Solubilization and Stabilization of an Anti-HIV Thiocarbamate, NSC 629243, for Parenteral Delivery, Using Extemporaneous Emulsions

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The *O*-alkyl-*N*-aryl thiocarbamate, I, (2-chloro-5-[[(1-methylethoxy)thioxomethyl]amino]benzoic acid, 1-methylethylester, NSC 629243, also known as Uniroyal Jr.) is an experimental anti-HIV drug with very low water solubility (1.5 μg/mL). Early clinical studies required an injectable solution at ≈15 mg/mL, representing a solubility increase of $\approx 10^4$-fold. Adequate solubilization of this hydrophobic drug was achieved in 20% lipid emulsions. Extemporaneous emulsions were prepared by adding a concentrated drug solution to a commercially available parenteral emulsion. Various methods of preparation to minimize drug precipitation during its addition and enhance redissolution of precipitated drug were evaluated. The stability and mechanism(s) of decomposition of NSC 629243 in both 20% lipid emulsions and in natural oil vehicles were examined. In lipid emulsions, the shelf life at 25°C varied from 1 to >10 weeks, depending on the extent to which air was excluded from the preparation. The shelf life of 50 mg/mL solutions in natural oils at 25°C varied from <1 to >100 days depending on the oil and its supplier. A qualitative correlation was found between the initial rate of oxidation and the peroxide concentration in the oil. The primary degradation product in both systems was shown to be a disulfide dimer, II, formed via oxidation. Oxidation was inhibited by vacuum-sealing of emulsion formulations or incorporation of an oil-soluble thiol, thioglycolic acid (TGA), into oil formulations. TGA may inhibit oxidation by consuming free radicals or peroxide initiators or by reacting with the disulfide, II, to regenerate the starting drug.

KEY WORDS: anti-HIV drugs; thiocarbamate; emulsion; solubilization; stabilization; disulfide exchange; oxidation; parenteral formulation; AIDS chemotherapy; Uniroyal Jr.

INTRODUCTION

The *O*-alkyl-*N*-aryl thiocarbamate, I [NSC 629243 or Uniroyal Jr. (1)], is a nonnucleoside reverse transcriptase inhibitor which was selected in early 1990 for preclinical development by the National Cancer Institute (NCI) as a potential candidate for the treatment of AIDS. As is usually the case, early clinical evaluation of this drug required an injectable intravenous formulation for assessment of toxicity and for determining the relative bioavailability of an orally administered dosage form. This compound is highly lipophilic, has no ionizable group, and hence has extremely low water solubility (1.5 μg/mL), which must be overcome in order to prepare such a parenteral formulation.

Highly lipophilic drugs with very low water solubility (1–10 μg/mL) may not be solubilized to the extent necessary (10^3- to 10^5-fold) to reach the relatively high concentrations (1–100 mg/mL) frequently desired in the early stages of preclinical and clinical evaluation by standard approaches such as aqueous cosolvents, pH adjustment of drugs with an ionizable group, complexation, or the use of surfactants. However, intravenous administration of lipophilic compounds is possible if they can be adequately solubilized in an oil/water emulsion. Drug-containing emulsions may be prepared extemporaneously by adding to the emulsion a concentrated solution of drug in a suitable solvent (2,3). Potential problems in developing these emulsions are the precipitation of the drug in the water phase before it can be incorporated into the oil phase and the slow redissolution of the precipitate.

This study addresses the development of an extemporaneous intravenous formulation of I prepared by adding a concentrated drug solution in a nonaqueous solvent to a commercially available lipid emulsion. Various procedures to minimize drug precipitation and to enhance the rate of redissolution of precipitated drug were studied. Stability studies were carried out both in emulsion formulations and in natural oils. Since >99.9% of the drug resides in the oil phase of lipid emulsions, its stability in natural oils was expected to resemble that in emulsions. Methods for inhibiting the oxidation of I to its disulfide dimer were also explored.

