

The pH changes of diluted dispersions of poly(N-isopropylacrylamide) below and over the LCST in the presence of chlorhexidine

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Summary

The pH of diluted aqueous dispersions of modified poly(N-isopropylacrylamide) with chlorhexidine was evaluated, at normalized skin surface temperature, as well below and over the lower critical solution temperature value. Three different poly-N-isopropylacrylamides were synthesized by surfactant free emulsion polymerization. They were evaluated in the terms of pH in the aqueous dispersions in the presence of chlorhexidine. The tendency was similar in all investigated systems at increasing temperature between 25°C and 45°C. The pH value decreased from the range between 9,87 - 9,94 down to the range 9,38 - 9,46. The course of pH decrease between the temperature of 32°C and 45°C was more radical, comparing to 25°C and 32°C, however in general the decrease was monotonic.

The systems with chlorhexidine tend to change the pH with temperature increase more radically, in the comparison to the chlorhexidine alone. The formulations applied on the skin surface or in the oral cavity should be evaluated in proper temperature spectrum.

Key words: chlorhexidine, microgels, N-isopropylacrylamide, thermosensitivity

INTRODUCTION

Thermosensitive polymers in the area between 32°C and 36°C have been specifically applied in the medical and pharmaceutical research, as they are close to the interesting range of physiological temperatures of the human body - 37°C of the internal body temperature, and 32°C of the body surface temperature. The microgel particles of poly(N-isopropylacrylamide) are widely known and extensively studied in many fields of biotechnology and medical sciences. They undergo a specific volume phase transition in the point of lower critical solution temperature (LCST), usually at ca. 32°C, when assessed in aqueous solutions [1-3]. In this group of polymeric material the network is stable and the size of the particles is in the range of nanometers and micrometers.

During the swelling, the diffusion is the controlling factor - the rate of the collapsing of the microgel is correlated to the size of pores in the network. On that way the medicinal agents may be delivered in the targeted manner to the desired place of activity [4,5]. The controlled delivery of medicinal agents topically to the skin surface may provide therapeutic levels where required and minimize the undesired systemic absorption of the drug. Binding of relatively large bioactive molecules with the use of thermosensitive polymers was researched i.a. for insulin and prednisone acetate [6,7]. Through the chemical modifications of polymers the release prolongation, or targeted drug delivery may be realized. On the other hand potential drug carriers may influence the natural pH of the body surface, and change the originally acidic environment to high pH values. This sophisticated systems, containing water, thermosensitive polymer and medicinal agent the pH values should be determined at different temperatures, to maintain the safety of preparation applied on the skin.

Between high number of local antiseptics used in common bacterial and fungal skin infections one of the most applied is the chlorhexidine. This bioactive component was evaluated in the terms of release from polymeric vehicles to enhance the antibacterial activity of the formulation [8, 9].

In this study the pH of diluted aqueous dispersions of modified poly(N-isopropylacrylamide) in the presence of chlorhexidine was evaluated, at

temperature assigned as standard human body surface temperature, and below and over the volume phase transition temperature value.

MATERIAL AND METHODS

Materials

Chlorhexidine was supplied by Sigma-Aldrich. Deionized water from the TKA DI 6000 unit (Germany) was used for all the preparations, and respective operations during the assessments. N-isopropylacrylamide 97%, respective initiators and co-monomer were obtained from commercial suppliers and used without further purification. The components used in the buffer solutions were of pharmacopoeial purity.

Applied polymers

pNIPAM microgels particles derived from former experiments in the field of polymer chemistry. They were synthesized by surfactant free emulsion polymerization (SFEP) in deionised water at 343 K, under an inert nitrogen atmosphere, according to the procedure evaluated by Pelton [10] and developed i.a. by Vincent [11] D'Emanuele and Dinarvand [12]. The shortened characteristics of the obtained polymers is presented in the following Table 1.

