The effect of PAMAM dendrimers with amine or hydroxyl terminal groups on the bioadhesive properties of hydrogels with clotrimazole

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Abstract: Polyamidoamine dendrimers (PAMAM) belong to a relatively new class of polymers that are evaluated as efficient systems for drug delivery due to their nanosize range, ability to improve solubility, permeability and pharmacological activity of many drugs. In this study, the influence of PAMAM dendrimers of generation 2 (G2) and 3 (G3) with -NH_2 or -OH terminal groups (PAMAM-NH_2 and PAMAM-OH, respectively) on the bioadhesive properties, viscosity and yield stress of polyacrylic acid polymer hydrogels with clotrimazole was investigated. We show that the addition of PAMAM-NH_2 dendrimers G2 and G3 into the hydrogel structures caused, in both cases, an about 2-fold decrease in viscosity of the hydrogels and a 1.3 and 1.4-fold decrease in the value of yield stress, respectively. We also found that PAMAM dendrimers with -NH_2 terminal groups induced the highest decrease in the work of adhesion between hydrogels and the examined adhesive layers.

Keywords: polyamidoamine dendrimers, hydrogels, adhesive properties, clotrimazole, viscosity, polyacrylic acid polymers.

Wpływ dendrymerów PAMAM z aminowymi lub hydroksylowymi grupami końcowymi na właściwości bioadhezyjne hydrożeli z klotrimazolem

Streszczenie: Dendrymery poliamidoaminowe (PAMAM) należą do nowej grupy polimerów badanych pod kątem ich zastosowania jako efektywne nośniki leków. Takim zastosowaniom sprzyjają wymiary nano, zdolność do poprawy rozpuszczalności i przenikania przez błony oraz zwiększania aktywności farmakologicznej wielu substancji leczniczych. W ramach pracy oceniano wpływ dendrymerów PAMAM generacji 2 (G2) i 3 (G3) zawierających końcowe grupy -NH_2 lub -OH (odpowiednio, PAMAM-NH_2 i PAMAM-OH) na właściwości bioadhezyjne, lepkość i granicę płynięcia hydrożeli z klotrimazolem, wykonanych z użyciem pochodnych kwasu poliakrylowego. Wykazano, że dodatek do struktury hydrożelowej dendrymerów PAMAM-NH_2 G2 i G3 powoduje około 2-krotne zmniejszenie ich lepkości oraz, odpowiednio, 1,3- i 1,4-krotne zmniejszenie wartości granicy płynięcia. Stwierdzono również, że dendrymery PAMAM z końcowymi grupami -NH_2 powodują największy spadek pracy adhezji pomiędzy hydrożelami a badanymi warstwami adhezyjnymi.

Słowa kluczowe: dendrymery poliamidoaminowe, hydrożele, właściwości adhezyjne, klotrimazol, lepkość, polimery kwasu poliakrylowego.

Hydrogels are hydrophilic, three-dimensional, polymeric networks that swell in aqueous media. They resemble natural tissues due to their soft consistency and high water content, which contributes to their biocompatibility and makes them useful materials for use as dermal drug delivery carriers [1, 2]. The properties of hydrogels depend on the characteristics of the gelling polymers. The most commonly used polymers for hydrogel formulations are carbomers – polyacrylic acid polymers crosslinked with polyalkenyl polyethers or divinyl glycol. Carbomer hydrogels have many benefits such as high viscosity at low concentrations, compliance with many active substances, thermal stability, patient acceptance and bioadhesive characteristics [3]. The presence of a large number of carboxylic groups, which provides the ability to form hydrogen bonds, contributes to the incremented adhesive contact. Therefore, polyacrylic acid polymers are frequently used in drug delivery systems that enhance drug bioavailability via lengthening the contact time of drug vehicles with the tissue (skin or mucosa) [4, 5].

Dendrimers are nanomolecules with globular shapes, internal cavities and various terminal groups. This relatively novel class of synthetic polymers includes many types of dendrimers: polyamidoamine (PAMAM), polyamidoamine-organosilicon (PAMAMOS), polypropy-

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leneimine (PPI), etc. [6, 7]. The first synthesized, and the most investigated family of dendrimers, are PAMAM, which have been studied as carriers in pulmonary, oral and transdermal drug delivery [8–11]. This class of dendrimers possesses the ability to improve the efficiency of dermal therapy due to their capability to enhance the permeability of the drug molecules through the membranes, improvement of drug solubility and its pharmacological activity [12–16].

