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# Chitosan/alginate nano-spheres for curcumin loading and delivery

S. Milenkova[1], B. Pilicheva[2], S.Tsoneva[3], M. Marudova[1*]

[1]*University of Plovdiv "Paisii Hilendarski", Faculty of Physics and Technology, 24 Tsar Assen str., 4000 Plovdiv, Bulgaria*
[2]*Medical University - Plovdiv, Faculty of Pharmacy, 15A Vassil Aprilov blvd, 4002 Plovdiv, Bulgaria*
[3]*University of Plovdiv "Paisii Hilendarski", Faculty of Chemistry, 24 Tsar Assen str., 4000 Plovdiv, Bulgaria*

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In this study curcumin-loaded chitosan-NaTPP nano-spheres were prepared by ionotropic gelation followed by chitosan-alginate polyelectrolyte complex formation for some of the samples. Dynamic light scattering and atomic force microscopic analysis showed that the particles were nearly spherical in shape with nano-sized diameters. The size of the spheres could be varied by changing the polymer and crosslinker concentrations. Fourier transform–infrared analysis revealed potential interactions among the constituents in the composite nano-spheres. It proved that the curcumin did not interact with the spheres and retained its chemical structure. The loading efficiency (%) of curcumin in the nano-spheres was above 60%. The *in vitro* drug release profile along with kinetics and mechanism of release from the nano-spheres were studied under simulated physiological conditions for different incubation periods. The release rate could be changed by varying the chitosan and NaTPP concentrations, as well as by coating the nano-spheres with chitosan-alginate complexes.

**Keywords:** drug-loading, nano-sized polyelectrolytes, chitosan, alginate, curcumin.

## INTRODUCTION

Nanomedicine introduces the nano-scale technology to the practice of medicine, namely for diagnosis, prevention and treatment of diseases and to gain an increased understanding of the underlying complex disease mechanisms [1]. One of the fastest growing trends in nanomedicine is nanopharmacy, which uses nano-structures as drug carriers. The nanometer size of these carrier systems allows efficient crossing of biological barriers, increased tissue tolerance, improved cellular uptake and transport, thus enabling efficient delivery of the therapeutic agents to the target sites like the liver, brain and solid tumours. Nanocarriers can be fabricated to protect drug molecules and preferentially target them to specific anatomical or cellular targets. This "targeting" is the key behind their popularity in drug delivery [2].

What is unique about nanomaterials is that often known physical and chemical properties are improved or changed, compared to bulk materials. For instance, nanocarriers have the potential to improve the solubility of lipophilic, poorly water-soluble compounds and to protect labile molecules from the biological environment [3, 4]. The main reason for these differences between nanoparticles and bulk materials is the much greater surface area-to-volume ratio of the nanoparticles.

In addition to these benefits, many drugs are facing the end of their patent protection and new nanoformulations may offer a commercial lifeline for these compounds.

Several strategies for the development of these vehicles were investigated, among which the design of biodegradable nanoparticles has drawn considerable interest. Biodegradable nanoparticles from naturally occurring polymers such as polysaccharides are attractive as drug delivery systems since they possess the biocompatibility, biodegradability and non-toxicity required for use in humans.

Biodegradable polymeric nanoparticles (NPs), with hydrophilic surfaces have been designed to have longer circulation periods in the blood. Such systems allow the control of the rate of drug administration which prolongs the duration of the therapeutic effect, as well as the targeting of the drug to specific sites.

Alginate (ALG) and chitosan (CS) are two biopolymers that have received much attention due to their availability and low cost [5] because of the ease of polyelectrolyte complexation between these two [6].

ALG-CS NPs are used as nanocarriers for controlled release of therapeutic proteins, poly-phenols and other drugs [7, 8].

* To whom all correspondence should be sent.
E-mail: marudova@uni-plovdiv.net

Curcumin is a low-molecular weight polyphenol extracted from the herb *Curcuma longa* (commonly known as turmeric). Curcumin possesses antioxidant [9], anti-inflammatory [10], anticarcinogenic, and antimicrobial [11, 12] properties, and suppresses proliferation of a wide variety of tumour cells.

