INFLUENCE OF SELECTED POLYMERS ON PHARMACEUTICAL: PROPERTIES OF MUCOSAL SYSTEMS CONTAINING ACYCLOVIR

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Abstract

The aim of this paper was to formulate the composition of a bioadhesive film obtained based on acyclovir incorporated into natural bioadhesive polymers (chitosan and guar gum) in different quantitative ratios. To this end, the author developed a technology for preparing films containing various quantitative ratios of chitosan to guar gum: 1:0.25; 1:0.50; 1:0.75; 1:1; and 1.2:0.50. The formulations were used to prepare mucoadhesive films containing 2% acyclovir. The dressings were tested with respect to their physicochemical properties and subjected to dissolution testing. A comparative analysis of Fourier-transform infrared (FT-IR) spectra of pure polymers, acyclovir, and films containing polymers in different quantitative ratios was conducted. The quantitative ratio of chitosan to guar gum significantly impacted the mechanical properties of films: texture, elasticity, tensile strength, swelling, and blur time of the testes carriers. The 1:1 formulation showed the highest mechanical strength and flexibility. The dressing containing 1.2% chitosan and 0.5% guar gum had the longest blur time. An increase in the chitosan content in the formulation significantly affected the drug dissolution parameters, which makes it possible to achieve the desired effect of extended-release time. The FT-IR spectra excluded the formation of drug-polymer interactions. Changes in the quantitative ratios of chitosan and guar game in the carrier's composition may impact the mechanical properties of the drug formulation and changes in the parameters of active substance release.

Keywords: *chitosan, guar gum, acyclovir, mucoadhesive film, carrier*

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Introduction

Oral mucosal lesions occur as a result of various stimuli that may contribute to oral inflammatory foci, infections, as well as ulcers and erosions. Viral infections may cause acute oral mucositis. There is a distinction between primary herpes stomatitis, recurrent herpes, and Zahorski herpangina. Herpes stomatitis is caused by the herpes simplex virus (HSV). The disease can occur on its own or be comorbid with other systemic diseases [1, 2].

If the lesion is local and limited to a small area, using a drug characterised by properties that make it possible for the active ingredient to remain at the application site for a longer time would be appropriate. Mucoadhesive systems, which are deemed of particular importance in the context of mucosa inflammation, are primarily tablets, buccal films (polymeric films), and high-viscosity gels. Mucoadhesive systems in the form of small, thin, and highly flexible films could provide comfort to patients and – thanks to their structure – limit the contact of the painful area with external factors, reducing pain.

The polymers used to obtain the films must be biocompatible and harmless to the body. Moreover, as the drug is in constant contact with the mucous membrane and its concentration is constantly diluted in the oral cavity, the drug formulation should be sufficiently stable with good mucoadhesive properties. Chitosan, combined with a polysaccharide (guar gum [GG]) to produce a mucoadhesive film with optimal binding properties, is a polymer of natural origin that could meet the above-mentioned conditions. Such combinations provide greater mechanical strength to the drug formulation, and by forming polymer-polysaccharide complexes, they modify the degree of dressing adhesion to the mucosa [3, 4]. Chitosan has adequate physicochemical parameters and a good viscosity level and has been proven to show antibacterial and antifungal activity. GG has good thickening and binding properties [5, 6].

The aim of the study was to obtain an optimal qualitative and quantitative composition of the hydrogel film for the treatment of oral cavity inflammation as well as aphthous ulcers, ulcers and erosions. To this end, the author incorporated acyclovir (ACV) as a therapeutic substance with anti-inflammatory, analgesic, and antiviral properties on mucoadhesive carriers containing chitosan and GG in various quantitative ratios. The obtained dressings were tested with respect to their physicochemical properties and subjected to dissolution testing to distinguish the form of the drug with the best parameters [7, 8].

