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Faculty of Pharmacy

Institute of Pharmaceutical Technology and Regulatory Affairs

Summary of the Ph.D. thesis

Development of buccal mucoadhesive polymer films with sodium alginate and cetirizine dihydrochloride in allergy therapy

Krisztián Pamlényi

pharmacist

Supervisors:

Dr. Katalin Kristó Ph.D.

Dr. habil. Géza Regdon Jr. Ph.D.

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University of Szeged

Doctoral School of Pharmaceutical Sciences

Head: Prof. Dr. Judit Hohmann D.Sc.

Educational Program: Pharmaceutical Technology

Head: Prof. Dr. Ildikó Csóka

Institute of Pharmaceutical Technology and Regulatory Affairs

Supervisors: Dr Katalin Kristó, Ph.D. and Dr. habil. Géza Regdon Jr,. Ph.D.

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	Dr. habil Andrea Vasas, Ph.D., Department of Pharmacognosy, University of Szeged

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1. INTRODUCTION

Buccal polymer films offer an alternative and innovative way to deliver an active pharmaceutical ingredient (API) to the systemic circulation without swallowing the dosage form. The characteristics of this dosage form can be exploited in many diseases, for example, in allergy, hypertensive crisis, Parkinson's disease, coma, treatment of children. The pharmaceutical industry has already started to focus on buccal films and recognized them as a potential drug delivery system, as evidenced by the availability on the market of a buccal film called Breakyl®, containing fentanyl.

Nowadays, the buccal drug delivery system is becoming an important alternative route in drug administration. This possibility of drug application has been untapped and poorly investigated. Buccal drug delivery systems have some bioadhesive solid dosage forms, such as bioadhesive tablets, gels, patches, and mucosal bioadhesive films. Patients do not have to swallow this dosage form, they should place the bioadhesive system onto the buccal mucosa of the mouth, and the API can be absorbed into the systemic circulation. Many research groups have already tried to formulate buccal polymers films with different APIs, such as omeprazole, miconazole, ondansetron and prednisolone, but they did not investigate the prepared films widely because it is not an official dosage form in the Pharmacopeia, so official rules and guidelines for polymer films cannot be found.

2. AIM OF EXPERIMENTAL WORK

In my research work, I prepared buccal mucoadhesive polymer films with SA and CTZ as a potential buccal drug delivery system.

In the first part of my work, my main goal was to prepare and investigate polymer films of different compositions without API. The aim of my work was to:

- evaluate the film-forming ability of SA
- optimize the prepared films with preformulation
- study the mechanical properties of films
  (thickness, breaking hardness, in vitro mucoadhesivity)
- investigate the effect of different amounts of GLY on the polymer system
- evaluate the chemical interactions between the components of films
  (FT-IR spectroscopy, TGA, DSC)
- develop a drug delivery polymer matrix based on SA for buccal application

- evaluate the data of the films with statistical analysis (factorial design) by mixed two-level and three-level factorial designs. With this analysis, we are able to predict the properties of certain compositions without measurement, so efficiency can increase.

In the second part of my research work, my main aim was to choose the API to be incorporated in the polymer film system. I chose CTZ as the drug for the films. The API can influence the bonds and interactions between the components of the films, as a result of which the properties of the films can be changed. Therefore, my further goals were as follows:

- investigate the mechanical properties of films (thickness, breaking hardness, in vitro mucoadhesivity, contact angle (CA) and surface free energy (SFE) measurement)
- study the effect of the GLY and CTZ on the polymer system
- evaluate the interactions between the API and the components of films (FT-IR and RAMAN spectroscopy, TGA, DSC)
- study the release of the API from the different compositions of films (dissolution test)
- evaluate the data of the films with statistical analysis (factorial design) by mixed two-level and three-level factorial designs.

In the last part of my scientific work, my main goal was to investigate the stability of the prepared films, as well as the relation of the promising compositions based on my earlier research work with the living cells. The knowledge of these properties is very important from the point of view of storage and application. The aims were the following:

- study the stability of the prepared film
    - mechanical stability (thickness, breaking hardness, in vitro mucoadhesivity)
    - physicochemical stability (FT-IR spectroscopy)
    - API content
- based on data from the literature, citric acid (CA) enhances permeation, therefore, it was incorporated into the films to improve permeation [43, 82].
- investigate the cytotoxicity of the polymer films
- examine the permeation of CTZ from buccal films across an artificial membrane
- evaluate the permeation of CTZ from the prepared films on the TR 146 buccal cell line

My final aim was to select the best composition with suitable mechanical and chemical properties, good stability, appropriate cytotoxicity and high permeability.

3. MATERIALS AND METHODS

3.1. Materials

Sodium alginate (SA) (Biochemica GmbH, Darmstadt, Germany) (10,000–600,000 g/mol) and hydroxy methylcellulose (HPMC) (Pharmacoat® 603, Shin Etsu Chemical Co., Ltd., Tokyo, Japan) were used as film-forming agents in the polymer film. Glycerol (GLY) 85% (w/w %) was added to the film as a plasticizer (Ph. Eur. 8.). Citric acid (CA) (Ph. Eur. 8.) was incorporated in the polymer film system as a permeation enhancer. Cetirizine dihydrochloride (CTZ) (Ph. Eur. 8.) was the API, which was a gift from ExtractumPharma Pharmaceutical Manufacturing, Marketing and Consulting Inc., Kunfehértó, Hungary. Mucin (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) (10 w/w %) dispersion was used in the in vitro mucoadhesion test.

3.2. Preparation of buccal films

The films were prepared at room temperature with the solvent casting method. As the first step of preparation, SA (1, 1.33, 1.5, 2, 3, 4 w/w %) was dissolved in distilled water and mixed (900 rpm) at room temperature. The solution was heated to 70 °C and mixed (900 rpm), and CTZ was then incorporated in the warm solution (70 °C, 0.5523 g/100 g solution), and the mixing was continued for 5 h. In certain cases, as the third step, HPMC (0, 0.66, 1, 1.5 w/w %) and CA were added to the solution with mixing without heating. In the fourth step, GLY (0, 1, 3, 5 w/w %) was added to the solution the following day. Mixing was decreased to 100 rpm for 3 h to help the air bubbles disappear from the solution.

The solution was cast on a glass surface in Petri dishes, with 10 g of solution/dish; then, it was dried at room temperature (24.4 ± 0.5 °C). The dried polymer films were removed from the surface and were placed in closed containers (24.4 ± 1 °C, 60 ± 2 % RH), and the other part of the prepared films was also placed in closed containers, but at 40 °C ± 2 °C, 75 % RH ± 5 % RH. The prepared films contained 10 mg of CTZ on an area of 4 cm^2, which is the therapeutic dose of this API. **Table 1.** shows the different compositions of the prepared polymer films.

