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Research Article

Effect of Sodium Salicylate on the Viscoelastic Properties and Stability of Polyacrylate-Based Hydrogels for Medical Applications

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Investigation was made into the effect exerted by the presence of sodium salicylate (0–2 wt.%), in Carbomer-based hydrogel systems, on processing conditions, rheological and antimicrobial properties in tests against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains, and examples of yeast (*Candida albicans*) and mould (*Aspergillus niger*). In addition, the work presents an examination of long-term stability by means of aging over one year the given hydrogels at 8°C and 25°C. The results show that 0.5 wt.% NaSal demonstrated a noticeable effect on the hydrogel neutralization process, viscosity, and antimicrobial properties against all of the tested microorganisms. The long-term stability studies revealed that hydrogels can maintain antimicrobial activity as well as viscosity to a degree that would be sufficient for practical use.

1. Introduction

Hydrogels are in use in various applications, including foodstuffs, pharmaceuticals, cosmetics, and medicine, due to the convenient physical properties they boast, their compatibility with a wide range of active ingredients, and the nontoxicity and biocompatibility they demonstrate [1]. The biodegradability and/or bioresorbability of certain developed hydrogels represent favourable characteristics for tissue engineering applications [2]. Indeed, some of the hydrogel systems exhibit an effective response to external physical (temperature) or chemical (pH, ionic strength) factors, in terms of rapid change in volume (swelling or shrinking) which is important for controlled delivery of an active substance or for biosensors construction [3].

Crosslinked polymers and copolymers of acrylic acid are compounds frequently utilized in the preparation of hydrogels, and they have been the object of both scientific and commercial interest. Such materials are known as Carbomers, which have proven applicable as additives (thickeners) or bases for incorporating cosmetically or medically active substances [4, 5].

A promising use of Carbomer is in dermatology as a hydrating agent for photorejuvenation therapy, involving intense pulsed light or laser treatments [6]. Carbomer hydrogels are applied prior to the laser treatment itself in order to hasten the passage of light. The expectation is for a synergetic effect to occur through combining the hydrogel, active substances, and therapeutic laser [7].

The active substances applicable in the hydrogels, for instance, pertain to antioxidants, aromatics, or antimicrobial agents that can, however, exert significant influence on the rheological properties, stability, and therapeutic effect of the hydrogel system [5, 8].

Sodium salt of salicylic acid (NaSal) is an interesting bioactive substance that has been primarily used as a preserving agent. Additionally, its anti-inflammatory properties lend it perspective with regard to therapeutic effects. Furthermore, salicylates have been studied as components for skin care creams and in dermatology as a keratolytic and antifungal agent; hence relevant safety data are already available for potential producers [9, 10]. Indeed, the connection of the Carbomer and NaSal has therapeutic potential.

Nevertheless, it is known that monovalent ions, including Na^{+1}, crucially affect the stability of hydrogel systems [11]. While low NaSal content fails to trigger the necessary preservation effect, high concentration can cause hydrogel systems to collapse. Despite this, the effect of NaSal concentration in Carbomer-based hydrogel on its processability, antimicrobial activity, and stability has yet to be studied in detail, and no concentration limits have been optimized.

The work presented herein describes the effect of NaSal at concentration (0–2 wt.%) on the rheological and antimicrobial properties of Carbomer-based hydrogel systems. In addition, elaboration of a long-term stability (1 year) assessment is given. The characteristics studied were observed on a rotary rheometer. The antimicrobial activities of the hydrogels were determined by the agar diffusion technique against Gram-positive and Gram-negative bacterial strains, as well as examples of a yeast and mould.

2. Experimental Section

2.1. Materials. Carbomer (Carbopol® 940 Polymer) was the product of Lubrizol Advanced Materials, Inc. (Cleveland, USA). Glycerol, sodium hydroxide (NaOH), and sodium salicylate (NaSal) (all analytical grade) were purchased from Penta (Prague, Czech Republic). Triethanolamine (TEA) (analytical grade) was supplied by P-Lab (Prague, Czech Republic).