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: ACV (Sigma-Aldrich Chemie GmbH, Germany); chitosan high molecular weight (CHIT) with a deacetylation degree of 75%, viscosity 800-2000 cP; GG, viscosity 5000 cP (Sigma-Aldrich Chemie GmbH); glacial acetic acid (FCC, J.T. Baker, USA); and aqua purificata as required by FP XII Poland.

2.2. Methods

2.2.1. Technology for the Preparation of Hydrogel Films With ACV

Appropriate amounts of chitosan were dissolved in 1% acetic acid. GG was dissolved in water. Polymer solutions were kept in the refrigerator for 24 h to enable the complete dissolution and swelling of polymers. Subsequently, GG gel and ACV suspension in an appropriate amount of glycerol were added to the chitosan solution in portions and mixed at the speed of 40 rpm for 20 min until the ingredients were combined entirely. The resulting gels were de-aerated using an ultrasonic bath for 30 min and poured by weight into 1 g round moulds (diameter of 1.6 cm and surface area of approximately 2 cm^2) and

5 g round moulds (diameter of 3.5 cm and a surface area of 9.6 cm^2). Then, they were dried in an air dryer for 48 h at 45°. The composition of the hydrogel films is presented in Table 1.

Table 1. Quantitative composition of the hydrogel films.

Formulation	CH [%]	GG [%]	GL [%]	ACV[%]
CH1+GG0.25+GL20+ACV	1.0	0.25	20	5.0
CH1+GG0.50+GL20+ACV	1.0	0.50	20	5.0
CH1+GG0.75+GL20+ACV	1.0	0.75	20	5.0
CH1+GG0.25+GL20+ACV	1.0	1.00	20	5.0
CH1.2+GG0.50+GL20+ACV	1.2	0.50	20	5.0

Note. In the first column, the number after each abbreviation indicates the percentage of that component in the film. Abbreviations: ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

2.2.2. Dynamic Viscosity Test

Rheological investigations were performed using a Rheotest 2 rotational viscosimeter (Medingen Dresden, Germany). The determinations were performed in the Ia and IIa ranges on a K-1 cone with a diameter of 36 mm and a 0.917 fissure at 37°C. The shear angle was measured using 12 shear rates in the ascending direction and 11 rates in the descending direction. All gels were tested three times, and the results are reported as the average of three measurements. The values of the shear stress and viscosity were calculated from measurements at 37°C using the following equations:

i. shear stress for the range Ia:

$$\tau = c \times \alpha_{(1\text{-}12)} = 85.0 \times \alpha_{(1\text{-}12)}$$

ii. viscosity for the range Ia:

$$\eta = \frac{\tau}{D(1-12)D(1-12)} \times 100 = \frac{85\alpha(1-12)85\alpha(1-12)}{D(1-12)D(1-12)} \times 100$$

iii. shear stress for the range IIa:

$$\tau = c \times \alpha_{(1\text{-}12)} = 820.2 \times \alpha_{(1\text{-}12)}$$

iv. viscosity for the range IIa:

$$\eta = \frac{\tau}{D(1-12)D(1-12)} \times 100 = \frac{820.2\alpha(1-12)820.2\alpha(1-12)}{D(1-12)D(1-12)} \times 100$$

For the above equations, τ is the shear stress (N/m^2), η is viscosity (mPa*s), α is shear angle, and D is the shear rate (1/s). Three trials were performed for each dressing,.

2.2.3. Examination of Water Adsorption by Hydrogel Carriers According to FP XII

The analysed films were weighed on an analytical scale. They were placed at the bottom of a tared beaker, to which 20 ml of purified water, heated to 37°, was added. The

samples were not mixed or shaken. After 3 min, the water was removed carefully, the beaker walls were dried, and then it was weighed on an analytical scale together with the swollen dressing [9]. For technological reasons, it is preferable for the dressing to exhibit a weak water adsorption capacity. Therefore, film swelling studies were conducted for the shortest possible time. For each dressing, three trials were performed.

