

Preparation of ursodeoxycholic acid by direct electro-reduction of 7-ketolithocholic acid

Xiangxi Yuan, Xiaolei Ma, and Xuejun Cao[†]

State Key Laboratory of Bioreactor Engineering, Department of Bioengineering, East China University of Science & Technology, 130 Meilong Rd., Shanghai 200237, China
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Abstract—A novel method of producing ursodeoxycholic acid was developed through direct electro-reduction of 7-ketolithocholic acid in a divided electrolytic cell. Titanium ruthenium mesh electrode was used as the anode, while high purity lead plate was used as the cathode. The process was optimized with regards to the electrolyte, temperature, concentration of methanol, current density and concentration of anolyte. When potassium bromide was used as the electrolyte, the saturated solution of 7-ketolithocholic acid in 85-93% (v/v) methanol, current density 9.52-28.6 A/m² and concentration of anolyte at 4-6% (w/w), the maximum percentage yield of ursodeoxycholic acid could be 47%. The method will provide a potential approach for large-scale production of ursodeoxycholic acid.

Keywords: 7-Ketolithocholic Acid, Chenodeoxycholic Acid, Ursodeoxycholic Acid, Direct Electroreduction, Potassium Bromide

INTRODUCTION

Ursodeoxycholic acid (3 α , 7 β -2-hydroxy-5 β -cholan-20-one, UDCA, Fig. 1) is an important clinical drug in the treatment of some diseases, such as primary biliary cirrhosis, primary sclerosing cholangitis, gallstones, carcinoma of rectum, viral hepatitis, alcoholic fatty liver diseases and non-alcoholic fatty liver diseases [1-8]. In early stage, ursodeoxycholic acid was prepared by bile acid from bear [9]. In 1954, Kanazawa et al. first synthesized ursodeoxycholic acid by reducing 7-ketolithocholic acid (3 α -hydroxy-7-oxo-5 β -cholan-20-one, 7K-LCA, Fig. 2) in acetone using sodium metal [10]. Claudio et al. [11] prepared UDCA by reducing 7K-LCA in tertiary alcohol using organic alkali metal. Kubota et al. [12] demonstrated that 7K-LCA could be reduced to UDCA in an alkaline environment using Pb/C as catalyst.

Because of the dangers of alkali metals, milder methods have

been proposed. Electrochemistry conversion is promising because of its convenience and green process. Hattori stated that 92.5% yield of UDCA could be obtained by hydrogenation catalyzed using Raney nickel as the catalyst at 0.5 MPa and 80 °C [13]. U.S. patent [14] reported that β configuration of the hydroxyl group could be generated by adding weak acid compounds such as tetramethyl urea. Japanese patent [15] reported that ursodeoxycholic acid could be prepared by electrolysis in short chain alcohol. Tian [16] applied catalytic transfer hydrogenation to convert 7K-LCA to UDCA with Raney nickel; the yield of UDCA is 94% at 40 °C and atmospheric pressure. However, these methods cannot successfully substitute the alkali metals reduction in industrial scale for UDCA production. Conventional chemical methods, biological methods, and electrolytic methods have some disadvantages of having multi-steps, high cost, harsh reaction conditions and high risk, while this electrochemical reduction method is convenient, green, low cost and mild conditions.

Thus, a stable, safe and convenient way needs to be found for the chemical synthesis of UDCA. This work aimed at developing a simple, efficient and economical method for generating UDCA from 7K-LCA.

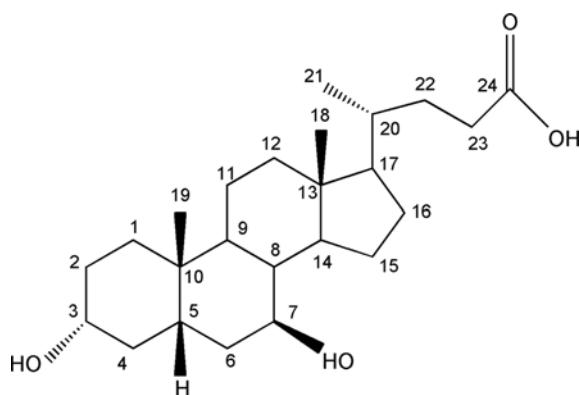


Fig. 1. UDCA.

