Potential of Liquid Membranes for Drug Overdose Treatment: *In Vitro* Studies

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Abstract □ The *in vitro* removal of six barbiturates from pH 2 donor solutions by liquid membranes with pH control was evaluated. More than 90% of amobarbital, phenobarbital, and secobarbital were removed within 10 min by the liquid membranes. Drug transport obeyed first-order kinetics initially, and Fick's law was obeyed. The transport rate of phenobarbital by a liquid membrane was temperature dependent. An Arrhenius plot revealed that the activation energy was 10 kcal/mole. The liquid membranes showed some instability in the presence of bile salts.

Keyphrases □ Liquid membranes—*in vitro* removal of various barbiturates from pH 2 donor solutions, effect of temperature, phase ratio, and bile salts □ Membranes, liquid—*in vitro* removal of various barbiturates from pH 2 donor solutions, effect of temperature, phase ratio, and bile salts □ Barbiturates, various—*in vitro* removal by liquid membranes from pH 2 donor solutions, effect of temperature, phase ratio, and bile salts

Human poisoning involving drug ingestion is common in the United States (1). Barbiturate poisoning accounts for 75% of suicides by drugs or 50% of all suicides by chemicals (2). Traditionally, acute poisoning treatment involves attempts to reduce drug absorption from the GI tract. Activated charcoal has been used for this purpose. Also, emetics and peritoneal dialysis have been recommended, although these methods have limitations.

Recently, liquid membranes (stable water-in-oil-in-water emulsions) were patented (3). They have many potential applications, *e.g.*, separation of hydrocarbon types (4), purification of waste water (5), and removal of uremia toxins (6). They also may have potential as an emergency treatment of a drug overdose (7). Preliminary *in vitro* studies indicated that liquid membranes were capable of rapid uptake of phenobarbital and aspirin from either pH 2 or 7 buffered donor solutions (7).

To understand the properties of liquid membranes in drug overdose treatment, *in vitro* studies were conducted under different temperatures and phase ratios of liquid membranes to the donor solutions. Since barbiturates are a common cause of drug overdoses, several barbiturates were employed as model drugs. The stability of liquid membranes in the presence of bile salts also was investigated.

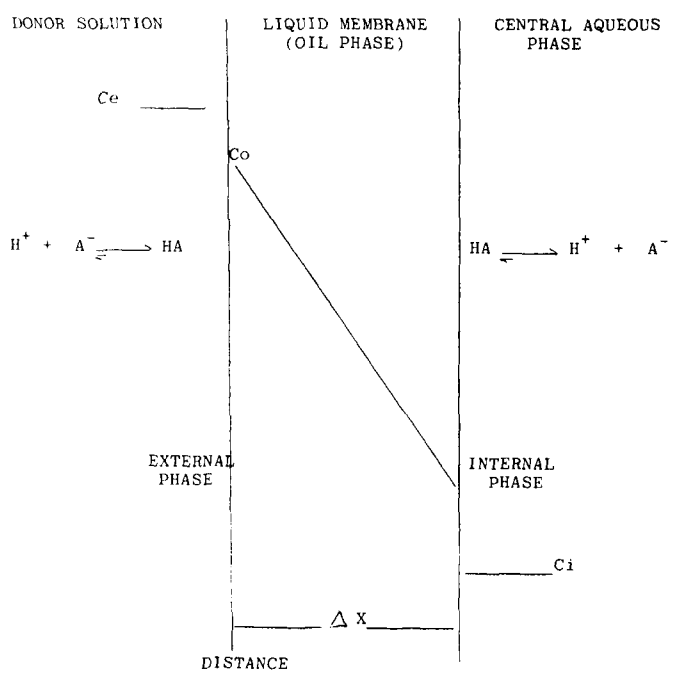

Figure 1—*Simplified scheme of drug transport through liquid membranes.*

THEORETICAL

The central aqueous phase of liquid membranes can be formulated to form a high capacity sink for the drug in the donor phase by (*a*) pH control, (*b*) plasma proteins to bind the drug, (*c*) activated charcoal, or (*d*) specific drug antibodies. The liquid membranes with pH control will be used as a model.