Composition of evaluated systems

The composition of evaluated systems was presented in the Table 2. Chlorhexidine was combined with the dispersion of synthesized polymer. The merging period was maintained for 24 h, at the room temperature.

pH measurements

The pH of the prepared gels were measured using the SevenMulti Metler Toledo device with attached ION segment, pH/mV/ORP. The pH electrode InLab

413, NTC, pH 0–14, 0–80°C, was used for the measurements, and five repetitions for every measurement were carried out, using deionized water.

Buffer solutions

For comparison of pH at different temperatures, standard buffer solutions were prepared [13]. Buffer of pH 9,18 and buffer solution of pH 10,01 were assigned as more close to the pH range evaluated in present research. Composition of buffers is attached in the Table 3.

RESULTS

LCST of the assessed systems was estimated to be in the range 32°C - 36°C °C. When the increased temperature was applied the pH of assessed polymeric dispersions decreased. In the case of PNM-I the pH decreased from ca. 9,87 at 25°C to ca. 9,74 at 32°C. After consequent increase of temperature up to 45°C the pH decreased to 9,46. Similar course was observed in the dispersion of PNM-II. The high pH value of 9,87 at 25°C decreased to 9,67 at 32°C, and than to 9,39. Also in the case of PNM-III, the pH values decreased from 9,94 to 9,46, with intermediate value of 9,73 at 32°C.

These measurements were compared to normalized buffer solutions of pH 9,18 and pH 10,01 at three different temperatures, and were depicted on the common graph - Figure 1.

For better evaluation of the samples, the area from Figure 1 was enlarged on the graph on Figure 2, and limited to the area between 25°C and 32°C. The bold dotted line represents the pH of chlorhexidine dispersion - the pH of that dispersion was in the range between 9,78-9,79. However the pH of dispersions of chlorhexidine combined with polymers decreased, and the difference between initial temperature (25°C) and intermediate temperature (32°C) was in the range of 0,10 - 0,20 pH unit.

DISCUSSION

Usually with the temperature change, the observed pH would also change, depending of the measured medium. As it is indicated in the following Equation 1 the pH is changing with the increase of the temperature.

$$\text{pH} = \text{pH}_s - (E - E_s)/k \quad \{\text{Eq. 1}\}$$

where: E - potential (in V) developed in the sensing electrode, E_s - standard potential (in V) of the electrode at known pH_s , k - the potential change of pH per pH unit expressed in Volts, according to Nernst equation.

The slight decrease of observed pH is usually observed when alkali buffers are used. Also in the case of low pH values, an increase in acidic buffers, when they undergo heating, is observed. During the sequential measurements of polymer dispersions with chlorhexidine, consequent decrease of pH was observed in the preliminary measurements. The measured differences were statistically significant, and confirmed in multiplied repetitions.

The proposed mechanism of evaluated changes in pH is the thermosensible characteristics of the polymers applied in the research. The particles of polymer are almost totally swollen below the so called LCST, and the chlorhexidine may exhibit its ionic activity. The chlorhexidine molecules may diffuse relatively fast in the aqueous environment of expanded polymer branches, and exhibit its ionic activity. This was confirmed in our research, as the initial pH is in the range of 9,87 - 9,94 at the temperature of 25°C, and decreases to 9,67 - 9,74 in the 32°C. For the non-combined chlorhexidine dispersion the pH in mentioned temperature range is stable - ca. 9,785. The proposed evaluation of the phenomena is given on the Figure 3.

When the temperature is below the LCST, the chlorhexidine (1) may relatively easy diffuse through the polymeric matrix (4), and dynamic equilibrium is maintained between the chlorhexidine entrapped in the polymeric matrix and the chlorhexidine present in aqueous environment (2). With the increase of temperature the polymeric net (4) is collapsing. The chlorhexidine (3) present in the polymeric matrix area may by more strictly bounded to the polymer branches, so the dynamic

equilibrium between the combined (3) and un-combined (1) chlorhexidine moves towards the combined fraction.