Curcumin is poorly soluble in aqueous solution, which remains a major barrier in its bioavailability and clinical efficacy. Being hydrophobic in nature, it is insoluble in water but soluble in ethanol, imethylsulfoxide, and acetone [13]. To increase its solubility and bioavailability, attempts have been made through encapsulation in liposomes, polymeric and lipo-NPs, biodegradable microspheres, cyclodextrin, and hydrogels [14, 15].

The present article deals with the immobilization of curcumin in chitosan nanoparticles coated with alginate. The effect of the polymer concentrations and of the use of crosslinking agents on the curcumin release kinetics were examined.

## EXPERIMENTAL DETAILS

### *Used material*

High molecular mass chitosan (600-800 kDa) was purchased from Acros Organics. Sodium alginate, sodium tripolyphosphate and curcumin were bought from Sigma-Aldrich (Germany). All other chemicals used were with analytical grade.

### *Synthesis of curcumin-loaded chitosan-TPP nanoparticles*

The preparation of nanoparticles by the ionotropic gelation method is based on the electrostatic interaction between oppositely charged molecules, such as polyanions and polycations. In the case of chitosan nanoparticles crosslinked with sodium tripolyphosphate (NaTPP), the amino groups in the chitosan molecule interact with the anionic groups of the tripolyphosphate salt.

The nanoparticles studied in the present research were prepared according to the following procedure: Different concentrations of chitosan (1 mg/ml or 2 mg/ml) were dissolved in 1% acetic acid solution in distilled water. 5 µl of TWEEN 20 were added to 1 ml of the resulting solutions. Curcumin with concentration of 0. 5 mg/ml - 2 mg/ml was dissolved in ethanol and added to the chitosan solutions. Finally, 100 µl of NaTPP solutions of different concentrations (0.2%, 0.4% or 0.6%) as a cross linker were dropwise added to the emulsified curcumin-chitosan solution. The obtained solutions were stirred for 30 min.

Some of the nanoparticles were further coated with sodium alginate. Solutions with different concentrations of sodium alginate (0.1%, 0.05%, 0.025%) in acetate buffer (pH = 5) were prepared for this purpose. For some samples, the alginate coating was crosslinked with $CaCl_2$.

### *Curcumin-loaded chitosan-TPP nanoparticles characterization*

The size of the formed complexes was determined using a NANOTRAC WAVE Particle Size, Zeta Potential and Molecular Weight Analyzer (Microtrac). The particle diameter was measured as the z-average size.

The shape, size and aggregation phenomena of curcumin-loaded chitosan-TPP nanoparticles were investigated by atomic force microscopy (AFM) AFM NANOSURF FLEX AFM (SWITZERLAND). A drop of the suspensions with nanoparticles was deposited on freshly cleaved mica. After 1 min of incubation the surface was gently rinsed with distilled water to remove unbounded nano-particles. The sample was dried at room temperature (25°C) for 24 h and mounted on the microscope scanner. The AFM images were collected in tapping mode with standard cantilever Tap190Al-G with 10 nm tip radius. The viewing field consisted of 256×256 pixels, revealing the morphology of 2.5 µm × 2.5 µm area from the sample surface. The line scan time was 1 s.

Next, the Fourier transform infrared (FTIR) spectra of curcumin, chitosan, alginate and curcumin-loaded chitosan-TPP nanoparticles were registered on a Vertex 70 spectrometer from 4000 $cm^{-1}$ to 400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ with 25 scans. Ultimately, the samples were mixed with pure potassium bromide (KBr) as the background and compressed into discs using a manual tablet press.

Thermal stability of the curcumin-loaded chitosan nanoparticles and the state of the loaded curcumin were assessed using DSC 204F1 Phoenix (Netzsch Gerätebau GmbH, Germany) based on the heat flux principle and cooled with an intracooler. The heat flo and the temperature ere calibrated ith indium standard (Tm 15 . C, ΔHm 28.5 J/g). The samples were hermetically sealed in aluminum sample pans. An empty pan, identical to the sample pan, as used as reference. The measurements ere performed under argon atmosphere at a heating rate of 10 C/min.

