CHITOSAN AND CHITOSAN-POLYETHYLENIMINE MICROSPHERES PREPARED BY MEMBRANE EMULSIFICATION AND THEIR APPLICATION FOR DRUG DELIVERY SYSTEMS

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Abstract

The aim of this paper was to prepare chitosan microspheres characterised by a narrow dispersion of dimensions, suitable for application for drug delivery systems (DDS). The materials, unmodified as well as functionalised using polyethyleneimine were then used for encapsulation of drug-mimicking dyes. The polymeric beads were prepared by one stage process of membrane emulsification. The encapsulation and then evaluation of the release of an active component was carried out using two cytostatics-mimicking dyes: methyl orange and Congo red. The efficiency of encapsulation changed with a type of microspheres and the type of a dye. The highest sorption towards dyes was revealed by PEI-modified chitosan microspheres. Methyl orange was released with the better efficiency than Congo red from all types of microspheres.

Keywords: chitosan, polyethyleneimine, microspheres, membrane emulsification, sorption, release

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1. Introduction

Many kinds of disease require long-term treatment. Pharmacological treatment of these diseases might be successful if an active substance can be maintained at a constant concentration in plasma. Researchers are constantly looking for new forms of drugs which would allow frequent dosage protocols to be overcome. The target is to achieve a predictable and reproducible release of an active substance over a long period of time. Synthetic and natural polymers have been considered as potential carriers for construction of drug delivery systems (DDS).Their application has reached a significant clinical level [1]. As examples, the following drug delivery systems can be mentioned: liposomes, dendrimers, systems of microemulsion/emulsion, micelles, gels, inorganic or organic particles (e.g. polymer) or biological systems (e.g. viruses) [2].

The materials for construction of DDS are biodegradable polymers, as they are often safer for the body and can protect an occluded active substance against rapid metabolic degradation. They should be biodegradable and give biocompatible or non-toxic products. These materials must simultaneously gradually release dispersed or dissolved drugs [3].

The naturally occurring polysaccharide chitosan has been shown to be amenable to functionalisation to produce a range of versatile materials with substantial potential for biomedical applications [4].Chitosan is a natural polysaccharide comprising copolymers of glucosamine N-acetylglucosamine [5] and can be derived by partial deacetylation of chitin. After cellulose, it is the most abundant natural polysaccharide on earth and can be obtained from the exoskeletons of marine crustaceans such as crabs, lobsters, shrimps and krill on a large scale. Chitosan is a copolymer of 2-amino-2-deoxy-D-glucose and 2-acetoamido-2-deoxy-D-glucose units linked with beta-(1→4)bonds [6]. Its properties depend largely on the degree of deacetylation and on its molecular weight. Because of chitosan's properties, such as non-toxicity, biocompatibility, biodegradability and mucoadhesion, it is very useful as a material for forming microspheres used as pharmaceutical carriers [7]. While chitosan provides these advantages, it has also some disadvantages that must be minimised. Chitosan has poor chemical stability and mechanical strength in acidic environments, which limits its application in adsorption processes. Crosslinking is an effective method to improve the stability and mechanical durability of chitosan materials in acidic solutions [8]. Nevertheless, the crosslinking reaction usually occurs on the amine groups of chitosan, leading to a decrease in adsorption capacity [9, 10]. Therefore, many researchers have tried to modify its properties. Recent studies have shown that this limitation can be overcome by the modification of chitosan with other functional materials [9], such as poly(ethylene glycol) (PEG) or polyethyleneimine (PEI) [9,11]. Because of the good biological activities of chitosan and PEG, a combination of chitosan and PEG may have beneficial effects on the biological characteristics of the modified chitosan materials [8]. Generally, most chemicals (dye, metals, etc.) are adsorbed by chitosan in a process where molecules are attracted by the positive charges on the amine groups of chitosan. Therefore, it is believed that the increase of the positive charge density on chitosan by grafting a cationic polymer, e.g. polyethyleneimine, can improve the adsorption capacity of chitosan [9].

Microspheres can be obtained from various polymers by crystallisation or by the removal of a solvent from emulsion or suspension. Sometimes, precipitative polymerisation is applied [12,13]. One interesting method for preparing monodispersed microspheres is the membrane emulsification process. The method in comparison to

emulsification by stirring provides fairly uniform beads whose size can be controlled mostly by the pore size of a membrane. Membrane emulsification is also characterised by lower energy than the classical stirring method [14].