Intriguing is the behaviour of chlorhexidine in the presence of polymers, when compared to the pure chlorhexidine dispersions, and should be further examined. The open question is, if the polymer, when present in the expanded form, influences the ionization of chlorhexidine, and enables higher dissolution of the species. When the temperature is increasing, the pH is consequently decreasing, and the rates K_A depicted on the Figure 3, may be ordered: $K_{A(25^{\circ}\text{C})} > K_{A(32^{\circ}\text{C})} > K_{A(45^{\circ}\text{C})}$. The presence of mesostructures was studied in dispersed macromolecules of microgels, where the space spanning networks were formed [14]. This occurrence may also influence the pH decrease the combined mixtures of poly-N-isopropylacrylamides and chlorhexidine.

The separate evaluation should be performed for the enlarged area of dependency between pH and temperature in the range 25°C and 32°C. The almost horizontal, dotted line on the Figure 2 represents the pH of chlorhexidine water dispersion. With the increase of the temperature the difference is in the range of 0,01 pH, whereas for the polymer - chlorhexidine systems the pH decreases in the range of 0,1-0,2 pH unit. This may be elucidated by the initial influence of the polymer on the ionic activity of the chlorhexidine in the aqueous dispersion.

According to Blackburn [15] the chlorhexidine has five ionization sites with pKa values between -4,46 and 10,15. The presence of polymer induces the increased ionic activity of chlorhexidine molecule at the 25°C temperature however with the increase of the temperature to 32°C the polymer particles are collapsing, and the influence on the ionization decreases. This may be schematically represented as on the Figure 4. The respective dissolution assessments should be performed to evaluate in details this hypothesis.

CONCLUSIONS

1. The presence of chlorhexidine influences the pH patterns of N-isopropylacrylamide polymers observed at various temperatures.

2. In the analyzed systems the tendency was similar in all systems, at increasing temperatures between 25°C and 45°C. The pH value decreased from the range between 9,87 and 9,94 down to the range between 9,38 and 9,46. The course

of pH decrease between the temperature of 32°C and 45°C was more radical, comparing to 25°C and 32°C, however in general the decrease was monotonic.

3. The systems with chlorhexidine tend to change the pH with temperature increase more radically, in the comparison to the chlorhexidine alone.

4. The formulations applied on the skin surface or in the oral cavity should be evaluated in proper temperature spectrum.

LITERATURE

- [1] Panayiotou M., Pohner C., Vandevyver C., Wandrey C., Hilbring F., Freitag R.: Synthesis and characterization of thermo-responsive poly(N,N'-ethylacrylamide) microgels. *React. Funct. Polym.* (2007), 67, 807-819.
- [2] Janczewski D., Tomczak N., Han M.Y., Vancso G. J.: Introduction of Quantum Dots into PNIPAM microspheres by precipitation polymerization above LCST. *Eur. Polym. J.* (2009), 45, 1912-1917.
- [3] Spevacek J.: NMR investigations of phase transition in aqueous polymer solutions and gels. *Current Opinion in Colloids and Interface Science.* (2009), 14, 184-191.
- [4] Ravaine V., Ancla C., Catargi B.: Chemically controlled closed-loop insulin delivery. *J. Control. Release.* (2008), 132, 2-11.
- [5] Saunders J. M., Tong T., Le Maitre C. L., Freemont T. J., Saunders B. R.: A study of pH-responsive microgel dispersions: from fluid-to-gel transitions to mechanical property restoration for load-bearing tissue. *Soft Matter* (2007), 3, 486-494.
- [6] Nolan C. M., Gelbaum L. T., Lyon L. A.: ¹H NMR investigation of thermally triggered insulin release from poly(N-isopropylacrylamide) microgels. *Biomacromolecules.* (2006), 10, 2918-2922.
- [7] Wei H., Zhang X. Z., Cheng H., Chen W. Q., Cheng S. X., Zhuo R. X.: Self-assembled thermo- and pH responsive micelles of poly(10-undecenoic acid-b-N-isopropylacrylamide) for drug delivery. *J. Control. Release.* (2006), 116, 266-274.
- [8] Manafi A., Hashemlou A., Momeni P., Moghimi H. R.: Enhancing drug absorption through third-degree burn wound eschar. *Burns.* (2008), 34, 698-702..
- [9] Bowker J. M., Stahl P. H., Preparation of water-soluble compounds through salt

