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# Gastroresistant gelatin films prepared by addition of cellulose acetate phthalate

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Gastroresistant capsules are obtained mostly by using modified-release fill in hard capsules, or by coating the gelatin shell with acid-resistant polymers. Modification of the material used at the stage when the capsule shell is produced would reduce the complexity and cost of introducing new products to the market. Gastroresistant gelatin films were obtained by using commercial cellulose acetate phthalate (aqueous dispersion Aquacoat® CPD). Only films casted from non-alkalized mixtures showed no visible disintegration at pH from 1.2 (simulated gastric fluid) to 4.5 (phosphate buffer). Elasticity of the dry films was comparable with the one determined for non-modified gelatin films, however tear resistance was 2-fold smaller, but still acceptable for practical application.

## 1. Introduction

Gelatin is a natural polymer widely used in pharmaceutical technology, primarily in production of hard and soft capsules. Its specific properties including temperature-related helix-coil transition allow formation of highly elastic and strong hydrogels. However, the properties are strictly dependent on many factors, that are difficult to control (Sobral and Habitante 2001). Despite of those problems alternative materials for production of capsules are used less frequently (Karim and Bhat 2008).

Gastroresistant hard gelatin capsules are obtained by using a modified-release fill (e.g. coated pellets) without modification of the capsule shell. The traditional way to produce gastroresistant gelatin shells was to crosslink the gelatin material with formaldehyde, but this is not considered further due to toxicological reasons (Reich 2004). Currently attempts of crosslinking the gelatin with enzymes or less toxic aldehydes and other polymers, with or without aid of elevated temperature and humidity conditions, are reported (Bigi et al. 2002; Biscarat et al. 2015; Boanini et al. 2010; da Silva et al. 2015; Ofner III et al. 2001; Vandelli et al. 2004). Crosslinking of gelatin shells leads, however, to the formation of a pellicle, an insoluble membrane, that results in delayed release, which is in fact independent of the pH of dissolution media and may cause failure in pharmacopoeial disintegration and dissolution tests of the product (Digenis et al. 1994).

In technology of soft capsules, gastroresistance is achieved by coating the capsules with acid-resistant polymers such as methacrylic acid – methyl acrylate copolymers (e.g. Eudragit L or S®) (Felton et al. 1995; Pagay 1994). This technology causes, however, many problems, that result from poor adhesiveness of the functional coating to the capsule shell (Pissinati and Oliveira 2003; Reich 2004). Modification of the shell composition would be a good alternative to coating because it simplifies production, eliminates many technological problems, reduces cost and time-to-market. However, a suitable gelatin-based material is not available yet and such a technology has not been developed. In patents that describe polymer film formulations composed of gelatin and acid-insoluble polymer (such as methacrylic acid and methyl acrylate copolymers or cellulose acetate phthalate), it is indicated that alkalization of the film-forming solution is necessary in order to obtain a water-soluble salt of the polymer, which is then homogenously dispersed in a gelatin film (Hassan 2012; Hassan et al. 2014). Only recently, gastroresistant films composed of gelatin and Eudragit L, NE or FS (acrylic and methacrylate acid copolymers) were prepared without alkalization (Teles et al. 2015).

Modified release non-gelatin hard capsule shells are available on the market and are produced by Capsugel (DRcaps®). These capsules are based on semi-synthetic cellulose derivatives. The commercial composition of "acid-resistant" capsule shell comprises hypromellose (HPMC), and the disintegration of these capsules is delayed by other mechanism than pH-related (www.capsugel.com, 2016). Combination of poloxamers with acid-resistant polymers was also proposed as suitable for enteric hard capsules (Benameur et al. 2015).

The aim of the present research was to obtain gastroresistant gelatin films by adding an acid-insoluble polymer, namely cellulose acetate phthalate (CAP), as a ready commercial dispersion. The disintegration of non-alkalized and alkalized formulation was compared. The mechanical properties of the films were determined in order to evaluate their applicability to produce capsules. Formulated gelatin-based films should have the potential to be optimized for both hard and soft capsule technology.

## 2. Investigations, results and discussion

### *2.1. Technical overview, disintegration time and microscopic observations*

Several alkalized and non-alkalized gelatin-CAP films were prepared (Table 1) using a procedure presented in Fig. 1.

