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Improved synthesis and characterization of bile acid esters: Organogelation and supramolecular properties

Jair García-Méndez [a], Adolfo López-Torres [b], María A. Fernández-Herrera [a,*]

[a] Departamento de Física Aplicada, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida. Km 6 Antigua Carretera a Progreso. Apdo. Postal 73, Cordemex, 97310 Mérida, Yuc, México
[b] Centro de Investigaciones Científicas, Instituto de Química Aplicada, Universidad del Papaloapan, Circuito Central Num. 200, Col. Parque Industrial, 68301 Tuxtepec, Oax, México

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ABSTRACT

Bile acid esters and their derivatives hold significant interest due to their applications in fields such as supramolecular chemistry, biomedicine, and nanomaterials. This study revisits the synthesis and characterization of esters derived from cholic, deoxycholic, and lithocholic acids using short-chain alcohols in combination with microwave-assisted heating. The synthesized esters were analyzed for their potential as gel-forming agents, and their organogelation properties were evaluated. Microwave-assisted synthesis offers rapid and efficient esterification, leading to high yields with improved selectivity. The organogels were characterized through techniques such as differential scanning calorimetry (DSC) and scanning electron microscopy (SEM), revealing distinct structural and thermal properties. The study highlights the potential of these bile acid esters in materials science and supramolecular chemistry, contributing to the development of novel functional materials.

1. Introduction

Esters and derivatives of bile acids (Fig. 1) are of interest due to their applications in fields such as biomedical sciences, nanomaterials, sensors, pharmacology, supramolecular chemistry [1–5], membrane-selective transporters [6–8], synthetic intermediates [9–13], antibacterial agents [14], polymerization hosts [15,16], and gelling agents [3,17]. Their versatility arises from unique properties like structural rigidity, stereochemistry, functionalization, biological activity, facial amphiphilicity, and self-assembly capability [5,18,19].

The esterification of fatty acids has been extensively studied, leading to the development of numerous methodologies. In contrast, only a limited number of approaches are available for the esterification of bile acids, primarily focusing on the synthesis of methyl esters. For instance, diazomethanes have been employed for this reaction; however, their preparation is hazardous, and the reagents involved are highly toxic [20]. Furthermore, the reaction exhibits poor selectivity, yielding 3- and 7-methoxy ethers as by-products. Later, alternative methods using mineral acids, such as HCl or H_2SO_4, as catalysts were introduced, resulting in improved yields [21]. Nevertheless, these reactions still produce dehydration by-products and ethers when applied to polyhydroxylated compounds. The use of mineral acid-derived salts, such as K_2CO_3 with Me_2SO_4, demonstrated promising results; however, these reactions required prolonged reaction times, elevated temperatures, and the use of volatile reagents during the work-up [22].

In the early 1980s, milder acids such as *p*-toluenesulfonic acid (*p*-TSA) [23] or acyl chloride (AcCl) [24] were explored as catalysts for the esterification of bile acids. These methodologies improved the process by providing higher yields, enhanced selectivity, and shorter reaction times. By the mid-1990s, the methylation of various bile acids was investigated using *p*-TSA as a catalyst, with heating provided by a household microwave oven [25]. This approach achieved conversion rates of 90–95% while maintaining selectivity and significantly reducing reaction times. However, by then, the repeatability of this technique was compromised due to the non-homogeneous heating produced by domestic microwave ovens, which do not effectively focus microwaves.

Bile acid derivatives have been extensively investigated in the field of supramolecular chemistry due to their inherent tendency to self-assemble. This property is of particular interest as it enhances our understanding of self-assembling systems, like phenomena observed in molecular crystal formation, protein folding, and cellular structures, all of which exhibit a wide range of functional self-assemblies. The study and comprehension of self-assembly are critical, as they provide insights into information encoded by nature (such as shape, surface properties,

* Corresponding author.
E-mail address: mfernandez@cinvestav.mx (M.A. Fernández-Herrera).

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charge, mass, polarity, etc.) within individual components, which dictate their interactions—primarily through non-covalent or weak interactions, including Van der Waals forces, electrostatic interactions, hydrogen bonds, coordination bonds, and hydrophobic interactions [26].

On the other hand, bile acid derivatives have demonstrated a variety of supramolecular architectures, including ionic receptors, dendrimer structures, crown ether macrocycles, molecular tweezers, and hydro- and organogels [27,28]. The gel-forming properties of bile acids were first identified in the 20th century, when it was observed that certain bile salts could form gels in physiological media, influenced by various factors [29–31]. Since then, numerous efforts have been made to synthesize, understand, and apply these entities in fields such as biology, luminescence, nanoparticle formation, and pharmaceuticals [5,32].

In this study, we revisit the synthesis of bile acid esters with the aim of optimizing and standardizing the reaction conditions for this important class of molecules, whose potential applications in materials science and supramolecular chemistry are significant. Building on our expertise in steroid chemistry, we describe the synthesis of a series of esters derived from cholic acid, deoxycholic acid, and lithocholic acid using short-chain aliphatic alcohols and microwave (MW) heating, followed by an investigation of their organogel-forming capabilities.

