for various initial chloride concentrations after 40 min contact time (from Refs. *34* and *35*).

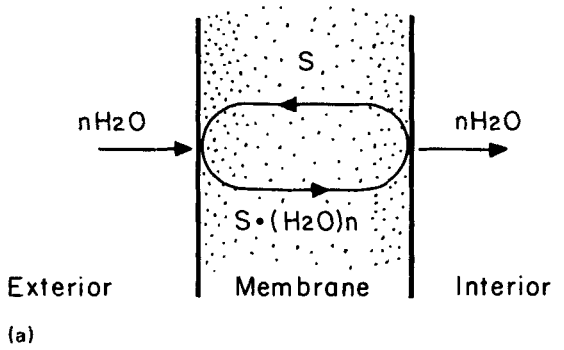


FIG. 15a. Mechanism for swell in LEM systems via hydrated surfactant (from Ref. *55*).

surfactant then diffuses to an interface between the oil and an aqueous phase of high salt concentration (low activity of water; usually the interior phase) and is dehydrated. Evidence supporting this mechanism includes NMR studies of surfactant hydration (*54*), the work on swell by Thien et al. (*55*), and the work of Colinart and coworkers (*53*).

The other mechanism is transport via reversed micelles (Fig. 15b). This mechanism is very similar to that of hydrated surfactant in that the micelle forms on the side of high a_w and is dehydrated on the low a_w side. Unlike hydrated surfactants, reversed micelles can accommodate small solutes as well as water (*56–58*). Thus, not only would these micelles transport water, but they could transport solute as well. This mechanism

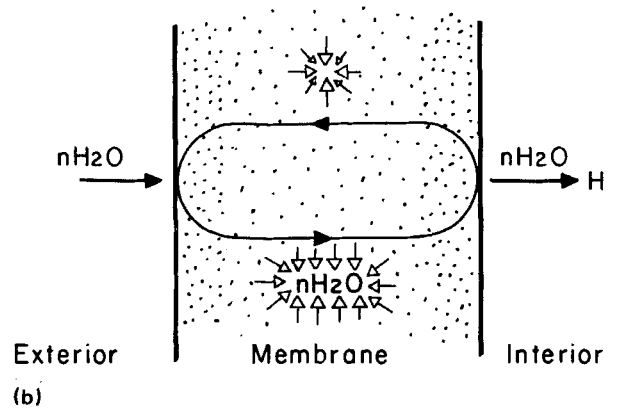


FIG. 15b. Mechanism for swell in LEM systems via reversed micelles (from Ref. *55*).

is supported by the data of several workers (*6*, *27*, *36*, *39*) who found that charged solutes transported across the membrane without carriers. In addition, there is indirect evidence that sorbitan monooleate, a surfactant used to stabilize many LEMs, promotes reversed micelle formation (*37*, *38*). D2EPHA, a chelating agent often used to transport cations (*39*), also promotes reversed micelle formation (*59*). Unfortunately, there is no direct evidence for the existence of reversed micelles in these systems.

The work of Colinart and coworkers (*53*) and Draxler and Marr (*60*) indicates that, regardless of mechanism, practically all surfactants and carriers mediate swell to some extent and thus osmotic swell should be anticipated in most LEM systems. There are few ways to avoid swell. Thien et al. screened surfactants for their hydration characteristics (*34*). Plucinski and coworkers (*36*) suggested the use of block copolymers that are incapable of forming micelles. Terry and colleagues increased the viscosity of the membrane, thus decreasing the rate of solute and water transport (*30*). Lorbach and Marr (*61*), in a novel approach, added nontransportable salts to the exterior phase in an effort to match the activity of water in the exterior and interior phases. Regardless of the approach to minimize swell, it is important that swell be considered in estimating the efficiency and optimizing the performance of LEM systems. This is especially true when the solute concentration in the interior phase is important.

