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 – 2016

The aim of the project ***New pH and temperature sensitive delivery systems based on renewable resources*** is to obtain the new release systems of some therapeutic agents with application in pharmaceutic/cosmetic or medicine fields. In the fist stage / 2015 of the project two activities were fulfilled: a) the study of the literature regarding the synthesis of new water-soluble polysaccharide derivatives with anionic/cationic groups; b) the synthesis of new polisaccharide derivatives with polymerizable groups. In the second stage / 2016 the proposal objectives were fully accomplished: *O1. Synthesis of new soluble polysaccharide derivatives with anionic/cationic groups; O2. Obtaining of micro-/nano-particles based on ionic polysaccharides.* In the following, the general information of the obtained results, which does not interfere with intellectual property rights, are presented.

O1. Synthesis of new soluble polysaccharide derivatives with anionic/cationic groups

T.1. Synthesis of new soluble cationic polysaccharide derivatives with controlled hydrophobicity

Polysaccharides with cationic groups present 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 native polymers with various reagents with ammonium groups or quaternary 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 [1], cellulose [2], chitin [3], chitosan [4], curdlan [5], dextran [6], pullulan [7], and starch [8]. These derivatives are widely used in different areas of interest including waters treatment, papermaking, food, pharmaceutical, and cosmetic industries.

Synthesis and characterization of curdlan with quaternary ammonium groups

The synthesis of curdlan with quaternary ammonium groups was conducted in homogenous conditions, by solving the polysaccharide in NaOH solution. The reaction products (NCurd_DMOctA and NCurd_DMEA) were purified by dialysis and recovered by freeze-drying.

The chemical structure of the new synthetized derivatives NCurd_DMOctA and NCurd_DMEA were investigated by FT-IR and MNR spectroscopy. The degrees of substitution (DS) were calculated from the coductometric date and correlated with the values obtained by integration the signals from the NMR spectra (Figure 1). The main characteristics of these new derivatives are presented in Table 1.

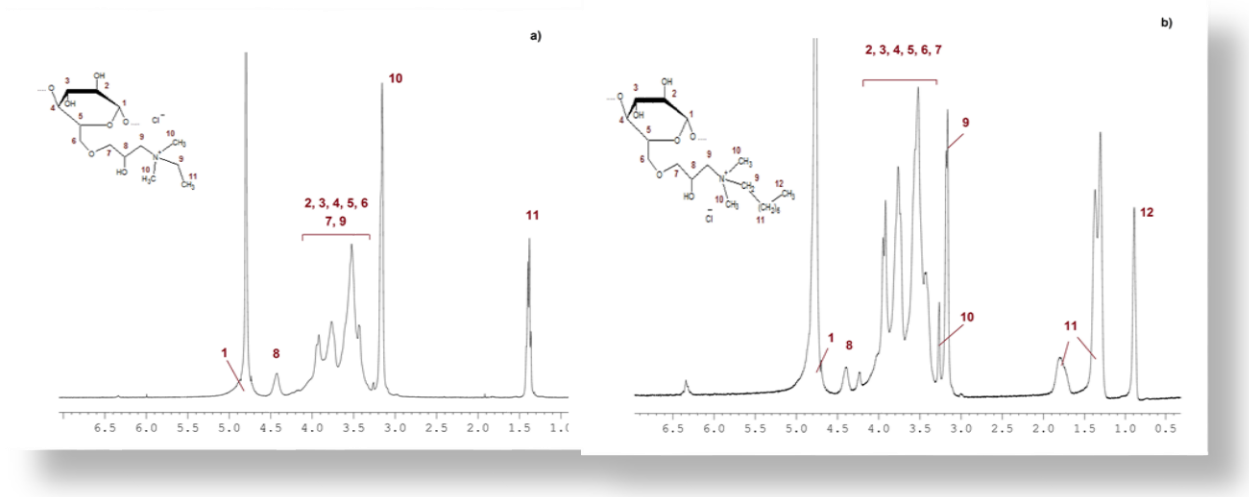

Figure 1. ^{1}H NMR spectra of NCurd_DMEA (a) and NCurd_DMOctA (b) derivatives in D_2O