The enteric capsule shell compositions reported by Hassan et al. (Pat. no. US 8685445, 2014) cover the preparation of a gelatin-based solution containing acid-resistant polymers, including CAP. In these compositions, the acid-insoluble ingredient is being salified using alkali compounds, like NaOH. However, we were unable to produce films insoluble in acidic pH if the gelatin–CAP mixture was alkalized. In the exemplary composition from the patent, the G:A ratio was 8:1, and sodium hydroxide as an alkalizing agent was used, (Hassan et al. 2014). In addition, for better reference, the G-CAP 3:1 composition was prepared, using NaOH proportionally to the CAP content. Unexpectedly, the films derived from alkalized solutions showed no resistance to acidic conditions

**Table 1: Composition [% w/w] of the Gelatin-Aquacoat (G-CAP) films (non-alkalized)**

| Ingredient | Gelatin : Aquacoat solids weight ratio | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3:1 | | 3:2 | | 1:1 | | 1:3 | |
| | Solution | Dry film | Solution | Dry film | Solution | Dry film | Solution | Dry film |
| Gelatin | 19.7 | 50.8 | 15.8 | 40.8 | 13.1 | 33.9 | 6.6 | 16.9 |
| Glycerol | 12.5 | 32.3 | 12.5 | 32.3 | 12.5 | 32.3 | 12.5 | 32.3 |
| CAP (Aquacoat) | 21.8 | 16.9 | 34.7 | 26.9 | 43.7 | 33.9 | 65.5 | 50.8 |
| Water | 46.0 | - | 37.0 | - | 30.7 | - | 15.4 | - |

Note: Since the Aquacoat commercial mixture contains water, the amount of additional water in the compositions is not consistent, and depends on amount of Aquacoat added to the formulation. The amount of Aquacoat in "Dry film" was calculated considering dry residue in the commercial product.

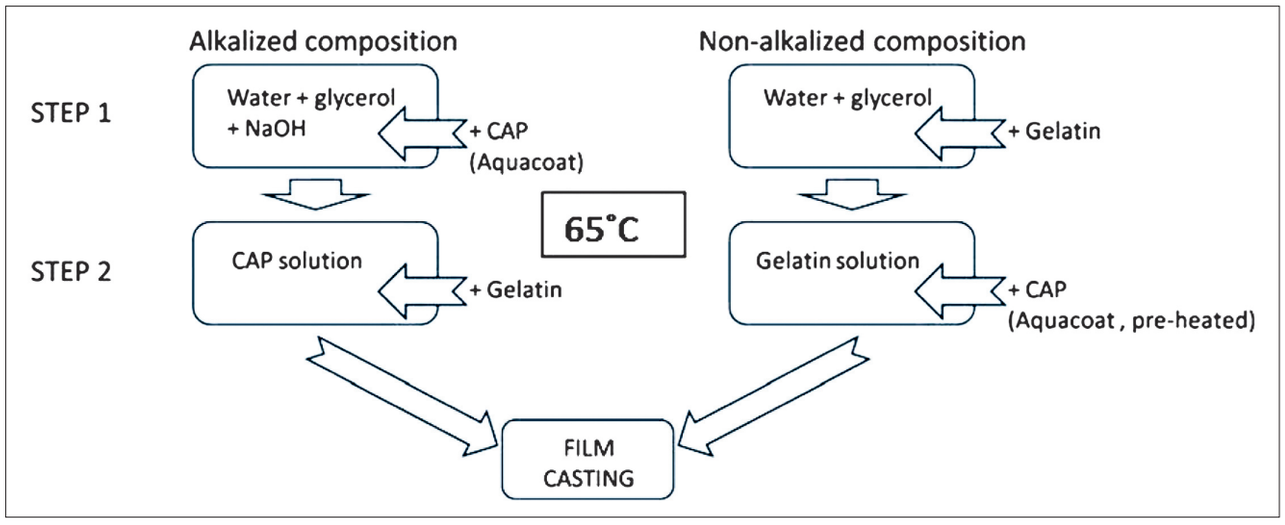


Fig. 1: Preparation of the modified gelatin films: with and without alkalization.

(Table 2, formulations 8:1A and 3:1A), even if microscopy indicates, that addition of NaOH leads to a homogeneous film (Fig. 2). Similar effects were observed when ammonium hydroxide was used for alkalization (data not shown). This may be explained by the fact that in the presence of alkali, CAP forms a salt, which is easily soluble in water, however the positive results reported in the above mentioned patent are contradictory. In view of the results described above, non-alkalized compositions containing gelatin and CAP aqueous dispersion were prepared (Fig. 1).

