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ORIGINAL PAPER

Synthesis of 7-ketolithocholic acid via indirect electrooxidation of chenodeoxycholic acid

Hongbin Zhao · He Tian · Yonghong Jin · Xuejun Cao

Abstract 7-Ketolithocholic acid was synthesized by indirect electrooxidation of chenodeoxycholic acid using the medium of Br^{-}/Br_2. Some important factors such as anode material, solvent, initial concentration of chenodeoxycholic acid, current density, electrolysis time have been investigated in detail. By comparing the experiment results, it can be concluded that productivity and current efficiency in the divided electrolytic cell are higher than that in the undivided electrolytic cell. The optimized process conditions have been found to be: the anode material PbO_2/Ti, the solvent acetonitrile, and initial concentration of chenodeoxycholic acid 23.8–31.8 mg/mL, current density 95.2–142.9 A/m^2. The current efficiency could reach 85% and the productivity of 7-ketolithocholic acid could reach 83%. The melting point, IR spectrum, ^1H NMR, ^{13}C NMR and EI-MS were used to characterize the product. The analytical results confirm that the product is 7-ketolithocholic acid.

Keywords Chenodeoxycholic acid · 7-Ketolithocholic acid · Bromide ion · Indirect electrooxidation · Ursodeoxycholic acid

1 Introduction

7-Ketolithocholic acid (3α-hydroxy-7-carbonyl-5β-bile acid, 7K-LCA, Fig. 1) is the precursor for preparing ursodeoxycholic acid (3α, 7β-2-hydroxy-5β-bile acid, UDCA, Fig. 2). Ursodeoxycholic acid is an important clinical drug in the treatment of gallstones, cholecystitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) [1–4]. In early stage, ursodeoxycholic acid was prepared by bile acid from animal. In 1950s, Kanazawa et al. [5, 6] reported that ursodeoxycholic acid was prepared by reducing 7-ketolithocholic acid in acetone using sodium metal.

Currently industrial production of ursodeoxycholic acid is based on the synthesis process reported by Kanazawa. Subsequently, researchers improved the process, and tried other methods [7–12]. Chemical synthesis has some disadvantages such as too many steps, harsh conditions and low yield. In the 1980s, researchers prepared ursodeoxycholic acid by using microorganisms. Hirano et al. [13] reported that chenodeoxycholic acid (3α, 7α-2-hydroxy-5β-bile acid, CDCA, Fig. 3) was converted into ursodeoxycholic acid in vitro by using human intestinal flora, and the total conversion yield of chenodeoxycholic acid reached 80–90%. Sutherland et al. [14] isolated *Clostridium limosum* from soil and applied it to conversion of chenodeoxycholic acid into ursodeoxycholic acid with the 80% yield. The researchers also tried to synthesize ursodeoxycholic acid by electrochemical methods. Japanese patent [15] reported that 7-ketolithocholic acid could be converted into ursodeoxycholic acid by electrochemical reduction in short chain alcohol. U.S. patent [16] reported that 7-ketolithocholic acid could be reduced by electrochemical method in the electrolyte containing short chain alcohol. By adding weak acid compounds such as dimethyl sulfoxide and tetramethyl urea, with ruthenized titanium or mercury electrodes, higher yield of ursodeoxycholic acid could be obtained. At present, there is very few reports related preparation of ursodeoxycholic acid by electrochemical method. The Synthesis of ursodeoxycholic acid

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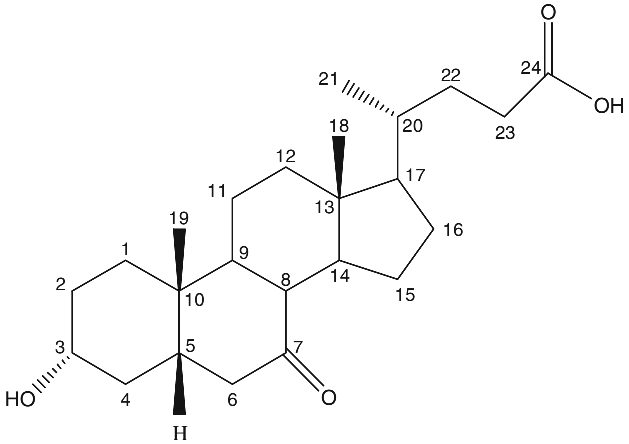


Fig. 1 7K-LCA

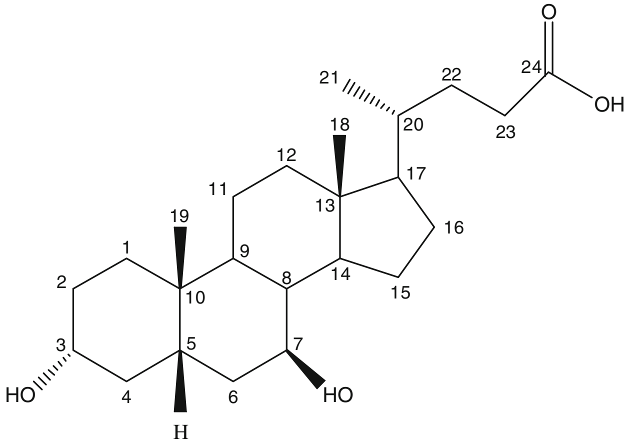


Fig. 2 UDCA

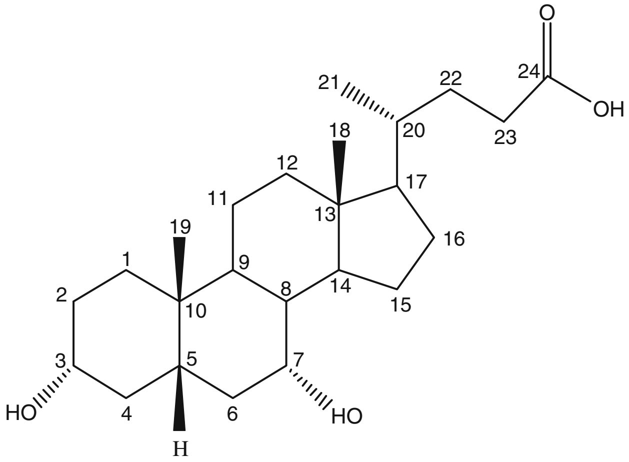


Fig. 3 CDCA

by electrochemical method is a relatively simple and safe operation process.

