Sorption activity of polyhexamethylene guanidine hydrochloride hydrogels towards extracts of medicinal plants

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Abstract. The study aims to obtain polyhexamethylene guanidine hydrochloride hydrogel compositions with extracts of medicinal plants growing in the Baikal region (Bergenia crassifolia, Calendula officinalis, Tussilago farfara) to evaluate sorption activity and consider the subsequent use of the compositions as an external wound healing agent. It has been shown that hydrogel sorbs mainly caffeinequinic acids in various compositions. It has been made an assumption about a link of sorption process with formation of secondary amino groups’ complex in the hexahydrotiazine cycle, which is formed as a result of gelation of PHMGhc, and carboxyl groups of extract substances. It is planned to study the wound healing activity of compositions by modeling skin damage of laboratory animals in future researches.

1. Introduction
Skin damage therapy is a complex measure and it requires careful adjustment of drugs depending on current phase of the wound process. There are different approaches used in creating multicomponent medicines for external application. Interest in water-swellable polymers has increased due to the emergence and development of a new direction in polymer chemistry - the creation of stimulus-sensitive polymeric materials that can respond to external influences in a predetermined way [1, 2]. The use of such materials in systems of controlled release [3, 4], encourages researchers to develop new and optimize the properties of already known polymers.

Several polymers of guanidine number with high antimicrobial activity and complexation ability with organic substances can be distinguished from a large number of gelation polymers [5]. It has been shown that hydrogels based on polyguanidines demonstrate ability to heal wounds as good as medical drug “Levomecole” and it may become possible to use this type of hydrogels for medical organic and synthetic drugs in skin damage therapy [6].

2. Materials and Methods
Polyhexamethylene guanidine hydrochloride (PHMGhc) was obtained by polycondensation (Figure 1) in melt of guanidine hydrochloride and hexamethylene diamine at a temperature of T=165°C for 3 hours. Guanidine hydro chloride was made by “Across Organics” and used without previous cleaning (99%,...
Tm=185-189°C, [H2O]≤0.2%). Hexamethylene diamine was cleaned by distilling method at a temperature of 205°C fraction was made at a temperature 202-205°C [5].

\[
\begin{align*}
\text{H}_2\text{N} & \xrightarrow{\underset{\text{Cl}}{\text{H}}} \text{C} \xrightarrow{\underset{\text{Cl}}{\text{H}}} \text{NH}_2 \\
\times x & \text{H}_2\text{N} - (\text{CH}_2)_6 - \text{NH}_2 & \xrightarrow{x\text{NH}_3} \text{C} \xrightarrow{\underset{\text{Cl}}{\text{H}}} \text{NH}_2
\end{align*}
\]

**Figure 1.** PHMGhc synthesis scheme.

PHMGhc hydrogel was obtained (Figure 2) by adding 10% formaldehyde solution (GOST-1652-89) to polymer solution (C=30 g/dl) in five-fold excess toward the summary of terminal amino groups PHMGhc [5]. The reaction took place at room temperature for one hour. Purification of hydrogels from sol fraction was made by multiple washing with distilled water in Buchner funnel. IR spectra were obtained using the equipment of the Collective Use Center of Buryat Scientific Center, Siberian branch, Russian Academy of Sciences.

\[
3\text{R} - \text{NH}_2 + 3\text{H_2C} = \text{O} \xrightarrow{-3\text{H}_2\text{O}} \text{R} = [\text{NH}_2 \text{(CH}_2)_6 \text{NH}_2]_n
\]

**Figure 2.** PHMGhc hydrogels synthesis scheme.

In the research, we used pharmacy materials such as leather bergenia leaves (Bergenia crassifolia (L.) Fritsch.), pot marigold flowers (Calendula officinalis L.) and coltsfoot leaves (Tussilago farfara L.) in the study. Plant extracts were prepared according to the pharmacopoeia article GPA.1.5.3.0006.15 of the State Pharmacopoeia in XIII edition named “Extractable assay test in medicinal plant materials and herbal medicines” (method 1). About 1g of powdered drug plant material (exact amount) was sifted through sieve with 1mm hole diameter and placed in a conical flask (capacity about 200-250 ml), after that it was added 50ml of water, flask was plugged with a stopper, weighed (accurate to ±0.01g) and left for 1 hour. The flask then was joined with reflux and heated up on boiling water bath. The flask with content after cooling was plugged with the same stopper and weighted. Loss in weight of the flask content was cured by the same solvent. Flask content was thoroughly shaken and filtered through dry paper filter into a dry flask with capacity about 150-200ml. 25 ml of the resulted filtrate was pipeted into a 50-70 ml porcelain casserole which was predried at a temperature of 100-105 °C to a constant weight. After that the content of a porcelain casserole was evaporated on water bath to dryness. Casserole with solid residue was dried to a constant weight at a temperature of 100-105°C, also it was cooled for 30 minutes in desiccator with anhydrous calcium chloride at the bottom and immediately weighted.