MATERIALS AND METHODS

Chemicals

NSC 629243, I (2-chloro-5-[[1-methyl-ethoxy)thioxomethyl]amino]benzoic acid, 1-methylethylester), was obtained from the National Cancer Institute (Bethesda, MD); the disulfide dimer, II, was isolated in our laboratory (see below). The intravenous fat emulsions utilized were (i) Intralipid 20% (KabiVitrum Inc., Clayton, NC), which was used 9–12 months before the expiration date; and (ii) Liposyn II 20% (Abbott Laboratories, North Chicago, IL), which was used 12–15 months before the expiration date. PEG 300 and USP sesame, soybean, cottonseed, and peanut oil were obtained from Spectrum Chemical Mfg. Inc. (Gardena, CA). Oils were also purchased from Croda Inc. (New York, NY) either with BHT added as a preservative (Super-Refined sesame oil, manufactured 4 months prior to study and referred to as "fresh") or without preservative (Super-Refined NP sesame, safflower, and soybean oil manufactured 2 years prior to study and referred to as "aged" oils). Sesame oil, triacetin, butylated hydroxyanisole (BHA), monothioglycerol, and thioglycolic acid (TGA) were obtained from Sigma Chemical Co. (St. Louis, MO). Butylated hydroxytoluene (BHT), vitamin E, EDTA, and *N*-acetylcysteine were obtained from Aldrich Chemical Co. (Milwaukee, WI). 2-Hydroxypropyl-β-cyclodextrin (Molecusol, MW 1540) was a gift from Pharmatec, Inc. (Alachua, FL). All other reagents and solvents were obtained from commercial sources and used without further purification. Aqueous solutions were prepared using deionized water.

Instrumentation

HPLC separations utilized a modular HPLC system

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consisting of two pumps (Model 110B, Beckman Instruments, Inc., Fullerton, CA), a pump controller (Beckman Model 421 A), a variable-wavelength detector (Model 480, Waters Chromatography Division, Millipore Corp., Milford, MA), an integrator (Waters Model 730 data module), and an autosampler (Waters Wisp 710B). Ultraviolet spectra were recorded on a Perkin Elmer UV-visible spectrophotomer (Lambda 7, Perkin Elmer Corp., Norwalk, CT). Fast-atom bombardment (FAB) mass spectra were recorded with a MAT 731 mass spectrometer (Finnigan MAT, San Jose, CA) from samples dissolved in a glycerol matrix. Ionization was induced with a 8-keV Xe beam produced by an Ion Tech FAB 11N ion source. Emulsion particle size measurements were made at 25°C by photon correlation spectroscopy on a Brookhaven Model BI-90 spectrometer (Brookhaven Instruments Corp., Holtsville, NY) using fixed-angle detection (90°) and a He–Ne laser light source (632.8-nm wavelength). The instrument was operated in the standard mode, which assumes a log-normal particle size distribution in the sample. Averages reflect at least 10 readings per sample.

Analytical Methods

The concentrations of I and II in various formulations were monitored by HPLC using an Applied Biosystems (Foster City, CA) Brownlee SPHERI-5 RP-18 column (5 μm, 220 × 4.6 mm). Method 1, used for monitoring both I and its degradation product (II), employed a mobile phase of 92/8 methanol/water and UV detection at 236 nm. The retention volumes were ≈4 mL (I) and ≈19 mL (II). Method 2, which was used for quantifying I and its aqueous hydrolysis products, utilized a mobile phase of 75/25 methanol/0.01 *M* $HN_4H_2PO_4$ adjusted to pH 3.0 and detection at 236 or 283 nm. Three peaks corresponding to aqueous solution degradation products eluted at retention volumes of 4.8 mL [ester hydrolysis product: (2-chloro-5-[[1-methylethoxy)thioxomethyl]amino]benzoic acid], 6.2 mL (thiocarbamate hydrolysis product: isopropyl 5-amino-2-chlorobenzoate), and 9.5 mL (carbamate analogue of I), while I exhibited a retention volume of ≈14 mL. Compound II did not elute under these conditions.

The peroxide concentrations in various vehicles were determined using an iodometric spectrophotometric procedure similar to that developed by Magill and Becker (4). One molecule of peroxide oxidizes two iodide ions and, in the presence of excess iodide ion, becomes triiodide, which has a maximum UV absorbance at 360 nm. To 9.8 mL of isopropyl alcohol (IPA) was added 100 μL of aqueous 5% potassium iodide and 100 μL of the vehicle of interest. The solution was allowed to stand in the dark for 3–5 hr to allow the reaction to come to completion and the absorbance at 360 nm was determined. External standards containing iodine in IPA with excess iodide were used to determine peroxide concentrations. The molar absorptivity of triiodide ion in IPA with and without added oil vehicle was determined to be 25,000 M^{-1} cm^{-1}, similar to the values of 24,000 and 27,000 M^{-1} cm^{-1} in aqueous systems without and with added surfactant, respectively, reported by Magill and Becker.