Selectivity

One of the most important issues in evaluating any separations technique is the selectivity of the technique. This is particularly true if one hopes to separate solutes from complex media such as fermentation broth. In LEM systems, the selectivity that is required of an LEM system can be brought about in different fashions, depending on the nature of the membrane. In Type I systems, for example, anything that is soluble in the oil phase can be transported across the membrane. Thus, the inherent "selection" of Type I membranes is for compounds that are at least slightly soluble in oil. In order to maintain a driving force for separation in these membranes, however, the reagent in the interior phase must convert the solute (with some specificity) into a nontransportable species. The overall selectivity for Type I systems can be summarized by stating that, at short times, solute solubility in the oil governs the selectivity. At long times the selectivity becomes governed by the interaction of the solutes with the interior reagent. This is beautifully shown by the data of

Terry et al. (*30*) in a comparison of the extraction of phenol and acetic acid in a phenol/acetic acid mixture (Fig. 16). The phenol is taken up faster than acetic acid, due to the high solubility of phenol in oil. But as time goes on, the acetic acid, being a much stronger acid than phenol, essentially "pushes" the phenol out of the membrane. This effect has been seen in other systems (*13*, *28*). In general, the selectivity of Type I systems that do not have specific driving forces is low. This indicates that Type I systems should be used primarily for dilute streams with few components.

The selectivity of Type II systems is primarily dictated by the selectivity of the carrier in the membrane. These carriers have traditionally been those developed for the extraction of metals in hydrometallurgical systems. This being the case, the selectivity of these carriers is inherently low. This low selectivity is demonstrated for quaternary ammonium salts by the work of Thien and coworkers (*34*) when they examined the

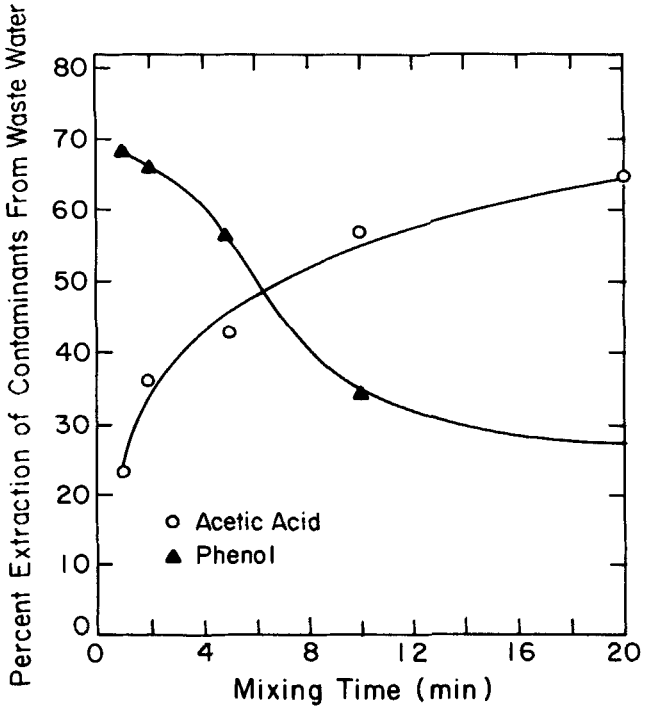


FIG. 16. Uptake of phenol and acetic acid as a function of time in an LEM system (from Ref. *30*).

separation of L-phenyalanine from a phenylalanine/sulfate mixture (Fig. 17). The sulfate acts as a strong competitor for the carrier. In further work, Thien et al. demonstrated that, for nonspecific carriers like quaternary ammonium salts, the hydrophobicity of the solute dictated the specificity of the membrane (*35*). Using current carriers, then, extremely limits the selectivity of Type II LEM systems. As stated by Schugerl (*62*), "At present, suitable carriers are still not available. Most commercial carriers were developed for chemical analytics and hydrometallurgical processes or for the reprocessing of nuclear fuels. Systematic investigations are necessary to develop highly specific carriers for the... separation of biotechnological products."

The last way in which the selectivity of the membrane can be enhanced is by the use of solute-specific driving forces. With these types of driving forces, only the solute (or its derivative) can build up to high concentrations. These driving forces can be of two types. The first type of specific driving force is that provided by catalytic conversion of the solute to a nontransportable form. These types of systems have been discussed earlier, and it suffices to say enzymatically-driven membranes can enjoy the extreme selectivity of the enzyme itself.

