ELSEVIER

International Journal of Pharmaceutics 130 (1996) 41–47

international journal of pharmaceutics

Ursodeoxycholic acid: improvement of dissolution behaviour and its HPLC determination

P. Giunchedi[a], S. Scalia[b], L. Maggi[a], U. Conte[a,*]

[a]*Department of Pharmaceutical Chemistry, University of Pavia, via Taramelli 12, 27100 Pavia Italy*
[b]*Istituto di Chimica Farmaceutica, Università di Catania, via A. Doria 6, 95100 Catania Italy*

Received 30 January 1995; revised 10 May 1995; accepted 30 June 1995

Abstract

The dissolution rate of a drug poorly soluble in water, ursodeoxycholic acid, was improved by using dissolution rate enhancers belonging to the group of cellulose and starch derivatives. Different techniques (mixing, milling and solvent evaporation) were utilized to prepare drug/carrier systems. The determination of the improved dissolution performance of the drug from the systems has been carried out by a modified in vitro dissolution test apparatus combined with HPLC analysis of the drug. The carriers and the techniques used for improving the dissolution rate, the dissolution apparatus and the HPLC method are proposed here to solve both the dissolution rate problems of the drug and its analytical determination.

Keywords: Water-insoluble drug; Ursodeoxycholic acid; Cholesterol gallstones; Improvement of drug dissolution rate; HPLC determination

1. Introduction

Ursodeoxycholic acid (UDCA) is a white, odourless, crystalline powder with a bitter taste. It is used as a drug for the dissolution of cholesterol gallstones (Bell et al., 1975; Bouchier, 1980; Dowling, 1983), because it reduces the cholesterol saturation of bile (Bergmann et al., 1984).

The use of UDCA for the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains has been demonstrated (Leuschner et al., 1981; Leuschner and Kurtz, 1987; Ward et al., 1984).

However in vivo studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects, and within the same subject (Parquet et al. 1985). More than 50% is lost in the stool (Stiehl et al., 1990) after a single oral dose of 300 mg.

This unfavourable in vivo behaviour is believed to be mainly due to the low solubility of UDCA in the intestinal neutral/basic environment (Igimi and Carey, 1980; Moroi et al., 1992). In fact after oral administration in capsule and tablet formulations, UDCA is slowly solubilized and passively absorbed in the small intestine (Roda et al., 1994). Owing to the low solubility of UDCA at intestinal

* Corresponding author.

SSDI 0378-5173(95)04249-A

pH (Igimi and Carey, 1980) its absorption is inefficient and dissolution is rate limited (Parquet et al., 1985; Roda et al., 1994). The solubility of UDCA increases only above pH 8.4, but this high pH is usually reached only postprandially with sustained duodenal and pancreatic secretion (Roda et al., 1994).

In previous works a water swellable polymer, cross-linked sodium carboxymethyl cellulose (croscarmellose sodium) (CMC-XL), was used as dissolution rate enhancer of model drugs insoluble in water (Giunchedi et al., 1990; Giunchedi et al., 1993).

The use of croscarmellose sodium together with polyvinylpyrolidone and lactose for the enhancement of the in vitro dissolution properties of UDCA has been recently described (Higginbottom et al., 1994). However the complexity of the preparation and the requirement for micronized drug are disadvantages of this formulation.

The aim of this work was the improvement of the dissolution rate of UDCA by using two different water swellable polymers as drug carriers: cross-linked sodium carboxymethylcellulose (CMC-XL) and sodium starch glycolate (PJ).

Drug/polymer systems were prepared with different techniques: ball milling, mixing and solvent evaporation. In vitro dissolution studies were carried out on the prepared systems, which were characterized by SEM and DSC analyses.

Since UDCA shows moderate absorption only in the short UV wavelength region (200–210 nm) (Scalia and Games, 1993), quantification of the amount released from the pharmaceutical preparations by simple UV spectrophotometry is hampered by possible interferences from formulation excipients and dissolution medium components (Anderson et al., 1990).

In order to overcome this problem, HPLC was used for the determination of the drug dissolution profiles.