Table 1. Composition of different SA- and HPMC-based films

Minta	SA	HPMC	GLY	CA	CTZ	Minta	SA	HPMC	GLY	CA	CTZ
	(w/w%)						(w/w%)				
1	2	0	0	-	-	25	1.33	0.66	1	-	-
2	3	0	0	-	-	26	1.33	0.66	1	-	+
3	4	0	0	-	-	27	1.33	0.66	3	-	-
4	2	0	1	-	-	28	1.33	0.66	3	-	+
5	2	0	3	-	-	29	1.33	0.66	5	-	-
6	2	0	5	-	-	30	1.33	0.66	5	-	+

Minta	SA	HPMC	GLY	CA	CTZ
	(w/w%)				
7	3	0	1	-	-
8	3	0	3	-	-
9	3	0	5	-	-
10	2	0	1	-	+
11	2	0	3	-	+
12	2	0	5	-	+
13	3	0	1	-	+
14	3	0	3	-	+
15	3	0	5	-	+
16	4	0	1	-	-
17	4	0	3	-	-
18	4	0	5	-	-
19	1	1	1	-	-
20	1	1	1	-	+
21	1	1	3	-	-
22	1	1	3	-	+
23	1	1	5	-	-
24	1	1	5	-	+

Minta	SA	HPMC	GLY	CA	CTZ
	(w/w%)				
31	1.5	1.5	1	-	-
32	1.5	1.5	1	-	+
33	1.5	1.5	3	-	-
34	1.5	1.5	3	-	+
35	1.5	1.5	5	-	-
36	1.5	1.5	5	-	+
37	2	1	1	-	-
38	2	1	1	-	+
39	2	1	3	-	-
40	2	1	3	-	+
41	2	1	5	-	-
42	2	1	5	-	+
43	3	0	1	+	+
44	3	0	3	+	+
45	1.5	1.5	1	+	+
46	1.5	1.5	3	+	+
47	2	1	1	+	+
48	2	1	3	+	+

3.3. Methods

3.3.1. Thickness

The thickness of the polymer films was measured with a screw micrometre (Mitutoyo Co. Ltd, Japan), sensitivity was 0.001 mm. Six points were selected randomly from all films (n = 6). The means and standard deviations (SD) were evaluated from these data.

3.3.2. Breaking hardness

Breaking hardness was tested with a self-developed device and software. The device and the software were developed at our institute [47, 88]. The device has two different types of sample holder as probes (needle-like probe, rod-like probe). The equipment has a fix disc and a vertically moving jowl. Force, force-displacement and time can be registered. The breaking hardness of the films can be examined with the needle-like probe (its area was 201 mm^2). At the beginning of the test, the sample was fixed on the bottom part of the equipment and the probe was lowered at constant speed (20 mm/min). The probe moved towards the film and finally it broke the film. The test was repeated six times (n = 6) for each film. Tablets, pellets, films can also be investigated with this equipment. The means and standard deviations were calculated.

3.3.3. In vitro mucoadhesivity measurement

Mucoadhesion was investigated with the same texture analyser with different settings and parameter modifications. In this study, a rod-like sample holder with a diameter of 5 mm was used. Polymer films was fixed on the surface of the sample holder. On the bottom part of the tester, a fixed disc with a diameter of 35 mm was applied, and 40 µL of freshly prepared mucin dispersion (10 w/w %) was spread on it. The rod-like sample holder went to the fixed, bottom disc and pressed the polymer film to the mucin-covered bottom disc with 30 ± 0.1 N for 30 s. Thereafter, the sample holder went back to the original place, and the force was decreased until the sample started to separate from the mucin, which can be seen as a well-defined, sharp peak in the force–time curve, indicating the in vitro mucoadhesion force of the films. The test was done six times (n = 6), and the means and standard deviations were calculated.

3.3.4. Contact angle and surface free energy (SFE) measurement

One drop of distilled water and diiodomethane was used to measure the contact angle (Θ) of polymer films for 15 s, with the circle fitting method, by using a contact angle apparatus (OCA20-DataPhysics Instrument GmbH, Filderstadt, Germany).

The means and standard deviations (SD) were calculated from 6 identical samples of each combination of films (n = 6). The means and standard deviations were used to calculate the surface free energy of films. Surface free energy was calculated with Wu's method.

3.3.5. FT-IR spectroscopy

An Avatar 330 FT-IR apparatus (Thermo-Scientific, Waltham, MA, USA) was used to analyse the Fourier-Transform Infrared Spectra of the raw materials and the prepared polymer films. The apparatus was equipped with a coupled Zn/Se horizontal attenuated total reflectance (HATR) unit. The range of wavelength was 600 to 4000 cm^{-1} during the investigation. The spectra were collected from 128 scans at the spectral resolution of 4 cm^{-1} with CO_2 and H_2O for correction.

3.3.6. Raman spectroscopy

To investigate the distribution of API, Raman spectra were acquired with a Thermo Fisher DXR Dispersive Raman (Thermo Fisher Sco. Inc., Waltham, MA., USA) equipped with a CCD camera and a diode laser operating at a wavelength of 780 nm. Raman measurements were carried out with a laser power of 24 mW at a 25-µm slit aperture size on a 2-µm spot size. The discrete spectra of the individual substances such as CTZ, HPMC, and SA, and different

compositions of polymer films were collected using an exposure time of 6 s, the number of exposures was 20, and the number of background exposures was 512. Smart background was used during the whole investigation. The applied spectral range was 3200–200 cm^{-1} with cosmic ray and fluorescence corrections.

3.3.7. Thermoanalytical measurement (thermogravimetric analyses (TGA), differential scanning calorimetry (DSC))

The thermoanalytical measurement of the prepared films was carried out with a Mettler-Toledo TGA/DSC1 instrument (Mettler Toledo, Switzerland). Small pieces of films (approximately 10 mg) were placed in aluminium pans (40 μL), and were inserted into the instrument. During the measurement, the start temperature was 25 °C and the end temperature was 500 °C. The heating rate was 10 °C/min. The samples were investigated in flowing nitrogen atmosphere, the flow rate was 50 ml/min. The curves were evaluated from the average of two parallel measurements with STARe software.

3.3.8. Dissolution test

Polymer films of a size of 2 cm × 2 cm (containing 10 mg of CTZ) were investigated in the dissolution test. The dissolution test was made by an Erweka DT700 dissolution basket tester at a mixing speed of 100 rpm. 900 mL of phosphate buffer (pH = 6.8) was used as the dissolution medium, and its temperature was 37 °C. Aliquots of 5 mL were analyzed in 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min with Genesys 10S UV-VIS (Thermo Fisher Scientific, USA) UV-spectrophotometry at λ = 207 nm.

3.3.9. Stability test

In my research work, stability studies were done according to ICH guidelines. The prepared films were placed in closed containers and stored at 40 °C ± 2 °C, 75 % RH ± 5 % RH for a period of 6 months. Different methods were applied to obtain information about the changes in the properties of the films. During the stability test, breaking hardness, in vitro mucoadhesivity and active agent content were analysed to determine changes in the physical properties of the films. Furthermore, the interactions I also examined with FT-IR spectroscopy, and the drug content was also detected during the 6-month period.

3.3.10. Cell viability test

In order to study the cell viability of CTZ films, a Guava® easyCyte™ 5HT (Luminex, Austin, TX, USA) flow cytometer was used for our experiments. TR-146 cells were collected from cell culture flasks with a trypsin–EDTA solution and redistributed into separate tubes, and 1 million cells were treated with 1 mL of CTZ solution (CTZ films dissolved in HBSS in equal concentration as in the case of the permeation tests). After 30 min of incubation, the cells were centrifuged, the test solutions were removed, and the cells were gently washed with cold HBSS and centrifuged again.

The supernatant was removed, and a 1 million cells/mL cell suspension was prepared with HBSS and then stained with 1 µL of 100 µg/mL propidium iodide solution. After 15 min, the suspensions were distributed on 96-well microplates in a volume of 200 µL (3 wells/group) and analysed. Propidium iodide was excited with a 488-nm laser and detected at the 525/30 nm channel (green parameter).

On the Forward and Side Scatter Patterns (FSC-SSC) scatterplot, the non-cellular events were excluded. The remaining events (8,000–10,000) were analysed on a scatterplot, and gates were created to determine stained (necrotic) and non-stained (living) cells. The experiment was carried out in triplicate. As negative control, HBSS was used, and cells treated with HBSS instead of the dissolved films were considered 100%, to which all treated groups were compared. As positive control, cells were treated with 1% Triton-X 100.

