Microporous and Mesoporous Materials 226 (2016) 71–78

Contents lists available at ScienceDirect

Microporous and Mesoporous Materials

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

Development of antimicrobial hybrid mesoporous silver phosphate–pectin microspheres for control release of levofloxacin

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form
10 December 2015
Accepted 22 December 2015
Available online 30 December 2015

Keywords:
Antimicrobial microspheres
Silver phosphate
Hybrid microspheres
Pectin
Levofloxacin

ABSTRACT

Pectin was used as nucleation agent in the synthesis of spherical mesoporous Ag_3PO_4 microspheres for the development of dual antimicrobial carrier containing a fluoroquinolone. The hybrid Ag_3PO_4–pectin microspheres were characterized by biophysical methods using optical, scanning and transmission electronic microscopies, X-ray diffraction and energy dispersive analysis, differential scanning calorimetry, laser diffraction, and molecular gas adsorption (Brunauer–Emmett–Teller). Scanning electron microscopy (SEM) images of silver phosphate microspheres without pectin displayed heterogeneous surface, bimodal size distribution between 0.9–1.0 μm and 1.5–1.8 μm with 20% and 25% population yield respectively and high amount of salt detritus. Meanwhile, SEM microphotographies of silver phosphate microspheres synthesized in presence of pectin showed a drastic change of particle morphology, homogenous surface, narrow size particle distribution in the 1.3–1.5 μm range with 90% population yield, 20–30% pore size increase and without debris. X-ray diffraction analysis of silver phosphate microspheres showed the same crystal profile in presence or absence of pectin suggesting no changes in the crystalline structure of Ag_3PO_4 by the addition of the biopolymer was made. Effect of silver phosphate hybrid microspheres loaded with levofloxacin tested against *Escherichia coli* and *Staphylococcus aureus* showed strong bactericidal activity compared with the bacteriostatic effect of free levofloxacin. The results are suggesting that hybrid Ag_3PO_4–pectin microspheres containing levofloxacin can be used as effective antimicrobial against several microorganisms, making them applicable to diverse medical devices and for antimicrobial control systems. The Ag_3PO_4–pectin hybrid microspheres are advantageous since they are synthesized by green chemistry methodology under standard laboratory conditions, do not requiring purification steps, the technique is highly reproducible and they are available for drug loading and specific target tailoring. The main advantage of Ag_3PO_4–pectin microspheres is the synergic antimicrobial activity of the silver ion and the antibiotic in the same microdevice acting as biocide matrix and also as carrier for levofloxacin.

1. Introduction

The emergency of multi-drug resistance (MDR) is a huge concern for human health at global scale [1]. For example, 450,000 new cases of MDR were reported in 92 countries only for tuberculosis in 2012. In particular, nosocomial microbial infections are serious obstacles everywhere for treatment of many microbial diseases [2]. Additionally, for many reasons the discovery, development and production of novel antibiotics were delayed over the last 30 years [3]. In order to solve the sanitary emergency, two main strategies for antimicrobials administration were established: the first one involves the development of novel and more effective drug carriers; and the second is the selection of two drug molecules with different microbiocide activity loaded together in the formulation [4].

Particularly, the interest of silver as antibacterial, used since ancient times, was recently rediscovered by the academy and extensively studied in last ten years [2,5]. The main advantages of

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http://dx.doi.org/10.1016/j.micromeso.2015.12.041
1387-1811/

silver devices are based on their low toxicity in humans, broad antimicrobial spectrum and low probability to produce bacterial resistance compared to traditional antibiotics. In fact, silver alone or combined with other molecules are currently used for controlling bacterial growth in a variety of applications, including dental implants, skin creams and patches, catheters, and clothes [6–8].

The strong silver biocide activity was attributed to several antimicrobial mechanisms such as damage of cell membrane proteins, blocking RNA transcription, disruption of DNA binding and replication [9]. Silver phosphate crystals with different morphologies synthesized in presence of detergent (Triton X-100) or polymers (polyacrylates or polyglycol 2000) were reported as bacteriostatic on *Escherichia coli* DH5α [10]. The antimicrobial mechanisms of silver phosphate crystals postulated by the authors were the membrane damage, ROS formation in the cytoplasm by the Ag^{1+} and interference on ATP synthesis by the silver salt [10]. Similarly, crushed code fish bones immersed into silver nitrate solution and dried showed high antimicrobial activity against *E. coli* and *Staphylococcus aureus* [11]. In another work, antimicrobial activity of hydroxyapatite containing silver was strongly correlated with the Ag^{1+} content. However, hydroxyapatite-silver particles higher than 45 nm or with high silver payload showed structural inhomogeneity and low antimicrobial activity against *S. aureus* and *Pseudomonas aeruginosa* [12]. Interestingly, no toxicity was found using calcium phosphate surfaces sprayed with silver nitrate on Chinese hamster V79 cell line [13]. Diverse silver phosphates morphologies and features such as amorphous to crystals structures showing from irregular spheroids to arrow-head morphologies in presence of ammonia (*i.e.* diamine–silver complex formation) were observed by SEM. In addition, polyhedral and cubic silver phosphate crystals were synthesized by modifying the reaction components and physicochemical parameters such as solvent polarity, the ratio of solvation (*e.g.* ethyleneglycol–water mixtures), temperature, etc [14–16]. Besides, SEM images of the reported silver phosphate salts showed medium to high polydispersity, and/or wide polymorphism and/or the presence of parasitic structures because of complicated intrinsic control of physicochemical experimental conditions. The particle polymorphism and high polydispersity are both serious drawbacks for the development of drug delivery carriers since it is not possible to establish a proper drug loading and controlled release profile.

