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RESEARCH PAPER

# Release Kinetics of Acyclovir from a Suspension of Acyclovir Incorporated in a Cubic Phase Delivery System

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**ABSTRACT**

*Acyclovir is a widely used agent in the treatment of herpes virus infections of the skin, but owing to its poor physicochemical properties in terms of bioavailability and suboptimal formulations, the treatment is far from optimal. The liquid crystalline cubic phase system has been reported to act as a bioadhesive drug delivery system. In the present study, acyclovir was suspended in a cubic phase of glycerol monooleate (GMO) and water 65%:35% w/w, and the phase behavior and release kinetics were examined. X-ray diffraction and differential scanning calorimetry (DSC) measurements demonstrated that the cubic phase containing 1%–10% (w/w) acyclovir retains its phase condition in the temperature range investigated (20°C–70°C). Acyclovir can be incorporated in high amounts (~40% w/w) without causing phase transition, as is shown in polarized light. This is probably because of its low solubility (~0.1% w/w) in the cubic phase. The release characteristics of acyclovir incorporated as a suspension (1%–5% w/w) into a cubic phase were investigated using Franz diffusion cells. Acyclovir was quantified by high-performance liquid chromatography (HPLC). The drug was readily released from the system, and the release increased with the initial drug load concentration. About 25%–50% was released after 24 h. The release is dependent on the square root of time, and the kinetics can be described*

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*by the Higuchi theory. The rate-limiting step in the release process is most likely diffusion. The suggested theory is further supported by identical release data obtained for micronized and nonmicronized acyclovir. The fluxes for 1% and 5% w/w were 380 and 900 $\mu g/h^{1/2}$, respectively. Comparison of the release rates of acyclovir delivered from a cubic phase and from the commercial product, Zovir® cream, showed the rate to be six times faster from the cubic phase. The results indicate that the cubic phase is a promising drug delivery system for acyclovir.*

***Key Words:*** *Acyclovir; Cubic phase; Glyceryl monooleate; Liquid crystals; Release kinetics*

## INTRODUCTION

Glyceryl monooleate (GMO), an amphiphilic lipid, forms various liquid crystalline phases in contact with water. The liquid crystals formed are dependent on the water content and the temperature. Drugs and excipients may also alter the phase diagram (1,2). With regard to drug delivery, most of the work has been dedicated to the cubic phase based on GMO. The structure of the cubic phase consists of curved lipid bilayers extending in three dimensions separated by two congruent networks of water channels (3,4). It is formed spontaneously in contact with water and stays in equilibrium with excess water. The cubic phase has a transparent, stiff, gel-like appearance, and it has recently proved to possess bioadhesive properties (5–8); e.g. it sticks effectively to the skin. Another important feature with regard to drug delivery is that it is biodegradable (9). The cubic phase has been reported to act as a drug delivery system for a number of drugs (10,11), and because of the amphiphilic nature of the cubic phase, both hydrophilic and lipophilic drugs can be incorporated. The cubic phase has a solubilizing effect on several drugs with low water solubility (e.g., miconazole) (12). However, most attention in the literature has been directed to water-soluble drugs in concentrations below about 5%, which in these concentrations, are soluble in the cubic phase system. Less attention has been paid to lipophilic drugs and drugs with low solubility in the cubic phase (11,13).

The release kinetics of water-soluble drugs delivered from the cubic phase have been demonstrated to exhibit a typical drug release that is square root dependent (2,11,14). The diffusion of drug molecules takes place through the water channels (2,11). The water pore diameter is about 5 nm (10). Acyclovir, [9-(2-hydroxyethoxy)methyl]-guanine, an acyclic analogue to the natural nucleoside 2′-deoxyguanosine, exemplifies a drug with poor physicochemical properties with regard to drug delivery. The water solubility is about 1.5 mg $ml^{-1}$ at 22°C, and the partition coefficient $P$ between octanol and 0.02 M phosphate buffer at pH 7.4 (22°C) is about 0.03. It is a widely used agent in the treatment of herpes virus infections (15).

Zovirax® cream and the synonym product Zovir® cream from Glaxo-Wellcome are the drugs of choice in the treatment of herpes virus infections of the skin. For local treatment, the creams should be applied 5–6 times a day. The frequency of administration results in poor compliance by the patient.