2. Materials and methods

Materials used were: ursodeoxycholic acid (UDCA), Erregierre Industria Chimica S.p.A., Bergamo, I; dvs = 66.55 μm (Coulter Counter, model TA II, Coulter Electronics Ltd., Luton, UK); m.p. 200–205°C; solubility in USP Intestinal Simulated Fluid (pH 7.5) at 25°C 459 mg/l (experimental data); cross-linked sodium carboxymethylcellulose (CMC-XL), croscarmellose sodium, type A NF (Ac-Di-Sol®, FMC Corp., Philadelphia, PA, USA); sodium starch glycolate NF (PJ), (Primojel®, Avebe, Veendam, The Netherlands).

2.1. Preparation of drug/polymer systems

Three techniques of preparation of drug/polymer systems were employed: ball milling, mixing and solvent evaporation.

2.1.1. Mixing technique

UDCA and CMC-XL were mixed with a Turbula apparatus (W.A. Bachofen, Basel, CH) at a speed of 30 rev./min, for 2 h.

2.1.2. Ball milling technique

UDCA and CMC-XL or PJ were milled in a ceramic ball mill for 2 h, at a speed of 30 rev./min.

2.1.3. Solvent evaporation

UDCA (5 g) was dissolved at room temperature in 75 ml of methanol. CMC-XL (10 g) was added, achieving a suspension which was magnetically stirred for about 10 min. The solvent was then evaporated (40–45°C) under reduced pressure, using a rotating evaporator (Rotavapor Büchi R110, Flawil, CH). The solid residual was kept in a dessicator under vacuum for 24 h, obtaining an aggregated powder that was subsequently deaggregated through a 25 mesh sieve.

The compositions of the drug/polymer systems prepared are reported in Table 1.

2.2. In vitro characterization of raw materials and polymeric systems

2.2.1. Scanning electron microscopy (SEM)

The shape and morphological characteristics of UDCA, CMC-XL, PJ particles and of all the systems prepared with the different techniques were analysed by scanning electron microscopy

Table 1
Compositions of drug/polymer systems

System	UDCA (%)	CMC-XL (%)	PJ (%)	Technique of preparation
$UXL1_{mill}$	33.33	66.66	-	Ball milling
$UXL2_{mill}$	50.0	50.0	-	Ball milling
$UPJ1_{mill}$	33.33	-	66.66	Ball milling
$UPJ2_{mill}$	50.0	-	50.0	Ball milling
$UXL1_{ev}$	33.33	66.66	-	Solvent evaporation
$UXL1_{mix}$	33.33	66.66	-	Turbula mixing

UDCA, ursodeoxycholic acid; CMC-XL, cross-linked sodium carboxymethylcellulose; PJ, sodium starch glycolate.

(JSM 35C, Japan Electron Optical Laboratory, Tokyo, Japan) at 10 kV.

2.2.2. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Mettler TA 3000, Zürich, CH) was used in order to assess the thermal behaviour of the raw materials, and of the different drug/polymer systems. Samples were scanned in aluminium pans, under static air atmosphere, at a heating rate of 10°C min^{-1}, in the temperature range 50–250°C.

2.2.3. In vitro dissolution tests

In order to evaluate the in vitro dissolution behaviour of UDCA alone and in the polymeric systems we used a modified USPXXII n.2 dissolution test apparatus (Conte et al., 1993; Giunchedi et al., 1993).

Three hundred mg of drug or a quantity of drug/polymer system corresponding to 300 mg of drug were placed in 5 l of USP Intestinal Simulated Fluid (pH 7.5, 37°C, 100 rev./min).

2.2.4. High performance liquid chromatographic (HPLC) determination of UDCA

The analytical determination of UDCA was carried out by a modification of the HPLC method described earlier (Scalia, 1990). The test solution was concentrated with solid phase extraction: this pre-treatment is requisite to obtain a suitable detection sensitivity.

At determined time intervals, 3-ml samples of the dissolution medium were withdrawn; the sample was replaced with an equal volume of fluid maintained at 37°C.

The test sample was filtered (0.22-μm filters Millipore®) and a portion of this solution (1–2 ml) was passed through a pre-conditioned (2 ml methanol and then 2 ml water) Isolute C18 cartridge (sorbent weight, 200 mg; International Sorbent Technology, Hengoed, UK) which was eluted successively with 2 ml water and 2 ml methanol. The last fraction, containing the UDCA, was reduced to dryness under a nitrogen stream, redissolved in 0.2 ml of mobile phase and a portion (20 μl) of this solution injected onto the HPLC column. The HPLC apparatus consisted of a modular chromatographic system (Model 880-PU pump, Model 880-02 solvent programmer and Model 875-UV variable-wavelength UV-VIS detector, Jasco, Tokyo, Japan) connected to a Rheodyne 7125 injection valve with a 20-μl sample loop (Rheodyne, Cotati, CA, USA) and a chromatographic data processor (Chromatopac CR3A; Shimadzu, Kyoto, Japan). The detector was set at 210 nm and 0.02 absorbance units full scale (a.u.f.s.).

