

The conductivity measurements applied for the evaluation of controlled release of chlorhexidine from thermosensitive N-isopropylacrylamide derivative microgels

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Summary

The aim of the work was the evaluation of the conductivity changes in aqueous environment, consisting of chlorhexidine, and N-isopropylacrylamide derivative microgel, during increasing the temperature between 25°C and 42°C, as a prerequisite to develop the this microgel for controlled release of chlorhexidine, when alterations in temperature are involved. Conductivity of studied systems underwent specific alterations, when temperature increased. For the system with polymer PNM I the values of conductivity were in the range 104,47 $\mu\text{S}/\text{cm}$ - 134,70 $\mu\text{S}/\text{cm}$, for temperature range 25°C and 42°C. In the case of PNM II - CX system, respective values reached 91,75 $\mu\text{S}/\text{cm}$ - 135,95 $\mu\text{S}/\text{cm}$. The lowest conductivity values were observed when PNM III - CX mixture was studied: 96,90 $\mu\text{S}/\text{cm}$ and 117,37 $\mu\text{S}/\text{cm}$.

When a complex of derivatives of N-isopropylacrylamide with chlorhexidine undergoes thermal alteration, there is a potential to obtain controlled release of chlorhexidine from the polymeric bead in the range between 25°C and 42°C. The affinity of chlorhexidine to the polymer may be assessed in this systems applying the conductivity measurements. The solubility of chlorhexidine in the polymeric systems should be in future evaluated, to determine role of this factor in the conductivity alterations.

Key words: chlorhexidine, microgel, N-isopropylacrylamide, pulsed release, thermosensitivity, conductivity

INTRODUCTION

Due to the potency against plaque bacteria, and the tendency to retention on the mucosal surfaces, chlorhexidine is considered to be one of the most effective antiplaque agent [1]. However other potential applications attracted the researchers. Rouse et al. analyzed the vaginal irrigation for the prevention of periparturient infection [2]. Chlorhexidine is advised for reduction of maternal and neonatal mortality and morbidity in the so called low-resource settings; the neonatal, and vaginal chlorhexidine applications demonstrated potential in reducing before mentioned maternal and neonatal mortality and morbidity, in concentrations applied for the irrigation between 0,25 and 1,00% [3].

The antimicrobial activity of chlorhexidine is widely used for the preoperative whole-body disinfection, when prophylaxis against wound infections is required [4]. Changes in skin flora composition were evaluated by many authors. According to that data, the daily scrub with chlorhexidine reduced the skin colonization by antibiotic resistant *Staphylococcus epidermidis* [5]. The application field of chlorhexidine was evaluated to skin disinfection procedure for insertion of peripheral catheters [6].

N-isopropylacrylamide derivatives, structured as spheres of dimension ca. 1 μm attract the researchers, according to the respective thermosensitivity, pH-sensitivity, etc. The new copolymers, consisting the N-isopropylacrylamide, and another co-monomer groups are now synthesized, to obtain particles with proper characteristics, including the chemical, physical, and biological properties. The deposition of biologically active components on the microgel carriers was widely studied.

The iron protoporphyrin IX molecule was bonded to new polymer developed on the basis of imidazole containing poly(N-isopropylacrylamide) microgel, to enable forming of soluble structures containing multiple iron centers [7]. Zhang and coworkers synthesized novel biodegradable thermosensitive microgel with structure similar to Pluronic F127, and evaluated the related technique for the encapsulation of three proteins, i.e. native hemoglobin of molecular weight ca. 67.000, bovine serum albumin

of molecular weight ca. 66.000, and insulin of molecular weight ca. 5.700 [8]. Thermosensitive poly(N-vinyl caprolactam) nanoparticles were used for binding of three model drugs, namely nadolol, propranolol, and tacrine. The applied bioactive molecules influenced the swelling of the synthesized polymer, and for nadolol and propranolol increased binding to polymer was observed, with the increase of the temperature of assessments [9]. The triggered delivery of doxorubicine was evaluated in release experiments [10]. According to authors statements this was the first system engineered to mimic the natural polymer network within the secretory granules, applying the phase transition behavior, in respective environment.

To study the release and the behavior of active compounds in the presence of different carriers, numerous methods are applied, including specific pharmacopeial guidelines [11]. However the evaluation of the bioactive molecules presence in the inner and outer phase of the analyzed system, may be performed by more sophisticated, but still simple methods.

