Carbohydrate Polymers 151 (2016) 441–451

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Development of a new antibacterial biomaterial by tetracycline immobilization on calcium-alginate beads

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ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 11 May 2016
Accepted 20 May 2016
Available online 24 May 2016

Keywords:
Calcium-alginate beads
Tetracycline
Immobilization
Antibacterial biomaterial
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

ABSTRACT

In recent years, increasing risk of infection, caused by resistant microorganism to antibiotics, has become the limelight discovery of new and natural antibacterial materials. Heavy metals, such as silver, copper, mercury and titanium, have antibacterial activity. Products, which improved these metals, do not have stable antibacterial property. Therefore, use of these products is restricted. The aim of this study was to immobilize tetracycline to alginate and improve an antibacterial biomaterial. For this purpose, calcium-alginate beads were formed by dropping to calcium-chloride solution and tetracycline was immobilized to beads using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide at optimum conditions. After immobilization, actualization of immobilization was investigated by analyzing ATR-FTIR spectrum and SEM images. Also, antibacterial property of obtained product was tested. Improved product demonstrated antibacterial property. It has potential for open wound, surgical drapes, bed and pillow sheath in hospitals and it may also be used for increasing human comfort in daily life.

1. Introduction

Modern life and working conditions provide a favorable environment for rapid growth of microorganism. The microorganism is located in the body, air, soil and all surfaces and they grow rapidly in case of providing mild conditions (De Faria et al., 2014). In recent years, increasing risk of infection, caused by resistant microorganism to antibiotics, has become the limelight discovery of new and natural antibacterial materials (Sohrabnezhad & Sadeghi, 2015). Requisition of comfort properties of advanced products that provided protection to internal and external factors that have high performance properties, increases day by day with advanced technology. Antibacterial products occupy an important place in terms of protection of human health, providing personal hygiene and comfort in functional properties improved products. Products, brought antibacterial properties, prevent the bacterial growth and provide hygiene as well as to prevent effluvia which generated by microorganisms (Yu, Wu, & Chen, 2015). Antibacterial materials commonly used in medical supplies, dental filling, food industry and areas which require elimination and preventing microbial growth and activity of microorganism (Sondi & Salopek-Sondi, 2004). Heavy metals, such as silver, zinc, copper, mercury and titanium, have antibacterial activity. Most of products, containing these metals, produced by encapsulation and impregnating of solution to the material. Therefore, these products lose their antibacterial properties via washing of material and use of these products is restricted. But, the developed product in this study has been demonstrated antibacterial property continuously because of performing of immobilization as covalently.

Alginate, which is commonly isolated from brown algae, is an anionic linear polysaccharide composed of two saccharides: epimeric β-D-mannuronate (M) and α-L-guluronate (G). The M and G monomers are covalently bonded through 1-4-glycosidic linkages and arranged into either homopolymeric blocks (MM and GG) or alternating blocks (MGMG) along the polymeric backbone (Goh, Heng, & Chan, 2012; Gong et al., 2011). In the presence of divalent cations, such as Ca^{2+}, alginate can form gels that can be used as carriers for drugs and bioactive food ingredients (Draget, Skjåk-Bræk, & Smidsrod, 1997; Reis, Neufeld, Vilela, Riberio, & Veiga, 2006). Alginate fibers are extensively used in wound care due to their potential bioactivity, non-toxicity, biocompatibility and relatively economical use, it has been widely used in biomedical applications such as wound dressing, scaffolds, and dental or surgical impression materials (Lian, Wu, Zhou, & Wong, 2011; Mikolajczyk, Boguń, Kurzak, & Szparaga, 2009). During the wound care process, the moist gel formed by ion exchange between Ca^{2+} and Na^{+} when alginate fibers interact with the wound exudates, thereby preventing fiber entrap-

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http://dx.doi.org/10.1016/j.carbpol.2016.05.073
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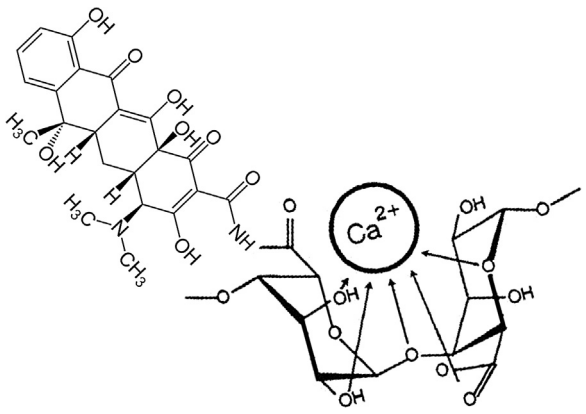

Fig. 1. Chemical structure of obtained product.

ment in the wound (Lihong et al., 2006; Murakami, Aoki, Nakamura, Takikawa, & Hanzawa, 2010).