3.3.11. Permeation test

The permeation of CTZ was studied in the Enhancer Cell across an artificial cellulose acetate membrane (Whatman®, SN:WHA10404106, pore size 0.2 µm, surface 2.54 cm^2). The films were put on the surface of the membrane and placed into the donor phase, which was 2 mL phosphate buffer (pH = 6.8), modelling saliva. The acceptor phase was also phosphate buffer (pH = 7.4, 300 mL), which simulated the pH of blood. The test was run in an Erweka DT700 dissolution basket tester at a mixing speed of 100 rpm. Aliquots of 5 mL were analysed at 15, 30, 60, 90, 120, 180 and 240 min with Genesys 10S UV–VIS (Thermo Fisher Scientific, Waltham, MA, USA) UV-spectrophotometry at λ = 207 nm wavelength.

For the in vitro permeation test, 1×10^5 cells were seeded on ThinCert® PET cell culture inserts with a pore size of 0.4 µm, a pore density of $2 \times 10^6/cm^2$ and a culturing surface of 33.6 mm^2. Each film was dissolved in Hank's balanced salt solution (HBSS) with pH = 7.2 before the experiment. The cell culture medium was removed from the inserts, and 400 µL of the dissolved films was added to the apical compartment (AC) and 1000 µL to the basolateral

compartment (BC). After 30, 60 and 90 min, 2 × 100 µL of solution was removed from the wells after gentle mixing. The removed volume was replaced with HBSS.

3.3.12. Statistical analysis

The collected data were analyzed with the factorial ANOVA method by Tibco Statistica v13.4.0.14 (Statsoft, Tulsa, OK, USA) software. The results were evaluated by mixed two-level and three-level factorial designs. The equations describe the relationship between the two factors (x_1–concentration of SA and x_2–concentration of GLY) and the five optimalization parameters (y_1–thickness; y_2–breaking hardness, y_3–mucoadhesion force, y_4–surface free energy, and y_5–dissolution).

The significance test of breaking hardness and in vitro mucoadhesivity was evaluated with Microsoft Excel (version 15, Redmond, Washington, USA) as software. A Two-Sample T-Test was applied. In all cases, the samples were compared to the composition without CTZ. In each case, we used a significance level $p < 0.05$.

In the stability study (breaking hardness, in vitro mucoadhesivity) the samples were compared to the freshly prepared sample (0 month). The apparent permeability values of the films were analysed with the Kruskal–Wallis test with Dunn's test as post hoc test. In each case, we used a significance level of $p < 0.05$.

4. RESULTS

4.1. Thickness and breaking hardness measurement

Thickness (y_1) of different compositions of films rises depending on the polymer and GLY concentration. The 3% polymer films are thicker than the 2% ones; therefore, the polymer concentration can increase the thickness of the films due to the higher amount of polymer. The type of polymer does not influence thickness notably; films with a higher amount of SA (without HPMC) are thicker than the 2:1 SA and HPMC films. The 2:1 SA and HPMC films are thicker than the 1:1 SA and HPMC films, but there is no significant difference between the SA films without HPMC (Samples 4-6, 10-12 and 7-9, 13-15), 1:1 SA and HPMC (Samples 19–24 and 31–36) and 2:1 SA and HPMC films (Samples 25–30 and 37–42). The c_{SA} (x_1) coefficients were very low in all cases, and they were not significant. C_{GLY} (x_2) can also influence thickness, and this was statistically significant in all cases. CTZ can also enhance thickness, which can be explained by the fact that CTZ increases the dry matter content of the films.

In my work, GLY was found to decrease the breaking hardness (y_2) of films. This observation is due to the fact that GLY can interact with other components of films (CTZ, SA, and HPMC), typically in the form of hydrogen bonds, and retains water. In addition, GLY can enhance the bonding distance, so films with a high GLY concentration can break more easily.

The average values (14.82 N and 9.55 N) were found to be lower in the case of 2% polymer concentration than in the case of 3% (25.32 N and 13.81 N). The total polymer increases breaking hardness because it can form a cohesive, strong, stable structure in the films due to the bonding of polymer chains.

Finally, it can be said that 2% polymer films with lower SA concentration (Samples 19–24) have low breaking hardness (less than 10 N), and these films break easily. This property is not acceptable from the aspect of application. The other film compositions have high breaking hardness and can be used properly for buccal application because they do not break from the force of the finger.

4.2. In vitro mucoadhesivity measurement

The mucoadhesion of the prepared films is presented in **Figure 1**. The CTZ-free films had high mucoadhesion force values (y_3), most of them more than 10 N and some samples above 15 N (Samples 3, 4, 7, 19, 21, 31, 33, 37, 39). The CTZ-containing samples had significantly lower mucoadhesion force, so CTZ decreases the mucoadhesion of polymer films, possibly due to the interaction between the carboxylic groups of SA, HPMC and CTZ molecules, thus fewer groups are able to bind to the mucin. These samples have moderate mucoadhesion, but they can be used for a buccal drug delivery system because this lower force is enough for buccal mucoadhesion. The increasing amount of polymers increased mucoadhesion due to the higher number of free binding groups in the system, which can bind to the mucin of the buccal mucous.

GLY can influence mucoadhesion force. The increasing amount of GLY decreased mucoadhesion force, probably because of the hydrogen bonds which can be formed between GLY and the film-forming polymers, and also due to the fact that no binding is formed with the chains of mucin because of the lower number of free chains in the polymer.

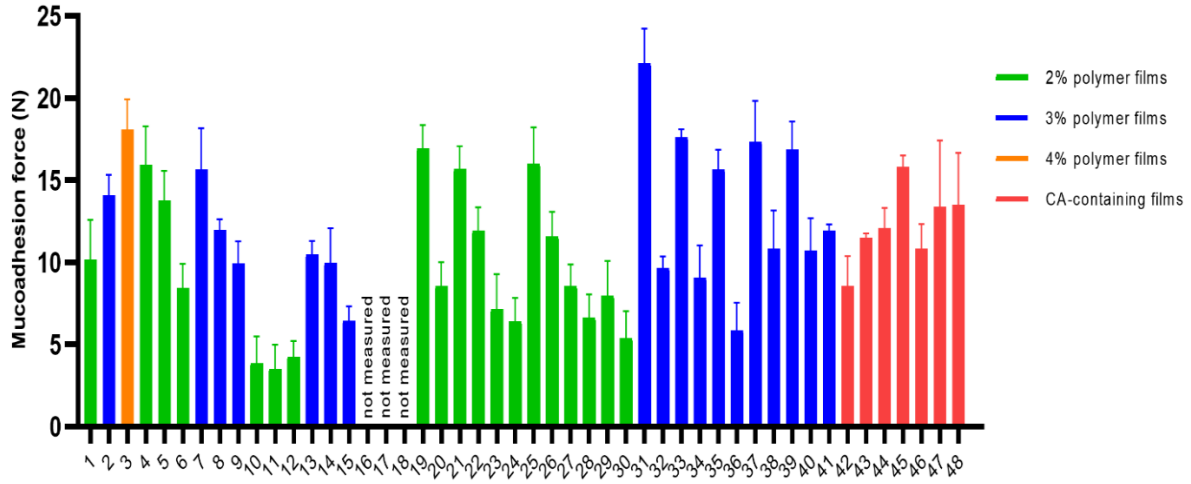

Figure 1. Mucoadhesion force of the prepared films

During statistical analysis, the mucoadhesion force of films of all compositions (CTZ-free and CTZ-containing) was found to be decreased significantly by GLY. Similarly, SA also decreased it in the CTZ-free films because SA has moderate mucoadhesion force, while HPMC has higher one, so a greater HPMC concentration can cause higher mucoadhesion in the films. The reason for this effect can probably be the interaction between the functional groups of SA and CTZ, thus SA will have fewer groups left capable of binding to the mucin (**Figure 1.**) as it can be stated in the FT-IR and in the Raman measurements as well.

