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Fractal and reactive dimensions of some ursodeoxycholic acid salts

Adamo Fini *, Giuseppe Fazio, Maria Angeles Holgado, Maria José Fernàndez-Hervàs

Facoltà di Farmacia, Istituto di Scienze Chimiche, Università di Bologna, Via S. Donato 15, 40127 Bologna, Italy

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Abstract

Six salts of ursodeoxycholic acid, a bile acid largely administered for the dissolution of cholesterol gallstones, were obtained as powders by lyophilization of their aqueous solutions. The salts were prepared using lithium, sodium and potassium hydroxide; and using arginine, pyrrolidine ethanol and Tris as organic bases. Thermal, microscopy and EDAX analysis were carried out to identify the nature of the salts. Using the parameters of fractal geometry, fractal surface dimension (D_S) and reactive dimension (D_R) to dissolution were also obtained. The very low values (2.02–2.15) for the fractal dimension (D_S) of the particle surface suggest that, except in the case of the lithium (2.29) and pyrrolidine ethanol (2.43) salts, the particles thus obtained are characterized by smooth and regular surfaces. The D_R for most salts were found to be higher than the corresponding D_S and in a very narrow range of values (2.79–2.84). This fact was briefly discussed and compared with previous results obtained with other surfactants. The behaviour of the salt with the Tris base was considered separately.

Keywords: Ursodeoxycholic acid salts; Lyophilization; Thermal analysis; Fractal analysis; Fractal and reactive dimensions

1. Introduction

Ursodeoxycholic acid (UDCA) is a minor component of the bile acid pool in man: it is a secondary 3α,7β-dihydroxy bile acid formed in the intestine from chenodeoxycholic acid by bacterial epimerization of the 7-OH, which converts from α to β orientation. Such an orientation makes the hydroxy group protrude towards the hydrocarbon surface of the steroid ring system, thus reducing the hydrophobic portion and imparting peculiar physical and chemical properties to the whole molecule (Hofmann, 1984). In its

* Corresponding author. Tel.: + 39 51 244540; fax: + 39 51 249770.

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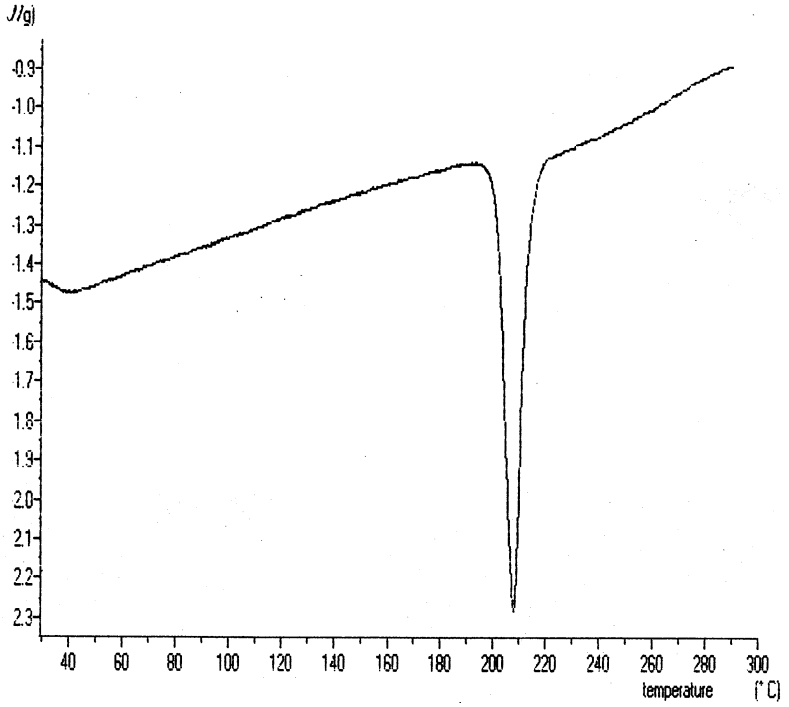

