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Research paper

Physicochemical stability of phospholipid-dispersed suspensions of crystalline itraconazole

Jane Werling, Sabine Graham, Heather Owen, Lakshmy Nair, Thomas Gonyon, Phillip W. Carter *

Baxter Healthcare, Medication Delivery and Technology Resources, Round Lake, IL, USA

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Abstract

The physicochemical stability of an aqueous, phospholipid-based dispersion of itraconazole microcrystals was studied as a model water-insoluble drug suspension. The particle size, phospholipid concentrations, free fatty acid (FFA) content, pH, and zeta potential of two test suspensions were followed over 63 days at 5 and 40 °C storage conditions. Hydrolysis of a control suspension containing Lipoid E80 led to rapid FFA formation, pH drop, and subsequent particle aggregation. In the second suspension, sodium oleate used in conjunction with Lipoid E80 significantly enhanced the suspension physicochemical stability. Oleate anions effectively (1) increased the anionic charge of the phospholipid surface layer, (2) buffered the suspension near pH 7, and (3) reduced the specific production of oleic acid as a phosphatidylcholine (PC) degradant. The observed hydrolysis rate constants $k_{obs} \sim 2 \times 10^{-7}$ (Lipoid only) and $k_{obs} \sim 5 \times 10^{-8}$ (Lipoid and oleate) were consistent with the pH dependent behavior reported for saturated soybean PC solutions. Mechanistically, FFA formed initially in the control suspension partitioned to the aqueous phase with limited influence on the phospholipid microenvironment at the itraconazole particle surface. Phospholipid stabilization of water-insoluble drugs was demonstrated with clear benefits from fatty acid anions as co-additives to influence the surface microenvironment, reduce hydrolysis kinetics, and enhance suspension physicochemical stability.

Keywords: Phospholipid; Hydrolysis; Physicochemical stability; Oleate; Crystalline suspensions; Itraconazole

1. Introduction

The use of phospholipids as surface-active agents enabling delivery of a variety of water-insoluble, pharmaceutically active compounds has been exploited for over 30 years. Oil in water emulsions for the intravenous delivery of total nutritional admixtures [1] or lipophilic drugs [2–4], liposomal drug formulations [5,6], microemulsions [7] and solid lipid nanoparticle delivery vehicles [8] have utilized phospholipids. Phospholipid technology has also been exploited for coating and stabilization of water-insoluble drug crystals [9–11]. While other surface-active excipients such as non-ionic polymers (poloxamers, poloxamines, polysorbates) and anionic surfactants (bile salts, alkyl sulfonates) are commonly used [12–14], the broadly understood biocompatibility of phospholipids remains a highly desirable attribute in developing stable and safe drug suspensions. Their acceptance by regulatory agencies, extensive history of use, and demonstrated safety make phospholipids useful excipients in formulations intended for oral, topical, and intravenous delivery.

From previous work on phospholipids (including applications for aqueous drug dispersions), chemical instability issues have been identified that impact overall dispersion physicochemical stability [15]. Phospholipids can easily undergo both oxidation and hydrolysis in aqueous systems. While oxidation can be mitigated to a large extent by atmospheric control, the hydrolysis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) to form their lyso

* Corresponding author. Baxter Healthcare, Medication Delivery and Technology Resources, 25212 W. Illinois Route 120, Round Lake, IL 60073, USA. Tel.: +1 11 847 270 2428; fax: +1 11 847 2705449.
E-mail address: phillip_carter@baxter.com (P.W. Carter).

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derivatives (LPC and LPE) and free fatty acids (FFA) can only be avoided by removal of water from the system. The lysophospholipids can be further hydrolyzed to form glycerophospholipids and additional FFA. Although the physiological effects of lipid degradation are beyond the scope of this work, it is noted that the degradation products, in particular the FFA, have been shown to correlate with toxicity in animal models, although the clinical incidence of toxic reactions to lipid emulsion formulations is low [16].

The PC hydrolysis kinetics are pseudo 1st order and are catalyzed under acidic and basic conditions, with a minimum rate near pH 6.5 [17]. For oil-in-water emulsions, the hydrolysis reaction generates FFA that decreases the bulk pH and increases the magnitude of the globule (or droplet) zeta potential, indicating increased negative surface charge. The negative charge is a consequence of FFA partitioning into the lipid layer at the oil–water interface. Indeed, studies investigating heat sterilization of emulsions found the increase in negative charge advantageous for emulsion stability [18,19]. A significant amount of previous work has demonstrated an increase in electrostatic repulsive force between droplets from incorporation of FFA anions into the lipid layer [20–22]. While other factors including phospholipid reorganization and subsequent phase transitions have also been suggested to be a stabilizing influence [23,24], increased inter-droplet electrostatic repulsion is considered a principal driver for minimizing flocculation, creaming, and oil separation rates associated with physicochemical instability.

The relationship between lipid hydrolysis and the physical stability of liposomes has also been studied in some detail. In addition to monitoring traditional changes in size and polydispersity, increased permeability of the liposome bilayers has been observed at a threshold hydrolysis level of 15% [25]. Furthermore, bilayers to which LPC was exogenously added showed differences in permeability compared to bilayers containing LPC and FFA as hydrolysis products, suggesting that FFA can mitigate the permeability-enhancing effect of LPC. Compositional changes in liposome bilayers due to hydrolysis have also been associated with lipid phase transitions [26]. The chemical stability of phospholipids has been shown to have a broad range of effects on the properties and physical stability of liposomes including vesicle size distribution, surface charge, permeability, phase and pH [15].

