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Polysaccharide-based self-assembling nanohydrogels: An overview on 25-years research on pullulan

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ABSTRACT

The aim of this overview is to review the evolution of the studies carried out, during more than 25 years, on nanohydrogels obtained by self-assembling of pullulan (PUL) using several hydrophobization strategies. After the first publications, mainly devoted to the preparation and characterization of PUL nanogels, a remarkable number of studies demonstrated how wide can be the field of applications within the main topic of biopharmaceutics. Numerous hydrophilic and lipophilic drugs were entrapped in the nanogel networks, consequently PUL nanogels have been proposed as delivery systems for single drugs and for combination therapies which allowed improvements of pharmacological activities and patient compliance. Furthermore, the large amount of water content allowed loading also proteins which could maintain their native structure and properties. Stimuli-sensitive and stealth PUL nanogel formulations allowed improving the performances of antitumor drugs. These nanohydrogels have also been studied for imaging techniques and for vaccines to be administered by injection and by mucosal application. The studies on PUL nanogels are still in progress and the perspectives for future researches are also addressed.

1. Introduction

Natural polysaccharides and their derivatives are indeed among the most employed and versatile polymeric materials that are actually used, or have been proposed, for hydrogel formulations [1].

In this sense it must be pointed out that polysaccharide hydrogels, whether chemically or physically based, show a wide variety of biomedical applications, ranging from their performances as drug carriers to their ability in living cell encapsulation; from bone and cartilage repair to the preparation of friendly scaffolds for tissue engineering. Furthermore, remaining within quite close areas of interest, polysaccharides are often present also in cosmetic and personal care products [2], are used as food additives [3] as well as in agriculture as fertilizer and for herbicide delivery [4], and have even been proposed in formulations for paper and painting cleaning [5,6].

The peculiar and diversified properties of polysaccharides can be related to the different primary structures and molecular weights that these macromolecules may have, as well as to the various types and number of reactive groups that can be present on each single repeating unit. These properties allow numerous chemical modifications that can be appropriately tailored according to the specific use that is assigned to these macromolecules.

It is well known that several natural polysaccharides are capable of forming gels in appropriate conditions. Typical examples are alginate, pectins and gellan, which are able to gel by means of the interactions between the carboxylic acid moieties and divalent ions (i.e., physical gelation by ion complexation); Locust Bean Gum and Xanthan whose synergistic interaction leads to gel formation [7,8] and the stable hydrogels that can be formed by borax interaction with the hydroxyl groups of some polysaccharides [9].

Furthermore, a large variety of bifunctional or multifunctional reagents have been used as crosslinking agents to chemically crosslink hydroxyl and/or other functional groups present on the native polysaccharide chains [10,11].

On the other side it must be pointed out that numerous native polysaccharides are not capable to form hydrogels; moreover, in many cases the performances of hydrogels obtained from natural polysaccharides need significant improvements for their practical applications. For this purpose appropriate chemical modifications can be carried out on the macromolecules. Furthermore, sometimes

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scientists must resort to multicomponent networks such as semi-Interpenetrating Polymeric Networks (semi-IPNs) and Interpenetrating Polymeric Networks (IPNs) [12].

These networks usually show physico-chemical properties that can remarkably differ from those of the polymeric constituents and their properties, according to specific needs, can be tailored by the choice of the nature and the concentration of the interpenetrating macromolecules [13].

Together with the methods used for polysaccharide hydrogel formation, schematically reported above, a relevant number of studies has been focused also to the size of the hydrogel networks for specific applications. Beside the bulk hydrogels, already studied since a long time, during last decades an ever-increasing attention has been devoted towards nanosized hydrogels (nanohydrogels/nanogels). Nanogels can be generally obtained following different techniques such as: polymerization of monomers in homogeneous or nano heterogeneous environments; chemical crosslinking of preformed monomers, top-down methods using lithography, template-assisted nanofabrication and physical self-assembly of interactive macromolecules.

Only few polysaccharides have been exploited for nanohydrogel formation (e.g. hyaluronic acid, chitosan, gellan, scleroglucan, mannans) using different strategies, but in this review the attention will be focused on a single polysaccharide, pullulan (PUL). A wide overview on PUL and its derivatives was recently published with information about the various and diversified reactions that can be carried on this polysaccharide for different applications [14]; however, in the present work, our interest is specifically oriented towards a well defined topic: the self-assembling strategy for PUL nanogel formation and the possible applications of these nanostructures in the field of biomedicine, whose interest and importance is attested by the relevant number of publications that appeared in recent years. Actually, macromolecular self-assembly leading to nanostructures is being investigated since many years [15] because such processes are rather common in nature and also because of the wide variety of practical applications that such systems may have [16], in particular in the field of pharmaceutics.