The microbial cultures *Escherichia coli* (CCM 4517), *Staphylococcus aureus* (CCM 4516), *Candida albicans* (CCM 8355), *Aspergillus niger* (CCM 8222), and *Pseudomonas aeruginosa* (CCM 478) were obtained from the Czech Collection of Microorganisms, Masaryk University (Brno, Czech Republic).

2.2. Methods

2.2.1. Hydrogel Preparation. Carbomer resin (powder, 0.8 wt.%) was dispersed in deionized water (23°C) and an appropriate amount of NaSal was added subsequently; the composition of said samples is shown in Table 1. Following this, the dispersion of Carbomer and NaSal was maintained to enable swelling at the temperature of 8°C for various time periods—from 3 to 120 hours. The mixture was neutralized with 10 wt.% NaOH aqueous solution under gentle stirring at 23°C. Optimal swelling time and supplementation with NaOH were investigated (see later in the text). Finally, the hydrogels were left to stabilize for 24 hours at 23°C. The samples were stored in the dark at 8°C. All the samples were conditioned for 6 hours at 23°C prior to further analyses.

2.2.2. Rheological Measurements. The viscoelastic properties of the hydrogel samples were investigated on a rotational viscometer (ARES 2000, Rheometrics Scientific, USA) equipped with the *RSI Orchestrator* software package. A 25 mm diameter parallel plate, which measured geometry with a gap of approximately 2 mm under slight strain (1%), was used to maintain measurements within the linear viscoelastic region. The samples were spread on the lower plate and upper one was moved down to make a required gap. Generally, at least 10 min waiting time to allow the hydrogel to reach the equilibrium temperature with the measuring system was kept before each measurement. Dynamic frequency sweep tests were carried out at the temperature of 29°C to determine storage (G') and loss (G'') moduli as a function of frequency, ω, from 0.1 to 100 rad·s^{-1} in dynamic frequency mode (angular frequency ω ranging from 0.1 to 100 rad·s^{-1}). Viscoelastic properties were also described by the complex viscosity (η^*) parameter calculated according to the following equation:

$$\eta^* = \sqrt{\left(\frac{G'}{\omega}\right)^2 + \left(\frac{G''}{\omega}\right)^2}. \quad (1)$$

Dynamic yield stress (τ_0) values were determined by extrapolating flow curves to zero shear rate and from the complex shear modulus, respectively, [12, 13].

A relative gel strength was defined as a function of G' value (at 0.4 rad·s^{-1}) and NaSal concentration [14].

Table 1: Compositions of the investigated hydrogel systems.

Sample designation	Carbomer (wt.%)	NaSal* (wt.%)
G1	0.8	0
G2		0.3
G3		0.5
G4		1
G5		1.5
G6		2

∗ related to 100 g of Carbomer (0.8 wt.%) and water mixture.

2.2.3. Stability Study. Investigation was made into the effect of NaSal concentration on gel stability in addition to antimicrobial properties. The aforementioned long-term stability tests were carried out in real time during product storage under recommended conditions (8°C and/or 25°C, in a dark place). The samples were kept at room temperature for 6 hours prior to taking measurements in order to maintain identical conditions during tests for viscosity and pH.

The viscosities of the samples in the stability study were measured on a Viscotester 6+ Haake device (Thermoelectron GmbH, Germany). The viscosity of the samples was measured in the glass beaker with diameter and height of 5 and 9 cm, respectively. A spindle type R6 (20 rpm) was used for the measurement in all cases. The pH measurements were gauged on a GPH014GL pH meter (Greisinger Electronic GmbH, Germany).