*Calculation of curcumin-loaded chitosan nanoparticles loading efficiency*

To obtain a nano-system with a maximum ratio of drug loading, different weight/weight ratios of chitosan/curcumin were tested. Therefore, various amounts of curcumin were dissolved in a certain amount of chitosan-TPP nanoparticles. Then the resulting system was centrifuged at 10000 rpm for 30 min and the supernatant of the centrifuged curcumin-loaded chitosan-TPP nanoparticles formulation was investigated for absorbance spectra by a spectrophotometer at 430 nm. The loading efficiency was calculated using the following equation:

$$E\% = \frac{(c_0 - c)}{c_0}.100\,, \qquad (1)$$

where $c_0$ is the total amount of curcumin, $c$ is the non-loaded curcumin.

*Investigation of the curcumin release kinetics*

The release of curcumin from the nanospheres was investigated by the diffusion method in dialysis bags. An accurately weighed amount of nanoparticles (equivalent to 34 mg of curcumin) was suspended in 1 ml of 100 mM acetate buffer (pH = 5) and then placed in the bag made the dialysis membrane. The bag was placed in phosphate buffer (pH = 7.4) medium in 100 ml volume containing 150 µl of TWEEN 20 at a temperature of 37 ° C and a shaking speed of 50 rpm. Samples of 500 µl ere taken at pre-established time intervals (15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h, 18 h, 24 h, 48 h, 72 h, 96 h) for spectrophotometric analysis, then an equivalent amount of the buffer used was added back to the release media.

*Investigation of the curcumin phase state*

For this purpose, a DSC 204F1 Phoenix differential scanning calorimeter (Netzsch Gerätebau GmbH, Germany) equipped ith an intracooler for cooling was used. The heat flux and temperature were calibrated to an indium standard ($T_m$ = 156.6 °C, $\Delta H_m$ = 28.5 J/g) at heating and cooling rates of 10 K/min as used in the experiments. Samples with a mass of 10 g were sealed in aluminum crucibles. An empty, hermetically sealed aluminum crucible identical to those of the specimens was used as a reference. Samples were heated from 20 °C to 300 °C with a heating rate of 10 K/min.

## RESULTS AND DISCUSSION

FT-IR spectra were analysed to identify the interactions between chitosan, sodium tripolyphosphate, alginate and curcumin.

The FT-IR spectra of chitosan, NaTPP and blank nano-spheres of chitosan crosslinked with NaTPP are presented in Fig. 1a.

The following bands are observed in the chitosan spectrum: 3454 $cm^{-1}$ (attributed to –OH group or to N-H group), 2872 $cm^{-1}$ (stretched –OH group), 1655 $cm^{-1}$ (C=O group), 1594 $cm^{-1}$ (characteristic N-$H_2$ group), 1382 $cm^{-1}$ (characteristic C-N group) 1154 $cm^{-1}$ (asymmetric C-O-C bond) [16].

The specific bands for NaTPP are: 1095 $cm^{-1}$ (P=O bond) and 901 $cm^{-1}$ (P-O-P group) [16].

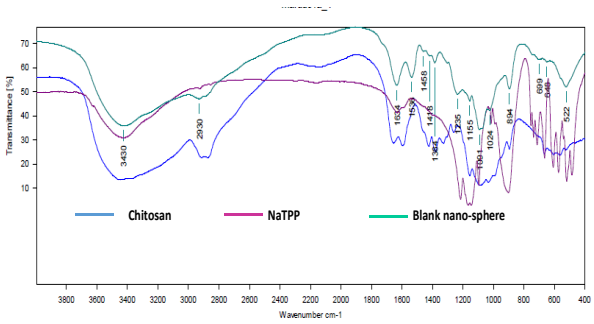


**Fig. 1a.** FT-IR spectra of chitosan, NaTPP and blank nano-spheres

In the blank nano-spheres spectrum, the characteristic chitosan peak at 1655 $cm^{-1}$ is shifted to 1634 $cm^{-1}$ and a new peak at 1536 $cm^{-1}$ appears due to the formation of bonds between the amino groups in the chitosan molecule and the phosphate groups in the anion. These results are consistent with the hypothesis of crosslinking chitosan by NaTPP.

The FT-IR spectra of chitosan, alginate and blank crosslinked chitosan spheres coated with alginate are presented in Fig. 1b.

