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Hydrolysis Kinetics of Phospholipids in Thermally Stressed Intravenous Lipid Emulsion Formulations

C. J. HERMAN AND M. J. GROVES

Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 115 South Sangamon Street, Chicago, Illinois 60607, USA

Abstract—A model 20% w/v emulsion, prepared with either a commercially available pharmaceutical grade soy oil or a highly purified grade of oil from the same origin and stabilized with a commercially available mixture of egg yolk phospholipids was passed through a Microfluidics homogenizer until the mean particle size fell below 500 nm diameter. Samples stored in sealed all-glass ampoules were thermally stressed over a temperature range of 5–90°C and samples taken at appropriate intervals for analysis by HPLC. Hydrolysis degradation kinetics were in conformation with the Arrhenius equation. The energy of activation for phosphatidylcholine was virtually identical for emulsions prepared with either pharmaceutical or purified oil (65 and 63 kJ mol^{-1}, respectively). For phosphatidylethanolamine itself the respective activation energies were 53 and 54 kJ mol^{-1}, suggesting that the source of the oil used in preparing the emulsions had no significance in the degradation processes of the resulting systems.

Sterile intravenous emulsions of triglyceride oils have been widely employed clinically as sources of calories and essential fatty acids for patients who are unable to consume or absorb food orally. In addition, these general systems have been used as vehicles for the injection of hydrophobic drugs such as diazepam.

Lipid emulsions were first administered parenterally in the 1940s and studies from that time suggested that natural mixtures of what we now know to be phospholipids provided the optimum stabilization of these systems (McKibben et al 1943, 1945). Following the pioneering studies of Wretlind (Schuberth & Wretlind 1961; Wretlind 1964) a commercial product, Intralipid, was introduced in the 1960s and this was followed by other commercially available products. Nearly all commercial systems are formulated with mixtures of phospholipids and contain 10, 20 or 30% w/v triglyceride oils from soy, safflower or cottonseed. The cottonseed oil system was later withdrawn following reports of toxicity attributed to the residual gossypol which is a natural contaminant of this oil. More recently mixtures of natural triglycerides with semisynthetic medium chain triglycerides have been employed.

Reports of toxicity from these intravenous emulsions attributable to the general physical or chemical properties of these systems have remained rare over the past two decades. This is unusual since these large volume parenteral systems are required to be terminally heat sterilized which must inevitably produce significant quantities of phospholipid degradation products such as the monoacyl- or lyso-derivatives. These materials are known to produce lysis of erythrocytes in-vitro (Saunders 1957; Weltzien 1979). Washington & Davis (1987) have also suggested that these lyso-compounds are implicated in emulsion droplet flocculation behaviour which may also provide a potential complication in the intravenous administration of these systems.

Study of the stability of intravenous emulsions requires both physical and chemical perspectives. The physical structure of these phospholipid-stabilized emulsion systems remains unresolved although commercial emulsions are known to contain oil droplets together with free liposomes (Groves et al 1985; Lutz et al 1990; Handa et al 1990). The phospholipids associated with the oil are most likely to be distributed at the oil/water interface. Physical studies are substantially limited to measuring changes in the droplet size distribution and state of aggregation since it is essential, in clinical use, that substantial numbers of large droplets in the μm diameter size range should not be administered to the patient in order to avoid interaction with the reticuloendothelial system or even produce emboli.

