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A Cubic-Phase Oral Drug Delivery System for Controlled Release of AG337

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ABSTRACT

AG337 is a novel thymidylate synthase inhibitor possessing potent antitumorogenic activity which was designed using protein structure-based techniques. It is currently undergoing clinical trials as both IV and oral formulations. Based on AG337's short in vivo half-life, an oral controlled-release formulation is desired. The feasibility of using cubic liquid crystalline phases formed from monoolein for controlled release of AG337 has been investigated in this study. AG337 (m.p. = 298°C) was triturated with glycerol and then dissolved in monoolein using mild heat. The resulting gel was liquified by further heating to 65°C, then cooled to RT to yield a clear viscous solution. Samples of the formulation were exposed to water for up to 48 hr at 25°C. Thermal analysis of this system was undertaken in order to determine the effect of hydration state on the liquid crystalline structure. The differential scanning colorimetry (DSC) profile of samples not exposed to water showed no distinct endo- or exothermic transitions. However, samples exposed to water exhibited multiple endothermic transitions from 80° to 120°C. These data demonstrate a thermal response to time-dependent water uptake in the formulation as might occur in vivo after oral dosing, due to changes in physical properties of the system. In vitro release rates of AG337 from this formulation were evaluated.

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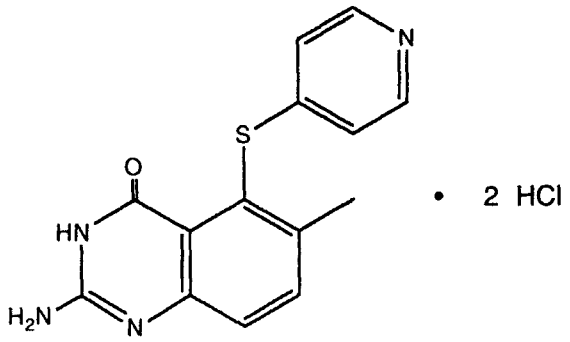
INTRODUCTION

AG337 (Scheme 1) is a novel thymidylate synthase inhibitor possessing potent antitumorogenic activity which was designed using structure-based techniques. It is highly water soluble and exhibits nearly 100% oral bioavailability in animal models. However, due to its short in vivo half-life (2–3 hr), an oral controlled-release formulation is desired.

Glyceryl monooleate is a polar compound with a waxy texture and a melting point of 34°–36°C. It is a distilled product of glycerolysis of refined erucic canola oil. In the presence of water, this monoglyceride is known to swell and form several lyotropic liquid crystalline structures in which the molecules are ordered in hexagonal, lamellar, or cubic arrays (1,2). The cubic-phase system is at equilibrium and forms a viscous gel in which water channels are surrounded by saddlelike lipid bilayers of the monoolein. These physical properties have given rise to the rationale for its potential to act as a controlled-release matrix for either lipophilic or hydrophilic drugs (3). Upon contact with an aqueous environment, the monoglyceride swells and forms a gel that will release a dissolved or dispersed drug by slow diffusion. In principle, the swelling mechanism should be unaffected by the pH of the bulk aqueous environment.



2-Amino-3,4-dihydro-6-methyl-4-oxo-5-(4-pyridylthio)-quinazoline dihydrochloride

$C_{14}H_{14}N_4Cl_2OS$

MW = 357.26

Partition Coefficient: log D @ pH 7.4 = 1.85

Scheme 1. Structure and selected physicochemical properties of AG337.

The objective of this work is to demonstrate the feasibility of a prototype controlled-release oral delivery system for AG337 utilizing a cubic lipid–water phase consisting of glyceryl monooleate. Specifically, the following characteristics were evaluated:

1. Ability to incorporate AG337 into glyceryl monooleate.
2. Thermal behavior by differential scanning calorimetry (DSC) or glyceryl monooleate with mixtures of AG337 and water, and with other additives such as glycerin.
3. Release rate profiles for AG337 from preformed cubic-phase gels and from gels formed in situ in the presence of simulated gastric fluid.