With the development of organic electrosynthesis, more and more organic chemicals can be prepared by direct or indirect electrochemical ways. When Br_2/Br^- was used as the medium to electrooxidize organic chemicals indirectly, Br^- was oxidized into Br_2 in the anode. Br_2 was reduced into Br^- when it oxidizes other materials. In this way, Br^- can be used in the form of recycle. This is a low cost and environmental friendly method. Gluconic acid salt was synthesized by using indirect electrooxidation of glucose with bromide ion was industrialized [17].

The purpose of this paper is to electrooxidize chenodeoxycholic acid into 7-ketolithocholic acid using the medium of Br^-/Br_2 indirectly. This is a promising industrial process.

2 Materials and methods

2.1 Materials

Chenodeoxycholic acid was from Chengdu Sichuan Dao Zhi Biochemistry Co. Ltd. (melting point: 144–145 °C, chromatography purity: 95%). Sulfuric acid, potassium bromide and acetonitrile were from Shanghai Ling Feng Chemical Reagent Co. Ltd. Methanol and ethanol were from Shanghai Zhen Xing chemical factory. All other reagents were analytical grade. HYD250-1 DC power with single regulator steady flow was from Shanghai Hu Yi Technology Co., Ltd. Electrolytic cells including H-divided cell and undivided cell were prepared by ourselves. Anode material consisted of ruthenized titanium mesh electrode was from Henan Xinxiang future water Chemical Co., Ltd., PbO_2/Ti mesh electrode was from the Xi'an Tai Electrochemical Technology Co., Ltd., The size of each titanium mesh is 30 mm × 150 mm, which was made from 1.5 mm diameter titanium wire plated with ruthenium oxide or lead oxide. The size of diamond-shaped mesh is 7 mm × 7 mm. Ru/Ti electrode was prepared according to Beer's patent [18]. The titanium mesh being first pickled in hot aqueous oxalic acid, subjected to ultrasonorous vibration in water, and dried. The solution (6.2 mL butyl alcohol, 0.4 mL 36% HCl, 3 mL butyl titanate and 1 g $RuCl_3$) was several times brushed on the titanium mesh, then the mesh thus treated was heated in the air at a temperature of 300–500 °C, for 1–5 min. PbO_2/Ti electrode was prepared according to literature [19]. The titanium mesh was treated as same as aforementioned. The solution was consisted of certain amount of $SnCl_4$ and Sb_2O_3 dissolved in hydrochloric acid, then mixed with butyl alcohol. It was brushed on surface of the titanium mesh, and heated at 120–140 °C for 15 min. Repeated this process 3–5 times, then the mesh was calcinated at 400–500 °C for 1 h. Prepared 700 mL electroplating solution containing 105 g $Pb(NO_3)_2$, 35 g $Cu(NO_3)_2 \cdot 3H_2O$ and 0.28 g NaF. Anodic deposition carried out at current 3 A for 0.4 h, then 1.5 A for 3.6 h, $PbCO_3$ was used to maintain pH 1–2. When electroplating completed, the mesh was rinsed with deionized water and dried. Graphite electrodes (plain sheet with 30 mm × 120 mm × 5 mm) was from Shanghai Yi-feng Carbon Co., Ltd. Cathode material consisted of stainless steel electrode with plain sheet of 30 mm × 150 mm. HK-101 cation exchange membrane consisted of fluoroplastics was from Shanghai Hua Kai Technology Company. The Membrane performance parameters are following: thickness 150–200 μm, ion-exchange capacity (titration) 0.8–1.5 meq/g, electrical conductivity (0.1 M NaCl) $(0.2\text{–}1.0) \times 10^{-2}\ \Omega\ \mathrm{cm}^{-1}$, the tensile strength (Tensile Tester for Paper) 140–180 $\mathrm{kg/cm^2}$. It should be immerged in 5–10% sulfuric acid for more than 14 h before use so that the membrane could be swelled extensively. Cyclic voltameter with an AUTOLAB PGSTAT30 Electrochemical Workstation (Eco Chemie B.V., The Netherlands) was used to measure electropolar potential. The three electrodes aforementioned are a working electrode, a stainless plain counter electrode and a saturated calomel reference electrode. LC-100 HPLC was from Shanghai Wu Hao Information Technology Co., Ltd.

2.2 Methods

2.2.1 *Electrooxidation of Br^- and oxidation of chenodeoxycholic acid*

During electrolysis, direct-current was used by using Ru/Ti, PbO_2/Ti or graphite electrodes as the anode and the stainless steel plate as cathode. HK-101 cation exchange membrane was used to divide a cell into an anode cell and a cathode cell. Anolyte was 0.053 mol/L KBr and a certain concentration of chenodeoxycholic acid dissolved in deionized water and organic solvents mixture, which was consisted of deionized water/organic solvents (acetonitrile, methanol or ethanol) with volume ratio of 13:50. Catholyte was 20% of dilute sulfuric acid solution. Both volume of anolyte and catholyte were 63 mL. During electrolysis in the undivided cell, the beaker was used as the electrolytic cell. Electrolyte was same as anolyte in divided cell process. Both the surface area of the anode plate and the cathode plate were $3.5 \times 3.0\ \mathrm{cm^2}$. All experiments were carried out at room temperature (about 25 °C).

The electrolysis was carried out in electrolytic cell until the anolyte became yellow. Then the anolyte was stirred and became colorless. Repeated the process and kept about 2 h total electrolysis time. The voltage variation ranged from 29 to 31 V (the initial voltage) to 17–20 V (the end of electrolysis). The process of reaction and the ending of reaction was detected by thin layer chromatographic (TLC) [20]. When the spot of chenodeoxycholic acid fade, reaction finished. Moving phase of TLC was chloroform/methanol/glacial acetic acid with volume ratio of 40:2:1. Chromogenic agent was 20% of phospho–molybdic acid of ethanol solution. Chromogenic temperature was about 100 °C. Chromogenic time was about 10 min. Content of 7-ketolithocholic acid was determined by high-performance liquid chromatography (Wu Feng LC-100). Flowing phase was acetonitrile/phosphate acid buffer (pH 3.0) with volume ratio of 70:30. HPLC was carried out at 1.0 mL/min, room temperature, with UV detecting at 208 nm. Retention time of 7-ketolithocholic acid is 4.15 min.