The goal of this paper was to establish a method for the preparation of monodispersed chitosan or chitosan-polyethyleneimine beads that could be used in the preparation of drug delivery systems. The potential use of the obtained materials as carriers for substances mimicking cytostatics – azo dyes – was investigated. For this purpose, two dyes were selected: methyl orange and Congo red. Their chemical structures are displayed in Figures 1 and 2, respectively. Both of these chemicals have groups characteristic for cytostatics; furthermore, they differ in molecular weight and hydrophilicity (Table 1).Such an approach allowed the effect of molecular mass and solubility of the chemical on utilisation of the prepared drug release systems to be checked.

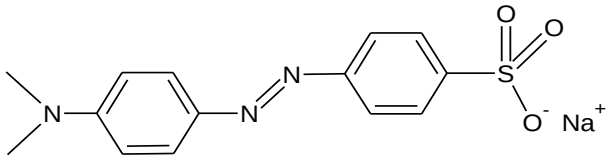

Figure 1. The structure of methyl orange.

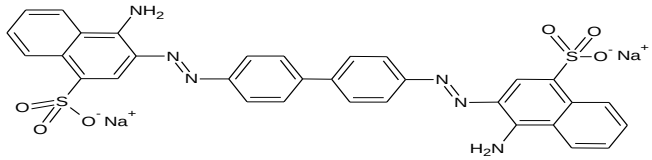

Figure 2. The structure of Congo red.

Table 1. Properties of the applied dyes

Dye	F. W.(g mol^{-1})	Solubility in water (g L^{-1})*	CAS number
Methyl orange (MO)	327.3	5 at 20°C	547-58-0
Congo red (CR)	696.7	10 at 20°C	573-58-0

*****Information given from the cards of characteristic of the Merck Millipore, producer of both of dyes used during the study.

2. Materials and methods

2.1 Materials

Chitosan with low (20 kDa) and medium (200 kDa) molecular weight with deacetylation degrees of 75–85%, glutaraldehyde 50% solution in water, Span 80 and Span 20, polyethyleneimine (PEI) (10 kDa) were supplied by Sigma-Aldrich. NaCl,

glacial acetic acid, ethanol and acetic acid were purchased from Avantor Performance Materials Poland Ltd. Two dyes, methyl orange (MO) and Congo red (CR),were acquired from Merck Millipore.

2.2 Preparation of chitosan solutions

Two types of chitosan were dissolved in 5 wt% aqueous solution of acetic acid, which contained 0.4 wt% of NaCl. The solution was additionally filtered to remove impurities. Then, the prepared solutions were subjected to a process of membrane emulsification. The content of chitosan in the solutionwas1.5 wt.%(see Table 2) [15].

2.3 Preparation of PEI-chitosan solutions

PEI was added to and mixed witht he prepared chitosan solution. The ratio of PEI:chitosan was maintained at 1:4 wt:wt (see Table 2).

2.4 Preparation of chitosan microspheres and chitosan-PEI microspheres

2.4.1 Preparation of water in oil emulsion

The membrane emulsification (ME) process was carried out in a commercial unit delivered by Micropore Ltd.(see Figure 3), which is described by Kosvintsev et al.[16].The system was equipped with a metal membrane, which had regularly arranged 20 μm pores. The compositions of the chitosan and oil phases are shown in Table 2. A volume of 50 mL of the chitosan phase was forced to flow through the membrane to the tubular reactor, which contained120 mL of the paraffin oil phase. The flow rate of the chitosan phase was set to1 mL min^{-1}. The rotation rate of the stirrer was set to 600 rpm. During this part of the study, the composition of the aqueous phase was varied: the molecular masses of chitosan with/without PEI were tested. Four trails were performed for each condition.

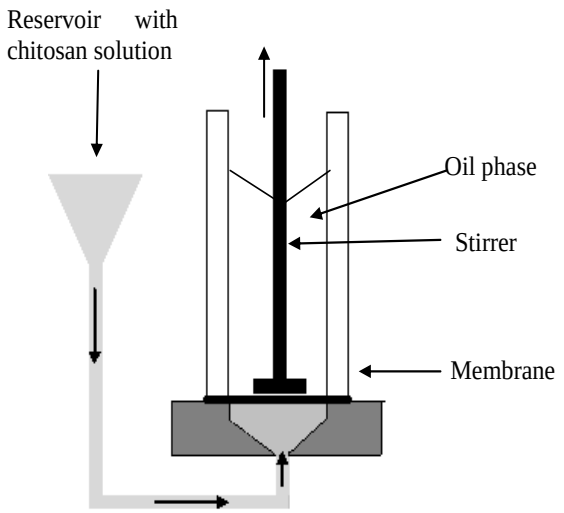

Figure 3. Scheme of Millipore.