2.2.4. Antibacterial Properties and Microbial Stability Assay. Antibacterial properties pertaining to NaSal content were determined by an agar diffusion test [15]. Mueller Hinton Agar (MHA) was used in plates of 90 mm diameter and 4 mm depth. The following organisms were tested: *Escherichia coli* CCM 4517 at the concentration 10^7–10^8 CFU/mL, *Staphylococcus aureus* CCM 4516 (10^7–10^8 CFU/mL), *Candida albicans* CCM 8355 (10^6–10^7 CFU/mL), and *Aspergillus niger* CCM 8222 (10^6–10^7 CFU/mL). The bacterial suspension was

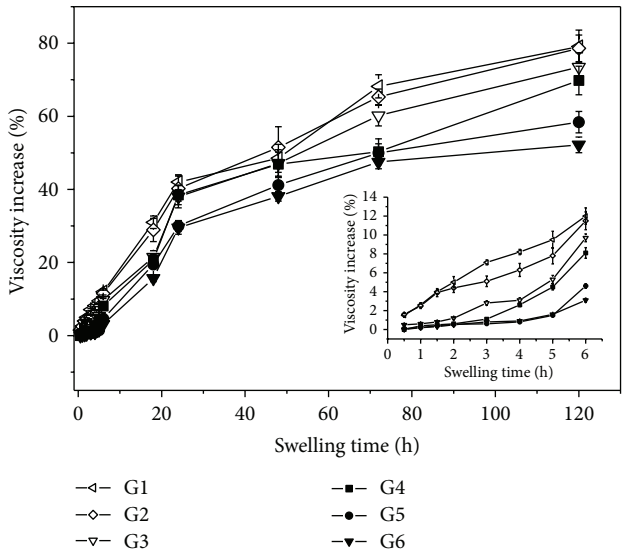

Figure 1: Swelling course of the hydrogels. The inserted figure shows the initial part of the swelling process.

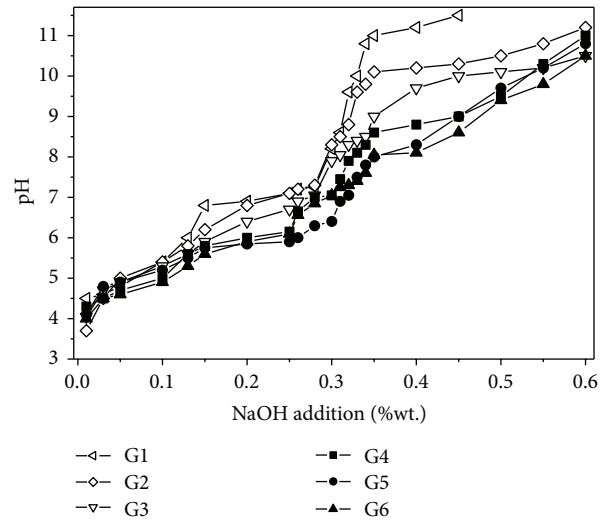

Figure 2: The pH of the hydrogels versus concentration of the neutralizer NaOH.

inoculated on the entire surface of MHA with a sterile cotton-tipped swab to enable it to form. Two holes of 8 mm diameter were made into each plate and these were filled with gel containing an active agent. Incubation time was 18–24 hours, while incubation temperature equalled 35°C for *S. aureus* and 37°C for *E. coli*. The representatives of yeast (*C. albicans*) and fungus (*A. niger*) were incubated at 25°C for 75 hours.

3. Results and Discussion

The swelling behaviour of the investigated compositions (G1–G6, see Table 1) expressed as the dependence of relative increase in hydrogel viscosity on duration of swelling is depicted in Figure 1. Said Figure 1 shows the initial swelling period (1–6 hours). The increase in viscosity is represented by the fraction for viscosity of hydrogel at a given time (η_t) over initial viscosity (η_0), according to

$$\text{viscosity increase}\,(\%) = \frac{\eta_t}{\eta_0} \times 100. \quad (2)$$

It is noticeable that interaction between water molecules and polymer chains is significantly reduced during the initial stage of the swelling process. In particular, compositions supplemented with NaSal at content above 1% (G4–G6) demonstrate no increase in viscosity after the first 3 hours. Generally, the assumption is that the higher the content of NaSal, the lesser the extent of swelling. Sodium and other multivalent salts are known to exert a negative effect on the viscosity and stability of Carbomer-based hydrogels. Thus, the viscosity of the hydrogel samples is limited by the presence of NaSal. Viscosity values continue to rise even after 120 hours; however, optimal processing parameters could be defined at 48 hours due to technological reasons. Herein, the swelling time of 48 hours was selected as the reference time for all samples presented further.