Current efficiency is the ratio of the theory electric quantity which is needed in the generation of 7-ketolithocholic to the actual electric quantity during electrolysis. $\eta_I = \frac{Q_t}{Q_p} \times 100\%$, Q_p is actual electric quantity, Q_t is theory electric quantity, which can be calculated according to Faraday's Law:

$$Q_t = \frac{m}{M} zF$$

where m is the mass and M is the molar mass of the generated 7-ketolithocholic, z is the electron transfer number, F is Faraday constant [21].

Productivity is the ratio of the consumed CDCA used to generate 7-ketolithocholic is divided by the total consumed CDCA in actual electrolysis process: $y = \frac{A_m}{A_t} \times 100\%$, A_m is the consumed amount of CDCA used to generate 7-ketolithocholic, A_t is the amount of total consumed CDCA in actual electrolysis process.

2.2.2 *Purification of 7-ketolithocholic acid mixture by column chromatography*

In order to determine the structure of 7-ketolithocholic acid, non-polar macroporous absorption resin HZ802 with polystyrene structure was used for purification of products to obtain pure product.

Resin was pretreated by using 95% ethanol and then was loaded in column (ϕ 300 mm × 20 mm). The column was equilibrated with 50% ethanol. Sample was dissolved in 50% ethanol solution (10 mL), then it was loaded on the column at flow rate of 0.2 mL/min. The elution was carried out with 60% ethanol solution and eluate was collected by automatic fractionator in liquid chromatography.

The eluate was analyzed by TLC, and then purified eluate containing 7-ketolithocholic acid was collected. Solvent was removed by rotary evaporation, and then purified 7-ketolithocholic acid sample was dried under vacuum (60 °C, 12 h).

3 Results and discussion

Chenodeoxycholic acid has two hydroxyls including 3-OH and 7-OH, and bromine mainly oxidizes 7-OH. Electrochemical oxidation with selectivity has important value. Here, 7-ketolithocholic acid was synthesized by indirect electrochemical oxidation of chenodeoxycholic acid in the medium of Br^-/Br_2 in divided and undivided electrolytic cell.

During electrolysis, Br^- is electrooxidized on the anode surface:

$$2Br^- \rightarrow Br_2(aq) + 2e \qquad (1)$$

$E^0 = 1.087$ V, in nonaqueous solution, $E^0 = 0.837$ (MeOH), $E^0 = 0.777$ (EtOH), $E^0 = 0.47$ (MeCN).

Chenodeoxycholic acid is oxidized into 7-ketolithocholic acid by Br_2.

$$C_{24}H_{40}O_4 + Br_2 \rightarrow C_{24}H_{38}O_4 + 2H^+ + 2Br^- \qquad (2)$$

Br^- produced is oxidized into Br_2 again on the anode surface and Br_2 produced oxidizes chenodeoxycholic acid into 7-ketolithocholic acid again. Thus, 7-ketolithocholic acid is continuously synthesized. The indirect electrooxidation reaction is a cycling process (Fig. 4).

Under certain conditions, it may generate oxygen on the anode surface, which is the side reaction:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e \qquad (3)$$

$E^0 = 1.229$ V.

It generates hydrogen on the cathode surface:

$$2H^+ + 2e \rightarrow H_2 \qquad (4)$$

In addition to the above reaction, side reactions maybe occur. 3-OH or 3-OH and 7-OH of chenodeoxycholic acid can be simultaneously oxidized by bromine. However, the main reaction is that bromine oxidizes the 7-OH of chenodeoxycholic acid because of the three-dimensional structure of chenodeoxycholic acid.

Br_2/Br^- used as oxidant has many advantages. Bromine is liquid with high solubility in the water at room temperature and moderate oxidation ability among the halogen elements. The Br_2/Br^- redox couple has been used for the selective oxidation of olefin and aldehyde compounds in the organic synthesis. The synthesis of 7-ketolithocholic acid using bromine ion by indirect electrooxidation of chenodeoxycholic acid is very useful. This process can reduce the cost of production and avoid the environment problem.

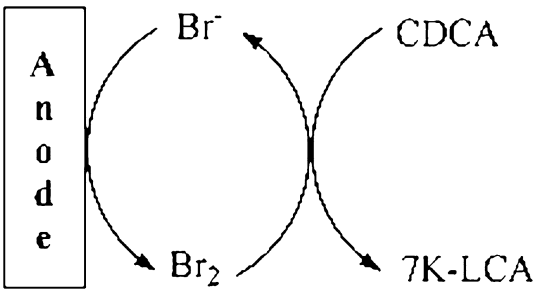


Fig. 4 Scheme of the indirect oxidation of CDCA by using Br^-

In addition, the cation (H^+ and K^+) will migrate to the cathode when continuous electrolytic process occurs. Then the exhaustion of the cation in anode chamber will result in stopping the oxidation cycle of Br^-. In this case, the method of intermittent electrolysis is used. Thus, in non-power conditions, the ion-exchange membrane is not selectivity. H^+ and K^+ will diffuse into the anode chamber as a result of concentration gradient so that electrolysis reaction will continue and realize the reaction cycle of Br^-.

The cyclic voltammograms of Ru/Ti electrode are shown in Fig. 5. It can be seen Br^- is oxidized to Br_2 by the Ru/Ti electrode at ca. 0.7 V (vs. SCE) and then the generated Br_2 oxidizes CDCA. CDCA can hardly be oxidized on Ru/Ti electrode, even when the equivalent K_2SO_4 is added. In contrast, oxidation current obviously increases after KBr was added. KBr plays two roles in this reaction. One is as the supporting electrolyte to delivery electrons. The other is to generate Br_2, which can oxidize CDCA into product, 7-ketolithocholic acid. Moreover, Br_2 is reduced into Br^- so that electrolysis reaction will continue and realize the reaction cycle.

