The Influence of Free Fatty Acid Formation on the pH of Phospholipid-Stabilized Triglyceride Emulsions

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Received November 2, 1992; accepted December 1, 1992
KEY WORDS: phospholipids; thermal degradation; triglyceride emulsions; pH; free fatty acids.

INTRODUCTION

The intravenous administration of triglyceride emulsions stabilized with phospholipid emulsifiers has been employed for parenteral nutrition for over 30 years (1). Terminally heat sterilized, these systems are required to be physically and chemically stable in order to avoid harming the patient (2). Nevertheless, slow hydrolysis of the phospholipids is known to occur after the initial sterilization-induced degradation (3).

It is implicitly assumed that the subsequent fall of product pH is due to the formation of free fatty acids (4,5). We have recently demonstrated that the initial hydrolysis of the phospholipids during the heat sterilization process paradoxically promotes physical stabilization of the emulsion system, most probably because of the formation of liquid crystalline structures at the oil/water interface (3,6). The principal degradation process is due to the hydrolysis of the diacylphosphatidylcholines and diacylphosphatidylethanolamines to their corresponding monoacyl (lyso-) derivatives and free fatty acid (FFA) moieties. In turn, the lyso derivatives can degrade to the corresponding glycerophosphoryl compounds, with the formation of additional FFA. FFA can also be formed by the hydrolysis of emulsified triglycerides to the corresponding mono- and diglycerides, although this reaction is believed to be relatively slow compared to the breakdown of the diacylphosphatidyl derivatives (3).

The emulsion systems are unbuffered and the formation of FFA will inevitably lower the pH from the initial value of 8.0 (1) over a period of time poststerilization. Håkansson (2) demonstrated that the degradation rate decreases until pH 6.5 is reached, after which there is again an acceleration of the degradation process. This effect has been confirmed by Grit *et al.* (7). Stabilization may, therefore, be improved by the addition of extraneous FFA, a suggestion made by Washington and Davis (4), who evaluated the effect induced by the addition of oleic acid to their emulsion systems. However, the value of this suggestion is unclear since, during the phospholipid hydrolysis process, lyso compounds are produced in addition to FFA and these materials also contribute to the emulsion stabilization process (3,6).

Measurement of pH could, therefore, provide an indirect method of determining the FFA content of phospholipid-stabilized emulsions. This concept was evaluated measuring the FFA content directly by potassium hydroxide titration of the degrading emulsion at the same time as taking the pH of the system with a glass electrode.

MATERIALS AND METHODS

Materials

Purified egg phospholipid, Asahi Injectable grade (lot 900-80201), was received as a gift from Austin Chemical, Chicago, IL. Dipalmitoyl phosphatidylethanolamine (Lot 1 60PE-45) (DPPE) and hydrogenated egg phosphatidylcholine (Lot HEPC −44) (HEPC) were purchased from Avanti Polar Lipids, Birmingham, AL. Pharmaceutical-grade and "superrefined"-grade soybean oils USP were received as gifts from Croda Inc., Edison, NJ. Glycerol, sodium hydroxide, potassium hydroxide, and potassium hydrogen phthalate were all used as received from Fisher Scientific, Itasca, IL. A Milli-Q ion-exchange water system was used.

Model Emulsion

The model emulsion was made to the following formula: soybean oil, 20 g; egg phospholipid, 1.2 g; glycerol, 2.25 g; and water to 100 mL.

The egg phospholipid was dispersed in the glycerol and about 95% of the water at 70°C, the oil mixed in, and the coarse emulsion passed through a Microfluidics Model 110T homogenizer at a pressure of 10,000 psig for a total of 10 times to ensure minimal particle size (8). The system was washed through the homogenizer and made up to volume with water, and the pH adjusted to 8.0 with 0.01 *N* sodium hydroxide and packed and sealed in 2-mL volumes in 2-mL glass ampoules. Gas sparging with nitrogen or oxygen was carried out for 15 min when required. Hydrogenated phospholipid-stabilized emulsions were prepared by the same method, using 0.24 g DPPE and 0.96 g HEPC instead of the 1.2 g egg phospholipids.

Samples were stressed and analyzed in replicate; $n = 4$.

pH Measurement

An Orion Model 811 glass electrode pH meter was used. Potassium chloride was added to counteract the ion adsorbing effects of charged droplets (9), a process also used in the USP XX11 for the measurement of the pH of Dextrose solutions. After experimentation to determine conditions required to give reproducible results, measurements were made following the addition of 50 μL of a saturated aqueous potassium chloride solution to 2 mL of emulsion sample.

Free Fatty Acid Measurement

FFA measurement of a solution of the emulsion sample

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0724-8741/93/0500-0774$07.00/0

was by direct titration to neutrality with 0.01 *N* potassium hydroxide solution standardized using potassium hydrogen phthalate, with phenolphthalein as indicator (USP XXII). The IUPAC method (10) specifies ethanol:diethyl ether (95:5) as the solvent of choice. However, for safety reasons, the solvent was changed to ethanol:chloroform (2:1), each titration being preceded by bringing the solvent to neutrality with 0.01 *N* potassium hydroxide solution.

