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SOME FORMULATION PROPERTIES OF LAPACHOL, A POTENTIAL ONCOLYTIC AGENT OF NATURAL ORIGIN

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ABSTRACT

Lapachol is a naphthaquinone of natural origin with reported oncolytic activity. However, earlier antitumor studies were inhibited by inadequate blood levels, allegedly due to formulation difficulties.

This present study shows that water solubility is markedly influenced by pH, varying from 1.5 μg/mL at pH 4.0 to 5 mg/mL at pH 10.0.

Evaluation of mixed solvent systems demonstrated that up to 30 mg/mL could be dissolved in polyethylene glycol (PEG) 400. Aqueous PEG 400 solutions of lapachol were stable at refrigerator temperatures but deteriorated when exposed to light or autoclaving. Aqueous ethanol or propylene glycol

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are realistic alternative solvent systems for injectable lapachol solutions that may be sterilized by autoclaving.

Phosphatide stabilized triglyceride emulsions are broken down by lapachol, suggesting an interfacial interaction between the phosphatide and the lapachol in the aqueous phase.

INTRODUCTION

Lapachol is a well-known naphthaquinone found in several plant species of the family Bignoniaceae used to treat cancer in South America and India[1,2,3]. Lapachol and related compounds have demonstrated antimalarial, antibacterial and antitrypanosomal activity[4,5,6]. Lapachol shows antitumor activity in the Walker-256, Murphy-Sturn and Sarcoma-180 tumor systems and in KB cell culture; related naturally occurring compounds and lapachol derivatives are active in other tumor systems[7,8]. Proposed mechanisms of antitumor action for lapachol include uncoupling of oxidative pphosphorylation[9], inhibition of glyoxalase metabolism[10], and inhibition of pyrimidine biosynthesis[11].

On the basis of its strong activity in the Walker-256 tumor system[3], lapachol entered the National Cancer Institute clinical testing program[12]. In human trials, oral lapachol administration caused nausea and vomiting. Prothrombin time prolongation was also observed, but could be counteracted with vitamin K administration. Significant antineoplastic effects were absent. It

was observed that despite oral doses of up to 50 mg/kg/day, plasma levels of lapachol never reached the level of 30 g/mL calculated to be critical for antitumor activity. Inadequate plasma concentrations were ascribed to lapachol's low water solubility and to possible deficiencies in human intestinal absorption of the drug[12]. The formulation difficulties posed by lapachol were never resolved, and the compound was dropped from the clinical testing program. Further complicating lapachol's formulation profile is its irritability during storage, resulting from the conversion of lapachol to dehydro-α-lapachone following exposure to light and air[13]. This present investigation re-examines the formulation properties of this potentially useful compound.

MATERIALS AND METHODS

Lapachol: Aldrich, Milwaukee, Wisconsin, lot number 545816-3.

In addition, commercial material prepared by the Laboratorio Farmaceutico do Estado de Pernambuco, Brazil, was supplied by Dr. N. R. Farnsworth (Program for Collaborative Research in the Pharmaceutical Sciences) as capsules containing 250 mg lapachol diluted with lactose. Lapachol was recovered by removal of lactose with water, filtration and recrystallization from absolute ethanol. Purity and identity of the compound was confirmed by standard melting point, mixed melting point, infrared and ultraviolet spectra, alone and mixed, and NMR and mass spectra methods using authentic lapachol as a reference material.

Solvents: polyethylene glycol (PEG) 400, glycerol, and propylene glycol, Fisher Scientific, Itasca, Illinois used as received. Absolute alcohol was obtained from Aaper Alcohol and Chemical, Shelbyville, Kentucky. Water/solvent systems were made by volume.

Water was triple glass distilled. Buffer systems were prepared from 0.1 M citric acid with appropriate amounts of 0.2 M disodium phosphate using a glass electrode pH meter, Fisher Model #815 MP.

Food grade soybean oil: Dominick's, Chicago, Illinois, lot number 73630-01550.

