Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 1421–1426
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

The synthesis of MP–CDCA conjugates and dissolution kinetics of model cholesterol gallstones

Rui-yu Gong [a], Zhi-liang Lü [a], Li-dong Zhang [a], Li-ping Du [a], Da Zhang [a], Xue-liang Qiao [b], Jian-rong Li [c,*]

[a] Pharmaceutical School of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[b] Department of Material Science and Engineering, Huazhong University of Science and Technology, Wuhan 430074, China
[c] Department of Pathogenic Biology of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Received 15 September 2007; received in revised form 28 November 2007; accepted 15 January 2008
Available online 7 February 2008

Abstract

The comb-like copolymers of polycarboxylic acid were synthesized and then reacted with chenodeoxycholic acid (CDCA) to obtain a series of conjugates, MP*n*–CDCA, where *n* is the number of the groups of oxyethylene in each graft chain. This was confirmed by infrared spectroscopy and thin-layer chromatography. We investigated the effects of dissolving model cholesterol gallstones with the MP*n*–CDCA conjugates in phosphate-buffered saline at pH 7.4. The dissolution rates of CDCA, MP40–CDCA, MP30–CDCA, MP20–CDCA and MP10–CDCA were 5.33, 5.717, 17.59, 6.868 and 9.615×10^{-7} kg m^{-2} s^{-1}, micellar solubilities were 0.2431, 3.095, 12.972, 5.248 and 5.790 kg m^{-3} and total resistances were 5.33, 5.717, 17.59, 6.868 and 9.615×10^{-7} kg m^{-2} s^{-1}, respectively. These studies suggested that the interfacial resistance was the dominant rate-determining factor in dissolving model cholesterol gallstones. Model cholesterol gallstones could be more effectively dissolved by increasing the steric interactive potential energy of side chains and ensuring that the hydrophilic–lipophilic properties of MP–CDCA are within an appropriate range. The micellar dissolution rates of model cholesterol gallstones by MP20–CDCA were significantly faster than by the other conjugates.
© 2008 Published by Elsevier Ltd. on behalf of Acta Materialia Inc.

Keywords: Model gallstone; Dissolving in vitro; Steric interactive potential energy; Kinetics

1. Introduction

Gallstone disease is a common digestive disease in the general population [1–8]. Major advances have been made in understanding the cholesterol gallstone problem. Although studies have been carried out to evaluate the thermodynamic equilibria existing in the bile acid–lecithin–cholesterol–water systems and their relation to cholelithiasis [9–13], there is relatively little information on the kinetics of gallstone dissolution. Oral chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) have been demonstrated to be effective in dissolving gallstones. Unfortunately, stone dissolution by oral CDCA and UDCA requires months to years of therapy and may be effective in only 50% of patients with radiolucent stones [14–16].

The present study deals with the chemical modification of CDCA by its conjugation with the comb-like copolymers of polyoxyethylene allyl methyl diether and maleic anhydride to produce MP–CDCA, and dissolution kinetics with respect to model cholesterol gallstones, which are drawing attention due to their superior performance in dispersing particles and maintaining dispersing stability characteristics [17]. Polyoxyethylene with non-toxic, non-immunogenic and amphipathic properties has been extensively used as chemical modifying reagents. So far, the purpose of our study was to determine: (a) whether or not the rate of cholesterol gallstone dissolution in vitro

* Corresponding author. Fax: +86 278 365 7829.
E-mail address: jrli@mails.tjmu.edu.cn (J.-r. Li).

1742-7061/$ - see front matter © 2008 Published by Elsevier Ltd. on behalf of Acta Materialia Inc.
doi:10.1016/j.actbio.2008.01.015

can be enhanced by MP–CDCA and (b) whether or not the dissolution rates of model gallstones in phosphate-buffered saline (PBS) are governed by diffusion in the bulk or by interfacial factors.

2. Materials and methods

2.1. Materials

Chenodeoxycholic acid, a BR-grade agent (greater than 92% pure), was purchased from Shanghai Guangmao Biochemical Co., Ltd. (Shanghai, China), after recrystallization twice by the method of Pope [18], and gave a single spot on thin-layer silica gel chromatography (chloroform:methanol:water, 65:28:4, v/v/v). Cholesterol (Beijing Aoboxing Bio-Tech Co. Ltd., Beijing, China) was recrystallized trice from hot 95% (v/v) ethanol, then was judged to be greater than 99% pure by thin-layer silica gel chromatography (hexane:diethylether:glacial acetic acid, 7:3:0.1, v/v/v). Polyoxyethylene allyl methyl diether (PEAO) was prepared according to an earlier study [19,20]. Cholesterol oxidase kits for measuring cholesterol [21] were obtained form Biosino Bio-technology & Science Inc. (Beijing, China). All the other reagents used were commercial grade.