Tabel 1. The main characteristic of new polysaccharidic derivatives

Sample	**FT-IR** new bands, cm^{-1}	**NMR** new signal, ppm	**DS**	
			RMN	Titration
NCurd_DMOctA	$-CH_2-$ to 2925 (from octyl radicals); –N–C– to 1466	0.889 for $-C^{12}\underline{H}_3$ 1.37-1.31 for $-C^{11}\underline{H}_2-$ 1.79 for $-N-C^{9}H_2-C^{11}\underline{H}_2-$ 3.26 for $-N-C^{10}\underline{H}_3$ 3.163 for $-N-C^{9}\underline{H}_2-$ 4.45 for $-C^{8}\underline{H}-$	0.45	0.36
NCurd_DMEA	–N-C to 1454 -1417	1.38 for $-N-C^{9}H_2-C^{11}\underline{H}_3$ 3.16 for $-N-C^{10}\underline{H}_3$ 4.45 for $-C^{8}\underline{H}-$	0.38	0.33

T.2. Synthesis and characterization of new derivatives polysaccharides with polymerisable groups

Curdlan with polymerizable groups (Curd_MA) was obtained by esterification reaction between curdlan maleic anhydride. The chemical structure of Curd_MA derivative was investigated by FT-IR and MNR spectroscopy. The DS was calculated from the potentiometric date and correlated with the values obtained by integration the signals from the NMR spectromety. The main characteristics of these new derivatives are presented in Table 2.

Table 2. The main characteristic of Curd_MA

Sample	**FT-IR** new bands, cm^{-1}	**NMR** new signal, ppm	**Degree of substitution**	
			RMN	Titration
Curd_MA	–COOH to 1729; $–COO^-$ to 1638; –CH=CH– to 823	6.59 - 6.10	0.80	0.75

T.3. The grafting of the temperature-sensitive groups

In order to obtain polyasccharide derivatives with theromsensitive groups without crosslinked samples, the synthesis was lead in two step: a) synthesis of thermosensitive oligomers; b) reaction with polysaccharidic chains (curdlan).

a) thermosensitive N-isopropylacrylamide oligomers (ONIP) with carboxilic end-groups were synthetised using 3-metcaptopropionic acid as chain transfer agent and azo-bis-isobutironitril as initiator. The ONIP oligomers with different molecular weight were obtained by varying the amount of chain transfer agent. In Table 3 are presented the main characteristics of the ONIP oligomers. These oligomers present low critical solution temperature (LCST), in aquous solutions. The values of LCST for ONIPs were determined by UV-vis measurements in phosphat buffer solution (pH=7.4).
b) reaction with polysaccharidic chains (curdlan): the temosensitive ONIP oligomers was grafted on the polysaccharidic chains of nativ/cationic curdlan by reacting of –COOH end-groups from ONIP with –OH groups from polysaccharidic chains. The new derivatives were also characterized by FTIR and ^1H NMR spectrometries. In FTIR spectra of new derivatives (Figure 3) were identified the characteristic bands:
    - for Curd-ONIP (curdlan withw termosensitive groups): for curdlan chains at 1368, 1079 și 890 cm^{-1} and for NIPAm units at1649 cm^{-1} - amide I, 1547 cm^{-1} - amide II, 2972 cm^{-1}, 1387 cm^{-1}, 1368 cm^{-1} – isopropil groups. Other peaks: at 1736 cm^{-1} for esteric bonds beween –OH group of polysaccharides and –COOH oligomer

end-groups; the peaks at 1460 and 1235 cm^{-1} can be attributed to the S-CH_2 linkage from oligomer;

- for cationic derivative of curdlan NCurd-ONIP: at 1736 cm^{-1} the esteric linkage and a wider large band at 1460-1370 cm^{-1} due to –CH_3 groups from the quaternary ammonium group.