Since the major PC hydrolysis reaction products are readily identifiable, much work has explored the effects of adding such degradants to the starting formulation. Oleic acid is one principal product of hydrolysis that when added to a commercial fat emulsion enhances globule stability by the electrostatic stabilization mechanism cited above [27]. However, the use of sodium oleate as a co-additive in a lipid emulsion drug delivery application stabilized particle size only at elevated pH values; near neutral pH conditions sodium deoxycholate was the preferred co-additive [28]. The specific FFA salt or anion type and associated microenvironment influence physicochemical behavior. The packing of fatty acids into different micellar and lamellar structures has been shown to alter their apparent pK_a [29]. Such changes in fatty acid pK_a have also been demonstrated when FFA is incorporated into phospholipid bilayers [30]. The stabilizing effects of anion type, phospholipid microenvironment, and overall composition remain an active area of investigation.

Extending phospholipid-based technology to the coating of crystalline, water-insoluble particles creates additional challenges due to the introduction of a solid surface with unique functionality, morphology, and topography. These new constraints are not addressed by the work utilizing liquid–liquid interfaces. Phospholipids and hydrolysis products in solid drug suspensions will partition between phases at the drug surface and solution [10]. Testing existing theories of phospholipid particle stabilization mechanisms at the solid–liquid interface will facilitate the development of more robust drug delivery formulations. Clear relationships between solution composition and physicochemical stability for water-insoluble drug suspensions have not been fully established.

This work examines the physical and chemical stability of a phospholipid-stabilized suspension of itraconazole crystals employed as a model water-insoluble drug dispersion. The utility and efficacy of itraconazole nanosuspensions have been described elsewhere [31]. A commercially available egg lecithin (Lipoid E80) was used as a phospholipid dispersing agent for itraconazole under test conditions with and without sodium oleate as a co-additive. The particle size, zeta potential, pH, and chemical compositions of the suspensions were measured over the course of 63 days at storage temperatures of 5 and 40 °C. While characterizing lipid phase behavior is beyond the scope of this work, understanding how lipid hydrolysis and fatty acid salts impact the phospholipid microenvironment on the surface of water-insoluble drugs has been largely unexplored. The key phospholipids and FFA of interest in this investigation are shown in Fig. 1.

2. Materials and methods

2.1. Chemicals

Itraconazole was purchased from DSM Pharma Chemicals (Parsippany, NJ). Lipoid E80 was purchased from Lipoid GmbH (Ludwigshafen, Germany) and consists of 80.0–85.0% phosphatidylcholine (PC) + lyso-phosphatidylcholine (LPC), 7.0–9.5% phosphatidylethanolamine (PE), and 2.0–3.0% sphingomyelin (SPM). The amount of LPC is not more than 3.0% and the amount of free fatty acids is not more than 0.05% (information provided from manufacturer). Sodium oleate (99.0%) was purchased from Fluka (St. Louis, MO). 1N sodium hydroxide and 1N hydrochloric acid were purchased from Ricca (Arlington, TX).

The solvents acetonitrile, chloroform, methanol, and hexane were HPLC-grade, purchased from Burdick and

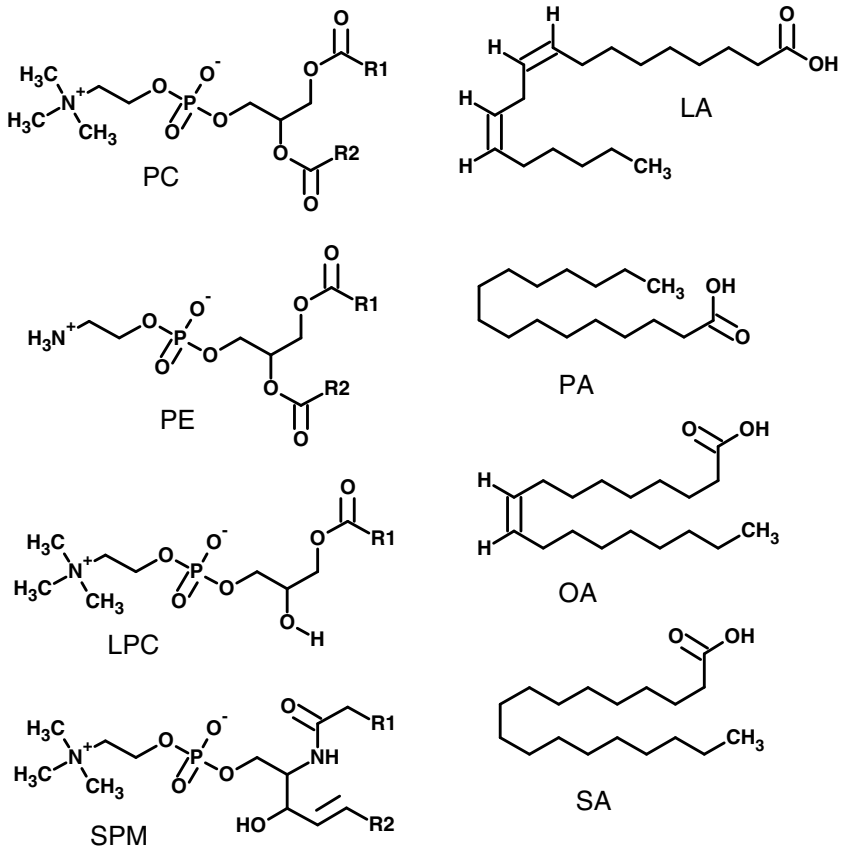

Fig. 1. Molecular structures of principal components in Lipoid E80 phosphatidylcholine (PC), phosphatidylethanolamine (PE), lyso-phosphatidylcholine (LPC), sphingomyelin (SPM), and molecular structures of major FFA hydrolysis products linoleic acid (LA), palmitic acid (PA), oleic acid (OA), and stearic acid (SA).

