Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2018, **28**, 598–600

Mendeleev Communications

Fabrication and drug release behavior of ionically cross-linked chitosan microspheres

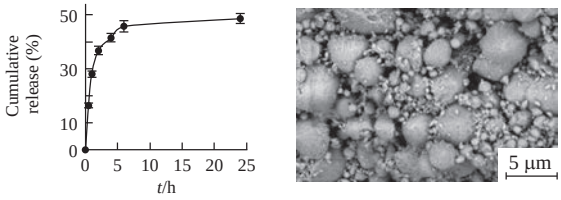
Natalia E. Sedyakina,*[a] Nataliya B. Feldman,[a] Sergey V. Lutsenko[a] and Grigory V. Avramenko[b]

[a] *Department of Biotechnology, I. M. Sechenov First Moscow State Medical University, 119991 Moscow, Russian Federation. Fax: +7 499 248 0181; e-mail: nsedyakina@mail.ru*

[b] *Department of Technology of Chemico-Pharmaceutical and Cosmetic Substances, D. I. Mendeleev University of Chemical Technology of Russia, 125047 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2018.11.011

The chitosan microspheres were prepared by an emulsion-ionic cross-linking method; the interaction between chitosan and an anionic cross-linker was studied, and the average size, surface morphology, insulin sorption and drug release profile of formulated microspheres were evaluated.



Controlled and sustained drug delivery systems have received extensive attention because they can overcome some drawbacks of traditional drug formulations.[1–5] Chitosan is a promising biomaterial for the design of drug carriers due to its nontoxicity, biodegradability, biocompatibility and mucoadhesive properties.[6] In addition, chitosan is suitable for chemical modification and capable of forming covalently or ionically cross-linked hydrogels.[7,8] Chitosan microspheres have gained significant research attention as oral protein and peptide delivery systems owing to the favorable properties of the polymer, the large specific surface area of particles and the ability to encapsulate drugs at high levels.[9–13] They protect proteins from degradation in the stomach environment and ensure their prolonged release in the small intestine.

The preparation of chitosan microspheres requires the use of a cross-linking agent such as glutaraldehyde, formaldehyde, glyoxal and genipin.[14,15] However, they are toxic and cause different undesirable effects.[16,17] To overcome these disadvantages, the ionic cross-linking interaction between the positively charged amino groups of chitosan and the negatively charged cross-linker counterions can also be performed. For example, the preparation of microspheres and microparticles *via* ionic cross-linking of chitosan with citric acid as a nontoxic cross-linking agent was reported.[18–21] Chitosan microspheres were obtained in polymer solution at pH 4.0. It is well known that there are low charge densities of citric acid at pH < 4.3 and chitosan at pH > 6.3.[22] Hence, the pH range of 4.3–6.3 is preferred for ion pair formation.

The aim of this study was to prepare microspheres as potential carriers for peptides and proteins using chitosan cross-linked with citric acid. The interaction between chitosan and citric acid at elevated pH was studied and the polymer-to-cross-linker ratios were chosen to produce the microspheres. The chitosan microspheres were prepared by an emulsification-crosslinking technique using polyglycerol-6-polyricinoleate-15 (PG-6-PR) as a nontoxic nonionic stabilizing agent. The properties of formulated microspheres such as average size, surface morphology, peptide sorption and drug release profile were evaluated.

The interaction of citric acid and chitosan at a high polymer solution concentration (10 g dm^{-3}) was studied.† The chitosan-to-citric acid mass ratio was varied from 0.3 to 4.0. The curve of the relative turbidity of the system (A/A_{max}) was plotted against the mass ratio of the components.[23]

The relative turbidity considerably increased as the polymer-to-cross-linking agent mass ratio was decreased from 1.0 to 0.6 (the chitosan monomer-to-citric acid molar ratio changed from 1.2 to 0.7) (Figure 1).

This fact indicated the formation of an insoluble complex due to electrostatic interaction between citrate and chitosan at a molar ratio of about 1 : 1. Based on the results obtained, the chitosan-to-citric acid mass ratios of 0.6, 0.8 and 1.0 (molar ratios of 0.7, 0.9 and 1.2, respectively) were chosen to produce the microsphere samples S1, S2 and S3, respectively. The microspheres were fabricated according to a previously described method.[13] The microsphere sample S0 containing no cross-linking agent was also prepared.

According to the FT-IR data,‡ there is a difference in the spectra of the samples (Figure 2). The cross-linked microspheres

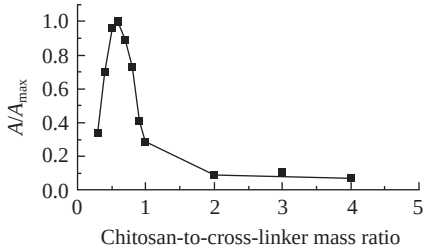


Figure 1 Dependence of relative turbidity (A/A_{max}) on chitosan-to-citric acid mass ratio in the system.