2. Experimental

2.1. General remarks

Optical rotations were measured at 24 °C using an MCP 500 Anton Paar polarimeter. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded in $CDCl_3$ on an Agilent DD2 600 spectrometer (^{1}H NMR at 600 MHz, ^{13}C NMR at 150 MHz). Chemical shifts are reported in parts per million (ppm) relative to residual solvent signals. All peak assignments were confirmed through two-dimensional NMR experiments (details provided in the Supporting Information). Spectra were processed using MestReNova software. High-resolution mass spectra (HRMS) were acquired via electrospray ionization (ESI) using a Waters Synapt G2-Si Q-TOF mass spectrometer. Infrared (IR) spectra were recorded with an Agilent Cary 630 FTIR spectrometer equipped with an attenuated total reflection (ATR) interface, covering the range 4000–600 cm^{-1}. Column chromatography was performed using a Teledyne Isco Combiflash system, and analytical thin-layer chromatography (TLC) was conducted on aluminum plates pre-coated with silica gel 60F-254.

Microwave-assisted reactions were carried out in a Monowave EXTRA 300 Anton Paar microwave reactor using 10 mL glass vials. Melting points were determined using an Electrothermal IA 9100 Digital Melting Point Apparatus with 0.8–1.1 × 90 mm glass capillary tubes. Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) analyses were performed with a JEOL JSM-7600F microscope equipped with a low-angle backscattered electron (LABE) detector, operating at 15 kV. X-ray diffraction (XRD) was conducted using a Bruker D-8 Advance Diffractometer with Cukα radiation (40 kV, 30 mA) under the following conditions: step time of 0.3 s and step size of 0.02°. Thermogravimetric analysis (TGA) was carried out on a Discovery TGA-5500, and differential scanning calorimetry (DSC) measurements were performed using a Discovery DSC-250 (TA Instruments).

2.2. General procedure for the synthesis of bile acid esters

All starting materials were purchased from Merck-Sigma-Aldrich (analytical grade) and used without further purification, except for cholic acid, which was purified prior to use via column chromatography on a *Combiflash* apparatus.

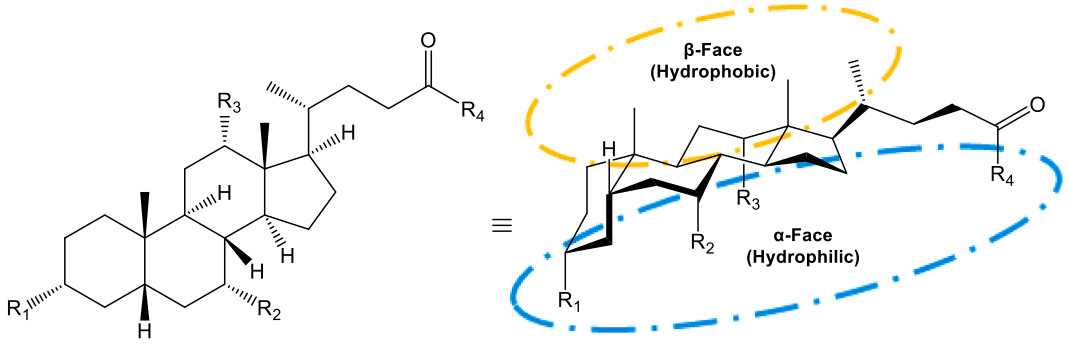
In a 10 mL microwave glass vial at room temperature, 5 mL of the corresponding alcohol (methanol (**a**), ethanol (**b**), isopropanol (**c**), or 1-butanol (**d**)) were added, followed by the dropwise addition of 0.4 mmol of acetyl chloride (AcCl) [17,24] to generate an anhydrous HCl solution. The mixture was stirred for 2 min, after which 0.12 mmol of the selected bile acid (cholic acid (**1**), deoxycholic acid (**2**), or lithocholic acid (**3**)) was introduced. The vial was then sealed and placed in the microwave reactor, with the temperature set to 140 °C for methanol and 160 °C for the other alcohols. The pressure reached 10.6 bar for methanol and 12.5 bar for the other alcohols. Optimized reaction times were determined to be 50 s for methanol, 75 s for ethanol and 1-butanol, and 120 s for isopropanol. The completion of the reaction was monitored by thin-layer chromatography (TLC).

Following the reaction, the excess alcohol was evaporated under reduced pressure, and the crude product was dissolved in CH_2Cl_2. The solution was washed with saturated $NaHCO_3$ (2 × 50 mL) and distilled water (2 × 50 mL). The resulting bile acid ester was purified by *flash* column chromatography and dried under high vacuum to obtain a white solid (isolated yields are provided in Table 1). Full characterization (mp, $[\alpha]_D$, FTIR, 1D and 2D NMR, and HRMS) for esters **1a-d**, **2a-d**, and **3a-d** is available in the Supporting Information section.

3. Results and discussion

3.1. Chemical synthesis

The methyl esterification of cholates and related compounds is well-



Cholic acid (**1**): $R_1 = R_2 = R_3 = R_4 = OH$
Deoxycholic acid (**2**): $R_1 = R_3 = R_4 = OH$; $R_2 = H$
Lithocholic acid (**3**): $R_1 = R_4 = OH$; $R_2 = R_3 = H$
Chenodeoxycholic acid: $R_1 = R_2 = R_4 = OH$; $R_3 = H$
Sodium glycocholate: $R_1 = R_2 = R_3 = OH$; $R_4 = NHCH_2CO_2Na$
Sodium taurocholate: $R_1 = R_2 = R_3 = OH$; $R_4 = NHCH_2CH_2SO_3Na$

Fig. 1. Most frequent bile acids and salts in mammals and their amphiphilic nature.

