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Protein release from alginate matrices ☆

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ABSTRACT

There are a variety of both natural and synthetic polymeric systems that have been investigated for the controlled release of proteins. Many of the procedures employed to incorporate proteins into a polymeric matrix can be harsh and often cause denaturation of the active agent. Alginate, a naturally occurring biopolymer extracted from brown algae (kelp), has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of biological agents. Alginate polymers are a family of linear unbranched polysaccharides which contain varying amounts of 1,4′-linked β-D-mannuronic acid and α-L-guluronic acid residues. The residues may vary widely in composition and sequence and are arranged in a pattern of blocks along the chain. Alginate can be ionically crosslinked by the addition of divalent cations in aqueous solution. The relatively mild gelation process has enabled not only proteins, but cells and DNA to be incorporated into alginate matrices with retention of full biological activity. Furthermore, by selection of the type of alginate and coating agent, the pore size, degradation rate, and ultimately release kinetics can be controlled. Gels of different morphologies can be prepared including large block matrices, large beads (>1 mm in diameter) and microbeads (<0.2 mm in diameter). In situ gelling systems have also been made by the application of alginate to the cornea, or on the surfaces of wounds. Alginate is a bioadhesive polymer which can be advantageous for the site specific delivery to mucosal tissues. All of these properties, in addition to the nonimmunogenicity of alginate, have led to an increased use of this polymer as a protein delivery system. This review will discuss the chemistry of alginate, its gelation mechanisms, and the physical properties of alginate gels. Emphasis will be placed on applications in which biomolecules have been incorporated into and released from alginate systems.

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1. Introduction

Alginate is a naturally occurring biopolymer that is finding increasing applications in the biotechnology industry. Alginate has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer. Alginate also has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of proteins and cells. These properties include: (i) a relatively inert aqueous environment within the matrix; (ii) a mild room temperature encapsulation process free of organic solvents; (iii) a high gel porosity which allows for high diffusion rates of macromolecules; (iv) the ability to control this porosity with simple coating procedures and (v) dissolution and biodegradation of the system under normal physiological conditions. This review will first describe the preparation, chemical structure and characterization of alginate. The different methods of gel formation and physical properties of the gels will then be discussed. Finally, specific examples of alginate systems and their application to protein delivery, nucleic acid delivery and cell encapsulation will be given.

2. Alginate chemistry

2.1. Sources of alginate

Commercial alginates are extracted primarily from three species of brown algae (kelp). These include *Laminaria hyperborea, Ascophyllum nodosum*, and *Macrocystis pyrifera*. Other sources include *Laminaria japonica, Eclonia maxima, Lesonia negrescens* and *Sargassum* species [1]. In all of these algae, alginate is the primary polysaccharide present and it may comprise up to 40% of the dry weight [2]. Alginate is found in the intracellular matrix where it exists as a mixed salt of various cations found in sea water such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Na^{+}. The native alginate is mainly present as an insoluble Ca^{2+} crosslinked gel [2]. Bacterial alginates have also been isolated form *Azotobacter vinelandii* and several *Pseudomonas* species [3].

2.2. Extraction and preparation

To commercially prepare alginates, the algae is mechanically harvested and dried before further processing except for *M. pyrifera* which is processed when wet. Alginate is then extracted from dried and milled algal material after treatment with dilute mineral acid to remove or degrade associated neutral homopolysaccharides such as laminarin and fucoidin. Concurrently, the alkaline earth cations are exchanged for H^{+}. The alginate is then converted from the insoluble protonated form to the soluble sodium salt by addition of sodium carbonate at a pH below 10. After extraction, the alginate can be further purified and then converted to either a salt or acid [2].

Since alginates are obtained from a natural source, a variety of impurities may potentially be present. These include heavy metals, endotoxin, proteins, other carbohydrates and polyphenols present in the kelp [4]. For applications in the food and beverage industry, low levels of these impurities do not pose a problem, but for pharmaceutical applications, particularly when alginate will be administered via the parenteral route, these impurities should be removed. Alginates of a pharmaceutical grade can now be obtained from several manufacturers including Kelco (Surrey, UK), ProNova Biopolymer (Drammen, Norway), Chemical MFG Corp. (Gardena, CA, USA) and Junsei (Tokyo, Japan).

2.3. Chemical structure

Alginates are a family of linear unbranched polysaccharides which contain varying amounts of 1,4′-linked β-D-mannuronic acid and α-L-guluronic acid residues (Fig. 1). The residues may vary widely in composition and sequence and are arranged in a pattern of blocks along the chain. These homopolymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interdispersed with regions of alternating structure (β-D-mannuronic acid–α-L-guluronic acid blocks) [5,6]. The composition and extent of the sequences and the molecular weight determine the physical properties of the alginates. The molecular variability is dependent on the organism and tissue from which the alginates are isolated. For example, alginates prepared from the stipes of old *L. hyperborea* kelp contain the highest content of α-L-guluronic acid residues while alginates from *A. nodosum* and *L. japonica* have a low content of α-L-guluronic acid blocks. Alginates do not have a regular repeating unit and the distribution of monomers along the polymer chain cannot be described by Bernoullian statistics.

Analytical characterization of alginates is more difficult than for other polysaccharides since acid hydrolysis can lead to destruction of the uronic acids. Circular dichroism spectroscopy has been used to match the linear spectra of the alginate to model samples of well