† The citric acid solutions were added to the chitosan solutions (pH 5.0) at different component ratios. Chitosan (82% deacetylation; number-average molecular weight [M_W], 200 kDa) from Bioprogress (Russia) was used. The samples were stored at room temperature for several days, vortexed (Vortex Mixer, VWR International, Germany) and diluted by a factor of 50. Optical density at 226 nm was determined for each sample with a UV-VIS spectrophotometer (Helios Zeta, Thermo Fisher Scientific, USA). The highest relative standard deviation did not exceed 10% (n = 3).

‡ The FT-IR spectra were recorded in KBr on an IR-380 spectrophotometer (Nicolet, Thermo Electron Corporation, USA).

(S1–S3) showed new peaks at 1730 and 1390 cm^{-1}, which were assigned to the C=O stretching vibrations of carboxyl groups and symmetric stretching vibrations of carboxylate ions, respectively. These results suggested the presence of ionized citric acid within a chitosan matrix.[24] Hence, observed changes can be attributed to ionic interaction between the protonated amine groups of chitosan and the carboxylate ions of citric acid.

The SEM studies[§] revealed that microspheres were heterogeneous in size and had a relatively smooth surface except for some wrinkles (Figure 3). For all the formulations obtained, the aggregation of microspheres during the steps of washing and drying occurred. The cross-linked samples (S1–S3) were nearly spherical in shape. Microspheres in S0 were formed despite the absence of a cross-linking agent from the system, most likely, due to the interaction of chitosan and the surfactant used. Uncross-linked microspheres were not spherical because of the deformation of their chitosan-surfactant shells in the course of separation and drying.[13] Compared to uncross-linked microspheres (7.2 μm), the mass-average diameter of the microspheres of S1–S3 (5.1–5.5 μm) decreased due to a higher density of the cross-linked polymer matrix.

To estimate encapsulation efficiency (EE, %), loading capacity (LC, %) and *in vitro* drug release from the cross-linked and uncross-linked microspheres, human insulin as a model drug was incorporated into the samples S2 and S0 by sorption.[¶] The encapsulation and release of a drug from the microspheres depend mainly on the swelling ability and the degree of degradation of microspheres, which were determined by the cross-linking density. To evaluate the effect of a chitosan matrix on insulin loading and drug release profile, the physicochemical characteristics of selected formulations were examined (Table 1).[††] The rise in the swelling ratio and the decrease in the erosion and the zeta-potential (ZP, mV) of S2 compared to those of S0 were attributed to the cross-linking of chitosan molecules and the formation of a cross-linked chitosan gel within microspheres owing to the presence of citrate.

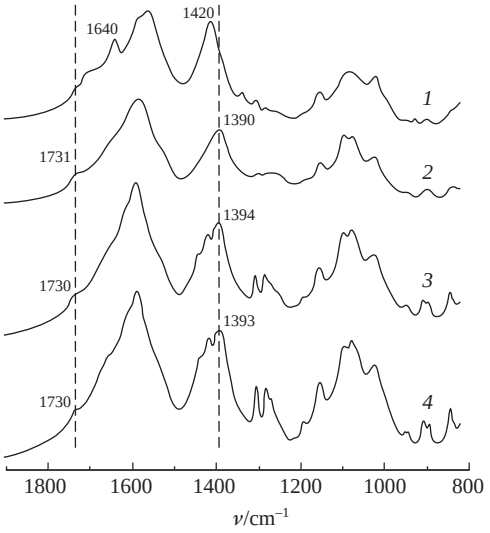


Figure 2 FT-IR spectra of chitosan microspheres prepared (*1*) in the absence of cross-linker (sample S0) and at the chitosan-to-citric acid ratios of (*2*) 0.6, (*3*) 0.8 and (*4*) 1.0 (samples S1, S2 and S3, respectively).

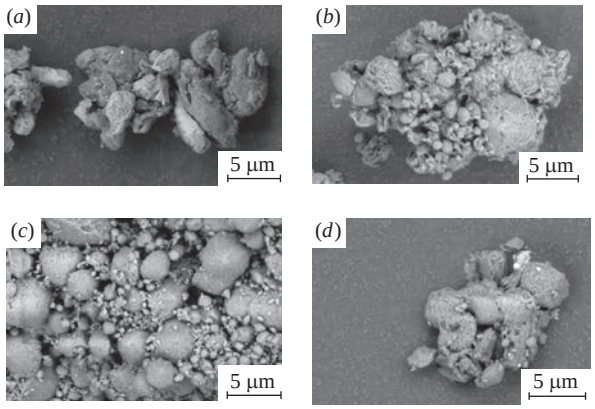


Figure 3 SEM microphotographs of chitosan microspheres prepared (*a*) in the absence of a cross-linker (sample S0) and at chitosan-to-citric acid ratios of (*b*) 0.6, (*c*) 0.8 and (*d*) 1.0 (samples S1, S2 and S3, respectively).