Preparation of the film-forming dispersion using the non-alkalized CAP (pH of the film-forming mass was 4.5) requires focusing on uniformity of the insoluble polymer particles. Uniformity can be obtained with proper stirring, because sedimentation is quick. Moreover, a highly heterogeneous mass, with large solid CAP particles, was obtained if CAP dispersion alone (Aquacoat CPD) was heated to 65 °C and higher. It was discovered that non-alkalized CAP dispersion, before it is added to a vessel with gelatin solution, should be very carefully pre-heated to a temperature not exceeding 60 °C. Such a procedure protects CAP from aggregation, even if the total mass is warmed afterwards to 65 °C. The effect of aggregation could be explained by the fact that above the glass transition temperature (Tg) the polymer chains become more movable what leads to easier formation of particle aggregates (Roxin et al. 1998). However, Tg value was 40 °C for CAP in the Aquacoat CPD and one could not expect that at 60 °C aggregation can be still avoided.

The microscopic structure of the film surface is presented in Fig. 2. Notable differences between the surface of the alkalized and acidic films (G-CAP 3:1) is shown. Solid particles are present in the films obtained without addition of alkali, resulting in a rough surface. The solid particles, that are most probably non-dissolved CAP, are also visible in non-alkalized films under UV light (330-380 nm). The film prepared from the alkalized material, with dissolved CAP, appears to be more uniform and have rather smooth surface (Fig. 2A).

Disintegration time of the dried films prepared with different gelatin to Aquacoat solids ratio is shown in Table 2. The alkalized formulations did not exhibit desired acid-resistant properties and disintegrated within 5 min in all tested media, even though the 8:1 patent-derived formulation was thicker than other tested formulations (340 µm *versus* 200-250 µm). Also the non-alkalized G-CAP 1:3 and 1:1 films disintegrated easily within 5 min in all tested media. Only 3:1 and 3:2 films showed the desired resistance to acidic conditions. These films did not show physical disintegration not only in SGF but also in pH 3.0 and pH 4.5 buffers. After 180 min nothing but swelling and whitening was observed. On the other hand, at pH 5.5 disintegration of these films was clearly visible after less than 30 min. The time required for disintegration shortened as the pH increased, so at pH 6.0 G-CAP 3:1 films disintegrated between 5 and 15 min, while at pH 6.8 disintegration occurred in the time range of 5 - 10 min.

The G-CAP 3:1 and 3:2 films obtained from an acidic (pH around 4.5) film-forming mixture exhibited good resistance during 3 h at pH up to 4.5. This is essential for good performance in vivo, because it is well known that gastric fluid pH may rise significantly after the meal intake (Koziolek et al. 2015) or in patients with various gastrointestinal conditions like achlorhydria (Zuleta et al. 2015). However, the G-CAP 3:2 films were soft and easy to damage after the disintegration test, in contrast to 3:1 formulation, which remained mechanically resistant. Therefore, the G-CAP 3:1 composition can be considered potentially useful for a functional gastroresistant capsule.

At pH 5.5, disintegration of G-CAP 3:1 films depended on thickness of the films. The 150 µm thick samples disintegrated within

**Table 2: Disintegration time of gelatin – Aquacoat (G-CAP 3:1, 3:2, 1:1, 1:3) non – alkalized films and G-CAP (8:1 A, 3:1 A) alkalized films.**

| Medium | Gelatin:Aquacoat ratio / film thickness* - non-alkalized films | | | | Gelatin:Aquacoat ratio / film thickness* - alkalized films | |
|---|---|---|---|---|---|---|
| | 3:1 / 248 µm | 3:2 / 225 µm | 1:1 / 237 µm | 1:3 / 277 µm | 8:1 A / 340 µm | 3:1 A / 193 µm |
| SGF (without pepsin) | No disintegration | No disintegration | 5 - 45 min | < 5 min | < 5 min | < 5 min |
| Citrate buffer pH 3.0 | No disintegration | No disintegration | < 5 min | < 5 min | < 5 min | < 5 min |
| Phosphate buffer pH 3.0 | No disintegration | No disintegration | < 5 min | < 5 min | < 5 min | < 5 min |
| Phosphate buffer pH 4.5 | No disintegration | No disintegration | < 5 min | < 5 min | < 5 min | < 5 min |
| Phosphate buffer pH 5.5 | 15 – 30 min | 5 – 15 min | < 5 min | < 5 min | < 5 min | < 5 min |
| Phosphate buffer pH 6.0 | 5 - 15 min | 5 – 15 min | < 5 min | < 5 min | < 5 min | < 5 min |
| Phosphate buffer pH 6.8 | 5 - 10 min | 5 – 10 min | < 5 min | < 5 min | < 5 min | < 5 min |

*average film thickness (n= 10)
Duration of the test was 180 min.