4.3. Contact angle and surface free energy (SFE) measurements

The contanct angle and SFE (y_4) measurements are very important and critical from the aspect of applying this pharmaceutical dosage form because the saliva has to spread on the surface of the films extensively in order for mucoadhesion to develop. The CTZ-free films exhibited medium SFE 51.99 mN/m and 57.81 mN/m average values, while the CTZ-containing films have significantly higher SFE, the average value was 69 and 76.11, so CTZ raises the SFE of films, particularly the polar part of SFE. CTZ-containing films also show significantly higher polarity, which may be caused by the carboxylic group of the CTZ molecule. From these results, it can be concluded that an extended conformer of CTZ can be found in the prepared films. Contact angle showed lower values in the CTZ-containing films. The SFE of CTZ-free films changed particularly as a function of increasing GLY concentration and only slightly as a function of the SA concentration. This observation was not true for the CTZ films because in those films, the SFEs had roughly constant values independently of the concentration of GLY and polymer concentration. In 2% polymer films with and without CTZ, SFE is increased by SA and GLY, while in films with 3% polymer API and without API, SFE is reduced by SA.

4.4. FT-IR spectroscopy

The polymer films were examined with FT-IR spectroscopy to identify the interactions between the components of the films. The carboxyl group of CTZ can be explored at 1739 cm^{-1} in FT-IR spectra (**Figure 2.**). In the spectra of the API, this peak can be separated sharply. However, in the spectra of the films, this peak is shifted and disappears, depending on the concentration of the polymer. In the 2% polymer films, the CTZ peak is shifted towards the larger wavenumber, but the intensity of the peak is smaller than in the case of the raw material. In case of the 3% polymer films, the peak disappears completely, which means that interactions can develop between CTZ and the SA and HPMC in the films. It can be asserted that hydrogen bondings are created between SA, HPMC, and the carboxylic group of CTZ. Due to the low polymer concentration, the films have fewer OH groups of SA and HPMC, which can create hydrogen bonding, so, in this case, the peak is only slightly shifted. However, the 3% polymer films have more OH groups, thus more hydrogen bonds can be created, which can cause a more remarkable structure change, so the peak can disappear completely.

With these findings, the FT-IR results sufficiently demonstrated the interactions that can occur in the films. The results of Raman spectroscopy also confirm these interactions.

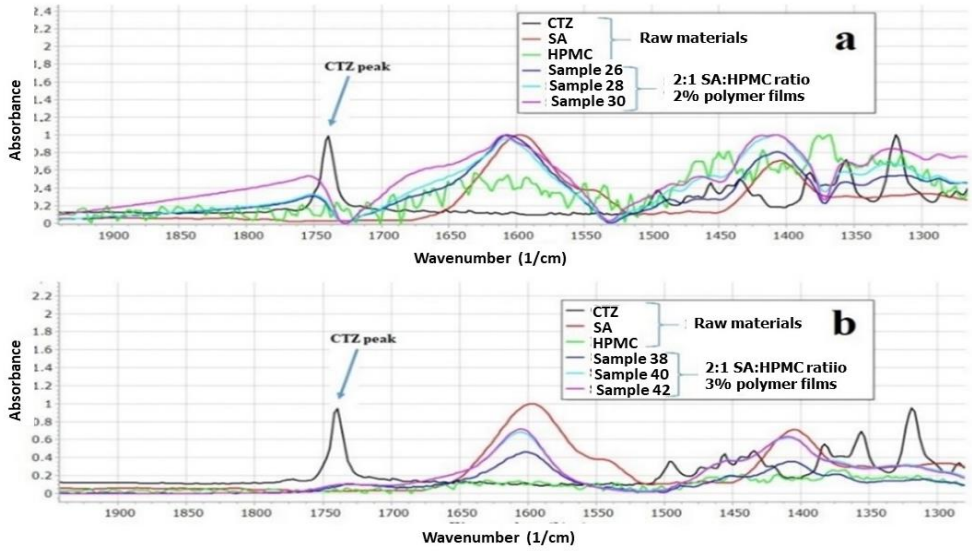

Figure 2. Individual FT-IR spectra of SA, HPMC, CTZ, and prepared films (**a**): 2% polymer films and (**b**): 3% polymer films

4.5. RAMAN spectroscopy

Based on the comparison of the spectra of the components, a CTZ peak was chosen at 1598.15 cm^{-1}. It is assigned to the C-C stretchings in the phenyl and chlorophenyl groups in the

chemical structure of CTZ. This sharp peak was chosen for the chemical mapping profile because it characterizes the API individually.

Chemical maps are shown in **Figure 3**. The distribution of CTZ was homogeneous in the polymer films with different GLY contents. The warm (red and orange) colors indicate a greater API content in the samples. In the film with 1% GLY content, the API can be in crystalline agglomerates (yellow, orange spots) in the samples. In the other two cases, especially in the case of the sample containing 3% GLY, the color distribution of the maps is very smooth. It means a possible molecular disperse distribution of the API in the film. As a conclusion, the water-soluble API is in a dissolved form in the films because a higher GLY concentration also means higher water content due to the moisturizing property of GLY. This property of the films can be disadvantageous because it can cause the physical instability of the samples. Besides, higher GLY concentrations can be harmful during the mucoadhesion of buccal films because GLY reduces the amount of mucoadhesive polymer chains which are able to bind.

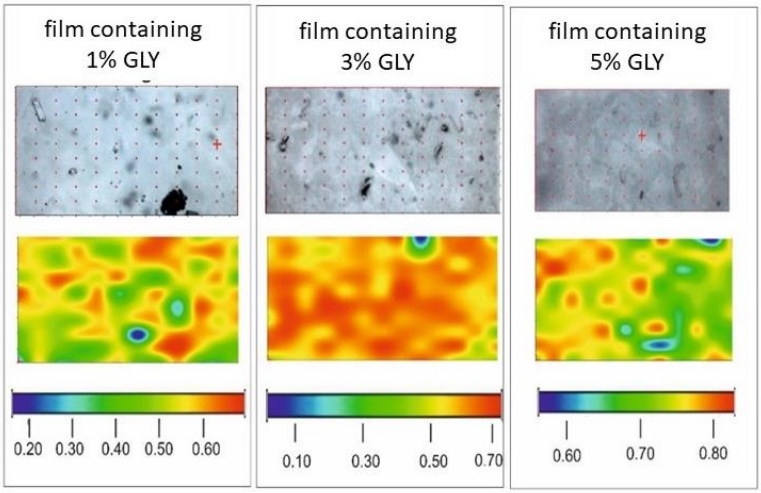

Figure 3. Chemical mapping of films with different GLY contents

4.6. Thermoanalytical measurement (thermogravimetric analyses (TGA), differential scanning calorimetry (DSC))

The mass loss of the film-forming agent is 11.84% until 180 °C, and 47.56% until 500 °C. The decomposition process starts from 75 °C. For GLY, the mass loss is almost similar until 180 °C, but it is more than 86% until 500 °C. The mass loss curve of CTZ reveals that decomposition starts above 200 °C, so CTZ can be said to be a thermostable API.

The DSC curve of SA shows an endothermic peak from 40 °C to 150 °C and an exothermic peak is visible from 200 °C to 280 °C. For GLY, 2 peaks can also be detected. The

decomposition of CTZ starts after the melting point, which can be seen at 223.32 °C, followed by the general decomposition of CTZ.