Jackson (Muskegon, MI), and used without further purification. Acetic acid (glacial, 99% purity) was purchased from EMD Chemicals Inc. (Gibbstown, NJ). The following lipid standards were obtained from Sigma (St. Louis, MO) for HPLC studies: phosphatidylethanolamine (PE), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC). The free fatty acid standards stearic acid (SA), palmitic acid (PA), oleic acid (OA), and linoleic acid (LA) were also obtained from Sigma and used for HPLC quantification.

2.2. Suspension preparation

Itraconazole (micronized powder with mean particle size of 11 micron) was dispersed in an aqueous phase consisting of 2.4% (w/v) Lipoid E80, pH 8.5 using an IKA Ultra-Turrax T25 mixer (Staufen, Germany) at 9500 rpm for 5 min. The 500 mL batch suspension was then homogenized using an Avestin EmulsiFlex-C55 piston-gap homogenizer (Ottawa, Canada) at 20–24 kpsi for approximately 350 passes. A final target mean particle size of 1 micron was chosen for this study and 350 passes was required to achieve the target particle size. The product was divided in half and a solution of 2% sodium oleate, pH 9.5 was added to one fraction (suspension B) with stirring to reach a target concentration of 0.22 wt% oleate. The final pH of Suspension B was 9.46. An equivalent volume of water was added to the other half (suspension A) to compensate for dilution effects, and the pH was adjusted to 9.40. All pH adjustment was performed using 1 N hydrochloric acid or 1 N sodium hydroxide. The nominally 1 wt% itraconazole batches were filled into 5 mL closed glass vials, and were stored at 5, 40, or −20 °C (frozen as a sample retain). The suspension compositions are displayed in Table 1.

2.3. HPLC analyses

2.3.1. Phospholipids

The HPLC system with evaporative light scattering detection (ELSD) for phospholipids analysis consisted of a Waters 2695 HPLC system (Waters, Milfred, MA) cou-

Table 1
Suspension compositions by weight percent in aqueous solution

Component	Suspension A (%)	Suspension B (%)
Itraconazole	0.96	0.96
Lipoid E80	2.2	2.2
Sodium oleate	0	0.22

pled with an Alltech ELSD 2000 Detector (Grace Davison, Deerfield, IL). Nitrogen gas (ultra-pure, >99%) used to operate the ELSD system was produced using a nitrogen generator manufactured by Grace Davison (Deerfield, IL). ELSD was operated in the impactor "OFF" mode and the drift tube temperature was set at 80 °C. The theory and operation of Alltech ELSD 2000 has been discussed in previously published literature [32]. Phospholipids were chromatographed using a 100 × 4.6 mm, Hypersil silica 3 μm column (Agilent Technologies, Palo Alto, CA). Mobile phase contained filtered distilled water, isopropyl alcohol and hexane. The flowrate was 1.5 mL/min under gradient conditions. The column temperature was maintained at 30 °C with a column heater. The injection volume was 25 μL. Samples for phospholipids analyses were prepared on a weight basis. Briefly, 1 mL of suspension was dissolved in 4 ml of methanol:chloroform (70:30 v/v) and brought to a final volume of 25 mL with water:isopropyl alcohol:hexane (2:58:40 v/v).

2.3.2. Free fatty acids

Free fatty acids were analyzed using a Waters 2695 HPLC system (Waters, Milfred, MA) coupled with a Corona® CAD® (Plus) (ESA Biosciences, Chelmsford, MA). Nitrogen gas (ultra-pure, >99%) used to operate the Corona® detector was produced using a nitrogen generator manufactured by Grace Davison (Deerfield, IL). The nitrogen pressure was maintained at 35 psi and the current output range was kept at 100 pA. Free fatty acids were separated on a 150 × 3.0 mm, Zorbax SB C18 3 μm column (Agilent Technologies, Palo Alto, CA). The mobile phase was prepared by combining water/acetonitrile and acetic acid. The flowrate was kept at 1.0 mL/min and operated under isocratic conditions. The column temperature was maintained at 30 °C with a column heater. The injection volume was 15 μL.

Samples for FFA analyses were prepared on a weight basis. Briefly, 1 ml of suspension was dissolved completely by adding 5 ml of sample diluent (50:50 mixture of 70/30 methanol/0.1 M acetic acid in acetonitrile). A fourfold dilution of each sample was also prepared and injected. Measurements were made in triplicate with a typical relative standard deviation less than 3%.