Separations were performed on a 5-μm Ultrasphere ODS column (150 × 4.6 mm i.d.; Beckman, Berkeley, CA, USA) eluted under isocratic conditions with a mobile phase constituted by methanol-acetonitrile-0.02 M aqueous sodium acetate (50:20:30 v/v/v) adjusted to pH 4.3 with phosphoric acid. The mobile phase was filtered through type GV filters and deaerated on-line by a model ERC-3311 automatic solvent degasser (Erma, Tokyo, Japan). Chromatography was performed at room temperature at a flow-rate of 1.1 ml/min. Quantification was carried out by the external standard method, using peak areas. Dissolution kinetics for each system were determined from the mean of five tests (S.D. within 3.7%).

3. Results and discussion

The different techniques used in the preparation of UDCA/CMC-XL systems play an important role in determining the morphological properties, as shown in Fig. 1. In fact $UXL1_{mix}$ (Fig. 1a) is characterized by the presence of CMC-XL fibers and UDCA crystals, both with no morphological change with respect to the raw materials (SEMs not reported); solvent evaporation leads to a remarkable change since the original shape of the drug particles has completely disappeared (Fig. 1b) and the surface of CMC-XL fibers are uniformally coated with a thin layer of drug (Fig. 1c).

Fig. 2 shows $UPJ1_{mill}$ morphological characteristics: PJ is present as roundish particles with a typical smooth 'pebble shape', while drug particles are characterized by a lower size (about 10 μm) with respect to the drug raw material (particles of about 100 μm), due to the milling process.

Fig. 3 presents the thermal behaviour of the two systems containing CMC-XL and prepared by milling, compared to the profiles of the components as raw materials. The drug is characterized by a sharp melting peak at about 204°C (Fig. 3a), while CMC-XL has a large endothermic band in the 50–100°C range, due to the loss of water present in the polymer (Fig. 3b). The presence of the drug in the two systems, $UXL1_{mill}$ (Fig. 3c) and $UXL2_{mill}$ (Fig. 3d), does not lead to any change either in the position or in the shape of the drug melting peak: no additional peak due to polymorphic transformation appears. However the heat involved in the melting process of UDCA in the two systems is different: in the case of UDCA:CMC-XL 1:2 system it is about 63% of the heat involved in the melting process of the drug alone, and about 70% in the case of UDCA:CMC-XL 1:1 system.

The thermal profiles of the two systems containing PJ are reported in Fig. 4 (c and d, respectively), compared to UDCA as pure drug (Fig. 4a) and to PJ as pure polymer (Fig. 4b). The PJ profile shows the 50–100°C endothermic band due to the loss of water, and an additional, unexpected short peak at about 168–170°C, probably due to an impurity of the material. Analogous peak was found in thermal studies carried out on sodium starch glycolate obtained by another manufacturer but it is not present in the DSC thermograms of corn starch; this is probably related to the preparation method of sodium car-