MATERIALS AND METHODS

Materials

AG337 bulk drug substance was manufactured at Agouron Pharmaceuticals, Inc. (San Diego, CA). Glycerol monooleate (Myverol® 18-99) was from Eastman Fine Chemicals (Kingsport, TN). Glycerin was obtained from Sigma Chemicals (St. Louis, MO).

UV Assay

An ultraviolet (UV) scan for AG337 dissolved in USP simulated gastric fluid without pepsin (SGF) showed absorbance peaks at 300.8, 227.6, and 203.6 nm. Samples with a known concentration of AG337 dissolved in SGF were measured at 300 nm. Regression analysis of absorbance versus concentration demonstrated linearity throughout the concentration range tested, and this working curve was used to convert absorbance of unknown samples to concentration.

Formulation

The model formulation of AG337 studied in glycerol monooleate is shown in Table 1. AG337 was triturated with glycerin (specific gravity ≈ 1.26) with mild heat to effect solution. Glyceryl monooleate was warmed to ~55°C until fluid. The AG337/glycerin solution was dispersed into the monoolein with stirring and mild heat until a homogeneous solution was obtained. The final solution was cooled to room temperature with stirring

Table 1

Model Formulation for AG337

Chemical Name	Theoretical Quantity (% w/w)
AG337 (Agouron)	1.8
Glycerin, USP	7.6
Glyceryl monooleate	90.6

to form a clear, light brown gel which was stored in the refrigerator at 2°–8°C prior to use.

Differential Scanning Calorimetry

Thermal analyses of each of the components in the formulation, alone and in combination, were performed by differential scanning calorimetry. In addition, thermal analyses of the final formulation were performed after exposure to water for 1 hr and 48 hr at 25°C. The calorimeter (Shimadzu DSC-50) was operated at a heating rate of 5°C/min with a nitrogen purge maintained throughout each run. The system was calibrated with ultrapure indium to within ±0.1°C of its m.p. (156.6°C) and ±1% of its DH_f (28.45 J/g). Samples were placed in sealed aluminum pans and scanned over a temperature range of 20°–200°C.

Release Rate Studies

Release rate medium was either USP simulated gastric fluid without pepsin (SGF; pH = 1.2) or USP simulated intestinal fluid without pancreatin (SIF; pH = 7.5). A weighed amount of formulation (500 mg) was carefully added to 10 ml of medium in a glass vial. The vial was capped, placed on an orbital shaker, and agitated at an intensity of 80 rpm. Vials were maintained at 37°C during the run. At predetermined times, medium was sampled by removing 2 ml from the vial and replacing with 2 ml of fresh, preheated medium. Samples were diluted with a known volume of double-strength SGF without pepsin and assayed by UV absorbance at 300 nm. A total of 16 studies were performed in SGF, and 3 studies were performed in SIF. Data were converted to cumulative % released based on a total drug load of 9.06 mg of AG337 per 500 mg of formulation.

RESULTS AND DISCUSSION

Thermal Analysis

AG337 did not exhibit any remarkable features on DSC except for a small endotherm at 131.55°C (see Fig. 1). Glyceryl monooleate exhibited melting at