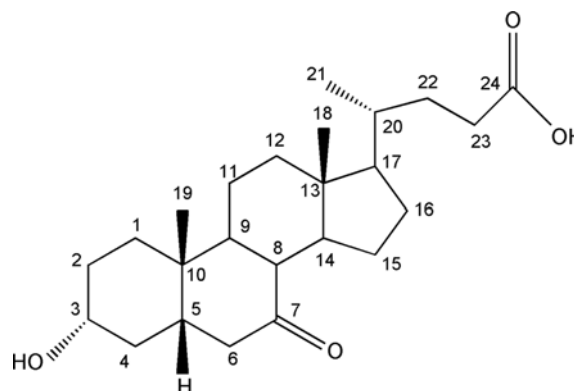


Fig. 2. 7K-LCA.

[†]To whom correspondence should be addressed.

E-mail: caoxj@ecust.edu.cn

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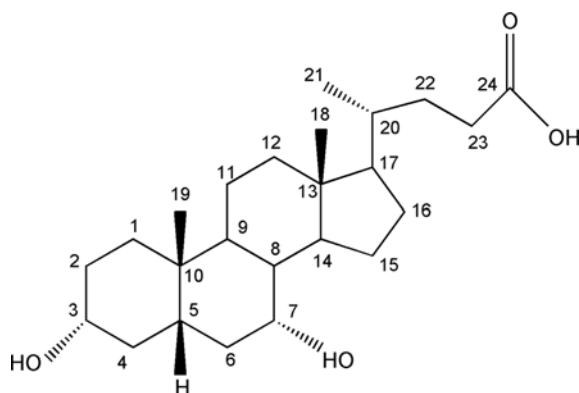


Fig. 3. CDCA.

MATERIALS AND METHODS

1. Materials

UDCA (99%) was purchased from Aladdin Chemistry Co. Ltd. 7K-LCA was prepared according to our previous work [17]. Methanol and potassium bromide were from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Acetonitrile of HPLC grade was purchased from Shanghai Xingke Biochemistry Co. Ltd. All other reagents were analytical grade, which were from Shanghai Ling Feng Chemical Reagent Co. Ltd. WY-30.5 DC power with single regulator steady flow was from Shanghai Starfish Instrument Co. Ltd. H-divided electrolytic cell was from Glass Instrument Factory of the East China University of Science and Technology (ECUST). Cathode material consisting of high purity lead plate electrode was from the Engineering Training Center of ECUST. The size of lead plate was 145 mm×30 mm. Anode material consisting of titanium ruthenium mesh electrode was from Henan Xinxiang Future Water Chemical Co. Ltd. The size of titanium ruthenium mesh electrode was 145 mm×30 mm. HF-101 cation exchange membrane of fluoroplastics was from Shanghai Hua Kai Technology Company.

2. Methods

2-1. Electrochemical Reduction of 7K-LCA

0.08 mol/L potassium bromide (or tetramethyl ammonium bromide) and 7-ketolithocholic acid were dissolved in a mixture of methanol and deionized water. The concentration of methanol in mixture was from 61% (v/v) to 100% (v/v). Anolyte was sulfuric acid solution at the concentration range of 2-14% (w/w). Both volumes of anolyte and catholyte were 130 mL. Electrolysis was carried out by using high purity lead plate as the cathode and the titanium ruthenium mesh electrode as the anode. HF-101 cation exchange membrane was used to divide the electrolytic cell into a cathode cell and

an anode. All experiments were carried out at a certain temperature.

The process of reaction was detected by thin layer chromatography (TLC). Mobile phase of TLC was chloroform/methanol/glacial acetic acid with volume ratio of 40 : 2 : 1. Chromogenic agent was 20% of phosphor-molybdic acid of ethanol solution. Chromogenic temperature was about 105 °C and chromogenic time was about 10 min.

Finally, the organic solvent methanol from catholyte was removed by using a rotary evaporator to obtain a solid sample. Then it was dissolved in NaOH solution, and pH was adjusted to about 2.0 by hydrochloric acid in order to get precipitates. The precipitate was collected by filtration, then dried in oven. The final product was detected by HPLC.