The inadequate effect of the creams is caused partly by the poor physicochemical properties of acyclovir with regard to absorption/transport and partly by suboptimal formulations (16,17). The transport problem of acyclovir may be solved by new formulation principles. By incorporating acyclovir into the cubic phase, which sticks to the skin, the number of daily applications could be reduced. Acyclovir is poorly soluble in the cubic phase (0.1% w/w, as found in this study), and it therefore exists as a suspension in concentrations above 0.1% w/w.

The aim of this study was to investigate the release kinetics of acyclovir delivered from a cubic phase and secondarily to compare the release rate with that of Zovir® cream. Further, the influence of the acyclovir concentration on the phase behavior was examined by X-ray diffraction and polarized light.

## EXPERIMENTAL

### Materials

Acyclovir, micronized and nonmicronized (Chemo Iberica, Spain), and GMO-90 containing a minimum of 95% monoglycerides, of which at least 88% is glyceryl monooleate (Danisco Ingredients, Grindsted Division, Denmark), were used. "GMO" used below denotes the above-mentioned glyceryl monooleate product. A cellulose membrane (Medicell International, Ltd.) was used. Zovir® cream (Glaxo-Wellcome) containing 5% micronized acyclovir was also used.

### Apparatus

Franz diffusion cells were used in the dissolution experiments. The liquid crystalline phases were determined in polarized light with a microscope (Leitz, Diaplane) equipped with polarization filters and a PE 60 Peltier stage controller (Linkam). A modified diffraction thermal pattern (DTP) camera was used for X-ray diffraction measurements. The source was an X-ray tube equipped with a copper anode emitting $K_\alpha$ rays at a wavelength of 1.5418 Å. The X-ray generator was a Phillips PW 1729. The DSC measurements were performed with a PerkinElmer Unix DSC model 7 differential scanning calorimeter.

The specific surface area of micronized and nonmicronized acyclovir was determined by the air permeability method (Ph. Eur., 3rd Ed. 2.9.14).

### Preparation of the Acyclovir Compositions

The GMO was gently melted at a maximum temperature of about 60°C, and the resulting liquid was cooled to about 40°C before it was mixed with other ingredients. Acyclovir was suspended in the melted GMO and mixed by stirring. Water was added to the homogeneous suspension under vigorous stirring or shaking. The mixture was then mixed to homogeneity with a mortar and pestle. The viscous mixtures were subjected to ultrasound treatment for about 1 h and stored in closed containers for at least 1 week before use to ensure that equilibrium had been reached.

### Release Experiments

The dissolution of acyclovir in various concentrations (1%–5% w/w) from cubic phases of GMO and Zovir® cream was determined using Franz diffusion cells with a diffusion area of 1.8 $cm^2$ and a receptor volume of 6.8 ml. The study was run at a temperature of 37°C, and a cellulose membrane was employed as a diffusion membrane. The membranes had a pore size of about 2.4 nm, and they retained particles with a molecular weight of more than 12,000–14,000. Before application, the membrane was pretreated and thoroughly rinsed with distilled water. An isotonic 0.05 M phosphate buffer solution of pH 6.5 was used as receptor medium, and this was stirred magnetically at about 100 rpm. Before the experiment, the cellulose membrane was allowed to equilibrate at 37°C for 30 min in the receptor medium. The membrane was placed in the diffusion cell, and about 300–350 mg of the composition to be tested was applied by means of a syringe or a scraper; care was taken to ensure homogeneous distribution of the composition over the total area of the membrane available for diffusion. The phosphate buffer was then loaded into the receptor part (time $t = 0$), and at appropriate intervals, 2-ml samples were removed and analyzed for the content of acyclovir. This relatively high volume was removed and replaced by fresh receptor medium to ensure sink conditions.

### Phase Determination

The liquid crystalline phase was identified by polarized light microscopy and by X-ray diffraction. The cubic phase is optically isotropic in polarized light.

