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Preparation of griseofulvin nanoparticles from water-dilutable microemulsions

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Abstract

Nanoparticles of griseofulvin, a model drug with poor solubility and low bioavailability, were prepared from water dilutable microemulsions by the solvent diffusion technique. Solvent-in-water microemulsion formulations containing water, butyl lactate, lecithin, taurodeoxycholate sodium salt (TDC) or dipotassium glycyrrhizinate (KG), 1,2-propanediol or ethanol were used. The formation of macroscopically homogeneous, stable, fluid, optically transparent, isotropic solutions (microemulsions) was investigated by constructing pseudo-ternary phase diagrams. In the presence of TDC or KG, microemulsion systems that remained transparent on water dilution could be obtained. The displacement of butyl lactate, with an excess of water, from the internal phase of the microemulsions containing the drug into the external phase, lead to successful fabrication of drug nanosuspensions. Nanoparticle size was dependent on microemulsion composition: using KG, griseofulvin nanoparticles below 100 nm with low polydispersity and an increased dissolution rate were obtained.

Keywords: Microemulsions; Griseofulvin; Nanoparticles

1. Introduction

Microemulsions are transparent, well characterized, thermodynamically stable and easily manufactured systems consisting of submicron-sized water pools dispersed within an immiscible organic (oil) phase or, vice versa, oil pools dispersed within a continuous aqueous phase (Kumar, 1999). The droplets are covered by a shell consisting of a suitable surfactant(s) and a cosurfactant, usually an alcohol. In recent years, microemulsions have been extensively used for many applications: as vehicles to increase local and systemic delivery of drugs and to enhance drug solubilization (Gasco, 1997); chemical and photochemical reaction media and in the preparation of nanosized particles for a number of other applications (Fendler, 1994). However, little has been done in the realm of crystallization of organic compounds.

Water-in-oil microemulsions containing solubilized aspartame have been prepared at elevated temperatures (Furedi-Milhofer et al., 1999). From such microemulsions, aspartame was crystallized by slow cooling to 5 °C, obtaining a new crystal form of the sweetener. Oil-in-water microemulsions containing stearic acid has been prepared at 70 °C and diluted with cold water, obtaining a very fine dispersion of the lipid (Gasco, 1993).

In a previous study (Trotta et al., 2001), nanosuspensions of mitotane, an anticancer dug normally administered by the oral route with very poor solubility

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and low bioavailability, were prepared from emulsions containing partially water-miscible solvents, such as benzyl alcohol or butyl lactate, by a solvent diffusion technique. The process is based on the water miscibility of these solvents. Upon transferring a transient oil–water emulsion into water, the drug dissolved in the organic solvent solidifies instantly due to the almost complete diffusion of the organic solvent from the droplets to the continuous phase. Using optimised formulations and homogenisation parameters, mitotane nanoparticles below 100 nm with very low polydispersity and high dissolution rates were obtained.

The aim of this study was to investigate the feasibility of preparing drug nanosuspensions from solvent-in-water microemulsions by the diffusion technique, using solvents, surfactants and cosurfactants accepted as having low toxicity. Butyl lactate, a partially water-miscible solvent (solubility 7.2%, w/w) with low toxicity (LD_{oral} 5 g/Kg) was used as internal phase of the microemulsions. Griseofulvin, an antifungal drug with poor solubility and low bioavailability, was used as model drug.

2. Materials and methods

2.1. Materials

Soybean lecithin (phosphatidylcholine 95%, Epikuron® 200) was obtained from Lucas Meyer (Hamburg, Germany) and used without further purification. Taurodeoxycholate sodium salt (TDC), butyl lactate, 1,2-propanediol, ethanol and griseofulvin were from Aldrich Chemical Co. (Dorset, UK). Dipotassium glycyrrhizinate (KG) was purchased from Maruzen Pharmaceutical (Hiroshima, Japan). All other chemicals were reagent grade and used as received. Water was freshly bidistilled.