Table 3 Main characteristics of ONIP oligomers

Sample	Mn, (g/mol)	LCST, °C	FT-IR new bands, cm^{-1}	NMR new signal, ppm
ONIP1	4.800	30,0	1650 (amide I); 1544 (amide II); 3200-3600 for -N-H; -O-H bends; 2973 for –CH_3 groups; 1713 for –COOH groups	1 - 2,2 and 3,9 for PNIPAM groups; 2,5 - 2,8 for 3-metcapto end-groups
ONIP2	2.400	30,5		

In ^{1}H NMR spectra of Curd-ONIP and NCurd-ONIP samples the characteristic signals at 1 and 2.2 ppm for ONIP were identified and the ratios between AGU and NIPAM oligomer units for each derivative were calculated (1:0.39 and 1:0.23 for Curd-ONIP and NCurd-ONIP, respectively). The thermosensitivity of derivatives was studied by varying the optical density with temperature, in phosphat buffer solution (in Figure 4 is presented for example the phase separation profile for NCurd-ONIP).

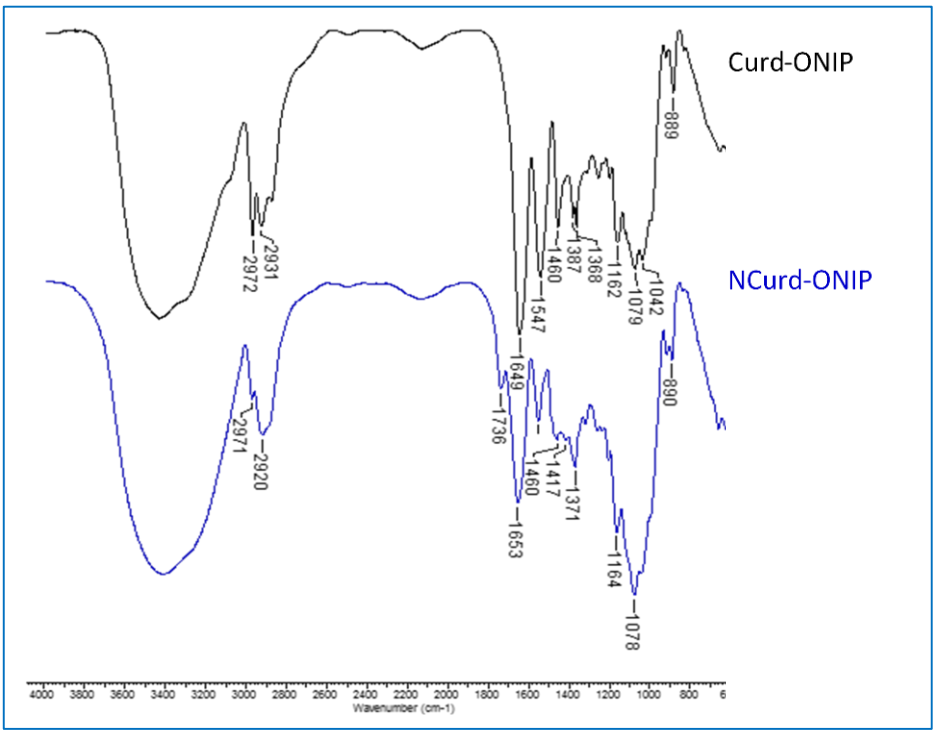

Figura 3. FTIR spectra of Curd-ONIP and NCurd-ONIP derivatives

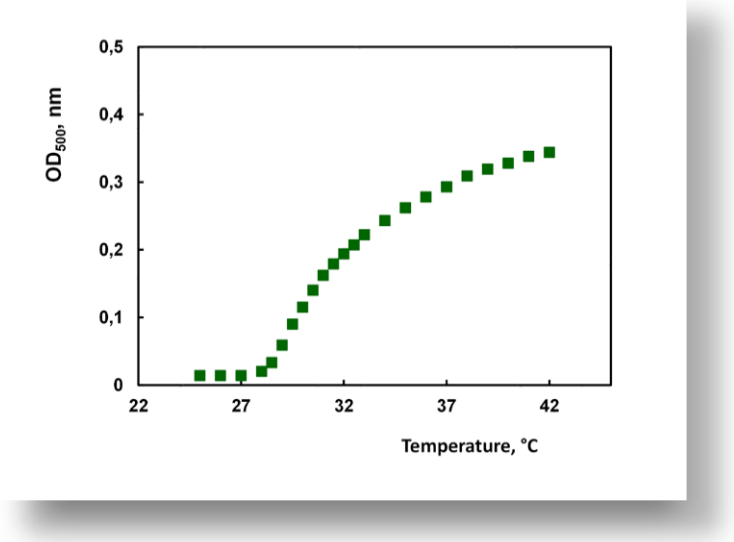

Figure 4. Phase separation profile for NCurd-ONIP in phosphate buffer solution (pH = 7.4)

O2. Obtaining of micro-/nano-particles/capsules based on ionic polysaccharides

Some major impediments to the efficient use of most drugs are their low insolubility (only 40% from the newly discovered drugs are soluble), high toxicity, high dosage, and nonspecific release. These downsides can be reduced by using as drug carriers the new micro/nano particles (MNP) based on polysaccharides. Polysaccharides (Pz), an important component of