- formation. [in:] *Pract. Med. Chem.* 3rd Edition, Ed. by Wermuth C. G., Elseviere, Academic Press, France, 2008, pages 747-766
- [10] Pelton R.: Temperature-sensitive aqueous microgels. *Adv. Colloid Interface Sci.* (2000), 85, 1-33.
- [11] Vincent B., Clarke J., Barnett K. G.: The flocculation of non-aqueous sterically-stabilised latex dispersions in the presence of free polymer. *Coll. Surf.* (1986), 17, 51-65.
- [12] D'Emanuele A., Dinarvan R.: Preparation, characterisation, and drug release from thermoresponsive microspheres. *Int. J. Pharm.* (1995), 118, 237-242.
- [13] Polish Pharmacopoeia, Vol. I, page 112, Warszawa, Ministry of Health, 2006.
- [14] Bischofsberger I., Trappe V.: Intriguing behaviour of gels formed by poly-N-isopropylacrylamide particles. [in:] *UK Polymer Colloids Forum*, University of Greenwich, London, 28-29 August, 2008. Abstract Book.
- [15] Blackburn R. S., Harvey A., Kettle L.L., Manian A. P., Payne J. D., Russell S. J.: Sorption of Chlorhexidine on Cellulose: Mechanism of Binding and Molecular Recognition. *J. Phys. Chem. B.* (2007), 111, 8775-8784.

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Tabela 1. Główna charakterystyka badanych polimerów

Table 1. Main characteristics of assessed polymers

Oznaczenie Assignment	Charakterystyka Characteristics
PNM-I	Inicjator z anionową grupą funkcyjną Initiator with anionic functional groups
PNM-II	Inicjator z kationową grupą funkcyjną Initiator with cationic functional groups
PNM-III	Komonomer z hydrofobową grupą funkcyjną Co-monomer with hydrophobic functional groups

PNM-I, PNM-II, PNM-III - polimery/polymers

Tabela 2. Skład badanych układów

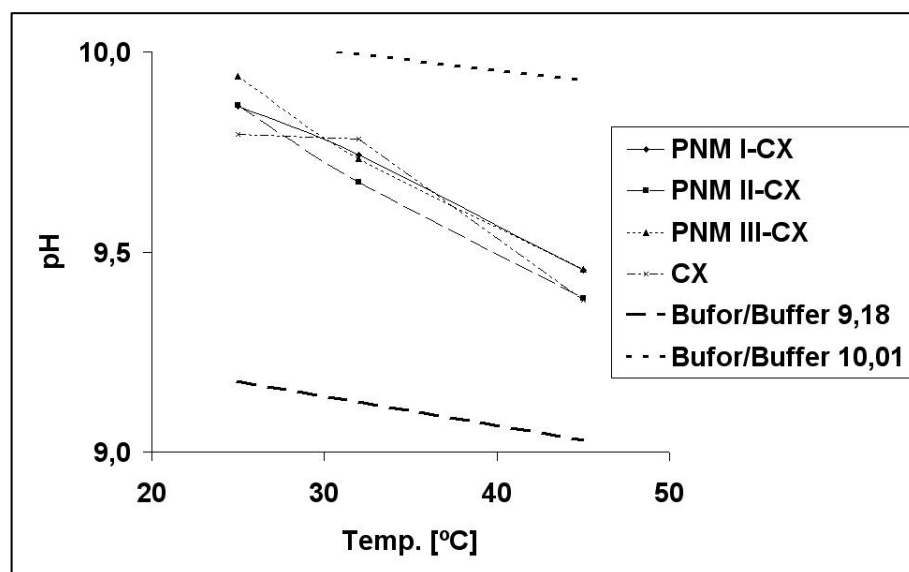
Table 2. The composition of evaluated dispersions