The results of the thermal behaviour of films can be found in **Figure 4.,** which shows the thermoanalytical curves of 3% polymer films. The decomposition of buccal films can take place in two steps. In the first step (until 180 °C), it is visible that films with the lowest GLY concentration (Sample 10) (it is not visible in **Figure 4.**) have the lowest mass loss, which can be explained by the lower water content of films with low GLY concentration. In the case of larger GLY and SA concentrations, which result in a higher water content, there is no significant difference in the mass loss of Samples 11-15.

In Figure 4., the same observations can be made in the case of the second stage of the decomposition process, but the mass loss values of the films with different compositions were higher, ranging from 62% to 70%.

Two characteristic peaks of SA can be seen in the DSC curves of films with different compositions (**Figure 4.**). The first peak shifted towards the higher temperature with increasing GLY concentration. This observation revealed a moderate interaction between the components of the films, which could indicate the presence of hydrogen bonds, which was also confirmed by the results of the FT-IR spectroscopy measurements. The exothermic peak of the DSC curves also showed a shift with increasing GLY concentration, the peak moved towards the lower temperature. This relationship was also observed in the case of films with 2 and 3% SA concentration. In summary, it can be said that the decomposition processes usually started at 70 °C, so the polymer films can be considered thermally stable up to this temperature.

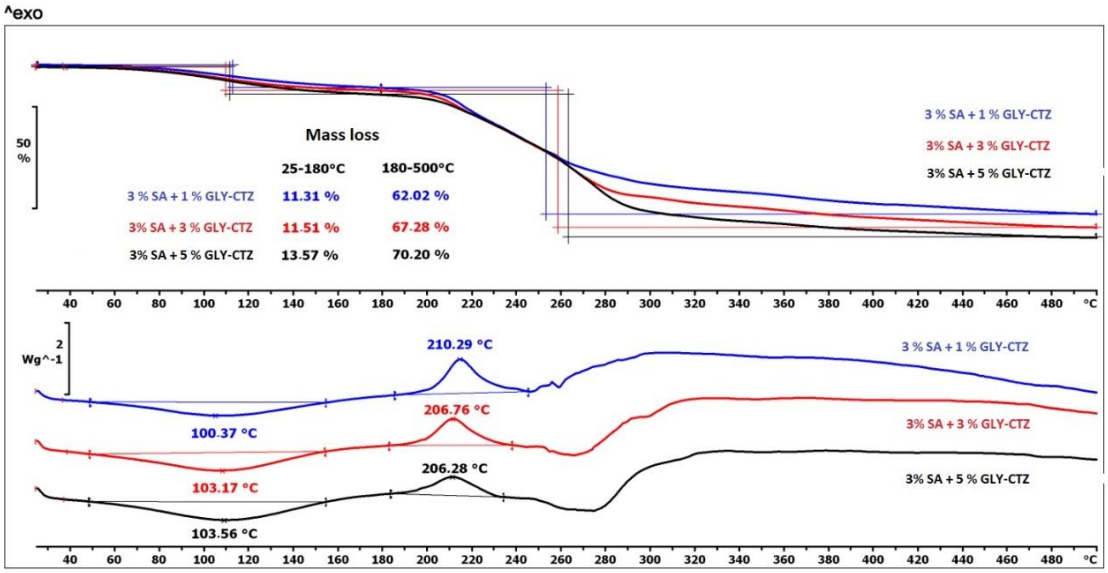

Figure 4. Thermal properties of the 3% polymer films as shown by TGA and DSC curves

4.7. Dissolution test

During the whole test (120 min), the total amount of CTZ dissolved from the different compositions of the films (y_5). In the first 20 min, almost 100% of the API can dissolve from the films with 2% polymer concentration (**Figure 5/a**). CTZ can dissolve from the thinner polymer layer faster than from thicker films, and on the other hand, in the case of thinner films, HPMC is able to dissolve more easily. Films with a lower SA concentration have faster API dissolution, which can be slowed down by GLY. When the SA and GLY concentrations were increased, dissolution became slower and in the first 20 min, the API cannot be released fully. 65% to 96% of the API dissolved from the 3% film compositions. It is noteworthy in the b" part of **Figure 5**. that, at the beginning of the test (first 5 min), the API dissolved faster from the films with equal polymer concentration. The reason for this is that in the case of 3% polymer concentration a stable, cohesive structure is formed between the chains of the polymers, GLY and API, as visible from the thickness and hardness tests and also supported by FT-IR spectroscopy measurement, therefore, GLY cannot affect dissolution significantly. Compared to 2% films, the effect of GLY is less than expected in 3% films.

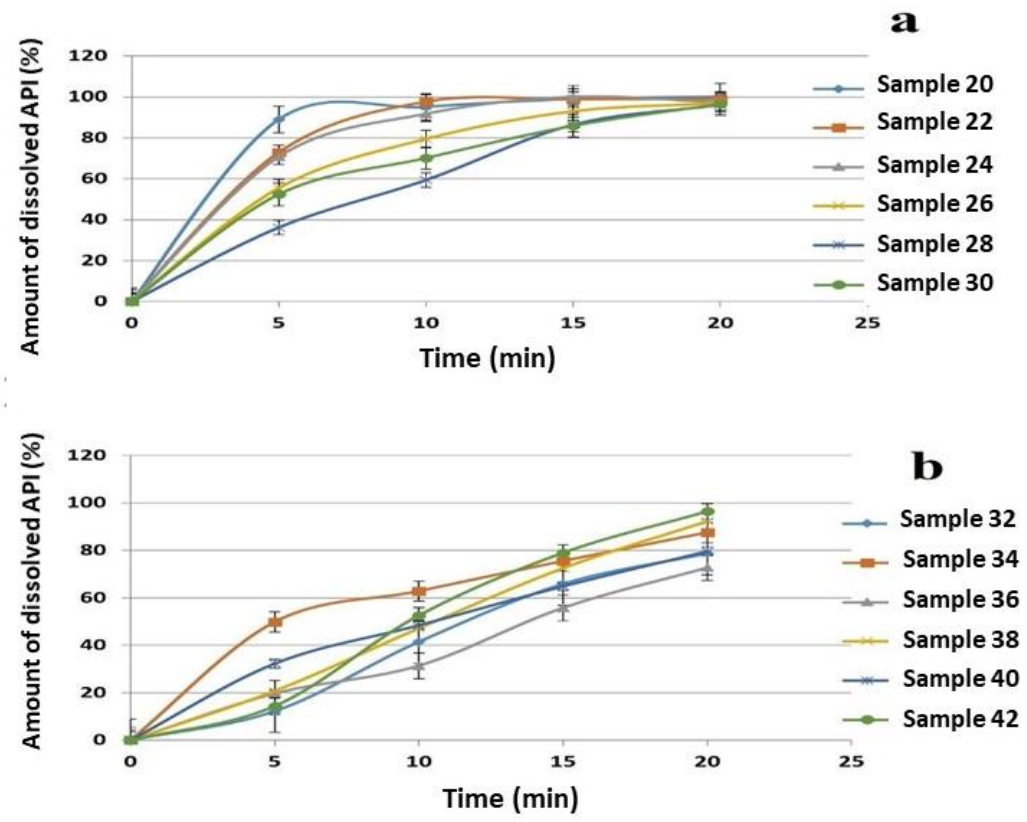

Figure 5. Dissolution curves of the prepared films in the first 20 minutes
(**a**: 2% polymer films, **b**: 3% polymer films)

4.8. Stability test

4.8.1. Breaking hardness measurement

The results of the breaking hardness measurements are presented in **Figure 6**. Breaking hardness decreased for every composition during the period. At the beginning of the investigation, the breaking hardness of the films containing CA was higher than that of the films without CA. At the same time, in the case of CA-containing films (Samples 43, 44, 45, 46, 47, 48,) the decrease in breaking hardness was higher than without CA (Samples 13, 14, 32, 34, 38, 40). As the GLY concentration increased, the breaking hardness of the films decreased. During storage, GLY can decompose, which can cause the decrease in the breaking hardness of the films, and due to this fact, the water content of the films also decreases. This assumption is also supported by the results of the FT-IR measurements. Because of these facts, it can be said that the films that do not contain CA and contain a low GLY amount have adequate stability in terms of breaking hardness (Samples 13, 32, 38).