Degradation Product Isolation and Characterization

HPLC analyses indicated the formation of a single degradation product in all oil and emulsion formulations. The product, II, was initially isolated as a crystalline solid which formed spontaneously in a sesame oil solution of I (100 mg/mL) which had been stored for 2 months at 50°C. The solution was cooled to room temperature and filtered to collect the crystals, which were rinsed with cold isopropyl alcohol. Compound II was also synthesized by dissolving 600 mg of I in 60 mL of acetone at room temperature followed by the addition of 250 mg I_2. After approximately 5 min, water (30 mL) was slowly added with constant stirring, resulting in the formation of a white precipitate. The reaction mixture was cooled to −5°C and allowed to stand for approximately 2 hr, after which the suspension was filtered, and the solid was washed with cold acetone and air-dried at 90°C for 2 hr (mp, 103.2–103.7°C; yield, 88%). The product was characterized by HPLC, scanning UV spectrophotometry, and fast atom bombardment (FAB) mass spectrometry. The reconversion of the product to I was also monitored (using HPLC method 1) at room temperature in a solution containing 75% methonol/0.01 *M* borate buffer (pH 9.0) in both the presence and the absence of a 30-fold molar excess (4.25×10^{-4} *M*) of cysteine.

Solubility Determinations

The solubility of I was determined in a variety of solvents by adding an amount of drug well in excess of its estimated solubility to the solvent of interest in a glass vial. The sealed vials were rotated in a 25°C water bath for 1–5 days. Aqueous samples were filtered using a glass syringe fitted with a 0.45-μm syringe filter, diluted properly with HPLC mobile phase, and analyzed by HPLC (method 2). Emulsions and oil samples were filtered using a glass syringe fitted with a 1.2-μm syringe filter, diluted properly with HPLC mobile phase (the oils were initially diluted by dissolving 5 μL in 1 mL of isopropyl alcohol), and again assayed by HPLC (using both method 1 and method 2).

Preparation of Extemporaneous Emulsions

Extemporaneous emulsions containing 15 mg/mL of thiocarbamate were prepared using the commercially available fat emulsion Liposyn II 20% or Intralipid 20% by sterile filtering a concentrated solution of I (254 mg/mL) in either DMSO or 10/90 DMSO/triacetin into the emulsions using a 0.2-μm sterile filter [Millex-FG (Teflon membrane) filter unit, Millipore Corp., Bedford, MA] and a 22-gauge needle. The tip of the needle was inserted under the surface of the emulsion during the addition of the drug solution and the rate of addition was either 0.051 mL/min using an infusion pump (Havard Apparatus Model 901) or 0.8 mL/min by manual addition. The emulsions were either mechanically shaken or magnetically stirred during the addition of the concentrated drug solution, and after this solution was added, the emulsions were either magnetically stirred or mechanically rotated at 20 rpm and at temperatures ranging from 25 to 50°C. In cases where the emulsions were stirred, a 100-mL bottle of Intralipid 20% or a 200-mL bottle of Liposyn II 20% was opened, a 1-in. stir bar was dropped into the emulsion, and the bottle was resealed under vacuum. The emulsion bottles with stir bars inserted were then autoclaved for 30 min at 125°C prior to the addition of the drug solution. Concentra-

tions of I were monitored by HPLC as a function of time until all of the drug was dissolved.

Stability Studies

The stability of I in emulsions was monitored at 25 and 37°C for periods up to 60 days. Samples were placed in controlled temperature ovens, and at predetermined time intervals a 10-μL aliquot was aseptically removed, diluted to 25 mL with mobile phase, and analyzed by HPLC. Average particle diameters in the emulsions stored at 25°C were measured after one month by photon correlation spectroscopy.

The stability of I in various oils, triacetin and PEG 300 was determined at concentrations of 5–100 mg/mL. Thioglycolic acid (TGA) was added as an antioxidant to some samples at a concentration of 0.1–1.0% (v/v). The samples were stored in screw-capped glass vials at −5, 5, 25, 37, or 50°C and monitored for drug concentration versus time by HPLC. Aliquots (5 μL) were dissolved in 1 mL IPA in a 25-mL volumetric flask and diluted to the mark with mobile phase. The resulting cloudy dispersions were injected directly onto the HPLC column and analyzed using both HPLC method 1 and HPLC method 2.

Stability studies were also conducted in sesame oil saturated with either solid EDTA, cysteine, or solutions of 0.1 *M* EDTA at pH 7 or 0.1 *M* cysteine adjusted to pH 9. When solutions were used, equal volumes of sesame oil and the solution were stirred overnight, then allowed to phase separate for 8 hr. The sesame oil was then centrifuged, separated from the aqueous phase, and further dried using molecular sieves. The recovered sesame oil was clear and colorless as it was prior to treatment.