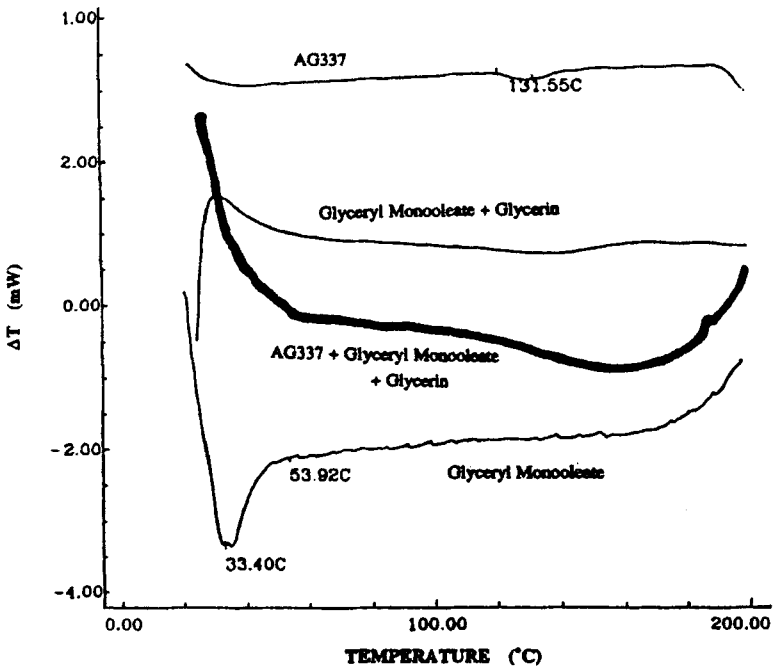


Figure 1. DSC thermograms of formulation components.

33.4°C. Mixtures of glyceryl monooleate/glycerin and AG337/glyceryl monooleate/glycerin exhibited melting below 33.4°C. These data suggest that the formulation is a solution at 33.4°C. However, since AG337 does not exhibit any remarkable features, it is difficult to determine what interactions, if any, are present.

The formulation not exposed to water shows no distinct endo- or exothermic transitions. However, the formulation exposed to water for 1 hr exhibits endo- and exothermic peaks at 89.04°C and and 127.6°C, respectively, as shown in Fig. 2. The latter peak may be due to a crystallization process in response to water uptake. The formulation exposed to water for 48 hr shows multiple endothermic peaks and transitions prior to the first endothermic peak, as shown in Fig. 3. Three of the endothermic peaks occur at about 106°C. These data suggest that melting is followed by a degree of crystalline formation, insofar as the thermal profile shifts to a lower temperature following each transition.

Release Rate Studies

The release profile in SGF (Fig. 4) is characterized by an early rapid release phase, with about 40% released in the first 15 min. Following this early rapid phase, the release rate decreases significantly over time. The maximum amount released in 24 hr was about 73%.

The release profile in SIF (Fig. 5) is characterized by an initial rapid rate of release up to about 90 min. Thereafter, the rate decreases with increasing time. It is notable, however, that less than 6% of the total drug load was released in the time frame tested, indicating that the formulation did not release significant amounts of drug in the more alkaline SIF medium.

These data suggest that two interdependent factors affect the release of AG337 from glyceryl monooleate:

1. The ionization state of AG337 relative to its pH–solubility profile
2. The dynamics of water uptake and the resulting phase transitions occurring in the glyceryl monooleate during the course of AG337 release

A proposed ionization scheme for AG337 is shown in Scheme 2. Since AG337 is a weak base, its solubility in acidic media, relative to its pK_a, is greater than its solubility in an alkaline media. The dependence of drug release on the pH of the release medium can be seen from the cumulative % release profiles in SGF and SIF, which are shown together on the same graph, Fig.

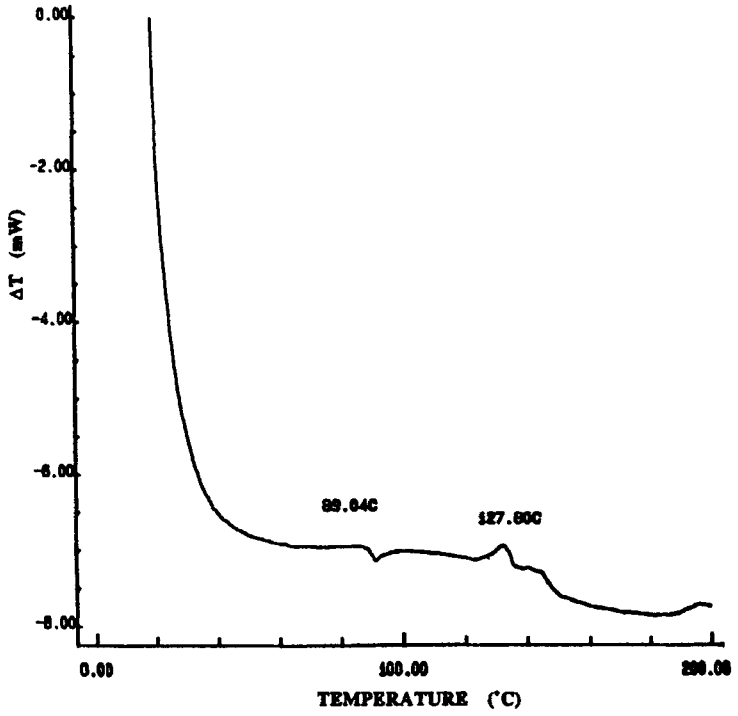


Figure 2. DSC thermograms of formulation after exposure to water for 1 hr.

