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Review

Determination of bile salt critical micellization concentration on the road to drug discovery

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ABSTRACT

With the discovery of the bile acid (BA)-activated nuclear and membrane receptors, the role of BAs as signalling molecules in important paracrine and endocrine networks has been fully documented in the last decade. Besides regulating their own synthesis and transport, BAs have been demonstrated being involved in triggering the adaptive response to cholestasis and other insults to liver. More to the point, their recognized ability to control the general energy-related metabolism and inflammation processes has contributed to justify the renewed interest towards this class of amphiphilic steroidal compounds.

All these evidences feed a continuing interest in the BA research aimed at designing and synthesizing new side chain- and body-modified derivatives endowed with improved biological and physico-chemical profiles, as well as with proper ADMET behaviour. In this context, the micellar aggregation of BAs, and the respective critical micellization concentration (CMC) value (determined on the BA sodium salt, BS), is considered a key parameter that needs to be determined in the preliminary phase of compound characterization, being implicated in cytotoxicity issues.

An extraordinary variety of different analytical techniques and methods have been proposed along the years with the aim of better identifying the start of the self-aggregation process of BS monomers. The unicity of the physico-chemical nature of such class of compounds can be invoked to explain this unusual interest. Accordingly, a number of both invasive and non-invasive approaches have been developed along with a limited number of indirect chromatographic-based estimation strategies. Worth to be mentioned among the non-invasive determination methods are those based on potentiometry, freezing point depression, surface tension, nuclear magnetic resonance, viscosimetry, turbidimetry, microcalorimetry, refractometry, conductimetry, spectrophotometry, cholesterol solubilization, and monoglucuronide solubilization. Dye solubilization- and fluorescence-based methods deserve instead credit among the invasive methodological approaches. Indirect chromatographic methods based on capillary electrophoresis and high performance liquid chromatography analysis also demonstrated to be profitably exploited for the CMC estimation, especially when a small amount of sample is available. The collection of literature data reveals that the CMC value of a given BS is markedly related to the method selected for determining it as well as to the experimental conditions applied during the analysis.

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1. Introduction

Over the last 10 years, the importance of bile acids (BAs) and their derivatives has increased considerably in both basic science and clinical applications for several reasons [1,2]. First, BAs are the main catabolic product of cholesterol, and they play a key role as endogenous body detergents in the absorption of dietary fat and solubilization of biliary cholesterol thanks to their amphiphilic nature [3,4]. Second, biliary secretion of BAs is determinant for the maintenance of bile flow and in preventing cholestatic diseases [5]. Third, it has been demonstrated that BAs are ligands for both nuclear and membrane receptors by which they modulate diverse signalling pathways [6], regulating their own homeostasis, fat glucose and energy metabolism [7], as well as inflammation processes [8]. Finally, certain BAs have proven to be employed as therapeutic agents: while ursodeoxycholic acid (UDCA, Ursofalk®) is commercially available as anticholestatic agent, a number of body [9,10] and side chain-modified analogs [11–18] represent the new avenue for BA-based drugs in a variety of preclinical and clinical settings, including liver and metabolic disorders [19].

In this scenario, the composite and complex nature of BA network and functions requires that new BA derivatives targeting these pathways are well defined in terms of pharmacological and physico-chemical properties. To this end, the study and the understanding of the relationships between their structure and properties are the critical factors which may facilitate, or sometime limit, their employment in drug discovery programmes. This makes of high priority the search for valuable analytical protocols, useful for the characterization of this important class of compounds based on the assumption that structurally diverse BA derivatives exhibit different physico-chemical profile, including the critical micellization concentration (CMC, determined on the BA sodium salt, BS). The presence and/or diverse orientation of the hydroxy groups, modifications of both steroidal nucleus and side chain induce different conformations of the BA scaffold thus affecting physico-chemical parameters directly related to detergency such as CMC and hydrophobic/hydrophylic balance.

Thus, due to its relevant physio-pathological implications, many efforts are still spent by scientists in order to refine (or even set-up) suitable experimental methodologies able to determine the CMC of BSs.

From a look of the concerning literature, it appears disconcerting the extraordinary variety and conceptual diversity of the analytical strategies which have been engaged through the years with this aim. The unicity of the physico-chemical nature of such class of compounds [18,20] can be invoked to explain this extraordinary interest. In this frame, to report the established IUPAC definition of CMC [21] is of aid to put into light the main dissimilarities among the BSs and the other classes of amphiphilic compounds: 'There is a relatively small range of concentrations separating the limit below which virtually no micelles are detected and the limit above which virtually all additional surfactant molecules form micelles. Many properties of surfactant solutions, if plotted against the concentration, appear to change at a different rate above and below this range. By extrapolating the loci of such a property above and below this range until they intersect, a value may be obtained known as the critical micellization concentration (critical micelle concentration) …'. Both the sentences reported in this definition do not actually fit the BS aggregation mechanism. (i) Unlike the conventional ionic surface active agents, the BS aggregation materializes over a broader concentration range ('stepwise aggregation process') [18,21]. This feature well justifies the more realistic concept of the 'noncritical multimer concentration' [22,23] which encodes for the non-cooperative and continuous (that is concentration dependent) association by the BS monomers. (ii) Owing to the polydispersity of the BS aggregates [24–27], many of the methods proposed for the determination of their CMC do not allow to sensitively detect subtle variations of the physico-chemical state of the system. In its train, the CMC value of these steroidal compounds is markedly related to the method selected for determining it along with the experimental conditions applied during the analysis [18,23,24]. The problem comes to be further complicated owing to the often unshared definition of BS micelle as well as the hierarchy of forces responsible for the monomeric association. In this scenario, the most trustworthy conceptual model to describe the micellization of the BSs is still that proposed by Small [18]. Briefly, the amphipatic nature is due to the existence of a hydrophilic concave side (α-face) and a hydrophobic convex side (β-face) (Fig. 1a and b).

Regardless the type of BS and the fixed experimental conditions (both affecting the aggregation process), some points of likeness can be however enlightened. Accordingly, as the first step, the monomers enter a contact through their hydrophobic β-surfaces (Fig. 1c), thus giving rise to the formation of the so-called primary micelles. In such a way, the hydrophilic concave α-faces are in contact with the solvent. 'Secondary micelles' are then formed by polymerization of the existing primary aggregates *via* intermicellar H-bonding among the exposed OH groups (Fig. 1d).

As previously advanced, many different techniques for determining the BS CMC value have been used so far. While some of these techniques use exogenous molecular probes or tracers (invasive methods) [22,23,28–42], others permit direct CMC value determination (non-invasive methods) [22,25,43–55].

The non-invasive approaches generally take the advantage to avoid the realization of 'secondary equilibria' between the detergent and the probe molecules. Such processes were noted to perturb the BS back-to-back contacts, thus affecting the start