2.2. Pseudo-ternary phase diagrams

Phase diagrams were constructed by titrating a series of surfactant/alcohol/solvent mixtures with water or with 0.1 or 0.025 M of TDC or KG (aqueous phase) at 25 °C. Lecithin was used as surfactant, butyl lactate as disperse phase and ethanol or 1,2-propanediol as cosolvent. The domain of existence of isotropic systems was determined at a constant lecithin:alcohol weight ratio of 1:1 (surfactant mixture).

Appropriate amounts of surfactant mixture and butyl lactate were weighed into glass ampoules. Sample were shaken for sufficient time to attain equilibrium and then progressively enriched with water or with an aqueous solution of TDC or KG, added drop by drop, and the amounts of added aqueous phase at which the transparent/opaque transition occurred were used to determine the phase domains. No attempt was made to distinguish among true solutions, micelles, bicontinuous structures, microemulsions, etc. and the domain of existence of transparent, isotropic systems was considered as the microemulsion phase, while the domain of existence of turbid systems was classified as the emulsion phase. For the purpose of this study, a schematic representation was sufficient as a guide to follow the evolution of phase equilibria.

By repeating this experimental procedure for other weight ratios of surfactant mixture to butyl lactate, pseudo-ternary phase diagrams were constructed.

2.3. Preparation of drug nanosuspensions

Microemulsions containing (w/w) 66.6% aqueous phase, 16.6% butyl lactate and 16.6% surfactant mixture were prepared at 25 °C. An excess of griseofulvin was added and the microemulsions with suspended drug stirred for 24 h, then filtered through 0.22 μm filter (Millipore, Bedford, MA, USA). The griseofulvin content in the microemulsions was determined by HPLC after appropriate dilutions.

Griseofulvin suspensions were prepared by adding 2 ml water, under magnetic stirring and at a constant rate of 10 ml/min, to 1 g griseofulvin saturated microemulsion. The average diameter, polydispersity index and *Z*-potential of griseofulvin suspensions were immediately determined by a laser light scattering technique (Brookhaven, New York, USA).

For dissolution studies, griseofulvin suspensions were washed by diaultrafiltration using a diaflo YM 100 membrane (cut-off 100,000 Da). The sample obtained from KG containing microemulsion was then lyophilised for DSC measurement. DSC was performed with a Perkin-Elmer differential calorimeter (Norwalk, CONN, USA) at a scan speed of 10 °C/min; the weight of the samples was in the 0.8–1 mg range.

2.4. Dissolution study

Diaultrafiltrated suspensions containing a known amount of griseofulvin were resuspended in 250 ml water to maintain sink conditions and incubated at 37 °C under gentle magnetic stirring at 300 rpm. At appropriate intervals, 5 ml aliquots were removed and replaced by 5 ml water; these aliquots were immediately filtered (cut-off 100 nm, Millipore) and assayed for griseofulvin concentration by HPLC. As reference systems for the dissolution tests, commercial drug, prewetted commercial drug (0.01% Tween 80) and a suspension, obtained by adding water to a butyl lactate solution of the drug, were used.

2.5. HPLC assay

The concentration of grisefulvin was determined by HPLC. The HPLC system consisted of a pump (LC10-AD), a VIS-UV detector (SPD-10, λ = 245 nm), a data station (Shimadzu, Kyoto, Japan) and a 15-cm C18 column (LiChrospher, Merck, Darmstadt, Germany). The mobile phase was methanol:water (70:30, v/v) and was delivered at a flow rate of 0.8 ml/min. The injection volume was 20 μl and the relative retention time was found to be 5.2 min.

2.6. Data report

Each set of experiments was repeated at least three times. Results were reported as mean ± standard deviation.

3. Results and discussion

The first object of this study was to formulate isotropic systems that form over a wide range of water contents. This means that the domain of existence of these systems can incline toward the aqueous corner in the isothermal pseudo-ternary phase diagram, allowing considerable dilution with water without destroying the microemulsion phase in favour of the turbid phase.