Table 2. Compositions of oil and water phases

Continuous phase
Paraffin oil: isooctane volume ratio:7:5 v/v(70mL:50 mL) Span 20 : 2.5 wt% (2.4 g) Span 80 : 2.5 wt%(2.4g)
Dispersed phase
1. Chitosan with molecular mass: **low (sample A)** or **middle (sample B).**Concentration of chitosan in 5% solution of acetic acid:1.5 wt% (1.5 g of chitosan in 98.5 g of acid solution then 50 mL of solution was taken for the emulsification process). 2. Solution of chitosan with molecular mass **low (sample A-PEI)** or **middle (sample B-PEI)** and polyethyleneimine (PEI) with molecular weight 10 kDa and with the weight ratio 1:4 PEI:chitosan (0.38 g of PEI was added into the prepared 100 g of chitosan solution).

2.4.2 Crosslinking process of chitosan

After each emulsification, the prepared water in oil emulsion was placed in a flask equipped with a mechanical stirrer. The crosslinking process was performed for 24 h at room temperature. The amount of glutaraldehyde was maintained at double the amount of chitosan in the emulsion in all cases. After the crosslinking reaction, the dispersion was filtered, and the microspheres were centrifuged twice with isooctane and twice with ethanol. Then the microspheres were dried and extracted with ethanol in a Soxhlet extractor for 24 h to remove the remaining solvents, glutaralaldehyde and other un-reacted chemicals. Finally, the microspheres were dried at 60°C, and after drying, the properties of materials were determined. The scheme of microsphere preparation is given in Figure 4.

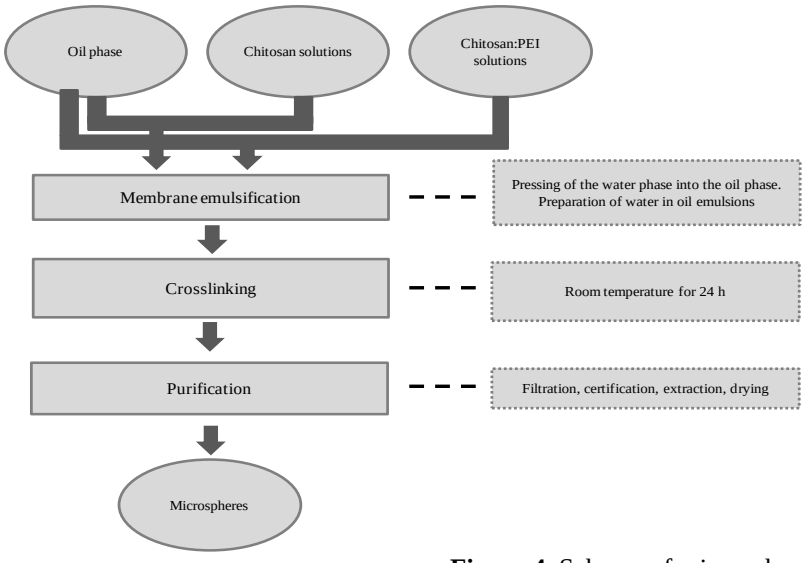

Figure 4. Scheme of microspheres preparation**.**

2.5 Analysis of microspheres

The average diameter of microspheres, as well as the SPAN number (Eq. 1),were detected by means of Mastersizer X (Melvern Instruments GmbH, Germany).

$$SPAN=(d_{90}-d_{10})/d_{50} \quad (1),$$

where:
d_{90}, d_{50},d_{10} are diameters for 90, 50 and 10 percent of the particle population.

Water regain (W_{H2O}) of the polymers was measured by the centrifugation method, in which ca. 1 g of swollen spheres was centrifuged for 5 minutes at 3000 rpm, weighed, dried at 105°C for 24 h and weighed again. Water regain was calculated as follows (Equation 2):

$$W_{H2O} = (m_w-m_d)/m_d\,, \quad (2),$$

where:
m_w(g) is the weight of the swollen polymer after centrifugation and m_d(g) is the weight of the dried polymer [17].

Nitrogen content (Z_N) in the polymer was measured by the Kjeldahl method after mineralisation of a sample (about 200 mg) in concentrated sulphuric acid containing copper sulphate and potassium sulphate [18]. The content of nitrogen was investigated in the samples before the sorption of dyes. All analyses were performed three times for each sample.

2.6 Evaluation of sorption properties

2.6.1 Sorption and release study

The studies on drug sorption and release was carried out using a two-step procedure, involving (1) batch sorption of a dye and (2) its release (desorption). For this purpose, the assay unit (Figure 5) was created.