The liquid crystalline state can be identified by low-angle X-ray diffraction. The characteristic X-ray diffraction pattern for the three liquid crystalline phases (lamellar, hexagonal, cubic) produces diffraction lines in the following order:

Lamellar: 1; 1/2; 1/3; 1/4

Hexagonal: 1; $1/\sqrt{3}$; $1/\sqrt{4}$; $1/\sqrt{7}$

Cubic: 1; $1/\sqrt{2}$; $1/\sqrt{3}$; $1/\sqrt{4}$; $1/\sqrt{5}$; $1/\sqrt{6}$; $1/\sqrt{8}$; other ratios may exist, such as 1; $\sqrt{3}/_4$; $\sqrt{3}/_8$; $\sqrt{3}/_{11}$

### Phase Transition Determined by Differential Scanning Calorimetry

The DSC measurements were performed at a heating rate of 3°C/min, and the scanning temperature was generally from 5°C to 70°C. Samples were kept in sealed aluminum pans (PerkinElmer BO14-3017), and empty aluminum pans were used as reference. Phase transitions cause only a relatively small change in enthalpy (18), and therefore the samples were optimized to about 25 mg. The prepared pans were sealed and stored at 5°C for 2 days prior to analysis.

### Solubility and Homogeneity Determinations

The solubility of acyclovir in the cubic phase of GMO was determined by microscopic examination of the acyclovir crystals at room temperature. The homogeneity of the formulations was inspected visually in the microscope, and the acyclovir concentration in samples taken from different parts of the bulk formulations were determined quantitatively.

### Quantitative Determination of Acyclovir by High-Performance Liquid Chromatography

Acyclovir was quantified by a reversed-phase HPLC procedure at room temperature. The stationary phase was a Nucleosil C-18 column (25 cm × 4.6 mm i.d.). For the quantification of acyclovir in the release experiments, the column was eluted with a mobile phase consisting of water:methanol (85%:15 % v/v), and the flow rate was 1.0 ml $min^{-1}$. The effluent was detected at 254 nm.

An extraction procedure was employed for the homogeneity experiments: About 100 mg lipid formulation was weighed and diluted to 50.00 ml with the mobile phase. The sample was diluted an additional 10 times with the mobile phase and filtered through a 0.2-μm filter. The mobile phase consisted in this case of water:methanol (20%:80% v/v). The solubility of GMO is enhanced in this medium with a relatively high part of methanol compared to the mobile phase used in the release experiments. This secures a recovery of about 100%. The flow rate was 0.8 ml $min^{-1}$, and the effluent was measured at 254 nm.

## RESULTS AND DISCUSSION

### Determination of the Solubility and Homogeneity of Acyclovir in GMO-Water Formulations and the Phase Condition in Polarized Light

Formulations containing GMO:water 65%:35% w/w with acyclovir (nonmicronized and micronized) added in a concentration of 1%–40% w/w resulted in cubic phase formulations, as shown in polarized light at 22°C and 37°C. The distribution of drug crystals of an initial loading level of 1%–5% in the cubic phase looked uniform in the microscope, and the homogeneity of the acyclovir content in the formulations was within ±10% of the theoretical value as determined by HPLC. The solubility of acyclovir in a GMO cubic phase was found to be about 0.1% w/w. Acyclovir added in high concentrations (~40% w/w) does not cause a phase transition, as is normally seen for drugs with a relatively high solubility (~5%) in the cubic phase (1,2). This may be because of the relatively low solubility of acyclovir in the cubic phase, which means that the drug molecules interfere with the lipid bilayers in the cubic system only to a minor degree.

### X-Ray Diffraction Measurements

Formulations containing GMO:water 65%:35% w/w (reference sample) and GMO:water 65%:35% w/w with acyclovir (nonmicronized and micronized) added in concentrations of 2.5%, 5.0%, and 10.0% w/w were examined by means of X-ray diffraction in a temperature scan in the range 20°C–70°C. Owing to technical irregularities, it was impossible to examine the formulations in the range 5°C–20°C. All the lipid-water formulations showed X-ray diffraction lines with a relative ratio corresponding to a cubic structure, and the cubic structure of the lipid phases was not affected by the presence of the drug. Only a minor part of the added drug (0.1%) is present in dissolved form, which reduces the ability of the drug to interfere with the lipid bilayers, thereby causing phase transitions.