2.3.3. Itraconazole

Itraconazole was analyzed using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) with UV detection at 210 nm. Itraconazole was separated on a Zorbax SB Phenyl, 5 μm, 250 × 4.6 mm column (Agilent Technologies) using a mobile phase containing 0.1% trifluoroacetic acid (Sigma–Aldrich, Milwaukee, WI) and acetonitrile. Itraconazole was eluted by a linear gradient with initial conditions of 60% 0.1% trifluoroacetic acid eluent A) and 40% acetonitrile (eluent B), increasing over 6 min to 20% eluent A/80% eluent B and holding for an additional 9 min. Total run time was 20 min. The injection volume was 5 μL.

Samples and standards for itraconazole analyses were prepared in a sample diluent containing a mixture of 0.15% trifluoroacetic acid and acetonitrile (60:40). Characterized itraconazole reference standard was prepared at a concentration of 0.1 mg/mL. Samples were prepared on a weight basis. Approximately 1 g of suspension was dissolved in 100 mL sample diluent.

2.4. Single particle optical sensing (SPOS)

Particle size analysis and distribution of each suspension at all intervals were determined using the AccuSizer™ Model 770 (Particle Sizing Systems, Santa Barbara, CA) equipped with the Pressure Assisted Liquid Sampler (PALS) and utilizing an LE 400 sensor in extinction mode previously calibrated with polystyrene latex spheres. Glass containers with magnetic stir bars were exhaustively rinsed with filtered distilled water prior to transfer of samples. The filled containers were then placed into the PALS unit and the background was assured to be below 0.01 particle/mL >2 microns prior to using the glassware for dilution. The pressure setting of the PALS system was set at 48 psi to produce a flow rate of 60 mL/min, corresponding to the calibration flow rate of the sensor.

The sample container was inverted 20 times and a suspension aliquot was removed with a calibrated autopipette, transferred to the filled beaker, and allowed to mix for 60 s on a magnetic stirring plate. Dilution factors ranged from 12,000 to 20,000 to achieve an acceptable level of cumulative particle counts within the targeted range of 1000–7000 counts/mL. This concentration of particles was selected to obtain a significant signal intensity while avoiding the coincidence limit of the sensor which is approximately 9000 particles/mL. Within particle counts that avoid coincidence limits, no significant variation in normalized counts was observed as a function of dilution factor for representative conditions.

2.5. Laser diffraction particle sizing for determining mean PSD

The Horiba LA-920 Laser Scattering Particle Size Distribution Analyzer (Horiba Instruments, Irvine, CA) was used to examine the particle size distribution. The laser diffraction testing was performed at all time intervals. After the sample container was inverted 20 times, sample aliquots were analyzed using filtered distilled water as a diluent with a relative refractive index of 1.22 and an imaginary term set to 0.1. A cell recirculation speed of 2 was used with no sonication during analysis.

2.6. Zeta potential measurements

Zeta potentials were measured using a Zetasizer Nano ZS with MPT-2 autotitrator (Malvern Instruments, Malvern, UK). Titration curves were generated using a titration sequence from pH 9.0 to 2.0 of a 15 mL aliquot

produced from 100 μL of 1 wt% particle suspension added to 40 mL of 1 mM NaCl diluent at pH 9.5. The mixture was titrated using 0.1 N HCl in increments of 1.0 pH units. The Smoluchowski model was used to generate zeta potential data from electrophoretic mobilities.

2.7. pH measurements

The pH of the suspensions was measured using a Thermo Orion pH meter (model 720A+) and an Orion 91-57 TriodeTM 3-in-1 pH/ATC electrode.

3. Results

PC hydrolysis was monitored by tracking phospholipid concentration profiles as shown in Table 2. The change in phospholipid concentrations indicated more extensive PC hydrolysis of suspension A compared to suspension B. After 63 days at 40 °C, the decrease in PC was a factor of 2.6 greater for suspension A compared to suspension B. The corresponding increase in LPC was a factor of 2.0 greater for suspension A compared to suspension B. The overall PE concentrations of each sample were significantly lower than PC levels and were approximately equivalent at each time interval.

The evolution of FFA concentrations resulting from PC hydrolysis is depicted in Table 3 along with the initial oleate added to suspension B. Consistent with the PC degradation results above, higher FFA concentrations were observed for suspension A compared to suspension B at sampling intervals $t = 28$ days and $t = 63$ days. The average relative increase in individual fatty acids (linoleic, palmitic, oleic, and stearic) produced was a factor of five greater for suspension A than for suspension B. Comparing the relative increases of FFA types between suspensions, oleic acid was produced to a slightly greater extent (6.8 times) and linoleic acid to a lesser extent (only 3.7 times) in suspension A compared to suspension B. Overall, the changes in PC, LPC, and FFA concentrations confirm that the rate of phospholipid hydrolysis was significantly faster in suspension A compared to suspension B.

While the introduction of fatty acids can be expected to lower solution pH, the magnitude of the effect will be dependent upon variables such as extent of FFA formation, formulation specifics, and sample storage conditions. Fig. 2 illustrates the fivefold increase in total free fatty acid produced from hydrolysis for suspension A compared to suspension B after 29 and 63 days. Suspension A achieved a final FFA concentration in excess of 20 mM over the 63-day time interval at 40 °C compared to the 4 mM total FFA generated for suspension B. In this instance, the increased FFA of suspension A does translate into a significantly lower pH near 4 by $t = 29$ days. The pH of suspension B decreases in a manner that appears to asymptotically approach pH 7. The differences in FFA production have created a disparity in bulk pH values where the oleate-free suspension A experiences an acidic regime by $t = 7$ days and suspension B maintained a pH > 7 throughout the 63 day storage at 40 °C.