Analysis: Ultraviolet absorption curves were recorded by scanning between 185-370 nm using a Perkin-Elmer model 200 spectrophotometer attached to a chart recorder. Standard curves were prepared using known amounts of lapachol in the appropriate solvent system. The absorbance was measured at 276 nm. Unknowns were analyzed by reference to the standard curves. All standard solutions were stored protected from light in the refrigerator prior to use and were used within four hours of preparation to minimize the influence of any possible deterioration.

Solubility: Lapachol was powdered and added in excess to approximately 3 mL of the appropriate solvent prior to sonication in a Heat Systems-Ultrasonics, Inc. ultrasonic bath for 1 hour. The suspension, protected from light, was then shaken at 25.0°C (±0.5°) for 24 hours in an

Eberbach water bath shaker. The sample was centrifuged and the supernatant assayed spectrophotometrically as described.

Stability: The stability of lapachol solutions was evaluated by comparing the U.V. absorbance spectra of the solutions with that of appropriate controls. Solutions were exposed to autoclaving in sealed glass ampules at 121°C for 20 minutes. Stability was also evaluated at ambient laboratory temperature (25°C); solutions were either protected from light or exposed to light.

Emulsions: Triglyceride emulsions were prepared by 30 seconds sonication of the following formulation, using a Branson Model Sonifier 350.

Lapachol	0.06 g
Soybean oil	10.0 g
Purified egg phosphatides (Hepar Industries, lot number EY10284)	1.2 g
Glycerol	2.5 g
Water to	100 mL

Two formulations were made by adjusting the pH of the emulsion to pH 6.0 and 8.5, respectively using 0.1M hydrochloric acid and sodium hydroxide solutions as required.

RESULTS

Solubilities.

The solubility curve for lapachol in aqueous buffer systems is shown in Fig. 1 and is representative of a typical salt-forming material.