Skład Composition	PNM-I [mg]	PNM-II [mg]	PNM-III [mg]	CX [mg]	Woda Water [g]
PNM-I-CX	2,5	-	-	2,5	30,00
PNM-II-CX	-	2,5	-	2,5	30,00
PNM-III-CX	-	-	2,5	2,5	30,00
CX dispersion/rozproszenie	-	-	-	2,5	30,00

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

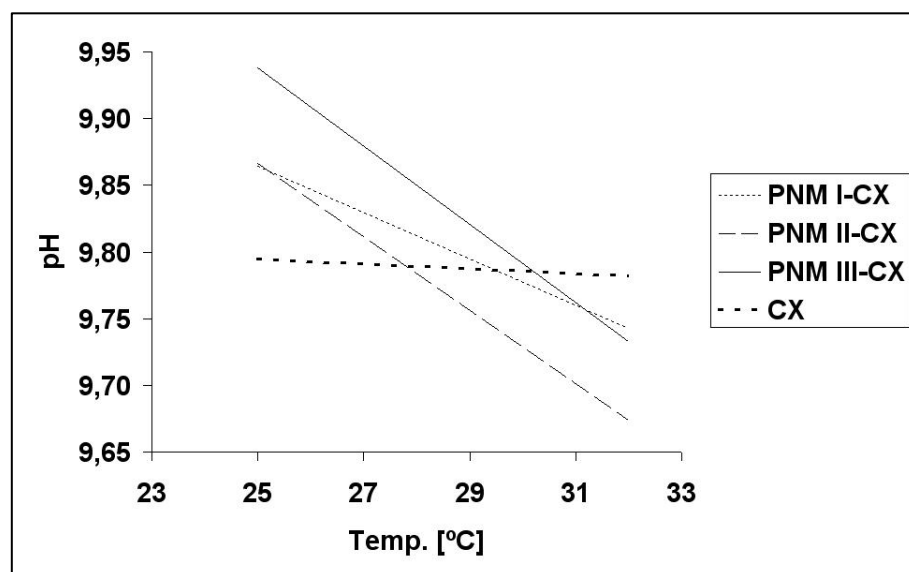
Tabela 3. Skład roztworów buforowych
Table 3. Composition of buffer solutions

Składniki Components	Roztwór buforowy Buffer solution pH 9,18	Roztwór buforowy/ Buffer solution pH 10,01
Tetraboran disodu/Disodium tetraborate [M]	0,01	-
Węglan sodu/Sodium carbonate [M]	-	0,0125
Wodorowęglan sodu/Sodium hydrocarbonate [M]	-	0,0125
Objętość roztworu wodnego/Aqueous solution volume [ml]	1000,0	1000,0