The average swelling coefficient of the tested complexes was calculated using the following formula:

$$Wp=(m_w-m_s)/ms \times 100$$

where W_p is the swelling coefficient [%], m_w is the mass of lyophilisate with absorbed water [g], and m_s is the mass of dry lyophilisate [g]. The test results are shown in Table 2.

2.2.4. Blur Time Test

Dressings measuring 1.6 cm in diameter were placed in beakers with the addition of 30 ml of water that was heated to 37°C and put in a thermostatic shaker (Memmert, Germany) at the speed of 100 rpm for 3 h. Three trials were performed for each dressing. The obtained blur times are presented in Table 2.

2.2.5. Hydrogel Carrier Texture Test

The TA.XT Plus single-arm texture analyser (Stable Micro Systems, Texture Technologies Corporation, USA) with a maximum load of up to 50 kg as well as the Texture Exponent 32 software were used to assess the flexibility and brittleness of the films. The author used an A/TGP measuring tip with two grips to stretch the film. The test was performed using film discs with a diameter of 35 mm and a surface area of 962 mm^2, which enabled stable fixation of the film between two grips placed 10 mm apart, with an applied force of 3.0 g and a stretching speed of 0.5 mm/s [10]. For each dressing, three trials were performed.

Values of the elongation percentage at the time of rupturing the dressing, as well as values of tensile resistance of the films were calculated.

$$\text{Tensile strength} = R_m=F/A_o \ [N/mm^2]$$

where:

F – straining force at which the film was wricked [N],

A_0 – initial surface area of the cross-section of the film [mm^2].

The percentage of elongation at the time of rupture [%] was calculated as:

$$(L - L_o)/L_o \times 100 \ [11]$$

where:

L_0 – length (diameter) of the dressing before the test,

L – length of the dressing after stretching, at the time of rupture.

The test results are presented in Table 3.

2.2.6. Dissolution of ACV From Bioadhesive Films

Dissolution of ACV was evaluated in accordance with Polish Pharmacopoeia 12 using the paddle method [9] and the Vankel VK7035 device (Varian Medical Systems, USA) with a Varian autosampler with a basket cap for releasing the medicinal substance from the films. The tests were conducted on films with an area of 9.62 cm^2 and an average weight

of 1.48 g. The films were placed in the basket and dipped in chambers containing 500 ml of purified water at 37°C and a mixer speed of 50 rpm. The trial was continued for 60 min. Three millilitre samples were collected at six intervals, namely after 10, 20, 30, 40, 50, and 60 minutes. The collected samples were filtered using filters with a 10 µm pore size. Six trials were performed for each dressing.

The collected samples were diluted and then their content was evaluated using JASCO V650 spectrophotometer and 1 cm cuvette at a wavelength of 253 nm. The drug concentration in samples and an average percentage of dissolved benzocaine were calculated using the linear regression equation for the drug y = 0.537x + 0.0162. A plot depicting the released dose of the drug depending on time is presented in Figure 1.

2.2.7. Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of polymers, ACV, and selected films with different polymer contents were obtained using a Perkin-Elmer Spectrum Two FTIR spectrometer (Perkin Elmer, USA). Spectra were recorded in the 4000-450 cm^{-1} wavenumber range with a resolution of 4 cm^{-1}, averaged over 32 scans. The spectra of polymers and selected films are shown in Figure 2.

3. Results and Discussion

3.1. Comparison of the Impact of Viscosity and the Swelling Coefficient on the Blur Time of the Dressings

The blur time of the dressings, their viscosity, and their capacity to swell are presented in Table 2.

Table 2. Viscosity parameters of hydrogels determined at 37°C and shear rates of 4860 [s^{-1}] and swelling, blurring parameters of the films.