The $d$ spacings of the GMO-water phases containing 5% w/w acyclovir were slightly higher than those found with the GMO-water phase without acyclovir (Table 1). This is presumably due

***Table 1***

*X-Ray Diffraction Data for Glyceryl Monooleate (GMO) Water Formulations Containing 5% w/w Micronized and Nonmicronized Acyclovir at 37°C*

| Formulation (% w/w) | X-ray *d* Spacing (Å) | Ratio | Structure |
|---|---|---|---|
| GMO:water: (65:35) | 53.2–43.4–26.8 | 1:0.82:0.50 | Cubic |
| GMO:water:acyclovir[a] (61.8:33.3:5.0) | 61.7–51.4–35.9–29.9 | 1:0.82:0.588:0.48 | Cubic |
| GMO:water:acyclovir[b] (61.8:33.3:5.0) | 61.7–51.4–35.9–29.9 | 1:0.83:0.582:0.48 | Cubic |

[a]Micronized quality.
[b]Nonmicronized quality.

to the increased volume of the aqueous phase containing acyclovir. The drug gives a series of X-ray short spacings—12.9 Å, 8.44 Å, 3.74 Å, and 3.42 Å—which is an indication of its crystalline state. These diffraction lines do not interfere with the diffraction lines of the lipid-water phase, which can be identified unambiguously. When the concentration of acyclovir was increased from 5% to 10%, the intensity of the corresponding X-ray diffraction lines also increased. No difference in the structure of the formulations containing micronized and nonmicronized acyclovir was found. The lipid-water formulations were all cubic in the temperature range 20°C–70°C. The cubic phase exists in different forms (e.g., gyroid and diamond), depending on the water content and temperature (3). However, the resolution of the applied method was not high enough to differentiate between the different types.

## Differential Scanning Calorimetry Measurements

DSC measurements do not alone provide information on the particular phases involved, but they are well suited to observe whether any phase transition occurs as a function of temperature. A DSC scan (5°C–70°C) was run on the GMO:water 65%:35% w/w (reference sample) and on the same formulation with the addition of 5% nonmicronized and 5% micronized acyclovir. The samples were stored at 5°C for 2 days to ensure equilibration of the sample. The lipids in the sample solidified at this temperature (gel state). The thermograms only showed a clear melting peak at about 16°C–17°C for both the reference sample and the samples containing 5% w/w acyclovir. The cubic phase is regenerated (reversible process) during the scan. No phase transition of the cubic phase was detected in the temperature area investigated. The results at 20°C–70°C are consistent with the results of the X-ray diffraction measurements.

## Release of Acyclovir from a GMO-Water Cubic Phase

The release of acyclovir from various GMO formulations was examined in 0.05 M isotonic phosphate buffer solutions at pH 6.5 (37°C) using Franz diffusion cells. The cellulose membrane used was considered to be a non-rate-limiting membrane (19,20). All compositions were suspensions of acyclovir as the saturation solubility of acyclovir in the compositions was determined to be about 1 mg $g^{-1}$. The release from 1%–5% acyclovir cubic phase formulations and Zovir® cream, containing 5% micronized acyclovir, was investigated. The influence of the particle size of acyclovir on the release rate of acyclovir was examined by comparing the release rates from 1% and 5% nonmicronized and micronized qualities.

Figure 1 gives the results of the release experiments of acyclovir in concentrations of 1%–5% w/w from a cubic phase and Zovir® cream. The release profiles show the cumulative release of acyclovir, and that release increases with the initial drug load in the range investigated. However, at concentrations of 3%–5% w/w acyclovir, no significant difference in the release rate was seen. At 24 h, about 25%–50% of the total amount had been released. The cubic phase is transparent, colorless, and highly viscous. Addition of acyclovir turns it white-gray and makes it more viscous. During drug release, the cubic phase in the area in contact with the receptor medium becomes transparent again as acyclovir is depleted from the system. Equilibrium between the cubic phase and the release medium occurs during