Composites were prepared by immersion of a 1g sample weight into solutions of plant extracts for different intervals (15, 30, 45, 60, 75 min.). Sample weights were removed and dried at an ambient temperature to a constant weight after hydrogel was held in plant extracts.

Quantitative analysis was carried out using a Milichrom A-02 microcolumn liquid chromatograph (Ekonova, Novosibirsk, Russia) on a ProntoSIL-120-5-C18 AQ column (2 × 75 mm, 5 μm; Metrohm AG, Herisau, Switzerland); moving phase: 0.2 M LiClO4 in 0.006 M HClO4 (A), MeCN (B); Gradient (% B): 0–6 min 10 - 20%, 6–9.3 min 20%, 9.3–10 min 20 - 30%, 10–14 min 30 - 35%, 14–16 min 35 - 55% (Bergenia crassifolia leaves); 0–5 min 11–18%, 5–9 min 18%, 9–10 min 18–20%, 10–12 min 20–25%, 12–16 min 25%, 16–20 min 25–100% (Calendula’s officinalis flowers); 0–27 min 5–10% (Tussilago’sfarfara leaves). Separation was performed at a flow rate of 150 μl/min and at a column’s temperature of 35°C. Detection was performed with UV-detector at a distance of 270 nm (Bergenia’scrassifolia leaves) and 330 nm (Calendula’s officinalis flowers and Tussilago’sfarfara leaves). Ratio-method was provided with such reference materials as a gallic acid (>96%), arbutin...
 (>97%), bergenit (>95%), 3-O- caffeoylquinic acid (>93%), 4-O- caffeoylquinic acid (>95%), 3,4-di-
O- caffeoylquinic acid (>92%), 4,5-di-O- caffeoylquinic acid (>94%), 3,5-di-O- caffeoylquinic acid
(>90%), 1,3-di-O- caffeoylquinic acid (>95%; all Sigma-Aldrich). Narcissine (>95%) and typhaneoside
(>92%) were extracted arlier [7, 8]. The results are depicted in mean of 5 parallel definitions (± standard
deviation, SD). Sample preparation was provided by transferring accurate weight of gel (80 mg) into the
Eppendorf tube (2 ml), adding 1 ml of water and treating by ultrasonic (50 kHz, 30 min, 40°C). After
all sample was centrifuged (6000 g, 20 min). Obtained extract was filtered through membrane filter
(0.45 μm) and used for assay (1 μl).

3. Result and Discussion
Previously, it was found that the hydrogel PHMGhch degrades upon prolonged exposure to water or an
acidic medium with pH <5, in the first case for 14 days, in the second for 3 hours. A hydrogel hydrolysis
scheme has been proposed, within which fragments of the starting polymer and formaldehyde are
formed in the form of hem-diol. The method of IR spectroscopy was a comparison of the hydrogel
before and after hydrolysis, the study showed that there are no pronounced differences (figure 3).

![Figure 3. IR Spectra: 1 hydrogel; 2-hydrolysis product.](image)

Using UV spectroscopy, attempts were made to detect traces of formaldehyde, however, the
sensitivity of the method was not enough, therefore, further work will be carried out to establish the
presence of formaldehyde in the solution after degradation of the hydrogel (figure 4) [9].

![Figure 4. Estimated PHMGhch hydrogel hydrolysis scheme.](image)

When designing drugs for external use, special attention must be paid to the prolonged action, as this
will primarily affect the effectiveness of the drug as a whole. The presence of the destruction property
of the PHMGhch hydrogel, together with its sorption activity, will positively affect the subsequent release of drugs to the wound surface, especially in the first phase of treatment, when the pH of the wound surface is shifted to the acidic side [9].

In [9], it was demonstrated that a hydrogel adsorbs an antibiotic well and can act as a carrier of drugs. The use of plant-derived compounds for the treatment of wound surfaces is widely used in world practice [10], therefore, their use in the design of complex agents for external use of prolonged action can well affect the effectiveness of therapy.

Medicinal plant extracts grown in the Baikal region area were used in the work (Bergenia crassifolia, Calendula officinalis, Tussilago farfara). Contented in those types of plant substances with observed antibacterial and antioxidant activity were caused the selection [11, 12].

Compositions were received by holding hydrogel in solutions of plant extracts for different intervals. During the sorption of Bergenia crassifolia extract compounds the largest values (82.45%) were reached for gallic acid held in solutions for 75 min, concentration of arbutin (23.86%) and bergenin (17.14%) was roughly the same for all the time (Table 1). In fact, gallic acid may be most often found in tannin esters with peroral and application anti-oxidant action [10]. High-gallic acid hydrogel should be good for wound-healing in damage skin experiments on laboratory animals.