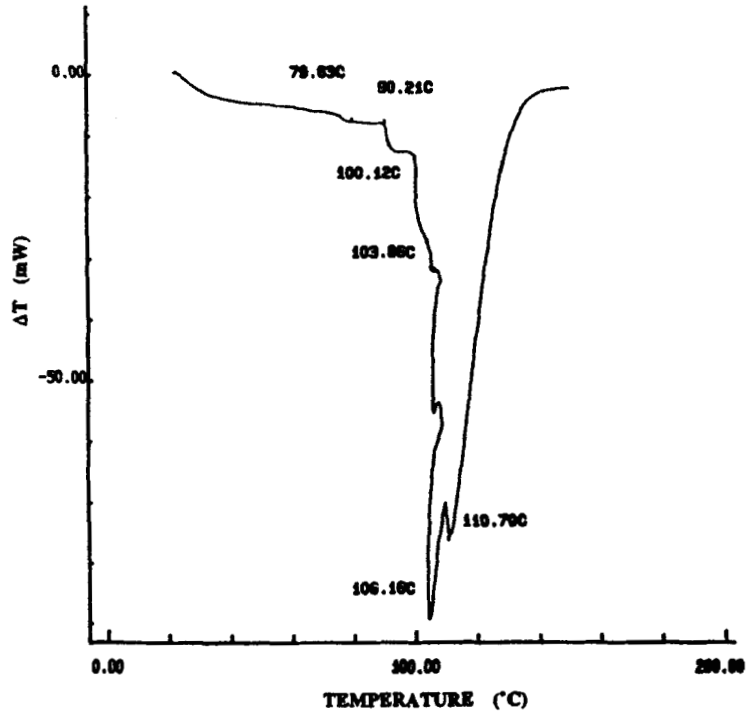


Figure 3. DSC thermograms of formulation after exposure to water for 48 hr.

6, for comparison. The rate and magnitude of AG337 released in SGF is much greater than in SIF. In the acidic SGF, the ionized and more water-soluble form of AG337 is present and will be more rapidly transported from the matrix into the medium. In the more alkaline SIF, the un-ionized and less water-soluble form of AG337 is present and release from the glyceryl monooleate matrix may be dissolution rate limited.

Significant interaction between AG337 and the release medium depends upon water uptake into the matrix. As an amphiphilic material, glyceryl monooleate can contain up to 40% (w/w) water (1). On visual ob-

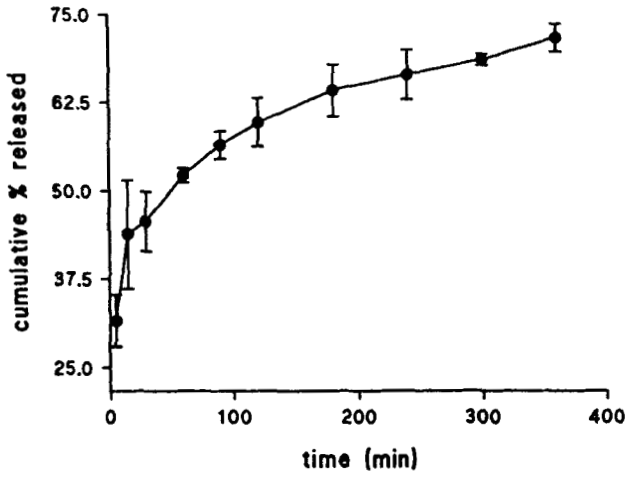


Figure 4. Release of AG337 from cubic phase system in simulated gastric fluid.