The tetracycline is an antibiotic utilized for the treatment and prevention of infectious diseases (Sassman & Lee, 2005; Tanis, Hanna, & Emmanuel, 2008). It is a bactericidal agent that acts by inhibiting the bacterial protein synthesis and presents activity against a wide range of microorganisms (Ocampo-Pérez, Rivera-Utrilla, Gómez-Pacheco, Sánchez-Polo, & López-Peñalver, 2012; Parolo, Savini, Valles, Baschini, & Avena, 2008). It is a broad-spectrum antibiotic, which has a broad antibacterial spectrum against both Gram (+) and Gram (−) microorganisms, including the species spirochete, actinomyces and mycoplasma (Michalova, Novotna, & Schlegelova, 2004).

We purposed to develop a biomaterial, which have continuously antibacterial property, in this study. For this purpose, tetracycline was immobilized to calcium-alginate. For this, first, calcium-alginate beads were formed by dropping to calcium-chloride solution and tetracycline was immobilized to beads by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) at optimum conditions. After immobilization, actualization of immobilization was investigated by analyzing ATR-FTIR spectrum and SEM images. Also, antibacterial property of obtained product was tested (Fig. 1).

2. Materials and methods

2.1. Chemicals

EDC was purchased from Merck, methanol, tetracycline, sodium alginate, calcium chloride, potassium phosphate was purchased from Sigma Chemical Co. (USA), nutrient agar and nutrient broth were purchased from Fluka.

2.2. Microorganism

Staphylococcus aureus (*ATCC 6538*) and *Escherichia coli* (*ATCC 8739*) were used for antibacterial tests.

2.3. Equipment

Equipment used in this study was given in Table 1.

2.4. Wavelength spectrum of tetracycline

Wavelength scanning of tetracycline was done using UV–vis spectrophotometer (Perkin Elmer Lambda 35 UV/VIS Spectrophotometer) for observing immobilization of tetracycline. Tetracycline was prepared in methanol. 4:3 methanol-phosphate buffer (pH 8.0 10 mM) was used as a blank.

Table 1
Equipment used in this study.

Equipment	Brand and Model
Precision Scales	AND GR SERIES
Magnetic Stirrer	IKA
pH Meters	HANNA HI 2211-02
Incubator	NEW BRUNSWICK SCIENTIFIC
Spectrophotometer	PERKIN ELMER
Micropipette	EPPENDORF RESEARCH PLUS
Water Bath	MEMMERT
Vortex	FISONS
Sterile Cabinet	ESCO CLASS 2 BSC
Autoclave	HIRAYAMA

Table 2
Optimization conditions for the immobilization of tetracycline to calcium alginate.

Optimization parameters	Values
Amount of EDC	0.01, 0.10, 0.50, 1.00, 5.00, 10.00, 25.00, 50.00 and 75.00 mg
pH	pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 (All of buffers were phosphate buffer)
Buffer concentration	10, 25, 50, 100 and 250 mM
Number of beads	3, 5, 10, 15 and 25 beads
Temperature	4, 15, 25, 37, 45, 55 and 80 °C
Shaking speed	0, 50, 100, 150, 200 and 250 rpm
Amount of initial tetracycline	2.50, 5.00, 7.50, 10.00, 12.50 and 18.75 mg
Time	0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 h

2.5. Preparation of calcium alginate beads

A solution of 50 ml 2% of the sodium alginate (M_A = 216.12 g/mol) and 100 ml 3% of the calcium chloride were prepared separately in deionized water. For preparation of calcium alginate beads, 2% of the sodium alginate solution was added drop wise using blunt needle to calcium chloride solution at 4 °C. The water-soluble sodium alginate was converted to water-insoluble calcium alginate beads and these beads incubated at 4 °C for 2 h with stirring for stabilization. All beads were washed with 50 ml deionized water once and 50 ml pH 5.0 25 mM phosphate buffer thrice to remove the excess of unbounded calcium chloride from the bead surfaces. One bead's size and weight were 2 mm and 10.10 mg respectively. The washed beads were stored at 4 °C.

2.6. Tetracycline immobilization to calcium alginate beads

10 of calcium alginate beads (101.4 mg), 1 ml of tetracycline (10 mg, in methanol) and 2 ml of EDC (25 mg, in pH 5.0 25 mM phosphate buffer) were added to the reaction medium and incubated at 15 °C and 150 rpm for 8 h.

2.7. Optimization of tetracycline immobilization

The examined parameters in optimization studies were given in Table 2.

Standard graph of tetracycline was prepared at 410 nm. After incubation, beads were taken from reaction medium and washed with 2 ml of 2:1 methanol-buffer solvent system four times. Tetracycline was determined in the sample, taken from washing water, at 410 nm.