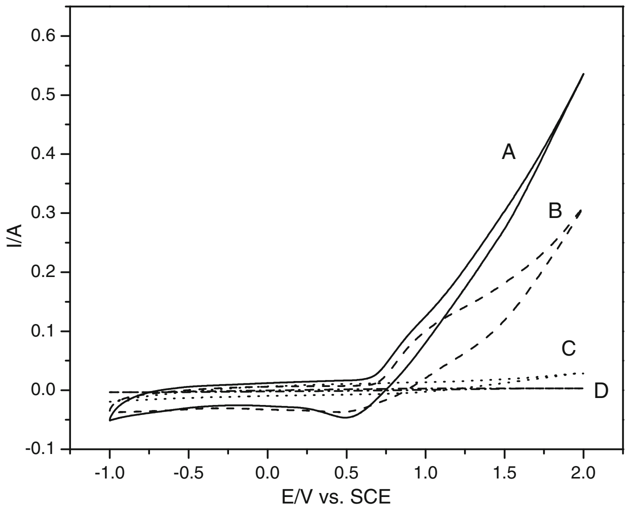


Fig. 5 Cyclic voltammograms of a Ru/Ti electrode. Speed: 50 mV/s; solvent: 63 mL mixture of acetonitrile and deionized water; electrolyte A: 0.053 mM KBr and 31.8 mg/mL CDCA (—); electrolyte B: only 0.053 mM KBr (···); electrolyte C: 0.027 mM K_2SO_4 and 31.8 mg/mL CDCA (· · ·); electrolyte D: only 31.8 mg/mL CDCA (- - -)

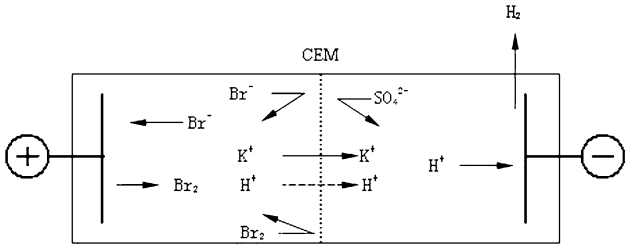


Fig. 6 Ions transport across the cation exchange membrane during the electrolysis

3.1 Influence of cation exchange membrane on the current efficiency and productivity

The ion transportation graph of HK-101 cation exchange membrane is shown in Fig. 6. CEM represents the cation exchange membrane. When electrolysis cell was working, membrane only allowed cation (H^+ and K^+) to pass through, while anion (Br^-) and small molecules (Br_2) could not reach the cathode chamber through the membrane. When electrolysis cell was power-off, cation (H^+ and K^+) returned to the anode chamber as a result of concentration gradient.

Current efficiency and productivity of 7K-LCA using divided and undivided electrolytic cells are showed in Table 1.

As is shown in Table 1, under the condition of same electrolysis, the current efficiency and productivity using the undivided electrolytic cell are lower approximately 35 and 15% than the divided electrolytic cell, respectively.

In the undivided electrolytic cell, hydrogen is generated in the cathode resulting in the alkalization of electrolyte. Under the condition of alkaline, Br_2 will become HBrO with stronger oxidation ability, and it would cause the 7-ketolithocholic acid to be further oxidized to result in other side reactions. Moreover, Br_2 also may be reduced on the cathode surface. In this way, Br_2 produced has no chance to oxidize the chenodeoxycholic acid. This is the main reason that the current efficiency decreases in undivided electrolytic cell.

Table 1 Influence of divided and undivided electrolytic cells on the current efficiency and productivity

	Divided electrolytic cell	Undivided electrolytic cell
Current efficiency (%)	84.2	48.2
Productivity (%)	76.8	68.4

Electrolyte: 0.053 mM KBr in 63 mL mixture of acetonitrile and deionized water, the concentration of CDCA: 31.8 mg/mL, PbO_2/Ti mesh anode, current density 95.2 A/m^2

In order to avoid some side reactions, we selected the H-model electrolytic cell to carry out the electrolysis experiment.

3.2 Influence of anode material on the current efficiency and productivity

In the three types of anode materials including Ru/Ti, PbO_2/Ti and graphite electrodes, Ru/Ti, PbO_2/Ti are coated metal anodes (Dimensionally stable anode, DSA), and the graphite plate electrode was used as control. The use of Ru/Ti would result in the following reactions:

$$RuO_2 + 2H_2O = RuO_4 + 4H^+ + 4e$$

or

$$Ru + 4H_2O = RuO_4 + 8H^+ + 8e$$

The generated RuO_4 would further decompose to:

$$RuO_4 + xH_2O = RuO_2 \cdot xH_2O + O_2$$

Bromide could penetrate the porous coating of RuO_2 hydrate to corrode titanium matrix [22]. However, in our experiment, the titanium matrix corrosion was not observed. Probably, the reaction rate constant of bromine with CDCA was higher than that with titanium in the system protecting titanium matrix. When graphite electrode was used, the graphite particles would disintegrate. However, perfluorinated cation-exchange membrane has non-stick surface and its membrane pore would not be fouled, so it would not interfere on the electrolysis reaction.

The cyclic voltammogram of three electrodes in H-model electrolytic cell is shown in Fig. 7. Solid line is the cyclic voltammogram of each electrolyte cyclic in the electrolyte containing CDCA. Dashed line is the cyclic voltammogram of each electrolyte cyclic in the electrolyte without CDCA. The two curves are very similar in three graphs. For Ru/Ti electrode, the current increased rapidly after the point with 0.7 V (vs. SCE) electrode potential, while for graphite electrode, the current increased after the point with 0.5 V (vs. SCE) electrode potential, and this point of the PbO_2/Ti electrode was about 1.0 V (vs. SCE) electrode potential value.

Br^- can be electrochemical oxidized on each of the three materials. While CDCA is chemical oxidized by Br_2, not be electro-oxidized into 7-ketolithocholic directly. This is indirect electro-oxidation of CDCA by means of Br_2/Br^-.