Lecithin, a naturally occurring, nontoxic and safe material, was used as surfactant in the formulation of microemulsions but, when used as sole surfactant, it was not capable of producing isotropic solutions of water and oil over a wide range of compositions.

Much dosage-form development activity has focused on the formulation of lecithin-based microemulsion, and some studies have shown that microemulsions containing pharmaceutically acceptable oils can be formulated using lecithin and a fourth component, which acts as cosolvent for both water and oil. There is extensive literature on the formation of microemulsions using alcohols, glycols, alcanoic acid, etc (Aboofazeli et al., 1994). In this study ethanol and 1,2-propanediol, alcohols normally used in pharmaceutical field, were used as cosolvents.

Generally, hydrophilic cosolvents are the best to produce a large optically-clear isotropic region, but a problem with using water-soluble cosolvents is the destruction of the microemulsion phase on dilution of the cosolvent to below effective levels.

A possible way of obtaining lecithin-based microemulsions that are stable at high water content could be the partial replacement of lecithin by a more hydrophilic amphiphile (Trotta et al., 1999); this because lecithin is too lipophilic to produce isotropic solutions of oil in water over a wide range of compositions. The critical packing parameter, which is the ratio between hydrocarbon volume, optimum head group area and tail length (Israelachvili et al., 1976), is rather too high to form a water-rich isotropic phase, and when used as the only surfactant lecithin tends to favour the formation of oil-rich isotropic phase.

In this study TDC or KG were used as second surfactant. TDC is a well known physiological molecule and its interaction with lecithin has been extensively reported in literature (Higichi et al., 1986). KG is a compound obtained by extraction with water from liquorice root, and because of its chemical stability, good solubility and emulsifying properties, it is widely used in cosmetics. The critical micelle concentration is reported to be 0.75%, w/w (Yonezawa et al., 1976). It is also used in internal and external drugs, as well as in sweeteners. In a previous study, a strong interaction was found between lecithin and KG, similar to that of TDC; by using this molecule, deformable liposomes able to cross intact skin could be obtained (Trotta et al., 2002).

Figs. 1–4 report the pseudo-ternary phase diagrams of systems containing butyl lactate/ethanol (1,2-propanediol)/lecithin and water or a water solution