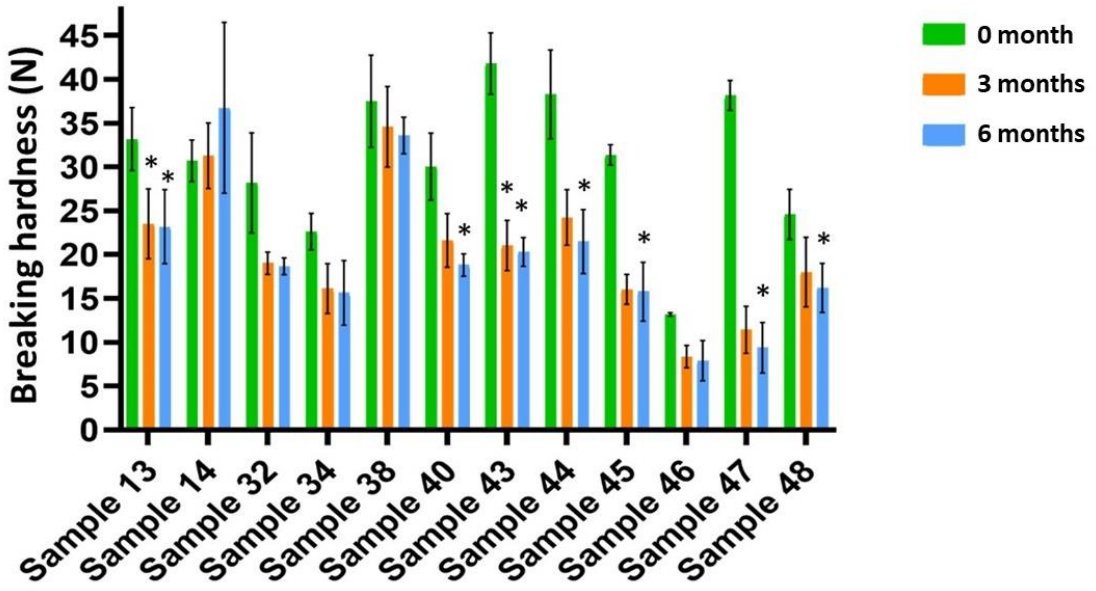

Figure 6. Breaking hardness of the prepared films (Samples 43, 44, 45, 46, 47, 48 contain CA) (* $p < 0.05$; $n = 6$). The samples were compared to the freshly prepared sample (0 month).

4.8.2. In vitro mucoadhesivity measurement

Mucoadhesion force increased for almost every sample during the 6-month period. It means that the films can connect to the buccal mucosa in a stronger way after storage time. This phenomenon can probably be explained by the fact that more free chains are formed during

storage, so carboxyl and hydroxyl groups can connect to the oligosaccharide chain of mucin on the buccal mucosa.

The GLY concentration can also influence the mucoadhesivity of films. Films with a higher GLY concentration may cause lower growth and growth rate in mucoadhesivity. This observation may be due to the fact that high amounts of GLY can build in the polymer film structure better, therefore during storage fewer chains are free which are able to bind to the mucin of the buccal mucosa.

4.8.3. Active agent content

Figure 7. shows the results of the change in content during 6 months of storage. As expected, a decrease can be observed for each sample under the forced condition during storage. The reductions in the amount of the API were higher at the beginning of the investigation (between 0 month and 3 months), while later (between 3 months and 6 months), the changes were smaller. The changes were larger for films containing CA, so it can be concluded that CA can decrease the stability of films. GLY can also enhance the decomposition of the API. It was found that films with a larger amount of GLY had a lower amount of API after 6 months than films with a low amount of GLY, so higher GLY concentrations in the films can also reduce film stability. As can be seen, Sample 13 and Sample 32 can preserve the required amount of the API (higher than 85%) after 6 months, while Sample 38, 45, 47 can preserve it after 3 months.

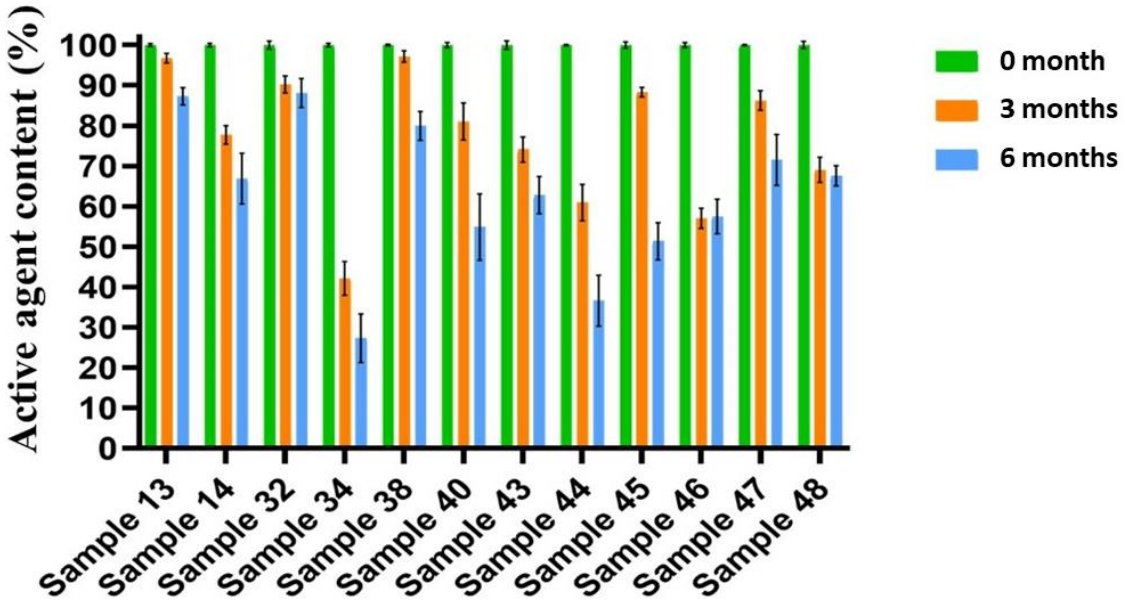

Figure 7. API content for different films during accelerated stability study (Samples 43, 44, 45, 46, 47, 48 contain CA) (n = 6).

4.8.4. FT-IR spectroscopy

In **Figure 8.**, the FT-IR spectra of the films can be seen. Part „A" of **Figure 8.** shows the film composition with the largest amount of API (Sample 13) after six months, while in the bottom figure the film composition with the least amount of API is presented (Sample 46). As can be seen, the carboxyl group of CTZ can be detected at 1739 cm^{-1} in the FT-IR spectrum. In the spectrum of CTZ as a raw material, this peak can be sharply distinguished. However, in the films, this peak is shifted to the higher wavenumbers. In case of Sample 13 (Part „A" of **Figure 8.**), the CTZ peak is shifted towards a higher wavenumber, but the intensity of the peak is smaller than in the case of the raw material. In Part „B" of **Figure 8.**, for Sample 46, the peak disappears completely after sixth months, which shows that the amount of the API decreased because of the forced condition during storage, and hydrogen bonds are formed between SA, HPMC and the carboxylic group of CTZ, which can cause more remarkable structural changes.

Overally, decomposition of API was faster in films containing CA than in films without CA. In addition, chemical interactions occurred between the components of the films.