2-2. Analysis of UDCA

UDCA was analyzed by reverse phase HPLC (RP-HPLC). HPLC (LC-20A, Shimadzu Corporation, Kyoto, Japan) with a UV detector using a C-18 column (Welchrom-C18 5 μm, 4.6 mm×150 mm, Welch Material Inc. Shanghai, China) was used for quantitative analysis of the reaction products at 208 nm.

Mobile phase was mixture of acetonitrile and phosphate acid buffer (pH=3.0) with volume ratio of 50 : 50 at a flow rate of 1.0 mL/min at 25 °C. 50% (v/v) methanol was used to clean syringe of SIL-20A autosampler (Shimadzu Corporation, Kyoto, Japan).

RESULTS AND DISCUSSION

Electrochemical reduction with selectivity has important application value. Here, ursodeoxycholic acid was synthesized by direct electrochemical reduction of 7-ketolithocholic acid under certain conditions.

During electrolysis, The C-7 carbonyl group of 7K-LCA reverted into 7β-hydroxy group on the cathode surface. (Scheme 1).

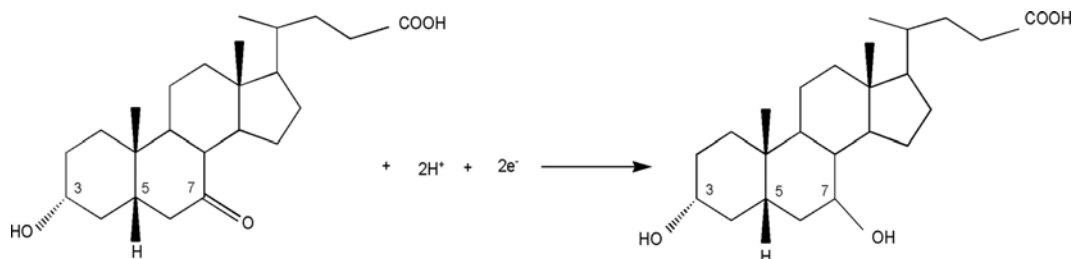
It may generate chenodeoxycholic acid and hydrogen on the cathode surface, which are the side reactions: (Scheme 2).

Meanwhile, H₂O is electrooxidized on the anode surface: (Scheme 3).

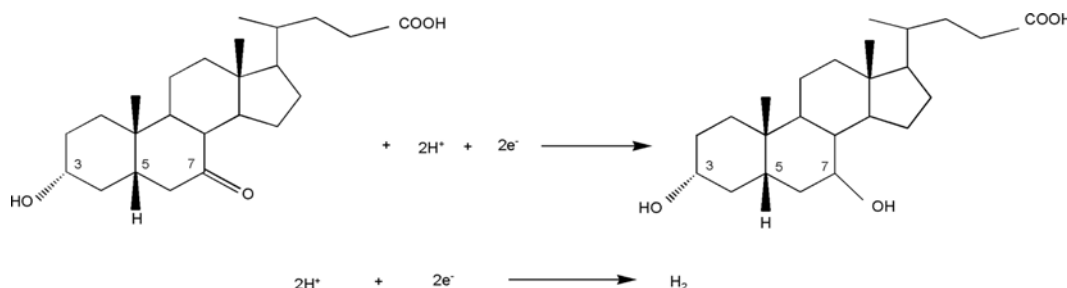
The cation H⁺ will migrate to the cathode because of the attraction of the cathode and the concentration gradient when a continuous electrolytic process occurs.

1. Influence of Different Electrolytes and Temperatures on the Yield of UDCA and Conversion Ratio of 7K-LCA

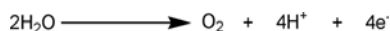
Fig. 4 shows the effect of different electrolytes and temperatures on the yield of UDCA and conversion ratio of 7K-LCA. In the divided electrolytic cell, the yield of UDCA using KBr as electrolyte increased from 23% to 43% with the increasing of temperature, while the yield of UDCA using tetramethyl ammonium bromide increased



Scheme 1.