Table 1
Isolated yields and reaction times for the esterification of bile acids and its comparative to conventional heating reported methods.

	Microwave synthesis			Reported conventional heating synthesis		
Compound	**Time**	**Catalyst**	**Yield**	**Time**	**Catalyst**	**Yield**
1a[a]	50 s	AcCl	95.2 %	1–3 min	*p*-TSA	90–95 %* [25]
1b[a]	75 s	AcCl	97.6 %	1 h	AcCl	90 % [17]
1c[b]	120 s	AcCl	79.1 %	1 h	AcCl	68 % [17]
1d[b]	75 s	AcCl	98.5 %	1 h	AcCl	73 % [17]
2a[a]	50 s	AcCl	96.4 %	1 h	HCl	38 % [35]
2b[a]	75 s	AcCl	93.9 %	–	–	–
2c[b]	120 s	AcCl	87.4 %	–	–	–
2d[b]	75 s	AcCl	77.0 %	–	–	–
3a[a]	50 s	AcCl	95.9 %	24 h	AcCl	98 % [36]
3b[a]	75 s	AcCl	86.1 %	–	–	–
3c[b]	120 s	AcCl	97.1 %	–	–	–
3d[b]	75 s	AcCl	87.9 %	–	–	–

a. The ratio bile acid:AcCl was 1:2.
b. The ratio bile acid:AcCl was 1:3.
* The reaction was performed in a domestic MW.
The dashes indicate that the data was not reported.

documented in the literature; however, the systematic study of bile acid esterification, particularly with detailed synthesis methods, remains underexplored. In this work, we investigated the microwave-assisted synthesis of a series of short-chain alkyl esters derived from bile acids (Scheme 1). Specifically, we employed three bile acids: cholic acid (**1**), deoxycholic acid (**2**), and lithocholic acid (**3**), in combination with short-chain aliphatic alcohols—methanol (**a**), ethanol (**b**), isopropanol (**c**), and 1-butanol (**d**)—using acetyl chloride (AcCl) as a catalyst.

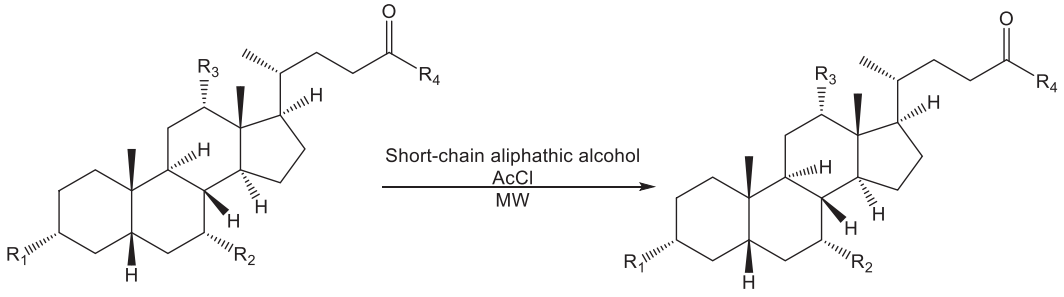
The synthesized esters were characterized using various analytical techniques, and their potential as gel-forming agents was evaluated. While conventional esterification methods typically rely on standard heating, we chose microwave-assisted synthesis due to its enhanced versatility and efficiency. This approach allows for rapid and uniform heating, which minimizes by-product formation—an advantage particularly relevant and popular in processes such as biomass conversion, waste treatment, pyrolysis, hydrolysis, and biodiesel production [33]. Microwave-assisted reactions offer several distinct benefits, including faster reaction rates, improved yields, high reproducibility, uniform heating, and reduced consumption of reagents [34].

The study began by comparing and performing the esterification reaction according to the methodologies described by Dayal et al. [23], Lillington et al. [24], and Nonappa et al. [17]. In the procedure reported by Dayal and co-authors, methanol was used as both the solvent and esterification agent, with *p*-TSA as the catalyst. This method was initially employed to synthesize methyl cholate (**1a**), which was obtained in good yields (~95 %). However, when *p*-TSA was used with longer alcohols (ethanol and isopropanol), the esterification reaction failed, yielding by-products instead of the desired esters. For the methodologies reported by Lillington et al. [24] and Nonappa et al. [17], AcCl was used as the catalyst. Using this approach, product **1a** was obtained with a yield like that reported by Dayal et al. [23], and the desired esters were successfully produced with ethanol and isopropanol in good yields. Based on these results, we selected AcCl as the catalyst for the entire study. All reactions were tested at different times and temperatures, always below the critical point, with the optimal time conditions summarized in Table 1.

In the cholate series (esters **1a-d**), the isolated yields of the esterification reactions showed that as the chain length of the linear alcohol increased (excluding isopropanol), the reaction produced higher yields, following the trend **1a** < **1b** < **1d**. The lowest yield was observed for compound **1c**, when isopropanol (a secondary alcohol) was used. This may be attributed to the higher acidity of primary alcohols compared to secondary alcohols. However, this trend did not hold consistently across all bile acids, as each series displayed distinct behavior.