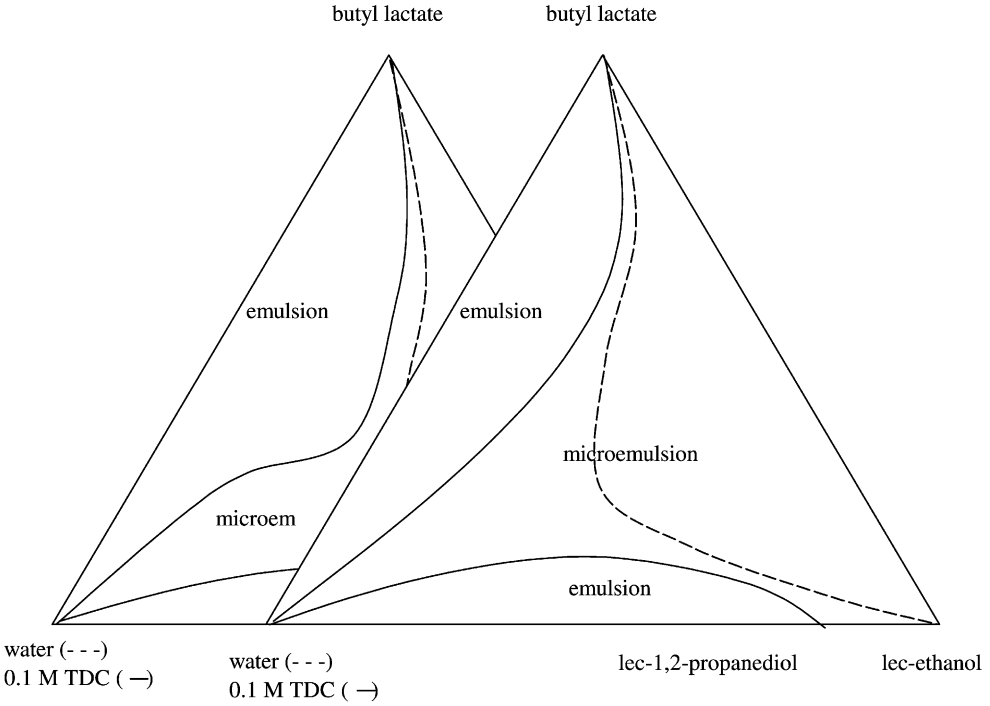

Fig. 1. Phase diagrams of systems containing water–TDC (0.1 M), lecithin–alcohol (1:1, w/w), butyl lactate (solid line) and water, lecithin–alcohol (1:1, w/w), butyl lactate (dotted line).

of TDC or KG at different concentrations (0.1 and 0.025 M).

All systems, regardless of the surfactant and co-solvent used, were capable of producing an isotropic phase. A comparison between phase diagrams shows that the trend of the changes induced by the presence of TDC or KG is that the isotropic realm shifts to the aqueous corner of the diagram. This could be ascribed to the incorporation of these molecules in the interfacial layer, reducing the packing parameter of lecithin by decreasing the effective hydrocarbon volume: the packing parameter could be shifted, as appropriate, to form oil–water microemulsions. For systems with appropriate compositions, because the isotropic region is connected to the water corner, any dilution with water phase will not induce transparent/turbid phase transformation.

The domain of existence of transparent systems, for formulations containing TDC or KG, was found to be quite similar, and an increase of this area was produced by increasing the concentration of second surfactant.

To prepare drug suspensions, microemulsions containing 66.6% aqueous phase (0.1 M TDC or 0.1 M KG), 16.6% butyl lactate and 16.6% surfactant mixture were prepared and characterized. After dilution of 1 g of this microemulsion with 2 ml water, the final concentration of the cosurfactant was about 0.025 M and that of water 88.6%, w/w.

The cosurfactant concentration was sufficient to avoid microemulsion/emulsion transformation, as can be seen in Figs. 3 and 4 where the transparent regions are connected to the water corner. The physico-chemical characterization of the microemulsions before and after water dilution was by droplet size determination. The values reported in Fig. 5 show that the mean diameters decrease as dilution increases and they were quite similar for the system containing TDC or KG. This experimental observation can be explained by the modification of the microstructure upon progression from microemulsions to swollen and pure micelles.

The microemulsions at 66.6% aqueous phase were then saturated with griseofulvin. The mean diameter