5 – 10 min while for films with a thickness of 250 µm this time was prolonged to 10 - 15 min and for the films 450 µm thick to 25 – 30 min.

To fully assess the gastroresistance of the G-CAP 3:1 and 3:2 formulations disintegration test involving enzymes and surfactants was carried out. The media simulating gastric and intestine fluids were: SGF + pepsin, FaSSGF (pH 1.6), FaSSIF (pH 6.5), FeSSIF (pH 5.0). The results show that the formulations are not susceptible to pepsin or to bile salts (Table 3).

**Table 3: Disintegration time of gelatin – Aquacoat (G-CAP 3:1 and 3:2) non – alkalized films using biorelevant media.**

| | G-CAP ratio | |
|---|---|---|
| Medium | 3:1 | 3:2 |
| SGF + pepsin | No disintegration | No disintegration |
| FaSSGF | No disintegration | No disintegration |
| FeSSIF | 10 min | 10 min |
| FaSSIF | 5 – 10 min | 5 – 10 min |

Duration of the test was 120 min.

### *2.2. Mechanical properties*

To characterize mechanical properties, tear resistance (TR) and % elongation at break (%EAB) were measured during drying process. For comparison, gelatin films of the same solid content and without the enteric polymer were prepared and tested (Gel film). Results are shown in Fig. 3. Noteworthy, differences in elongation at break and water content between G-CAP 3:1 film and the reference Gel film were visible after 30 min of drying. Differences in water content between the two investigated films were reduced after 24 h of drying. However, after 24 h, differences in mechanical properties, namely in TR values were still notable, although elasticity (EAB%) was similar. Smaller mechanical resistance of the modified gelatin film was not sufficient for the manufacturing process of soft capsules. However, visual observation of the dry film allows to consider G-CAP 3:1 films as a material which can be potentially used to form hard capsule shells.

### *2.3. Conclusions*

Gastroresistant gelatin films can be manufactured with addition of CAP commercial dispersion, without further alkalization. The best results were obtained for G-CAP films with 3:1 gelatin to Aqua-

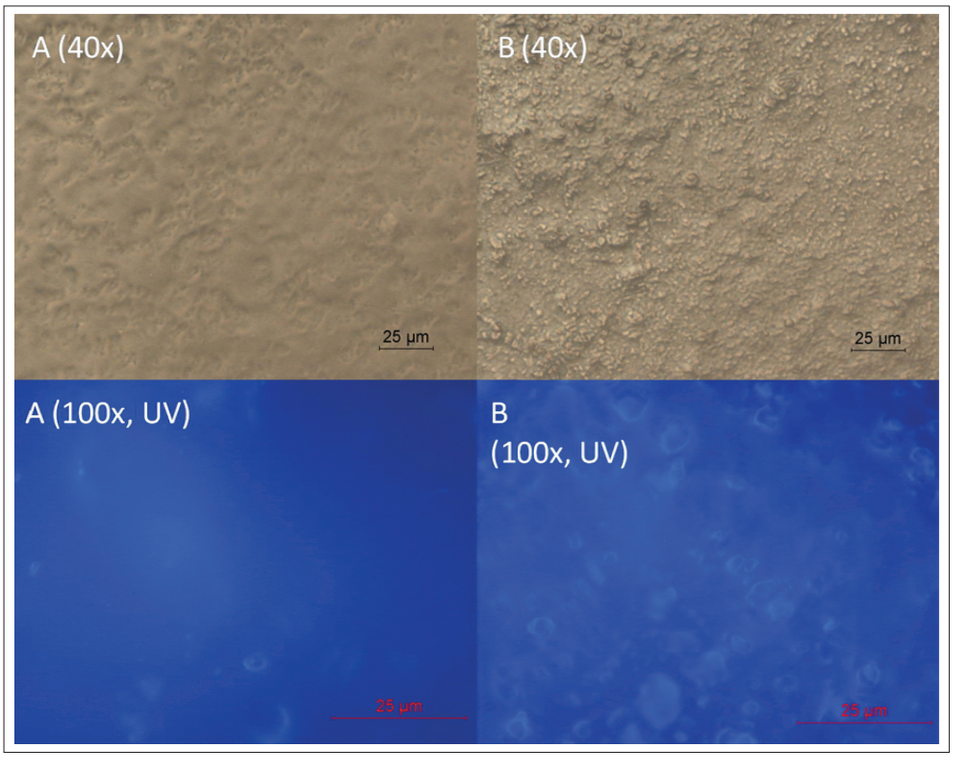


Fig. 2: Microscopic image (visible light and UV) of films made from alkalized (A) and non-alkalized (B) mixtures