For the deoxycholate series (esters **2a-d**), an inverse trend was observed: as the chain length increased, the yields decreased. Notably, a pattern emerged including the nonlinear alcohol isopropanol, following the trend **2a** > **2b** > **2c** > **2d**. In contrast, for the lithocholate series (esters **3a-d**), no clear relationship was evident between the chain length or the nature of the alcohol and the isolated yields (**3c** > **3a** > **3d** > **3b**). Overall, the yields obtained across all reactions were consistently high, with the same catalyst employed throughout. Variations in yield trends may be attributed to the influence of different substituents, which likely affect reaction efficiency and outcomes.

Analyzing the reactions of each bile acid with the various alcohols individually, it is evident that for the reaction with methanol, yields were comparable, with minimal variation (**1a** ≈ **2a** ≈ **3a**). For the esterification with ethanol, yields decreased as the number of hydroxyl substitutions on the bile acid decreased, following the trend **1b** > **2b** >



Bile acids:
1. $R_1 = R_2 = R_3 = R_4 = OH$
2. $R_1 = R_3 = R_4 = OH$; $R_2 = H$
3. $R_1 = R_4 = OH$; $R_2 = R_3 = H$

Bile acid esters:
1a. $R_1 = R_2 = R_3 = OH$; $R_4 = OCH_3$
1b. $R_1 = R_2 = R_3 = OH$; $R_4 = OCH_2CH_3$
1c. $R_1 = R_2 = R_3 = OH$; $R_4 = OCH(CH_3)_2$
1d. $R_1 = R_2 = R_3 = OH$; $R_4 = O(CH_2)_3CH_3$
2a. $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = OCH_3$
2b. $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = OCH_2CH_3$
2c. $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = OCH(CH_3)_2$
2d. $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = O(CH_2)_3CH_3$
3a. $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = OCH_3$
3b. $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = OCH_2CH_3$
3c. $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = OCH(CH_3)_2$
3d. $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = O(CH_2)_3CH_3$

Scheme 1. General reaction scheme for the synthesis of bile acid methyl esters (isolated yields are provided in Table 1).

3b. Interestingly, for the esterification with isopropanol, the opposite trend was observed, yielding higher results with bile acids that had fewer substitutions on the steroidal base (**3c** > **2c** > **1c**). Lastly, esterification with 1-butanol exhibited no discernible trend (**1d** > **3d** > **2d**). In principle, if any biological assay were to be performed with the synthesized esters, as the aliphatic chain grows, the activity described by the SAR should decrease. This is because the activity of the aliphatic hydroxyl groups is influenced by the addition of –CH or –CH_2 groups in the chain. In addition, the presence of hydrogen bonds also affects the SAR. For example, a tertiary hydrogen surrounded by water is less reactive than a secondary hydrogen. When a tertiary hydrogen is abstracted, it is stabilized due to its surrounding chemical environment, which includes three neighboring functional groups, reducing its activity.

3.2. NMR characterization

Methyl derivatives: For compound **1a** (methyl cholate), three signals corresponding to protons adjacent to hydroxyl groups at positions C-3, C-7, and C-12 were observed. C-3 appears as a multiplet at 3.43 ppm and the signals at 3.83 and 3.96 ppm correspond to C-7 and C-12, respectively. The methyl group attached to the ester is detected as a singlet at 3.65 ppm. The carbonyl group (C═O) was identified at 175.0 ppm. Carbons attached to hydroxyl groups at C-12, C-7, and C-3 appeared at 73.2, 68.6, and 72.0 ppm, respectively, with the methyl ester signal at 51.6 ppm. In **2a** (methyl deoxycholate), two signals for the protons adjacent to hydroxyl groups at C-3 and C-12 were observed at 3.59 and 3.96 ppm, respectively, along with the methyl ester singlet at 3.64 ppm. The ester carbonyl was detected at 174.9 ppm, while C-12 and C-3 were found at 73.2 and 71.8 ppm, respectively, with the methyl ester at 51.5 ppm. For compound **3a** (methyl lithocholate), the proton at C-3 is shown at 3.62 ppm, while the methyl ester appears at 3.66 ppm. The carbonyl ester was found at 174.9 ppm, and C-3 at 72.0 ppm, with the methyl ester at 51.6 ppm. Across all three compounds, the methyl groups at C-21, C-19, and C-18 were found at lower frequencies, with Me-21 appearing as a doublet and Me-19 and Me-18 as singlets.