Formulation	Shear stress [N/m^2]	Average viscosity of hydrogels [mPas]	average swelling factor [W_p]	Blur time Tav [min] ± standard deviation
CH1+GG0.25+GL20+ACV	708.05	19.22	3.633	109 ± 15.04
CH1+GG0.50+GL20+ACV	1275.00	42.76	3.367	134 ± 8.14
CH1+GG0.75+GL20+ACV	1700.00	57.75	3.132	170 ± 20.11
CH1+GG1.0+GL20+ACV	2351.95	63.57	2.411	193 ± 17.39
CH1.2+GG0.50+GL20+ACV	1289.45	46.73	3.086	220 ± 3.06

Note. In the first column, the number after each abbreviation indicates the percentage of that component in the film. Abbreviations: ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

At the shear value of 4860 s^{-1}, the hydrogel with the lowest GG content (CH1+GG0.25+GL20) showed the lowest shear stress (τ), i.e. 708.05 N/m^2, and its viscosity (η) was 19.22 Pas. Gel viscosity was the lowest among all tested gels. An increase in the GG content was accompanied by an increase in viscosity. At the shear value of 4860 s^{-1}, the hydrogel with the highest content of GG (CH1+GG1.0+GL20) showed the highest shear stress (τ) of 2351.95 N/m^2, and its viscosity (η) was 63.57 Pas. The above analysis revealed that an increase in the GG concentration in the gel causes a significant increase in the values of the rheological parameters. Comparing the CH1+GG0.50+GL20 and CH1.2+GG0.50+GL gels, there is a slight difference in the values of their rheological

parameters. By analysing gels with different chitosan contents, the author concludes that adding chitosan slightly improves gel viscosity.

Water adsorption tests presented in Table 2 prove that together with an increase in the added polymers (GG and chitosan), there is a decrease in water adsorption of a given carrier. The swelling coefficient of the formulation with the lowest content of the gum, i.e. 0.25% (CH1+GG0.25+GL20), was 3.633, while in the case of the formulation with the highest content GG, i.e. 1% (CH1+GG1+GL20), the coefficient was 2.411, the lowest among all tested formulations. Increased addition of polymers in formulations causes thickening of the polymer network, which may be associated with lower water adsorption capacity. For technological reasons, it is preferable for the dressing to have a lower water adsorption capacity.

The blur time was investigated to determine the approximate duration of the activity of dressings on the oral mucosa. An increased GG content in the films extended blur time. In the case of films with the lowest GG content (CH1+GG0.25+GL20), the blur time was 109 min, and it increased to 193 min for dressings with the highest GG content (CH1+GG1+GL20). There was a longer blur time when comparing the CH1+GG0.50+GL20 carrier with a lower chitosan content with the CH1.2+GG0.50+GL20 carrier with a greater chitosan content. Analysis of the above data indicates that adding polymer strengthens the film's structure and delays the process of hydrogen blurring. The swelling coefficient, blur time, and viscosity are closely related and characterise the physicochemical properties of drug formulations. The increase in hydrogel viscosity along with the increased addition of polymers corresponded to a decrease in the swelling coefficient and extension of blur time. This means that increased crosslinking in hydrogels characterised by poor water absorption improves their stability and makes them harder to blur at the application site.

3.2. Effects of GG and Chitosan Addition on the Texture Parameters of Hydrogel Dressings

Based on the results presented in Table 3, the author concluded that all films had high tensile strength and satisfactory elongation exceeding 23%. Dressings with a higher GG content (i.e. 0.75% and 1%) were characterised by high strength values of 6.1×10^{-3} N/mm ^{2} and 8.0×10^{-3} N/mm^2, respectively, as well as high results for film ductility, with elongation of 52.143% and 61.140%, respectively. The presence of chitosan in the carrier's composition results in significant improvement in mechanical parameters of the film, which could indicate the formation of a crosslinked and stable structure between chitosan and GG.

Table 3. Hydrogel film texture test results.

