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In vitro release of L-phenylalanine from ordered mesoporous materials

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ABSTRACT

The applicability of different types of mesoporous materials as pharmaceutical carriers for L-phenylalanine was evaluated. Silicas, such as SBA-15, SBA-16 and KIT-6 were synthesised by hydrothermal method with tetraethyl orthosilicate as the silica source and triblock copolymer P123 as a template. XRD and TEM studies confirmed an ordered hexagonal structure of SBA-15 and a cubic structure of SBA-16 and KIT-6. The materials are characterised by well-developed specific surface areas and large pore volumes. Adsorption of L-phenylalanine over various mesoporous silicas was studied from solution of different pH (5.6–9.4). The greatest sorption capacity was observed at pH 5.6, which is close to the isoelectric point of L-phenylalanine (pI = 5.48). The amount of L-phenylalanine adsorbed on the mesoporous materials decreases at pH 5.6 in the following sequence: KIT-6 > SBA-15 > SBA-16 that was strongly related to the average pore diameter of the samples. The structural and textural features of the silicas seem to be responsible for the different L-phenylalanine release rate. Additionally, it was found that L-Phe release rate exhibited the pH sensitivity. These phenomena allow control of the experiment according to the needs.

1. Introduction

Mesoporous silicas have attracted the attention of many scientists all over the world as delivery vehicles of biologically active substances [1,2]. Their applications as carriers for various molecules has been under intensive *in vitro* and recently also *in vivo* research. Mesoporous materials are interesting to be applied in delivery systems because of their outstanding features such as high surface area, great pore volumes, well–ordered, tunable pores and nontoxicity [3–10]. It has been proved that these materials are capable of carrying high dosages of various drugs in mesopores [11–13]. Several studies have proved that different biologically active molecules can be loaded and successfully released from mesoporous materials [14–17]. It has been suggested that the pore diameter of the carrier should be adapted according to the released molecule size [18]. Therefore, in this study we try to access the applicability of various mesoporous materials for loading and release of L-phenylalanine. L-phenylalanine is the form of amino acid that occurs naturally in proteins in human body [19]. It plays essential role as a precursor in the biosynthesis of L-tyrosine and is crucial in biochemical processes regarding the synthesis of several neurotransmitters (such as L-dopa, dopamine, epinephrine, thyroxine and melanin). Furthermore, it can be converted through several pathways to phenylethylamine that is suggested to elevate mood and have an influence on the synthesis of brain neuropeptides [19]. Phenylalanine has potential antidepressant and analgesic effects and it seems to be useful in the vitiligo treatment. On the other hand, it is thought that L-phenylalanine can exacerbate symptoms of phenylketonuria [20] and dyskinesia in some schizophrenic patients. Clinical trials have resulted in mixed results and more research is required. In some cases L-phenylalanine is essential for human body, therefore we have developed a carrier for this active compound that enables its slow-rate delivery.

To the best of our knowledge, there has been no article regarding the potential use of ordered mesoporous silicas for amino acids release. Furthermore, we have chosen L-phenylalanine as a model for protein release because it has both aromatic hydrophobic region as well as hydrophilic functional groups (Fig. 1). The main aim of this study was to analyse the effect of ordered mesoporous materials such as SBA-15, SBA-16 and KIT-6 on the loading and release of L-phenylalanine.

2. Experimental procedure

2.1. Sample preparation

A highly ordered SBA-15 sample was synthesized using the triblock copolymer, $EO_{20}PO_{70}EO_{20}$ (Pluronic P123, BASF) as template and tetraethyl orthosilicate (TEOS, Aldrich) as the silica source, following the synthesis procedure reported by Zhao et al. [21]. The starting composition was 0.0017 mol of P123: 0.10 mol TEOS: 0.60 mol HCl: 20 mol H_2O. In a typical synthesis, 1.1 g TEOS was added dropwise to 19.0 ml of 1.6 M HCl containing 0.5 g of P123

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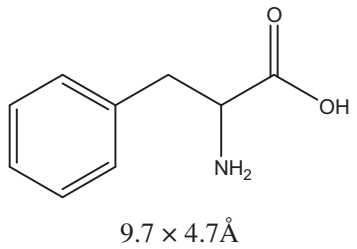

Fig. 1. Chemical structure of phenylalanine with its cylindrical size approximations.

at 35 °C. The mixture was stirred with a magnetic stirrer until TEOS was completely dissolved. Then, the mixture was placed in an oven for 24 h at 35 °C and subsequently for 6 h at 100 °C. The white solid product was filtered without washing and dried at 100 °C for 24 h in air oven. Finally, the product was calcined at 550 °C in air to remove the template.