The results of these stability studies are expressed in terms of "shelf life," which is the t_{90} or the time when 90% of the initial amount of drug still remains. The actual numbers were the time when the concentration of I dropped to 90% of its initial value or extrapolation to 90% remaining assuming first-order degradation.

RESULTS AND DISCUSSION

Solubilization of I

The solubility of I in a variety of solvent systems is shown in Table I. This compound's solubility in water was found to be extremely low (1.5 μg/mL), well below the target solution concentration of 15 mg/mL. Since the molecule has no ionizable functional groups, pH adjustment had no effect on its aqueous solubility. Classical cosolvent approaches or complex formation using the water-soluble cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (HPCD), failed to yield the magnitude of increase in solubility required (≈10^4-fold). Approximately exponential increases in drug solubility were attained with increases in the volume fraction of organic solvent in various cosolvent systems, resulting in high solubilities in systems containing >50% of the organic solvent component, consistent with the expected behavior for highly lipophilic compounds in cosolvent systems (5). However, increases of only 2–3 orders of magnitude were possible in those solvent systems which would be considered pharmaceutically acceptable for intravenous injection. Complex formation with HPCD was also highly favorable, but extrapolation of the binding data obtained at low concentrations of HPCD, assuming 1:1 complexes, to the maximum attainable HPCD concentration of 1 g/mL [i.e., the aqueous solubility limit of HPCD (6)] yielded an estimated solubility of <3 mg/mL, still well below the target solution concentration.

Table I. Solubility of I in Various Solvents at 25°C

Solvent	Solubility (mg/mL)
Water	0.0015 (CV = 10%, *n* = 3)
40% EtOH/water	0.294[a]
40% PG/water	0.044[a]
PG/EtOH/water (20/30/50)	0.42 (CV = 3%, *n* = 2)
0.05 *M* HPCD	0.20[a]
Liposyn II 20%	16.3 (CV = 3%, *n* = 6)
Intralipid 20%	14.2 (CV = 9%, *n* = 6)
Intralipid 20% with 5% DMSO	15.7 (CV = 3%, *n* = 3)
Intralipid with 5% 10/90 DMSO/triacetin	18.7 (CV = 4%, *n* = 2)
Soybean oil	91 (CV = 5%, *n* = 2)
Sesame oil	105 (CV = 6%, *n* = 2)
DMSO	380
Triacetin	300

[a] Single point determination.

Because I is highly lipophilic, as demonstrated by its relatively high solubility in natural oils such as soybean oil or sesame oil (≈100 mg/mL), solubilization via incorporation into a parenteral lipid emulsion appeared to be a feasible approach. Experiments in 20% lipid emulsions (Liposyn II 20% and Intralipid 20%) confirmed that solubilities of approximately 15 mg/mL were possible. As the solubility in 20% lipid emulsions was found to be approximately 20% of that in the pure natural oils, it is likely that I resides primarily in the internal oil phase, with little contribution to the overall solution concentration provided by interfacially bound drug. In emulsions which also contained 5% of the organic solvent used for incorporating drug into the emulsions (i.e., DMSO or 10/90 DMSO/triacetin), the solubility of I increased significantly. This was particularly evident when the solvent was 10/90 DMSO/triacetin, where incorporation of triacetin into the internal oil phase increased its apparent solubilizing capacity by ≈30%.

Methods for Preparation of Extemporaneous Emulsions of I

To complete the early stages of evaluation of a drug candidate which can be solubilized for intravenous injection only by incorporation into an emulsion, preparation of extemporaneous formulations by adding a concentrated solution of the drug to a commercially available parenteral fat emulsion may be advantageous (2,3). A potential difficulty in the preparation of extemporaneous emulsions is the possible precipitation of drug prior to its incorporation into the oil phase and slow redissolution of the precipitate once formed.

Drug precipitation may occur if the drug concentration in the aqueous (external) phase during the addition process or for a period of time after addition exceeds its saturation solubility. Apart from considerations of the kinetics of crystal growth, three rate processes are likely to govern the external phase concentration–time profile: (i) the rate of addition of the concentrated drug solution into the emulsion, (ii)

the rate of dissolution of the solvent vehicle containing drug, and (iii) the rate of drug partitioning into the internal oil phase. Decreasing the rate of drug addition and selecting a solvent for the drug which dissolves slowly in water would be expected to reduce precipitation. The rate of solute transport into emulsion droplets is likely to be aqueous diffusion controlled (7) and, therefore, governed by the diffusion coefficient of the solute, the total surface area of the emulsion particles, and the solute activity gradient from the external phase to the internal phase.