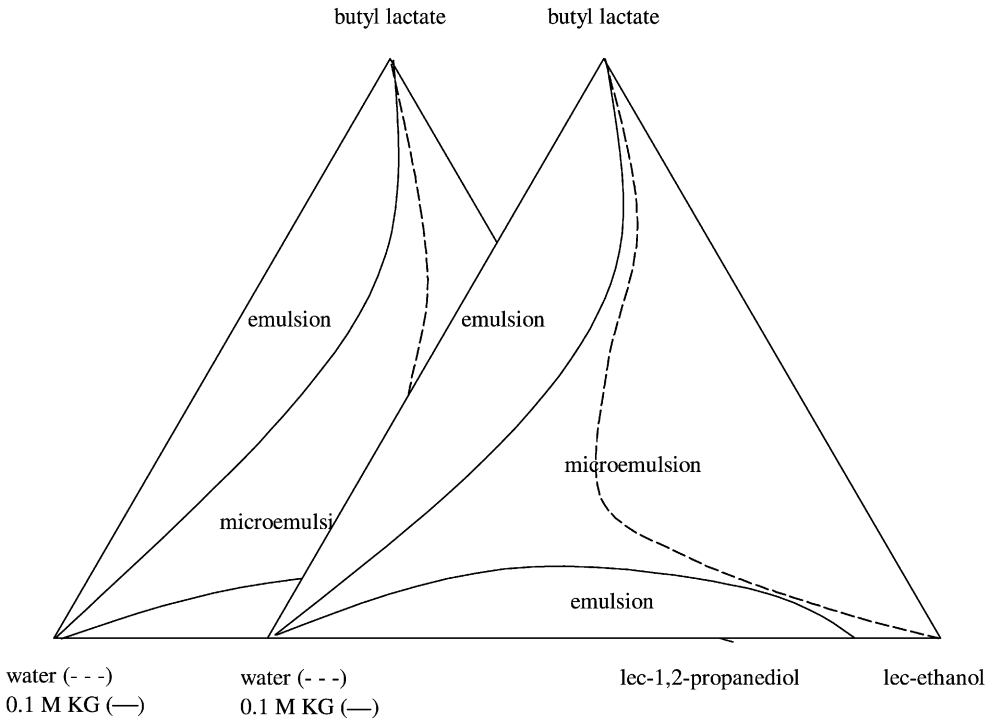

Fig. 2. Phase diagrams of systems containing water–KG (0.1 M), lecithin–alcohol (1:1, w/w), butyl lactate (solid line) and water, lecithin–alcohol (1:1, w/w), butyl lactate (dotted line).

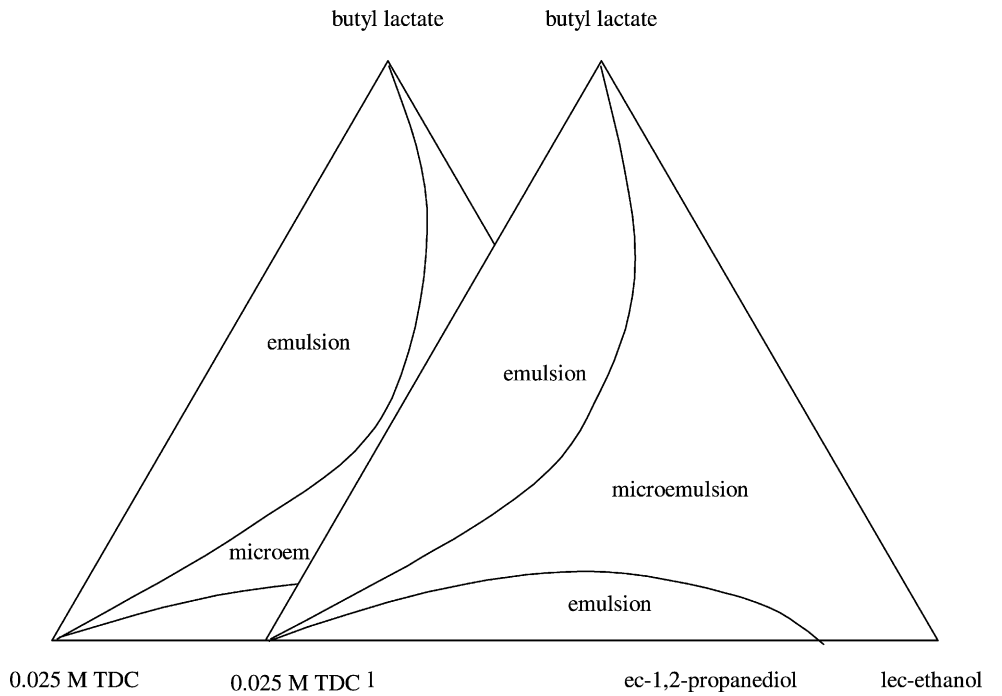

Fig. 3. Phase diagrams of systems containing water–TDC (0.025 M), lecithin–alcohol (1:1, w/w), and butyl lactate.

