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# URSODEOXYCHOLIC ACID FOR ORAL ADMINISTRATION: A FORMULATORY STUDY OF A SUSPENSION

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## ABSTRACT

Ursodeoxycholic acid (UDCA), a hydrophilic bile acid effective in dissolving cholesterol gallstones, is usually administered by capsules and tablets. However, oral solid dosage forms are generally inadequate for pediatric needs, since, in order to individualize the preparations, hospitals have to prepare, from capsules, doses corresponding to age and weight of the children. Aim of the present paper was to produce and characterize UDCA suspensions for a personalized regimen of therapy. Four different suspensions were prepared by dissolving preservatives, sodium chloride sweeteners and/or rheology modifiers in hot pure water. After cooling 2.5% (w/v) of UDCA powder was added. After production the formulations were subjected to UDCA content analysis by HPLC, rheological measurements and stability test. The physical and chemical stability of UDCA containing suspensions were investigated for 28 days after production. For physical stability the rate of sedimentation, the height of the sediment and the ease of redispersion, were measured. Particularly, the sedimentation volumes of the four suspensions were between 88 and 95%. The results demonstrated that all UDCA suspensions are characterized by good chemical stability (nominal concentration over 96%). The results indicated that suspensions could be proposed as alternative formulations for the pediatric administration of UDCA.

**Keywords:** Ursodeoxycholic acid (UDCA); oral dosage form; suspensions.

### Introduction

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that under normal circumstances represents a small fraction of the bile acid pool in humans. It is effective in dissolving cholesterol gallstones in appropriately selected patients.[1] Ursodeoxycholic acid improves serum alkaline phosphatase and aminotransferase levels in primary biliary cirrhosis, but its effects on rates of liver transplantation are less certain.[2-4]

In addition, obstetric cholestasis is an uncommon condition causing elevated blood bilirubin and bile acid level during the third trimester of pregnancy probably caused by a combination of genetic sensitivity of the bile duct membranes and increased hormone levels during pregnancy.[5,6] Treatment focused on reducing the bile acid levels to alleviate the mother's symptoms and to help decrease complications for the baby is, unfortunately, no perfect.[7] Current standards of treatment include the bile resin binder, cholestyramine, to reduce bile acid levels and antihistamines to control itching. It has been demonstrated that UDCA dramatically improved measured levels of bilirubin and liver enzymes.[4,8] Since further evidence is needed to better establish the safety and usefulness of the drug, this drug is currently considered for more severe cases occurring later in pregnancy.

Although the administration of UDCA is mainly made by capsules and tablet formulations, oral solid dosage forms are generally inadequate

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for pediatric needs, also because hospitals have to prepare, from capsules, doses corresponding to age and weight of the children in order to individualize the preparations. In addition the use of capsules for pediatric use can determine (a) a variable bioavailability depending on powder granulometry, (b) the necessity to open the dosage form in the case of administration to small children and (c) difficulty in swallowing tablets or capsules.[9] In addition, after oral administration, the suspended drug is already in a wetted state and is presented in the GI fluids in a finely divided form, thus increasing its absorption rate into the blood stream with respect another solid dosage form. In this view the replacement of capsules and tablets by oral liquid preparations able to supply various doses, such as suspension, is here proposed.

The limited use of UDCA suspension could probably be ascribed to the disgusting taste of the drug, that results in a low compliance of the patient. However, disagreeable taste, could be masked using suitable sweeteners or flavors while preparing the formulation. Among formulations intended for oral administration, suspensions represent an ideal dosage form for children. Moreover, drugs in suspension are chemically more stable than in solution, thus increasing the storage time of the formulation. In spite of these advantages, suspensions can be physically instable. As largely described in literature, physical instability is controlled by the addition of (a) flocculating agent able to enhance particle 'dispersibility' and (b) viscosity enhancer that reduce the rate of sedimentation in the flocculated suspensions.[10-12]

Aim of the present paper is thus to produce and characterize a UDCA suspension for a personalized regimen of therapy.

## Experimental

Suspensions were prepared as follows. 70 ml of pure water was heated to 100°C, and then preservatives and sodium chloride were added. Sweeteners and/or rheology modifiers were added after cooling to 90°C. Afterwards the solution was cooled to room temperature, then surfactants were added and the solution was diluted to the final volume. Finally 2.5% (w/v) of UDCA powder was added to the vehicle.