Chemically, it is important to characterize the main components of the natural phospholipid mixtures known to produce satisfactory emulsions. These materials are diacyl-derivatives of phosphatidylycholine (PC) and phosphatidylethanolamine (PE), together with minor components such as the corresponding lyso-derivatives of both PC and PE. In addition phosphatidylinositol, phosphatidylserine, phosphatidic acid and cholesterol are also found in the commercial mixtures used to prepare emulsions. It is accepted that the mixture of the phospholipids made from avian egg yolks, and in some cases soy beans, are more effective as emulsifiers than the individual components (Yeadon et al 1958; Hansrani 1980; Rydhag & Wilton 1981) but there is less agreement about the exact mechanism involved in this stabilization process. The lysophospholipids are present at low levels in the natural mixtures of phosphatides and it is believed that these levels increase during emulsification, sterilization and prolonged storage of the emulsion. In the pure form they have been shown to associate with various aggregation structures from micelles to bilayers, depending on their fatty acid chain lengths and the headgroup type (Stafford et al 1989). When associated with specific diacyl phospholipids in vesicular dispersions, the lyso-compounds have been demonstrated to reside in the bilayers (Kumar et al 1989) although this phenomenon has yet to be demonstrated for emulsion systems in which the overall levels of lyso-components have been determined. The reported toxicity of pure lyso-compounds (Saunders 1957) has been attributed to their deter-

Correspondence: M. J. Groves, The Institute for Tuberculosis Research, University of Illinois at Chicago (M/C 964), 115 South Sangamon Street, Chicago, Illinois 60607, USA.

gent-like properties (Weltzien 1979), with adsorption to, and subsequent penetration of, the erythrocyte membrane. The compromised cellular bilayer changes ion permeability and eventually lyses to release its contents. Weltzien (1979) has demonstrated that relatively low concentrations of lyso-PC with either palmitic acid or stearic acid acyl components are capable of inducing significant amounts of haemolysis in blood cells in-vitro. These levels of lyso-PC are certainly present even in freshly prepared emulsion systems but reports of clinical toxicity are rare. Part of the explanation for this may lie in the suggestion by Saunders (1957) that the lyso-PC and the parent PC are themselves closely associated to form a complex from which the lyso-derivative is not readily available. More recently Kumar et al (1989) suggested that the lyso-compounds were associated with the vesicular material in liposomal systems which would also have the effect of removing free lyso-lipid from the system.

The lyso-derivatives are produced by hydrolysis of the parent phospholipid and, in their turn, the lyso-compounds undergo a secondary hydrolysis to the parent glycerophosphoryl derivatives. Both reactions produce free fatty acids (FFA), thereby lowering the pH of the system. Increased FFA concentrations have been associated with an increase of the droplet surface zeta potential (Washington & Davis 1987). Indeed, those authors suggested that enhanced stability could be achieved by the addition of FFA to the emulsion although, in reality, an increase in FFA could not be achieved without a concomitant increase in the corresponding lysophospholipids. Spooner et al (1990) have recently reported that oleic acid partitions equally between the bilayered structures and the droplets when added to an emulsion system. On the other hand, Ekman et al (1988) suggested that the FFAs were located primarily at the surface in a ratio of approximately 12:1 of the triglyceride (triolein) core. Effects induced by the addition of FFAs to emulsion systems have been studied by Yeadon et al (1958) and Rubino (1990) and involve an increase in aggregation rates, especially in the presence of calcium ions.

It will be evident that the stabilization mechanisms in phospholipid stabilized emulsions are by no means clearly established although some investigators such as Yeadon et al (1958) and Hansrani (1980) have confirmed that the sterilization process, itself inevitably increasing the amounts of lyso-derivatives present in the emulsions, actually increased the stability of the product. Accordingly, we have attempted to determine the effective kinetics of the PC and PE degradation processes in practical intravenous emulsion systems based on the use of the commercially available purified phospholipid mixtures prepared from avian egg yolks. Our evaluation followed the successful development of an HPLC method for the measurement of PC and PE together, with their respective lyso-derivatives (Herman & Groves 1991). Previous investigators have explored the specific reactions under various conditions (Marriott 1969; Grit et al 1989).

Materials and Methods

Materials

Asahi injectable grade purified egg phospholipid was received as a gift from Austin Chemical, Chicago, IL, USA. Phospholipid standards were egg lecithin (phosphatidylcholine, PC), lot 19252; phosphatidylethanolamine (PE), lot 19363; lysophosphatidylcholine (lyso-PC), lot 18891; lysophosphatidylethanolamine (lyso-PE), lot LA 17105, from Matreya Inc., Pleasant Gap, PA, USA. Glycerophosphorylcholine (lot 89F8446) and glycerophosphorylethanolamine (lot 60H8365) were purchased from Sigma Chemical Company, St Louis, MO, USA.