SBA-16 mesoporous silica was prepared exactly as reported recently by Ryoo et al. [22] using triblock copolymer in a ternary copolymer-butanol-water system and low-acid concentration. The aqueous mixture of Pluronic F127 copolymer ($EO_{106}PO_{70}EO_{106}$, Aldrich) with 1-butanol (Aldrich, 99%) was applied to create a mesotructure to achieve an ordered self-assembly of the silica source tetraethyl orthosilicate (TEOS, Aldrich). Typically, 3 g of the copolymer F127 was dissolved in a solution of 144 g water and 5.94 g of hydrochloric acid (Chempur, 35%). After 30 min 9 g of the co-surfactant 1-butanol was added. After 1 h stirring 14.2 g TEOS was added to the solution. At a constant temperature of 45 °C the mixture was further stirred for 24 h. The mixture was then placed in an oven for 24 h at 100 °C. The molar gel composition of the synthesis mixture was 0.00024 F127: 0.12 $C_4H_{10}O$: 0.068 TEOS: 0.057 HCl: 8 H_2O. The white solid product was filtered without washing and dried at 100 °C for 24 h in air oven. At last, the product was calcined at 550 °C in air to remove the template.

KIT-6 sample was synthesized as follows: 4.0 g of Pluronic P123 (BASF) was dissolved in 144 g of distilled water and 7.9 g of hydrochloric acid (Chempur, 35%) solution upon stirring at 35 °C [23]. After complete dissolution, 4.0 g of 1-butanol was added immediately. After 1 h stirring, 8.6 g of TEOS was added to the homogeneous clear solution. The mixture was kept under vigorous and continuous stirring at 35 °C for 24 h. Subsequently, the reaction mixture was aged at 100 °C for 24 h under static condition. The molar gel composition of the synthesis mixture was 0.0007 P123: 0.054 $C_4H_{10}O$: 0.041 TEOS: 0.076 HCl: 8 H_2O. The product was filtered without washing and dried at 100 °C for 24 h in air oven. Finally, the sample was calcined at 550 °C in air to remove the template.

2.2. Powder X-ray diffraction (XRD)

The materials obtained were characterised by X-ray diffraction (XRD) using a D8 Advance diffractometer (Bruker) (Cu Kα radiation, $\lambda = 0.154$ nm), with a step size 0.02° in the low-angle range.

2.3. Nitrogen sorption

Characterization of the pore structure of samples obtained was performed on the basis of low-temperature nitrogen adsorption–desorption isotherms measured on a sorptometer Quantachrome Autosorb iQ. Prior to adsorption measurements, the samples were degassed in vacuum at 300 °C for 2 h. Surface area and pore size distribution were calculated by BET and BJH methods, respectively. Total pore volume and average pore diameter were determined as well.

2.4. Transmission electron microscopy (TEM)

For TEM measurements, powdered samples were deposited on a grid with a perforated carbon film and transferred to a JEOL 2000 electron microscope operating at 80 kV.

2.5. L-phenylalanine adsorption

L-phenylalanine solution of concentration 30 mmol/l was prepared by dissolving amino acid in potassium phosphate buffer solutions (pH 5.6–9.4). In each adsorption experiment, 0.2 g of a mesoporous silica (SBA-15, SBA-16, KIT-6) was suspended in 50 ml of L-phenylalanine solution. The resulting mixture was continuously stirred in a closed batch at room temperature (48 h). The amount of amino acid adsorbed was calculated by subtracting the amount found in the supernatant liquid after adsorption from the amount of amino acid present before addition of the adsorbent by UV absorption at the λ_{max} of L-phenylalanine, 257 nm.

2.6. L-phenylalanine release experiment

The appropriate mesoporous material containing L-phenylalanine was placed in the Enhancer cell. In order to maintain appropriate experiment conditions and steady surface area, the mesoporous materials with adsorbed amino acid were sandwiched between two porous synthetic nets.

In vitro release studies were performed with the use of an USP Apparatus 2 (Varian Vankel 7010). Potassium phosphate buffer at pH 5.6, pH 7.2 and pH 9.4 was used as the receiving medium. The medium (200 ml) was maintained at 32.0 ± 0.5 °C and stirred at 100 rpm. The samples were filtered through 35 μm HDPE Full-Flow filters and the concentration of the dissolved L-phenylalanine was monitored by UV–Vis spectrophotometer at 257 nm. The absorbance of the sample aliquots was used to assess the amount of compound release at each time point. In addition, the reference standard solutions of L-phenylalanine was prepared in the appropriate receptor fluid in order to generate the standard curve of absorbance versus concentration.

2.7. Kinetics calculations

In order to obtain the release rate of L-phenylalanine from mesoporous materials the amount of the substance which was released per time unit was taken into account. The release of amino acid in

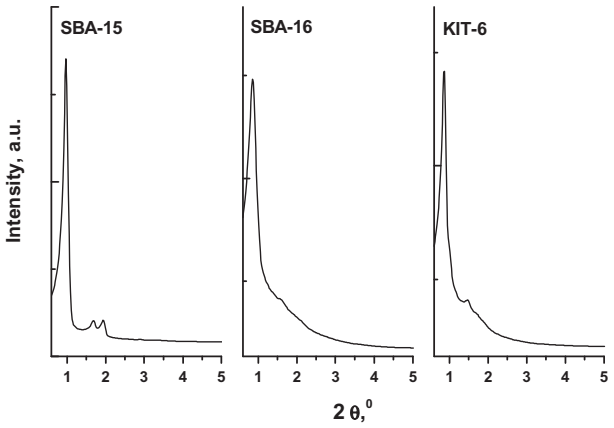

Fig. 2. Low-angle X-ray diffraction patterns of ordered mesoporous silica.