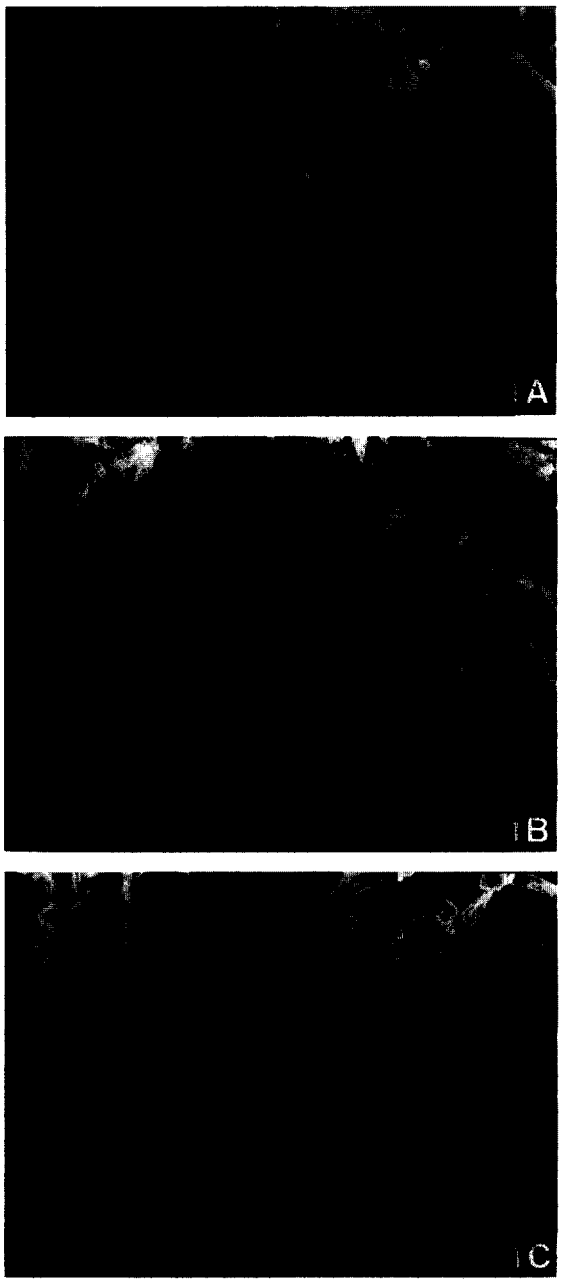

Fig. 1. Photomicrographs (SEM) of: (a) $UXL1_{mix}$ (120 ×); (b) $UXL1_{ev}$ (150 ×); (c) $UXL1_{ev}$ (800 ×).

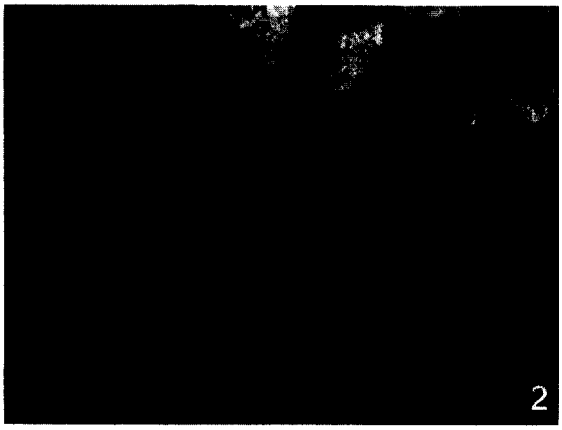

Fig. 2. Photomicrograph (SEM) of $UPJ1_{mill}$ (700 ×).

boxymethyl derivatives of starch. As for the CMC-XL systems, the presence of UDCA in the PJ systems does not lead to any variation in the position/shape of the melting peak, but the heat involved in the melting process of the drug is different (and much lower) with respect to the drug alone: about 45% for UDCA:PJ 1:2 and for

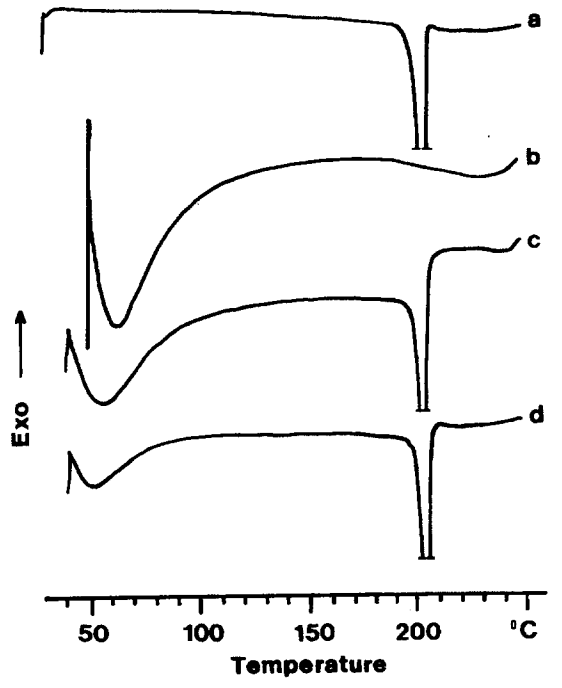

Fig. 3. DSC profiles of: (a) UDCA; (b) CMC-XL; (c) $UXL1_{mill}$; (d) $UXL2_{mill}$.