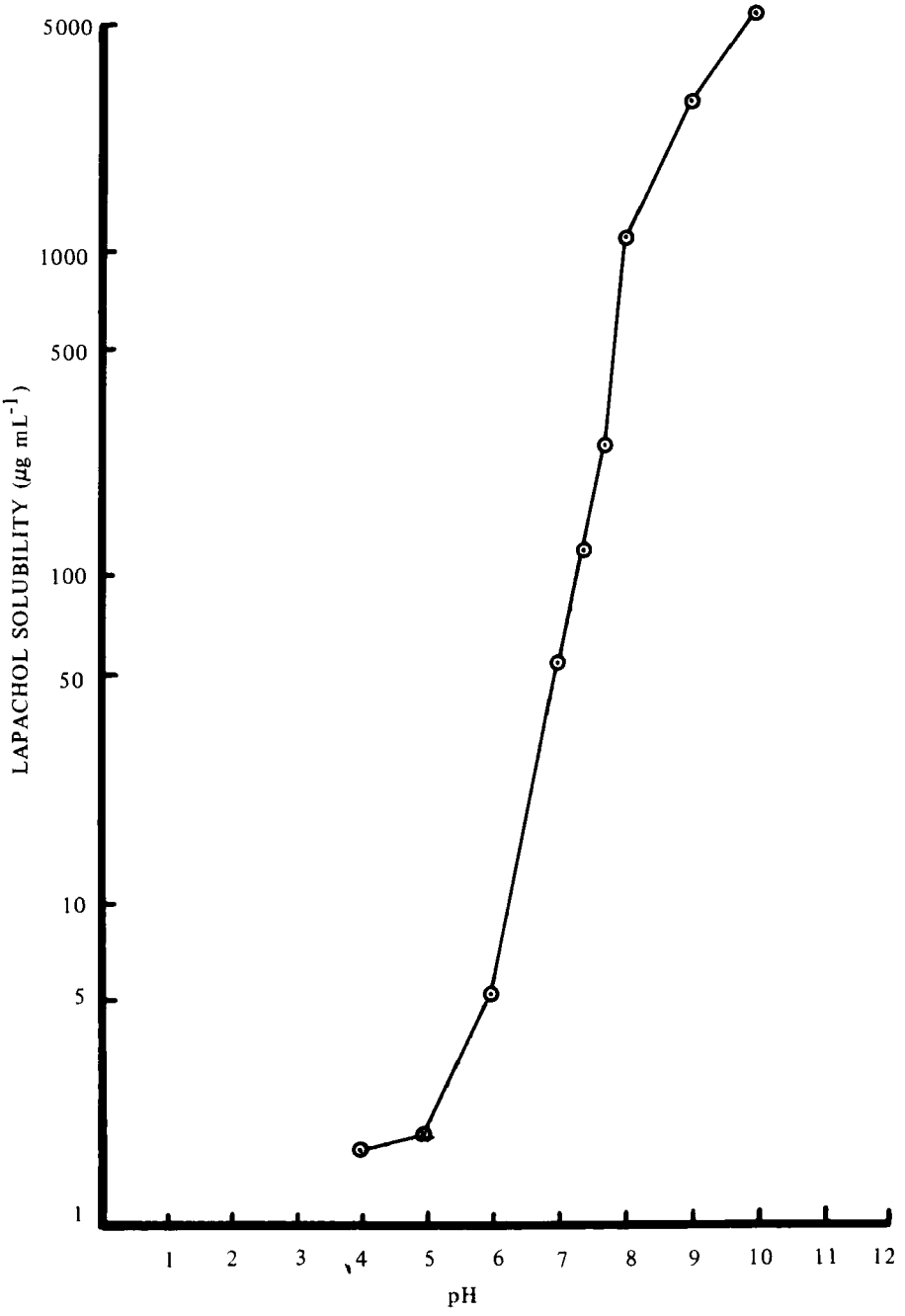

FIGURE 1

Aqueous solubility of lapachol at 25°C vs. pH.

TABLE 1

Saturated Solubilities of Lapachol at 25°C in Various Solvents

	Solubility (mg/mL)
Water (pH 5.0)	0.002
(pH 8.0)	1.1
Ethanol	16.0
Glycerol	0.06
PEG 400	31.0
Propylene Glycol	4.4
Soybean Oil	6.9

Saturated solubilities of lapachol in various solvents are shown in Table 1.

In general, aqueous solvent mixtures demonstrated the Hildebrand-Scott[14] semilogarithmic relationship between solvent concentration and concentration, Yalkowsky et al.[15]. Parameters are shown in Table 2. However, glycerol/water and propylene glycol/pH 7.4 buffer systems did not fit this relationship. The respective solubility curves are shown in Fig. 2. The glycerol may affect the apparent pH of this solvent system in a nonlinear fashion which would account for the curvature observed. The considerable enhancement of solubility induced by propylene glycol in water buffered at pH 7.4 is sufficient for the solubility to exceed the saturated propylene glycol