The stability of a Carbomer-based hydrogel is also strongly dependent on pH, which is usually adjusted by NaOH to the figure for pH in the range 7–8.5 that provides the most stabile hydrogel structure [11, 12]. This pH value makes it suitable for dermatological applications. A dependence of pH on supplementation with NaOH (expressed as the content of NaOH related to 100 g of hydrogel compositions G1–G6) is presented in Figure 2. Initially, adding the neutralizer (NaOH) triggered a slight increase in pH followed by a steep rise in pH, in the range 0.25–0.35 wt.%. The behaviour of the samples at higher contents of the neutralizer (0.36–0.6 wt.%) can be split into two groups. While the pH for compositions with NaSal concentrations at 0, 0.3, and 0.5 wt.% (G1, G2, and G3) did not change much, compositions with NaSal content at 1, 1.5, and 2 wt.% were characterized by intense rise in pH values. In parallel with the swelling experiment (Figure 1), Figure 2 clearly displays the effect of increasing NaSal concentration. Samples containing NaSal possess lower pH at the same level of addition of neutralizer in comparison with the composition without NaSal (G1).

The pH of hydrogels, influenced by adding the neutralizer, is linked with viscosity, as presented in Figure 3. The results clearly demonstrate the noticeable effect of NaSal on reducing the viscosity of the resultant hydrogel system. Furthermore, pH adjustment does not exert a positive influence on increase in viscosity, as is usual for NaSal-free composition (G1).

The thickening mechanism of the neutralizer is based on alteration of coiled polymer chains into an uncoiled form through transforming acidic Carbomer into a salt through the action of a neutralizing agent, NaOH. An alternative neutralizer, such as triethanolamine, is also known to be effective [16]. When an additive in ionized form is used to modify the Carbomer system, a negative effect can occur. Monovalent ions cause diminished thickening. However, multivalent ions (Ca^{2+}, Al^{3+}) potentially cause insoluble precipitate to form, with subsequent collapse of the hydrogel systems [11].

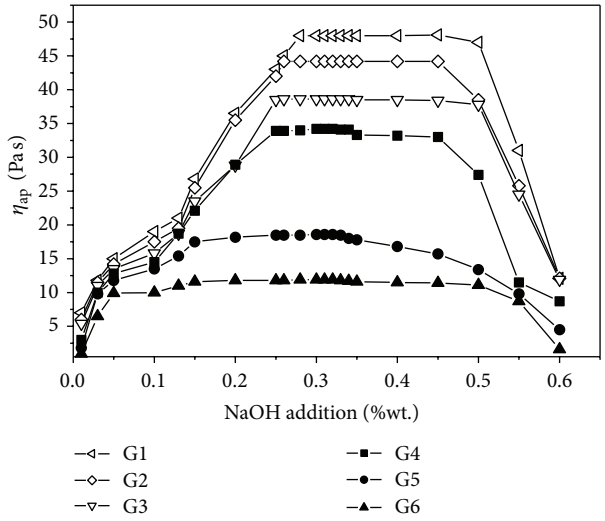

Figure 3: Apparent viscosity of the hydrogels versus concentration of the neutralizer NaOH.

