Colloids and Surfaces A: Physicochem. Eng. Aspects 362 (2010) 135–139

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Preparation of azithromycin microcapsules by a layer-by-layer self-assembly approach and release behaviors of azithromycin

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ARTICLE INFO

Article history:
Received 13 January 2010
Received in revised form 17 March 2010
Accepted 5 April 2010
Available online 13 April 2010

Keywords:
Azithromycin
Microcapsule
Layer-by-layer self-assembly
Polyelectrolyte
Dissolution

ABSTRACT

A novel preparation method of azithromycin (AZI) microcapsules based on hollow polyelectrolyte (PE) microcapsules, which were prepared by layer-by-layer self-assembly onto the surface of silica microsphere (SiO_2) followed by core dissolution has been investigated. The prepared AZI/PE microcapsules with an average diameter 1.2 μm possess homogeneous size and regular spherical shape. FTIR spectra and XRD patterns indicated that AZI molecular structure was not changed and AZI crystal state changed from monohydrate to dihydrate. The drug release experimental results showed an obvious improvement in the dissolution rate of the prepared AZI/PE microcapsules in comparing with AZI raw material drug powder.

1. Introduction

Azithromycin (AZI) is a semi-synthetic acid-stable macrolide antibiotic with a 15-membered azalactone ring. It shows a broad spectrum of bacteriostatic activity, and is proved to be clinically effective against not only Gram-positive but also Gram-negative bacteria and atypical pathogens, which make up the deficiency of erythromycin, the first macrolide used clinically as an antibiotic [1]. Although AZI is derived from erythromycin, it differs by the insertion of a methylsubstituted nitrogen on the lactone ring at position 9-a of the large macrolactone ring. This modification produces an enhanced spectrum and potency against bacteria, and a superior stability in acidic environment [2]. Besides, AZI is available in immediate oral or intravenous release, and has a longer half-life period, fewer side-effects, and higher concentration in tissue than erythromycin, and can even be applied to children or pregnant women [3]. So AZI and other newer macrolides, such as larithromycin, dirithromycin and roxithromycin, are regarded as an "advanced-generation" for erythromycin [4]. As for bacteriostatic mechanism, like erythromycin, it appears to bind to the same receptor, 50 S ribosomal subunits of susceptible bacteria and suppresses protein synthesis [1]. AZI plays a leading role in the treatment or prophylaxis of common respiratory tract infections, skin structure infection and several other clinical diseases, such as opportunistic infections in AIDS, toxoplasmosis, pediatric infections, urethritis, cervicits, among others [5,6].

However, the clinical application of AZI is limited by its low bioavailability as a result of its poor solubility in water and poor gastrointestinal response, as diarrhea for instance [5]. According to Noyes–Whitney equation [7], the dissolution rate of a given drug particles is proportional to the particles' specific surface area. Therefore, one promising way to improve the solubility and dissolution behavior of AZI is to reduce the particle size, thus leading to an increased specific surface area and an augmented dissolution rate [5]. Particles size reduction methods include mechanical comminution, reprecipitation, high-pressure homogenization, ultrasonic emulsification solvent diffusion method, spontaneous emulsification solvent diffusion method, etc. [8]. However, each of these methods has its own drawback. In the process of mechanical comminution, impurities are brought in inevitably, and the distribution of drug particles size is difficult to control. As for high-pressure homogenization, ultrasonic or spontaneous emulsification solvent diffusion method, although the AZI particles size can be reduced to μm or nm level, the resulting AZI particles are easily polluted owning to the introduction of a surfactant or an emulsifier. In addition, these methods do not deal much with the microstructure of AZI particles and require a great deal of energy.

The present study introduces a novel method for the preparation of AZI/PE microcapsules. This method is based on hollow PE microcapsules and difference of AZI solubility in water and in alcohol solution. Pharmaceutical microspheres and microcapsules are highly favored in drug delivery and controlled release systems for

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doi:10.1016/j.colsurfa.2010.04.006

their convenience for subsequent processing and the potential of incorporating with other materials (i.e., vaccines, drugs, or inorganics) in the core or on the surface [9]. Hollow PE microcapsules are prepared by sequential adsorption of oppositely charged polyelectrolyte, also known as layer-by-layer self-assembly, onto the surface of a template core of 0.1–10 μm in diameter followed by the removal of the core [10]. The size of the template core is determinant for the size of the AZI/PE microcapsules, and some other properties of AZI/PE microcapsules can be controlled according to the need by choosing proper template core of different type or diameter, such as SiO_2, melamine formaldehyde, polystyrene latex, erythrocytes, and $MnCO_3$ particles. Furthermore, owning to the repulsive interaction of the external polyelectrolyte with the same charge, the AZI/PE microcapsules will disperse easily in the solution, resulting in a higher dissolution rate to same extent [11]. Then hollow PE microcapsules with AZI alcohol solution is slowly added into water under mixing conditions provided by a magnetic stirrer. As a result of the difference of AZI solubility in water and alcohol solution, called good-poor solvent strategies, AZI is precipitated on the base of the hollow PE microcapsules [9], thus AZI/PE microcapsules can be produced.