Clotrimazole (CLO) – an imidazole derivative – is frequently used in the topical treatment of dermatological and vaginal diseases. However, its lipophilic properties and instability in acidic pH are restricting factors in the development of pharmaceutical dosage form with CLO [17, 18]. Promising drug delivery systems for CLO are hydrogels because CLO exhibits a greater level of diffusion from hydrophilic vehicles than from hydrophobic bases.

To the best of our knowledge, there are no reports describing the effect of PAMAM dendrimers on the adhesive characteristics of hydrogels. Therefore, the aim of this study was to examine the influence of PAMAM dendrimers on the bioadhesive properties of hydrogels with CLO using shaved rat skin, gelatin disc and porcine vaginal mucosa. Moreover, the effect of PAMAM dendrimers on the rheological characteristics of hydrogels were studied.

EXPERIMENTAL PART

Materials

Clotrimazole (CLO) was a gift from Aflofarm (Pabianice, Poland).

Polyamidoamine (PAMAM) dendrimers G2 and G3 with -NH_2 or -OH surface groups (PAMAM-NH_2 and PAMAM-OH, respectively), gelatin type B from bovine skin and polyethylene glycol sorbitan monooleate (Tween® 80) were purchased from Sigma Aldrich (Steinheim, Germany).

Potassium dihydrogen phosphate, sodium hydroxide, ethanol and propylene glycol were provided by Chempur (Piekary Śląskie, Poland).

Propyl *p*-hydroxybenzoate and methyl *p*-hydroxybenzoate were purchased from Caelo Caesar & Loretz GmbH (Hilden, Germany).

Crosslinked polyacrylate polymer with the trade name Carbopol® 980 was a gift from Azelis Poland (Poznań, Poland).

Porcine vaginal mucosa from large white pigs weighing about 200 kg were obtained from the veterinary service (Turośń Kościelna, Poland). Shaved rat skin (excised from the dorsal region of male Wistar rats weighing 150––200 g) was collected according to a protocol approved by the Ethics Commission of the Medical University of Białystok, Poland. Samples of porcine vaginal mucosa and shaved rat skin were stored at -20 °C and before the experiment were defrosted and cut into 5 mm diameter pieces.

Water was distilled and passed through a reverse osmosis system Milli-Q Reagent Water System (Billerica, MA, United States).

Preparation of hydrogels

All hydrogels were prepared using a mechanical stirrer model DT 200 (Witko, Poland). Carbopol® 980 was dispersed in the water and stirred for 45 min and then the mixture was neutralized by the addition of 20 wt % solution of NaOH until a gel appeared. The propyl *p*-hydroxybenzoate and methyl *p*-hydroxybenzoate were dissolved in propylene glycol. Tween® 80 and ethanol were added to the hydrogel base. Finally, CLO in an aque-

T a b l e 1. Composition of the prepared hydrogels

Component	Symbol of hydrogel				
	H-0	H-1	H-2	H-3	H-4
	Content of component, g				
Clotrimazole (CLO)	1.0				
Carbopol® 980	0.35				
Sodium hydroxide solution (20 wt %)	0.5				
Propylene glycol	10.0				
Ethanol	10.0				
Tween® 80	2.0				
PAMAM-NH_2 G2	–	–	–	0.3	–
PAMAM-NH_2 G3	–	–	–	–	0.3
PAMAM-OH G2	–	0.3	–	–	–
PAMAM-OH G3	–	–	0.3	–	–
Methyl *p*-hydroxybenzoate	0.1				
Propyl *p*-hydroxybenzoate	0.1				
Water up to	100.0				

ous solution of PAMAM dendrimers was mixed with the hydrogel base. CLO is stable in alkaline medium, but in acidic medium it hydrolyzes to (*o*-chlorophenyl)diphenyl methanol and imidazole. Therefore the pH of all preparations was set at 6.8 [17]. The prepared hydrogels were stored for six months at 25 ± 0.5 °C and relative humidity of *RH* = 60 ± 5 %. The composition of the prepared hydrogels is shown in Table 1.