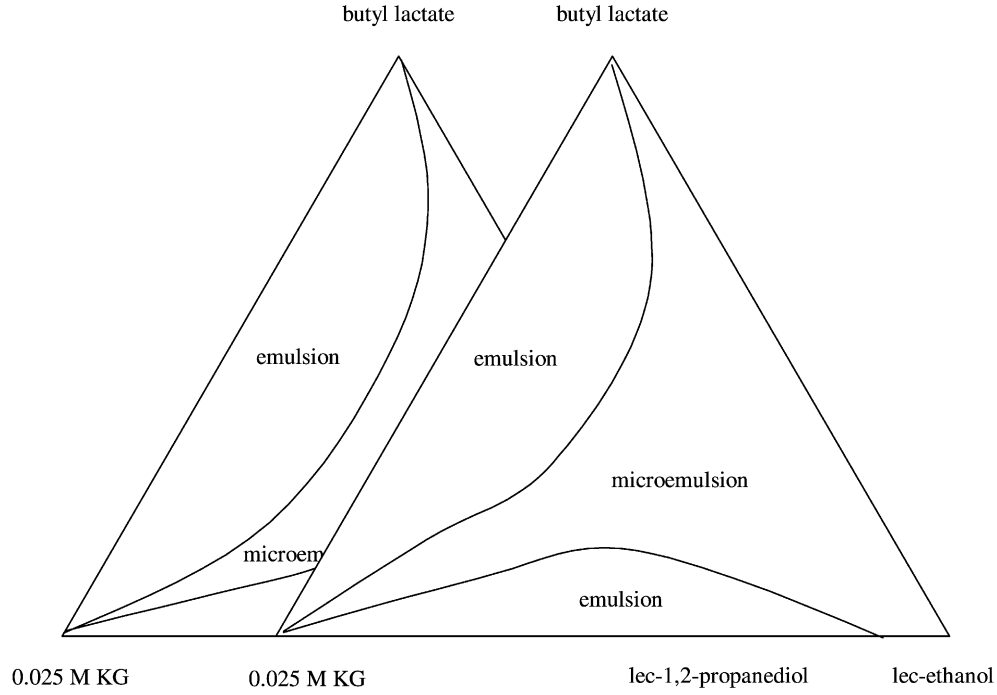

Fig. 4. Phase diagrams of systems containing water–KG (0.025 M), lecithin–alcohol (1:1, w/w), and butyl lactate.

of the drug-loaded microemulsions was 20–25 nm and they did not change significantly compared to the free-drug microemulsions.

The solubility of griseofulvin in the microemulsions ranged from 21 to 23 mg/ml, while the water solubility of the drug was 12 μg/ml: this indicated that most griseofulvin was in the disperse phase of the microemulsions. If a water-insoluble drug is dissolved in the disperse phase, the subsequent dilution with additional water extracts most of the disperse phase, converting the organic solvent droplets into solid particles.

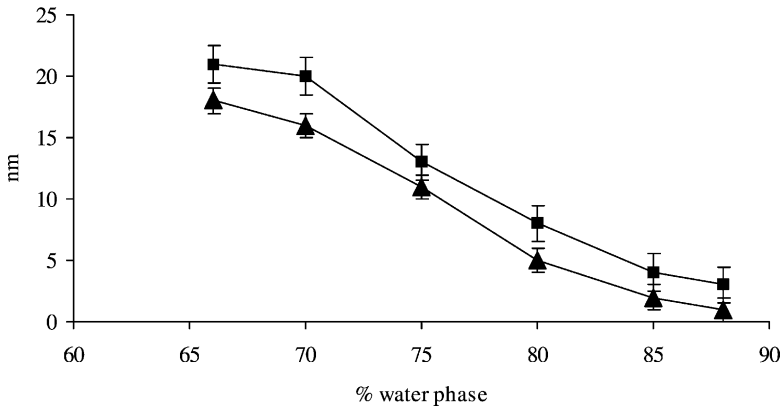

Fig. 5. Mean droplet size of the microemulsion containing 66.6% aqueous phase, 16.6% lecithin–ethanol (1:1, w/w), 16.6% butyl lactate as a function of water addition: (▲) 0.1 M KG, (■) 0.1 M TDC.