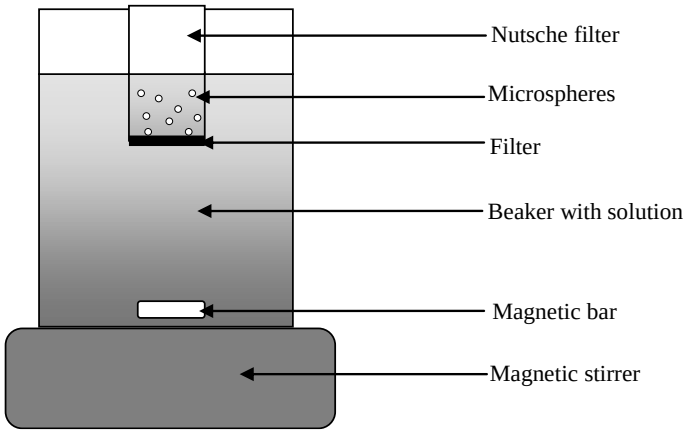

Figure 5. Scheme of sorption/release device.

2.6.2 Batch mode sorption

The sorption of dyes (methyl orange or Congo red) was studied in a batch mode with 4 mg L^{-1} dye solutions at 22 ± 2°C. During all sorption studies, two kinds of dye solution were used: i) methyl orange water solution at a concentration of 4 mg L^{-1} and ii) Congo red water solution at a concentration of 4 mg L^{-1}. About 0.02 g of sorbent was shaken with 20 mL of the solution for 24 hours. After that time, the resins were filtered and the concentration of each dye was determined spectrophotometrically: at 465 nm for methyl orange (MO) or at 497 nm for Congo red (CR) according to the calibration curve of MO or CR. The uptake of dye was calculated according Eq. 3.:

$$q_i = \frac{(c_0 - c_t) \cdot V}{m} \qquad (3),$$

where:
q_i (mmol g^{-1}) is the amount of dye (methyl orange or Congo red) adsorbed at a given time (t), c_0, c_t (mmol L^{-1}) is the liquid-phase concentrations of a dye at the beginning or at a given time (t), respectively, V (L) is a volume of a solution, m (g) is the mass of dried polymers used.

On that basis, the distribution coefficient of sorption, $logK_{i,}$ was calculated as a ratio of the amount of dye adsorbed on 1 g of resin and the amount of dye at equilibrium in 1 mL of solution (Eq. 4) [19]:

$$Ki = \frac{q_i \cdot d}{c_{eq}} \qquad (4),$$

where:
q_i (mmol g^{-1}) is the amount of dye (methyl orange or Congo red) adsorbed at equilibrium, c_{eq} (mmol L^{-1}) is the liquid-phase concentration of dye at equilibrium, d (g L^{-1}) is the density of solution.

Additionally, the encapsulation efficiency (*EE*) was calculated according to Eq. 5:

$$EE = \frac{c_0 - c_{eq}}{c_0} \cdot 100\% \qquad (5),$$

where:
c_0 and c_{eq} (mmol L^{-1}) are the liquid-phase concentrations of a dye at the beginning or at the equilibrium state, respectively.

The process of sorption was performed three times for each sample, allowing the determination of the error of the obtained results displayed together in corresponding tables.

2.6.3 Drug release study

The release of the adsorbed drug-mimicking dyes from the prepared microspheres was carried out in aqueous solution of sodium chloride, 4 wt%, at 37°C. The concentration of NaCl was altered to match a typical physiological fluid (0.9%). This is because patients with cancer, especially those who have been subjected to long-term

treatment, often experience changes in the electrolyte concentrations of their physiological fluid. For this reason, the present study aimed to test the release of an active component in such "extreme" conditions

The samples with adsorbed dye were placed again into the mesh filter and located into the beaker as shown in Figure 5. Then, a20 mL portion of NaCl solution was added. At selected time intervals, 2.0 mL of the solution was taken, and the amount of released methyl orange or Congo red in the sample was determined spectrophotometrically. After concentration analysis, the sample was returned to the beaker. The process of release was investigated three times for each sample.

2.6.4 Sorption kinetics

Sorption kinetics was determined for all samples. The typical procedure was as follows: 20 mg ± 0.1 of microspheres were placed into a mesh filter. After this step, the polymer in the mesh filter was put into a beaker equipped with a magnetic stirrer. To the beaker, 20 mL of a solution of methyl orange or Congo red was added, and the solution was mixed gently. The concentration of the solutions was 4 mg L^{-1} in both cases. Next, the 3 mL samples of the so-prepared suspension was taken each 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 120, 150, 180, 240, 300, 360, 420, 480, 720, 1080, 1440 min. to determine the dye concentration and then sample was returned to the system. The uptake of a dye was calculating according to Eq. 3. The scheme of the sorption-release system is given in Figure 5.When sorption was completed, a saturated sorbent was rinsed with deionised water, dried and used in the release process. The process of kinetics was investigated three times for each sample.