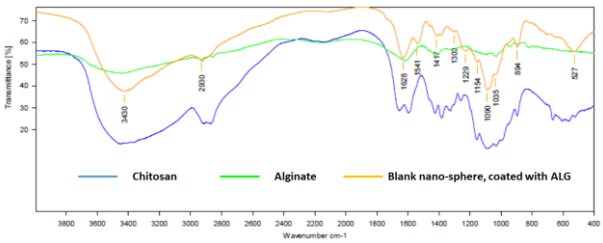


**Fig. 1b.** FT-IR spectra of chitosan, alginate and blank nano-spheres coated with alginate

The characteristics peaks of alginate are: 1618 $cm^{-1}$ (asymmetric vibration of $COO^-$ group), 1420 $cm^{-1}$ (symmetric vibration of $COO^-$ group) and 1031 $cm^{-1}$ (stretching of the C-O-C bond).

Because of the complexation between chitosan and alginate, peak shifting of the asymmetric and symmetrical vibrations of the alginate groups is observed at 1628 $cm^{-1}$ and 1417 $cm^{-1}$, respectively. The chitosan-characteristic group at 1594 $cm^{-1}$ is shifted to 1541 $cm^{-1}$ [17].

Fig. 1c shows the spectra of non-immobilized and immobilized in the chitosan nano-curcumin. The characteristic curcumin bands are observed at: 1603 $cm^{-1}$ (C = C), 1510 $cm^{-1}$ and 1430 $cm^{-1}$ (C-C), 1282 $cm^{-1}$ (C-O), 1207 $cm^{-1}$ (C-O-C) [18]. Comparing the spectra of non-loaded and loaded curcumin, no change in the characteristic bands of the immobilized curcumin are observed. Therefore, it could be assumed that when curcumin is loaded in the chitosan nanoparticles, it does not participate in any chemical interactions.

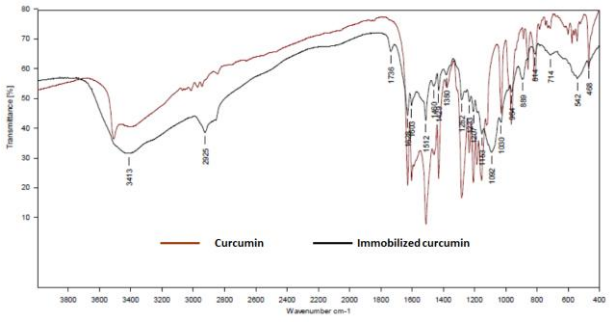


**Fig. 1c.** FT-IR spectra of non-loaded and loaded in the chitosan nano-spheres curcumin

The nano-spheres investigated in the present study were formed by ionotropic gelation of chitosan in the presence of NaTPP crosslinking agent. In order to optimize the composition and particle size, different chitosan concentrations (0.1% and 0.2%) and crosslinker concentrations (0.4%, 0, 6%, 0.8%) were used.

The particle sizes were determined by the Dynamic Light Scattering method. In all samples, together with the individual particles, aggregates were also observed. The average particle diameters are shown in Fig. 2.

Based on the experimental results, at the same concentration of chitosan, the increase in crosslinker concentration results in a reduction of the mean particle size (except for sample 3). A possible cause of diminution could be the formation of a dense structure due to the high concentration of cross-linking areas. Thus, more stable structures could be produced.

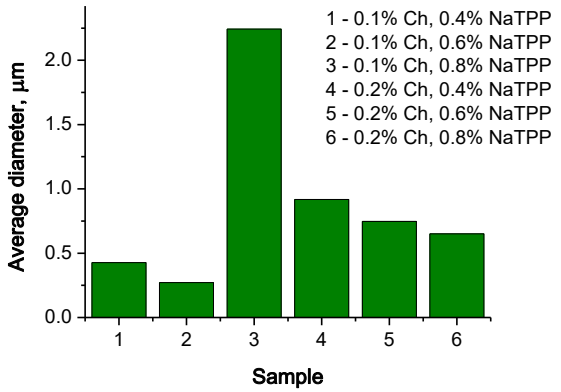


**Fig. 2.** Average sizes of chitosan nano-spheres with different chitosan and crosslinker concentrations

Stabilization of the particles could be achieved by forming a shell of the oppositely charged polyelectrolyte, resulting in a polyelectrolyte complex. Such a coating was achieved with the negatively charged sodium alginate.

