New pH and temperature sensitive delivery systems based on renewable resources

Project PN-RU-TE-2014-4-0437

Contract No.**: 93** from **01/10/2015**

Acronym: **SensCurdSyst**

Scientific Report – 2015

I. Literature study regarding the synthesis of water-soluble polysaccharide derivatives with anionic/cationic groups

A lot of papers and patents from the last decade in the field of controlled drug release highlight the significant progress of researchers in medical/pharmaceutical fields. In medical terms, drug administration meant the route whereby the drug comes in contact with the body. As drug delivery method, the drug administration has a significant effect on its efficiency. The drug carriers can have very different nature and origin: based on soluble or insoluble natural or synthetic polymers, lipoproteins, liposomes, micelles. The controlled drug delivery systems based on micro/nano particles/capsules assume the existence of a polymer support in which the therapeutic agent (drug, enzyme, protein, vitamin, et al.) is linked by physical and/or chemical interactions. The increase of the efficiency and the reduction of the frequency of drug administration may be achieved by gradual, controlled, targeted release using a suitable drug delivery system. A polymer can be used as drug carrier if poses three main features: biocompatibility, biodegradability, and non-toxicity. All these requirements are fulfilled by natural polymers as for example polysaccharides or biopolymers.

Polysaccharides, an important component of the life world, are natural renewable polymers found in a large variety of vegetable and animal resources or can be biosynthesized by microorganisms. The origin of polysaccharides confers the important basic characteristics which could be exploited in the obtaining of biomaterials with medical, pharmaceutical, and cosmetic applications [1]. Recent research has shown for some polysaccharides or their derivatives specific properties such as: antiviral [2], anti-tumour [2-4], anti-cancer [5], and non-toxicity, allowing them to be used as excipients in drugs formulation [6]. The ionic derivatives of polysaccharides are, for example, used as support matrix for the immobilisation of enzyme/protein [7], as potential substituent of erythrocyte, as anti-tartar agents or anti-dental caries in toothpaste [8].

From the large number of the polysaccharides, the project team has focused on a less studied class such as the β-glucans class. These are renewable non-cellulosic polysaccharides synthesized by microorganisms, bacteria or fungi, with anhydroglucose units in the principal chain. The members of the family of β-glucans are: ● the linear glucans, with anhydroglucopiranosic units (AGU) linked by α or β (1,3)-bonds; or ● the branched glucans with AGU linked in (1,6), (1,4) and/or (1,2) positions [9,10]. Some biological activities of these polysaccharides useful for medical, pharmaceutical or industrial applications are described in literature [1,11,12]. However, their applicability is limited due to their insolubility in water, generally attributed to the existence of extensive intra/intermolecular hydrogen bonds and the helix or triple-helix structure [13]. This disadvantage can be eliminated by the introduction of functional groups (ionic groups).

1. Water-soluble derivatives of β-glucans

1.1. Anionic water-soluble derivatives of β-glucans

Some medicinal applications need fungal glucans readily water-soluble, while many glucan preparations from fungal raw materials comprise mostly insoluble polysaccharides or their complexes. To improve the solubility of such preparations specific chemical modifications can be used. Carboxymethylation, sulphation, phosphorylation as well as some other modifications are common ways to prepare water-soluble derivatives of fungal glucans. Introduction of carboxymethyl and/or sulphate groups into β-D-glucan improved its water solubility significantly and enhanced the stiffness of the chains. Moreover, such modifications of fungal glucans may induce or significantly enhance specific biological activities. The effectiveness of polysaccharide modification is usually monitored by NMR spectroscopy and by determination of the substitution degree (DS).

Carboxymethylation of glucans is usually made by the reaction of glucan suspension with chloroacetic acid in alkaline conditions. Ukai et al. [14] described the preparation and analysis of the carboxymethyl derivatives of scleroglucan and (1,3)-β-D-glucan from *Agrocybe cylindracea*. Based on GC-MS analysis authors concluded that distribution of the carboxymethyl substituents depends on the type of glucan and its conformation. Wiater et col. [15] prepared carboxymethylated derivatives of (1,3)-β-D-glucans from various fungal sources and described their biological activities. Water-soluble carboxymethylated derivatives of insoluble (1,3)-β-D-glucan from the sclerotium of *P. cocos* [16], and branched (1,3),(1,6)-β-D-glucans from *S. cerevisiae* [9] has been reported.

Sulphation of fungal glucans is commonly achieved by the reaction with chlorosulphonic acid–pyridine or sulphur trioxide–pyridine complexes in dimethyl sulphoxide medium. The reaction takes place preferably at O-6, but is also possible at O-2, O-3 and O-4 positions.