Figure 8 shows the effect of different anode materials on current efficiency and productivity.

As is shown in Fig. 8, during electrolysis in a divided electrolytic cell, all of three anode materials have little influence on the productivity, but have great influence on the current efficiency. When PbO_2/Ti mesh electrode was used, the highest current efficiency with 84.2% was

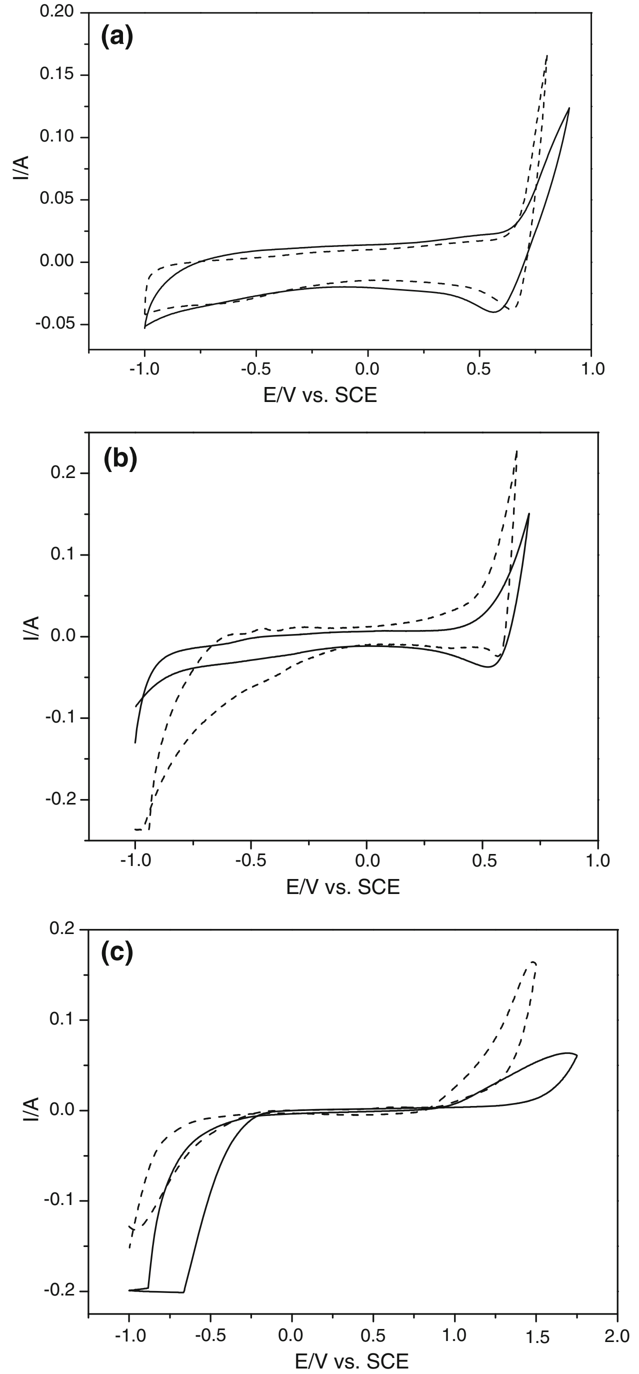


Fig. 7 Cyclic voltammograms of three electrodes in divided H-model electrolytic cell. Speed: 50 mV/s. Electrolyte in anode cell: 0.053 mM KBr and 31.8 mg/mL CDCA in 63 mL mixture of acetonitrile and deionized water (—), 0.053 mM KBr in 63 mL mixture of acetonitrile and deionized water (- - -). **a** Ru/Ti electrode; **b** graphite electrode; **c** PbO_2/Ti electrode

obtained. While ruthenized titanium mesh electrode was used, the highest productivity with 78% was obtained. This is mainly because electrical oxidation activity of PbO_2/Ti mesh electrode is higher than that of other two anode materials. The high current efficiency of Br^- electrooxidation is attributed to the lower oxidizing potential in the neutral solution, the higher oxidation current and the mild oxidizing condition. Therefore, the PbO_2/Ti mesh electrode was selected as the anode material in later study.

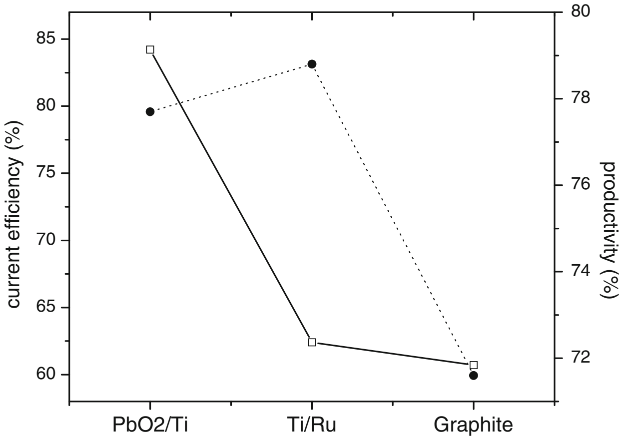


Fig. 8 Influence of different anode materials on the current efficiency and productivity. Electrolyte in anode cell: 0.053 mM KBr in 63 mL mixture of acetonitrile and deionized water; the concentration of CDCA: 31.8 mg/mL; current density: 95.2 A/m^2; current efficiency (☐) and productivity (●)

3.3 Influence of solvent on the current efficiency and productivity

Figure 9 shows the effect of different solvent on current efficiency and productivity of 7K-LCA in a divided electrolytic cell.