3. Results and discussion

The main objective of the study was to select parameters of the process of preparing narrow dispersed chitosan microspheres, which can be used for the formation of DDS. During the study, the composition of the continuous phase was the same as selected during our earlier work [15]. As the oily phase, a paraffin oil and isooctane mixture with the volume ratio of 7:5 was used. A1:1 mixture of Span 80 and Span 20, with concentration of 2.5 wt% each, was added as a stabiliser [15]. Four different samples of microspheres were prepared. The first type were unmodified particles obtained from chitosan with low (sample A) or medium (sample B) molecular weights. The next type of samples were prepared from solutions of chitosan and PEI. In all cases, the weight ratio of PEI to chitosan was 1:4 wt:wt. After emulsification, the prepared emulsions were taken into a reactor and the crosslinking process was performed. The amount of glutaraldehyde was double the amount of chitosan in the aqueous phase. After each process of microsphere preparation, the efficiency of the process was calculated according to the theoretical mass of microspheres that should be obtained after the process. The results are presented in Table 3,and are the average of five trials. As can be seen, the process efficiency did not depend on the conditions of microsphere preparation, as the average value in all cases was about 75–80%. Some microspheres were lost during the cleaning procedure, particularly the particles with the lowest diameters. This could be the reason for lower productivity in the processes.

In the membrane emulsification process, the greatest influence on the size of the prepared droplet and hence microspheres (d) was the pore size of the membrane (d_m). This can be described by a linear relationship $d = x \cdot d_m$, where x can range typically from 2 to 10 [14]. In our study, one membrane with a nominal pore diameter of 20 μm was

used, and the obtained beads had about 2.5 time larger diameters than the size of the pores. The addition of PEI into the water phase had no effect on the size of the prepared microspheres or on the polydispersity index. The results of the dynamic light scattering (DLS) analysis are given in Table 3.

The prepared sorbents were characterised by the content of nitrogen and water regain typical for the description of a sorbent(see Table 3). The nitrogen content was investigated before encapsulation of the dyes. During the study, it was observed that unmodified materials had about half the nitrogen content, and both unmodified chitosan samples were characterised by the same content of N, which was about 3.0 mmol g^{-1} and the same values of water regain (about 1.1 g of water per 1 g of dry sample). For materials prepared from the solution of chitosan and PEI, the nitrogen content and value of water regain was higher than for their analogues without modification. This indicates that modification was complete, resulting in more nitrogen groups.

Table 3. Properties of chitosan microspheres with and without modification

Sample	$M_{chitosan}$	Modification	Efficiency (%)	W_{H2O} (g g^{-1})	Z_N* (mmol g^{-1})	$d_{average}$ (μm)	*SPAN*
A	low	no	75±5	1.1±0.1	2.9±0.1	47±5	0.8±0.1
A-PEI	low	yes	79±6	1.9±0.1	5.9±0.1	50±7	0.9±0.1
B	medium	no	80±6	1.2±0.1	3.0±0.1	45±6	1.0±0.1
B-PEI	medium	yes	82±6	2.0±0.1	5.9±0.2	48±4	1.0±0.1

$M_{chitosan}$–molecular weight of chitosan, W_{H2O}–water regain (calculated from Eq. 2), Z_N–content of nitrogen*****, nitrogen content was investigated before encapsulation of dyes into the spheres, $d_{average}$ – the average diameter of particles, ***SPAN*** –index of polydispersity calculated from Eq. 1, **Efficiency** – the amount of obtained microspheres relative to the theoretical calculated mass of microspheres.

3.1 Evaluation of sorption properties

The sorption properties of the prepared materials were investigated to evaluate their usefulness for loading of dyes as the model of a cytostatic drug. Two dyes with different molecular weights – methyl orange (327.3 g mol^{-1}) and Congo red (696.7 g mol^{-1})– characterised by different hydrophilicity were used (see Table 1). The calculated uptake of dyes and the distribution coefficients of methyl orange and Congo red are shown in Table 4. The unmodified materials showed a slightly lower sorption capacity towards dyes than the modified microspheres. This probably results from the amount of nitrogen in the materials. The modified samples have double the nitrogen content and hence double the ion exchange groups in the polymeric matrix. The biggest differences between the encapsulation efficiency (see Table 4: the values of the uptake of dyes) was in the case of Congo red between modified and unmodified materials. The equilibrium capacity was about double for the modified chitosan microspheres.