Ethyl derivatives: For compounds **1b** (ethyl cholate), **2b** (ethyl deoxycholate), and **3b** (ethyl lithocholate), the ^{1}H NMR spectra show a quartet corresponding to the –OCH_2- group from the ester. This signal appears at 4.12 ppm for compound **1b**, and at 4.10 ppm for both **2b** and **3b**. Protons adjacent to hydroxyl groups are also detected. In compound **1b**, a broad signal at 3.96 ppm (C-12) and 3.84 ppm (C-7), and a multiplet at 3.43 ppm (C-3) are present. For compound **2b**, a singlet at 3.96 ppm (C-12) and a multiplet at 3.56 ppm (C-3) were observed. In **3b**, a multiplet at 3.61 ppm (C-3) was detected. The ethyl ester methyl group is a triplet at 1.24 ppm for compounds **1b** and **3b**, and at 1.23 ppm for **2b**. The Me-21 (doublet), Me-19, and Me-18 (singlets) are also identified. In the ^{13}C NMR spectra, the carbonyl group (C-24) was observed at higher frequencies: 174.5 ppm for compounds **1b** and **3b**, and 174.4 ppm for **2b**. The hydroxyl-bearing carbons for compound **1b** were detected at 73.2 ppm (C-12), 68.6 ppm (C-7), and 72.0 ppm (C-3). In compound **2b**, the signals for C-12 and C-3 were observed at 73.2 and 71.8 ppm, respectively, while in compound **3b**, C-3 was detected at 72.0 ppm. The methylene carbon from the ester appears at 60.3 ppm in all compounds, while the methyl groups from the ester chain are found at 14.4 ppm for compounds **1b** and **3b**, and at 14.3 ppm for **2b**.

Isopropyl derivatives: For **1c** (isopropyl cholate), **2c** (isopropyl deoxycholate), and **3c** (isopropyl lithocholate), the ^{1}H NMR spectra show a septet corresponding to the –OCH- group of the ester at ~ 4.99 ppm. Hydroxyl group-adjacent protons in **1c** are seen at 3.96 ppm (C-12), 3.84 ppm (C-7), and 3.44 ppm (C-3). For **2c**, the signals for C-12 and C-3 appear at 3.96 and 3.59 ppm, respectively, while in **3c**, a multiplet at 3.61 ppm (C-3) was observed. The isopropyl chain methyl groups appear as doublets at ~ 1.21 ppm across all three compounds, with signals for Me-21 (doublet), Me-19, and Me-18 (singlets). In the ^{13}C NMR spectra, the carbonyl carbon (C-24) appears at 174.0 ppm for all compounds. Hydroxyl-bearing carbons were detected as follows: for **1c**, at 73.2 ppm (C-12), 68.6 ppm (C-7), and 72.0 ppm (C-3); for **2c**, at 73.3 ppm (C-12) and 71.9 ppm (C-3); and for **3c**, at 72.0 ppm (C-3). The methine signal from the ester was found at 67.5 ppm for compounds **1c** and **2c**, and at 67.4 ppm for **3c**. The ester methyl groups were observed at 22.0 ppm for compounds **1c** and **2c**, and at 23.5 ppm for **3c**.

Butyl derivatives: For **1d** (butyl cholate), **2d** (butyl deoxycholate), and **3d** (butyl lithocholate), the ^{1}H NMR spectra show a triplet corresponding to the –OCH_2- group at ~ 4.05 ppm. Protons adjacent to hydroxyl groups are detected for compound **1d** at 3.97 ppm (C-12), 3.85 ppm (C-7), and 3.44 ppm (C-3). For **2d**, the C-12 proton appears at 3.96 ppm, and C-3 at 3.59 ppm. In compound **3d**, a multiplet at 3.60 ppm (C-3) was observed. The butyl ester methyl groups appear as triplets at ~ 0.92 ppm in all compounds. Signals for Me-21 (doublet), Me-19, and Me-18 (singlets) are also present. In the ^{13}C NMR spectra, the carbonyl group was detected at 174.6 ppm for compounds **1d** and **3d**, and at 175.5 ppm for **2d**. Hydroxyl-bearing carbons for compound **1d** were observed at 73.2 ppm (C-12), 68.6 ppm (C-7), and 72.1 ppm (C-3). For compound **2d**, the signals for C-12 and C-3 were at 73.2 ppm and 71.8 ppm, respectively, while for **3d**, C-3 appeared at 71.9 ppm. The methylene signal from the ester appears at 64.3 ppm for compounds **1d** and **2d**, and at 64.2 ppm for **3d**, while the ester methyl groups were found at ~ 13.8 ppm across all compounds. The methyl groups at C-21, C-19, and C-18 were present at low frequencies in all spectra.

3.3. Gelation studies

Gelation tests were conducted for all esters using a series of aliphatic and aromatic solvents, such as hexane, dichloromethane, diethyl ether, *p*-xylene, toluene, and benzene, across a concentration range of 0.4 – 3.0 % w/v. Each solution was sonicated, and the optimal results for each ester are summarized in Table 2. After several experiments with 12 esters, three organogels were successfully formed, one for each bile acid derivative (Fig. 2): **1a** in *p*-xylene (2.0 % w/v), **2a** in hexane (3.0 % w/v), and **3b** in hexane (2.5 % w/v). Full characterization (XRD, SEM, and DSC) for the three organogels from **1a**, **2a**, and **3b** is available in the Supporting Information section.

Organogel-melting temperatures were determined using the inverted tube test and compared to differential scanning calorimetry (DSC) data (Table 3, Fig. 3). The DSC thermograms indicate that the **1a**/*p*-xylene organogel (a) is the most thermally stable of the three. It exhibits a crystallization transition at 12.4 °C, corresponding to the solvent melting point (m.p. = 13.2 °C). The organogel-melting process begins at approximately 49 °C, peaks at 59.8 °C, and concludes at 66.5 °C, consistent with the inverted tube test results.