In order to rule out the possibility that the drug itself (or a drug degradant) was changing over time, itraconazole concentration was measured for both initial and $t = 63$ day samples stored at 5 and 40 °C. The concentration of itraconazole was unchanged over the course of the study

Table 2
Phospholipid concentrations in wt% determined by HPLC

Sample	Phospholipid	$t = 0$	$t = 28$ days	$t = 63$ days	$\delta[A]/\delta[B]^*$
A no oleate	PC	1.68	0.79	0.56	2.6
	PE	0.17	0.11	0.11	1.2
	lyso-PC	0.11	0.34	0.48	2.0
B with oleate	PC	1.63	1.40	1.20	–
	PE	0.16	0.11	0.11	–
	lyso-PC	0.10	0.14	0.24	–

$^*\{([A]_{t=63} - [A]_{t=0})/([B]_{t=63} - [B]_{t=0})\}$ calculated for each phospholipid.

Table 3
Free fatty acid concentrations in wt% determined by HPLC

Sample	Fatty acid	$t = 0$	$t = 28$ days	$t = 63$ days	$\delta[A]/\delta[B]^*$
A no oleate	Linoleic	<0.005	0.0297	0.066	3.7
	Palmitic	<0.005	0.0957	0.211	5.1
	Oleic	<0.005	0.0704	0.178	6.8
	Stearic	<0.005	0.0439	0.101	5.0
B with oleate	Linoleic	<0.005	0.007	0.018	–
	Palmitic	<0.005	0.016	0.041	–
	Oleic	0.22	0.244	0.248	–
	Stearic	<0.005	0.008	0.020	–

$^*\{([A]_{t=63} - [A]_{t=0}/([B]_{t=63} - [B]_{t}=0)\}$ calculated for each fatty acid.

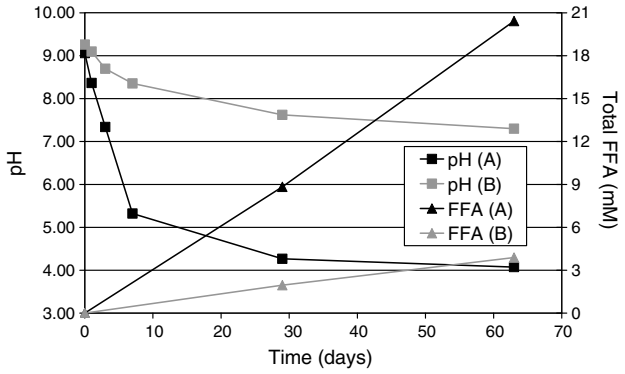

Fig. 2. The pH and FFA concentration produced by PC hydrolysis for suspension A and B as a function of storage time at 40 °C. Suspension A shows a more significant drop in pH and a higher level of FFA formation than suspension B. Suspension B pH appears to approach pH 7 asymptotically. The total FFA molarity is determined by summing the calculated molarity for each FFA shown in Table 3 (minus the initial oleate concentration in suspension B).

regardless of storage temperature, confirming the chemical stability of the water-insoluble drug under these test conditions. Thus, physicochemical effects were due solely to the lipid degradation.

In addition to the chemical changes described above, changes in particle size distribution are illustrated in Fig. 3 as a function of time at 40 °C. In suspension B, the initial mean particle size was maintained throughout the 63-day, 40 °C stress condition. In contrast, suspension A mean particle size increased by $t = 29$ days and more than doubled by $t = 63$ days from its initial size. The shape of the PSD was examined by comparing D10, D50, and D90 intervals of the cumulative distribution. The shape of suspension B PSD was essentially unchanged, and suspension A PSD became broader with D90 showing the largest increase in size. The SPOS measurement showed a slightly greater sensitivity for monitoring changes in agglomeration compared with mean size measurements of laser diffraction, as evidenced by the increase in suspension A large particle counts at $t = 3$ days and $t = 7$ days relative to the change in mean particle size. To summarize, the time

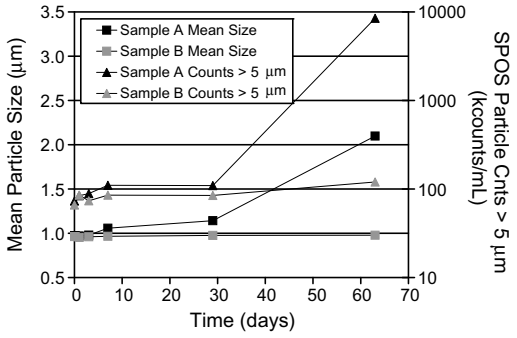

Fig. 3. The particle size distribution characteristics of suspension A (control) and B (with added oleate) as a function of storage time at 40 °C. Mean particle size by laser diffraction and large particle counts by SPOS increased significantly for suspension A after 63 days.

dependent particle size data showed suspension B to be more stable than suspension A with respect to particle agglomeration and growth at elevated temperatures.