Table 4. Sorption properties of chitosan microspheres with and without modification

Sample	q_{MO} [mg g^{-1}]	q_{CR} [mg g^{-1}]	$logK_{MO}$	$logK_{CR}$	EE_{MO} [%]	EE_{CR} [%]
A	2300±150	8300±320	3.6±0.03	3.5±0.01	91±2	86±3
A-PEI	3200±170	9700±350	3.8±0.02	3.7±0.02	97±2	96±2
B	1900±170	5500±270	3.6±0.02	3.6±0.02	88±3	87±2
B-PEI	2600±180	11000±350	3.8±0.02	3.7±0.03	96±2	96±2

q_i – the uptake of dye (i) at equilibrium (calculated from Eq.3 and recalculated according to the molecular weight of the appropriate dye), K_i – distribution coefficient (calculated from Eq. 4), EE_i–encapsulation efficiency (calculated from Eq. 6), ***i***–MO or CR, **MO** – methyl orange, **CR** –Congo red.

The next part of the sorption properties study was the investigation of the encapsulation process according to time. Figures 6a and 6b show the kinetics of methyl orange and Congo red uptake. It can be noted that the process of sorption depends on the type of sample and type of dye. The maximum uptake of dyes for sample A (low molecular weight of chitosan) and B (medium molecular weight of chitosan) was reached after5 hours for both dyes. For samples A-PEI and B-PEI, the maximum uptake of dye was related to the type of adsorbed substance and the type of material. It should be noted that modification increased the sorption capacity and accelerated the sorption process. As was mentioned above (see Table 4), better sorption properties of the modified samples are associated with a greater number of ion-exchange groups.

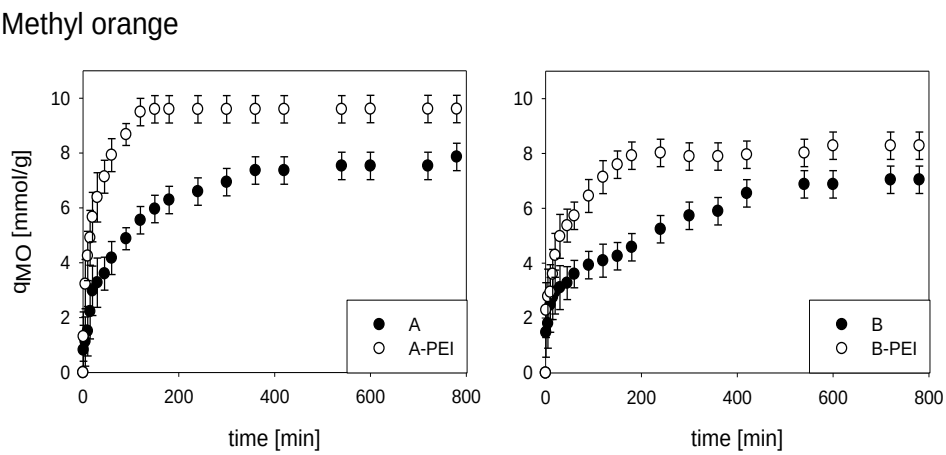

Figure 6a. Kinetic sorption of methyl orange for prepared samples

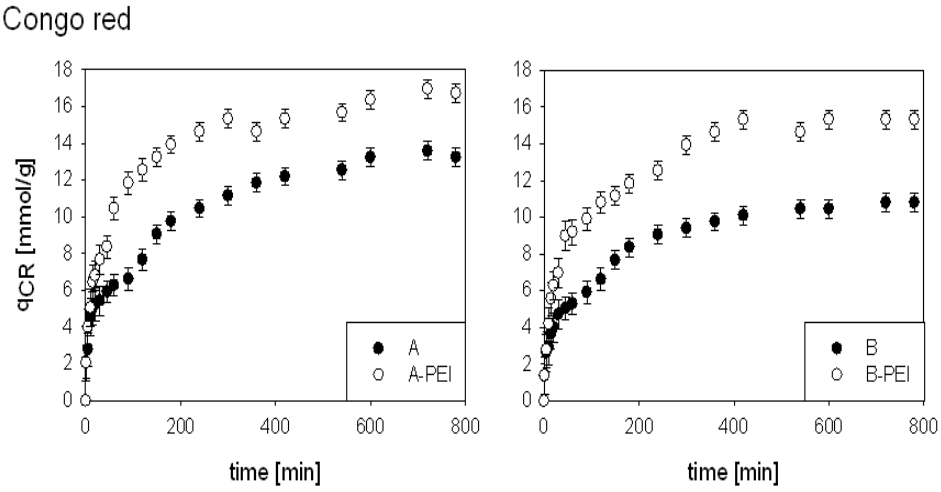

Figure 6b. Kinetic sorption of Congo red for prepared samples.

Sorption kinetics is the feature that describes the sorption efficiency. In order to examine the rate controlling mechanism, the diffusion model and conventional kinetics model were applied.