Water-soluble sulphated derivatives were obtained from various fungal glucans: (i) (1,3)-β-D-glucans originating from mycelia of *P. cocos* [17] and from basidiocarps of *Lentinula edodes* [18]; (ii) (1,4),(1,6)-β-D-glucans from *Gastrodia elata* Bl [19]; (iii) various branched (1,3),(1,6)-β-D-glucans from *Pleurotus tuber-regium* [20], *Russula virescens* [21], *Sacharomyces cerevisiae* [22], botryosphaeran [23], Grifolan [24], and lentian [25]. Depending on the polysaccharide structure and reaction conditions, the products showed DS in the range of 0.17–1.74. Huang et al. [26,27] described phosphorylation of water-insoluble (1,3)-α-D-glucan from mycelia of *Poria cocos* with H_3PO_4 in solution of LiCl and urea in dimethylsulfoxide. Also, the water-soluble phosphated derivatives were obtained in our laboratory by reaction of curdlan with phosphorous acid in molten uree [28].

1.2. Cationic water-soluble derivatives of β-glucans

Polysaccharides with cationic groups have some important characteristics as for example: hydrophilicity, biodegradability, biocompatibility, and bacteriostatic properties, very useful for bio-applications. These cationic polysaccharides can be obtained by the reaction of the native polymers with various reagents with ammonium groups. The commercial reagents, glycidyltrimethylammonium chloride or 3-chloro-2-hydroxypropyltrimethylammonium chloride, are the most used to prepare quaternary ammonium salts of polysaccharides, such as

agarose [29], cellulose [30], chitin [31], chitosan [32], curdlan [33], dextran [34], pullulan [35], and starch [36]. The water-soluble glucans derivatives with amino or ammonium groups are also reported in the literature [37,38].

1.3. Soluble β-glucans derivatives with polymerizable groups and thermosensitive groups

Grafting of thermosensitive polymeric chains on polysaccharides can be done by different methods: a) coupling reactions of thermosensitive polymers on the polysaccharide chains; b) radicalic polymerization of the monomers in the presence of polysaccharides using initiators that create free radicals on the polysaccharide chain; c) atomic transfer radical polymerization where a polysaccharide derivative is used as macroinitiator; d) introduction of polymerizable groups on the polysaccharide chain followed by monomers polymerization.

a) Coupling reactions of thermosensitive polymers on the polysaccharide chain

Poly(N-isopropylacrylamide) (PNIPAM) or thermosensitive copolymers of NIPAM with amine or carboxyl terminal groups were obtained by radical polymerization in the presence of a chain transfer agent. Oligomers with therminal amino groups were coupled on polysacchaides with carboxylic groups as sodium alginate [39,40], carboxymethyl guar-gum [41] in the presence of activating agents (carbodiimides). Thermosensitive oligomers with carboxyl terminal groups were also coupled on cationic derivatives of dextran [42]. Another approach is the introduction of thermosensitive oligomers with $–NH-NH_2$ terminal groups on the chitosan chain previously activated with 4-nitrophenyl chlorophormate [43,44]. Thermosenssitive copolymers based on poly(ethyleneoxide) (PEO) and poly(propylenoxide) (PPO) were also grafted on the polysaccharide chains. Thus, statistic or block copolymers of PEO and PPO with $–NH_2$ terminal groups were coupled on nanocrystaline cellulose with carboxyl groups introduced by TEMPO oxidation [45], or on carboxymethyl pullulan 48]. PEO-b-PPO-b-PEO (Pluronic) with carboxyl terminal group, obtained by reaction with succinic anhydride, was also coupled on chitosan [49]. All these coupling reactions were performed in the presence of activators used in the amidation reaction from the peptide synthesis.

b) Radical polymerization of monomers in the presence of initiators that create free radicals on the polysaccharide chain

Radical polymerization was used as a grafting method of thermosensitive polymers on polysaccharide chains. Thus, N-isopropylacrylamide (NIPAM) was polymerized in the presence of guar gum [50], carboxymethylchitosan [51], hydroxypropylcellulose [52], carrageenan [53], cellulose [54,55], pullulan [56], and N-vinylcaprolactam was polymerized in the presence of dextran [57]. The initiation take place by gamma-irradiation, by the decomposition of usual chemical initiators (peroxydes or azo-compounds), or by the reduction of cerium ions that determine the disentanglement of the glicozydic cycle from polysaccharide with the formation of a macro radical. In order to remove the obtained polymers that are not bound on the polysaccharide chain, the reaction products must be washed with a solvent for the polymer, but not for the grafted polysaccharide (e.g acetone or methanol for the removal of PNIPAM).