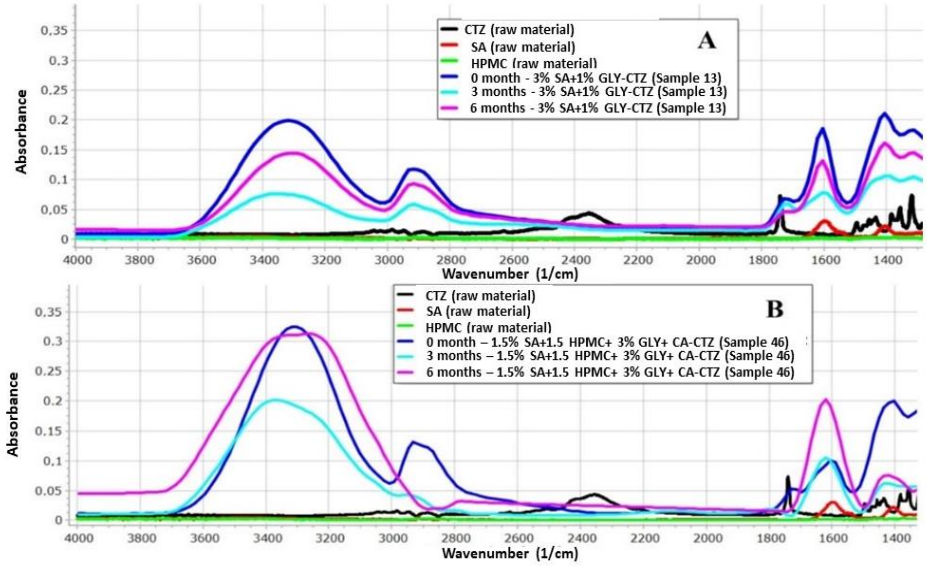

Figure 8. Results of the FT-IR spectra of the prepared films (Part „A" Sample 13, Part „B" Sample 46)

4.9. Cell viability test

The TR-146 cells were treated with the dissolved films, in the concentration of 0.35 mg/mL CTZ in HBSS as a solvent. **Table 2.** shows the results of the cytotoxicity test according to flow cytometry stained with propidium iodide. It can be seen that some formulations had a high impact on cell viability. Samples 34 and 47 showed more than 60% cytotoxicity. However, Samples 14, 32 and 48 had almost no cytotoxic effect. The other samples had a cell viability

value between 55 and 87%. Cellular transport experiments were performed only with Samples 13, 14, 32, 38, 43, 46 and 48, which had cytotoxic values over 60%.

Table 2. Cytotoxicity of the prepared films according to flow cytometry. The experiment was carried out in triplicate. (Samples 43, 44, 45, 46, 47, 48 contain CA)

Samples	Cell viability compared to control (%)
13	67.1 ± 2.7
14	92.8 ± 0.4
32	99.3 ± 0.8
34	33.6 ± 0.7
38	87.2 ± 0.5
40	56.4 ± 4.6
43	78.7 ± 0.6
44	56.3 ± 1.0
45	55.9 ± 0.2
46	87.7 ± 0.1
47	17.4 ± 0.3
48	91.3 ± 1.5
Triton X	0.2 ± 0.1

4.10. Permeation test

4.10.1. Permeation test across artificial membrane

Figure 9. shows the results of the permeation test across an artificial cellulose membrane. From most compositions, more than 40% of the API can permeate to the acceptor compartment. The SA concentration can influence the amount of the permeated API. A higher amount of the API can permeate to the acceptor compartment from the films with a higher SA concentration than from the ones with a lower SA concentration. This correlation is shown in **Figure 9.**, where Sample 13 (black curve) has a higher permeation rate than the comparable samples (Sample 32—green curve, Sample 38—brown curve). The same correlation can be observed for Sample 14 (red curve) and Sample 40 (blue curve). Furthermore, it can also be stated that the GLY concentration did not influence the permeation rate and speed of the API. These results can be considered good because the entire amount of the permeated API can have an effect compared to per os tablets because of the first-pass effect of the liver.

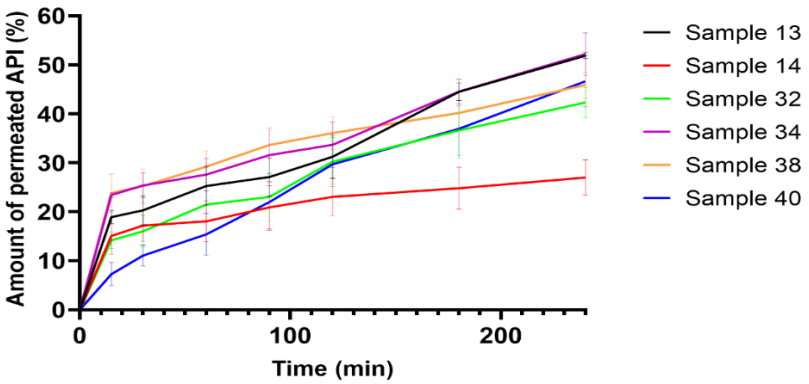

Figure 9. Permeation curves of polymer films on artificial membrane (n = 6)

4.10.2. In vitro permeation test

TR-146 cells are an accepted model for the in vitro testing of buccal absorption. The prepared films were dissolved in HBSS, and their cellular transports were observed for 90 minutes. Sample 43 had exceptionally high permeation, while Sample 48 had the lowest value. The presence of CA had no direct effect on cellular transport. Moreover, films with HPMC content had smaller permeability.

Most films had a linear transport rate through the cells between 30 and 90 minutes. With the exception of Sample 43, all films had a faster transport rate in the first 30 minutes, after which the transport slowed. The dissolution and the transport of CTZ from all films were stable, with no observed dose dumping.

5. SUMMARY AND PRACTICAL USEFULNESS

In my research work, I focused on the development of buccal film as a potential drug delivery system in allergy. I used SA as a novel film-forming agent to prepare buccal films. First, I studied the film-forming ability of SA without API. To learn about the properties of SA-based films, I incorporated CTZ as an API in the polymer film matrix with other excipients. I characterized the prepared films with several methods to get to know the films in depth. I investigated the physical properties of the films, such as thickness, breaking hardness, in vitro mucoadhesivity, contact angle and surface free energy. I also studied the chemical behavior of the films with FT-IR, RAMAN spectroscopy and thermoanalytical measurements (TGA, DSC). I tested the release of API from the films and the permeation of API both across artificial membrane and on buccal cell line (TR146). I also obtained information about the cell viability of the films with a cytotoxicity test. Finally, I learned information about the stability of my films, which is important from the point of view of transport, storage and application. Based on

the results, it can be said that I formulated buccal polymer films with CTZ successfully. The mechanical properties of the films can be changed within a wide range. SA, GLY and CTZ can increase the breaking hardness of films. SA enhances the mucoadhesivity of films, while GLY and CTZ decrease it. A chemical interaction can be found between SA, HPMC, GLY and CTZ, which can be manifested mainly in the form of hydrogen bonds. The API is distributed in the films completely homogeneously and, depending on the GLY concentration, it can be found in the form of crystalline and molecular disperse distribution. The films are stable up to 70 °C, therefore heating is possible during preparation and drying, so the process can be faster. In the first 20 minutes, more than 80% of the API can dissolve from most compositions. More than 40% of the API can permeate from the donor compartment examined through the artificial membrane. The speed of CTZ transport was nearly constant from all compositions, and none of the components influenced it significantly on the buccal cell line. Samples 14, 32, 8, 38, 46 and 48 have appropriate and acceptable cytocompatibility. The stability test revealed that CA, GLY can enhance the reduction of the API significantly. I found some formulations which can preserve the API content according to pharmacopeia expectations (Samples 13, 32, 38, 45, 47) after 3 or 6 months. Finally, I developed a fast-dissolving polymer film from SA and found a promising (optimal) composition, Sample 32, which is suitable and possible to apply as a buccal drug delivery system.