Scheme 2.



Scheme 3.

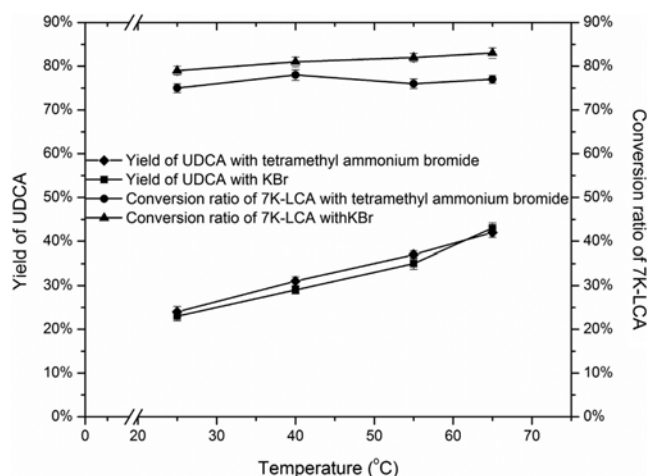


Fig. 4. Influence of different electrolytes and temperatures on the yield of UDCA and conversion ratio of 7K-LCA. The saturated solution of 7K-LCA in 130 mL mixture of methanol and deionized water; current density: 18.51 A/m²; the concentration of anolyte: 5.6% (w/w).

from 24% to 42%. Due to less steric hindrance, the conversion ratio of 7K-LCA with potassium bromide as electrolyte is about 5% higher than that with tetramethyl ammonium bromide. The conductivity can be increased by electrolyte. Compared with tetramethyl ammonium bromide, potassium bromide was selected as electrolyte because of its lower cost, lower moisture absorption in industrial preservation, lower toxicity and the ability to match the electro-oxidation process of CDCA to 7K-LCA. We can conclude from the experiment results that a higher temperature is beneficial for increasing the amount of UDCA. However, a higher temperature results in the volatilization loss of methanol, which causes methanol/water disproportion. Finally, the reaction temperature selected is between 60 °C and 65 °C.

2. Influence of Concentration of Methanol on Yield of UDCA

Fig. 5 shows the effect of concentration of methanol on the yield of UDCA.

It can be seen that the yield of UDCA increases at first and then decreases with the concentration of methanol. The yield of UDCA achieves a maximum value of 42% when the concentration range of methanol is between 85% (v/v) and 93% (v/v). 7K-LCA could

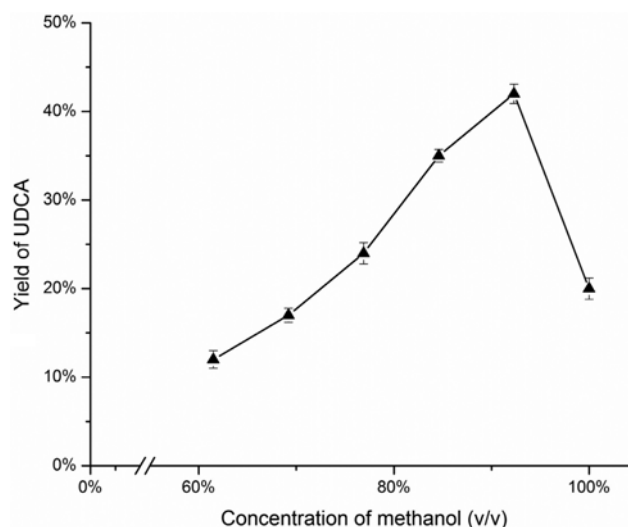


Fig. 5. Influence of the concentration of methanol on the yield of UDCA. Electrolyte in cathode cell: 0.08 mol/L KBr in 130 mL mixture of methanol and deionized water; the saturated solution of 7K-LCA; current density: 18.51 A/m²; the concentration of anolyte: 5.6% (w/w).

not be dissolved in water, but has good solubility in methanol. As the concentration of methanol increases from 61.5% (v/v) to 92.3% (v/v), the yield of UDCA increases correspondingly, and after reaching 92.3% (v/v) it drops drastically. 7K-LCA needs to uptake two H⁺ ions to produce the end product UDCA but transfer of H⁺ ions is limited by water contents. So, it is very difficult to occur in pure methanol or at very low water contents. From Fig. 5, the uptake of H⁺ ions was hindered when methanol concentration is higher than 92.3% (v/v). Therefore, a proper proportion of methanol and water is the key to a successful electrolytic reaction.