Basket method is adopted to compare the dissolution of commercial AZI raw material drug and AZI/PE microcapsules [12]. To better represent the adsorption of AZI by blood or tissue, the screen used to restrict the drug particle is substituted by a semi-permeable membrane (the molecular weight cut-off of the membrane ranges from 12,000 to 14,000), which simulate the partial pervasion properties of a cell membrane. Phosphate buffer solution (pH = 6.0) and hydrochloric acid solution (pH = 2.0), which is analogous to the pH of gastric acid, is used as the dissolution medium. The results of dissolution show that the prepared AZI/PE microcapsules have an apparent improvement in the dissolution velocity compared to AZI raw material drug powder.

2. Experimental

2.1. Materials

AZI was purchased from Beijing Taiyang Pharmaceutical Co., Ltd. (Beijing, PR China). Silica microspheres (SiO_2) used here had an average diameter of 1.2 μm, and were synthesized by Template Mechanism as described elsewhere [13]. Poly (allylamine hydrochloride) (PAH, Mw = 70,000) and 4-poly (styrene sulfonate sodium) (PSS, Mw = 70,000) were purchased from Sigma–Aldrich. All other reagents used for buffer and standard solution preparation were purchased from various commercial sources and were of analytical grade. The water used in all the experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system.

2.2. The preparation of hollow PE microcapsules and AZI/PE microcapsules

Polyelectrolyte microcapsules were obtained by alternate adsorption of three bilayers of PAH/PSS onto the surface of silica microspheres via layer-by-layer self-assembly technique. Typical adsorption conditions were 1 mg mL^{-1} PAH in 0.5 mg mL^{-1} NaCl, and 2 mg mL^{-1} PSS in 0.5 mg mL^{-1} NaCl. The adsorption time was 15 min. After each adsorption step, the unadsorbed PE is removed by repeated centrifugation and washing. The PAH/PSS multilayer film is formed by the alternate adsorption of oppositely charged polyions, beginning with the deposition of positively charged PAH onto the negatively charged silica particles. The 3–5 bilayers PAH/PSS were then deposited on the silica microspheres. The silica microspheres template cores were removed by exposure to a hydrofluoric acid (HF)/ammonium fluoride (NH_4F) buffer (pH = 5) for 5 min. Following several washing cycles, the decomposition products of the silica sphere were discarded, and then hollow PE microcapsules were obtained [13].

The appropriate quantity of hollow PE microcapsules with certain concentration AZI alcohol solution were washed by alcohol once to wash the AZI outside the PE microcapsules, then were slowly added into appropriate volume of water under magnetic stirring (1000 rpm). The mixed solution transformed from clear to opalescent within a few seconds. AZI/PE microcapsules were obtained after washing by water [9].

2.3. Dissolution test

The AZI concentration was measured by colorimetry after adding the AZI solution to sulfuric acid solution (85 → 100) for coloration.

Plot the AZI solution standard curve. First, a series of AZI solutions at different concentrations, 40, 80, 120, 160, 200, 240, 300 and 360 μg mL^{-1}, was prepared, then in accordance with the Chinese Pharmacopoeia, a precise volume of 5 mL of the abovementioned AZI solutions was mixed uniformly with exact volume of 5 mL sulfuric acid solution (85 → 100). The mixture was cooled at room temperature for 30 min, and then was scanned within the ultraviolet to visible light spectrum by using an ultraviolet-visible spectrophotometer. The maximum absorbance wavelength of 482 nm and the corresponding average absorbance were adopted for linear regression to draw the AZI solution standard curve [14]. Based on the relationship between the AZI standard solution concentration and the corresponding absorbance at 482 nm, a linear equation for AZI standard curve was obtained: $A = 0.00621C + 0.061$ (C, μg mL^{-1}), $n = 8$. The correlation coefficient calculated from the linear equation was Adj. R-Square = 0.99792, which showed a good linearity.

Dissolution test for AZI raw material drug powder and AZI/PE microcapsules. The modification of basket method, which meant screen was replaced by semi-permeable membrane, was introduced to test the dissolution of AZI raw material drug powder and AZI/PE microcapsules. According to the Chinese Pharmacopoeia, phosphate buffer (0.1 mol L^{-1} disodium hydrogen phosphate with appropriate volume HCl, pH = 6.0 (±0.05)) is as dissolution medium under magnetic stirring (100 rpm) at room temperature [14]. After the addition of semi-permeable membrane carrying certain AZI into the dissolution medium, a series of 5 mL dissolution medium volumes were collected to measure the AZI concentrations in the medium at proper intervals by using the ultraviolet-visible spectrophotometer method and the AZI solution standard curve to determine the AZI dissolution rate. After each sampling, 5 mL fresh dissolution medium was added to keep the dissolution volume unchanged. The relation curve of AZI concentration released in dissolution medium and release time was obtained through the AZI standard curve.

The release of AZI in gastric acid was also simulated by substituting the phosphate buffer by HCL solution (pH = 2). A similar method to the one discussed above was used to test the AZI dissolution.

2.4. Characterization

The surface morphology of AZI was studied by using JSM-6360LV scanning electron microscopy (SEM, JEOL, Japan). All the transmission electron microscope (TEM) images were obtained using a model JEM-100CX (JEOL, Japan) system operated at 120 kV. AZI raw material drug size was analyzed by LS230 laser particle size analyzer (Beckman Coulter, USA). The group and structure was studied by IFS28 Fourier transform infrared spectroscopy (FTIR, Bruker, Germany). The X-ray powder diffraction (XRD) data were recorded on a Rigaku D/max 2550 VB/PC diffraction using nickel-