In this study the extent of drug precipitation was monitored by observing the drug concentration in solution just after adding the concentrated drug solution (the zero-time points in Fig. 1). Addition of a solution containing 250 mg/mL of I in DMSO (Fig. 1c) appeared to result in more precipitation during the addition than when the drug was in 10/90 DMSO/triacetin (Fig. 1b). Drug-loaded droplets of 10/90 DMSO/triacetin dissolved more slowly in water than DMSO, due to the limited solubility of triacetin in water. This may have allowed more time for drug uptake into the lipid emulsion droplets prior to crystallization.

The rates of drug redissolution were monitored versus time while emulsions were either stirred magnetically or mechanically rotated at 20 rpm at temperatures ranging from 25 to 50°C. Some representative redissolution profiles are depicted in Fig. 1. These profiles were fitted by Eq. (1), which describes an apparent first-order approach to the final emulsion concentration.

$$y = y_o + (100 - y_o) \cdot (1 - e^{-kt}) \quad (1)$$

where y is the percentage dissolved at time t, and y_o is the percentage dissolved immediately after drug addition (t_o). Apparent first-order rate constants for redissolution, k, were calculated by fitting the data in Fig. 1 to Eq. (1) by nonlinear least-squares regression analysis. Values for y_o and k are listed in Table II. The relative values of k indicated that magnetic stirring at 50°C was most effective for obtaining rapid redissolution. Once drug precipitation has occurred, the rate of drug incorporation into the oil phase, which depends on the solute concentration gradient from the external phase to the surface of emulsion particles, will be determined partially by the rate of dissolution of solid drug into the external phase. Higher temperatures increase the water solubility of I, and thereby the driving force for uptake into emulsion droplets, and also increase the drug's diffusion coefficient. Stirring may be more effective than rotation in decreasing the aqueous boundary layer thickness surrounding solid particles, which would facilitate dissolution.

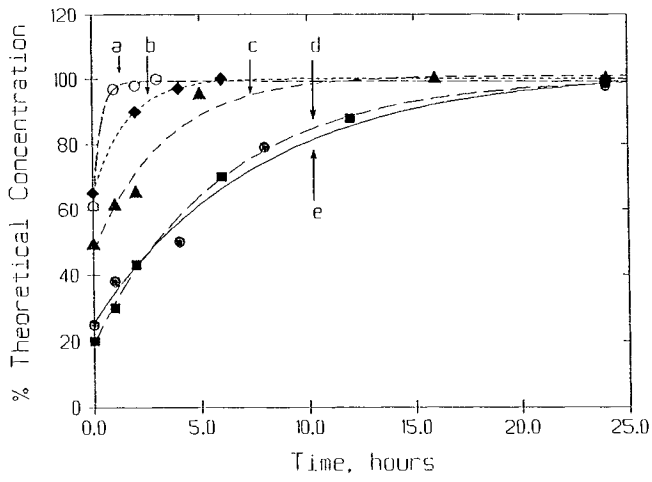


Fig. 1. Drug concentration (% of equilibrium concentration) in extemporaneous emulsions (20% lipid) vs time after addition of drug concentrate using various methods of preparation: (a) 10/90 DMSO/triacetin added at 0.8 mL/min with stirring at 50°C, followed by stirring at 50°C; (b) 10/90 DMSO/triacetin added at 0.051 mL/min with shaking at 25°C, followed by 20 rpm rotation at 50°C; (c) same as b except using DMSO; (d) DMSO added at 0.8 mL/min with stirring at 25°C, followed by 20-rpm rotation at 37°C; (e) same as d except rotation at 25°C.

These emulsion incorporation studies illustrate the need to verify the solution concentration of drug when preparing extemporaneous emulsions, even when the theoretical drug concentration is well below the equilibrium solubility of the drug in the emulsion. Depending on the method of drug addition, temperature, and degree of agitation of the emulsion, several hours or days may be necessary to reach complete solubilization of highly lipophilic, water-insoluble drugs. As an extra precaution, extemporaneous emulsions containing drug for parenteral administration should be filtered during their intravenous administration to prevent any residual solid drug particles from entering the bloodstream.