3. Influence of Current Density on the Yield of UDCA and Conversion Ratio of 7K-LCA

Fig. 6 shows the effect of different current density on the yield of UDCA and conversion ratio of 7K-LCA in the divided electrolytic cell.

The yield of UDCA and conversion ratio of 7K-LCA increase at first and then decrease with the current density change from 9.52 to 28.6 A/m² and the optimal value is at about 19 A/m². With less current density, only a small amount of H can pass the membrane, which results in low reaction efficiency. However, when the current density was over 19 A/m², many bubbles appeared on the cathode surface, which indicates the produced hydrogen reaction happened.

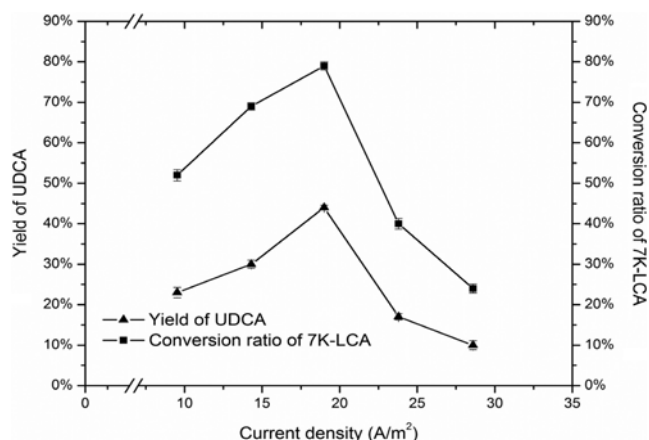


Fig. 6. Influence of current density on the experimental results. Electrolyte in cathode cell: 0.08 mol/L KBr in 130 mL mixture of methanol and deionized water; the saturated solution of 7K-LCA; the concentration of anolyte: 5.6% (w/w).

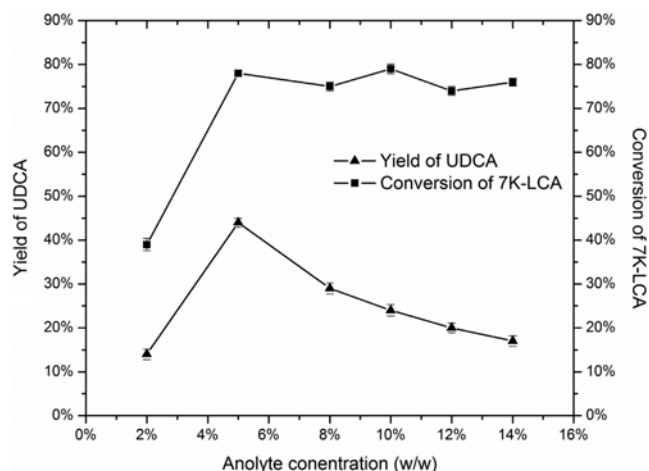


Fig. 7. Influence of the concentration of anolyte on the experimental results. Electrolyte in cathode cell: 0.08 mol/L KBr in 130 mL mixture of methanol and deionized water; the saturated solution of 7K-LCA; current density: 18.51 A/m².

4. Influence of Concentration of Anolyte on the Yield of UDCA and Conversion Ratio of 7K-LCA

Fig. 7 shows the effect of different concentrations of sulfuric acid on the yield of UDCA and conversion ratio of 7K-LCA in the divided electrolytic cell. The yield of UDCA increases with the concentration of anolyte from 2% (w/w) to 5% (w/w), and then decreases with concentration of sulfuric acid. The conversion ratio of 7K-LCA increases at first and then keeps relatively stable. The concentration of sulfuric acid corresponding to maximal yield of UDCA is located between 4% (w/w) and 6% (w/w). When the concentration of sulfuric acid is lower than 2% (w/w), the amount of H^+ through the exchange membrane from anode to cathode is too little to meet the needs of the electro-reduction in the catholyte. When the concentration of sulfuric acid is higher, H^+ can be transferred from anode to cathode because of the concentration gradient and the attraction of cathode, which leads to pH value of catholyte too low to generate β configuration of the hydroxyl. That shows a certain relationship between configuration of the hydroxyl and pH of the electrolyte.