The following experiments demonstrated the size of nanoparticles coated with different concentrations of alginate (0.025%, 0.05%, 0.1% - Fig 3. In two of the studied samples, the coating was further stabilized by crosslinking with $CaCl_2$.

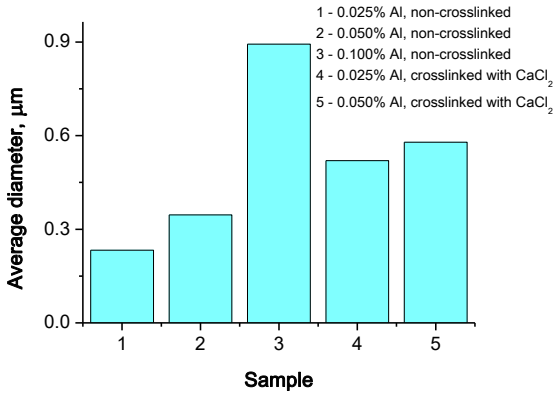


**Fig. 3.** Average sizes of chitosan nano-spheres coated with alginate

Increasing the concentration of sodium alginate results in an increase in size of the coated spheres (samples 1, 2 and 3). The crosslinking of the coating causes further enlargement of the dimensions. This is most likely due to the accumulation of more alginate because of the pairing of molecules by the egg box model.

Shape, size, and aggregation of curcumin-loaded nano-spheres were studied by atomic-force microscopy. Fig. 4 shows a microphotograph of the surface of uncoated and coated nanoparticles, the cores of which are formed from 0.1% chitosan crosslinked with 0.6% NaTPP.

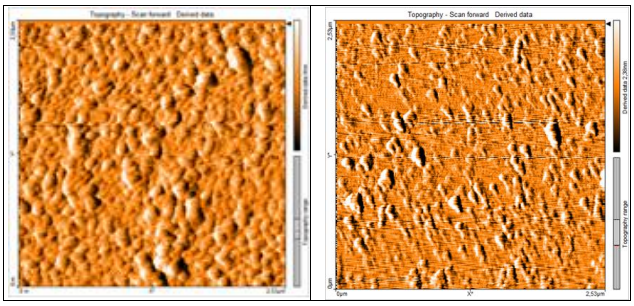

**Fig. 4.** AFM images of non-coated (left) and coated (right) nano-spheres.

It is clear from the presented images that there is a uniform distribution of the particles on the area under investigation. Both individual nanoparticles and particle aggregates, the presence of which is also accounted for by the dynamic lightning process, can be considered. In addition, there is a difference in the morphology of the two types of spheres - the non-coated are almost ideally spherical in shape, and the coated are oval.

In order to determine the optimum composition of the nano-spheres with the highest percentage of loaded curcumin, initial experiments were performed by varying the crosslinker (NaTPP) concentration at constant concentrations of chitosan and curcumin. The optimal concentration was found to be 0.6%, and then experiments with different curcumin concentrations were conducted.

The values for the encapsulation efficiency are presented in Table 1.

**Table 1**. The encapsulating efficiency (%) of curcumin-loaded chitosan-TPP nano-spheres.

| Chitosan, mg/ml | Curcumin concentration, mg/ml | Encapsulation efficiency (%) |
|---|---|---|
| 1 | 0.20 | 85 |
| 1 | 0.15 | 84 |
| 1 | 0.10 | 75 |
| 1 | 0.05 | 62 |

The data in the table and the experiments at the other NaTPP concentrations indicate that the curcumin encapsulation efficiency is high (it is above 60% for all samples tested). This result is in good agreement with the results presented for similar systems by other authors [19]. The highest percentage of loading was observed at the highest initial curcumin concentrations. A concentration of 0.2 mg/ml was chosen to study the kinetics of the curcumin release.

The curcumin release from the nano-spheres was carried out in phosphate buffer at pH 7.4. Fig. 5 presents the results of the release from nano-spheres containing different concentrations of chitosan (0.1% or 0.2%) cross-linked with different concentrations of NaTPP. Biphasic release of curcumin was found for nano-spheres obtained from 0.1% chitosan and crosslinked by 0.6% NaTPP. First, a large amount of medicine was released at a high rate (the so-called burst effect), followed by a delay. The burst release was completed within the first 10 h.