Complementary information describing particle surface composition was obtained from zeta potential measurements. Figs. 4 and 5 show the time dependent itraconazole particle charge behavior resulting from changes in the phospholipid microenvironment, as monitored by pH dependent zeta potential measurements. Both suspensions A and B started with an isoelectric point (IEP) near pH 3.5 that shifted to pH 2.3 by $t = 63$ days consistent with an increase in anionic surface charge due to incorporation of negatively charged surface species. While suspension B exhibited the same initial and final IEP as suspension A, two clear distinctions can be made from zeta analyses. First, suspension B shows a more negative zeta potential at pH > 7 at early storage times compared with suspension A, reflecting differences in the initial phospholipid surface composition. Second, suspension B also exhibited a more uniform and higher rate of increase in anionic surface charge at earlier time intervals ($t < 29$ days) compared to suspension A.

A more detailed comparison of the pH 5–9 range is presented in Fig. 6 to highlight some of the key differences between suspension zeta potential characteristics. At time-zero, the zeta potential values for suspension B is clearly more negative compared with suspension A at pH > 7. Similarly after 63 days at the 5 °C storage condition, suspension B also exhibited a more negative surface

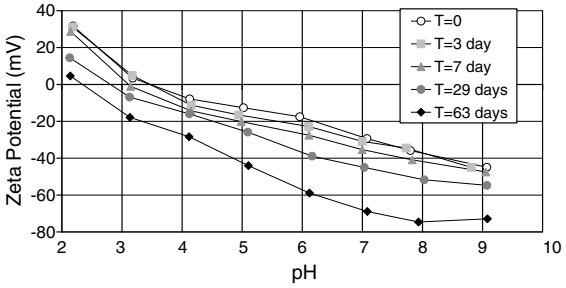

Fig. 4. The pH dependent zeta potential for suspension A over the course of 63 days at 40 °C. Suspension aliquots were diluted in 1 mM NaCl initially at pH ~ 9.5 and titrated with HCl to pH ~ 2.

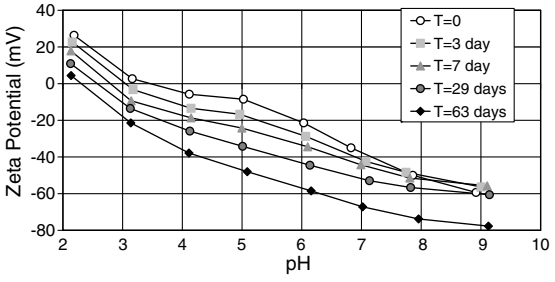

Fig. 5. The pH dependent zeta potential for suspension B over the course of 63 days at 40 °C. Suspension aliquots were diluted in 1 mM NaCl initially at pH ~ 9.5 and titrated with HCl to pH ~ 2.

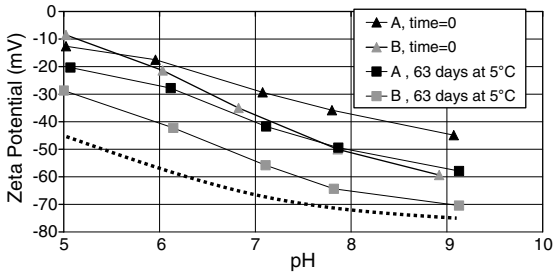

Fig. 6. The pH dependent zeta potential comparing suspension A with suspension B at initial conditions and after 5 and 40 °C storage conditions. Suspension aliquots were diluted in 1 mM NaCl initially at pH ~ 9.5 and titrated with HCl to pH ~ 2. The oleate-containing suspension B showed lower zeta potential values at $t = 0$ and for suspensions stored for 63 days at 5 °C compared to oleate-free suspension A control. The dashed line represents the equivalent pH dependent behavior observed for both suspensions after 63 days storage at 40 °C.

charge than suspension A and across a broader pH range in this case. Clearly, the presence of oleate anions in formulation B created more negative surface charge under conditions where limited PC hydrolysis is observed. In contrast to these differences at early storage intervals, after 63 days at the 40 °C storage condition, the shape of the titration curves is essentially equivalent for suspension A and suspension B represented by the dashed line in Fig. 6. The time dependent particle charge results reflect changes in particle–phospholipid surface composition stemming from hydrolysis and equilibration phenomena. These changes in particle surface properties cannot be simply inferred from HPLC, pH, or particle size measurements and are further analyzed in the discussion section below.

A summary of the key physicochemical measurements and analyses for the two drug suspensions is compiled in Table 4. Initial and final particle size, surface charge, and solution composition measurements reveal the time and temperature dependent behavior. Not surprisingly, suspension A and suspension B stored at 5 °C showed improved physicochemical stability compared with the 40 °C storage condition. At 5 °C the particle size distribution for each suspension was essentially unchanged, maintaining a mean particle size less than 1 micron and SPOS large particle counts (>5 μm) near initial values of 66–73 kcounts/mL. The suspension IEP pH and overall dispersion pH did not decrease to the same extent at 5 °C storage compared to the 40 °C storage conditions described above. The more stable pH is a consequence of minimal FFA production (below HPLC detection limits) for suspension A and suspension B. Consistent with these findings, minimal degradation of PC and PE was observed, and the initial concentration of LPC remained unchanged at the lower temperature condition of 5 °C. The 63-day stability data clearly show that the 5 °C storage condition minimizes both PC hydrolysis and particle growth kinetics in the presence and absence of oleate anions.

