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Xanthoceraside hollow gold nanoparticles, green pharmaceutics preparation for poorly water-soluble natural anti-AD medicine

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ABSTRACT

In order to increase the solubility of poorly water-soluble natural product, xanthoceraside, an effective anti-AD compound from *Xanthoceras sorbifolia Bunge*, and maintain its natural property, the xanthoceraside hollow gold nanoparticles were successively prepared by green ultrasonic method with silica spheres as templates and HF solution as selective etching solvent. Hollow gold nanoparticles and drug-loaded hollow gold nanoparticles were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The solubilities of xanthoceraside loaded on hollow gold nanoparticles were increased obviously from 3.0 μg/ml and 2.5 μg/ml to 12.7 μg/ml and 10.7 μg/ml at 25 °C and 37 °C, respectively. The results of XRD and DSC indicated that the reason for this increase was mainly due to the amorphous state of xanthoceraside loaded on the hollow gold nanoparticles. In summary, the method of loading xanthoceraside onto hollow gold nanoparticles was a green and useful strategy to improve the solubility and dissolution of poorly water-soluble natural products and worth to applying to other natural products.

1. Introduction

In our current daily life, with the increasing attention to green and healthy life, natural medicines have obtained more and more people's favor because of their green resources without chemical contamination, higher efficacy from multi-factored and multi-targeted properties, as well as less side effects. While, on the other hand, most natural products are always accompanied by a variety of insufficient, such as poor solubility (Zhou et al., 2014; Wang et al., 2011), low bioavailability (Tu et al., 2012; Liu et al., 2015), bad biocompatibility, and limited chemical stability etc. (Gokuladhas et al., 2014), which have to be overcome by different means of preparation. As we all know that the amount of drug formulation excipients is often larger than the amount of drug itself in preparations, so the formulation excipients often significantly affect the natural properties of natural medicines. Using inorganic material of non-chemical pollution as formulation excipient and carrier material for natural product is a green and good choice, because no residual organic solvents produced during the preparation. During our continuously attempt and study on those inorganic materials, metal gold aroused our great attention. Many studies have revealed that gold nanoparticle is a highly biocompatible pharmaceutical carrier material (Shukla et al., 2005; Connor et al., 2005; Hemant et al., 2013; Yang et al., 2016; Venkatpurwar et al., 2011; Elbialy et al., 2015) because of its chemically inert property, which could guarantee the original biological activity remaining unchanged. Therefore, gold nanoparticle is a good carrier for natural product medicine.

With the continually prolonging of the human being's life time, more and more elder people are suffering from Alzheimer's disease (AD), a devastating neurodegenerative disorder with a relentless progression. The production and aggregation of the amyloid beta (Aβ) protein are widely believed to trigger the development of AD. Our previous phytochemical studies on anti-AD natural products have resulted in the isolation and determination of a series of characteristic angeloyl (Ang)-substituted triterpenoids, among

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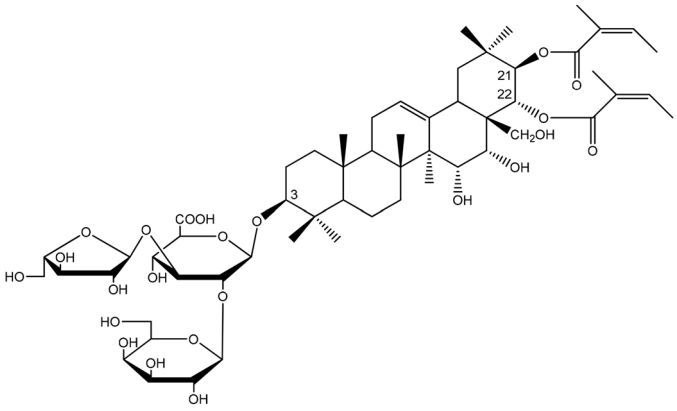

Fig. 1. The structure of xanthoceraside.

which xanthoceraside (Fig. 1) was recently found to be the major determinant against Aβ-induced AD in mice (Ling et al., 2011). The biological activities of this triterpenoids on AD are prominent (Liu et al., 2014; Chi et al., 2009, 2013; Lu et al., 2012) and significantly stronger than that of the synthetic donepezil (Liua et al., 2013; Jin et al., 2014a,b). It is abundant in the fruit husks of *Xanthoceras sorbifolia* Bunge and the extraction and separation processes have been relatively skillful (Wang et al., 2012). Because of its excellent therapeutic effect, xanthoceraside is most likely to be a potential drug for AD treatment, but its low water solubility, poor bioavailability are the key problems to prevent it from becoming a clinic drug. Therefore, in order to ensure the natural property of xanthoceraside and increase the bioavailability and dissolution of this poorly water-soluble compound, the inorganic gold nanoparticles were used as the carrier materials for this natural derived anti-AD compound. In this paper, a green ultrasonic method with silica spheres as templates and HF solution as selective etching solvent was used for the preparation of xanthoceraside hollow gold nanoparticles, which were further characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Herein, the details of the study work, as long as the potential mechanisms will be discussed comprehensively.