In previous studies an approach was made to assess the release of chlorhexidine and lidocaine from ionic [12] and non-ionic gel vehicles [13]. According to the bibliographical data, the drug release from the carrier to the acceptor compartment may be assessed by drug - selective electrode, where a procaine selective membrane was applied [14].

There are also numerous techniques arising on the background of electrochemical methods, which are applied in biosystems based on the nano-science [15]. The conductometrical analysis per se was evaluated with good results for drug release assessments, in the case of controlled drug delivery systems of naltrexone hydrochloride and morphine hydrochloride [16]. The conductometric methods were also applied with success for the assessment of complexation constants of alprostadil - alphacyclodextrin complex [17]. In last work of Abad-Villar et al. the conductivity measurements were involved to detect the chlorhexidine in eye drops preparations [18].

The aim of the work was the evaluation of the conductivity changes in aqueous environment, consisting of chlorhexidine, and N-isopropylacrylamide derivative microgel, during increasing the temperature between 25°C and 42°C, as a prerequisite to develop the this microgel for controlled release of chlorhexidine, when alterations in temperature are involved.

MATERIALS AND METHODS

Materials

Polymers

pNIPAM microgels obtained in previous works in the field of polymer science. The polymers were synthesized by surfactant free emulsion polymerization (SFEP) in de-ionized water at 343 K, under an inert nitrogen atmosphere. The basic property of the polymers is presented in the Table 1.

Chlorhexidine was supplied by Sigma-Aldrich. Water was purified in the TKA DI 6000 system (Germany), and in this form used for all the experiments. N-isopropylacrylamide >99%, initiators and co-monomer were received from commercial suppliers.

Composition of assessed systems

The composition of evaluated systems was presented in the Table 2. The respective mass of chlorhexidine was composed with synthesized polymer in homogenous mixture. The binding interval was maintained for 24 h, at 22°C.

The conductivity evaluation

The conductivity was evaluated in studied polymeric dispersions, at maintained temperatures: 25°C, 32°C, and 42°C. The conductivity of pure dispersion of chlorhexidine was measured in concentrations applied in the systems. The SevenMulti Metler Toledo, containing conductivity segment TDS/SAL/resistivity with the respective sensor InLab 730, NTC, 0,001-1000 mS/cm, of range -5 °C – 100 °C was used.

RESULTS

Conductivity of studied systems underwent specific alterations, when temperature increased. They are presented in column 3 of Table 3. For the system with polymer PNM I the values of conductivity were in the range 104,47 μ S/cm - 134,70 μ S/cm, in

the range between 25°C and 42°C. In the case of PNM II - CX system, respective values reached 91,75 $\mu\text{S/cm}$ - 135,95 $\mu\text{S/cm}$. The lowest conductivity values were observed when PNM III - CX mixture was studied. The data between 96,90 $\mu\text{S/cm}$ and 117,37 $\mu\text{S/cm}$ were observed for this system.

Respective values for CX only, dispersed in water, were much lower. 46,62 $\mu\text{S/cm}$, 38,08 $\mu\text{S/cm}$, and 41,18 $\mu\text{S/cm}$ values were noted, when 25°C, 32°C, and 42°C temperatures were applied.

In column 4 of the Table 3, basing on the consideration that the conductivity in this systems has additive characteristics, respective theoretical, calculated values are gathered. When PNM I - CX system was heated from 25°C, through 32°C, to 42°C, the values should increase in following manner - 55,15 $\mu\text{S/cm}$, 48,33 $\mu\text{S/cm}$, 64,42 $\mu\text{S/cm}$. For the PNM II - CX system respective values were 58,03 $\mu\text{S/cm}$, 62,74 $\mu\text{S/cm}$, and 85,81 $\mu\text{S/cm}$. In the case of PNM III - CX the values interestingly were ordered as: 58,21 $\mu\text{S/cm}$, 51,39 $\mu\text{S/cm}$, 63,26 $\mu\text{S/cm}$.

The difference between assessed instrumentally and estimated theoretically data are presented as absolute values and as increase in percents, in final two columns, 5 and 6.