the life world, are the natural renewable polymers occurring from a large variety of vegetable and animal resources or are biosynthesized by microorganisms. The origin of Pz confers the important basic characteristics which not found in other classes of polymers and could be exploited in the obtaining of biomaterials with medical, pharmaceutical, and cosmetic applications. In the following section, the synthesis of two type of MNP obtained by new ionic derivatives is briefly presented (synthesised in the ***Objective O1.***)

T.5. Obtaining of micro-/nano-particles based on ionic polysaccharides.
T7. Characterisation of micro-/nano-particles based on ionic polysaccharides

1. Synthesis and characterization of microparticles based on monobasic cellulose phosphate

Monobasic cellulose phosphate microspheres (PCellMS) were prepared by chemical cross-linking with epichlorohydrin using the water-in-oil (w/o) inverse (mini)emulsion. The cross-linked microparticles were recovered by filtration and then washed for the removal of residuals. Finally, the microspheres were completely dried by overnight exposure to 60°C, under vacuum. The microspheres

morphology and size particles were evaluated by scanning electron microscopies, when a spherical structure of microparticles and the size distribution between 10 – 20 μm were observed (Figure 5).

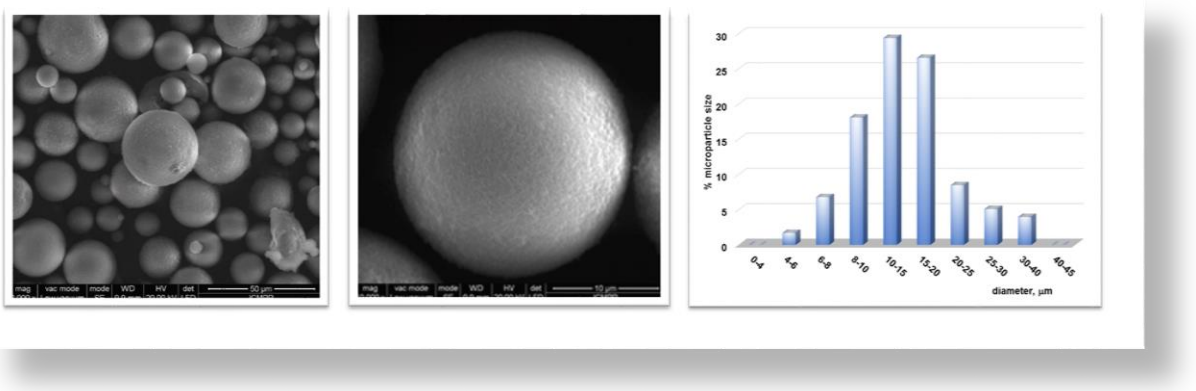

Figure 5. ESEM photographs of PCellMS. General view, surface detail, and the corresponding size distribution