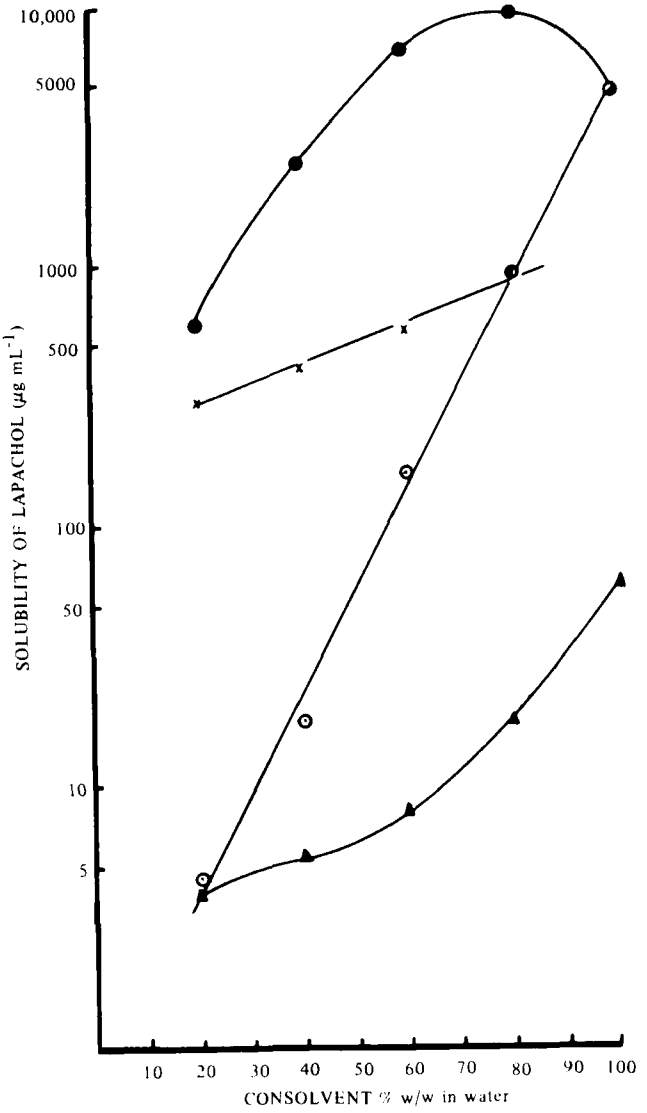

FIGURE 2

Solubility of lapachol at 25°C in water or aqueous buffer pH 7.4/glycerol and water or aqueous buffer pH 7.4/propylene glycol systems.

Key: ▲—▲ water-glycerol

x - x aqueous buffer pH 7.4/glycerol

⊙ - ⊙ water - propylene glycol

●—● aqueous buffer pH 7.4/propylene glycol

TABLE 2

Lapachol Hildebrand-Scott Solubility Parameters for Mixed Solvent Systems at 25°C

Log y = log a + bx

where y = concentration of lapachol
a = intercept
b = slope (solubility parameter)
x = concentration of solvent in water

Solvent System	(a)+	(b)	r^2*
PEG 400/water	1.0	0.10	1.0
Ethanol/water	7.6	0.08	0.99
Propylene glycol/water	0.7	0.09	1.0
Glycerol/pH 7.4 buffer	205.0	0.02	1.0

+represents solubility in water (variable according to pH, Fig. 1)

*coefficient of determination

solubility. This enhancement accounts for the deviation from linearity experienced in this case.

Stabilities.

The ratio of the absorbance at 276 nm and 254 nm for control and test solutions was a convenient indicator of stability. Data are shown in Tables 3 through 6. It is evident that within the observed

TABLE 3

Absorbance Ratios (Test:Control) at 254 and 276 nm for Lapachol Solutions Stored in the Dark Under Refrigerator Conditions (4-5°C) for 48 and 164 Hours

Solvent System	Wavelength 254 nm 48 hours	164 hours
PEG 400	1.00	1.04
PEG 400:water 50:50	0.99	1.02
Propylene glycol	0.98	1.01
Propylene glycol:water 50:50	0.98	0.96
Ethanol	0.93	1.14
Ethanol:water 50:50	1.02	1.04
Glycerol	1.03	1.04
Glycerol:water 50:50	0.94	0.92
Buffer pH 7.7	1.02	1.03

Solvent System	Wavelength 276 nm 48 hours	164 hours
PEG 400	1.01	0.99
PEG 400:water 50:50	0.98	0.98
Propylene glycol	1.00	1.02
Propylene glycol:water 50:50	0.96	0.96
Ethanol	1.09	0.83
Ethanol:water 50:50	1.01	1.06
Glycerol	1.08	1.05
Glycerol:water 50:50	1.00	1.01
Buffer pH 7.7	1.01	1.01

TABLE 4

Absorbance Ratios (Test:Control) at 254 and 276 nm for Lapachol Solutions Stored at Ambient Room Temperature (25°C) Protected From Light for 48 and 164 Hours

Solvent System	Wavelength 254 nm 48 hours	164 hours
PEG 400	1.03	0.99
PEG 400:water 50:50	1.01	0.97
Propylene glycol	1.05	1.02
Propylene glycol:water 50:50	1.02	1.00
Ethanol	1.07	1.09
Ethanol:water 50:50	1.01	0.94
Glycerol	1.04	1.05
Glycerol:water 50:50	ND	0.85
Buffer pH 7.7	0.98	1.03