DISCUSSION

Thermosensitive polymers, in the form of small spherical particles are characterized by specific property, which enables expanding and collapsing of the polymeric net, when temperature is altered in evaluated system. The alteration in volume of the particle is reflected by alteration in solubility or in so called phase volume. According to that fact, when Lower Critical Solution Temperature (LCST) or Volume Phase Transition Temperature (VPTT) is exceeded the microparticle collapse. Consequently also the functionals, present in the microparticle should be less available, as they are "hidden" inside of the particle. Hence the collapsing, and expanding of the microsphere at different temperature conditions should influence the binding and the release of the drug, or other small molecules in/from the polymeric bead. Actually, in this study the influence of temperature was assessed as factor influencing the conductivity, which reflects the presence of very sparingly soluble drug - chlorhexidine base in the system containing thermosensitive polymer.

According to the Fig. 1, the conductivity of PNM - CX systems increases with the increase of temperature. In former research we identified the tendency of aqueous

dispersions of studied polymers to express increased conductivity when heated [19]. The increase had similar course like the exponential function, with the exception of PNM II polymer, where the increase was much higher at 42°C. This information leads to the suggestion, that the increased temperature influences the activity of ionic groups in the PNM II microgel. That information should lead to the application of the thermosensitive polymer in development of the controlled drug delivery system.

The increase in the conductivity of applied systems in the course of extending temperature was compared between values calculated theoretically and assessed during measurements. The informations from table 3, column 3 were compared to values from the column 4. The regularity was observed in all the cases, i.e. the conductivity predicted, calculated was ca.50% lower than that assessed in the measurements of respective samples. For PNM I - CX mixtures, the measured conductivity at 25°C was 47% higher than that calculated.

According to that it may be assumed, that in this conditions, part of the chlorhexidine is better solubilized than in the case of dispersion of pure chlorhexidine base, or ionization of the total system increased. Also at higher temperatures, 32°C and 42°C, the measured values are 59% and 52% higher, comparing to the theoretically predicted, so there is increase in the presence of ionized substances in the dispersion. We should mention, that this interaction was observed, nevertheless the polymer was synthesized with acidic initiator or alkali initiator, however in the case of PNM I it is slightly higher. The PNM II - CX system behaves similarly - the conductivity is regularly higher in the measurements, comparing the predicted and measured values. Regarding the alkali character of the initiator applied in the synthesis, it would be logical result of the interaction between the polymer and drug, that the increase in conductivity is slightly lower, comparing to PNM I - CX system. This happened actually, and in our assessments the resulting values for that system were: 36,75 $\mu\text{S}/\text{cm}$, 49,53 $\mu\text{S}/\text{cm}$, and 36,88 $\mu\text{S}/\text{cm}$ respectively for the temperatures 25°C, 32°C, and 42°C. The difference in range of 10%, could be assigned to the reversed ionization of the chlorhexidine in the presence of polymer synthesized with the use of alkali initiator.

The data from present experiments, will be further developed, including the influence of the increased lipophilicity of PNM III on the conductivity of the system with chlorhexidine. As it is seen from the Table 3, column 6, the conductivity data for 25°C, 32°C, and 42°C may be interpreted as intermediate level between PNM I - CX and

PNM II - CX. We can conclude, that both lipophilicity, and acidity influence the ionization of the PNM III - CX system.

The relations in a microgel particle, according to the alterations in temperature are depicted on Fig. 2. The $K_{(25)}$, $K_{(32)}$, and $K_{(42)}$ values reflect in this study the affinity of the assessed substance to the microgel in altered temperatures, when the conductivity is measured. Actually the K values differ slightly in various systems, depending both on the applied system and temperature of evaluation. When PNM I - CX, PNM II - CX, and PNM III - CX systems are considered, the values are in following pattern: $K_{(25)} > K_{(32)} < K_{(42)}$. However comparing the affinity of chlorhexidine to the polymers following order should be evaluated: $K_{(PNM II)} > K_{(PNM III)} > K_{(PNM I)}$. The important factor, which should be in future additionally analyzed is the solubility of chlorhexidine in evaluated systems.

CONCLUSIONS

1. When a complex of derivatives of N-isopropylacrylamide with chlorhexidine undergoes thermal alteration, there is a potential to obtain controlled release of chlorhexidine from the polymeric bead in the range between 25°C and 42°C.
2. The affinity of chlorhexidine to the polymer may be assessed in this systems applying the conductivity measurements.
3. In the case of cationic initiator involved in the synthesis, according to the observed conductivity changes the N-isopropylacrylamide polymer may be involved in devices, which release chlorhexidine in controlled manner, when increased temperature is applied.
4. Also the N-isopropylacrylamide derivatives synthesized with anionic initiator, and characterized by increased lipophilicity, may exhibit the controlled release during cooling the polymeric beads.
5. The solubility of chlorhexidine in the polymeric systems should be in future evaluated, to determine role of this factor in the conductivity alterations.