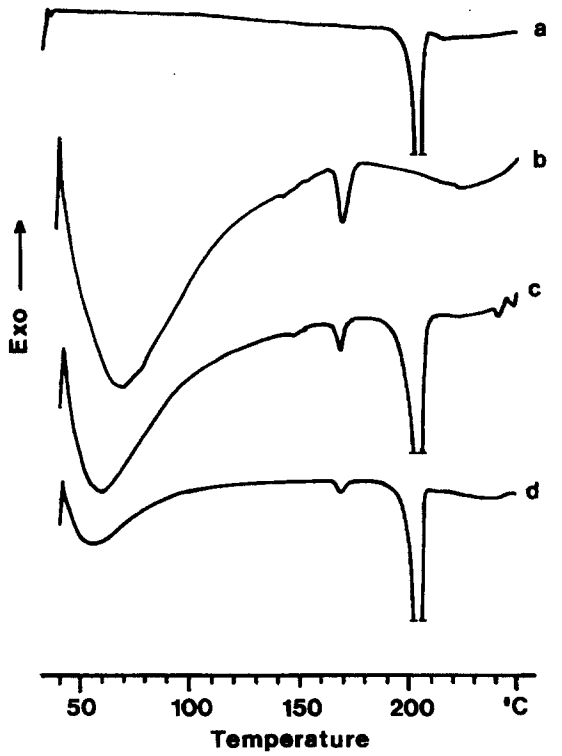

Fig. 4. DSC profiles of: (a) UDCA; (b) PJ; (c) $UPJ1_{mill}$; (d) $UPJ2_{mill}$.

UDCA:PJ 1:1 systems. Finally, Fig. 5 shows the thermal profiles of the UDCA:CMC-XL 1:2 systems prepared with the three different techniques: milling (Fig. 5a), mixing (Fig. 5b) and solvent evaporation (Fig. 5c). The only difference is, again, in the heat involved in the melting process of the drug and not in the position/shape of the melting peak: about 79% for mixed system, 56% for solvent evaporated system and 63% for milled system.

The dissolution profiles of the milled systems containing CMC-XL in the two different ratios, compared to the profile of the drug alone are reported in Fig. 6. The presence of CMC-XL leads to a remarkable improvement of the dissolution rate of the drug in a rank order corresponding to the amount of polymer in the systems: in 15 min about 100% of dissolved drug is achieved in the case of the system with the higher polymer content ($UXL1_{mill}$), vs. about 70% in the case of the system with the lower content ($UXL2_{mill}$).

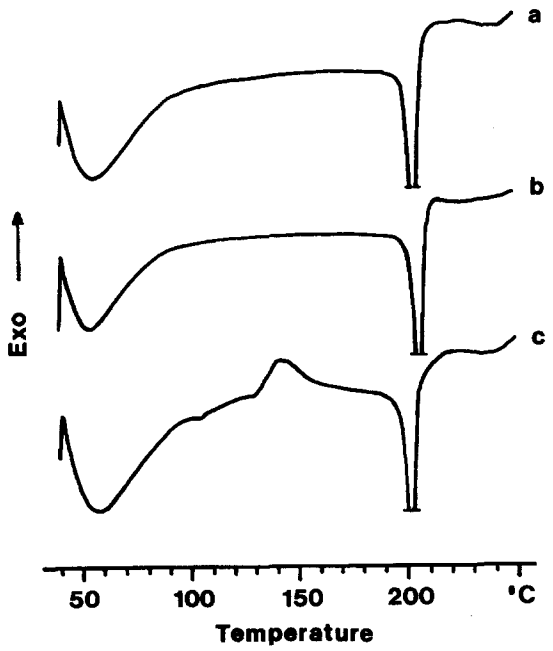

Fig. 5. DSC profiles of: (a) $UXL1_{mill}$; $UXL1_{mix}$; $UXL1_{ev}$.

UDCA raw material shows only about 12% of dissolved drug within the same time period.

When PJ is used as dissolution rate enhancer (Fig. 7), a good dissolution behaviour of UDCA is obtained from the two drug/polymer systems (about 75% of dissolved drug in 15 min), but the two different ratios of polymer lead to almost the same results as those achieved with $UXL2_{mill}$.