Methods of testing

The viscosity was examined using a programmable viscometer RV DV-III Ultra Brookfield (Brookfield, Middleboro, MA, USA) with a cone-plate device (CP-51, 40 mm diameter, 30 μm gap) at a shear rate 8 s^{-1} (25 ± 0.5 °C). Measurements were made in triplicate and new samples were loaded for each run. Before measurement, the samples were left to relax at least 2 min after loading.

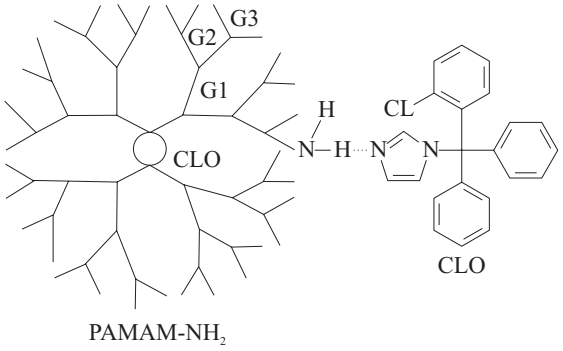
Yield stress was examined using a programmable viscometer RV DV-III Ultra Brookfield (Brookfield, Middleboro, MA, USA) with a V73 spindle at 25 ± 0.5 °C. Measurements were made in triplicate. The influence of PAMAM dendrimers on the viscosity and yield stress were analyzed directly and six months after preparation.

Evaluation of adhesiveness was performed using TA.XT plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) and three different models of adhesive layers: shaved rat skin (excised from the dorsal region), porcine vaginal mucosa and gelatin discs. The experimental parameters of the process were chosen during preliminary tests and set as follows: pretest speed 0.5 mm/s, test speed 0.1 mm/s, contact time 180 s, post test 0.1 mm/s and applied force 1 N. Gelatin discs were prepared by pouring a 30 wt % aqueous solution into a Petri dish. The tests were conducted at 32 ± 1 °C. Each adhesive layer was adhered to an upper probe. The adhesive properties were determined as the work of adhesion – calculated from the area under the force versus distance curve and expressed in μJ.

The quantity of all determined variables were expressed by the mean values and standard deviations. Statistical analyses were performed by ANOVA and conducted using STATISTICA 10.0 software. Differences between groups were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

PAMAM dendrimers are classified as highly defined globular polymers with a large number of surface groups and a compact molecular structure. Active substances can be encapsulated into their interior cavities and/or attached to the surface groups. Scheme A shows an example of the possible interactions between PAMAM-NH_2 and CLO. This unique structure of PAMAM dendrimers make them useful in pharmaceutical applications as solubility, antimicrobial or antifungal activity enhancers [19–21]. In our previous studies [22, 23], it was shown that PAMAM dendrimers enhanced the solubility, antifungal activity and the *in vitro* release of CLO from hydrogels. Therefore, in the designed formulations, PAMAM dendrimers were used as a cosolvent for CLO – a drug commonly used to treat fungal infections. To avoid potential skin irritation, the concentration of PAMAM in hydrogels was set at 3.0 mg/g [24].



Scheme A. Possible interactions between PAMAM-NH_2 and clotrimazole (CLO): encapsulation into interior cavities or/and attachment to the aminesurface groups

The rheological properties of dosage form can affect the characteristics of the final product, for example, viscosity and yield stress influence the spreadability and contact time of active substances with skin or mucosa. Moreover, yield stress helps in estimating the squeezing action that is needed to discharge the semi-solid form from the tube [25].