Quali-quantitative analysis of UDCA in the suspension was performed by reverse phase HPLC using a JASCO HPLC system, a Rheodyne 7125 sample injection valve (Rheodyne, USA) (equipped with a 20-ml loop), an LC 313 UV detector set at 260 nm. Samples were chromatographed on a 250 x 4.6 mm stainless steel column packed with 5 mm particles (Model Hypersil BDS C-18, Supelco, USA), eluted isocratically at room temperature using 50 mM sodium acetate (pH 4.3)-methanol (1:4, v/v) at a flow rate of 1 ml/min. The UDCA content of sample solutions was obtained by comparison of a standard UDCA solution.

Rheological measurements were carried out using a RI:2:M rotational viscometer (Rheology International Ltd., Shannon, Ireland). This instrument fits a cylindrical coaxial system with variable diameters conforming to DIN system. 14, 14w and 24 geometry spindle/cylinder couples and shear rate range between 1 and 165 $sec^{-1}$ have been used. Measurements were conducted at room temperatures.

The morphology of the powder in suspension was evaluated after 3 cycles of 24 hours thermal heating and cooling by observation on optical microscopy using an optical microscope Diaphot (Nikon, Japan).

## Results and Discussion

Four different suspensions (summarized in Table 1) were prepared as described in the experimental section. Particularly, the suspensions are characterized by the presence of polyols (i.e. mannitol and sorbitol) and a monosaccharide (i.e. fructose) (S1, S2, S4) or a disaccharide (i.e. sucrose) (S3) in order to mask the disgusting taste of UDCA. As wetting agents, Tween 20 (S2, S3) or Pemulen TR-2 (S4) are employed. The presence of Tween 20 leads to the production of opaque suspensions while the presence of Pemulen allows the obtaining of transparent and tight suspensions (data not shown). In addition, the presence of surfactants as wetting agents confers stability to the suspensions due to the reduction of floating phenomena. After production, suspensions were checked for UDCA content by HPLC method showing a nominal concentration over 96% (Table 1).



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***Table 1.*** *Composition of the suspensions used in the present study*

| Component | Suspension 1 (% w/v) | Suspension 2 (% w/v) | Suspension 3 (% w/v) | Suspension 4 (% w/v) |
|---|---|---|---|---|
| Sodium Chloride | 0.05 | 0.05 | 0.05 | 0.05 |
| Sodium Saccharinate | 0.8 | 0.8 | 0.8 | 0.8 |
| Fructose | 3.2 | 3.2 | - | 3.2 |
| Mannitol | 16.0 | 16.0 | - | 16.0 |
| Sucrose | - | - | 43.0 | - |
| Sorbitol | 26.0 | 26.0 | - | 26.0 |
| Agar | 0.4 | 0.4 | 0.4 | 0.4 |
| Pemulen | - | - | - | 0.2 |
| Propyl-p-hydroxybenz | 0.12 | 0.12 | 0.12 | 0.12 |
| Methyl-p-hydroxybenz | 0.03 | 0.03 | 0.03 | 0.03 |
| Tween 20 | - | 0.1 | 0.1 | - |
| Flavour | 0.1 | 0.1 | 0.1 | 0.1 |
| Water | to 100 ml | to 100 ml | to 100 ml | to 100 ml |
| UDCA[a] (mg/ml) | 25 | 25 | 25 | 25 |
| Recovered UDCA[b] | 97.49±1.69 | 96.71±1.43 | 97.01±2.52 | 96.20±1.98 |

[a] amount of UDCA poured in the formulation (nominal concentration). [b] percentage of recovery of UDCA with respect to total amount of drug poured in the formulation ±SD

The stability of suspension was evaluated by checking (a) the UDCA content, (b) the volume of the sediment and (c) the crystal growth during 28 days. The choice of 28 days for the stability analyses of the suspensions was made considering that for extemporaneous suspensions this length of time is much longer with respect to the period of common use of this type of formulation (usually around 10 days).

The results, reported in Table 2, demonstrated that all UDCA suspensions are characterized by good stability since the nominal concentration of the drug is maintained over 96%.

***Table 2.*** *UDCA[a] stability within the suspensions prepared in the present study*

| Day | Suspension 1 (% w/v) | Suspension 2 (% w/v) | Suspension 3 (% w/v) | Suspension 4 (% w/v) |
|---|---|---|---|---|
| 1 | 97.49±1.69 | 96.71±1.43 | 97.01±2.52 | 96.20±1.98 |
| 7 | 97.66±0.58 | 96.80±1.84 | 97.40±1.20 | 96.90±1.70 |
| 14 | 96.30±2.12 | 95.15±1.43 | 96.50±1.90 | 96.35±0.07 |
| 21 | 96.50±0.19 | 94.80±1.70 | 96.12±0.92 | 96.70±0.85 |
| 28 | 96.46±2.70 | 96.97±1.02 | 97.90±0.33 | 96.45±0.49 |

[a] amount of UDCA poured in the formulation (nominal concentration) is 25 mg/ml; [b] percentage of recovery of UDCA with respect to total amount of drug poured in the formulation. Data are the mean of three different determinations ±SD

The physical stability of a suspension is normally assessed by the measurement of its rate of sedimentation, the final volume (height) of the sediment and the ease of redispersion. The obtained sedimentation volumes of the four suspensions were between 88 and 95%. The high sedimentation volume is related to the slow rate of settling which give rise to the formation of a deflocculated system. This behavior is very important since it is possible to take from container a uniform dose of the drug, reducing the risk of inaccurate administration of the drug itself. Therefore a deflocculated suspension with a sufficiently high viscosity to prevent sedimentation would be an ideal situation.