The novelties of my Ph.D. work are the following:

- Chemical interactions can be found between the components of films, which are mostly hydrogen bonds, and it was demonstrated that these interactions can determine and influence the physical properties of the films as well as the release of the API, so the use of analytical methods during development is unavoidable and indispensable.
- The amount of GLY does not only affect the physical properties and stability of the films (among many parameters), but can also influence the chemical form of the API in the films.
- Although CA can increase the permeation of the API, its application in films is not recommended as it greatly reduces the stability of the films.
- The investigation of polymer films was successfully performed on the TR146 buccal cell line in order to model, under in vitro conditions, the amount of the API that passed through the cells, which is able to exert an effect immediately after entering the systemic circulation. The transport of API from my buccal films through the cells was linear and a sufficient amount passed through, under 30 minutes more than 60%.

PUBLICATIONS RELATED TO THE THESIS

1. **Pamlényi, K.**, Kristó, K., Jójárt-Laczkovich, O., Regdon jr., G.:
„Formulation and Optimization of Sodium Alginate Polymer Film as a Buccal Mucoadhesive Drug Delivery System Containing Cetirizine Dihydrochloride”.

   Pharmaceutics 13, 619 (2021) doi: 10.3390/pharmaceutics13050619.

   Indep. citations: 10 Q1 IF = 6.525 (2021)

2. **Pamlényi, K.,** Kristó, K., Sovány, T., Regdon jr., G.:
„Development and evaluation of bioadhesive buccal films based on sodium alginate for allergy therapy”
   Heliyon 8, e10364 (2022) doi: 10.1016/j.heliyon.2022.e10364)

   Q1 IF = 3.776 (2021)

3. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Fenyvesi, F., Kristó, K.:
„Stability, permeability and cytotoxicity of buccal films in allergy treatment”
   Pharmaceutics 14, 1633 (2022) doi: 10.3390/pharmaceutics14081633

   Indep. citations: 1 Q1 IF = 6.525 (2021)

PUBLICATIONS NOT RELATED TO THE THESIS

1. Litauszki, K; Kiserdei, É; **Pamlényi, K.**; Szarka, Gy; Kmetty, Á; Kovács, Zs.:
„Controlled Drug Release from Laser Treated Polymeric Carrier”.
   J. Pharm. Sci. 111, 3297-3303 (2022) doi: *10.1016/j.xphs.2022.08.018*

   Indep. citations: 2 Q2 IF = 3.784 (2021)

2. **Pamlényi, K.**; Kristó, K; Nemes, D., Bácskay, I.; Regdon jr., G:
„Formulation and characterization of pramipexole containing buccal films for using in Parkinson’s disease”
   Eur. J. Pharm. Sci. Editor decision: “revision”, we are currently doing it

   Q1 IF = 5.112 (2021)

PRESENTATIONS RELATED TO THE THESIS

1. **Pamlényi, K.,** Sovány, T., Kristó, K., Regdon jr., G.: *„Formulation of an innovatve buccal mucoadhesive drug delivery system with sodium alginate polymer film”*, XII. Central European Symposium on Pharmaceutical Technology and Regulatory Affairs conference, Szeged, Sep 2018 – **presenter** (poster presentation)

2. **Pamlényi, K.,** Kristó, K., Regdon jr., G.: „*Szájnyálkahártyán alkalmazható, Na-alginát tartalmú mukoadhezív polimer film előállítása és vizsgálata”,* 2018. évi Tudományos Diákköri Konferencia, *Szeged, Nov 2018* - **presenter** (oral presentation)

3. **Pamlényi, K.,** Kristó, K., Regdon jr., G.: *„Szájnyálkahártyán alkalmazható, Na-alginát tartalmú mukoadhezív polimer film előállítása és vizsgálata”,* XXVI. Tudományos Diákköri Konferencia, Marosvásárhely, Apr 2019 – **presenter** (oral presentation)

4. **Pamlényi, K.,** Kristó, K., Regdon jr., G.: „*Szájnyálkahártyán alkalmazható, Na-alginát tartalmú mukoadhezív polimer film előállítása és vizsgálata”,* XXXIV. Országos Tudományos Diákköri Konferencia Debrecen, May 2019 - **presenter** (oral presentation)

5. Regdon jr., G, Módra, Sz., **Pamlényi, K.,** Sovány, T., Kristó, K.: *„Formulation, structural and thermal analysis of an innovative buccal mucoadhesive film drug delivery system from two different polymers”*, 2nd Journal of Thermal Analysis and Calorimetry Conference (2ndJTACC+V4 2019), Budapest, Jun 2019 - **co-author**

6. **Pamlényi, K.,** Kristó, K., Regdon jr., G.: *„Bukkális szájnyálkahártyán alkalmazható innovatív, nátrium-alginát alapú gyógyszerhordozó rendszer kialakítása és termoanalitikai vizsgálata”* Gyógyszertechnológiai és Ipari Gyógyszerészeti Konferencia, Siófok, Sep 2019 – **presenter** (poster presentation)

7. **Pamlényi, K., Kristó, K., Regdon jr., G.:** II. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, *„Development and characterization of sodium alginate polymer film as a buccal mucoadhesive drug delivery system”,* Szeged, Jan 2020 - **presenter** (oral presentation)

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10. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Preparation and investigation of permeability and physical-chemical properties of buccal films with sodium alginate”*, III. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2021 - **presenter** (oral presentation)

11. **Pamlényi, K.**, Kristó, K., Regdon jr., G.: *„Nátrium-alginát alapú bukkális mukoadhezív polimer film gyógszerforma formulálása”* Kárpát-medencei Fiatal Magyar Kutatók Konferenciája (EFOP-3.10.1-17-2017-00001), Budapest, Mar 2021 - **presenter** (oral presentation)

12. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Investigation of stability and permeability of buccal films based on sodium alginate”*, XIII. Central European Symposium on Pharmaceutical Technology and Regulatory Affairs conference, Gdansk, Sep 2021 - **presenter** (oral presentation)

13. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Cetirizin tartalmú nátrium-alginát alapú polimer filmek stabilitási és permeabilitási vizsgálata”*, XIV. Clauder Ottó emlékverseny, Budapest Nov 2021 – **presenter** (oral presentation)

14. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Stability and permeability properties of sodium alginate buccal films”,* IV. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2022 - **presenter** (**oral** presentation)

15. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Pramipexolt tartalmazó innovatív bukkális polimer film formulálása és vizsgálata”*, V. Fiatal Technológusok Fóruma, Budapest, May 2022 – **presenter** (oral presentation)

16. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Parkinson-kór kezelésében alkalmazható, pramipexolt tartalmazó innovatív bukkális polimer film formulálása és vizsgálata”*, SZTE Gyógyszerésztudományi Kar 2021. évi ÚNKP díjazottjainak tudományos előadóülése, Szeged, Jun 2022 – **presenter** (oral presentation)

17. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.:, *„Preparation of buccal films in Parkinson's Disease”* 9th BBBB Conference on Pharmaceutical Sciences, Ljubljana, Slovenia, Sep 2022 - **presenter** (oral presentation)

18. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.:, *„Formulation of Buccal Films in Parkinson's Disease”,* V. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2023 - **presenter** (oral presentation)

19. **Pamlényi, K.,** Regdon jr., G., Nemes, D., Bácskay, I., Kristó, K.: *„Evaluation of Stability and Cell Line Studies of Alginate Films Containing Cetirizine as Anti-Allergic Agent”,* V. International Conference on PharmScience Research & Development Las Vegas, NV, USA, Feb 2023 - **presenter** (oral presentation)

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