Fig. 1. Thermogram of ursodeoxycholic acid.

nonionized form it is less soluble than its parent epimer, forms micelle-like aggregates at a higher concentration and has a lower partition coefficient (Fini, 1989), diplaying an overall higher hydrophobicity. For its superior physiological properties it is widely administered as a drug for the solubilization of cholesterol gallstones (Makino et al., 1975). In this respect its administration as a powder has some problems either due to the low solubility and dissolution rate, when in the form of an acid, or to the acid–base reaction occurring to the salt form at the gastric level that limits bioavailability. Therefore any increase in knowledge about this compound and its derivatives will positively contribute to improving its use as a gallstone solubilizer.

We prepared six salts of this bile acid using inorganic hydroxides (LiOH, NaOH, KOH) and organic bases (arginine (Arg), pyrrolidine ethanol (PE) and trismethylol amino methane (Tris)) and analyzed them by differential scanning calorimetry (DSC). The fractal dimension of the powder surface (D_S) and the reactive dimension to dissolution (D_R) (Farin and Avnir, 1987) were determined to highlight the parameters affecting the dissolution of these salts. Since the dimension values of the powder are affected by the mode of crystallization or of the preparation of the sample (Fini et al., 1996a,b, 1997), all salts were processed in the same way: their concentrated aqueous solutions were lyophilized all together and the powders analysed as obtained for the fractal and reactive dimensions. This was particularly important, since in a previous paper (Fini et al., 1996b) it was found that the nature of the particle surface of the sodium cholate samples was clearly affected by the type of process used for their preparation.

2. Experimental

2.1. Materials

UDCA was a gift from Professor A. Roda (University of Bologna, Italy). Thermal analysis

Table 1
Chemical and physical properties of UDCA and comparison with its epimer chenodeoxycholic acid

	m.p.[a] (°C)	ΔH[a] (J/g)	S[b](mM)	pK_a^c	ST[d]	CMC[e]*	CMC[e]**	Log P^f_{HA}	*Log* P^g_{A-}
UDCA	209	92[a1]	9	5.08	48	19	7	3.00	2.20
Chenodeoxycholic acid	169	65[a2]	27	5.07	46	9	4	3.28	2.25

[a] Melting point and associated enthalpy: [1] present paper; [2] work in progress; [b] solubility at 25° and pH = 3.0; [c] p$K_a = -\log K_a$ (K_a, acidity constant in water at 25°C); [d] surface tension at CMC in dyn cm^{-1}; [e] critical micellar concentration in water (*) and in 0.15 M NaCl (**); [f]and [g] P = octanol–water partition coefficient of nonionized, HA [f] and of the ionized A^- [g] form (Roda et al., 1990). [a, b, c, g] refer to the acidic form; [d, e, g] refer to the ionized form (sodium salt).

revealed that the sample was pure and was used as received (Fig. 1). Some chemical–physical parameters of the unionised and ionised forms for UDCA are shown in Table 1 in comparison with those of its epimer, chenodeoxycholic acid (Fini, 1989).

Sodium hydroxide and potassium hydroxide were commercial samples from Carlo Erba (Milan, Italy). Lithium hydroxide, PE, Arg and Tris were purchased from Fluka (Buchs, Switzerland). All the samples were of analytical grade. PE was distilled at reduced pressure prior to use.

2.2. Preparation of the salts

UDCA (5 mmol) were suspended in 10 ml water under stirring; equivalent amounts of the bases and hydroxides, dissolved in 10 ml water, were added dropwise. At the end, a small excess of UDCA was added in order not to leave unreacted bases or hydroxides in the system and the mixture was left overnight under stirring. After filtration to eliminate the excess unreacted UDCA, the limpid solutions were frozen at −20°C and lyophilized at 10^{-2} mmHg.

