Separation and Purification Technology 155 (2015) 20–25

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

The relationship between swelling and hydrolysis of Trichosanthin (TCS) in aqueous ionic liquids

Chang Yang, Hang Song *, Shun Yao, Yan Wang, Yi Xie

Department of Pharmaceutical and Biological Engineering, Sichuan University, Chengdu 610065, PR China

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 12 January 2015
Accepted 14 January 2015
Available online 4 February 2015

Keywords:
Trichosanthin
Ionic liquids
Swelling
Hydrolysis

ABSTRACT

The swelling of Trichosanthin (TCS) particles in aqueous ionic liquids (ILs) was observed to discover the mass transfer mechanisms in natural products-solvent system. The hydrolysis behaviour of TCS accompanied with swelling was investigated to explore the balance of the two processes. The results of Colour Electron Microscopy (CEM), scanning electron microscope (SEM), thermo gravimetric analysis (TGA) and Fourier Transform Infrared (FTIR) spectroscopy showed that swelling expended the internal structure with solvent permeation. While 2.5 mol/L [HMIM][HSO_4] (10 ml) was employed, the equilibrium absorbency of TCS (60–80 mesh) could reach 1.85 at 80 °C. The IL was reused for 5 times, both the equilibrium absorbency and hydrolysis degree of TCS were approximately consistent. The hydrolysis degree would decrease sharply with the increase of equilibrium absorbency. In conclusion, the relationship between swelling and hydrolysis was competitive inhibition. This finding could be applied in related process of TCS and would lay the foundation of the further study in leaching balance.

1. Introduction

The skeletons of nature products rich in organic matters are ubiquitous of pores on the surface [1]. Solvent partially penetrates into the skeleton by swelling when they are mixed. Swelling is a normal phenomenon occurring between the solid state of nature products and the liquid state of solvent. At first, the solvent molecules slowly diffuse into the polymer to produce a swollen gel. If the polymer–polymer intermolecular force is great, the cross linking, crystallinity, or strong hydrogen bonding, this is all what happens. But, if related forces are overcome by the introduction of strong polymer–solvent interaction, the dissolution of the polymer can take place as the second stage [2]. Macro and micro morphological were both changed under the participation of solvent, which would affect the physical and chemical properties and transfer behaviour [3,4].

As a green solvent, ionic liquid (IL) has been widely applied in various fields of chemistry [5]. Compared with water and traditional organic solvents, they have low vapour pressure, high solvent capacity, chemical stability and tenability [6]. ILs have been applied in many aspects, such as extraction [7,8], hydrolysis [9,10], and separation [11,12] in nature products. ILs not only can permeate into the plant cell wall and dissolve the active ingredients, but also can destroy the structure of polymers and make it more efficiently hydrolyzed into smaller molecules [13].

Trichosanthin (TCS) is a single-chain ribosome inactivating protein (RIP) isolated from the root tubers of the Trichosanthes kirilowii Maxim, which has been used as a drug for termination of pregnancy [14]. The aim of this study was to explore the relationship between swelling and hydrolysis of TCS in aqueous ionic liquids. The swelling of TCS particles in aqueous ionic liquids (ILs) was observed to discover the mass transfer mechanisms in natural products-solvent system. The hydrolysis behaviour of TCS accompanied with swelling was studied to explore the balance of the two processes. Various methods have been applied to indicate the phenomenon of swelling and hydrolysis, such as SEM, TG and FTIR Spectrometer. Four types of iminazole acidic ILs were chosen in this study, including [PsMIM][H_2PO_4] [15], [HMIM][HSO_4] [16], [BMIM][H_2PO_4] [17] and [HMIM][Cl] [18]. The recovery and reuse of [HMIM][HSO_4] have also been considered.

2. Experimental

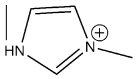
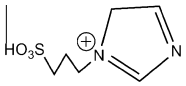
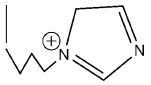
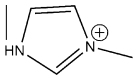
2.1. Materials

All ionic liquids used in this study were synthesised refer to the previous achievements [15–18], and their purity is all above 98% determined by high performance liquid chromatography. The structures and pH values were presented in Table 1. Radices trichosanthis

* Corresponding author. Tel./fax: +86 028 85405221.
E-mail address: chenyangruoxue@yeah.net (H. Song).

http://dx.doi.org/10.1016/j.seppur.2015.01.033
1383-5866/

Table 1
Structures and pH values of the ionic liquids.