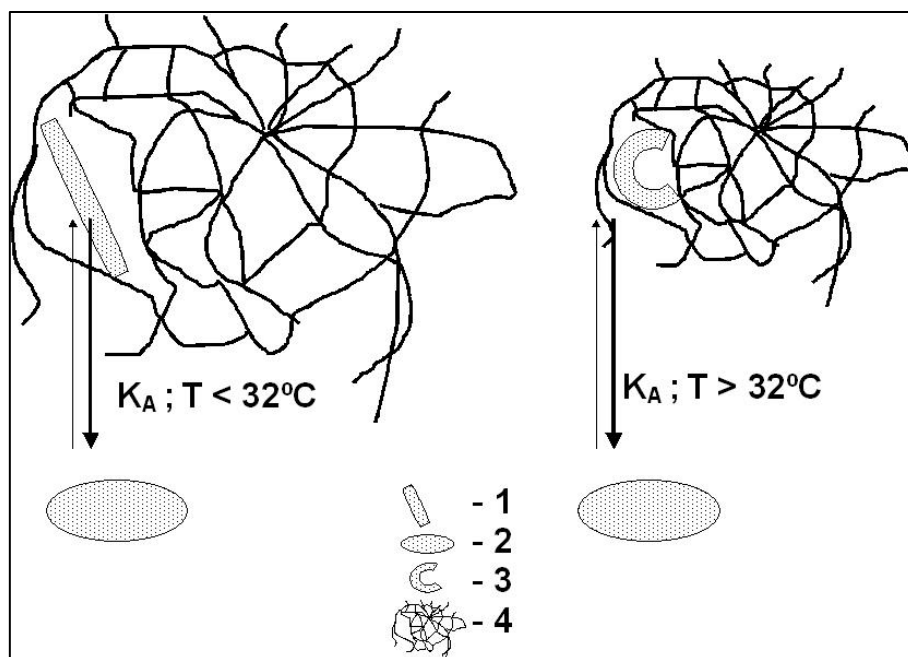
Ryc. 1. Wpływ temperatury na odczyn rozproszeń badanych polimerów N-izopropylakrylamidu i chlorcheksydy

Fig. 2. The pH of dispersions of synthesized polymers and chlorhexidine at different temperatures



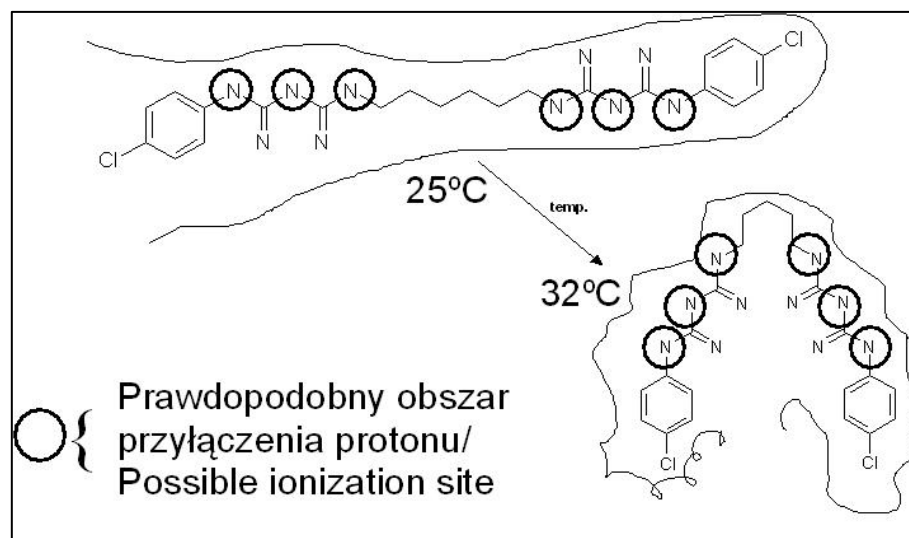
Ryc. 2. Wpływ temperatury na odczyn rozproszeń badanych polimerów
N-izopropyluakryloamidu i chlorheksydyny – powiększony obszar

Fig. 2. The pH of dispersions of synthesized polymers and chlorhexidine at different temperatures – the enlarged area



Ryc. 3. Schematyczne przedstawienie równowag dynamicznych w obrębie systemu polimer-chlorheksydyna - objaśnienia w tekście

Fig. 3. Schematic representation of dynamic equilibria in the system polymer-chlorhexidine – details in the tekst



Ryc. 4. Możliwy wpływ polimeru na jonizację cząsteczki chlorheksydyny

Fig. 4. The possible influence of polymer on the ionization of the chlorhexidine molecule