Pharmaceutical grade and "super refined" grades of Soy Oil USP were obtained as gifts from Croda US Inc., Edison, NJ, USA, and were used without further purification.

Glycerol USP was obtained from Fisher Scientific, Itasca, IL, USA, as were all the chromatographic reagent grade solvents 2-propanol, n-hexane, methanol, chloroform and acetonitrile.

All materials were used as received.

Emulsification procedure

A model 20% lipid emulsion was made from 1·2 g purified egg lecithin, 2·25 g glycerol, with 20 g soy oil and Water for Injection to 100 mL. The lecithin was dispersed by stirring in the glycerol and most of the water followed by addition of the oil phase. The pH was adjusted to 8·5 with 0·01 M sodium hydroxide, made up to the final volume with water and the system homogenized through a Microfluidics Model 110Y homogenizer until the mean particle diameter fell below 500 nm, determined using an HIAC/Nicomp photon correlation spectrophotometer.

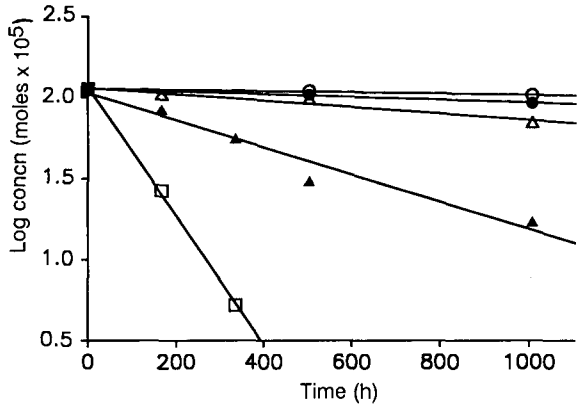

FIG. 1. Degradation of PC in a soy oil emulsion stabilized with a mixed phospholipid system. ○ 25, ● 37, △ 50, ▲ 70, □ 90°C.

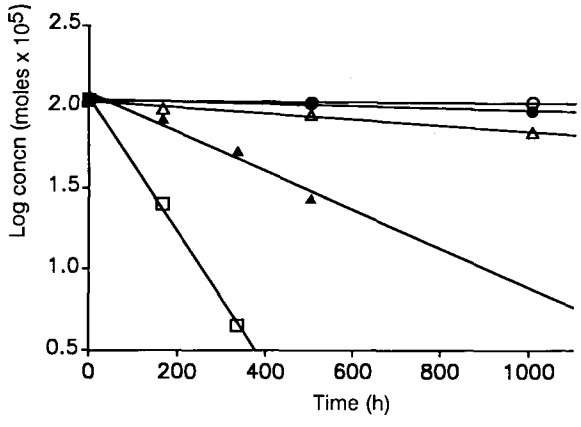

FIG. 2. Degradation of PC in a refined soy oil emulsion stabilized with a mixed phospholipid system. ○ 25, ● 37, △ 50, ▲ 70, □ 90°C.

Table 1. Conditions used in HPLC for analysis of phospholipids passed through a Lichrosorb Si 60 10 μm column.

Phospholipid	Solvent system
PC and PE	2-Propanol:n-hexane:water 5·2:3·8:1·0
Lyso-PC	2-Propanol:n-hexane:water 3·2:1·1:1·0
Lyso-PE	2-Propanal:n-hexane:water 5·5:3·8:1·0
GPC and GPE	Methanol:water 3:2

Portions were filled into 2 mL glass ampoules, sealed and autoclaved initially for 20 min at 121°C. Controls were not sterilized.