4. Discussion

The physicochemical stability of water-insoluble itraconazole particles dispersed with Lipoid E80 is controlled by the time dependent changes in the composition of solution constituents and particle surface chemistry. Phospholipid hydrolysis/degradation reactions coupled with partitioning equilibria drive observed differences in bulk pH and particle microenvironments. The first order rate constants, k_{obs}, for PC hydrolysis at 40 °C, were calculated using the simple relationship expressed by Eq. (1) between initial and final PC concentrations.

$$k_{obs} = \frac{\ln\left(\frac{[PC]_0}{[PC]_t}\right)}{\text{time}} \quad (1)$$

The calculated k_{obs} values were found to be 2.0×10^{-7} and 5.6×10^{-8} s^{-1} for suspensions A and B, respectively. Despite significant pH variation in the first 7 days of storage, the k_{obs} values are consistent with previous measurements for saturated soybean PC solutions at pH 4 and 7.5 [17], corresponding to approximate pH values of suspensions A and B over the majority of storage time at 40 °C. Thus, the difference in rates of hydrolysis is dictated primarily by the observed dispersion pH, and maintaining bulk

Table 4
Dispersion properties as a function of storage temperature after 63 days compared with initial values

Measurement	Suspension A			Suspension B		
	Initial	$T = 5$ °C	$T = 40$ °C	Initial	$T = 5$ °C	T = 40 °C
Mean Particle size (microns)	0.97	0.99	2.10	0.96	0.95	0.98
Particles > 5 μm (kcounts/mL)	73	74	8500	66	63	120
IEP (pH units)	3.5	3.1	2.3	3.4	2.8	2.3
Dispersion pH	9.23	6.93	4.07	9.36	8.39	7.30
PC hydrolysis k_{obs} (s^{-1})	NA	1.1×10^{-8}	2.0×10^{-7}	NA	1.4×10^{-8}	5.6×10^{-8}
Linoleic acid (wt%)	<0.005	<0.005	0.066	<0.005	<0.005	0.018
Palmitic acid (wt%)	<0.005	<0.005	0.211	<0.005	<0.005	0.041
Oleic acid (wt%)	<0.005	<0.005	0.178	0.22	0.22	0.248
Stearic acid (wt%)	<0.005	<0.005	0.101	<0.005	<0.005	0.020
Phosphatidyl choline (wt%)	1.68	1.58	0.56	1.63	1.51	1.20
Phosphatidyl ethanolamine (wt%)	0.17	0.15	0.11	0.16	0.12	0.11
Lyso-phosphatidyl choline (wt%)	0.11	0.11	0.48	0.10	0.11	0.24

dispersion pH values around 6.5 will be most beneficial for physicochemical stability because this is where base and acid catalyzed mechanisms are least efficient.

More interesting is the result that adding sodium oleate to the itraconazole suspension significantly enhanced physicochemical stability. While the incorporation of oleate anions into the phospholipid surface layer is well known to improve electrostatic stabilization, oleate's role as a buffering reagent is less widely understood. The physicochemical stability of suspension B is due in part to oleate's role as a pH 7 buffer capable of maintaining a pH regime that minimizes hydrolysis. The pK_a of free oleic acid is approximately 5.0, and therefore individual aqueous oleate molecules do not provide any significant buffering capacity above pH 6.0. However, oleate anions present in phospholipid vesicles have demonstrated a pK_a near 7 due to the influence of (1) neighboring anionic charges, and (2) low dielectric constant in the microenvironment [29,30]. In the present work, the initial oleate anion concentrations in suspension B are approximately 7.5 mM, and the zeta potential results confirm initial, significant oleate anion incorporation into the phospholipid microenvironment of the itraconazole crystals. The 3.9 mM total FFA produced after 63 days for suspension B represents about half of the initial sodium oleate concentration and does not exceed the suspension buffering capacity. Without pH 7 buffering capacity, suspension B would have decreased well below pH 6 with this amount of FFA. Understanding the concentration, partitioning, microenvironment, and molecular structure relationships between carboxylic acid functionalized molecules and their effective pK_a have yet to be fully delineated. "Engineered" buffers like the oleate-enriched phospholipid suspension have the threefold benefit of (1) reducing the rate PC hydrolysis, (2) maintaining significant particle electrostatic charge, and (3) minimizing salt concentrations.

For suspension A, the degradation of PC produced significant amounts of FFA that lowered the solution pH, reduced particle charge, decreased electrostatic repulsion between particles, and facilitated particle agglomeration. More specifically, total FFA concentrations were generated in excess of 20 mM (63-day, 40 °C) producing a final suspension pH near 4 where the net particle charge is less than 50% its pH 7 value. The pH drop for this suspension could be mitigated with traditional buffering reagents like hydrogen phosphate, or with more unique oleate-rich phospholipids (as discussed above). The FFA generated *in situ* during hydrolysis offers no buffering capacity, because the stoichiometry of the PC hydrolysis reaction generates a carboxylic acid group, not a carboxylate salt. Therefore, suspension A lacks sufficient FFA conjugate base, and no significant buffering capacity can be observed even if FFA was incorporated into the phospholipid layer.