c) *Atomic transfer radical polymerization (ATRP)* was used for the introduction of PNIPAM grafts with controlled length on dextran [58], pullulan [59], or cellulose [60] chains. This method consists in the introduction of new groups with halogens (Br, Cl) on the

polysaccharide chain, so the polysaccharide acts as a macro initiator in the polymerization reaction that takes place in the presence of catalysts based on Cu.

d) *The introduction of polymerizable double bonds* on the polysaccharide chain allows further grafting of thermosensitive polymers. The most used modifying agents are glicidyl methacrylate, acrylic acid, acryloil chloride, and maleic anhydride. The derivatization of polysaccharide such as dextran [61-63], cellulose [64,65] or hemicellulose [66,67] with maleic anhydride was used in order to introduced a polymerizable group, that allows the subsequent grafting or cross-linking. By direct derivatization of chitosan with maleic anhydride, the amine group is blocked by amidation reaction [68], but the protecting of amine group before the reaction with maleic anhydride, lead to the obtaining of esthers that retain the cathionic properties of chitosan [69]. The grafts of PNIPAM, subsequently introduced by polymerization of NIPAM, confer thermosensitivity to the new product, demonstrated by the existence of a low critical solution temperature (LCST).

II. The synthesis of new polysaccharide derivatives with polymerize groups. Preliminary results

The synthesis of polysacharideswith polymerize groups

The synthesis of curdlan (Curd-MA) and cellulose (Cell-MA) with polymerizable groups was conducted in homogenous conditions, by solving the polysaccharide in different media: LiCl/DMAc and LiCl/DMF.

The reaction products were purified by dialysis, till the conductivity of the dialysis water was lower than 6 µS/cm. The Curd-MA and Cell-MA were recovered from aqueous solution by freeze-drying and white powders were obtained.

Analysis and Characterization of polysaccharide derivatives

The chemical structure of the new synthetized derivatives Curd-MA1, Curd-MA2, and Cell-MA were preliminary investigated by FT-IR and MNR spectroscopy. The substitution degrees (DS) were calculated from the potentiometric date and correlated with the values obtained by integration the signals from the NMR spectra. The main characteristics of these new derivatives are presented in Table 3.

Tabel 3. The main characteristic of new polysaccharidic derivatives

Sample	**FT-IR** new bands, cm^{-1}	**NMR** new signal, ppm	**Degree of substitution**	
			RMN	Titration
Curd-MA1	–COOH to 1726; $–COO^-$ to 1580; –CH=CH– to 822	6.59 - 6.10	0.34	0.41
Curd-MA2	–COOH to 1726; $–COO^-$ to 1584; –CH=CH– to 819	6.64 - 5.99	0.84	0.90
Cell-MA	–COOH to 1725; $–COO^-$ to 1580; –CH=CH– to 823	6.62 – 6.27	1.38	1.20

Conclusions

In the first stage of the project two activities were fulfilled:

- ✓ the study of the literature regarding the synthesis of new water-soluble polysaccharide derivatives with anionic/cationic groups, and
- ✓ the synthesis of new polisaccharide derivatives with polymerizable groups.

The first activity included an extensive study regarding the water-soluble derivatives of β-glucans and their characterization and application. Concerning the carboxymethylated, sulphonated and phosphorylated glucan derivatives, only few researchers were found in literature. These detivatives could be used as anti-HIV, anti-clotting and antioxidants agents. On the oher hand, glucan derivatives with polymerizable or thermosensitive groups were not found. In this respect, in the second activity, few syntheses which involved the transformation of curdlan using maleic anhydride were made. Some preliminary results about the new compounds were also performed. The structure was established using FT-IR and ^{1}H NMR spectroscopies, while the degree of substitution was determined by two methods: ^{1}H NMR spectroscopy and potentiometric titration. The degree of substitution calculated from the potentiometric titration is in good accordance with that determined from ^{1}H NMR signals integration. The two curdlan derivatives and one of cellulose, both containing maleat polymerizable groups has the degree of substitution between 0.3 and 1.38. For the further applications, the new derivatives will be purified more in order to remove the traces of unreacted compounds or secondary products. The purified products will be used in chemical reactions for the insertion of thermosensitive groups (Stage 2 / 2016). The resulted derivatives will be used for the obtaining of microparticles or microcapsules able to encapsulate active principles, to achieve new drug delivery systems for therapeutic purposes.

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