No.	Ionic liquid	Cation	Anion	pH value[a]
A	[HMIM][HSO_4]		HSO_4^-	2.07
B	[PSMIM][H_2PO_4]		$H2PO_4^-$	2.14
C	[BMIM][H_2PO_4]		$H_2PO_4^-$	3.12
D	[HMIM][Cl]		Cl^-	5.54

[a] pH values were measured in the 5 mmol/100 g aqueous solution at 30 °C by PHS-25 (Leici, Shang Hai, China).

were purchased from local drug store of Chengdu, Sichuan province, China, which were identified by Xinying Li (researcher from Southwest Nationalities University). Then they were smashed into a series of particles with different internal diameters (20–100 mesh). After drying for 24 h, the samples were stored at low temperature away from light.

2.2. Swelling of TCS

TCS particles were placed into 3 ml EP tube with pinholes (200 mesh gauze in lining), and then embedded into 5 ml EP tube filled with IL solutions. After being sealed for 10 h in 50 °C, 3 ml EP tube was taken out for centrifugal operation and equilibrium absorbency was determined, while the solutions in 5 ml EP tube were analysed by UV spectrophotometer (Puxi, Beijing, China) after dilution. The flow sheet of the process was shown in Fig. 1.

2.3. Hydrolysis of TCS

The degree of hydrolysis (DH) was determined by photometric ninhydrin method [19,20]. It can be calculated according to the following equation:

$$\mathrm{DH} = \frac{M_a - M_b}{M} \times 100\% \quad (1)$$

where M_a (mg) is the mass of –azyl after hydrolysis; M_b is the mass of –azyl before hydrolysis; M is the mass of TCS.

2.4. Analytical methods

2.4.1. Colour Electron Microscopy (CEM)

The cells of TCS were captured with BX53F CEM (OLYMPUS, Tokyo, Japan). Ninhydrin was added as a chromogenic agent to illustrate the change of TCS after swelling.

2.4.2. Scanning Electron Microscopy (SEM)

The micro morphological of TCS was captured with JSM-7500F SEM (JEOL, Tokyo, Japan). In this case, the samples were coated with gold using the sputtering technique.

2.4.3. Thermo gravimetric analysis (TGA)

The thermo stability of TCS samples was characterized by 209-F3 TGA (Tarsus, Netzsch, Germany). Approximately 8 mg of each sample was heated from 30 °C to 800 °C at a heating rate of 10 °C/min. All of the measurements were performed under a nitrogen atmosphere with a gas flow of 20 ml/min in order to prevent any thermoxidative degradation.

2.4.4. Fourier Transform Infrared (FTIR) spectroscopy

The spectra of the TCS samples were recorded on L1600300 FT-IR spectrometer (PerkinElmer, Waltham, USA) in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1}. The samples were ground into powder and blended with KBr followed by pressing the mixture into ultra-thin pellets.

2.4.5. Gravimetric analysis (GA)

The gravimetric analysis of TCS particles after swelling was measured by PRACTUM224-1CN electronic balance (Sartorius, Beijing, China). The samples were treated by centrifugal operation to eliminate the influence of free water.

2.4.6. Ultraviolet spectroscopy (UV)

The degree of hydrolysis was determined by TU-1810 UV spectrum (Puxi, Beijing, China). The solutions after swelling were diluted into appropriate concentration for UV analysis. Glycine was treated as standard amino acid, and the standard curve was obtained as following equation (R^2 = 0.9999):

$$Y = 0.0274X + 0.045 \quad (2)$$

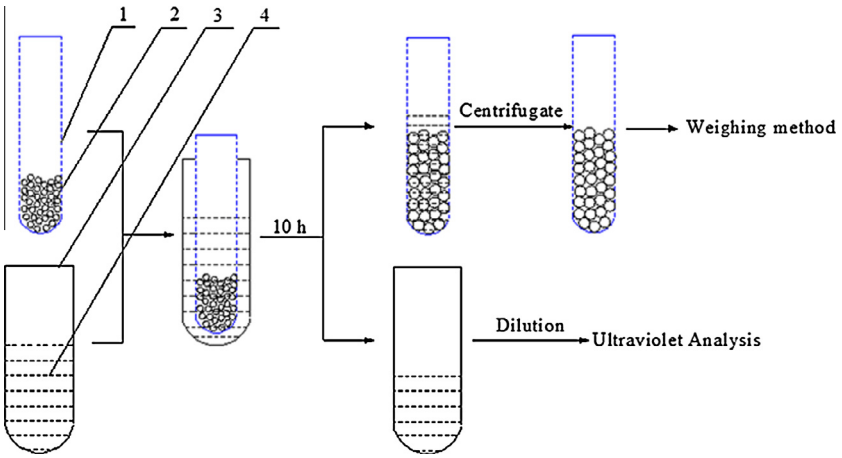


Fig. 1. The swelling process of Trichosanthin particles in ionic liquid solutions 1. EP tube (3 ml) with pinhole; 2. Trichosanthin particles; 3. EP tube (5 ml); 4. Ionic liquid solutions.