Thermal Stress

Thermal stress was applied by filling heating block chambers (Dry Baths, Fisher Scientific, Itasca, IL; 60 chambers per block, each 12 mm diameter and 50 mm deep) with oil and immersing the 2-mL ampoules containing the emulsion at the desired temperature, covering the blocks with aluminum foil to minimize thermal fluctuation. Temperatures were determined with calibrated mercury-in-glass thermometers placed at random in the block chambers.

In general, all ampouled emulsion samples were initially sterilized at 121°C (F_o = 18) using a Getinge BioF_oOE autoclave. Unautoclaved controls were stored at 5°C prior to evaluation.

RESULTS AND DISCUSSION

The rate of pH change and the rate of FFA formation are compared in Fig. 1 for a model emulsion prepared with the pharmaceutical grade of soy oil. Results obtained using the "superrefined" grade of oil were similar. It is evident that the rate of formation of FFA is slower than the rate at which the pH drops, suggesting that some other factors are involved. As shown in Fig. 2, emulsions prepared with unsaturated or saturated acyl groups on the phospholipid moieties and sparged with nitrogen prior to sterilization and storage changed pH at almost identical rates. However, it should be noted that these two emulsions had markedly different hydrolysis rates, (3), which suggested that the lowering of

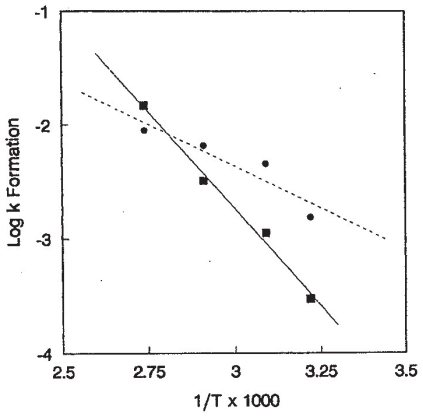

Fig. 1. The rate of FFA formation (by KOH titration) and the fall of pH (glass electrode) in a 20% soy oil emulsion stabilized with egg lecithin after sterilization and storage over the range 25–90°C. pH (slope = −3.42), ——■——; FFA (slope = −1.47), ---●---. *K*, rate of formation (slope of change against time); T, absolute temperature (kelvins).

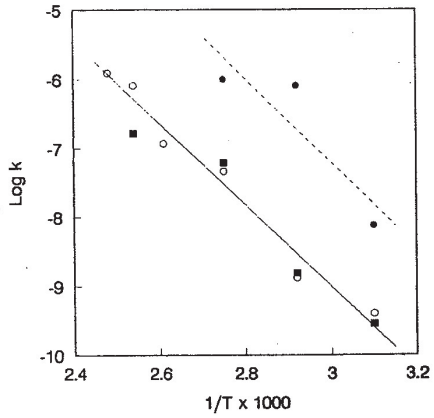

Fig. 2. The rate of pH change in a 20% soy oil emulsion stabilized with egg lecithin or saturated phospholipids and sparged with nitrogen or oxygen before sterilization and storage over the range 25–90°C. Control: egg phospholipids, nitrogen sparged (estimated slope = −5.93), ——○——; saturated phospholipids, nitrogen sparged (estimated slope = −5.30), ■; egg phospholipids, oxygen sparged (estimated slope = −6.11), ----●----.

pH was due to some other factor not necessarily associated with phospholipid hydrolysis. As noted earlier, free fatty acids can also arise by hydrolysis of triglycerides but the total (titratable) FFA in the system, irrespective of its source, is clearly unable to account for the relatively rapid lowering of pH.

When sparged with oxygen, the pH change in the control emulsion was considerably increased (Fig. 2), and this observation suggests that the effect of oxygen on the emulsion pH was more pronounced than that produced by the heat-induced hydrolytic degradation reaction.

The solubility of gases in liquids is described by Henry's law (11):

$$P_A = X_A K_a$$

where P_A is the vapor pressure of a solution containing solute A, X_A is the mole fraction of A, and K_a is Henry's law constant. Thus, intuitively, since the K_a for gases in nonaqueous solvents is generally higher than the corresponding value in water, it would appear that the oxygen is likely to preferentially dissolve in the oil phase of the emulsion. Atkins (11) discussed this issue in relation to benzene at standard temperature and pressure and observed that, in all cases, the gas was more soluble in the benzene than in the water.

Based on this consideration, it seems feasible to suggest that residual oxygen may remain dissolved in the triglyceride oil phase after preparation and manipulation of the emulsion. Some of the triglycerides contain unsaturated acyl centers, which could, therefore, become partially oxidized, although not necessarily to the point where they would be titratable with alkali and estimated as FFA. These moieties would affect the pH of the unbuffered system, in addition to the effects produced by the FFA resulting from hydrolysis of phospholipid and triglyceride entities. The evident complexity of the physical and chemical structure of phospholipid-

stabilized emulsions makes this suggestion difficult to confirm at present.

ACKNOWLEDGMENTS

Our joint thanks are due to Mr. Peter Cade and his colleagues at Croda (US) Inc. for partial support on this project.

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