The kinetics data were fitted to diffusion models derived from Fick's second law (equations 6 and 7) [20,21].

$$k_a t = -\ln(1-\frac{q_t}{q_e}) \qquad (6)$$

where:

q_t and q_e represent the amount of adsorbed species (mmol g^{-1}) at any time, t, and at equilibrium time, respectively, and k_a represents the sorption rate constant (min^{-1}).

The sorption rate constant k_a (min^{-1}) can be calculated from the plot of $-\ln(1-\frac{q_t}{q_e})$ vs. time.

$$k_b t = -\ln(1-(\frac{q_t}{q_e})^2) \qquad (7)$$

where:

k_b is the sorption rate constant (min^{-1}), q_e and q_t are the amount of adsorbed species (mmol g^{-1}) at equilibrium and at time t.

The sorption rate constant k_b (min^{-1}) can be calculated from the plot of $-\ln(1-(\frac{q_t}{q_e})^2)$ vs. time.

Tables 5a and 5b give the calculated values of k_a and k_b and the correlation coefficients. Analysis of k_a and k_b shows for which material the process of sorption was faster. It could be observed that the values of k were larger for the sorption of methyl

orange compared with the *k* calculated for Congo red. This means that for the investigated samples, the sorption equilibrium was reached faster for methyl orange. This can be explained by differences in the molecular weight of the two dyes: the smaller dye has the greater possibility of getting through the pores of the sorbents. The analysis of kinetics also confirmed that the sorption on the modified samples ran faster than on their analogues without modification. The analysis of the correlation coefficients show the kind of diffusion that mostly controlled the process. For all investigated samples and for both dyes the correlation coefficients (R^2)were higher in the case of the particle diffusion model. Hence, this process determined loading on a sorbent.

Table 5a. Analysis of kinetic studies for methyl orange

Sample	$k_a t = -\ln(1-\frac{q_t}{q_e})$		$k_b t = -\ln(1-(\frac{q_t}{q_e})^2)$	
	k_a	R^2	k_b	R^2
A	$5.0 \cdot 10^{-3}$	0.990	$4.0 \cdot 10^{-3}$	**0.996**
A-1	$1.7 \cdot 10^{-2}$	0.963	$1.3 \cdot 10^{-2}$	**0.990**
B	$6.1 \cdot 10^{-3}$	0.990	$5.0 \cdot 10^{-3}$	**0.995**
B-1	$1.7 \cdot 10^{-2}$	0.971	$1.0 \cdot 10^{-2}$	**0.990**

Table 5b. Analysis of kinetic studies for Congo red

Sample	$k_a t = -\ln(1-\frac{q_t}{q_e})$		$k_b t = -\ln(1-(\frac{q_t}{q_e})^2)$	
	k_a	R^2	k_b	R^2
A	$4.5 \cdot 10^{-3}$	0.970	$3.8 \cdot 10^{-3}$	**0.993**
A-PEI	$8.0 \cdot 10^{-3}$	0.979	$6.6 \cdot 10^{-3}$	**0.994**
B	$3.7 \cdot 10^{-3}$	0.976	$2.6 \cdot 10^{-3}$	**0.991**
B-PEI	$6.5 \cdot 10^{-3}$	0.972	$5.9 \cdot 10^{-3}$	**0.995**

3.1.1 Drug release study

For the DDS sample, the process of sorption is not sole one which decided about their utility. The drug release process, when the sorbent frees the loaded drug species, is very important. For this reason, the kinetics of desorption was also studied. The release curves for methyl orange and Congo red are shown in Figures 7a and 7b.