The purity of the final powders was examined by DSC and their morphology was examined by scanning electron microscopy (SEM).

The final powders were sieved (Retsch, type Vibrio) and the following fractions were collected: 50–100, 100–150, 150–200, 200–250, 250–300, 300–350 μm and used for the determination of the fractal and reactive dimensions.

2.3. SEM

A very thin coat of carbon was applied to each sample examined at different magnifications and the micrographs taken (Philips, XL30, The Netherlands) are shown in Fig. 2A–F. During this examination, EDAX analysis was also carried out on the same powder particles.

2.4. DSC

Thermal analysis using DSC methods was performed using an automatic thermal analyzer system (Mettler FP80HP central processor and FP85 TA cell, Switzerland). A data processing system (Mettler FP89HT, Switzerland) was connected to the thermal analyzer. Sealed and holed aluminium pans were used for all the experiments. Temperature calibrations were made using indium as a standard. An empty pan, sealed in the same way, was used as a reference. All the samples were run at a rate of 10°C min^{-1}, from 30 to 300°C. Table 2 shows the temperature peaks (°C) and the enthalpy change (J/g) associated with the melting of the salts prepared with the organic bases.

2.5. Fractal Analysis of the particle surface

The analysis was carried out according to the increment method (structured walk method) and was followed automatically during the SEM inspection (Fernàndez-Hervàs et al., 1994; Fini et al., 1996c).

At a given magnification, SEM selects a step length (δ_1) to measure the perimeter of the particle