Table 1 Characteristics of the chitosan microspheres.

Sample	D_m/μm	ZP/mV	Swelling ratio (%)	Erosion (%)	EE (%)	LC (%)
S0	7.2 ± 0.7	7.3 ± 1.8	132.7 ± 9.5	66.1 ± 3.7	5.1 ± 0.2	6.8 ± 0.5
S2	5.5 ± 0.5	–0.05 ± 0.01	148.3 ± 3.3	51.2 ± 5.0	35.8 ± 3.5	34.0 ± 1.2

The microspheres of S2 showed a greater ability to sorb the peptide compared to uncross-linked formulation due to a higher swelling ratio (see Table 1). The *in vitro* release of insulin from the samples was studied.[‡‡] The uncross-linked microspheres produced a faster initial burst release of the peptide (Figure 4).

These results can be explained by a higher degree of degradation of the sample S0 and the formation of a gel within the cross-linked sample S2. The slower release of insulin from the cross-linked microspheres was observed, and approximately 50 wt% of the peptide was released into the PBS within 24 h. In contrast, the chitosan microspheres prepared at a lower pH value demonstrated the complete release of the peptide in the same time as that reported previously.[11] The reason is probably related to an increased efficiency of the capture of insulin molecules by a more tightly cross-linked polymer matrix of the microspheres S2. The prolonged peptide release is an important characteristic in the development of effective drug delivery systems.

These results indicate that the citrate cross-linked chitosan microspheres are a promising platform for the development of systems for the long-term delivery of peptide and protein drugs.

[§] The surface morphology and size of the microspheres were estimated using a raster electron microscope (MIRA 3, Tescan, Czech Republic). Mass-average diameters of particles (D_m, μm) were calculated using the equation $D_m = \sum n_i D_i^4 / n_i D_i^3$, where n_i is the number of particles with diameter D_i (μm).

[¶] The encapsulation efficiency and loading capacity of each sample were examined by suspending ~30 mg of microspheres in a 1.55 g dm^{-3} solution of insulin (Actrapid HM, Novo Nordisk, Denmark) in phosphate-buffered saline (0.01 M PBS, pH 7.4). The suspension was stored for 48 h at room temperature, and the loaded microspheres were then separated by centrifugation and dried in a desiccator at 40 °C. The drug content of the supernatant was determined by Bradford's protein assay. EE and LC values were calculated as the encapsulated insulin divided by the total peptide amount used for entrapment or the weight of dry microspheres, respectively.

[††] Swelling ratio and erosion of the batches were determined by a slightly modified method described previously.[25] The unloaded microspheres were incubated in PBS (pH 7.4) at room temperature for 6 and 48 h, respectively. Then, the particles were separated by centrifugation. In the first case, they were reweighed after wiping of excess of liquid with a tissue paper. To evaluate the mass loss, the collected microspheres were washed twice with distilled water by centrifugation, dried in an oven at 40 °C for 24 h and reweighed. The swelling ratio and erosion were calculated as the mass change divided by weight of initial dry microspheres. Zeta-potential measurements were performed with Zetasizer Nano ZS (Malvern Instruments, UK). The samples were prepared according to reported procedure.[26]

[‡‡] The microspheres were incubated in 0.01 M PBS (pH 7.4) at 37 °C in a thermostatic shaker under mild agitation (100 rpm). At predetermined time intervals the supernatant was collected by centrifugation. The amount of insulin released from the microspheres was determined by the Bradford's protein assay.

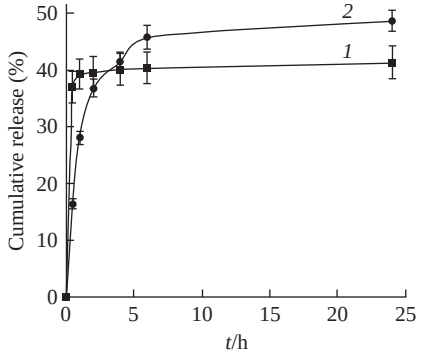


Figure 4 *In vitro* percentage cumulative release of insulin from the (*1*) uncross-linked S0 and (*2*) cross-linked S2 chitosan microspheres.

The FT-IR spectra were recorded using the equipment of the Mendeleev Center for Collective Use.