The results of the viscosity investigations are listed in Table 2. It was found that the examined hydrogels exhibited non-Newtonian characteristics – the viscosity decreased when the shear rate increased. The addition of PAMAM-NH_2 dendrimers G2 and G3 resulted in an about 2-fold decrease in the viscosity of hydrogels (H-3 and H-4), while PAMAM-OH dendrimers G2 and G3 did not lead to such a significant reduction in the hydrogels' viscosity (H-1 and H-2). It is worth noting that all the examined PAMAMs used at a concentration of 3.0 mg/g did not influence the viscosity of the prepared hydrogels during six months of storage. Similar trends were observed during yield stress examinations, with the results collected in Table 3. PAMAM-NH_2 dendrimers G2 and G3 caused about a 1.3 and 1.4-fold decrease in the value of yield stress, respectively. The yield stress of all examined hydrogels did not change significantly after six months of storage.

The addition of PAMAM-OH at a concentration of 3.0 mg/g evoked no significant changes in the hydrogels' pH values. In hydrogels with PAMAM-NH_2, only a slight increase in pH values was observed (from pH = 6.8 in H-0 to pH = 6.9 in H-4).

The bioadhesive properties of the polymers enable the hydrogels to adhere to the biological surface involving skin (adhesion process) and mucosa (mucoadhesion process) and in consequence elongate the retention time of

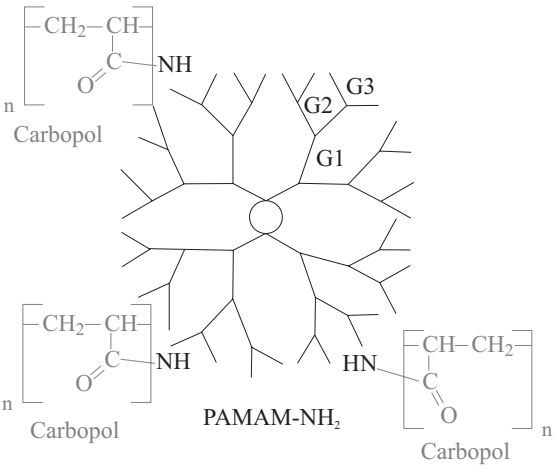
T a b l e 2. The influence of PAMAM-NH_2 and PAMAM-OH G2 and G3 on the viscosity of carbomer hydrogels with CLO

Symbol of hydrogel	The mean value of viscosity ± standard deviation, mPa · s	
	directly after preparation	after six months storage (25 ± 0.5 °C, *RH* = 60 ± 5 %)
H-0	24 292 ± 215	24 202 ± 180
H-1	20 488 ± 236	20 394 ± 198
H-2	17 405 ± 142	17 387 ± 136
H-3	14 014 ± 148	13 906 ± 139
H-4	11 312 ± 151	11 279 ± 142

T a b l e 3. The influence of PAMAM-NH_2 and PAMAM-OH G2 and G3 on the yield stress of carbomer hydrogels with CLO

Symbol of hydrogel	The mean value of yield ± standard deviation, mPa · s	
	directly after preparation	after six months storage (25 ± 0.5 °C, *RH* = 60 ± 5 %)
H-0	69 540 ± 116	69 437 ± 121
H-1	63 390 ± 144	63 288 ± 122
H-2	58 110 ± 159	58 174 ± 160
H-3	54 880 ± 113	54 799 ± 110
H-4	49 740 ± 125	49 822 ± 115

the dosage form at the site of application [26]. Hydrogels possess the ability to deform and an internal mechanism to scatter energy leading to beneficial adhesive features. The results of experiments carried out using three different models of adhesive layers (shaved rat skin, gelatin disc and porcine vaginal mucosa) are shown in Fig. 1. The largest decrease in the work of adhesion between hydrogels and examined models was caused by the addition of PAMAM-NH_2 into the hydrogel structures. Interestingly, no significant differences between the examined generations were noted. It was also observed that PAMAM-NH_2 dendrimers stronger declined mucoadhesive properties (evaluated as the interaction between hydrogels and gelatin discs or porcine vaginal mucosa) than adhesive characteristics (measured as interaction between hydrogels and shaved rat skin). A decrease in the work of adhesion was correlated with the viscosity of the hydrogels – hydrogels with lower viscosity were characterized by weaker bioadhesiveness. The probable cause of these changes is the reaction of amine or hydroxyl terminal groups of PAMAM dendrimers with carboxylic groups of the carbomer and, in consequence, disturbance of the polymer network. It is worth noting that no changes of hydrogel bioadhesiveness were observed after six months of storage at 25 ± 0.5 °C. The mechanism of the bioadhesion process is not fully understood and can be explained by