The zeta potential data at early storage times ($\leqslant$7 days) indicated modest FFA incorporation into the surface microenvironment for the oleate-free suspension A. Therefore, it is postulated that FFA is preferentially partitioned to the aqueous phase at early times driving the solution pH below 5. At lower pH and higher total FFA acid concentrations, the FFA partitions more significantly into the phospholipid–particle surface layer where after 63 days at 40 °C, the microenvironment of particles from each suspension appeared equivalent via surface charge measurements.

The importance of partitioning equilibria is further underscored by the more inhibited rate of oleic acid formation in suspension B compared to suspension A (Table 4). This suggests that the oleic acid-rich microenvironment either suppresses the specific forward reaction or contributes to the reverse condensation reaction. Another possibility is a pH dependence in FFA product distributions. Nontheless, the overall rate of PC degradation is consistent with previous studies, and the presence of FFA salts may influence the relative composition of degradation products. In fact, the second half of the 40 °C storage for suspension A showed a lower relative PC degradation compared with the first half despite having a lower bulk pH that should enhance hydrolysis. Overall, sodium oleate has three independent mechanisms to influence physicochemical stability: pH buffering, electrostatic stabilization, and reaction equilibria. The electrostatic stabilization and reaction equilibria mechanisms would not be obtained from traditional pH buffers.

As an alternative to pH control of hydrolysis, lower storage temperatures also slowed PC degradation significantly. At 5 °C storage conditions, k_{obs} was closer to 1×10^{-8} s^{-1} for each suspension indicative of the anticipated slower rate of hydrolysis. Although there was minimal FFA generated at these low temperatures, the zeta potential measurements showed significant increases in oleate anion incorporation for suspension B. Suspension A shows very little change in particle surface charge characteristics at the lower temperature further supporting FFA partitioning into solution during early stages of PC hydrolysis. The incorporation of oleate anions can occur over relatively long time intervals in the absence of significant amounts of FFA produced by PC hydrolysis. Quantifying the differences between bulk solution composition and particle surface composition with respect to phospholipids and fatty acids remains an opportunity for improved understanding.

In the present study, there is a large excess of solution PC relative to PC adsorbed to itraconazole particles. A one-off experiment from a centrifuged sample found the ratio of (PC in supernatant)/(PC in total suspension) to be approximately 0.95. TEM micrographs of itraconazole suspensions show PC to act as a wetting agent depositing 1−3 monolayers on the itraconazole crystalline surface [33]. Using the laser diffraction determined specific surface area ($\sim$8 m^2/g) and approximate PC head group area of 0.75 nm^2, an itraconazole/PC mass ratio of 75 is calculated for monolayer coverage. Since the actual mass ratio in this study is closer to 0.5, there is about a 150-fold excess of PC. Even assuming multilayer coverages, PC vesicles will be in solution by virtue of the excess in concentration. Previous

work showed no significant change in vesicle particle size up to 23% PC hydrolysis despite changes in bilayer permeability at 15% hydrolysis [23]. The specific contribution of free PC vesicle hydrolysis on particle stability is beyond the scope of this work.

In addition to free vesicle effects, it is unclear whether there are stability advantages to pH buffering of the microenvironment relative to the bulk solution using traditional buffers. Broader structure-activity relationships that include (1) direct determination of surface composition, (2) process variables, and (3) bulk solution composition are needed to further understand and enhance stabilization.

5. Conclusions

The physicochemical stability of an aqueous suspension of itraconazole formulated with Lipoid E80 phospholipid was influenced by the compositional changes induced by PC hydrolysis. The control suspension instability was observed at 40 °C storage conditions where PC degradation and FFA generation decreased the pH to a point of reduced particle charge and reduced interparticle electrostatic repulsions; particle aggregation results. When sodium oleate is used in conjunction with Lipoid E80, phospholipid incorporated oleate anions (1) increased the negative charge of the particle surface layer, (2) buffered the suspension near pH 7, and (3) reduced the specific production of oleic acid as a phosphatidylcholine byproduct. These mechanistic differences produced a more stable itraconazole suspension in the presence of oleate anions.

The observed PC hydrolysis rate constants $k_{obs} \sim 2 \times 10^{-7}$ (control) and $k_{obs} \sim 5 \times 10^{-8}$ (oleate) were consistent with the pH dependent behavior reported previously for liposomes. Therefore, the introduction of a unique hydrophobic, solid surface did not significantly perturb the known aqueous hydrolysis reactions of PC. In addition, the pH dependent particle charge characteristics were identical for the control and oleate-containing suspensions stored at 40 °C after 63 days despite unique suspension pH, compositions, and particle size characteristics. Thus, particle stability relies on both bulk solution and particle surface compositions. The 5 °C storage condition favors sodium oleate incorporation when phospholipid hydrolysis rate constant is reduced to $k_{obs} \sim 1 \times 10^{-8}$. Low temperature storage of oleate-containing phospholipid suspensions is predicted to have the longest, stable "shelf-life" of dispersed hydrophobic microparticles.

The results demonstrate that phospholipid hydrolysis kinetics and mechanisms proposed at liquid–liquid interfaces are consistent with the results obtained at the solid–liquid junction. Additives can influence the physicochemical stability through their effect on the phospholipid microenvironment, pH buffering capacity, and equilibrium reactions. Minimizing PC hydrolysis kinetics is essential for stabilizing water-insoluble drug suspensions. Designing formulations to gain functionality from degradants is an equally important concept.

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