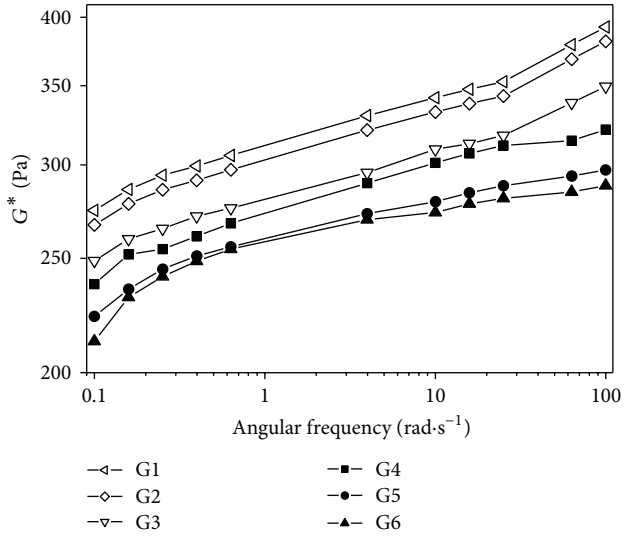

Figure 4: Complex shear modulus (G^*) versus angular frequency dependence of the hydrogels.

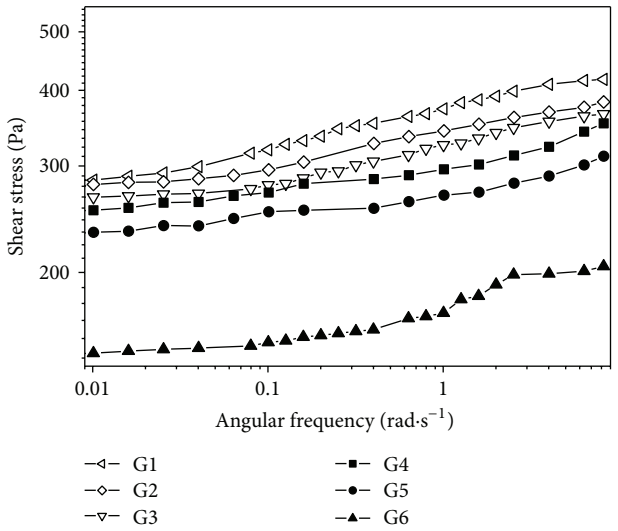

Figure 5: Shear stress versus angular frequency dependence of the hydrogels.

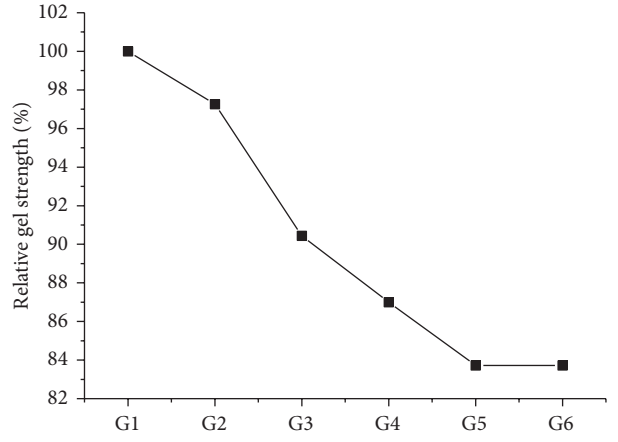

Figure 6: Relative gel strength as a function of the hydrogel composition and NaSal content.

Table 2: Dynamic yield stress and gel strength of the hydrogels.

Sample	Dynamic yield stress (Pa)
G1	283 ± 5
G2	271 ± 4
G3	265 ± 5
G4	252 ± 5
G5	232 ± 3
G6	145 ± 5

The effect of NaSal concentration on Carbomer hydrogels, specifically their rheological properties, is shown in Figures 4 and 5, which depict dependencies of complex shear modulus (G^*) versus angular frequency and shear stress versus the same, respectively. The parallel increase in shear stress with shear rate reveals the characteristics of Bingham fluid with yield stress, as presented in Table 2 together with the characteristics of gel strength. In accordance with the results presented above, NaSal degrades the rheological properties of hydrogels.