The observed burst effect is probably due to the desorption of the drug located on the surface of the nano-spheres or to the initial swelling and dissolution of the chitosan matrix. Similar results have been reported by other authors [20]. The burst effect can be recognized as a positive effect that guarantees an initial therapeutic concentration of the drug.

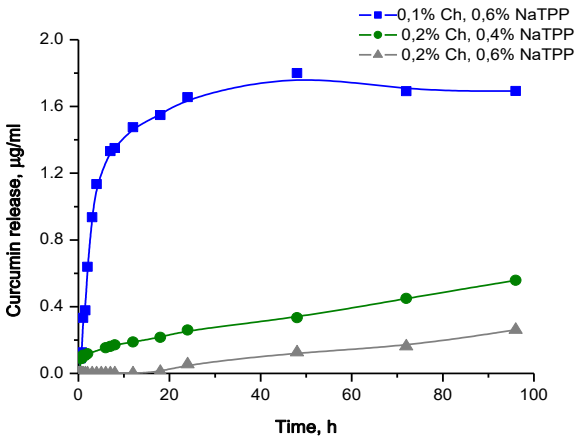


**Fig. 5.** Effect of chitosan and crosslinker concentration on the curcumin release

Increasing the concentration of the chitosan solution from which the nano-spheres were formed from 0.1% to 0.2% results in a drastic change in the curcumin release curve – Fig. 6. In this case, no drug is released during the first 20 h, then the amount of drug released increases linearly over time. A possible reason for the observed behavior is the much larger particle size obtained under these conditions. Generally, the release of a drug from large-sized structures is slower due to the relatively smaller surface contacting the release medium [21].

A change in the concentration of the crosslinking agent did not affect the release behaviour, but only changed the release rate.

To elucidate the release mechanism, the experimental results were described with different kinetic models.

For nano-spheres obtained from a 0.2% solution of chitosan, the release obeys a zero-order kinetic model, with values of the specific zero-order release coefficient of $K_0$ = 0.0005 µg/ml.h for spheres cross-linked with 0.4% NaTPP and $K_0$ =

0.0003 μg/ml.h for spheres cross-linked with 0.6% NaTPP.

The most appropriate kinetic model describing the release from the nano-spheres obtained from 0.1% of chitosan is the Weibull model:

$$M = M_0\left[1 - e^{-\frac{(t-T)^b}{a}}\right], \qquad (2)$$

where $M$ is the amount of released drug as a function of time, $t$; $M_0$ is the total amount of drug being released; $T$ accounts for the lag time measured as a result of the dissolution process; parameter $a$ denotes a scale parameter that describes the time dependence, while $b$ describes the shape of the dissolution curve progression. The model parameters are presented in Table 2.

**Table 2**. Weibull model parameters for curcumin release kinetics from non-coated and alginate-coated nano-spheres

| Model parameters | $M_0$ | b | a |
|---|---|---|---|
| Non-coated spheres, *0.1%* Ch, *0.6 %* NaTPP | 1. 9± 0.04 | 1.05±0.01 | 4.72±0.66 |
| Coated, *0.1%* Ch, *0.6 %* NaTPP, *0.025%* Al | 1. 7± 0.08 | 0. 7± 0.06 | 6.1±0.61 |
| Coated and crosslinked, *0.025%* Al. | 0.85±0.06 | 0.59±0.05 | 6.31±0.49 |
| Coated, *0.050%* Al | 0.7±0.01 | 0. 7± 0.05 | 6.7 ± 0.58 |

Fig. 6 presents curcumin release profiles from coated chitosan nano-spheres obtained from 0.1% of chitosan crosslinked with 0.6% NaTPP. The presence of alginate coatings does not change the release behavior and the experimental results are fitted to the Weibull model (Table 2).