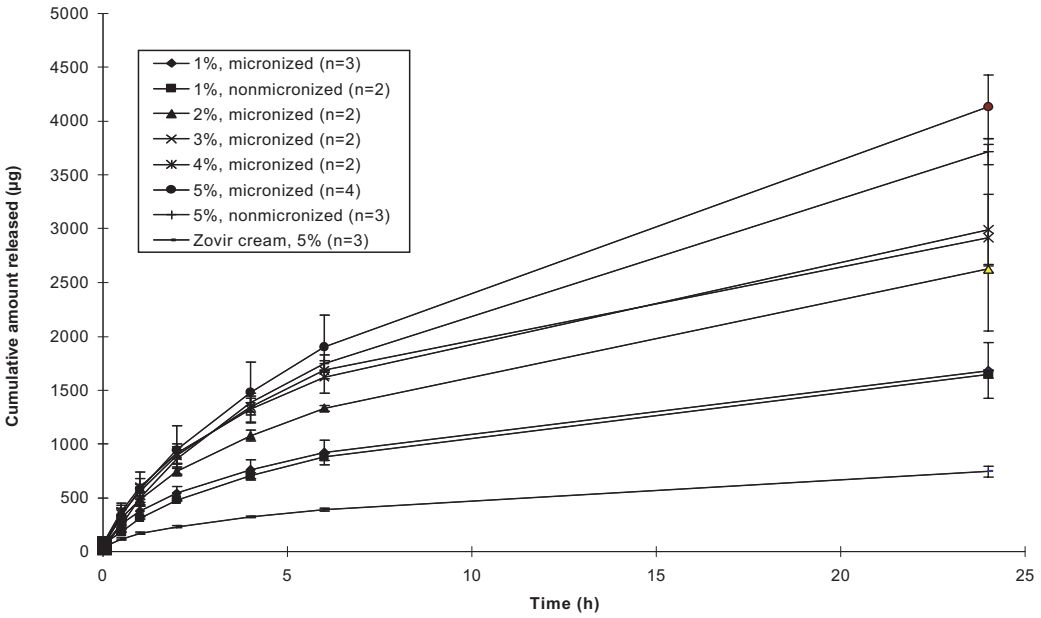


**Figure 1.** Release profiles of acyclovir delivered from a cubic phase (GMO:water 65%:35% w/w) and Zovir® cream into an 0.05 M isotonic phosphate buffer solution, pH 6.5 (37°C). Standard deviations are indicated by bars.

the experiment, and the cubic phase will be fully swelled (about 40% water) (3). At the end of the experiments, the formulations were removed from the cells and examined in polarized light at 37°C. The results showed that, in all cases, the cubic phase was conserved during the release experiments. This indicates that the drug delivery system has a rather high physical stability under the test conditions. The physical stability of the cubic phase on the skin remains to be evaluated. The ionization constants for acyclovir have been calculated to be 2.4 ($pK_{a1}$) and 9.1 ($pK_{a2}$) (21). No major differences in the release rate within physiologically relevant pH values (5–8) should therefore be expected.

## Release Kinetics

Generally, the release of drug from a system (e.g., the cubic phase) in which the initial drug-loading concentration is greater than the saturation solubility of the drug can be assumed to follow a three-stage process involving (1) dissolution of suspended drug in the system, (2) diffusion of dissolved drug through the system, and (3) transfer of drug across the cubic phase/release medium interface.

The release of a drug from a suspension of semisolids is often controlled by diffusion of the drug through the matrix, as described by Higuchi (22). Higuchi's equations can be outlined as follows:

$$Q = [D(2A - C_s) \times C_s \times t]^{1/2}$$

where $Q$ is the cumulative amount of drug released, $C_s$ is the solubility or saturation concentration of drug in the matrix, $A$ is the total concentration of dissolved and undissolved drug in the matrix, and $D$ is the diffusion coefficient of the drug in the matrix. The factor $[D(2A - C_s) \times C_s]^{1/2}$ is the release rate constant $k$ for drug release.

A plot of $\sqrt{t}$ as a function of the cumulative amount of acyclovir released $Q$ (Fig. 2) resulted in straight lines with the slope $k$, thus indicating that the release rate can be described by the Higuchi equation, and that the main controlling parameter is diffusion, probably through the water channels (2,11). The ensuing rate constants ($\mu g/h^{1/2}$) are shown in Table 2.

