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Synthesis of carboxymethylcellulose nanoparticles using various coiling agents

Introduction

Carboxymethylcellulose (CMC) is one of the major derivatives of cellulose, which is a naturally occurring polysaccharide and one of the best renewable resources available to mankind. CMC occurs as the sodium salt (Fig. 1) and is produced in reaction of alkali cellulose with sodium monochloroacetate.

Due to many advantages such as non-toxicity, biodegradability and biocompatibility, CMC is widely used as raw material in various industrial applications including pharmaceutics, cosmetics, food, etc. [*Li et al., 2011*]. As a polymer, which can be easily modified, CMC has additionally great potential for biomedical applications [*El-Hag et al., 2008*]. The use of CMC for the synthesis of nanoparticles (NPs), which can be applied as anticancer drugs carriers is very promising. The reason that makes NPs so attractive for medical purposes is based on their unique qualities, such as size and their surface to mass ratio which is significantly larger than for other particles. Additionally, the polysaccharide NPs can carry not only medicines, but may also serve as carriers of other compounds like vaccines, proteins, nucleic acids, probes, genes etc. [*de Jong and Borm, 2008*]. The possibility of chemical modification of polysaccharides and their ability to bind various molecules contribute to improving the therapeutic effect and better targeting. This also allows to obtain NPs with appropriate surface charge what is very important in order to avoid uptake by the macrophages system and premature removal from the body. A very important task is to determine the ideal size of the NPs used as drug carriers in order to ensure the highest potential for prolonged circulation in human body. The size of resulting NPs should be large enough to avoid uptake in the liver, but small enough to avoid filtration in the spleen. Therefore, it was concluded that the most desirable size range of the nanoparticles is from 50 to 100 nm.

The aim of the experiments was the synthesis of carboxymethyl cellulose NPs using various coiling agents and different reagents proportion. Additionally, we wanted to investigate the influence of oxidation degree of CMC on NPs size.

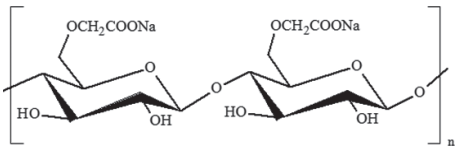

Fig. 1. Unit structure of NaCMC

Experimental

Materials

Carboxymethylcellulose sodium salt (M_W = 90,000 Da) was received from *Aqualon*^{TM} by *HERCULES*. Sodium metaperiodate, benzylamine, octylamine and hexylamine were purchased from *Sigma Aldrich*. Alanine was purchased from *POCh S.A.*

Carboxymethylcellulose oxidation

CMC was oxidized with sodium metaperiodate to obtain some aldehyde groups along the polysaccharide chain. Sodium metaperiodate oxidization of CMC to dialdehyde carboxymethyl cellulose (DCMC) was carried out as follows: 10 g of CMC was dissolved in 400 ml deionized water and then sodium metaperiodate was added to the CMC solutions. The resulting mixture was stirred for 1 h in dark at room temperature. After 1h ethylene glycol was added to quench the reaction. The oxidized product, was extensively dialyzed against deionized water using dialysis membrane bag (MWCO 12,000÷14,000 Da), in room temperature for 3 days (water was changed every 24 hours). Obtained product was dried at 60°C to constant weight. The amount of aldehyde groups in oxidized CMC was estimated using hydroxylamine hydrochloride method [*Zhao and Heindel, 1991*]. Unmodified CMC was used as a control. The CMC was oxidized in three different oxidation degrees.

Nanoparticles synthesis

Synthesis of polysaccharide NPs is based on the reaction between aldehyde groups in DCMC chain and amino groups in aliphatic amine or drug. To prepare NPs, 0,1 g of oxidized CMC was dissolved in 5 ml deionized water and heated at 30°C. Appropriate amount of aliphatic amine was added, to introduce a hydrophobic groups along the DCMC chain. The mixture was stirred at 30°C for 30 min. Then *pH* of the solution was slowly changed with 0.1 M sodium hydroxide to *pH* 10 and after that mixture *pH* was adjusted at 7.4. The obtained solution was dialyzed against deionized water for 30 min. The resulting NPs were further lyophilized. NPs with different CMC oxidation degree and different aliphatic amine type were synthesized. The ratio of added amine were calculated relative to a drug, that in future studies will be bound to the NPs. The amount of the drug will constitute 5 weight percent of DCMC used in the reaction. The proportion 1:1, 2:1, 3:1, 5:1, 7:1 and 10:1 of aliphatic amine to drug (mol : mol) were applied. To substitute the remaining unreacted aldehyde groups and reduced free aldehyde groups toxicity, alanine was added.