For the assessment of crystal growth, an accelerated stability testing was performed. By exaggerating the temperature fluctuations of a formulation under normal storage conditions, it should be possible to compare the physical stability of a series of suspensions. Particularly, the formulations were subjected to four thermal cycles consisting of storage at 52°C for 7 hours followed by freezing at 4°C for 17 hours; afterwards samples were observed by optical microscope.

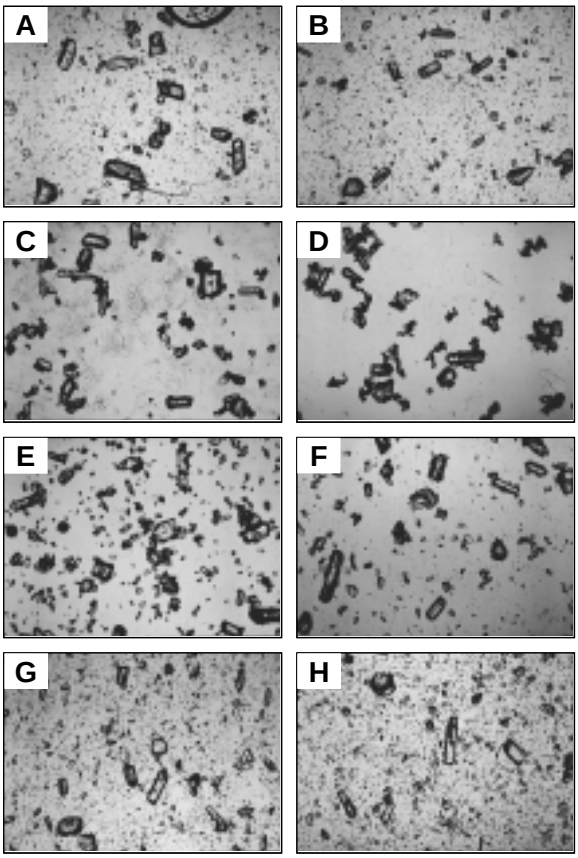


**Figure 1.** Light microscopy photographs of S1 (A, B) S2 (C, D) S3 (E, F) and S4 (G, H) before (A, C, E, G) and after (B, D, F, H) thermal cycling.

From the analysis of the results reported in Figure 1, it can be observed that after preparation S1 and S2 show many crystals of different size; whilst after thermal cycling the size of the large crystals was quite reduced but the size and number of smaller crystals do not seem to be changed. Nevertheless, the presence of Tween 20 in S2 induces a reduction in the number of the smaller crystals with respect to the untreated suspension. On the contrary, after thermal cycling the size of the larger crystals does not appreciably change. Concerning S3 (characterized by the presence of sucrose), the reduction in size of smaller crystals with respect to the corresponding untreated suspension is evident. Whilst for S4 there is no evidence of both crystals increase or disappearance of smaller one, probably due to the presence of the polymeric surfactant Pemulen in the vehicle that makes it tighter and allows a more efficient protection to the crystals.

In order to characterize UDCA suspensions, a series of steady stress sweep tests both for placebo and UDCA containing forms were performed. These tests gave information about the viscosity values as a function of shear rate.[13] As a general rule, the presence of UDCA causes a slight decrease of viscosity values except for S1, the only one without surfactant. Nevertheless, both placebo and UDCA containing formulations exhibit Newtonian behavior.

In conclusion the characterization of UDCA suspensions here described demonstrated that the presence of surfactants as wetting agents confers stability to the suspensions due to the reduction of floating phenomena, whilst the presence of the drug slightly reduces suspension viscosity. Particularly, after 28 days, UDCA suspensions maintained the nominal concentration of the drug over 96%; the sedimentation volumes of the four suspensions were between 88 and 95%; the presence of Tween 20 or sucrose gave rise to the reduction of the number of smaller crystals and the presence of Pemulen TR-2 do not affect crystal size after thermal cycles.

Based on the above, the present study indicates that suspension could be proposed as alternative formulation for the pediatric administration of UDCA.

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