A simplified schematic diagram of the removal of acidic drug from the donor phase (pH 2) by the liquid membrane with pH control is shown in Fig. 1. Drug transfer from the donor solution to the central aqueous phase is accomplished by solution of the drug in the membrane and diffusion in the direction of the concentration gradient. Since a membrane made from a hydrocarbon is virtually impenetrable to ions, only the uncharged species in the donor phase can be dissolved in the membrane and transported through it to the central aqueous phase where an appropriate trapping agent is present. In this study, a pH 12 buffer solution was used as this trapping agent. According to pH partition theory, the acidic unionized species transported from the donor solution will be ionized at this high pH in the central aqueous phase and be unable to diffuse out of the membrane.

The uptake rate of the drug from the donor solution by a liquid membrane is discussed using the following symbols:

C_e = concentration of drug in the donor phase
C_e^o = concentration of drug in the external, *i.e.*, oil, phase of the membrane
C_i = concentration of drug in the internal, *i.e.*, aqueous, phase of the membrane
P = apparent partition coefficient of unionized drug (HA) between oil and aqueous phases
D = diffusion coefficient of drug in the membrane
ΔX = membrane thickness
A = area of contact between the donor solution and liquid membrane (this is a function of the volume of the liquid membrane used when the stirring speed is kept constant)

According to Fick's law, the transport of unionized drug across the membrane is:

$$\frac{dC_o}{dt} = -DA\frac{\Delta C}{\Delta X} = \frac{-DA}{\Delta X}(C_o - C_i) \qquad \text{(Eq. 1)}$$

Since $C_e = C_o/P$, then:

$$\frac{dC_e}{dt} = \frac{1}{P}\frac{dC_o}{dt} = \frac{-DA}{\Delta X}\frac{1}{P}(PC_e - C_i) \qquad \text{(Eq. 2)}$$

When a trapping agent, pH 12 buffer, is used in the central aqueous phase, the unionized species transported is ionized at this pH and C_i is negligible.

Equation 2 can be written as:

$$\frac{dC_e}{dt} = \frac{DA}{\Delta X} C_e = kC_e \quad \frac{dC_e}{dt} = \frac{-DA}{\Delta X} C_e = -kC_e \qquad \text{(Eq. 3)}$$

By integration:

$$C_e = C_e{}^o e^{-kt} \qquad \text{(Eq. 3a)}$$

According to Eq. 3, first-order kinetics are followed if the transport is governed by simple Fickian diffusion; the rate constant k is a function of the diffusion coefficient of drug used, the area of contact between the donor phase and liquid membrane, and the membrane thickness.

The dependence of the first-order rate constants with temperature could be expressed by the Arrhenius equation:

$$k = Ae^{-E_a/RT} \qquad \text{(Eq. 4)}$$

where k is the transport rate constant, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature.

EXPERIMENTAL

Materials and Methods—Three different liquid membranes, A, B, and C, were made with a 1:1 ratio of oil to aqueous phase. Liquid Membrane A was made from an isoparaffinic oil phase[1] with 0.1 *N* NaOH as the central aqueous phase. Membranes B and C were both made from 83% of the isoparaffinic oil and 15% of a normal paraffinic oil of lower viscosity[2], but pH 12 buffer was used as the central aqueous phase for B and pH 2 buffer was used as the central aqueous phase for C.

The barbiturates were barbital[3], amobarbital[4], phenobarbital sodium[5], pentobarbital sodium[6], secobarbital sodium[7], butabarbital[8], and ring ^{14}C-labeled pentobarbital[9] (0.1 mCi/ml). Standard pH 2 and 6 donor solutions were made from potassium chloride–hydrochloric acid and monobasic potassium phosphate–sodium hydroxide buffers, respectively.

The drugs were dissolved in either pH 2 or 6 buffer. Appropriate amounts of the liquid membrane were mixed with the drug solution in a beaker and stirred at a constant speed with a magnetic stirrer. The whole system was maintained at required temperatures (±1°) using a water bath.