Nanoparticles size determination

The diameter and the size distribution of the resulting NPs were measured using *Nanosight LM 10 – HS* instrument (*Malvern Instruments*, UK). The measurement is based on an analysis of the rate of Brownian motion of particles in liquid, with *Nanoparticles Tracking Analysis* (NTA) software. The size distribution of NPs and their concentration in solution are calculated using the *Stokes-Einstein* equation. All prepared samples were measured in triplicate.

Results and discussion

Carboxymethylcellulose oxidation

For the oxidation of carboxymethyl cellulose, sodium metaperiodate was used. This reaction is based on the specific cleavage of the C2 – C3 bond of glucose units. The result is the formation of two aldehyde groups per one glucose unit [*Li et al., 2011*]. The reaction scheme is shown in Fig.2.

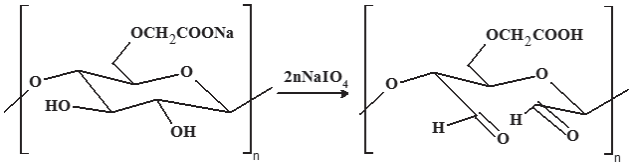

Fig. 2. CMC oxidation scheme

The amount of $NaIO_4$ added to the reaction was calculated to obtain theoretical oxidation degree of 15%, what means that 15% of glucose rings along the polysaccharide chain will be opened and 30% of aldehyde groups will be formed (one glucose formed two aldehyde groups). The number of aldehyde groups in the resulting DCMC was determined using a hydroxylamine hydrochloride method. The received

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degree of oxidation was similar to theoretically predicted and reached 16% ± 0.5. DCMC with 10% and 20% oxidation degree were oxidized and the received oxidation degrees were 10% ± 0.7 and 22% ± 0.4 respectively.

Nanoparticles synthesis

Polysaccharide NPs are made during the reaction between amino groups in aliphatic amine or drug and aldehyde groups present along the oxidized CMC chain forming *Schiff* base. The reaction scheme is shown in Fig. 3. During the slow change of the *pH* to 7, due to hydrophobic–hydrophilic interactions NPs are formed, what was verified using light scattering technique. After reaction, resulting NPs were lyophilized to dry powder and redissolved in water. Due to self-assembly of molecules the same size distribution was reached.

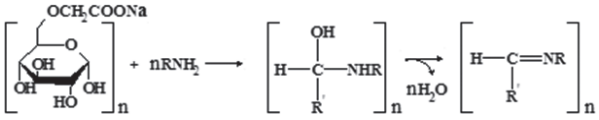

Fig. 3. The reaction of polysaccharide nanoparticles synthesis

Nanoparticles size determination

Nanoparticles formed by the reaction described above, were characterized by *Nanosight LM 10-HS* instrument. The analysis result, showing the size distribution of the NPs is depicted in Fig.4.

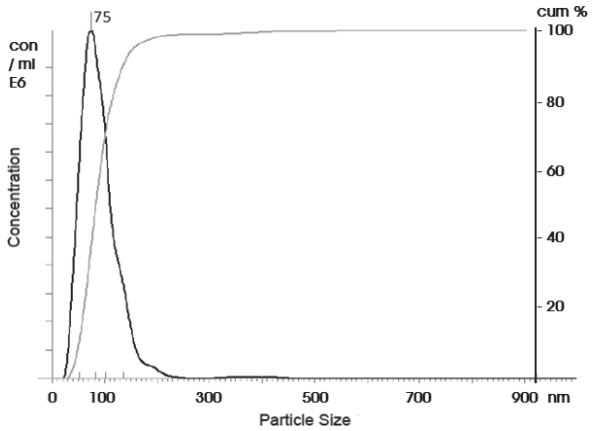

Fig. 4. Diameter distribution obtained by *Nanosight* analysis method

To evaluate the effect of type and amount of aliphatic amine and CMC oxidation degree on the size of NPs, a series of experiments were conducted.