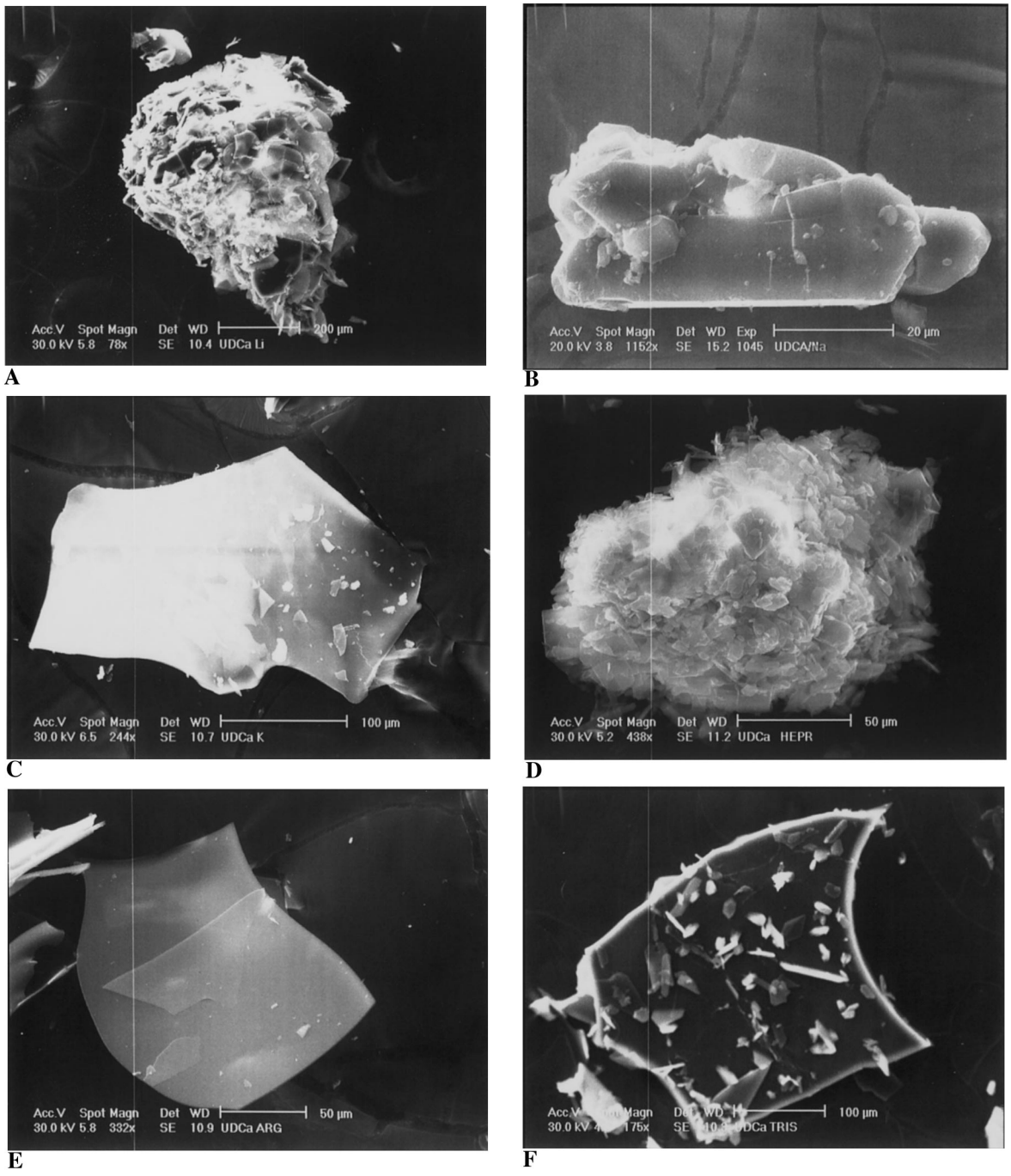

Fig. 2. Micrographs of salt particles between UDCA and (A) Li; (B) Na; (C) K; (D) PE; (E) Arg; (F) Tris.

Table 2
Thermal parameters for UDCA salts with organic bases

Base	Peak temperature (°C)	ΔH (J/g)
Arg	143	4.97
PE	147	67.6
Tris	119	13.2

($L_{\delta 1}$); a larger step $\delta_2 > \delta_1$ will give a perimeter $L_{\delta 2} < L_{\delta 1}$, because fewer details of the particle border are considered. The operation is repeated from five to ten times for each particle for up to 50–100 particles for a given fraction of the salt powder, until the magnification is so low that the resulting data lack meaning. The mean size of the particle was the same for all salts examined.

The fractal dimension of the particle contour (D_l) was calculated from the slope of the Richardson plot ($\ln L_\delta$ versus $\ln \delta$): the fractal dimension of the surface was obtained as usual: $D_S = (D_l + 1)$ (Fini et al., 1996c) and is shown in Table 3.

The method adopted in this paper is listed as one of the possible methods suggested for the determination of the D_S values (Farin and Avnir, 1987), even though D_S could be more properly determined using gas adsorption. The use of a large number of particles examined together with SEM micrographs of the particle surface make it possible to validate the D_S values simply obtained starting from D_l.

2.6. Reactive dimension to dissolution

To determine the reactive dimension to dissolution of the UDCA salts the selected fractions of the sievings were examined for the dissolution rate.

The dissolution assay was carried out in a basket apparatus (Turu Grau, model D-6, Barcelona, Spain), according to USP XXIII, using 500 ml bidistilled water at 37 ± 0.05°C. The rotational speed was kept constant at 50 rpm and 50 mg of each sample were used. The sink conditions were maintained constant throughout each assay. Dissolution of the sample was followed by measuring the conductance of the solutions using a digital conductivimeter (Crison, model Micro, M-220) linked to a chart recorder and an IBM compatible PC, the system provides one datum per s. The dissolution profiles as a function of the mean size of the particles were as expected.

To evaluate D_R we used the dissolution efficiency (E_d), an amodelistic parameter, calculated from the dissolution profiles obtained for each fraction. The E_d is defined as the ratio, in terms of percentage, between the area under the curve for the dissolution obtained after a prefixed time and the area of the rectangle having as dimension the ordinate corresponding to 100% dissolution and the same time abscissa.