At appropriate intervals, the mixture of liquid membrane and donor solution was sampled and filtered through a filter paper[10]. An appropriate volume of the filtrate was made alkaline with 1 *N* NaOH, and the absorbance was determined[11] at 255 nm. A standard curve of the absorbance as a function of drug concentration was prepared for each experiment. A blank also was run each time by using the same buffer solution without drug. The radioactivity assay for carbon-14 was accomplished by using the internal standard method to correct the color quench of sodium glycocholate[12].

The first-order rate constants for drug removal were calculated by using a log–linear least-squares fitting program. The regression coefficients for all studies were >0.95.

Partition Coefficient Studies—Accurately weighed (W_{LM}) liquid Membrane C was stirred with 100 ml of a barbiturate (six were tested) in pH 2 buffer solution at 37°. Samples were withdrawn and assayed. When an equilibrium was reached, the concentration of barbiturate in the donor solution was equal to that of the central aqueous phase of the liquid membrane. Therefore, the barbiturate concentration in the oil phase could be calculated as:

$$C_{oil} = \left[(C_e)(V_e - C_e)\left(V_e + \frac{W_{LM}}{2}\right) \right] / (W_{LM}/2) \qquad \text{(Eq. 5)}$$

The barbiturate uptake rates in pH 2 buffer by Membrane B, whose central aqueous phase, a pH 12 buffer, served as a sink for the barbiturates, also were determined at 37°.

Phase Ratio Studies—Pentobarbital sodium in pH 2 buffer and Membrane A were utilized. The ratios of the weight of Membrane A to the volume of pentobarbital solution (pH 2, 500 mg/liter) ranged from 0.1 to 1.0. The uptake rates of pentobarbital sodium with different phase ratios were investigated at 37°.

Temperature Effect—The uptake rates of a 500-mg/liter phenobarbital solution (pH 2, 100 ml) by 50 g of Membrane B were studied at 22, 30, 37, 45, and 50°.

Effects of Bile Salt—The pentobarbital sodium uptake in pH 6 buffer at 37° by Membrane B in the absence of bile salt and in the presence of 0.5 and 2% sodium glycocholate was studied; 0.1 ml of ^{14}C-labeled pentobarbital was added to a 500-mg/liter pentobarbital solution in pH 6 buffer with 0.5 or 2% sodium glycocholate. The radioactive assay was used for systems containing sodium glycocholate.

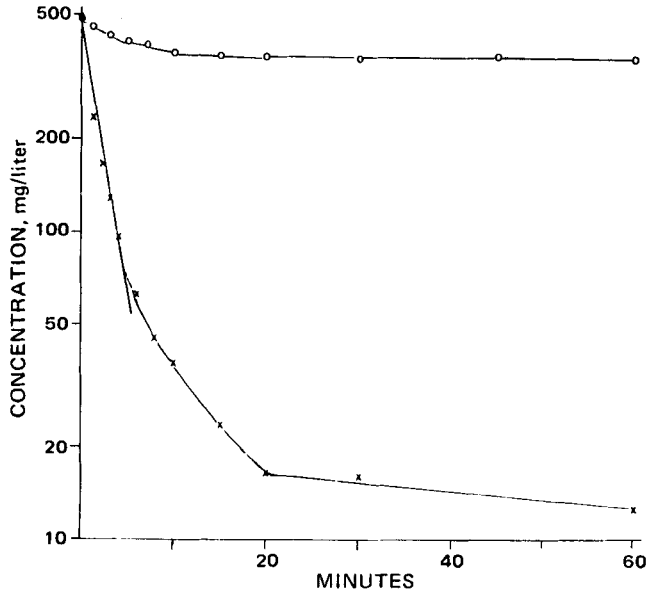

Figure 2—*Pentobarbital transport by liquid Membranes B and C from donor solution. Key: ×, Membrane B (pH 12 buffer); and ○, Membrane C (pH 2 buffer).*

RESULTS AND DISCUSSION

Partition Coefficient Studies—The semilogarithmic plot of pentobarbital concentration in pH 2 buffer after treatment with liquid Membrane B is shown in Fig. 2. It indicates that first-order kinetics were followed from time zero to 4 min. Because the pentobarbital concentration in the central aqueous phase was negligible in comparison with that in the donor phase during that time, Eq. 3 was obeyed. However, the drug concentration in the donor phase decreased with increasing drug concentration in the central aqueous phase. Therefore, C_i was no longer negligible relative to C_o, and Eq. 2 should be used.