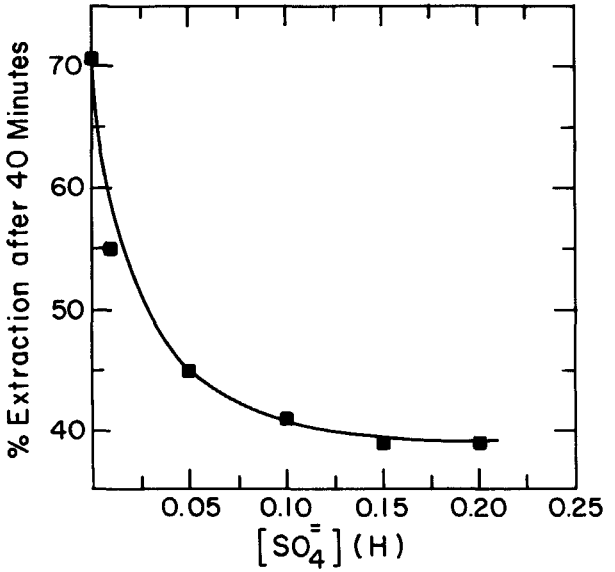


FIG. 17. L-Phenylalanine extraction after 40 min contact time as a function of the initial exterior phase sulfate concentration (from Ref. *34*).

The second type of specific driving force is that of the specific reagent. Unlike the nonspecific driving forces used in systems like that outlined in Fig. 2b, these interior reagents react with only specific solutes. Halwachs and coworkers (*28*) and Yagodin et al. (*29*) demonstrated the benefit of these driving forces. In addition, Chiang et al. (*7*) suggested the use of solute-specific antibodies as driving forces. Other possibilities include the development or exploitation of solute-specific chemistries.

DIRECTIONS

Those working in the field of LEM-mediated separations have come far in demonstrating the usefulness of LEMs, particularly in the field of bioseparations. Until now, as is witnessed by the systems discussed above, many different LEM-mediated bioseparations have been examined. Few of these systems have been examined in depth, and even fewer have been examined with development of a commercial bioseparation process in mind. In terms of the criteria for an "optimal" downstream process, as well as developing credible and realistic LEM-based processes in bioseparations, future research should be directed in the following areas.

Emulsion Formulation

The formulation of emulsions to minimize breakage and swell, as well as to maximize flux rates and solute concentration, must be carefully considered. Surfactants should be screened and categorized by their hydration characteristics and their ability to impart stability to the membrane. Solvents should also be classified by their solute compatibility and their viscosity. Consideration should be given to the fact that many of these systems will come into contact with solutes or exterior phases whose final fate is in food products or direct placement in the human body. Attention should be paid to the toxicity of the components used in membrane formulations.

Selectivity

Without specificity, LEM techniques cannot hope to compete with currently established techniques. As mentioned above, new carriers must

be developed that are specific for the desired biological products. In addition, new chemistries and biological reagents must be examined and developed.

Realistic Source Phases

The fluids which will act as source phases for the biochemicals to which LEM systems may be applied are complex media. Not only do they possess inorganic salts and particulates, but they will also contain cell metabolites, cellular products, natural surfactants, and products very similar in structure or chemical properties to the solute of interest. These unknown components may cause unforeseen problems during separation. In addition, while many fermentations, for example, are considered to be dilute in product, the product concentration is often much higher than that found for solutes in traditional metal separations with LEMs. Studies of LEM bioseparation systems should, as quickly as is prudent, be conducted on the real system as opposed to "simulated media." To show the extent to which this is practiced, Table 3 identifies the concentrations and source phases used in the studies discussed above. The data indicate that only about half of the studies have worked with real systems. Even more importantly, essentially none of the studies dealt with realistic concentrations of solute (some were off by an order of magnitude). LEM systems are particularly challenged by high solute concentrations in the exterior phase, both in terms of capacity and