D_R was calculated from the slope of the plot $\ln E_d$ versus ln (mean size) of the particles fraction. Table 3 lists the D_R values obtained for the different salts.

3. Discussion

The influence of the counterion in the salts of acidic drugs is widely recognized, mainly with regards to the solution behaviour (Fini et al., 1996d), moreover, since the nature of the salt also affects the properties of the drugs in the solid state, especially the crystal structure, it could also be interesting to evaluate their influence on the external structure of the particles resulting from a common crystallization process. In fact, the morphology of the particles largely depends on the

Table 3
D_S and D_R for UDCA salts

Dimension	Salt (value ± S.D.)					
	Li	Na	K	PE	Arg	Tris
D_S	2.29 ± 0.02	2.15 ± 0.02	2.06 ± 0.04	2.43 ± 0.02	2.02 ± 0.02	2.09 ± 0.04
D_R	2.85 ± 0.02	2.84 ± 0.01	2.82 ± 0.03	2.79 ± 0.05	2.79 ± 0.05	2.50 ± 0.01

experimental conditions during crystallization, as was also demonstrated in a previous paper concerning the fractal dimension of sodium cholate (Fini et al., 1996b). To eliminate possible differences related to this aspect, which depends on the nature of the solvent or the cooling rate or the extent of saturation, all the salts were prepared from aqueous solutions with the same starting concentration and lyophilised in only one batch.

Lyophilization was chosen among other techniques for preparation of the final samples for a variety of reasons. First of all, crystallization of the different salts could have made it necessary to use different solvents or mixtures, thus introducing undesirable variables. But more importantly, this technique allowed us to use a common process suitable for all present purposes and also useful for possible future comparisons. This method of producing powders permits the analysis of solid particles, without any further processing apart from sieving. The final differences in the resulting powder particles could therefore be attributed to the nature of the counterion, thus eliminating cross influences of other factors (Fini et al., 1996a).

While the only difference among alkali metal UDCA salts consists of the increasing radius of the cation, the organic bases mainly differ in their hydrophilic–hydrophobic balance: Arg possesses a charged moiety, Tris has three hydroxy groups on the molecule, PE was recently found useful in increasing the solubility of the poorly soluble diclofenac, an antiinflammatory drug (Fini et al., 1991). All the inorganic counterions are used in drug administration; Arg is also a physiological aminoacid; Tris is used in biological buffers and, even some adverse effects have been described (Perrin and Dempsey, 1974), it was used to prepare salts of an antiinflammatory drug (Gu and Strickley, 1987), finally PE was recently used in preparing a salt with diclofenac for oral administration (Maggi et al., 1990).

Micrographs of the inorganic salts of Fig. 2 (A, B and C) show a regular morphology for most salt particles, which have the appearance of crystalline structures: the edges of the particles are very well-defined and the particle surfaces also appear clean and smooth. The Na salt shows well-defined borders which allow the identification of polygonal facets. In the case of organic salts, UDCA–Arg and UDCA–Tris are formed by well-shaped single particles with a smooth appearance. On the contrary, UDCA–PE has particles apparently formed by the agglomeration or stratification of smaller particles.

The different salts were also analyzed by EDAX and DSC techniques.

EDAX analysis was performed during the microscopy examination. When the electron beam of SEM hits the particles, an emission of X-rays was induced, whose frequency is a function of the elements present in the compounds. So, except for the Li salt, whose atomic weight is too low for this type of analysis, the Na and K salts were identified by the presence of typical peaks in the EDAX spectrum. While the salts with organic bases had the common presence of the N-peak; this peak was not significant, because it was too close to the O and C peaks.

Lyophilization allows the preparation of well-dehydrated particles: no endotherm related to loss of water is present in DSC thermograms of the salts from organic and hydrophilic bases. Moreover, even under sieving, the powders do not form agglomerates of larger size and rugged surface, as was reported in the case of sodium cholate (Fini et al., 1996b).