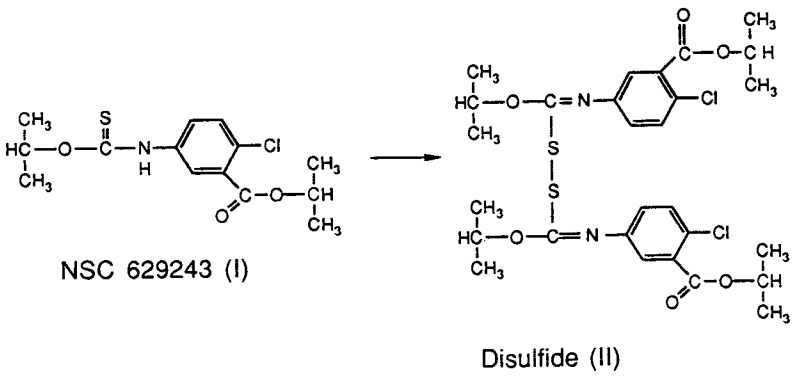
Degradation Product Characterization

Several lines of evidence suggested that the product formed from the thiocarbamate in natural oils and emulsion formulations (see below) was the disulfide dimer, II (Scheme I). By HPLC, there was no appearance of any hydrolysis products and the single decomposition product exhibited a longer reverse-phase HPLC retention time than I, consistent with its expected increased lipophilicity. The wavelength of maximum absorbance, λ_{max}, was dramatically shifted from a value of 283 nm for I to ca 248 nm for II, suggesting an alteration in the C=S functionality. The FAB mass spectra of I and II were almost identical except for a peak at 629 in the mass spectrum of II, approximately twice the molecular weight of the parent compound. A large ion peak at 316, the molecular weight of I, was observed in the spectra of both compounds, indicating that I was formed from II in the ionization chamber, presumably as a result of the weak disulfide bond in II that generates I upon cleavage.

Table II. Redissolution Rate Constant (k) and Initial Concentration (y_o) of I in Extemporaneous Emulsions Obtained from Fitting the Data in Fig. 1 According to Eq. 1

Preparation method (see Fig. 1)	k (hr^{-1})	y_o (% dissolved at t_o)
A	2.69 (0.48)[a]	61.0 (1.0)[a]
B	0.614 (0.029)	65.0 (0.5)
C	0.296 (0.081)	47.0 (4.7)
D	0.162 (0.009)	19.5 (1.0)
E	0.127 (0.038)	25.7 (4.8)

[a] Values in parentheses are standard deviations.



Scheme I. Proposed reaction of NSC 629243, I, in oil formulations and in emulsions leading to the formation of the disulfide dimer, II.

Since disulfides undergo exchange in the presence of a thiol, exposure of II to a thiol-containing reagent such as cysteine would be expected to result in the formation of I. The reaction of II in 72/25 methanol/0.01 *M* borate buffer at pH 9.0 in the absence of cysteine resulted in the formation of I, but the conversion was slow, with a t_{90} of ≈0.5 hr. In the presence of a ≈30-fold molar excess of cysteine the reaction was complete within 10 min, resulting in quantitative conversion of II to I, providing further evidence that the degradation product II is the disulfide dimer shown in Scheme I.

Stability of I in Lipid Emulsions and in Natural Oils

The stability of drugs which are susceptible to oxidation is an important concern in lipid emulsion formulations because the natural oils used as the internal oil phase may contain trace quantities of peroxides or other free radical initiators which may promote oxidation. Since ≥99.9% of I resides in the internal oil phase, studies of the stability of I were conducted in natural oil vehicles as well as in emulsion formulations.

Natural Oils. As shown in Table III, the stability of I in natural oils was highly variable, depending on the oil, the supplier, and the date of manufacture. The only degradation product detected in all of these oils was the disulfide dimer, II. The variability of the shelf life can be seen in the sesame oil data (Table III), where the shelf life at 25°C varied from <1 to >100 days. A qualitative correlation was observed between shelf life and peroxide concentration. Thus the aged Croda sesame oil without preservative had a peroxide concentration of 0.0061 *M* and exhibited the poorest drug stability, while the fresh Croda sesame oil with preservative had a peroxide concentration of 0.0012 *M* and exhibited the best drug stability of all the sesame oils tested. The drug concentration in these experiments was 50 mg/mL (0.16 *M*), while the highest observed peroxide concentration is 0.0061 *M*. Thus, it appears that peroxides did not react directly with I but probably served as initiators of the oxidation.

The kinetics of the reaction in both the presence and the absence of antioxidants were distinctly biphasic, with an initial rapid loss of drug followed by a much slower phase (Figs. 2 and 3). This kinetic behavior may suggest that a reactant is consumed in the rapid initial phase and only slowly replenished in the subsequent phase of the reaction. Further studies are in progress to more completely explain these complex kinetics.