Increasing content of NaSal in the systems leads to reduction of the observed rheological characteristics including the dynamic yield stress. These reductions are relatively small up to 0,5 wt.% NaSal (6.3% yield stress reduction in comparison to pure hydrogel, G1). On the contrary 2 wt.% of NaSal (G6) caused significant drop of the dynamic yield stress (about 49% decrease).

Similar effect of NaSal can be observed in relative gel strength parameter that is depicted as a function of NaSal content in Figure 6. Small additions of NaSal (G3) cause its reduction up to 10%. Hydrogels with 1 and 2 wt.% of NaSal (G5 and G6) showed relative gel strength decrease above 15%. These results have reverse trend compared to work of Farres

Table 3: Antimicrobial activity of freshly prepared samples G1–G6 expressed as inhibition zone diameter (mm).

Sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Staphylococcus aureus*	*Aspergillus niger*
G1	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
G2	0.5 ± 0.0	1.0 ± 0.3	0.5 ± 0.0	4.5 ± 0.5	0.5 ± 0.3
G3	1.0 ± 0.3	1.5 ± 0.3	1.0 ± 0.5	6.5 ± 0.5	0.5 ± 0.3
G4	1.5 ± 0.3	2.0 ± 0.5	1.5 ± 0.3	9.0 ± 1.0	1.0 ± 0.3
G5	3.5 ± 0.3	4.5 ± 0.5	2.0 ± 0.3	11.5 ± 1.0	1.5 ± 0.3
G6	5.5 ± 0.5	6.0 ± 0.5	2.5 ± 0.3	15.0 ± 1.0	2.0 ± 0.3

Table 4: Antimicrobial activity of samples G1–G6 after 1 year of storage at 8°C expressed as inhibition zone diameter (mm).

Sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Staphylococcus aureus*	*Aspergillus niger*
G1	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
G2	No inhibition	0.5 ± 0.3	No inhibition	1.0 ± 0.5	No inhibition
G3	No inhibition	0.5 ± 0.3	No inhibition	2.5 ± 0.5	No inhibition
G4	1.0 ± 0.3	1.5 ± 0.5	1.0 ± 0.3	3.5 ± 1.0	0.5 ± 0.3
G5	2.0 ± 0.3	3.0 ± 0.5	1.0 ± 0.3	7.5 ± 1.0	1.0 ± 0.3
G6	4.0 ± 0.5	3.5 ± 0.5	2.5 ± 0.3	8.0 ± 1.0	1.5 ± 0.3

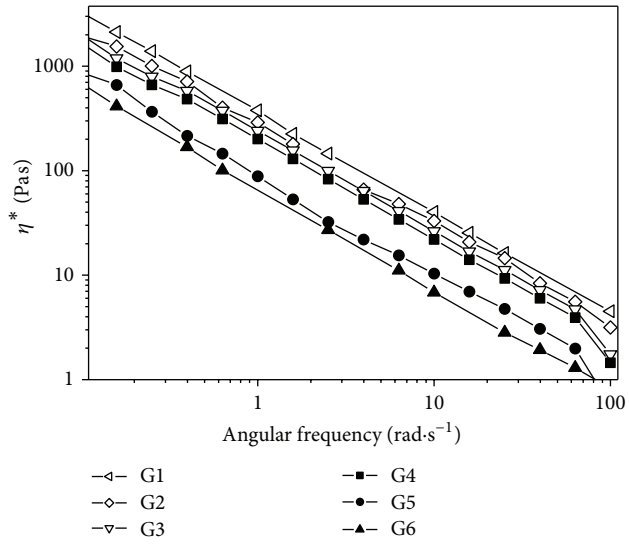

Figure 7: Complex viscosity versus angular frequency dependence of the hydrogel systems.

et al. who reported rheology of alginate fluid gels produced by in-situ calcium release. Specific interactions of calcium are caused of calcium junction into calcium chelates.