The rate of dissolution of acyclovir in the cubic phase is apparently sufficient to replace the amount of acyclovir released. The suggested theory is further supported by the identical release data for micronized and nonmicronized substances (Fig. 3). The specific surface areas measured were 1.95 and 0.35 $m^2 g^{-1}$, respectively, which gives a factor 6

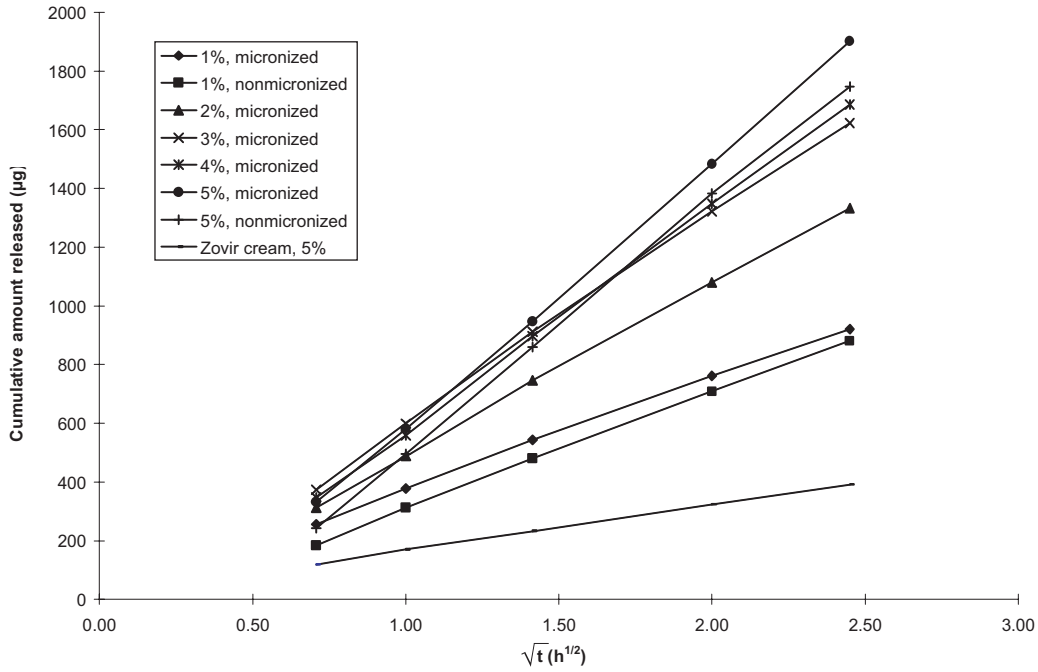


**Figure 2.** Comparison of release profiles of nonmicronized and micronized acyclovir delivered from a cubic phase (GMO:water 65%:35% w/w) into an 0.05 M isotonic phosphate buffer solution, pH 6.5 (37°C). Standard deviations are indicated by bars.

***Table 2***

*Release Rate Constants of Acyclovir Released from a Cubic Phase and Zovir® Cream*

| Acyclovir Concentration (% w/w) | $k$ (µg $h^{1/2}$) Mean | ± SD | $n$ | Correlation Coefficient |
|---|---|---|---|---|
| 1[a] | 380 | 50 | 3 | 0.9996 |
| 1[b] | 400 | — | 2 | 0.9997 |
| 2[a] | 590 | — | 2 | 0.9998 |
| 3[a] | 720 | — | 2 | 0.9996 |
| 4[a] | 770 | — | 2 | 0.9999 |
| 5[a,c] | 900 | 101 | 5 | 0.9999 |
| 5[b,c] | 870 | 31 | 3 | 0.9999 |
| 5[a] Zovir® cream | 160 | 11 | 3 | 0.9998 |

[a]Micronized quality. The specific surface area was determined to be 1.95 $m^2 g^{-1}$.
[b]Nonmicronized quality. The specific surface area was determined to be 0.35 $m^2 g^{-1}$.
[c]Student $t$ test was performed on the release rate constants at 5% loading level; $P_{a/b} > 0.1$ (no significant difference).

difference. These findings further show that the dissolution rate is of minor significance in the release process. If the dissolution of the suspended drug is the rate-limiting step for drug release, then the release of drug becomes time independent; that is, the release data can be fitted to a zero-order release model (23,24).