As is shown in Fig. 9, during electrolysis in a divided electrolytic cell, acetonitrile, methanol and ethanol were used as solvents. All of three solvents have little effect on productivity, but have a significant effect on current

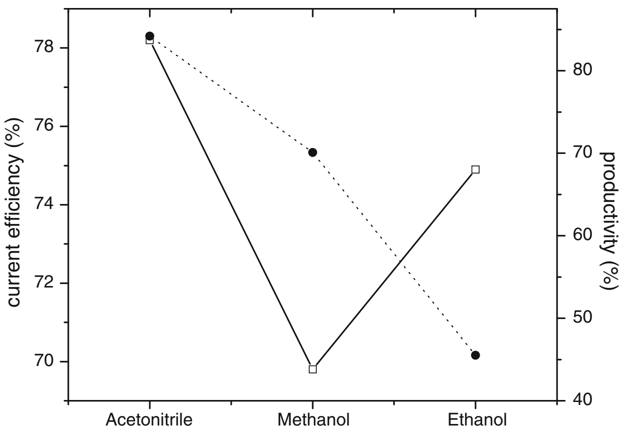


Fig. 9 Influence of different solvents on current efficiency and productivity. Electrolyte in anode cell: 0.053 mM KBr in 63 mL mixture of organic solvent and deionized water; the concentration of CDCA: 31.8 mg/mL; PbO_2/Ti mesh anode; current density 95.2 A/m^2; current efficiency (☐) and productivity (●)

efficiency. When acetonitrile was used as the solvent, there was highest productivity and current efficiency with 76.5 and 84.8%, respectively. Probably, acetonitrile has the highest polarity among selected solvents. When ethanol was used as the solvent, lowest current efficiency with 45.5% was obtained. Different solvents have different conductivity. Movement of reaction particle is rather quick in acetonitrile due to its highest polarity and lowest viscosity. When acetonitrile was used, there were higher electrode reaction rate and current efficiency. On the contrary, ethanol has the lowest polarity and highest viscosity, then the current efficiency is much lower.

3.4 Influence of concentration of chenodeoxycholic acid on current efficiency and productivity

Figure 10 shows the effect of different concentration of chenodeoxycholic acid on current efficiency and productivity of 7K-LCA in a divided electrolytic cell.

As is shown in Fig. 10, during electrolysis in the divided electrolytic cell, the current efficiency and productivity increase first and then decrease with concentration of CDCA. The two values can achieve 84.2 and 77.1% respectively. The current efficiency and productivity have a sharply decreasing tendency when concentration of CDCA is over 30 mg/mL. This is because chenodeoxycholic acid has low solubility in acetonitrile. At higher concentration, the some amounts of CDCA may precipitate because of the volatilization of solvent. The higher concentration may have effect on conductivity of solution, thus increases of the cell voltage would waste electrical energy, and reduce the current efficiency. The concentration of CDCA should be maintained in the range of 23.8–31.8 mg/mL.

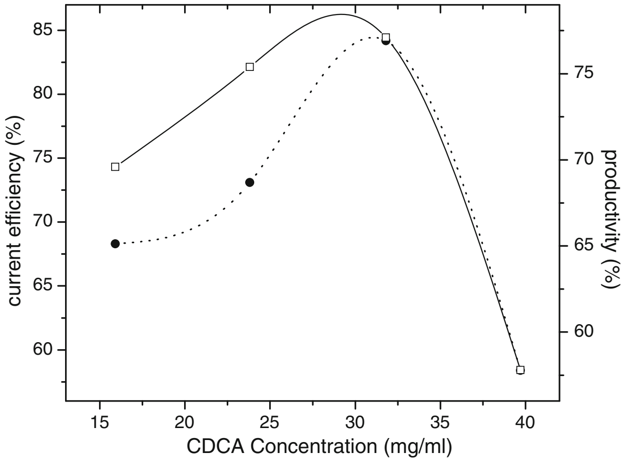


Fig. 10 Influence of concentration of chenodeoxycholic acid on current efficiency and productivity. Electrolyte in anode cell: 0.053 mM KBr in 63 mL mixture of acetonitrile and deionized water; PbO_2/Ti mesh anode; current density 95.2 A/m^2; current efficiency (☐) and productivity (●)

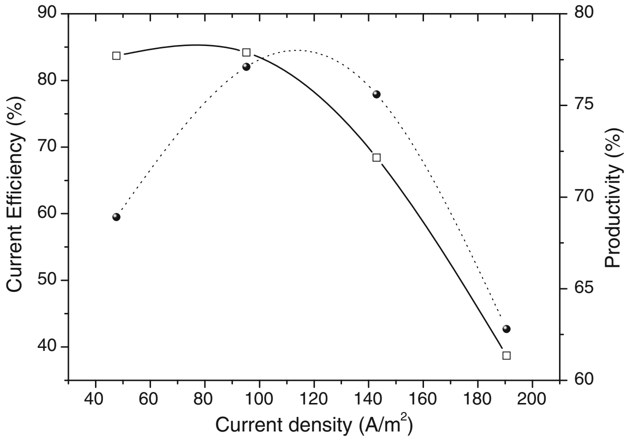


Fig. 11 Influence of current density on current efficiency and productivity. Electrolyte in anode cell: 0.053 mM KBr in 63 mL mixture of acetonitrile and deionized water; the concentration of CDCA: 31.8 mg/mL; PbO_2/Ti mesh anode; current efficiency (☐) and productivity (●)