In contrast, the **2a**/hexane and **3b**/hexane organogels (b and c thermograms, Fig. 3) were less stable, as evidenced by their lower melting onset temperatures. The DSC thermograms show a gradual slope, possibly indicating partial network melting at 59.2 and 24.5 °C, respectively. A sharper transition follows, representing complete organogel melting at 65.3 and 44.1 °C, respectively, which also aligns with the inverted tube test.

The greater stability of the methyl deoxycholate (**2a**) organogel compared to the ethyl lithocholate (**3b**) organogel may be attributed to the additional hydroxyl group in methyl deoxycholate, which likely contributes to a more robust network through hydrogen bonding.

3.4. Morphology of xerogels

To examine the gel morphologies, scanning electron microscopy (SEM) was conducted for the xerogels of compounds **1a**, **2a**, and **3b**. These xerogels were prepared by allowing the solvent to evaporate at ambient conditions, followed by drying under high vacuum. The micrographs revealed distinct structural arrangements for each xerogel. The SEM image of the **1a**/*p*-xylene xerogel [2000x; EDS (weight%): C = 87.31, O = 12.69] displayed a flake-like structure with pores of varying

Table 2
Gelation tests for bile acid esters[a].

Solvent	1a	1b	1c	1d	2a	2b	2c	2d	3a	3b	3c	3d
toluene	v	v	v	s	s	s	s	s	s	s	s	s
benzene	v	s	s	s	s	s	s	s	s	s	s	s
p-xylene	g	v	s	s	s	v	s	s	c	c	c	c
pyridine	s	s	s	s	s	s	s	s	s	s	s	s
hexane	v	s	p	s	g*	v	s	s	v	g**	s	s
dichloromethane	s	s	s	s	s	s	s	s	s	s	s	s
diethyl ether	g[x]	g[x]	p	p	s	s	p	s	s	c	s	s

(g = gel, v = viscous solution, s = soluble, p = precipitate, c = crystalize).
[a] Concentration 2.0 w/v % otherwise indicated.
* Concentration 3.0 w/v %.
** Concentration 2.5 w/v %.
[x] unstable gel (evaporation occurs due the highly volatile nature of solvent).

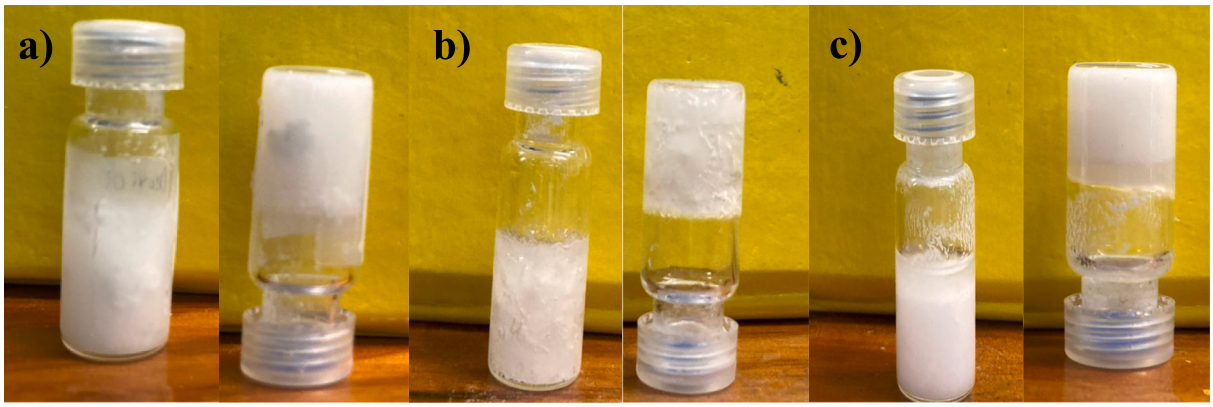


Fig. 2. Organogels obtained for: a) methyl cholate (**1a**)/*p*-xylene (2.0 w/v%), b) methyl deoxycholate (**2a**)/hexane (3.0 w/v%) and c) ethyl lithocholate (**3b**)/hexane (2.5 w/v%).

Table 3
Organogel melting temperatures from the inverted tube test and DSC measurements.

Organogel	Melting temperature (inverted tube)	Melting temperature (DSC)
Methyl cholate (**1a**)/*p*-xylene (2.0 w/v%)	67.1 °C	59.8 °C
Methyl deoxycholate (**2a**)/ hexane (3.0 w/v%)	66.3 °C	65.3 °C
Ethyl lithocholate (**3b**)/ hexane (2.5 w/v%)	38.7 °C	44.1 °C

sizes across the surface, forming a porous network (Fig. 4a). The xerogel of **2a**/hexane [EDS (weight%): C = 85.38, O = 14.62] showed a complex entanglement, with rod- or column-like structures contributing to a porous matrix. At higher magnifications (10,000–20,000x), the arrangement resembled stacked crystals or tablet-like structures, with some regions containing small fibers or columns (Fig. 4b). In the case of the xerogel **3b**/hexane [EDS (weight%): C = 90.73, O = 9.27], a fibrillar network of varying sizes and shapes was observed. Upon closer examination, the structure consisted of columnar formations, similar in appearance to stacked tongue depressors (Fig. 4c).