Figure 2 also shows pentobarbital transport from pH 2 donor solution by Membrane C, with pH 2 buffer as the central aqueous phase. Liquid Membrane B could effectively remove 90% of pentobarbital in the donor solution in 7 min, while Membrane C only removed 20% of the drug. At equilibrium, only 27% of pentobarbital was transported into Membrane C.

Table I—Comparison of the Fractions of Drugs Remaining in the Donor Phase after Treatment with Liquid Membranes B and C

Barbiturate	B				C,
	10 min	30 min	60 min	155 min	$C_e/C_e{}^o$
Barbital	0.68	0.46	0.35	0.19	0.82
Butabarbital	0.14	0.05	0.05	—	0.79
Phenobarbital	0.35	0.18	0.10	—	0.77
Amobarbital	0.04	0.03	0.02	—	0.75
Pentobarbital	0.07	0.03	0.03	—	0.73
Secobarbital	0.03	0.02	0.02	—	0.69

[1] Exxon S100N.
[2] Exxon Norpar 13.
[3] Gane's Chemical Works.
[4] Eli Lilly, Indianapolis, Ind.
[5] Mallinckrodt Chemical Works.
[6] Abbott, Chicago, Ill.
[7] Ruger Chemical Co.
[8] McNeil Laboratories, Fort Washington, Pa.
[9] New England Nuclear.
[10] Whatman No. 42.
[11] Perkin-Elmer spectrophotometer.
[12] Amend Chemical Co.

Table II—Apparent Partition Coefficients, Dissociation Constants, and Transport Constants for Six Barbiturates

Barbiturate	Dissociation Constant, pKa[a]	Apparent Partition Coefficient	Transport Rate Constant, min^{-1}
Barbital	7.86	0.00	0.037
Butabarbital	8.01	0.07	0.188
Phenobarbital	7.37	0.21	0.116
Amobarbital	7.87	0.36	0.662
Pentobarbital	8.03	0.50	0.401
Secobarbital	7.90	0.83	0.727

[a] From Ref. 8.

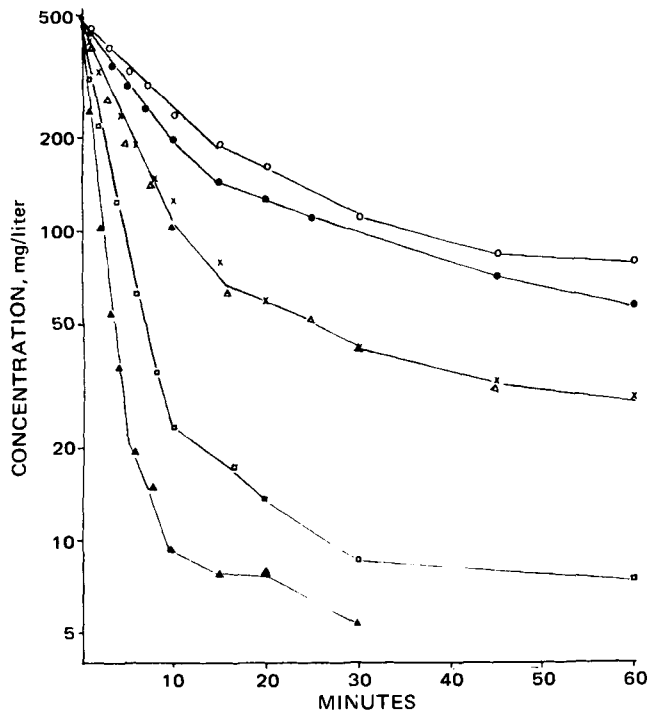

Figure 3—*Pentobarbital uptake by liquid Membrane A. Different phase ratios of the amount of liquid membrane to the volume of donor phase are presented. Key (phase volume ratio): ○, 0.1; ●, 0.14; × and △, 0.20; □, 0.50; and ▲, 1.00.*

The percentages of six different barbiturates remaining in the donor solution at 10, 30, 60, and 155 min after they were treated with Membrane B, as well as the final fraction (at equilibrium) of the same drug treated with Membrane C under the same experimental conditions, are listed in Table I. Ninety-five percent of the barbiturate except barbital and phenobarbital was removed from the donor phase in 30 min by B, while only 18–31% of the drug was trapped inside C.