2. Materials and methods

2.1. Materials

Ammonium hydroxide (25%), ethanol absolute, hydrofluoric acid (40%), potassium carbonate, tetraethylorthosilicate (TEOS) (99%), (3-aminopropyl) triethoxysilane (APTES), trisodium citrate dehydrate, sodium borohydride (98%), formaldehyde solution (37%) and chloroauric acid were purchased from Sinopharm Chemical Ragent Corporation. 3-Aminopropyltriethoxysilane was purchased from Aladdin Industial Corporation. Nutshells of *X. sorbifolia* were provided by CAS Shenyang Institute of Ecology.

2.2. Preparation of xanthoceraside

The air-dried nutshells of *X. sorbifolia* Bunge (15.0 kg) were chopped and extracted with 120 l (×3) of 70% aqueous EtOH under reflux for 2 h. After evaporation of the combined EtOH extracts *in vacuo*, the resultant residues (~5000 ml) were extracted with chloroform (5000 ml × 3) and *n*-butanol (5000 ml × 3) respectively. 750.0 g of *n*-butanolic extracts was chromatographed on silica gel column (1000 mm × 100 mm i.d.) using a gradient CH_2Cl_2–MeOH system (100:0–0:100, v/v) and xanthoceraside was isolated from the fraction of CH_2Cl_2–MeOH 100:25 by further recrystallization. Molecular ion peak $[M+Na]^+$ at *m/z* 1163.3, fragment ion peak $[M-132]^+$ at *m/z* 1008.4, $[M\text{-}132\text{-}162]^+$ at *m/z* 846.8 and $[M\text{-}132\text{-}162\text{-}176\text{-}H2O]^+$ at *m/z* 652.9 were given by ESI–MS. NMR data of xanthoceraside are listed in Table 1.

Table 1
NMR data of xanthoceraside.[a]

No.	δ_C δ_H	No.	δ_C δ_H
1	39.7	GluA-1	105.2 4.89 q (7.3)
2	25.7	2	78.9
3	89.9	3	86.4
4	39.0	4	71.7
5	55.7	5	77.3
6	18.9	6	172.0
7	36.4	Gal-1	104.9 5.32 q (7.5)
8	41.1	2	73.5
9	47.2	3	75.2
10	37.0	4	69.8
11	24.0	5	76.7
12	125.5 5.48 brs	6	61.9
13	143.7	Ara-1	111.2 6.03 s
14	47.8	2	83.6
15	67.6	3	77.7
16	73.4	4	85.5
17	48.4	5	62.4
18	41.5	Ang-1	167.8
19	47.0		168.2
20	36.8	2	129.0
21	78.6 6.70 d (10.2)		129.2
22	73.6 6.32 d (10.2)	3	137.4 5.96 q (7.0)
23	28.0 1.26 s		136.6 5.76 q (6.6)
24	16.8 1.16 s	4	15.9 2.09 d (7.2)
25	15.8 0.81 s		15.7 1.93 d (6.6)
26	17.6 0.98 s	5	21.0 2.00 s
27	21.3 1.84 s		20.7 1.72 s
28	63.2 3.73,3.50 d (10.2)		
29	29.6 1.09 s		
30	20.3 1.31 s		

[a] Measured at 300 MHz; obtained in Pyridine-d_5 with TMS as an internal standard; chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses.

2.3. Preparation of hollow gold nanoparticles

Hollow gold nanoparticles were synthesized by the method (Abdollahi et al., 2013) with some suitable modifications. To prepare monodispersed silica spheres, 100 ml ethanol, 5 ml deionized water, 8.5 ml ammonium hydroxide and 1.5 ml TEOS were mixed and stirred at 40 °C for 3 h. Then 0.1 ml APTES were added and stirred overnight. The modified particles were finally separated by centrifugation and dispersed in deionized water. 1 ml of 1% $HAuCl_4$ solution, 2 ml of 1% trisodium citrate and 1 ml of fresh 0.075% $NaBH_4$ in 1% trisodium citrate were added into 100 ml deionized water and the mixture was stirred for about 10 min to form the gold nanospheres. 10 ml of the modified particles were added to 100 ml of the gold nanospheres, stirred for 5 min and stood for 2 h. Then the gold decorated silica particles were obtained by centrifugation and dispersed in deionized water. In order to make the gold seeded silica shell grow, 1 ml of gold seeded silica solution and 80 μl formaldehyde were added into 20 ml of gold hydroxide solution which was obtained from mixing 1.5 ml of 1% $HAuCl_4$ solution and 100 ml of 2 mM potassium carbonate aqueous solution and stirring overnight. The solution above was centrifuged and dispersed in deionized water. The last step was to etch the silica templates using HF. 14 ml of 2.5vol% HF solution was added into 20 ml of sonicated solution and the mixture was stirred magnetically for 5 min in presence of 0.015 g trisodium citrate. The hollow gold nanoparticles were obtained by centrifugation.