TABLE 3
Survey of Exterior Phases Used in the Literature

Ref.	Solute	Concentration used	Commercial concentration	Real source
18	*L*-Phe	15 g/L	30[a]	Yes
24	Pen G	NA	NA	Yes
28	Phenol	94 mg/mL	94 mg/mL[b]	Yes
29	Cholesterol	2 g/L	2 g/L[b]	Yes
30	Acetic acid	1 g/L	30 g/L[a]	No
31	Acetic acid	1 g/L	30 g/L[b]	No
36	*L*-Valine	0.2 g/L	30 g/L[c]	No
39	*L*-Lysine	1 g/L	40 g/L[a]	No

[a]Ref. *64*.
[b]From author's data.
[c]Ref. *65*.

continued flux rates. It thus becomes imperative that, if one wishes to develop an accurate idea of an LEM's efficiency for a given system, that system must use real source phases at realistic concentrations.

Product Recovery

Once the solute has been separated into the interior phase, the product must be recovered. Few authors have dealt with the issue of product recovery in the LEM literature, other than those conducting pilot-plant studies. Yagodin et al. (*39*) discussed the thermal breaking of emulsions and the subsequent crystallization of L-lysine from emulsion interiors. They did not, however, discuss the economics of the process (especially the energy intensive method of thermal demulsification), nor did they provide a commercial process flow sheet. Thien et al. (*35*), while qualitatively discussing the commercial operation of the process and providing a flow sheet for a commercial scale process, neither attempted actual crystallization nor process economics. No authors have discussed where LEM operations should belong in the train of downstream operations. If LEM processes are to be used early in downstream processing for crude separation and concentration, it must be demonstrated *for biochemicals from the native source* that LEM operations can compete economically with well-established commercial processes. If LEM techniques are to be used later in the downstream processing, it must be shown that adequate and specific separation can be achieved. In addition, it must also be shown that loss of product of added value will be acceptable. It is clear that an economic analysis based on relaible LEM pilot-plant data for biochemical separations must be carried out if LEM processes are ever to gain acceptance as a commercially viable downstream operation.

Process flow sheets for the recovery of biochemicals using LEMs are not much different than those proposed for the separation of heavy metals. An example of a process flow sheet for the separation of L-phenylalanine from fermentation broth was presented by Thien et al. (*35*) and is recreated in Fig. 18. As can be seen from the figure, the flow diagram uses a simple mixer-settler train followed by electrostatic coalescence of the emulsion and reuse of the membrane and interior phase components. Recovery of the amino acid from the interior phase takes advantage of decreased solubility of amino acids at isoelectric pH's.