The influence of the preparation technique on the drug dissolution behaviour, using CMC-XL (1:2 drug:polymer weight ratio), is reported in Fig. 8. Milling leads to the best results. Solvent evaporation is more effective than the mixing technique, which is less efficient (Fig. 8).

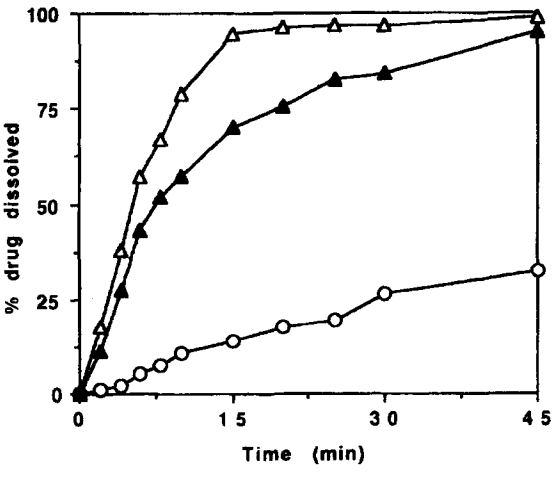

Fig. 6. In vitro dissolution profiles in USP phosphate buffer (pH 7.5) of: UDCA (○); $UXL1_{mill}$ (△); $UXL2_{mill}$ (▲).

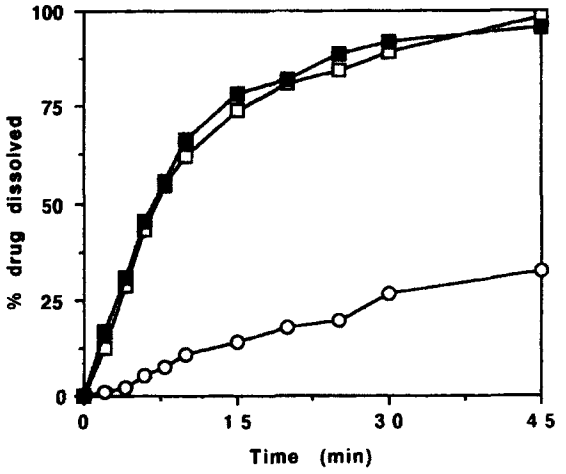

Fig. 7. In vitro dissolution profiles in USP phosphate buffer (pH 7.5) of: UDCA (○); $UPJ1_{mill}$ (△); $UPJ2_{mill}$ (▲).

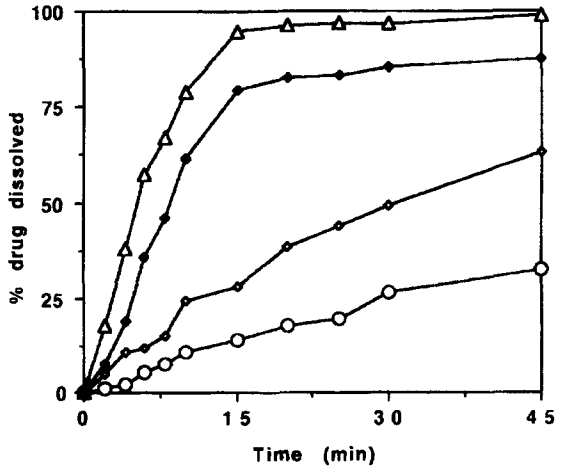

Fig. 8. In vitro dissolution profiles in USP phosphate buffer (pH 7.5) of: UDCA (○); $UXL1_{mill}$ (△); $UXL1_{mix}$ (◇); $UXL1_{ev}$ (◆).

4. Conclusions

A remarkable improvement of the drug dissolution rate is achieved by the systems prepared using cross-linked sodium carboxymethylcellulose or sodium starch glycolate.

Particularly promising is the use of sodium starch glycolate because it gives good dissolution results even in the lower weight ratio.

Milling and solvent evaporation techniques are the most effective among the different techniques of preparation employed. However the milling method has the advantage over solvent evaporation because it does not involve the use of any organic solvent.

For all these reasons the milled systems developed in this study can be considered promising for the preparation of oral dosage forms containing UDCA.