2.2. Synthesis

2.2.1. PEAO–MAn (MP)

Maleic anhydride (MAn, 0.1 mol) was added to the solution of polyoxyethylene allyl methyl diether (APEO-*n*, 0.1 mol, *n* is the number of the oxyethylene group in graft chain) in toluene, heated to 80° and well stirred in a three-necked flask under a nitrogen atmosphere. When MAn was solved completely, the azobisisobutyronitrile (AIBN, 0.009 mol) as an initiator, dissolved in 1×10^{-4} m^3 toluene, was added dropwise slowly over a period of 30 min. The mixture was stirred for 4 h at 80°. The cold mixture was filtered by gentle suction and washed with toluene. The filtrate was dried by azeotropic distillation with toluene for 8 h. The product, MP, as brown waxy solid, was obtained by vacuum distillation under nitrogen atmosphere; yield 0.098–0.101 mol (98–101%).

2.2.2. MP–CDCA

CDCA (0.095 mol) dissolved in tetrahydrofuran (THF) was added to the solution of MP (0.1 mol) in THF, well stirred at room temperature for 45 min. The temperature of the reaction system was raised because of heat producing. The crude product (yield: ~100%, ~0.095 mol, according to CDCA) was a deep brown waxy solid and was purified by column chromatography (silica gel, chloroform:methanol:water, 65:28:4, v/v/v).

2.3. Preparation of model gallstone

For the dissolution experiments, thin ($2 \pm 0.2 \times 10^{-6}$ m) disks of cholesterol were prepared by compressing a certain amount of cholesterol crystals in an evacuated stainless steel die, 6.0×10^{-6} m (i.d.) under a pressure of 1.5×10^{7} kg m^{-2} using a single punch tablet press (Shanghai Pharmaceutical Mechanism Factory, Shanghai, China). The exposed surface area of the resulting pellets was about 9.42×10^{-5} m^2.

2.4. Dissolution of model cholesterol gallstone

The 200 mmol dm^{-3} solutions of dissolving model gallstone were prepared as follows: conjugates and CDCA were dissolved in 0.1 mol dm^{-3} phosphate buffer, by stirring, pH 7.4, respectively. In each experimental group, the model gallstones were placed in flasks containing 5.0×10^{-5} m^3 of the above solutions for 1 week. The flasks were placed in a constant temperature water bath and gently agitated every 4 h for 10 min. Model gallstones placed in 0.1 mol dm^{-3} phosphate buffer (pH 7.4) for the same period as above were used as controls. The model gallstones were removed from the flasks at every day, the solution was sonicated for exactly 10 s and 1.0×10^{-5} m^3 aliquot was taken at suitable intervals for cholesterol analysis. The model gallstones were washed with distilled water, vacuum dried at 60° for 45 min and weighed.

2.5. Chemical analysis

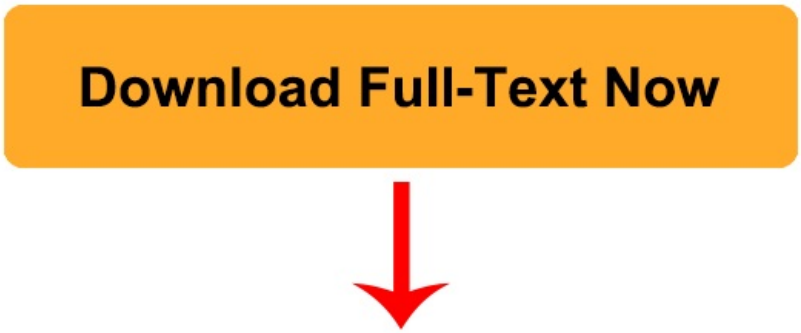
IR spectra of the products were determined on a FTIR spectrometer (Spectrum One; Perkin-Elmer Co., America) at room temperature using KBr disk pellets. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates. These were developed using the solvent system chloroform:methanol:water 65:28:4 (v/v/v) and detected by spraying the plates with 10% phosphomolybdic acid in ethanol and heating at 110° for 10 min. The cholesterol concentration of the sample solution was determined using cholesterol oxidase kits.

3. Results and discussion

3.1. The synthesis of MP–CDCA

The comb-like copolymers of polycarboxylic acid were synthesized with polyoxyethylene allyl methyl diether (PAO-*n*) and MAn (Fig. 1). These comb-like copolymers were then reacted with CDCA, resulting in MP–CDCA; this gave a single spot on thin-layer silica gel chromatography (chloroform:methanol:water, 65:28:4, v/v/v) and the yield is shown in the Table 1. The conjugates MP10–CDCA, MP20–CDCA, MP30–CDCA, MP40–CDCA are formed. It is well known that maleic anhydride (MAn) and allyl compound do not form homopolymers under normal conditions, but they easily react with each other under certain conditions to obtain an alternating copolymer [22]. MAn is an electron-deficient monomer, referred to as an acceptor monomer (*A*), and allyl compound is an elec-

ID	Title	Pages
1574	The synthesis of MP–CDCA conjugates and dissolution kinetics of model cholesterol gallstones	6



http://fulltext.study/article/1574

FullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study