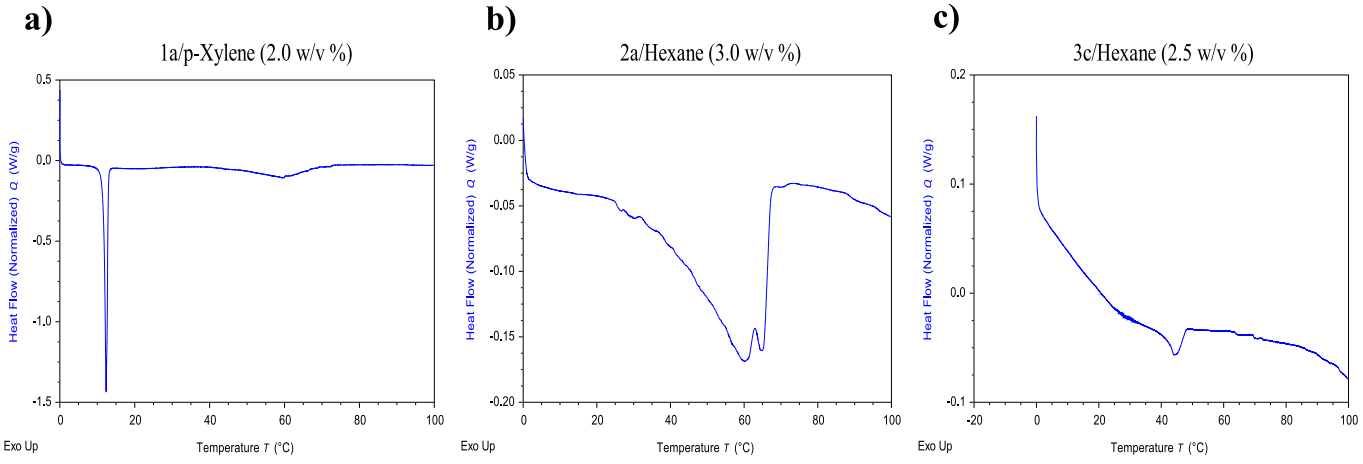


Fig. 3. DSC thermograms for **1a**/*p*-xylene, **2a**/hexane and **3b**/hexane organogels.