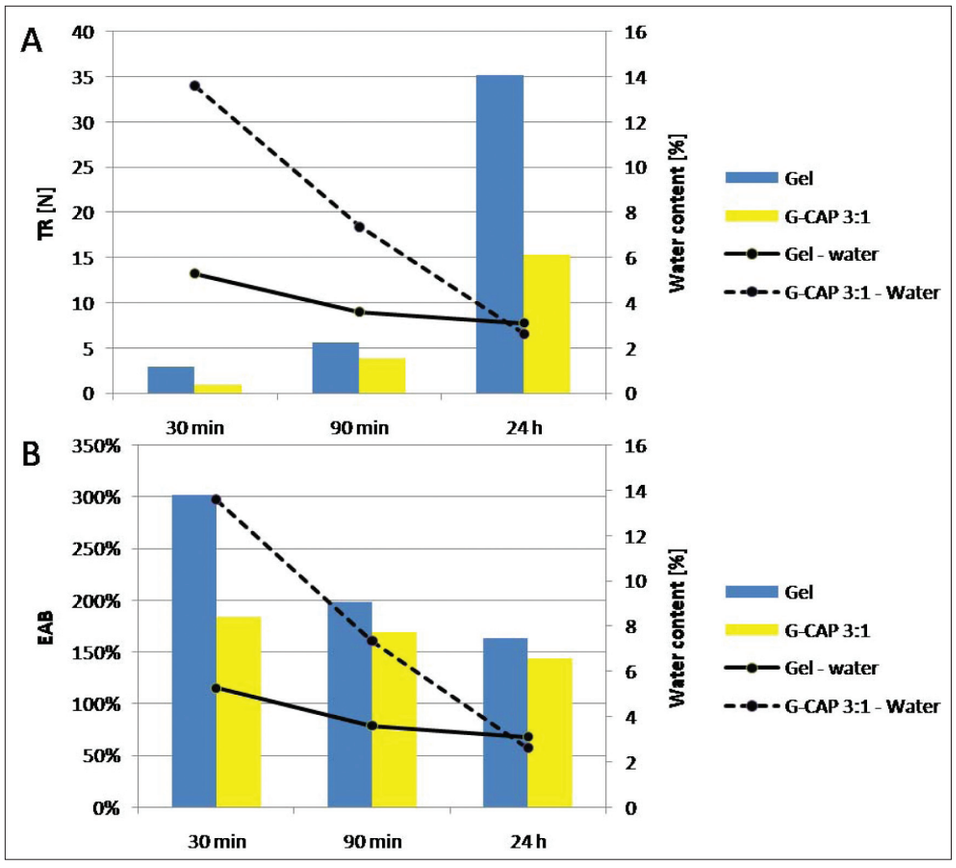


Fig. 3: The effect of drying time on the tear resistance (A) and % elongation at break (B) of investigated films: G-CAP 3:1 – sample of non-alkalized G-CAP film; Gel – reference non-modified gelatin film. The lines demonstrate water content in the films during the test.