Formulation	Average force required to break F [N]	Tensile strength [N/mm^2] ± standard deviation	Average increase in dressing length [mm]	Average percentage of dressing elongation [%]
CH1+GG0.25+GL20+ACV	3.913	$4.1 \times 10^{-3} \pm 0.335 \times 10^{-3}$	16.760	47.886
CH1+GG0.50+GL20+ACV	5.328	$5.5 \times 10^{-3} \pm 0.299 \times 10^{-3}$	17.130	48.934
CH1+GG0.75+GL20+ACV	5.904	$6.1 \times 10^{-3} \pm 0.450 \times 10^{-3}$	18.250	52,143
CH1+GG01+GL20+ACV	7.734	$8.0 \times 10^{-3} \pm 0.850 \times 10^{-3}$	21.399	61.140
CH1.2+GG0.50+GL20+ACV	6.225	$6.5 \times 10^{-3} \pm 0.218 \times 10^{-3}$	19.372	55.349

Note. In the first column, the number after each abbreviation indicates the percentage of that component in the film. Abbreviations: ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

3.3. Dissolution of ACV from Mucoadhesive Films

After 30 min, the average percentage of released drug dose was highest for the film with a 1:1 chitosan-to-GG ratio (CH1+GG1+GL20+B), with a result of 83.98%. Analysis of drug release from films with a constant chitosan content and varying GG content revealed very small differences in the percentage of the released dose: 79.95% for 0.25% GG, 75.59% for 0.5% GG, and 77.87% for 0.75% GG (Figure 1). The release rate constants of ACV from dressings with the above compositions were as follows: 0.0499, 0.0482, and 0.064 4min^{-1} (Table 4).

After 30 min, ACV release from films differed significantly depending on the chitosan content when the GG content remained (the CH1+GG0.50+GL20+ACV and CH1.2+GG0.50+GL20+ACV formulations). The average percentage of released drug dose from the film with 1% chitosan content was 75.59%, while for films with 1.2% chitosan content, the average percentage of released drug dose was 65.95% (Figure 1). The rate constant for the release of active ingredient from dressings containing 1% chitosan was 0.067 min^{-1}, while for the carriers with 1.2% chitosan, the rate constant was 0.0354 min^{-1} (Table 4).

An increase in the chitosan-to-GG ratio in films reduced the rate of release, increased the half-release time, and decreased the average percentage of released drug dose per minute. The rate of the process of release of ACV from mucoadhesive dressings over time is consistent with first-order kinetics. The obtained release results, which are presented in Table 4, show that ACV release from the tested films follows first order kinetics, which is confirmed by high correlation coefficients of the trend line of the dependence of ln (100% – % released) on time.