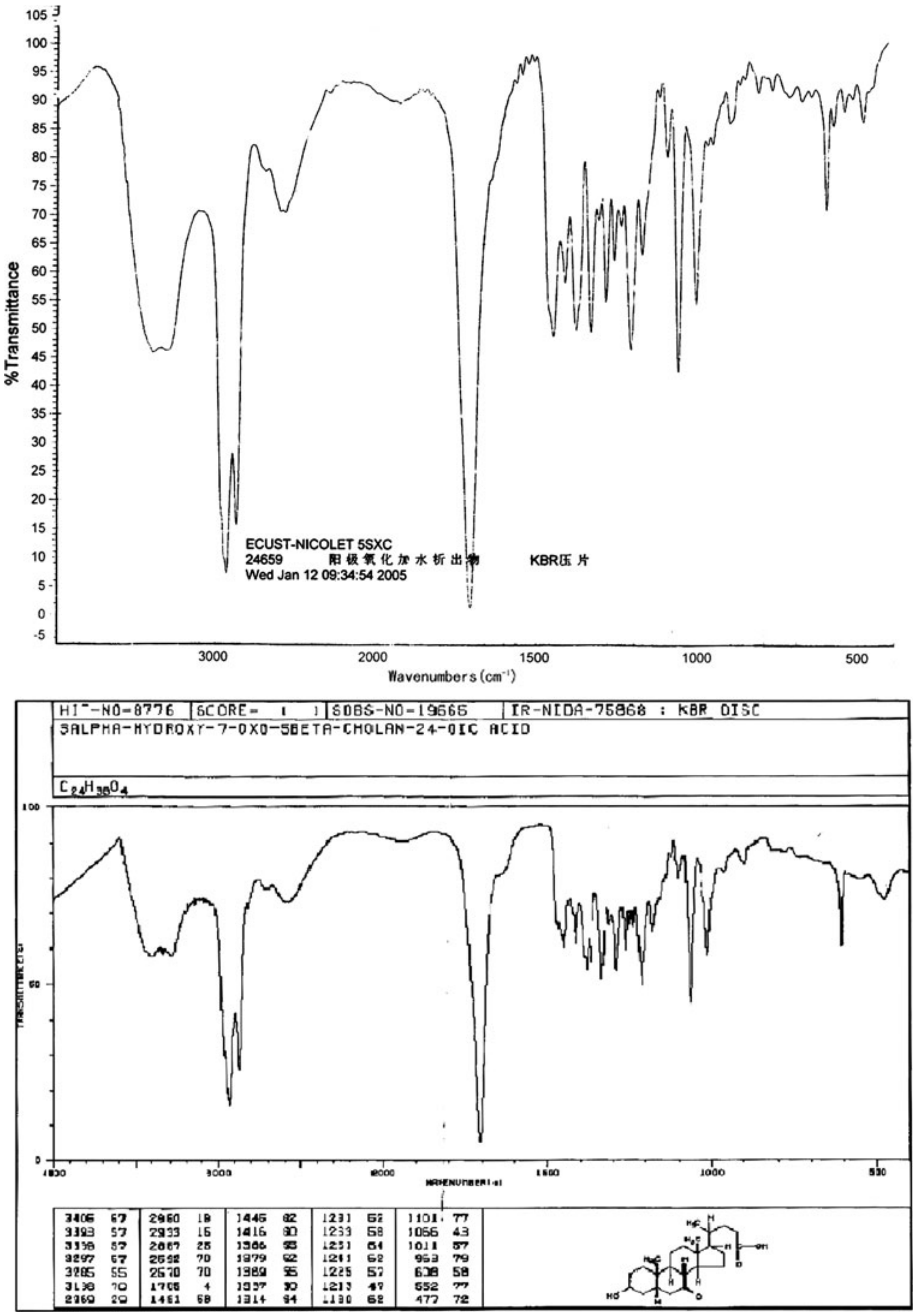
3.5 Influence of current density on the current efficiency and productivity

Figure 11 shows the effect of different current density on current efficiency and productivity of 7K-LCA in a divided electrolytic cell.

As is shown in Fig. 11, during electrolysis in a divided electrolytic cell, the productivity increase first and then decrease with the current density from 95.2 to 142.9 A/m^2. The current efficiency was 62.8–77.1% in above current density range. Obviously, the current efficiency is influenced by current density. The current efficiency increases first and then decrease sharply with rise of the current density. It generated some oxygen in the anode when the current density is greater than 142.9 A/m^2. The oxygen in the surface of cathode impedes the reaction between the electrode and the reactant. Thus it affects the conversion rate of chenodeoxycholic acid to result in low current efficiency and productivity of 7K-LCA. In addition, cell voltage increases with the rise of current density causing the additional consumption of energy. If the current density is too lower, the oxidation potential of anode is too low to achieve the oxidation potential required. In order to obtain the high current efficiency and productivity, the current density should be controlled in the range of 95.2–142.9 A/m^2.

3.6 Characterization of product

The melting point of product is 200–203 °C (Pharmacopoeia of China, 2005). It is consistent with the literature.



HI⁻-NO-8776 | SCORE= ( ) | SDBS-NO-19665 | IR-NIDA-75868 : KBR DISC

3ALPHA-HYDROXY-7-OXO-5BETA-CHOLAN-24-OIC ACID

$C_{24}H_{38}O_4$

3406	67	2960	18	1446	82	1231	62	1101	77
3393	57	2933	16	1416	80	1233	58	1066	43
3356	57	2867	25	1366	93	1251	64	1011	57
3297	67	2652	70	1379	92	1241	62	953	79
3285	55	2670	70	1389	95	1225	57	638	58
3138	70	1705	4	1337	90	1213	47	652	77
2969	29	1461	68	1314	94	1130	62	477	72

Fig. 12 The infrared spectrum of the product and the standard infrared spectrum of 7K-LCA

The product is characterized by using IR, MS, ^{1}H NMR spectra and ^{13}C NMR.

As is shown in Fig. 12, The IR spectrum demonstrated that the $(CH_3)_2CH$– or $(CH_3)_3C$– (1380 cm^{-1}), –CO– (1080 cm^{-1}), –CH– (2750–3000 cm^{-1}) and –COOH (1700 cm^{-1}) groups exist in the chemical structure of the product. The infrared spectrum of product is consistent with the standard infrared spectrum (SDBS: NIMC, the database of Japanese organic spectrum).

The mass spectrometry shows that the molecular weight of product is 390.6.

It is known that in the hydrogen spectrum (^{1}H NMR), the calculated chief chemical shifts for the various positions are δ(33H, m, $-CH_2-$, $-CH_2CH_3$, $-CH_2CH_2-$) = 0.5–1.8, 2.1,