The effect of added antioxidants on the stability of I in sesame oil was investigated. The aged sesame oil was used in these screening studies, for if I could be stabilized in this vehicle, which exhibited the worst stability and the highest peroxide concentration, then it should be stabilized in other oils as well. The results are summarized in Table IV. The antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are nearly insoluble in sesame oil and did not improve the stability of I. Vitamin E (0.1%) improved the stability but there was still ≈15% disulfide formation after 1 day at 50°C. EDTA and the thiol containing amino acid cysteine were studied either by saturating sesame oil with solid disodium EDTA or cysteine or by saturating the sesame oil with solutions of EDTA at pH 7 or cysteine at pH 9. The results of these studies are shown graphically in Fig. 2. EDTA and cysteine improved the stability of I, but not to the extent required. These limitations may be due to

Table III. Estimated Shelf Lives of I (50 mg/mL Initial Concentration) and Peroxide Concentrations in Various Vehicles at −5 and 25°C

Vehicle	Brand	Shelf life (days) −5°C[a]	Shelf life (days) 25°C	Peroxide conc. (*M*)
Sesame	USP, Spectrum	35	30	0.0016
Sesame	Sigma	10	2	0.0039
Sesame	Croda, aged[b]	3	<1	0.0061
Sesame	Croda, fresh[c]	—	>100	0.0012
Safflower	Croda	<1	<1	0.0026
Soybean	Croda	<1	<1	0.0042
Soybean	USP, Spectrum	>100	>100	0.0010
Cottonseed	USP, Spectrum	>100	>100	0.00063
Peanut	USP, Spectrum	>100[d]	>100	0.00032
Triacetin	Sigma	>210	>210	<0.00003
PEG 400	USP, Spectrum	>240	>240	<0.00003

[a] All oil formulations freeze at −5°C but become clear when warmed up to room temperature.
[b] Manufactured 2 years prior to study, without preservative.
[c] Manufactured 4 months prior to study, with BHT added as the antioxidant preservative.
[d] Some I remained undissolved when warmed to room temperature but dissolved with mild heating.

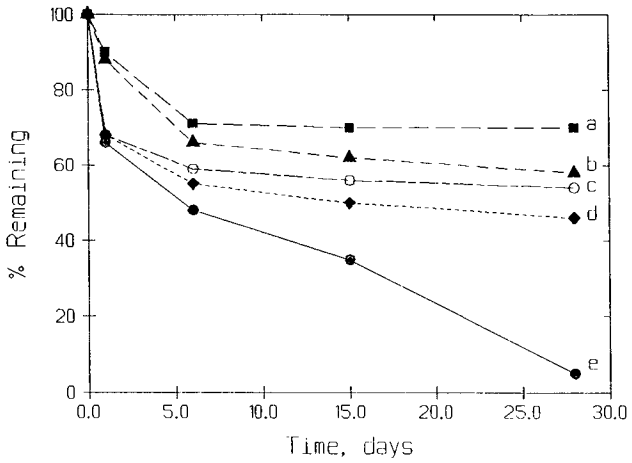


Fig. 2. Concentration of I versus time in aged sesame oil in the presence or absence of antioxidant at 50°C. (a) Saturated with 0.1 *M* cysteine at pH 9; (b) saturated with 0.1 *M* EDTA at pH 7; (c) saturated with solid EDTA; (d) saturated with solid cysteine; and (e) no antioxidant.

the difficulty of achieving sufficiently high concentrations of these oil-insoluble additives in sesame oil.

Since washing with an aqueous cysteine solution yielded the greatest improvement of the antioxidants tried initially, we postulated that a thiol with greater solubility in sesame oil would stabilize I to a greater extent. Additional thiols studied were monothioglycerol, thioglycolic acid (TGA), and *N*-acetylcysteine. The solubilities of thioglycerol and *N*-acetylcysteine were less than 1% (v/v) in sesame oil, but TGA was soluble at ≥1% (v/v). As shown in Table IV, none of the thiols were sufficiently effective at the 0.1% level. However, as the percentage of TGA increased, the stability increased, and in the presence of 1.0% TGA, I was very stable. A long-term stability study of I in USP sesame oil containing 1% TGA showed that the shelf life was more than 1 year at 5 to 37°C (102, 100, and 98.5% remaining after 1 year at 5, 25, and 37°C, respectively) and about 1 year at 50°C (88.8% remaining after 1 year at 50°C). Decomposed formulations were clear, but often slightly yellow. The mechanism by which TGA prevented the oxidation of I is not yet