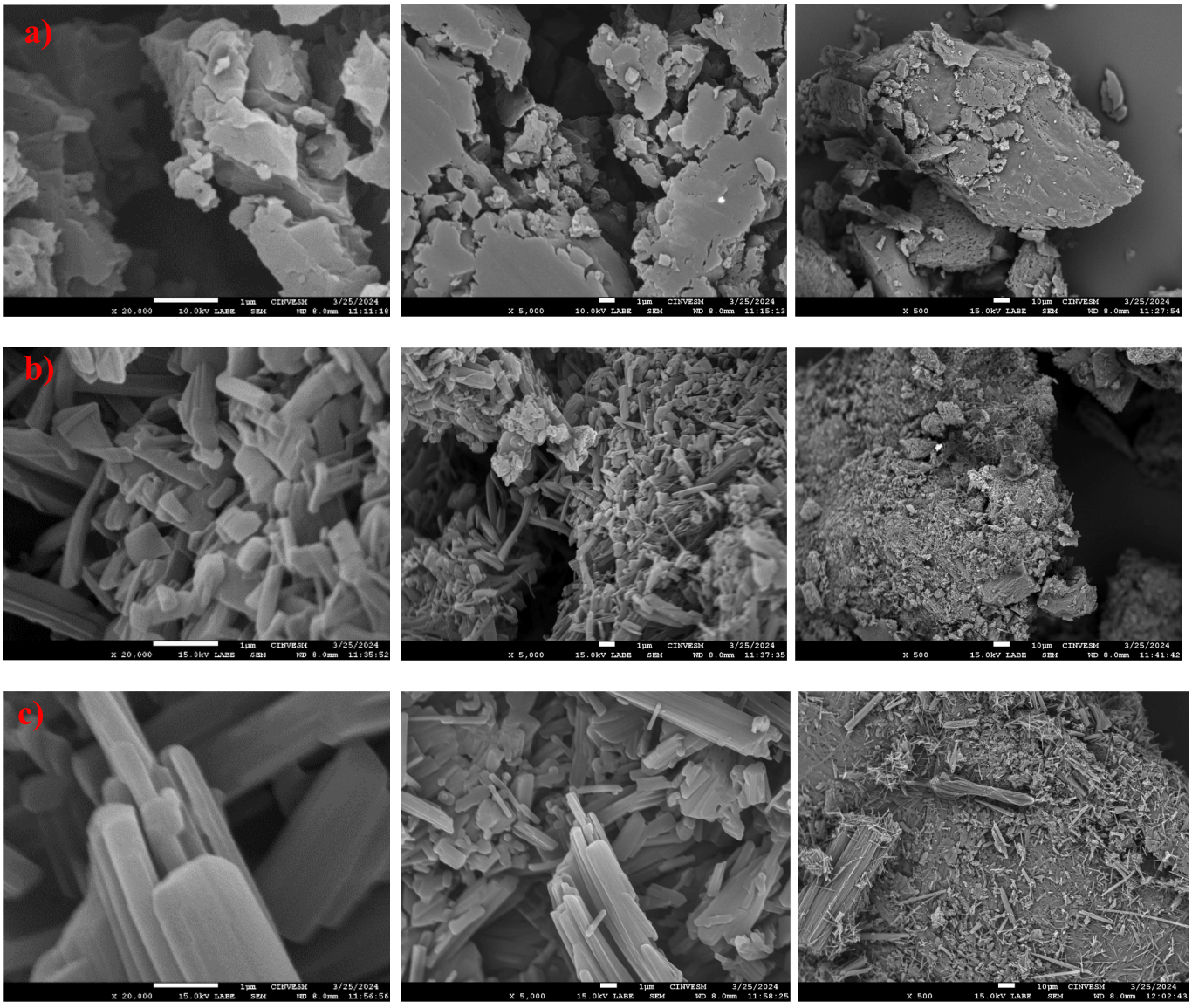


Fig. 4. Scanning electron micrographs of xerogels obtained from **1a**/*p*-xylene, **2a**/hexane, and **3b**/hexane at magnifications of 20,000x, 5,000x, and 500x (from left to right).

3.5. XRD experiments

XRD spectra were recorded for the pure esters and compared with those of the xerogels obtained from **1a**, **2a**, and **3b** (Fig. 5). Notably, ester **1a** changes its crystalline structure when transitioning from the pure form to the xerogel obtained in *p*-xylene. For ester **2a**, the pure form exhibits an amorphous arrangement. However, upon gelation in hexane, a crystalline structure of **2a** emerges, as observed in the SEM

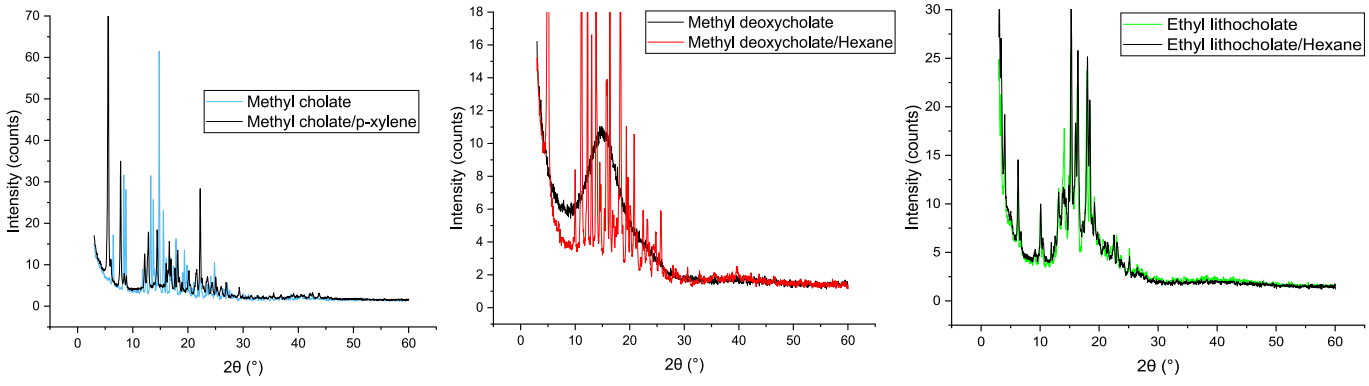


Fig. 5. XRD diffractograms for bile alkyl-esters **1a**, **2a**, and **3b**, compared with the spectra of the corresponding xerogels.

micrographs. In contrast, compound **3b** retains its crystalline arrangement both in the pure form and when gelled in hexane.

4. Conclusions

This study successfully revisits and optimizes the esterification of bile acids using short-chain aliphatic alcohols under microwave-assisted conditions, revealing significant advancements in reaction efficiency and product yields. By employing cholic acid, deoxycholic acid, and lithocholic acid as substrates, and AcCl as a catalyst, the optimized methodology offers an improvement over conventional heating techniques, minimizing by-product formation and enhancing uniform heating. These findings contribute valuable insights into the systematic synthesis of bile acid esters, which had previously been underexplored, particularly for non-methyl esters. Besides, the work further highlights the organogel-forming capabilities of the synthesized esters, with specific bile acid esters displaying notable self-assembling behavior in various solvents. The formation of stable organogels with distinct thermal and morphological properties showcases their potential in supramolecular chemistry, nanomaterials, and biomedical applications. Morphological analyses of xerogels through SEM and structural characterization via XRD reveal the influence of bile acid structure and alcohol type on gel properties, suggesting opportunities for tailoring material properties for specific applications. Overall, the combination of microwave-assisted synthesis and organogel formation offers a versatile and efficient approach to produce bile acid derivatives with potential applications in materials science, pharmaceuticals, and self-assembly systems. Further studies into the molecular interactions governing gel formation and the scalability of the synthesis will enhance the utility of these compounds in practical applications.

CRediT authorship contribution statement

Jair García-Méndez: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Adolfo López-Torres:** Writing – review & editing, Software, Resources, Methodology, Investigation. **María A. Fernández-Herrera:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2025.109560.

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