In recent years, mesoporous silica-based materials, *e.g.* MCM-41 or SBA-15, loaded with wide range of antimicrobials from silver nanoparticles, antibiotics to lytic enzymes were explored for the development of microbiocide devices against fastidious and antibiotic resistant microorganisms [17,18]. The main advantages of mesoporous materials are the ability of absorb and contain many molecules with different molecular weights and physicochemical properties keeping their biological activities by tailoring pore diameter and/or chemical structure. The tuning of mesoporous structures is determining the interactions within the loads and consequently the controlled release kinetics [19].

Devices based on silver salts and polymers could improve antimicrobial activity against pathogens but only few reports were found in the literature and the mechanisms are not fully understood [20,21]. Biopolymers such as alginate, cellulose, pectin among others were lately reported as potential “green carriers” for drug delivery [22]. Natural polymers are providing excellent platforms for molecular loading and release because of gelling properties, and also considered smart molecules since they are sensitive to environmental conditions (*e.g.* pH, temperature, ionic strength). Most of biopolymers are non-toxic, degradable, showing high structural diversity and some of them are used in food or food supplements (*e.g.* fibers). Also, biopolymers displayed wide variety of functional groups easy to tailor by Green Chemistry techniques. Particularly, pectins are water-soluble food-grade polysaccharides synthesized in plant cell walls. Pectins are linear poly-α-(1,4)-D-galacturonic acids chains partially methoxylated. Pectins are slowly degraded in humans by the intestinal flora, which can be considered advantageous for the development of intestinal drug delivery carriers [4]. In this sense, the use of biopolymers as nucleation agents for the development of hybrid inorganic matrices carrying biocides is a feasible alternative (*e.g.* encapsulation of ciprofloxacin in a matrix composed of $CaCO_3$ and carrageenans) [23]. Similar approach was reported for the synthesis of silver phosphate antimicrobial particles in presence of chitosan and hyaluronic acid, but the particles are displaying wide particle size distribution, and no biocide was incorporated into the device [24].

Fluoroquinolones are among the most used broad-spectrum antibiotics for Gram(+) and Gram(−) bacteria. The main mechanism of fluoroquinolones antimicrobial activity is based on the inhibition of topoisomerases (*e.g.* DNA gyrase and topoisomerase type IV) causing DNA breakage and consequently microbial cell death. Levofloxacin is a fluoroquinolone used to treat many bacterial infections (*e.g.* chronic bronchitis, sinusitis, conjunctivitis, and penicillin-resistant strains of *Streptococcus pneumonia*, etc.), urinary tract and abdominal infections, but also skin and soft tissue infections. However, some adverse effects of levofloxacin like gastrointestinal problems such as abdominal discomfort, anorexia and diarrhea were reported in clinical studies [25]. Additionally, fluoroquinolones are showing unwanted secondary side effects because of molecular stacking attributed to the aromatic ring structure and low solubility in aqueous media [26]. These main properties of fluoroquinolones are reducing the antibiotic biodisponibility and became toxic because of crystal formation inside the body [27].

The aim of the present work was to develop a simple and reproducible aqueous method for the synthesis of Ag_3PO_4 microspheres (AgP-Ms) in presence of pectin and loaded with levofloxacin as novel dual-antimicrobial device. The novel hybrid AgP-Ms were characterized by biophysical methods using optical, scanning and transmission electronic microscopies (OM, SEM and TEM), X-ray techniques like diffraction (XRD) and Energy Dispersive Analysis (EDAX), Differential Scanning Calorimetry (DSC), Laser Diffraction (LD), and molecular gas adsorption (Brunauer–Emmett–Teller, BET and Barrett–Joyner–Halenda, BHJ). Finally, the antimicrobial activity of AgP–pectin microspheres containing levofloxacin was tested against the Gram(+) *S. aureus* and the Gram(−) *E. coli* in agar plates, and also the microbial viability and minimal inhibitory concentrations (MIC) were determined in liquid medium.

2. Experimental

2.1. Materials

High Methoxylated Pectin (HMP, ED 74%, average Mη 1.60×10^5 Da) was kindly provided by C.P. Kelco (Buenos Aires, Argentina). All other reagents used were of analytical grade purchased from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany) or from local suppliers. Deionized water was prepared using reverse osmosis equipment Aqual 25 (Brno, Czech Republic) and further purified by using a MiliQ Direct QUV apparatus equipped with a UV lamp.

2.2. Silver phosphate microspheres synthesis

Silver phosphate microspheres (AgP-Ms) containing or not high methoxylated pectin (HMP) were made by precipitation. Briefly, 9.0 ml of 100 mM $AgNO_3$ solution was quickly added to 9.0 ml of