Different types of hydrophilic and amphiphilic polymer chains have been used for the preparation of nanogels suitable for diagnosis and/or therapy; in the case of polysaccharides, the spontaneous association leading to a nano-hydrogel is often achieved by an appropriate hydrophobic derivatization of these macromolecules [17], according to the general scheme of the process reported in Fig. 1.

According to Kabanov and Vinogradov [16] a nanogel can be defined as "an aqueous dispersion of hydrogel particles formed by physically or chemically cross-linked polymer networks of nanoscale size". Actually, when the self-assembling approach is used, these nano-structures are sometimes defined also as micelles, due to a certain similarity with surfactant micelles bearing a hydrophobic core and a hydrophilic shell [18], and in some studies the value of a critical micelle concentration has been identified and reported, as in the case of one of the first papers on hydrophobized PUL, where the more general term of nano-particles was used.

Throughout this paper the terms nanohydrogel/nanogel and nanoparticle will be used as synonyms while the word micelle will be reported only when the authors of the mentioned articles specifically selected this word to indicate the presence of single or multiple microdomains within the nanostructure.

2. The long story of pullulan

After some papers on the synthesis of cholesterol-modified pullulan (CHP) employed for liposome coating [19,20], and just one year after the wide overview on molecular self-assembly as a strategy for the preparation of nanostructures reported in 1991 in Science [15], J. Sunamoto et al. [21,22] gave preliminary information on self-aggregation of palmitoyl and cholesterol derivatives of several native polysaccharides such as amylopectin, dextran, mannan and PUL. It was evidenced that the critical concentrations leading to the polymer aggregates were strictly dependent on the degree of substitution of the hydrophobic moiety. Cholesterol-bearing PUL showed a stronger binding for hydrophobic guest molecules and a higher colloidal stability compared with the corresponding palmitoyl-bearing one; for this reason most of the following studies were carried out on CHP. The authors described also the physical cross-linking of cholesterol hydrophobized PUL for the formation of nanoparticles (hydrogels) capable to form a complex with various globular and soluble proteins such as hemoglobin, peroxidase, myoglobin, and cytochrome c.

While in some preliminary approaches CHP was prepared *via* the aminoethyl-carboxymethyl-derivative of the polysaccharide followed by the condensation with cholesteryl chloroformate, an improved procedure, which involved the synthesis of cholesteryl *N*-(6-isocyanatohexyl) carbamate and the subsequent condensation with PUL, was proposed in order to increase the stability of the nanogels. In most cases, the self-aggregated nanoparticles have been obtained by sonication in water but they could be prepared also by water dilution of a CHP solution in DMSO. The characterization of the system was carried out by size exclusion chromatography and the critical aggregation concentration was determined by fluorometry. The size and density of the hydrogel

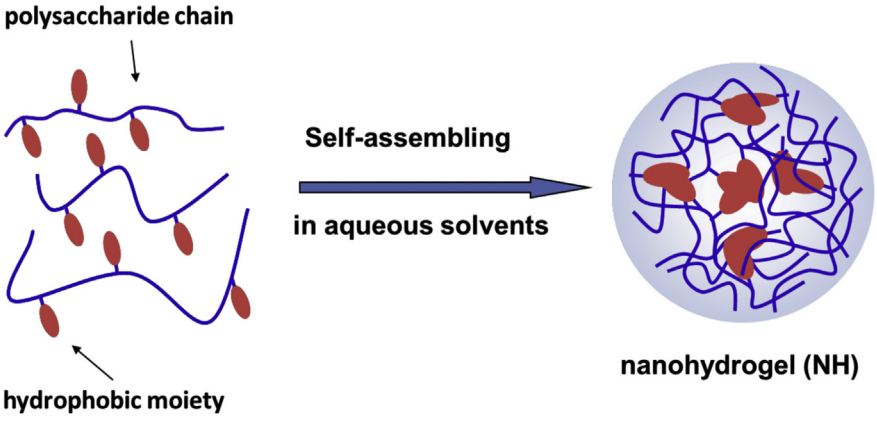

Fig. 1. Scheme of the self assembling process for the nanohydrogel formation by polysaccharide hydrophobization (modified from Ref. [17]).