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This research was financed by a Marie Curie Transfer of Knowledge Fellowship of the European Community 6th Frame Program under contract no. MTKD-CT-2005-029540-POLYSURF, and made at the University of Maribor.

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Tabela 1. Charakteryzacja zastosowanych w badaniach mikrożeli

Table 1. Characterization of microgels applied in the present study

Rodzaj polimeru Polymer assignation	Struktura grupy funkcyjnej Structure of the functional
PNM I	$\text{H}-\text{O}-\text{S}(\text{O})_2-\text{O}-\text{R1}$
PNM II	$\begin{array}{c} \text{H}_2\text{N} \quad \text{CH}_3 \\ \diagdown \quad / \\ \text{HC}-\text{C}-\text{R1} \\ \diagup \quad \diagdown \\ \text{H}_2\text{N} \quad \text{CH}_3 \end{array}$
PNM III	$\begin{array}{c} \text{H}_3\text{C} \quad \text{CH}_3 \\ \diagdown \quad / \\ \text{C}-\text{NH} \\ \diagup \quad \diagdown \\ \text{H}_3\text{C} \quad \text{R1} \\ \quad \quad \quad \text{O} \end{array}$

Tabela 2. Skład badanych systemów polimerowych

Table 2. The composition of assessed systems

Skład Composition	PNM I [mg]	PNM II [mg]	PNM III [mg]	CX [mg]	Woda Water [g]
PNM I - CX	5,00	-	-	5,00	60,00
PNM II - CX	-	5,00	-	5,00	60,00
PNM III CX	-	-	5,00	5,00	60,00
CX	-	-	-	5,00	60,00

PNM I, PNM II, PNM III - polimery/polymers, CX - chlorheksydyna/chlorhexidine

Tabela 3. Przewodnictwo badanych układów, w których zastosowano polimery i chloroheksydyne

Table 3. Conductivity of evaluated systems with polymers and chlorhexidine

Temperatura Temperature [°C]	Typ Type	Przewodnictwo/Conductivity [μS/cm]					
		1*	2	3	4	5	6
		Mikrożel Microgel	CX	Wartości zmierzone CX + mikrożel Measured values CX + microgel	Wartości obliczone CX + mikrożel Estimated values CX + microgel	Różnica Poz. 4 i 3 Difference It. 4 and 3	Przyrost Increase [%]
25	PNM-I	8.73	46.4 2	104.74 ±0.77 SD	55.15	49.59	47.34
32	PNM-I	10.25	38.0 8	118.35 ±0.79 SD	48.33	70.02	59.17
42	PNM-I	23.24	41.1 8	134.70 ±0.24 SD	64.42	70.28	52.17
25	PNM-II	11.61	46.4 2	91.75 ±1.04 SD	58.03	33.72	36.75
32	PNM-II	24.66	38.0 8	124.32 ±0.84 SD	62.74	61.58	49.53
42	PNM-II	44.63	41.1 8	135.95 ±0.61 SD	85.81	50.14	36.88
25	PNM-III	11.79	46.4 2	96.90 ±0.79 SD	58.21	38.70	39.94
32	PNM-III	13.31	38.0 8	105.29 ±0.57 SD	51.39	53.90	51.19
42	PNM-III	22.08	41.1 8	117.37 ±0.71 SD	63.26	54.11	46.10

PNM I, PNM II, PNM III - polimery/polymers, CX - chloroheksydyna/chlorhexidine,

*dane opracowane i przedstawione wcześniej, przy analizie systemów zawierających chlorowodorek lidokainy/

data considered and presented in former research which involved the use of lidocaine hydrochloride, SD - odchylenie standartowe/standard deviation