Thermal analysis gave reliable results for the organic salts, that show endotherms between 100 and 200°C, attributed to melting, while for inorganic salts no melting peak could be observed in this temperature range. In this last case the nature of the salt was confirmed by the EDAX spectra.

Particles of all the salts prepared have regular contours: under these conditions the D_S of isolated particles was low, near 2.0, a reference value for very smooth and regular surfaces, as is also shown in the micrographs (Fig. 2). The fractal dimension of the surface is a suitable and reliable descriptor of the surface roughness. As documented by the micrographs, the PE and Li salts show small surface irregularities, with respect to other samples. This is also confirmed by the relatively higher D_S values found for the above mentioned salts, in agreement with the direct SEM imaging of the particle surface.

From Table 3 two points are evident: (a) the D_R values are higher than the corresponding D_S ones; (b) the D_R are practically the same for all the compounds examined, except the UDCA–Tris salt.

$D_R > D_S$ values have been reported (Farin and Avnir, 1987) as indicating the existence on the particle surface of sites where dissolution occurs more efficiently than expected. However SEM micrographs show that the particles of most salts have a regular surface, without the presence of formations, such as cracks or fissures, or agglomerations, well-known to activate the dissolution process. According to previous reports (Fernàndez-Hervàs et al., 1994; Fini et al., 1996b) it can be suggested that the main factor responsible is not the nature of the surface, but the nature of the dissolving compounds. In fact UDCA, like other bile acids, behaves as a surfactant in the ionized form and forms micelle-like aggregates, above a given concentration (CMC). Although it is reported that the CMC for a given bile salt depends on the nature of the cation (Carey and Small, 1969), differences are limited. According to the Noyes–Whitney theory the concentration of the solute is high in the diffusion layer, around the dissolving particle, tending to saturation, so it can easily overcome the CMC, at least in this volume.

In the case of the dissolution of a surface active compound specific parameters play an additional role in the process. Surfactants decrease the interfacial tension between the dissolution medium and the dissolving particle surface, thus increasing the dissolution rate. Furthermore, the formation of aggregates slows down the achievement of the saturation in the diffusion layer, even though the increase of the mass of the solute aggregate hinders its diffusion toward the bulk solution. This last aspect probably is not really important, since bile salt micelles are known to possess a low aggregation number. In particular this could be true for UDCA salts, because, as reported above (Fini, 1989) the presence of the 7-βOH reduces the aggregation ability of the UDCA anions.

Under these conditions the reactive dimension to dissolution is less dependent on the particle surface, but tends to be leveled at the highest values and only very small differences could be found among these salts. Similarly high D_R values were observed with some samples of sodium cholate (Fini et al., 1996b), having very different surfaces, and with samples of the diclofenac–PE salt (Fernàndez-Hervàs et al., 1994), suggesting that this could be a rather general behaviour in the case of surfactants.

If this is true and confirmed by further cases, a general conclusion can be drawn: in the case of surfactants, dissolution is not governed by the nature of the particle surface, as is the case in heterogeneous reactions. The chemical form of the surfactant (i.e. the nature of the counterion) would appear to be a secondary aspect, provided that it assures a sufficiently high solubility.

Finally the Tris salt behaves a little differently to all the others. Possibly this can be attributed to the high number of hydroxy groups, that extensively interact with water molecules of solvation, lowering the diffusion coefficient of the solute. The possibility that this salt has a lower solubility than the other ones cannot be ignored either. This aspect is not supported by the thermal parameters, which suggest a rather low interaction in the solid state, as documented by low values of both the melting point and the associated enthalpy change. However a low solubility in water was surprisingly observed also in the case of the diclofenac–Tris salt (4 mM), that, despite the high hydrophilicity of the cation, showed a much lower solubility in water than that of the sodium salt (30 mM) (Fini et al., 1996a).

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