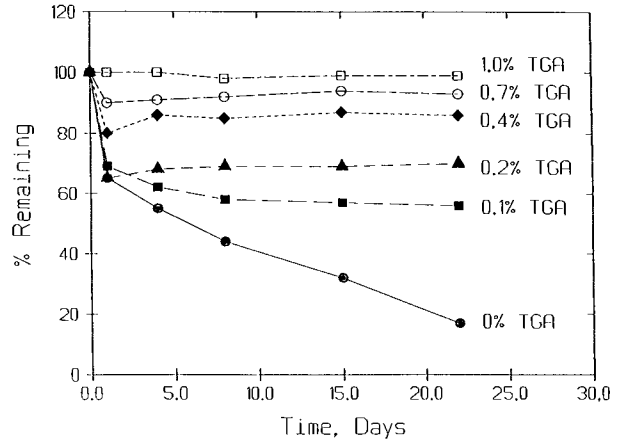


Fig. 3. Concentration of I versus time in aged sesame oil solutions containing various concentrations of thioglycolic acid at 50°C.

Table IV. Stability of I in Aged Sesame Oil[a] with and Without Added Antioxidant

Antioxidant	% remaining at 1 day and 50°C	Shelf life at 50°C (days)
None	76	<1
0.02% BHA	77	<1
0.02% BHT	78	<1
0.10% BHT	72	<1
0.10% vitamin E	85	<1
0.01 *M* EDTA, pH 7[b]	87	<1
0.005 *M* cysteine, pH 9[b]	86	<1
0.1% thioglycerol	65	<1
0.1% *N*-acetylcysteine	61	<1
0.1% TGA[c]	70	<1
0.2% TGA	66	<1
0.4% TGA	80	<1
0.7% TGA	89	[d]
1.0% TGA	100	>360

[a] Croda Super-Refined NP manufactured 2 years prior to this study.
[b] Solutions used to saturate the sesame oil (see Materials and Methods).
[c] Thioglycolic acid.
[d] Percentage remaining reached a plateau at ca. 90% (see Fig. 3).

known. TGA may serve as a free radical scavenger by undergoing oxidation itself or it may react with the disulfide dimer formed to regenerate I.

Twenty Percent Emulsions. Table V illustrates the stability of extemporaneous emulsions containing 5.0–15 mg/ml of I which were prepared from either Liposyn II 20% or Intralipid 20%. When these emulsions were prepared and stored in vacuum-sealed bottles, >98% of the initial drug concentration remained after 60 days of storage at 25°C. Emulsions prepared and stored exposed to the air exhibited substantially diminished shelf lives (≈2 weeks).

Although the chemical stability of I in extemporaneous emulsions was excellent when care was taken to exclude oxygen, a few small yellow droplets had collected on the top of the emulsions after 1 month at 25°C. After 60 days the emulsions appeared the same, suggesting no further phase separation. The mean particle size in emulsions, as determined by PCS, exhibited no significant change with time. An Intralipid 20% emulsion containing drug exhibited a mean diameter of 364 ± 7 nm after 3 days and 350 ± 6 nm after 31 days at 25°C. A lot of Liposyn II 20% with drug added exhibited a mean particle size of 275 ± 5 nm after 3 days and

Table V. Shelf Life of I in Lipid Emulsions at 25 and 37°C

Emulsion	mg/mL	Shelf life (days) 25°C	Shelf life (days) 37°C
Intralipid 20%	15	>60[a]	—
Liposyn II 20%	15	>60[a]	—
Intralipid 20%	15[b]	>14[c]	>14[c]
Liposyn II 20%	12.5[b]	8–10	4–8
Liposyn II 20%	5.0[b]	8–10	4–8

[a] More than 98% of drug remaining.
[b] Stored exposed to air.
[c] No detectable loss of drug after 14 days.

271 ± 4 nm after 31 days at 25°C. Particle sizes for Intralipid 20% and Liposyn II 20% without drug were 466 ± 6 and 261 ± 1 nm, respectively. Differences in mean particle size in Intralipid and Liposyn emulsions have been reported previously (8). The reason for this difference is unknown. The significant size decrease which occurred on the incorporation of drug into Intralipid 20% also occurs when a nonaqueous vehicle (e.g., DMSO) without drug is added to an Intralipid 20% emulsion (8). The cause of this phenomenon is under investigation.

These stability studies demonstrate that thiocarbamates may undergo oxidation to form disulfide linked dimers. This reaction was found to be particularly important in solutions of the thiocarbamate, I, in natural oils and in lipid emulsion formulations. The rate of oxidation in oils was found to vary dramatically, depending on the identity, age, and source of the natural oil, and could be nearly completely inhibited using 1% thioglycolic acid. In lipid emulsions, exclusion of oxygen was essential for long-term stability. Further studies of the mechanism of disulfide dimer formation from thiocarbamate-containing drugs are in progress in these laboratories.

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