## Comparison of the Release Rate of Acyclovir Delivered from a Cubic Phase and Zovir® Cream

To compare the release rate of acyclovir from Zovir® cream and a cubic phase, both containing 5% acyclovir, the release of acyclovir from these formulations was examined as described above (Figs. 1 and 3). Comparison of the rate constants (see Table 2) shows that the release rate of acyclovir was about 5–6 times faster from the cubic phase than from the Zovir® cream. Zovir® cream is an

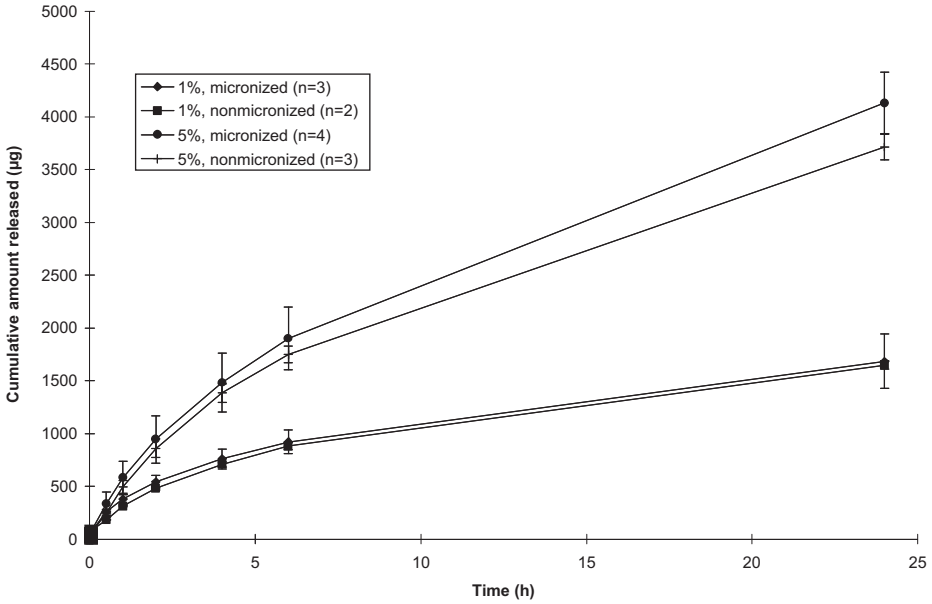


**Figure 3.** Cumulative amount of acyclovir released from cubic phase formulations and Zovir® cream as a function of the square root of time for initial drug-loading concentrations of nonmicronized and micronized qualities of acyclovir.

oil-in-water emulsion, and it consists of cetyl and stearyl alcohols, sodium lauryl sulfate, paraffin liquid, poloxamer 407, propylene glycol, petrolatum, and water. The solubility of acyclovir in the aqueous phase of the oil-in-water cream is supposed to be about 3 mg/ml according to Jones and White (25) and about 1 mg/ml in the cubic phase. Despite the apparent lower solubility in the cubic phase, the release rate is faster from this kind of vehicle. Factors like drug partition between formulation and the receptor phase and the microstructure of the formulations may influence the release rate. The results indicate that the cubic phase may be a suitable vehicle for acyclovir.

## CONCLUSION

Results of X-ray diffraction measurements show that the cubic phase in which 2.5%–10% w/w acyclovir has been suspended (nonmicronized and micronized) retains its phase condition in the temperature range examined (20°C–70°C). This is supported by a DSC temperature scan (5°C–70°C). As evidenced by polarized light, acyclovir can be incorporated into the cubic phase in relatively high concentrations (~40% w/w) without causing any phase transition, which may be due to the relatively low solubility of acyclovir in the cubic phase (~0.1% w/w).

Release experiments with Franz diffusion cells demonstrated that acyclovir suspended in a GMO cubic phase is readily released into an aqueous medium despite its relatively low solubility in water and in the cubic phase. No difference in the release rate between nonmicronized and micronized acyclovir was seen. The release kinetics followed the Higuchi theory; consequently, the release is most likely controlled by diffusion. The release rate is about six times faster from a cubic phase than from Zovir® cream. The results indicate that the cubic phase might be a promising drug delivery system for acyclovir.

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