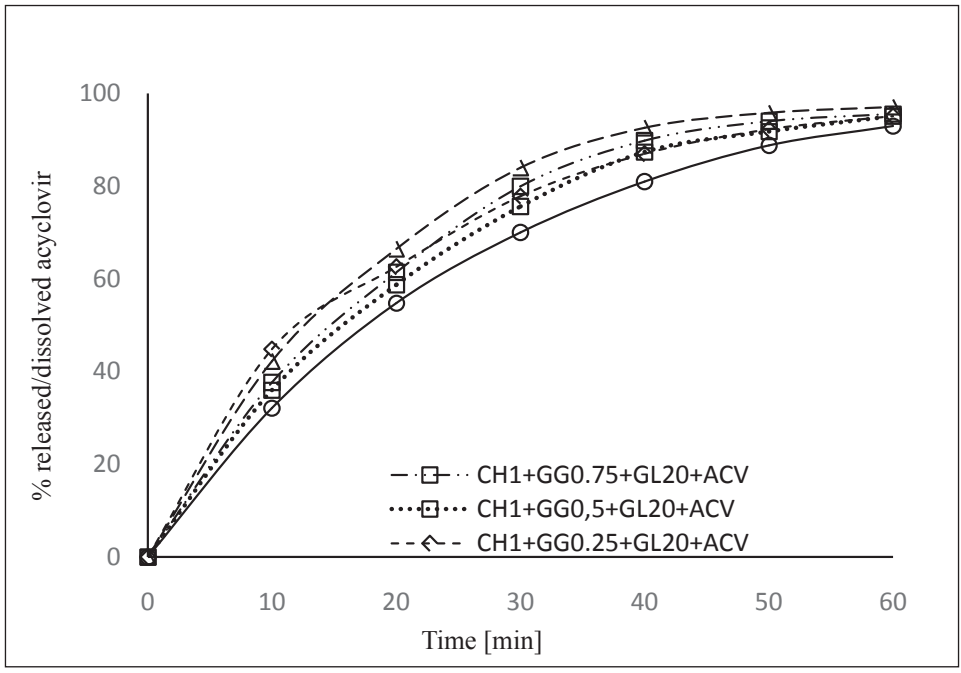

Figure 1. ACV release from hydrogel films. The number after each abbreviation indicates the percentage of that component in the film. Abbreviations: ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

The prolonged release time of active ingredient from films is beneficial in the treatment of oral diseases, as it means that the time during which the drug remains in contact with the site of the lesion is longer, which improves the therapeutic effect of the film.

Based on the above analysis, changes in the GG content in films do not affect the kinetics of drug release from bioadhesive dressings. On the other hand, changing the quantity of chitosan in the formulation significantly affects drug dissolution parameters, and increasing its concentration leads to the desired effect of prolonged release time.

Table 4. Release rate constant [K] and semi-liberation rate (at T0.5) of ACV from the hydrogel films.

Formulation	Equation describing the kinetics of the release profile	Release rate constant K [min^{-1}]	Semi-liberation rate at T0.5 [min]	Correlation coefficient R^2
CH1+GG0.25+GL20+ACV	y = 0.0284x + 3.7029	0.0499	13.88	0.9754
CH1+GG0.50+GL20+ACV	y = 0.0386x + 4.2586	0.0482	14.37	0.9854
CH1+GG0.75+GL20+ACV	y = 0.0347x + 3.9956	0.0644	10.76	0.9860
CH1+GG01+GL20+ACV	y = 0.0438x + 4.0204	0.0670	10.34	0.9797
CH1.2+GG0.50+GL20+ACVvV	y = 0.0279x + 4.0623	0.0354	19.58	0.9912

Note. ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

3.4. Analysis of FT-IR Spectra of Chitosan, GG, and Their Combinations

The FT-IR spectra for chitosan, gum and their combinations are presented in Figure 2. The FT-IR spectrum of GG is characterised by a wide absorption band at 3300 cm^{-1}. It may correspond to the O-H bonds present in its structure. Vibrations caused by C-H bonds are visible at 2900 cm^{-1}. The clear absorption band at 1000 cm^{-1} may correspond to stretching vibrations caused by the C-O-C bond.

The FT-IR spectrum of chitosan is also characterised by a wide band at 3300 cm^{-1}, which identifies stretching vibrations caused by the N-H and O-H bonds. A band at 2900 cm^{-1} is also noticeable; similarly to GG, it corresponds to the C-H bonds present in chitosan. A maximum in the spectrum band is also visible at approximately 1650 cm^{-1}; this is characteristic of the C=O bond, which forms a part of amide groups (-$CONH_2$). Stretching vibrations caused by the C=C and C-OH bonds can be seen at approximately 1400 cm^{-1}. Clear vibrations are also visible at 1000 cm^{-1}; they are characteristic of the C-O-C bonds [12].

The ACV spectrum presents a wide band at 3400 cm^{-1}, which may be caused by O-H bonds, and at 3200 cm^{-1}, which corresponds to N-H bonds. A characteristic band at 1650 cm^{-1} is noticeable; this band stems from C=O bonds which form amide groups (-CONH2). Intense vibrations at the wavenumber range of 1700-700 cm^{-1} are visible; they are a characteristic band for ACV, which corresponds, among others, to C=N bonds (at approximately 1487 cm^{-1}) and C-N bonds (at 1185 cm^{-1}) [13].