Solvent System	Wavelength 276 nm 48 hours	164 hours
PEG 400	0.99	0.94
PEG 400:water 50:50	1.00	0.95
Propylene glycol	1.07	1.04
Propylene glycol:water 50:50	1.01	0.96
Ethanol	0.97	0.94
Ethanol:water 50:50	1.00	1.05
Glycerol	1.05	1.10
Glycerol:water 50:50	ND	1.12
Buffer pH 7.7	1.01	1.01

ND = not determined

TABLE 5

Absorbance Ratios (Test:Control) at 254 and 276 nm for Lapachol Solutions Stored at Ambient Room Temperature Exposed to Light for 48 and 164 Hours

Solvent System	Wavelength 254 nm 48 hours	164 hours
PEG 400	0.95	0.84
PEG 400:water 50:50	0.86	0.54
Propylene glycol	0.98	0.90
Propylene glycol:water 50:50	0.94	0.80
Ethanol	1.05	0.90
Ethanol:water 50:50	0.81	0.84
Glycerol	0.96	0.88
Glycerol:water 50:50	0.91	0.74
Buffer pH 7.4	1.02	1.03

Solvent System	Wavelength 276 nm 5 hrs	48 hrs	164 hrs
PEG 400	1.05	1.10	1.24
PEG 400:water 50:50	0.97	0.88	0.58
Propylene glycol	1.05	0.99	0.91
Propylene glycol:water 50:50	0.97	1.03	1.07
Ethanol	1.00	0.93	0.84
Ethanol:water 50:50	1.00	1.13	1.07
Glycerol	0.97	1.41	1.92
Glycerol:water 50:50	1.00	1.06	1.18
Buffer pH 7.4	1.00	1.01	1.01

TABLE 6

Absorbance Ratios (Test:Control) at 276 nm for Autoclaved Lapachol Solutions (121°C for 20 minutes)

Solvent System	Ratio at 276 nm
PEG 400	0.34
PEG 400:water 50:50	0.36
Propylene Glycol	1.0
Propylene Glycol:water 50:50	1.05
Glycerol	0.97
Glycerol:water 50:50	0.97
Buffer pH 7.4	1.04

repeatability of better than 10% for the assay, lapachol solutions are stable under most conditions. However, a marked spectral shift is seen for unbuffered aqueous and non-aqueous solutions when exposed to daylight suggesting deterioration. PEG 400 solutions also appeared to deteriorate following autoclaving, as shown in Fig. 3.

Emulsions.

Although lapachol was theoretically present at a level sufficient to be completely soluble in the triglyceride phase, both the pH 6.0 and the pH 8.5 emulsions had partially cracked within a few hours of preparation. In addition, the color of the aqueous phase demonstrated that lapachol was present in the water component of the emulsions. These observations