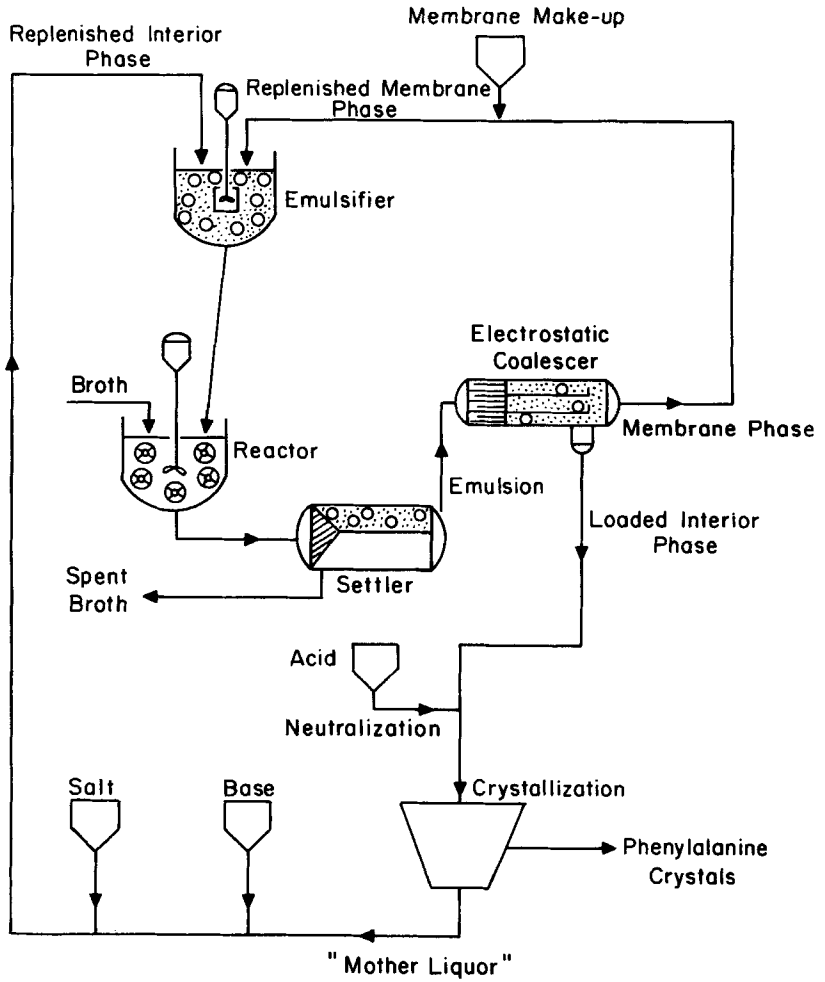


FIG. 18. Process diagram for LEM-based recovery of amino acids (adapted from Ref. 9).

Applications

While the considerations above have been general in nature, it is important that specific, well thought out applications for LEM processes be examined. For most types of biochemical separations, there are well-established, cheap, and efficient methods of bioseparation. LEM technology, regardless of how efficient it is, will be hard pressed to displace conventional technology for easily separated products. Systems for study should be chosen with care, attention being paid to systems where the unique features of LEM systems can give significant advantage over currently used separation processes. These applications include, but not exclusively, the separation of biochemical zwitterions, the production of stereospecific compounds from racemic mixtures using *recoverable enzymes,* general processes where enzyme immobilization would be advantageous, and feedback inhibited fermentations. Being that LEMs function best when the solute is present in dilute concentrations, LEM systems should be applied to fermentation products that are produced in low titer.

In addition, new applications of LEMs should be examined. For example, there are few ways to separate proteins by liquid–liquid extraction. LEMs, as discussed up to this point, cannot be used to extract proteins because the proteins would denature when transported through the solvent. The use of reversed micelles has been suggested as a liquid–liquid process that could be used to extract proteins (*63*). Unfortunately, reversed micelle solutions, as do most extraction processes, have limited capacity on a per volume basis (*63*).

The idea that reversed micelles may be present in LEMs was discussed above. A possibly new application of LEMs would be to purposely incorporate reversed micelles in the membrane phase to shuttle the protein across the membrane (Fig. 19). This system would have the advantages of both LEMs and reversed micelles: quick separations, high capacity on a per volume basis, ability to be operated continuously and with little broth pretreatment, and ability to concentrate the protein while separating.

CONCLUSIONS

While it seems that there is much left to do to establish the use of LEM technology in the commercial downstream processing of biochemicals, the versatility and applicability of LEM technology has been demon-

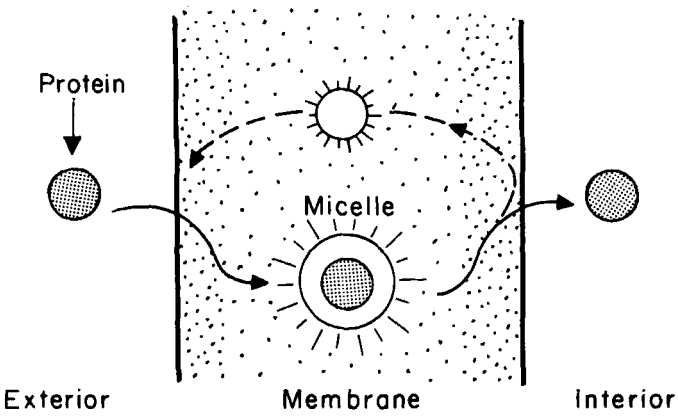


FIG. 19. Combination reversed micelle/LEM system for protein extraction.

strated in the work to date. As more work is conducted and economic data collected on LEM-based systems, the advantages of such systems, namely that these systems can be continuous, that they are easily scaled and require little pretreatment, and that they can concentrate as they separate, may allow LEM-based separation technology to find an increasingly important role in the downstream processing of biochemicals.

Acknowledgments

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