Thermal stressing

Ampoules were placed in 12 mm diameter holes in aluminium heating blocks (Dry Baths, Fisher Scientific, Itasca, IL, USA) and heated at predetermined conditions for periods of 200 to 1000 h before opening for analysis.

Analytical procedures

The HPLC analytical procedure was based on that described by Herman & Groves (1991). Intact emulsion samples were lyophilized and extracted with methanol and the phospholipids adsorbed onto a solid phase extraction column. Following elution from the column with methanol, phospholipids were separated on a silica column and detected by flame ionization.

Four hundred μL of each emulsion sample was frozen in screw-capped glass vials and lyophilized to remove the aqueous phase. Soluble phospholipids and fatty acids were dissolved in 5 mL methanol, the methanol removed under a nitrogen stream and the extract dissolved in 5 mL chloroform before passing through a Bakerbond solid phase extraction 1°/2° amino (NH_2/NH) cartridge (J. T. Baker, Phillipsburg, NJ, USA). Adsorbed phospholipids were eluted with 5 mL methanol. The eluate was evaporated to dryness under a nitrogen stream and redissolved in 500 μL chloroform and 100 μL methanol before placing on a Lichrosorb Si 60 10 μm 4·6 × 250 mm column (Alltech Inc, Deerfield, IL, USA). The HPLC system consisted of a Waters Model U6K manual injector, a Waters Model 600 Multisolvent Delivery System, a Tremetrics (formerly Tracor) Model FID 945 flame ionization detector and a Waters Model 745 Data Module.

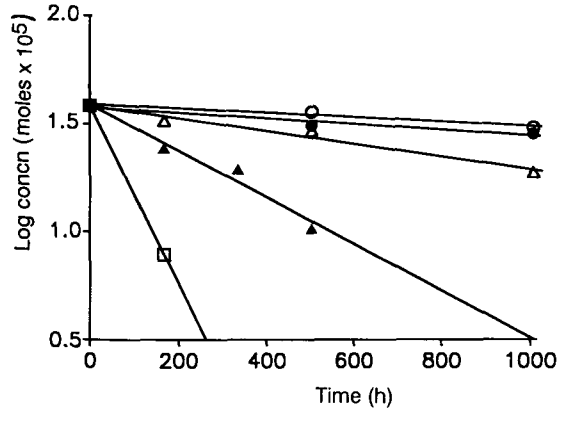

FIG. 3. Degradation of PE in a soy oil emulsion stabilized with a mixed phospholipid system. ○ 25, ● 37, △ 50, ▲ 70, □ 90°C.

Table 2. Phospholipid activation energies.

Phospholipid	Emulsion oil phase	E_A (kJ mol^{-1})
PC	Soy oil USP	63·0
PE	Soy oil USP	53·2
PC	Refined soy oil	65·3
PE	Refined soy oil	54·5
PC liposomes	(Grit & Crommelin 1990)	≈90
PC liposomes	(Grit et al 1989)	57·2

Calibration and validation of the system demonstrated that the method is capable of detecting PC, PE and their respective lyso-derivatives at the 1–10 μg level according to the column loading (Herman & Groves 1991). Conditions for the measurement of these phospholipids and their glycerophosphoryl derivatives are shown in Table 1.

Duplicate vials were sampled and analysed.

Results

Over the temperature range of the experiment (5–90°C), the degradation processes of both PC and PE could be seen to demonstrate first order hydrolysis rates, fitting Arrhenius kinetics (Figs 1–4). Energies of activation were determined

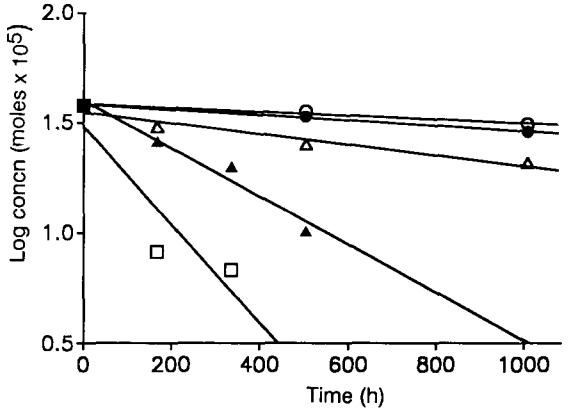

FIG. 4. Degradation of PE in a refined soy oil emulsion stabilized with a mixed phospholipid system. ○ 25, ● 37, △ 50, ▲ 70, □ 90°C.