The influence of the type and amount of aliphatic amine was determined by the synthesis of NPs with CMC oxidized in 15%, using different types of amines (benzylamine, octylamine and hexylamine) in various proportions of aliphatic amine to drug (mol: mol): 1:1, 2:1, 3:1, 5:1, 7:1 and 10:1. The amount of amine were calculated relative to a drug, that in future studies will be bound to the NPs. Obtained results are shown in Fig. 5.

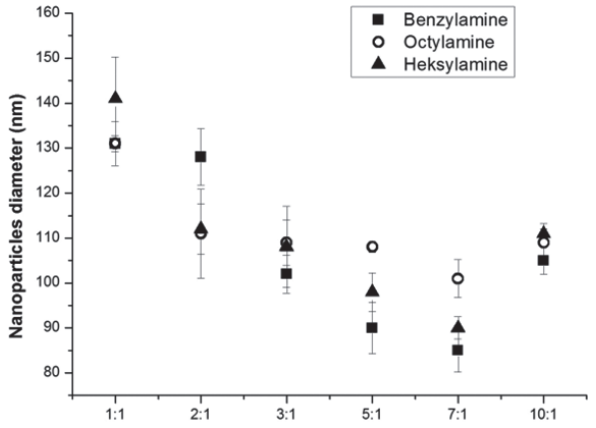

Fig. 5. Influence of the proportion of aliphatic amine:drug on nanoparticles diameter for DCMC with 15% oxidation degree

Obtained results show that benzylamine is the aliphatic amine, which allowed to obtain NPs similar in size to the desired range (50÷ 100 nm). The results also show that the most appropriate ratio of aliphatic amine to drug, resulting in formation of NPs with the best diameter (about 80 nm) is 7:1.

The influence of CMC oxidation degree was studied by synthesis of NPs with CMC with three oxidation degree – 10, 15 and 20%, with all three aliphatic amine (benzylamine, octylamine and hexylamine) in the ratio 7:1. The results are shown in Fig.6. For all used aliphatic amine the best results were obtained for a CMC with degree of oxidation of 15%.

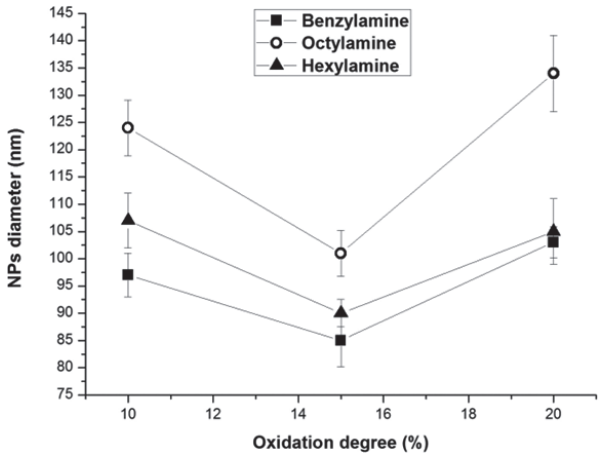

Fig. 6. Changes in nanoparticles diameter with aliphatic amine type (ratio of 7:1 [amine mol: drug mol]): benzylamine (■), octylamine (○), hexylamine (▲) depending on the degree of oxidation of CMC

Conclusion

The aim of this study was to investigate the influence of the type and amount of aliphatic amine (benzylamine, octylamine, hexylamine) and different CMC oxidation degree (10%, 15%, 20%) on NPs size. The results showed that the most appropriate amine for the synthesis of polysaccharide NPs is benzylamine and the best proportion of aliphatic amine to drug (mol : mol) is 7:1. The resulting NPs have the desired size (below 100 nm) in order to properly function as a carrier of anticancer drugs. As regards the degree of oxidation of CMC, the best results were obtained for CMC oxidized at 15%.

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