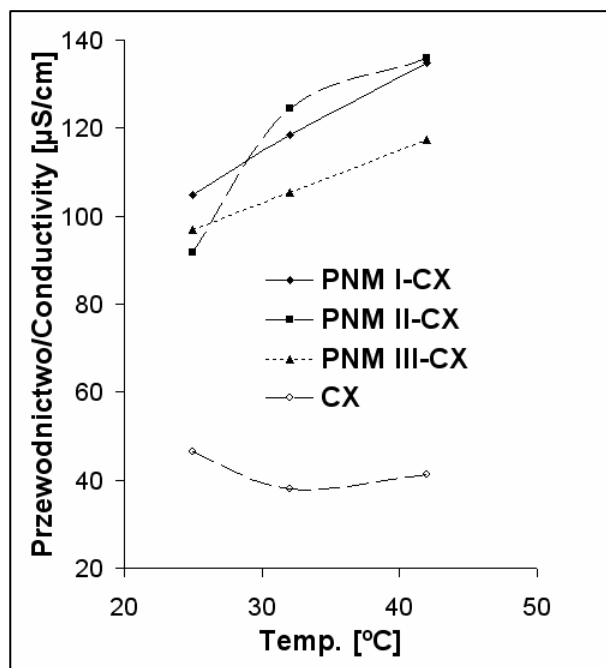
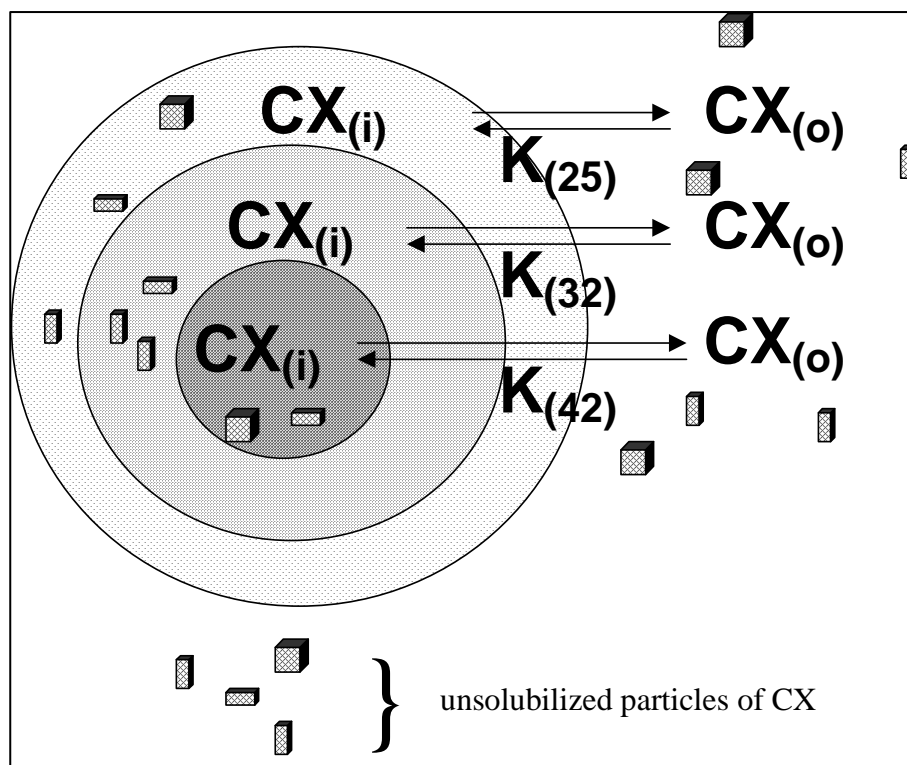


Fig. 1. Wpływ temperatury na przewodnictwo badanych systemów zawierających polimery i chlorheksydyne, oraz na samą chlorheksydyne w układzie wodnym, PNM I - CX, PNM II - CX, PNM III - CX: odpowiednie mieszaniny polimerów i chlorheksydyny, CX: chlorheksydyna

Fig. 1. Influence of temperature on the conductivity of assessed systems containing polymers and chlorhexidine, PNM I - CX, PNM II - CX, PNM III - CX: respective mixtures of polymers and chlorhexidine, CX: chlorhexidine



Ryc. 2. Wizualizacja wiązania chlorheksydyny z mikrożelami, w zależności od temperatury pomiaru. $CX_{(i)}$ - chlorheksydyna związana z mikrożelem, $CX_{(o)}$ - chlorheksydyna poza mikrożelem, gęstość zacienienia odpowiada zmianom morfologicznym zachodzącym w trakcie podwyższania i obniżania temperatury rozproszenia mikrożeli

Fig. 2. Visualization of the influence of temperature on the binding of chlorhexidine with microgels. $CX_{(i)}$ - chlorhexidine bonded to microgels, $CX_{(o)}$ - chlorhexidine outside the microgels, density of the dots represents the alterations during increasing and decreasing the temperature