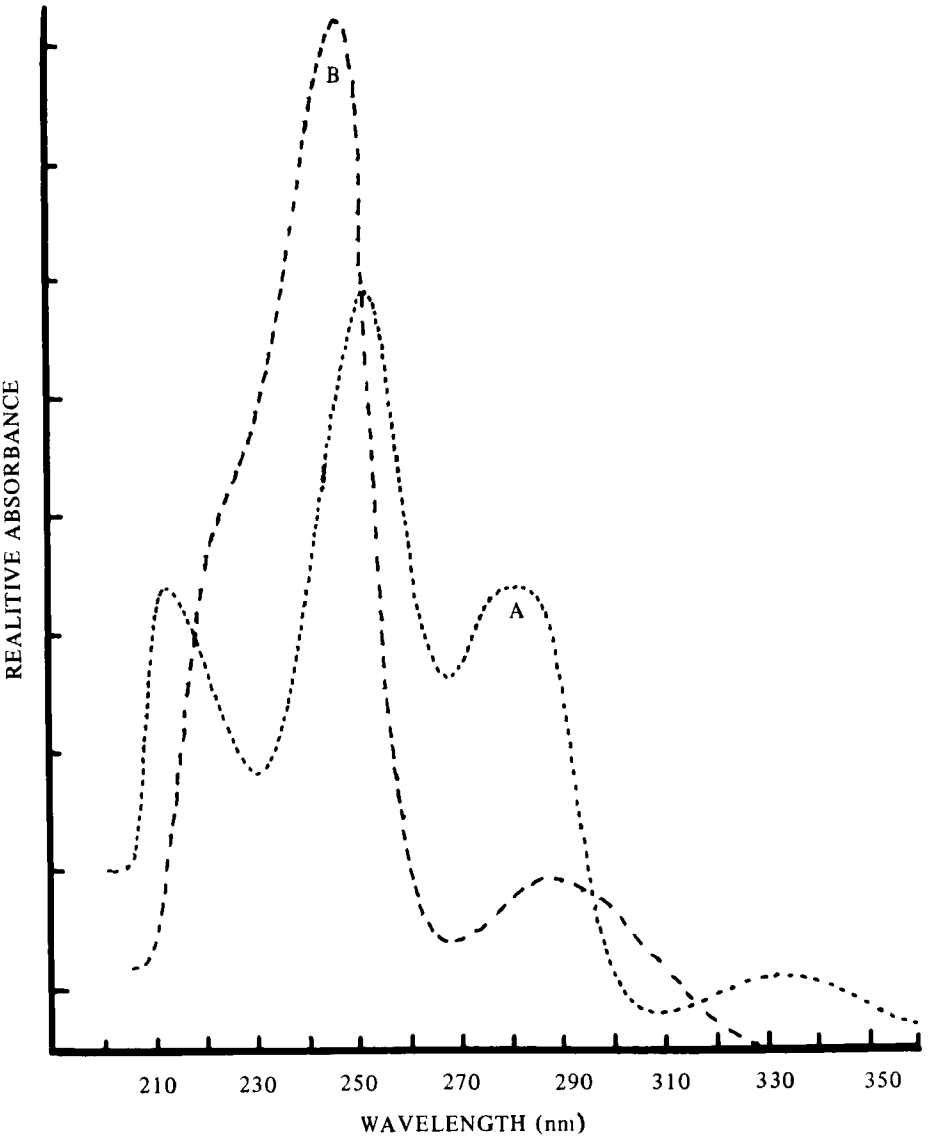

FIGURE 3

Absorbance spectra of lapachol solutions in PEG 400 (100%).

A before autoclaving

B after autoclaving

suggest that the compound may interfere with the interfacial stabilization mechanism promoted by the phosphatides[16].

DISCUSSION

Although lapachol has a water solubility curve typical of a compound with an ionizable hydroxyl, the solubility at physiological pH 7.4 is low (~0.1 mg/mL) and cannot be improved sufficiently by buffering to pH's acceptable for intravenous injection. Although physiologically acceptable solvents increase the solubility considerably, when solutions of lapachol in these solvents are diluted in water the compound is precipitated out as coarse crystals. However, if the dilution is made into aqueous buffer at pH 7.4 the compound stays in solution, suggesting that there should be no precipitation during intravenous administration.

The optimum solvents are propylene glycol or ethanol admixed with aqueous buffer, allowing a concentration containing ~10 mg/mL to be prepared for transport. The drug would be administered to the patient by slow intravenous drip using saline or dextrose as a diluent.

A higher concentration of lapachol may be obtained by using aqueous buffered PEG 400 solutions. However, the labile nature of the compound in this system suggests that a sterile filtration process and doubtful stability may be concomittant disadvantages.

Organ targeting by the administration of lapachol dispersed in an intravenous emulsion

system[16] is possible although the lapachol interferes with the interfacial stabilization of the emulsion. However, addition of lapachol to a sterile, preformed, emulsion would appear to be feasible and requires further evaluation. Additional emulsion stabilizers may also improve the system.

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