The viscoelastic properties of the compositions tested are presented in Figure 7 as the dependence of complex viscosity (η^*) on angular frequency, where a significantly decreasing trend is observable for samples G1–G6. Nevertheless, the increase in Na^+ ions concentration, originating from the presence of NaSal, brought about reduction in η^* alongside a rise in angular frequency (ω). This behaviour is typical for physically crosslinked polymer networks where, after a certain yield, stress is achieved.

The antimicrobial activity of the prepared compositions, observed by measuring inhibition zones, is presented in Table 3 for freshly prepared hydrogels and in Table 4 for those after 1 year of storage at 8°C. NaSal is known to be an effective antimicrobial agent against Gram-positive and Gram-negative bacterial strains, yeasts, and moulds. The results for freshly prepared hydrogels indicate that even 0.3 wt.% of NaSal exerts some effect against all the tested microorganisms. Indeed, composition G2 (0.5 wt.% NaSal) previously demonstrated its proven antimicrobial properties. The hydrogels stored for one year at 25°C had noticeably diminished antimicrobial activity (Table 5). However, sample G4 (1 wt.% NaSal) continued to show relatively suitable antimicrobial activity even after a year from its preparation.

Table 5: Changes of pH, viscosity, and NaSal concentration of the hydrogels after 1 year of storage at 8 and 25°C.

	G1	G2	G3	G4	G5	G6
$\Delta pH_{25°C}$ (%)	−27	−34	−35	−36	−45	−46
$\Delta pH_{8°C}$ (%)	−17	−18	−20	−24	−31	−38
$\Delta\eta_{25°C}$ (%)	−97	−36	−45	−56	−65	−75
$\Delta\eta_{8°C}$ (%)	−70	−21	−28	−43	−58	−64
$\Delta C_{NaSal,25°C}$ (%)	−54	−55	−52	−54	−54	−55
$\Delta C_{NaSal,8°C}$ (%)	−39	−38	−39	−39	−39	−38

Besides antimicrobial activity, the aging of the given hydrogel compositions was tested as regards pH and η and dependence on storage temperature (8°C and 25°C for 1 year). Since NaSal was expected to be deactivated by reacting with either of the components of the hydrogel systems (polymer, neutralizer), its concentration in the hydrogel samples was also subjected to study. The NaSal was determined by applying UV-VIS spectrometry at a fixed 515 nm wavelength using 5 mM $Fe(NO_3)_3$ in 12 mM H_2SO_4 and a colouring agent [17].

The results expressed as negative changes to the relevant characteristics are shown in Table 5. Indeed, the effect of storage temperature is noticeable. Storage at ambient temperature can completely eradicate all important characteristics, for

example, pH, η, and NaSal content, while loss of antimicrobial activity might also be anticipated. Despite reducing the characteristics studied, storage at 8°C could be considered sufficient for maintaining the quality of the hydrogel over the long term. Nevertheless, greater NaSal content (up to 1 wt.%) in the hydrogel composition brings about parallel reduction in the parameters studied. Supplementing the hydrogel systems with more NaSal (above 1 wt.%) does not bring further enhancement of properties.

4. Conclusions

This study investigated the effect exerted on rheological and antimicrobial properties through the presence of sodium salicylate (NaSal, 0–2 wt.%), in a hydrogel matrix based on a chemically crosslinked copolymer of acrylic acid (Carbomer). The results show that even small concentrations of NaSal noticeably affected hydrogel neutralization, viscosity, and antimicrobial properties against Gram-positive and Gram-negative bacterial strains, yeasts, and moulds. Long-term stability studies (1 year) revealed that hydrogels can maintain antimicrobial activity as well as viscosity at a level potentially sufficient for practical use, despite diminishment in the all properties observed. It was also found that NaSal reduced the effects of aging.

In addition to NaSal representing a potential active agent for cosmetic or medical applications, description was given on its contribution to the processing, final properties, and stability of the given hydrogel systems. Its optimal concentration in the Carbomer matrix was determined as 1 wt.% as a three-way compromise between the necessary therapeutic (antimicrobial) effect, hydrogel properties (pH and viscosity), and stability requirements.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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