Scheme B. Possible interactions between PAMAM-NH_2 and Carbopol

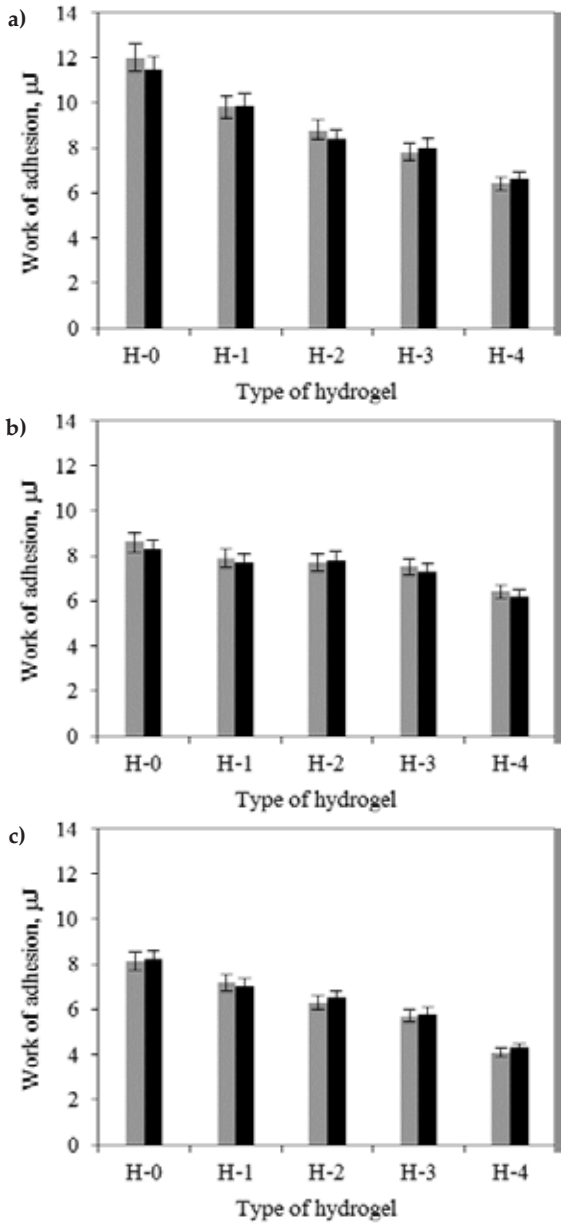


Fig. 1. The influence of PAMAM-NH_2 and PAMAM-OH G2 and G3 on the bioadhesive properties of carbomer hydrogels with CLO measured directly after preparation (grey bars) and after six months storage at 25 ± 0.5 °C and *RH* = 60 ± 5 % (black bars) for various adhesive layers: a) gelatin discs, b) shaved rat skin, c) porcine vaginal mucosa

several theories. According to diffusion theory, bioadhesion strength depends on the formation of an interpenetration layer between dosage form and skin or mucosa. The interaction of terminal groups of PAMAM dendrimers with carboxylic groups of carbomer could inhibit the creation of this layer and, in consequence, lower the work of adhesion as shown in Scheme B [3]. Furthermore, the bioadhesion process can be explained by the formation of hydrogen bonds between the adhesion layer and carbomer. Changes in the carbomer hydrogel network might diminish the number of hydrogen bonds, which disable the interaction between the polymer and evaluated models, finally decreasing the bioadhesive properties of designed hydrogels.

CONCLUSIONS

PAMAM-NH_2 or PAMAM-OH dendrimers G2 and G3 influence the viscosity, yield stress and bioadhesive properties of carbomer hydrogels containing CLO. It was noted that the effect evoked by PAMAM-NH_2 dendrimers was significantly higher. Furthermore, PAMAM-NH_2 poorer the mucoadhesive characteristics than adhesive properties. The probable reason for this change in rheological and bioadhesive characteristics of hydrogels is the interaction of amine terminal groups of PAMAM with carboxylic groups of the carbomer.

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