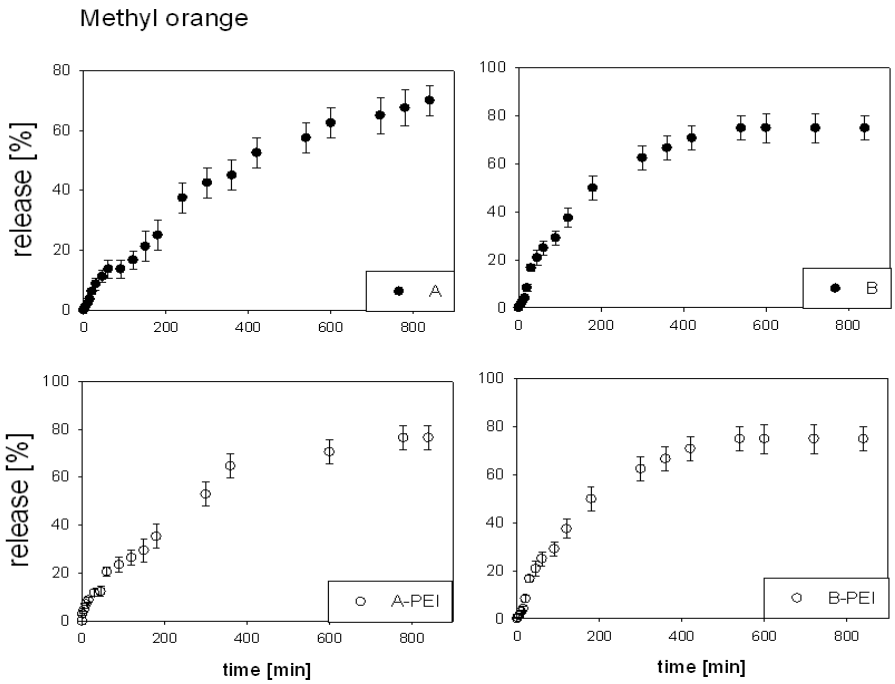

Figure 7a. Percentages of methyl orange release from the prepared microspheres

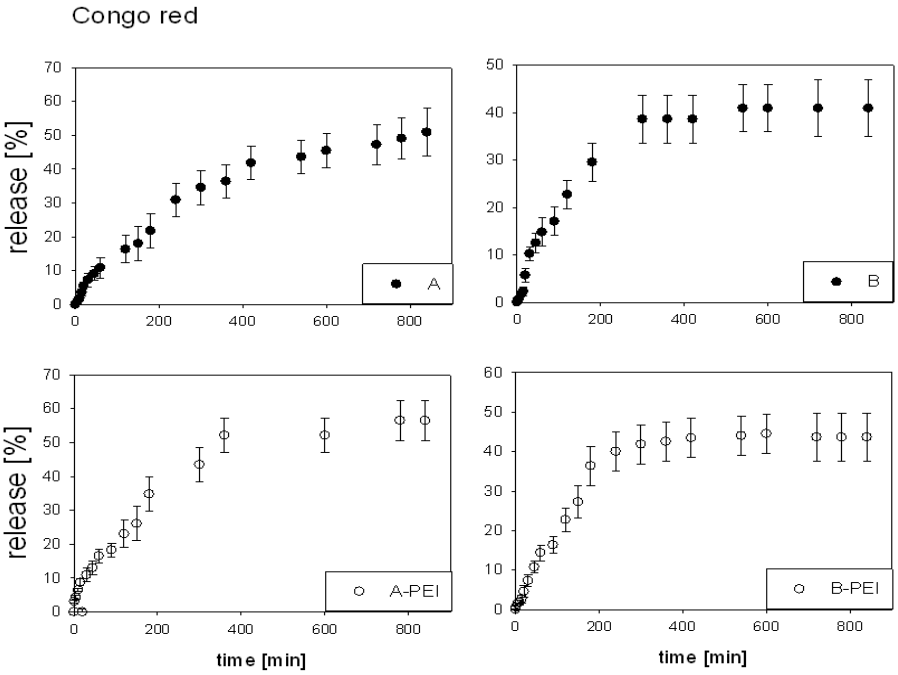

Figure 7b. Percentages of Congo red release from the prepared microspheres

As expected, the release process was related to the type of dye and the type of sorbent. All materials released methyl orange with double the efficiency of Congo red. The microspheres obtained from chitosan were less effective in freeing dyes than their PEI-modified analogues (see Table 6). In the cases of unmodified chitosan microspheres, the process of loading active substances and its release had a comparable rate.

Table 6. Percentages of methyl orange or Congo red release

Sample	$M_{chitosan}$	modification	type	% of MO release	% of CR release
A	low	no	-	70±4	51±4
A-PEI	low	yes	PEI	76±4	57±4
B	medium	no		75±4	41±4
B-PEI	medium	yes	PEI	79±4	44±4

MO – methyl orange, **CR** – Congo red.

4. Conclusions

The membrane emulsification method for microsphere preparation allowed chitosan microspheres and chitosan-polyethyleneimine microspheres with relatively narrow particle size distribution to be obtained. The addition of PEI into chitosan solutions had no influence on the size or polydispersity of the prepared beads. The materials synthesised from the mixture of chitosan and polyethyleneimine had greater values of nitrogen content and took more water than their analogues prepared form neat chitosan. The molecular weight of chitosan did not affect the sorption/desorption processes. However, the loading and release of a dye strictly depended on its molecular weight, i.e. the studied methyl orange was encapsulated and released more efficiently than Congo red. The modified materials were slightly more effective during loading and during the release processes. The results indicate that microspheres prepared from the mixtures of chitosan and PEI could be used as drug carriers.

5. Acknowledgements

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