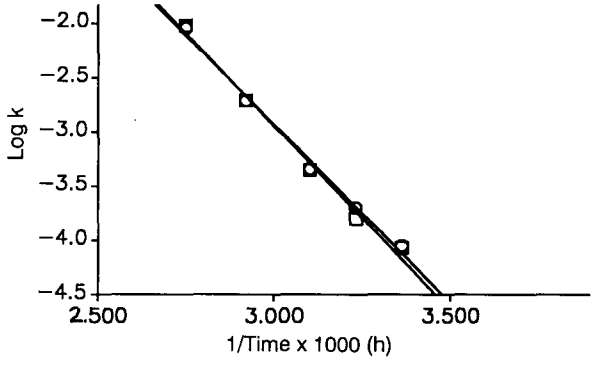

FIG. 5. The degradation rate of phosphatidylcholine as a function of temperature measured over the range of 25–90°C. ○ Soy oil emulsion, □ refined soy oil emulsion.

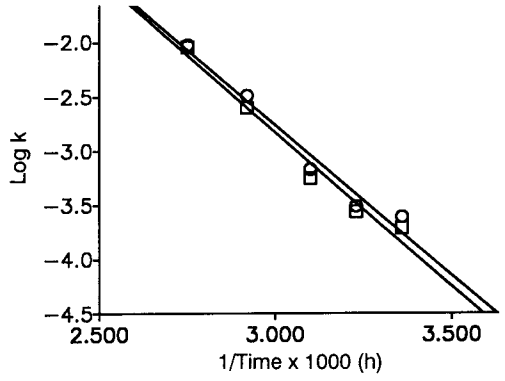

FIG. 6. The degradation rate of phosphatidylethanolamine as a function of temperature measured over the range of 25–90°C. ○ Soy oil emulsion, □ refined soy oil emulsion.

for both PC and PE (Figs 5, 6) and data are reported in Table 2. The results obtained with emulsions prepared with either the pharmaceutical grade soy oil or the ultra-refined oil were essentially similar.

Discussion

The results of this study confirm previous investigations that PC and PE degrade by first order hydrolysis processes and follow Arrhenius kinetics when heated by autoclaving and stressed at various temperatures. Heat exposure in small ampoules will be faster than in bottles during sterilization of commercial product but, for the purpose of this exercise, we believe the experiments reported here are relevant.

The energies of activation are virtually unaffected by the source of the oil. The energy of activation for PC obtained on buffered liposomal dispersions reported by Grit et al (1989) was consistently lower than our values (Table 2). In part, an explanation for this discrepancy may lie in the use of a UV detection method by Grit et al, as this method is not sensitive to saturated fatty acid derivatives. Using refractive index detection Grit & Crommelin (1990) have recently reported values almost twice their earlier estimates. It might be anticipated that dispersed systems such as liposomal bilayers would be more readily exposed to interactions with water than emulsion systems in which some, at least, of the interfacial phospholipid is protected from the aqueous environment.

In practice, phospholipid-stabilized emulsions are not buffered and rarely contain significant quantities of electrolyte. This may be relevant to the catalysis of the buffered liposomal dispersions of PC observed by Grit & Crommelin (1990).

The secondary degradation process in which lyso-PC is hydrolysed to glycerophosphatidylcholine was evident at temperatures in excess of 70°C, although the corresponding ethanolamine derivative could not be detected under the conditions of the experiment. This reaction needs to be taken into account when determining the constitution of the emulsions systems following thermal insult or prolonged storage.

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