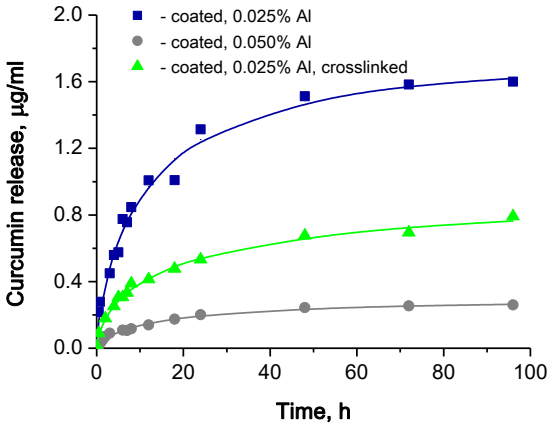


**Fig. 6**. Effect of alginate coating on the curcumin release

The application of an alginate coating decreases the rate of release. Curcumin release rate is manifested in the different values of the parameter $a$ in the Weibull model, which is about 1.5 times greater for the coated spheres (Table 2). The presence of coating leads to a delay in the diffusion of curcumin, which rn causes a lower concentration of curcumin released over the time the systems are tested. This is most likely due to the occurrence of a polyelectrolyte complex between chitosan and alginate, which is difficult to be dissolved in water, and, as a result, the diffusion coefficient of curcumin across the spheres decreases.

At the lowest concentration of the alginate coating, the parameter $M_0$, taking into account the concentration of the released drug, is of the highest value. Increasing the concentration of the coating or the presence of cross-linking results in a significant decrease in the concentration of the released drug. The release time parameter for all three models has very similar values.

The thermal stability of the nano-spheres and the phase state of curcumin were investigated by the differential scanning calorimetry (DSC) method.

The resulting thermogram is presented in Fig. 7.

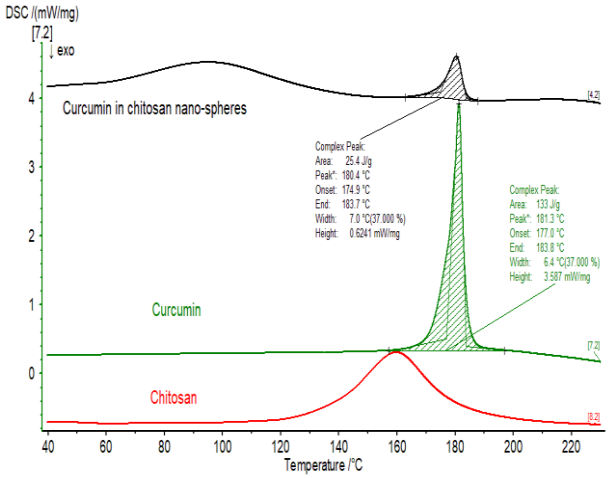


**Fig. 7.** DSC thermograms of non-loaded and loaded in chitosan nano-spheres curcumin

In non-loaded state, curcumin is a crystalline substance characterized by a melting point of 181.3 °C and a specific melting enthalpy of 129.2 J/g.

In loaded state, curcumin's melting peak changes shape, becoming lower and wider, i.e. curcumin crystals melt in a wider temperature range. This fact makes it possible to assume a structure of heterogeneous in size and quality crystals that occurs under unfavorable crystallization conditions. It can be estimated by melting enthalpy that only about 30% of curcumin in the nano-spheres is in the crystalline state. Thus,

the loading of curcumin in the chitosan nano-spheres alters its phase state from crystalline to predominantly amorphous, thereby increasing its bioavailability.

Based on the obtained DSK curves, it can be further concluded that the nanospheres are stable from room temperature up to 300 °C, ith no phase transitions (other than curcumin) or thermal degradation observed within this temperature interval.

## CONCLUSION

Curcumin-loaded chitosan nano-spheres crosslinked with NaTPP were prepared in this study. Some of the spheres were coated with alginate, resulting in a chitosan/alginate polyelectrolyte complex. The average size of the nano-spheres was found to be strongly influenced by the concentration of chitosan and the cross-linking agent, as well as by the presence of alginate coating. The nano-spheres of cross-linked chitosan had a spherical shape and those covered with alginate were oval. Chitosan nano-spheres could be successfully used as a drug delivery system for curcumin. The loading of curcumin into the nanospheres did not lead to chemical interactions between chitosan and curcumin. The percentage of the loaded curcumin was relatively high and, depending on its initial concentration, varied between 62% and 85%. The loaded curcumin was predominantly amorphous, which increased its bioavailability. Increasing the concentration of chitosan in the nanoparticles resulted in a delayed drug release process. Alginate coating impeded the process of curcumin release from the matrix.

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