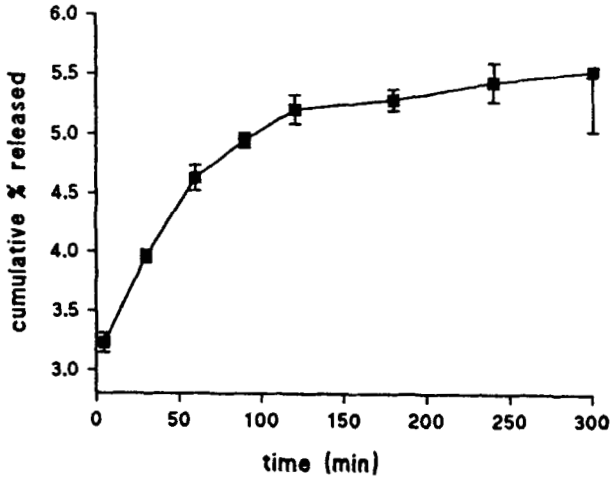
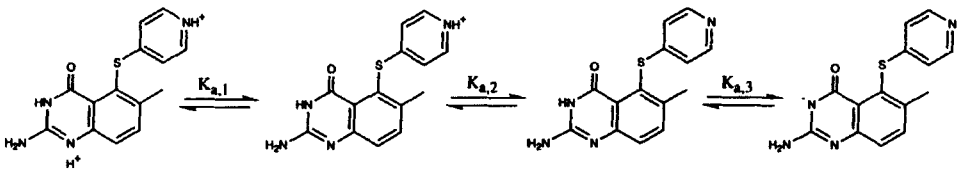


Figure 5. Release of AG337 from cubic phase system in simulated intestinal fluid.



Scheme 2. Proposed ionization scheme for AG337.

servation, the formulation appeared to form a viscous mass upon addition to the release medium. However, AG337 was rapidly leached from the formulation early in the process of water exchange between glyceryl monooleate and the aqueous phase. After approximately 1 hr the release rate decreased, which may be related to the onset of a phase transition in glyceryl monooleate. Glyceryl monooleate then became increasingly capable of controlling the release rate over time.

CONCLUSIONS

Water uptake by the formulation and physical changes associated with water uptake are dependent on time. In the initial phases of water uptake, the formulation is viscous and thermal analysis indicates some physical change as evidenced by the appearance of an endo- and exothermic peak. After longer periods of water exposure, appearance does not change but thermal analysis again indicates that water uptake has further changed the physical properties of the system.

Initial contact between the formulation and an acidic release medium leads to rapid release of AG337, until a condition is reached where the glyercyl monooleate intrinsically controls AG337 release through phase transitions which are dependent upon the dynamics of water uptake. During the early phase of release, the water-soluble form of AG337 is more easily leached from the carrier system. When the release medium is alkaline, there is an early burst effect, but the amount released is very low.

The release rate and the amount released is dependent upon the pH of the release medium, at least under the conditions of this experiment where the drug was incorporated as a solution in glycerin into glyceryl monooleate.

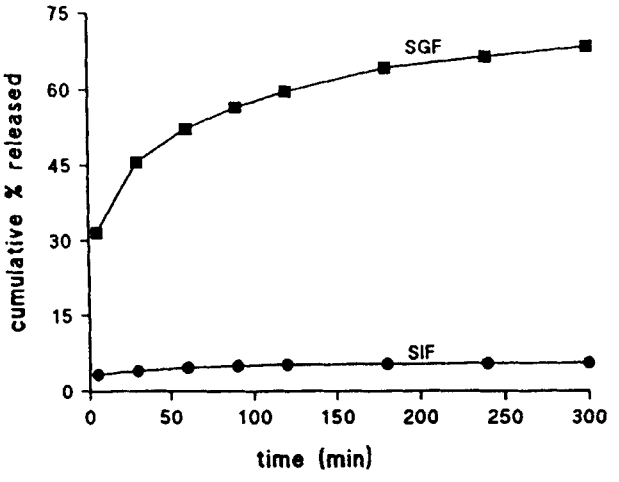


Figure 6. Release of AG337 from cubic-phase system in SGF and SIF.

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