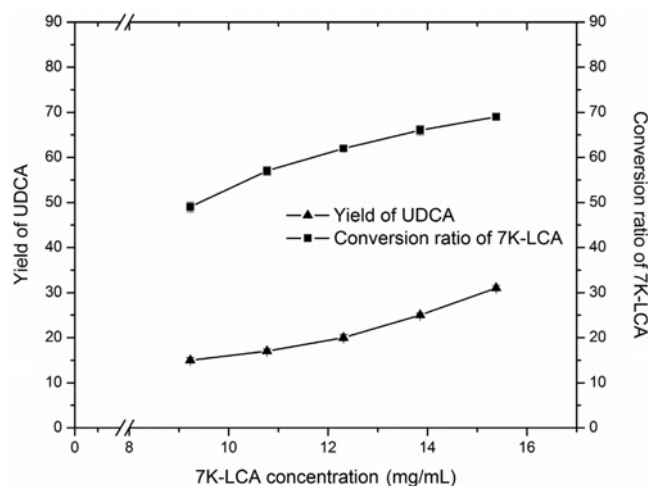


Fig. 8. Influence of the concentration of 7K-LCA on the yield of UDCA and conversion ratio of 7K-LCA. Electrolyte in cathode cell: 0.08 mol/L KBr in 130 mL mixture of methanol and deionized water; the concentration of anolyte: 5.6% (w/w); current density: 18.51 A/m².

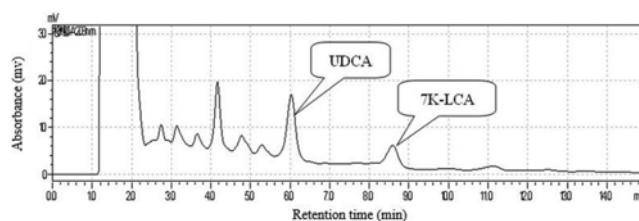


Fig. 9. HPLC chromatogram of the product. Flowing phase was mixture of acetonitrile and phosphate acid buffer (pH=3.0) with volume ratio of 50 : 50 at a flow rate of 1.0 mL/min at 25 °C.

5. Influence of Concentration of 7-Ketolithocholic Acid on the Yield of UDCA and Conversion Ratio of 7K-LCA

Fig. 8 shows the effect of different concentration of 7-ketolithocholic acid on the yield of UDCA and conversion ratio of 7K-LCA. The yield of UDCA and the conversion ratio of 7K-LCA increase gradually with initial concentration of 7K-LCA. The former slope increases gradually, while the latter slope decreases. The higher the initial concentration of 7K-LCA is, the more products that can be obtained. 7K-LCA solubility depends on the proportion of methanol/water solution. That shows the saturated solution of 7K-LCA is the best choice.

6. Result of HPLC

As is shown in Fig. 9, the retention time of UDCA and 7K-LCA is 6.0 min and 8.59 min, respectively. Many impurity peaks could be seen between 2.0 min and 6.0 min because some of the impurities were contained in plant samples and others might have been produced by the electro-reduction process. The yield of UDCA can reach 47% by calculating according to an external standard.

CONCLUSIONS

Ursodeoxycholic acid is synthesized by direct electro-reduction of 7-ketolithocholic acid in a divided cell. It is concluded that in

this article:

Under the conditions of the cathode material with high purity lead plate, the anode material titanium ruthenium mesh electrode, electrolyte potassium bromide, the saturated solution of 7K-LCA in 85-93% (v/v) methanol, current density 9.52-28.6 A/m², and concentration of anolyte at 4-6% (w/w), the amount of UDCA reach 47%. Compared with chemical and biological preparative processes, the electrochemical process has advantages of low cost, less environmental pollution and production safety.

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