Table 1
Photon correlation spectroscopy diameter, polydispersity index, after and before diaultrafiltration, and *Z*-potential of griseofulvin suspensions

Solvent	Surfactant mixture	Cosurfactant	Size (nm)		*Z*-potential (mV)
			After	Before	
Butyl lactate	Lecithin–ethanol	TDC	162 ± 15 (0.12)	240 ± 32 (0.18)	−31
Butyl lactate	Lecithin–1,2-propanediol	TDC	212 ± 24 (0.15)	286 ± 44 (0.18)	−36
Butyl lactate	Lecithin–ethanol	KG	85 ± 12 (0.09)	98 ± 15 (0.11)	−38
Butyl lactate	Lecithin–1,2-propanediol	KG	103 ± 13 (0.08)	116 ± 18 (0.09)	−32

Table 1 reports the mean particle diameter, polydispersity index and *Z*-potential, determined by laser light scattering, of griseofulvin suspensions before and after the diaultrafiltration process. For all systems examined, the use of KG produced finer particles than those obtained using TDC. Considering that the mean diameter and the solubility efficiency were about the same for the microemulsions containing TDC or KG, the mechanism by which the use of KG produced finer particles than those obtained using TDC is not clear, and other parameters such as cosurfactant mobility, critical micelle concentration, etc. should be considered.

The suspension sizes and *Z*-potential values did not change significantly after diaultrafiltration for the systems containing KG; however, a significantly increase in size and polidispersity index occurred for the suspensions obtained from microemulsions containing TDC. The griseofulvin suspensions obtained from microemulsions containing lecithin alone, which gave an initial emulsion phase, produced very poor results.

The dissolution profiles of griseofulvin from diaultrafiltrated suspensions are reported in Fig. 6, together with those from reference systems. The dissolution rate of griseofulvin was markedly enhanced in the systems obtained by the solvent diffusion technique, compared to the dissolution rate of the drug from commercial nonwetted or wetted products and that of the suspension obtained by direct precipitation from butyl lactate drug solution. The fastest dissolution rate was for the suspension obtained from microemulsion containing KG, which also had showed the smallest particle size.

To verify whether the fast dissolution of griseofulvin from the suspension obtained via the solvent diffusion procedure was due to polymorphism, DSC measurements were carried out. The endothermic peaks of griseofulvin obtained from microemulsion and of commercial product were located at the same temperature (215 ± 1 °C) and of similar intensity, 106 ± 3 and 94 ± 4 J/g, respectively, indicating no significant change in the crystallinity of the drug; the

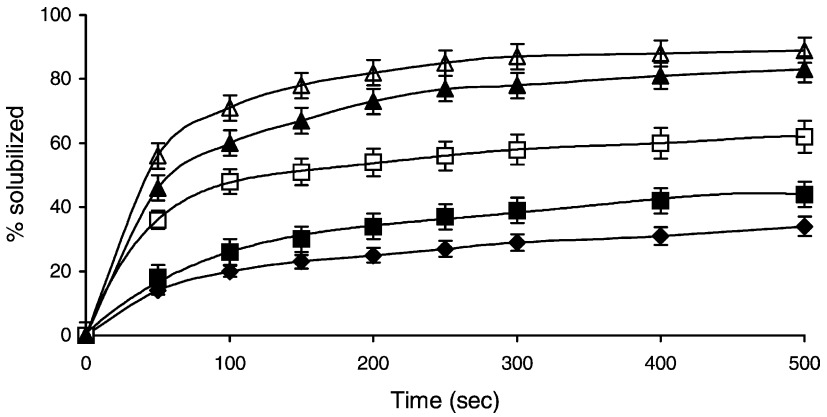

Fig. 6. Griseofulvin dissolution profiles of the commercial product (◆), commercial wetted product (■), suspension obtained from butyl lactate solution (□), suspension obtained from TDC (▲) and KG (Δ) containing microemulsions.

increase in dissolution rate was thus attributed to an increase in the available surface area of the particles.

4. Conclusions

The microemulsion-diffusion technique using pharmaceutically acceptable solvents, such as butyl lactate, led to successful fabrication of drug nanosuspensions. Using optimised formulations, griseofuvin nanoparticles below 100 nm with low polydispersity were obtained. Dissolution rates of griseofulvin particles obtained by the solvent diffusion procedure were higher than that commercial product.

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