A comparison of spectra of dressings that contain both polymers and ACV reveals that the characteristic vibrations which appear in the spectra of films containing ACV constitute characteristic maxima of polymers in the range of 1200-900 cm^{-1}. Increasing the GG and chitosan concentration enhances the peaks characteristic of the particular polymer. Analysis of the FT-IR spectra of films did not reveal the formation of unidentified peaks, which could indicate the formation of new physicochemical structures.

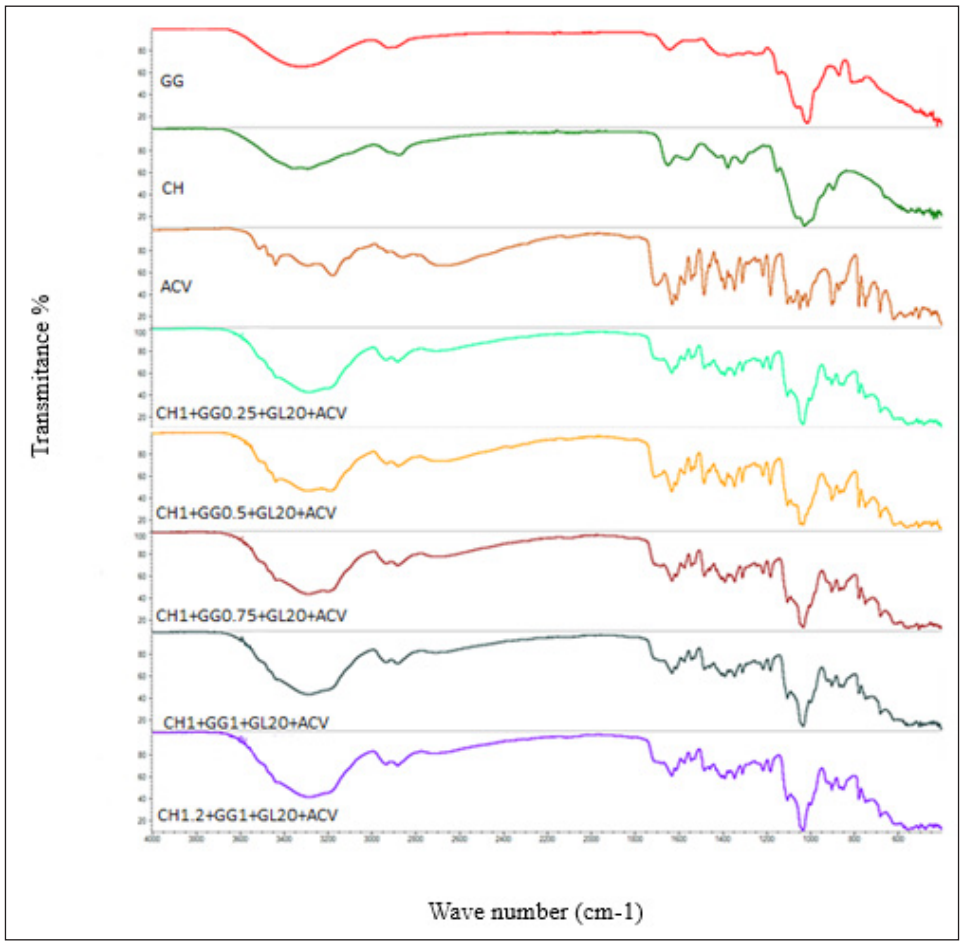

Figure 2. Fourier-transform infrared spectra of chitosan, GG, ACV, and selected films with different polymer contents. The number after each abbreviation indicates the percent of that component in the film. Abbreviations: ACV, acyclovir; CH, chitosan; GG, guar gum; GL, glycerol.

4. Conclusions

1. The film containing 1% chitosan and 1% GG exhibited the best parameters regarding the strength and elasticity.
2. The film containing 1.2% chitosan and 0.5% GG had the best pharmacokinetic parameters.
3. GG significantly impacts the mechanical resistance of the film, while chitosan impacts the texturometric properties and the dissolution of ACV.
4. Analysis of the FT-IR spectra of the prepared films did not reveal any new chemical structures in dressings.

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