Acknowledgements

This work was partially supported by a grant of M.U.R.S.T. (Ministero Università e Ricerca Tecnologica, Italy). The authors wish to thank Prof. G.P. Bettinetti (Dipartimento Chimica Farmaceutica, Università di Pavia, Italy) for DSC studies, Mrs M.C. Sacchi for figure preparation and Dr. A. Callegari (Dipartimento Scienze della Terra, sezione Mineralogia, Petrologia e Geochimica, Centro Grandi Strumenti, Università di Pavia) for SEM analyses.

References

Anderson, N.H., Johnston, D. and Vojvodic, P.R., Dissolution testing using continous multicomponent UV analysis to correct for excipient interference, *J. Pharm. Biomed. Anal.*, 8 (1990) 987–989.

Bell, G.D., Dowling, R.H., Whitney, B., and Sutor, D.J., The value of radiology in predicting gallstone type when selecting patients for medical treatment, *Gut*, 16 (1975) 359–364.

Bergmann, K., Epple-Gutsfeld, M., and Leiss, O., Differences in the effects of chenodeoxycholic acid and ursodeoxycholic acid on biliary lipid secretion and bile acid synthesis in patients with gallstones. *Gastroenterology*, 87 (1984) 136–143.

Bouchier, I.A.D., The medical treatment of gallstones. *Annu. Rev. Med.*, 31 (1980) 59–77.

Conte, U., Giunchedi, P., Maggi, L. and La Manna, A., Erodible matrices containing hydroxypropyl-β-cyclodextrin for linear release of a water-insoluble drug (diazepam). *S.T.P. Pharma Sciences*, 3 (1993) 242–249.

Dowling, R.H., Cholelithiasis: medical treatment. *Clin. Gastroenterol.*, 12 (1983) 125–178.

Giunchedi, P., Conte, U. and La Manna, A., A swellable polymer as Carbamazepine dissolution rate enhancer. *Boll. Chim. Farm.*, 129 (1990) 17–20.

Giunchedi, P., Maggi, L., Conte, U. La Manna, A., Linear extended release of a water-insoluble drug, carbamazepine, from erodible matrices. *Int. J. Pharm.*, 94 (1993) 15–22.

Higginbottom, S., Malinson, C.B., Burns, S.J., Attwood, D., and Barnwell, S.G., Ursodeoxycholic acid: Effects of formulation on in vitro dissolution. *Int. J. Pharm.*, 109 (1994) 173–180.

Igimi, H. and Carey, M.C., pH-solubility relations of chenodeoxycholic acid and ursodeoxycholic acid: physical-chemical basis for dissimilar solution and membrane phenomena. *J. Lipid Res.*, 21 (1980) 72–90.

Leuschner, U. and Kurtz, W., Treatment of primary biliary cirrhosis and cholestatic disorders with ursodeoxycholic acid. *Lancet*, ii (1987) 508.

Leuschner, U., Leuschner, M., and Hubner, K., Gallstone dissolution in patients with chronic active hepatitis. *Gastroenterology*, 80 (II) (1981) 1208–1209.

Moroi, Y., Kitagawa, M., and Itoh, H., Aqueous solubility and acidity constants of cholic, deoxycholic, chenodeoxycholic, and ursodeoxycholic acids. *Lipid Res.*, 33 (1992) 49–53.

Parquet, M., Metman, E.H., Raizman, A., Rambaud, J.C., Berthaux, N. and Infante, R., Bioavailability, gastrointestinal transit, solubilization and fecal excretion of ursodeoxycholic acid in man. *Eur. J. Clin. Invest.*, 15 (1985) 171–178.

Roda, A., Roda, E., Marchi, E., Simoni, P., Cerré, C., Pistillo, A. and Polimeni, C., Improved intestinal absorption of an enteric-coated sodium ursodeoxycholate formulation. *Pharm. Res.*, 11 (1994) 642–647.

Scalia, S., Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges. *J. Pharm. Biomed. Anal.*, 8 (1990) 235–241.

Scalia, S. and Games, D.E., Determination of free bile acids in pharmaceutical preparations by packed column supercritical fluid chromatography. *J. Pharm. Sci.*, 82 (1993) 44–47.

Stiehl, A., Raedsch, R., and Rudolph, G., Acute effects of ursodeoxycholic and chenodeoxycholic acid on small intestine absorption of bile acids. *Gastroenterology*, 98 (1990) 424–428.

Ward, A., Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S., Ursodeoxycholic acid: A review of its pharmacological properties and therapeutic efficacy. *Drugs*, 27 (1984) 95–131.