The effect of the initial pH solution on the water retention of microspheres was also studied and presented in Figure 6. These experimental data were included in the manuscript with title "Preparation and adsorption studies of phosphorylated cellulose microspheres" submitted in 2016 at *Cellulose Chemistry and Technologies* journal.

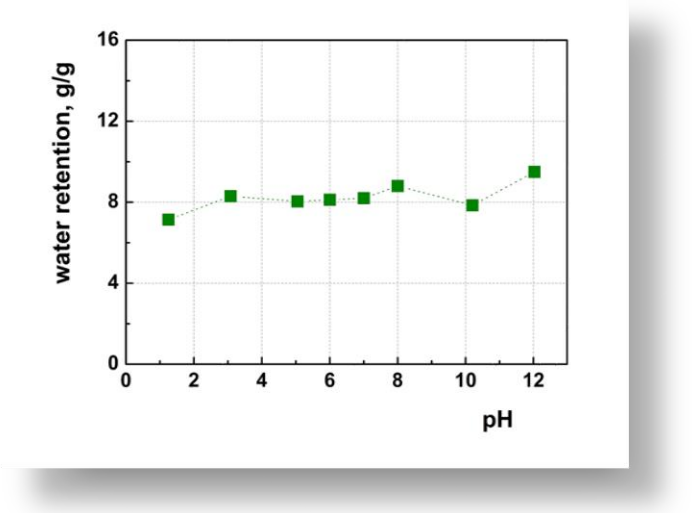

Figure 6. The pH effect on the water retention of PCellMS

2. Synthesis and characterization of microparticles based on ionic curdlan

Hydrogels were prepared by free radical polymerisation of NIPAM in the presence of Curd_MA without a low molecular cross-linker, but also in the presence of N,N′-methylenebis-acrylamide. The morphology of the particles was evaluated by scanning electron microscopy (Figure 7). The porosity of the hydrogel proved to be relatively high (8-15 $\boldsymbol{\mu}$m).

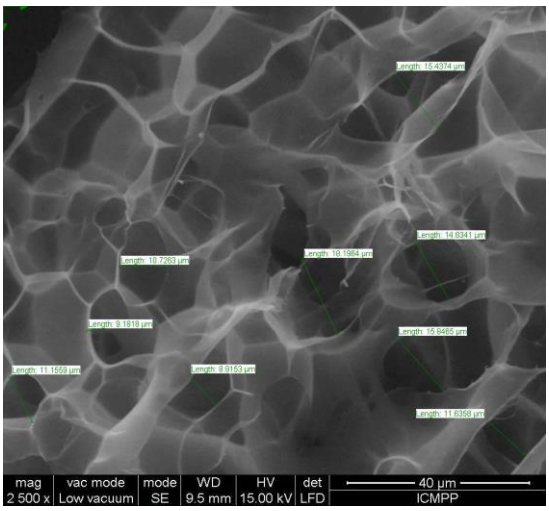

Figure 7. Scanning electron microphotographs of Curd-MA-PNIPAM microparticles dried under vacuum and lyophilized

The influence of the pH on the swelling the microparticles was studied, revealing that the dissociation of the remaining carboxylic groups from Curd_MA have the great influence of the micropaticles behavior. The thermosensitive character of the microgels was investigated following the influence of the temperature on the swelling degree but also on the turbidity (optical density) of the swollen microgels. The volume phase transition temperature was between 31 and 37°C, increasing with the increase of the pH and with the decrease of the ionic strength.

The obtained results in this stage / 2016 have been disseminated through publication of two scientific articles and were presented at two Scientific Symposia (three oral presentation and two posters)

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