Table II shows the partition coefficients, P, calculated from Eq. 6, the first-order transport rate constants, k, and the dissociation constants, pKa (8). There is no obvious linear relationship.

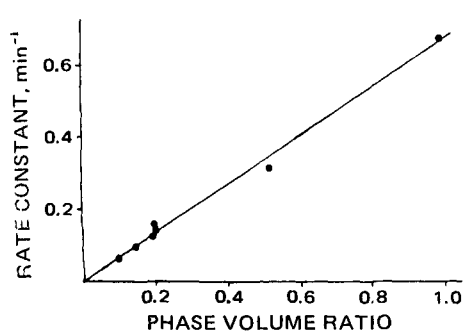

Figure 4—*Transport rate constants as a function of the relative amount of liquid membrane present.*

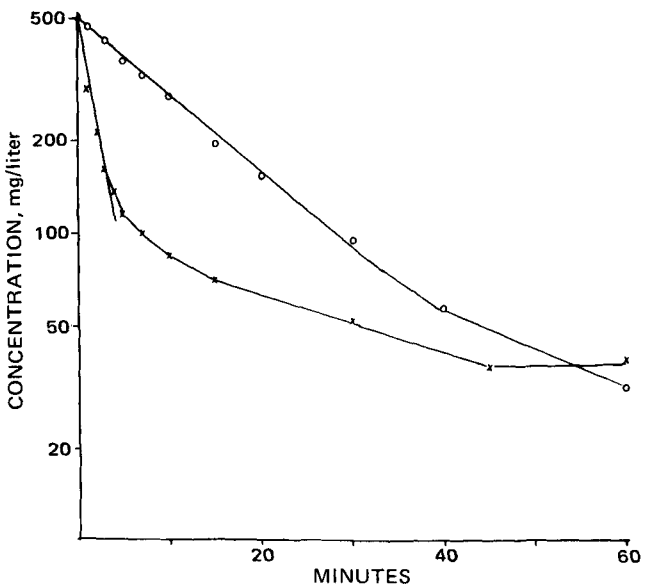

Figure 5—*Pentobarbital transport by liquid Membrane B at 50° (×) and 22° (○).*

Phase Ratio—Figure 3 shows the semilogarithmic plots of the pentobarbital concentrations in the donor phase after treatment with different amounts of Membrane A. Initially, all plots followed monoexponential decay kinetics with different rate constants. After equilibrium, the pentobarbital concentration in the donor phase also was a function of the amount of liquid membrane used. A plot of the first-order transport rate constants of pentobarbital as a function of the phase ratios of liquid membrane used to the volume of the donor phase gave a straight line with a regression coefficient of 0.99 (Fig. 4). Therefore, the transport rate of pentobarbital from the donor phase was directly dependent on the phase ratio.

Temperature—The semilogarithmic plots of the phenobarbital concentrations in a donor phase of pH 2 treated with Membrane B at 22 and 50° are shown in Fig. 5. The same studies were also carried out at 30, 37, and 45°. The results indicated that the higher the temperature, the faster the phenobarbital transport rate, but the final equilibrium concentrations of phenobarbital were very similar in all studies.

The plot of the logarithm of the first-order transport constants of phenobarbital as a function of reciprocal temperatures is presented in Fig. 6. A straight line with a regression coefficient of 0.97 was obtained.