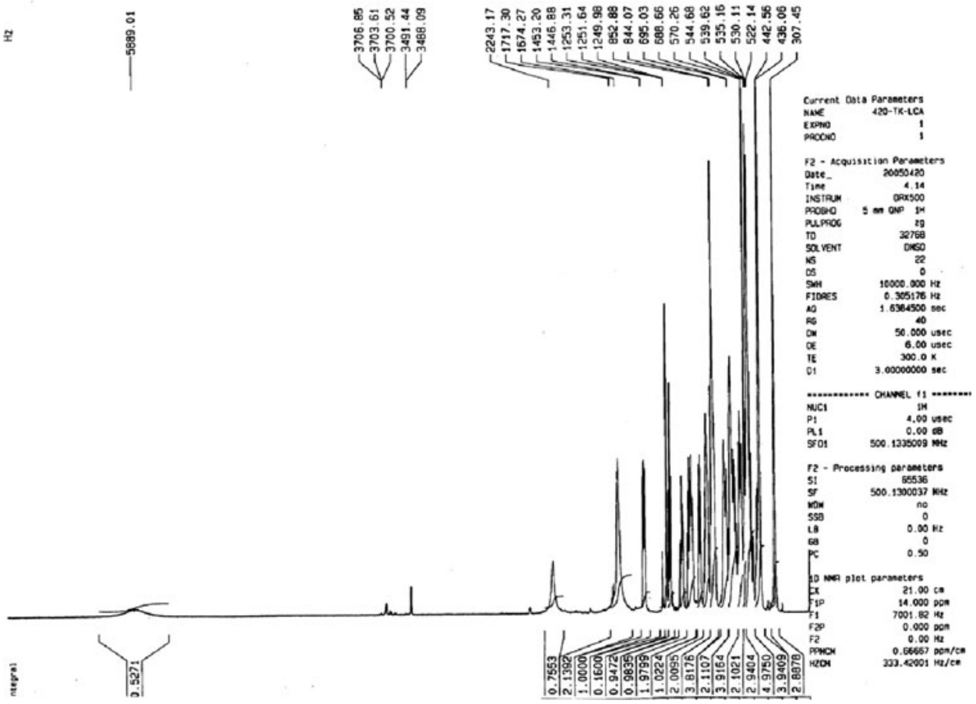


Fig. 13 ^{1}H NMR spectrum of the product

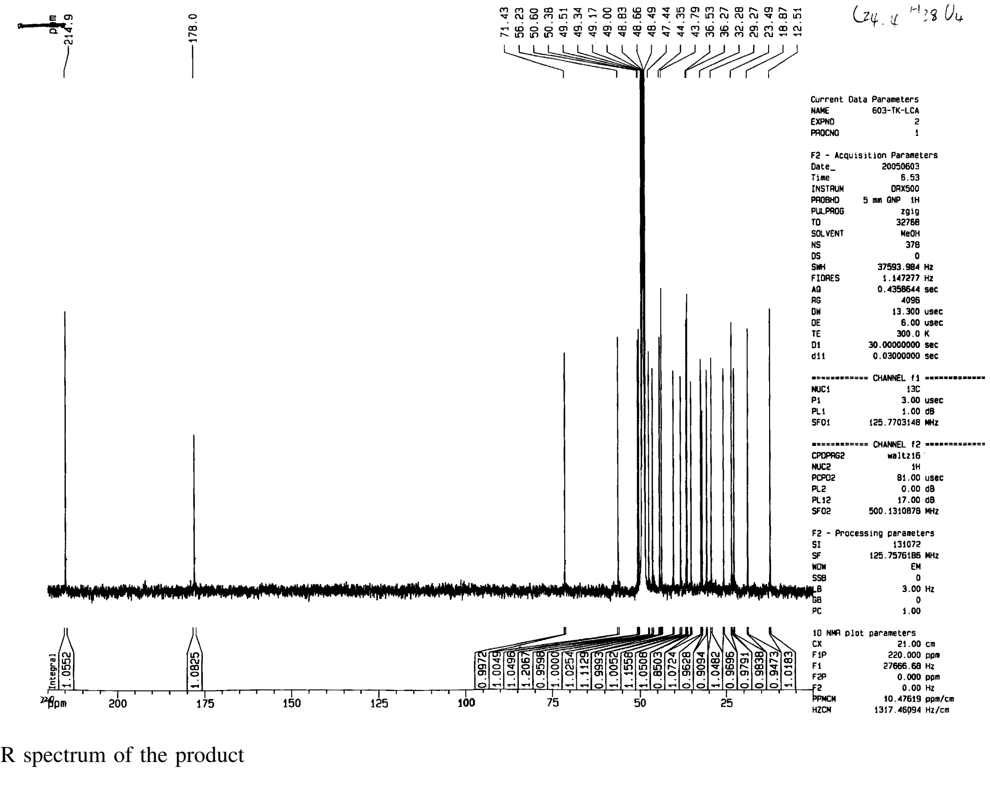


Fig. 14 ^{13}C NMR spectrum of the product

2.2, δ(1H, d, –CH–) = 1.9, δ(1H, t, –COCH) = 2.4, δ(1H, q, –$CH_2CH_2CH_2$–) = 2.8, δ(1H, s, –OH) = 4.5, δ(1H, s, –COOH) = 11.4. The 1H of total integral is 38 (Fig. 13).

It is known that in the carbon spectrum (^{13}C NMR), the calculated chief chemical shifts for the various positions are δ(C7) = 214.9, δ(C24) = 178.0, δ(C3) = 71.4,

$\delta(\mathrm{C18}) = 12.5$, $-\delta(\mathrm{C19}) = 23.5$. The 13C of total integral is 24 (Fig. 14).

From the above spectrums we can conclude that the product is 7-ketolithocholic acid and its chemical formula is $C_{24}H_{38}O_4$.

4 Conclusions

The 7-ketolithocholic acid, the precursor of ursodeoxycholic acid is synthesized by indirect electrooxidation, in which Br^-/Br_2 was used as the medium, and the relative inexpensive chenodeoxycholic acid as the raw material. It is concluded that in this article:

(1) In the same electrolysis condition, the current efficiency and productivity in the undivided electrolytic cell is approximately 35 and 15% lower than in H-model divided electrolytic cell.

(2) Under the conditions of the anode material PbO_2/Ti, the solvent acetonitrile, initial concentration of 7-ketolithocholic acid at 23.8–31.8 mg/mL, and current density 95.2–142.9 A/m^2, the current efficiency could reach 85% and the productivity could reach 83%.

(3) The product purified is characterized by using IR, MS spectra, ^{1}H NMR spectra and ^{13}C NMR. The analytical results show that the product is 7-ketolithocholic acid, and its chemical formula is $C_{24}H_{38}O_4$.

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