**Table 1.** The concentration of arbutin, gallic acid and bergenin in the extract of the roots of Bergenia crassifolia, μg / ml ± SD (n = 3).

| Compound | Sample | 0       | 1       | 2       | 3       | 4       | 5       |
|----------|--------|---------|---------|---------|---------|---------|---------|
| arbutin  |        | 273.65 ± | 205.62 ± | 206.29 ± | 207.62 ± | 212.28 ± | 209.84 ± |
|          |        | 5.19    | 3.91    | 4.33    | 3.52    | 4.45    | 3.98    |
| gallic   |        | 6.61 ± 0.14 | 3.29 ± 0.07 | 2.72 ± 0.06 | 2.23 ± 0.04 | 1.82 ± 0.03 | 1.16 ± 0.02 |
| acid     |        | 391.82 ± | 339.25 ± | 328.54 ± | 325.74 ± | 315.60 ± | 313.96 ± |
| bergenin |        | 8.62    | 7.12    | 7.88    | 6.18    | 6.94    | 6.27    |

In case of sorption of Calendula officinalis extract compounds were found compositions sorbed by hydrogel maximally (3-caffeoylquinic acid, narcissine, typhenaeside) (Table 2). It has been shown that after holding hydrogel in extract solution for 45 min the concentrate of substances achieves plateau. Composition shown by anti-oxidants and against this background it can be possible to predict the multiplying external wound-healing effect.

**Table 2.** Concentration of 3-O-caffeoylquinic acid (3CQA), typhaneoside and narcissin in the extract (solution) of Calendula officinalis flowers, μg / ml ± SD (n = 3).

| Compound | Sample | 0       | 1       | 2       | 3       | 4       | 5       |
|----------|--------|---------|---------|---------|---------|---------|---------|
| 3CQA     |        | 26.73 ± 0.53 | 4.51 ± 0.09 | 4.96 ± 0.10 | 2.79 ± 0.05 | 2.93 ± 0.05 | 2.82 ± 0.05 |
| typhaneoside | 64.95 ± 1.22 | 16.67 ± 0.33 | 17.20 ± 0.34 | 10.68 ± 0.21 | 11.74 ± 0.23 | 11.45 ± 0.22 |
| narcissin |        | 50.77 ± 1.09 | 10.62 ± 0.20 | 11.80 ± 0.24 | 6.41 ± 0.12 | 7.41 ± 0.14 | 7.45 ± 0.14 |

Sorption of Calendula officinalis extract by hydrogel has common character with creating the compositions of Tussilago farfara extract (Table 3). Tussilagos family plants have high antioxidant and antimicrobial activity [13]. It was reached by occurrence of high concentrated caffeyolquinic acids.

Probably, such a high sorption of carboxyl-contained compounds by a hydrogel is due to complexation by protonation of the secondary amino groups of the hexahydrotiazine ring, formed after crosslinking of the terminal amino groups PHMGhch and also partial binding to the positively charged carbon atom of guanidine group. Future researches will be aimed at studying of PHMGhch hydrogel’s complex formation with carboxyl-contained compounds involving the use of physico-chemical analysis methods.
4. Conclusion
As a result of the work, it was found that the polyhexamethylene guanidine hydrochloride hydrogel obtained by crosslinking the terminal amino groups of the branched polymer exhibits high sorption activity against carboxyl-containing compounds of medicinal plant extracts (Bergenia crassifolia, Calendula officinalis, Tussilago farfara). The HPLC method was used to determine the composition of compounds whose concentrations dominate during hydrogel sorption: caffeilchinic acids, gallic acid, arbutin, bergenin, typhaneoside, narcissin. All these compounds exhibit high antioxidant and antibacterial activity, which will accelerate the wound healing process during experiments with the external use of the compositions under conditions of modeling damage to the skin. The nature of sorption activity will be studied in subsequent studies.

At the moment, an experiment was conducted using a composition based on PHMGch and Bergenia crassifolia in the treatment of experimental animals with thermal skin burn. Preliminary results showed intensive damage healing, manifested in the inhibition of inflammatory processes with purulent necrotic complications and inhibition of fiber destruction and, as a result, the formation of granulation tissue. The study of the total antioxidant activity and leukocyte number showed that the use of the hydrogel composition leads to the normalization of these parameters and indicates its effectiveness. Thus, it was shown that the composition of the hydrogel PHMGch and Bergenia crassifolia leads to the normalization of a number of parameters of blood biochemistry and the restoration of all layers of the skin of rats and ends with the complete closure of the skin defect. The full results of the study are planned for publication in our next articles.

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