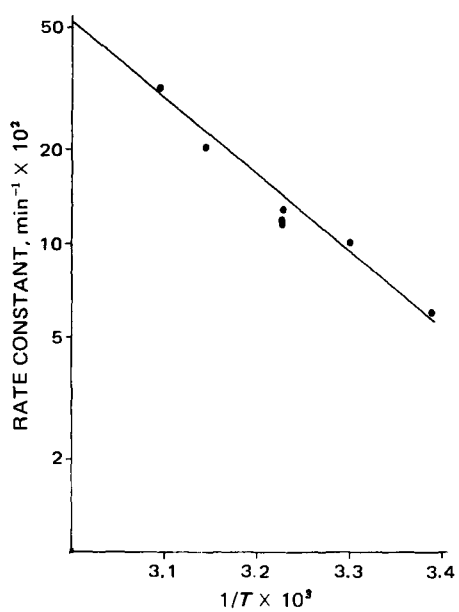

Figure 6—*Semilogarithmic plot of transport constant as a function of the reciprocal of absolute temperature.*

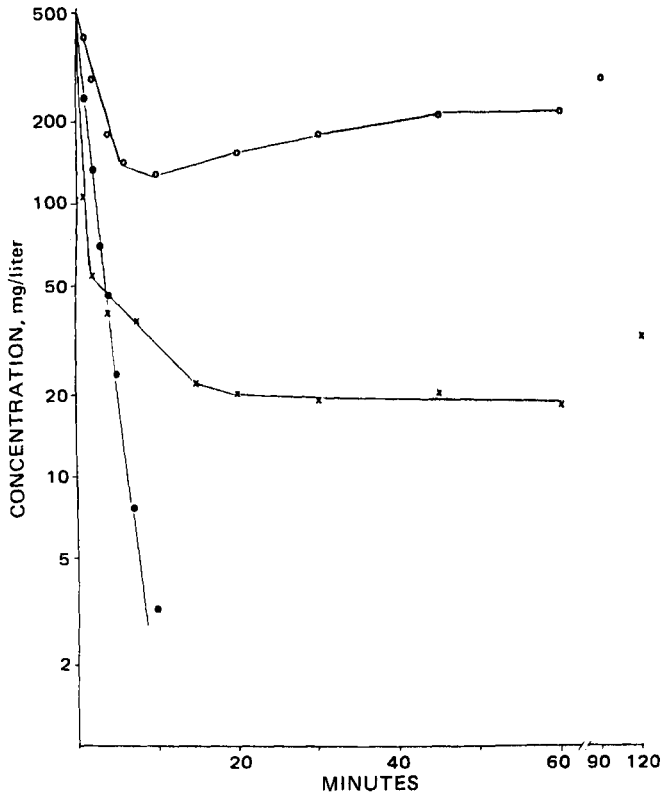

Figure 7—*Pentobarbital transport from pH 6 buffer solution by liquid Membrane B. Key:* ●, *no bile salt present;* ×, *0.5% bile salt present; and* ○, *2% bile salt present.*

From the Arrhenius equation, the activation energy of the system was calculated to be 10.8 kcal/mole (45 kjoules/mole).

Effect of Bile Salt—Figure 7 shows pentobarbital uptake in pH 6 buffered donor solution in the presence of 0.5 and 2% sodium glycocholate. Pentobarbital uptake by Membrane B in the presence of 0.5% sodium glycocholate (which corresponds to the approximate concentration of the total conjugated bile salts in the upper jejunum of fasting humans) was initially faster than in the system without bile salt. This faster pentobarbital transport may perhaps result from the bile salt changing the permeability of the liquid membrane to the drug. It has been observed that bile salt alters the permeability of intestine to drugs (10). However, after 4 min, the transport rate of pentobarbital tended to decrease.

When 2% sodium glycocholate [the approximate total concentration of conjugated bile salts commonly found in the jejunum after fat digestion (11)] was added to the liquid membrane system, pentobarbital transport was much slower and reached a maximum in 10 min. Thereafter, the pentobarbital concentration in the donor phase tended to increase. Even though these *in vitro* studies showed that bile salt may adversely affect the liquid membrane in the intestine, *in vivo* studies may give different results. Because there is food present in the intestine, which interacts with bile salts, the free bile salt concentration may well be less than in these *in vitro* studies.

Further studies will be conducted in animals to evaluate the effectiveness and applicabilities of these liquid membranes.

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