References

1 A. O. Elzoghby, *J. Control. Release*, 2013, **172**, 1075.
2 N. G. Balabushevich, N. V. Borzenkova, V. A. Izumrudov, N. I. Larionova, O. A. Bezborodova, E. R. Nemtsova and R. I. Yakubovskaya, *Appl. Biochem. Microbiol.*, 2014, **50**, 206 (*Prikl. Biokhim. Mikrobiol.*, 2014, **50**, 232).
3 L. Ya. Zakharova, R. R. Kashapov, T. N. Pashirova, A. B. Mirgorodskaya and O. G. Sinyashin, *Mendeleev Commun.*, 2016, **26**, 457.
4 A. S. Joshi, C. C. Patil, S. S. Shiralashetti and N. V. Kalyane, *Drug Invent. Today*, 2013, **5**, 229.
5 A. V. Sybachin, O. V. Zaborova, K. M. Imelbaeva, V. V. Samoshin, V. A. Migulin, F. A. Plamper and A. A. Yaroslavov, *Mendeleev Commun.*, 2016, **26**, 276.
6 T. A. Sonia and C. P. Sharma, *Adv. Polym. Sci.*, 2011, **243**, 23.
7 A. S. Kritchenkov, S. Andranovitš and Yu. A. Skorik, *Russ. Chem. Rev.*, 2017, **86**, 231.
8 A. V. Odinokov, D. Yu. Dzhons, A. V. Budruev, A. E. Mochalova and L. A. Smirnova, *Russ. Chem. Bull., Int. Ed.*, 2016, **65**, 1122 (*Izv. Akad. Nauk, Ser. Khim.*, 2016, 1122).
9 V. R. Sinha, A. K. Singla, S. Wadhawan, R. Kaushik, R. Kumria, K. Bansal and S. Dhawan, *Int. J. Pharm.*, 2004, **274**, 1.
10 S. Jose, J. F. Fangueiro, J. Smitha, T. A. Cinu, A. J. Chacko, K. Premaletha and E. B. Souto, *Eur. J. Med. Chem.*, 2013, **60**, 249.
11 L. Y. Wang, Y. H. Gu, Z. G. Su and G. H. Ma, *Int. J. Pharm.*, 2006, **311**, 187.
12 A. A. Yaroslavov, A. A. Efimova, F. D. Mulashkin, G. N. Rudenskaya and G. G. Krivtsov, *Mendeleev Commun.*, 2018, **28**, 140.
13 N. E. Sedyakina, A. N. Zakharov, A. F. Krivoshchepov, A. P. Pribytkova, Y. A. Bogdanova, N. B. Feldman, S. V. Lutsenko and G. V. Avramenko, *Colloid Polym. Sci.*, 2017, **295**, 1915.
14 K. C. Gupta and F. H. Jabrail, *Carbohydr. Res.*, 2006, **341**, 744.
15 J. Karnchanajindanun, M. Srisa-ard and Y. Baimark, *Carbohydr. Polym.*, 2011, **85**, 674.
16 D. P. Speer, M. Chvapil, C. D. Eskelson and J. Ulreich, *J. Biomed. Mater. Res.*, 1980, **14**, 753.
17 C. Nishi, N. Nakajima and Y. Ikada, *J. Biomed. Mater. Res.*, 1995, **29**, 829.
18 J. Varshosaz and R. Alinagari, *Iran. Polym. J.*, 2005, **14**, 647.
19 X. Z. Shu and K. J. Zhu, *Int. J. Pharm.*, 2002, **233**, 217.
20 I. Orienti, K. Aiedeh, E. Gianasi, V. Bertasi and V. Zecchi, *J. Microencapsulation*, 1996, **13**, 463.
21 N. E. Sedyakina, A. O. Silaeva, A. F. Krivoshchepov and G. V. Avramenko, *Mendeleev Commun.*, 2018, **28**, 70.
22 X. Z. Shu, K. J. Zhu and Weihong Song, *Int. J. Pharm.*, 2001, **212**, 19.
23 M. A. Krayukhina, N. A. Samoilova, A. S. Erofeev and I. A. Yamskov, *Polym. Sci. Ser. A*, 2010, **52**, 243 (*Vysokomol. Soedin., Ser. A*, 2010, **52**, 394).
24 G. Sailakshmi, T. Mitra, S. Chatterjee and A. Gnanamani, *J. Biomater. Nanobiotechnol.*, 2013, **4**, 151.
25 K. G. H. Desai and H. J. Park, *J. Microencapsulation*, 2005, **22**, 377.
26 C. Zhang, Y. Cheng, G. Qu, X. Wu, Y. Ding, Z. Cheng, L. Yu and Q. Ping. *Carbohydr. Polym.*, 2008, **72**, 390.

Received: 24th April 2018; Com. 18/5554