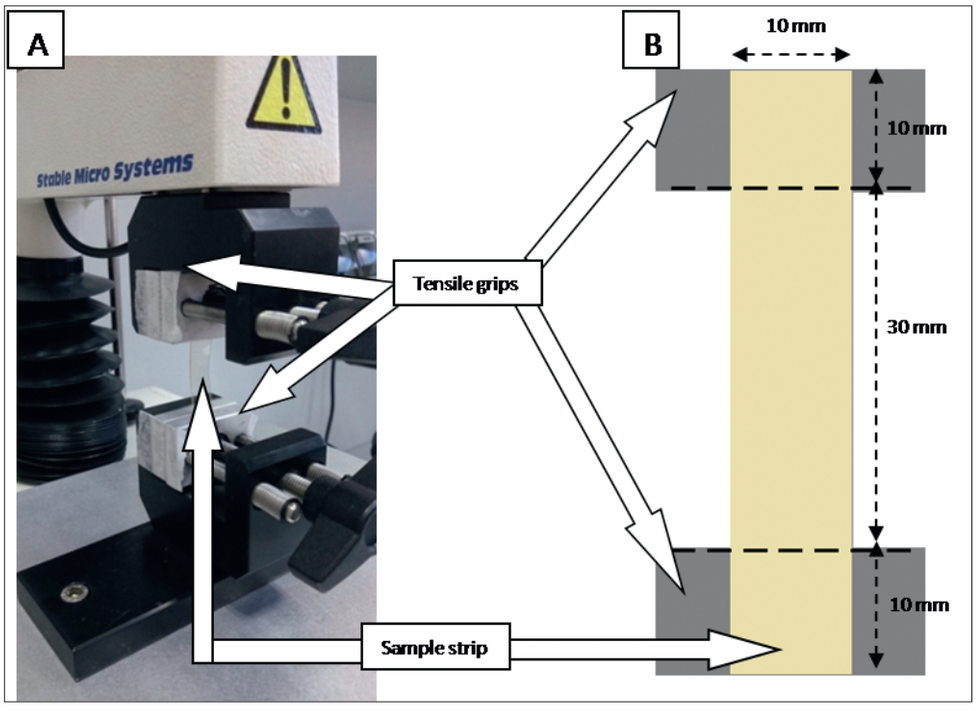


Fig. 4: Uniaxial tensile test: A- photo of test setup, B – scheme of test setup.

coat ratio. Because of the satisfying disintegration behavior, and promising mechanical properties, the described formulations may be applicable in the technology of gastroresistant hard capsules. Further research will be carried out in order to modify the mechanical behavior of the material and to optimize the formulation for the use in production of soft capsules by a standard process employing shell-producing and encapsulating industrial devices.

## 3. Experimental

### *3.1. Materials*

Bovine gelatin produced by Lapi Gelatine (Empoli, Italy) was kindly provided by Curtis Health Caps (Poznań, Poland). Aquacoat® CPD (FMC Biopolymer, Philadelphia, USA) was a dispersion of cellulose acetate phthalate (CAP) in water with poloxamer (total content of solids – 30% w/w) and was a gift from IMCD Polska (Warsaw, Poland). Glycerol (99.5%) was purchased from Glackonchemie (Merseburg, Germany), sodium hydroxide from POCh (Gliwice, Poland) and triethyl citrate from Fluka (Buchs, Switzerland). Purified water was produced with Elix Essential Water Purification System (Merck Millipore, Darmstadt, Germany). Disintegration media were: simulated gastric fluid (SGF), citrate buffer pH 3.0 and phosphate buffers pH 3.0, 4.5, 5.5, 6.0 and 6.8. All fluids were prepared according to European Pharmacopeia (Ph. Eur. 8.). For further disintegration tests the following media were chosen: FaSSGF (Fasted state simulated gastric fluid), FeSSIF (fed state simulated intestinal fluid) and FaSSIF (fasted state simulated intestinal fluid) prepared from SIF Powder (Biorelevant.com, London, UK), SGF with pepsin was prepared according to European Pharmacopeia 8.0 using pepsin from Fluka (Buchs, Switzerland).

### *3.2. Preparation of films*

Formulations consisting of gelatin, aqueous CAP dispersion Aquacoat®CPD, glycerol and water were prepared (G-CAP films). The gelatin to Aquacoat solids weight ratios were 3:1, 3:2, 1:1 and 1:3. Compositions are shown in Table 1 and Fig. 1 shows steps of the film preparation. The non-alkalized mixtures were prepared by adding gelatin into a hot (65 ˚C) mixture of glycerol and water. After dissolving the gelatin, a pre-heated (to 60 ˚C) Aquacoat CPD was added and mixing was performed until homogenous mass was obtained. The alkalized films were prepared by adding the Aquacoat to a mixture of glycerol, water and NaOH (mass ratio for CAP and NaOH was 2.5:1) and heating to 65 ˚C. Only after complete dissolving of the CAP (a transparent solution) the gelatin was added and mixing was continued until homogenous mass was obtained. Films were made by casting 50 g of the mixture on a glass plate (15 cm x 15 cm) with a TLC Plate Coater (Camag, Muttenz, Switzerland). The films were dried for 90 min in an air dryer at temperature 20-25 °C (RH 40-55%). Next, they were kept over a silica gel (RH 15-25%) for 24 h. For better characteristics of the modified films in comparison to a non-modified gelatin film, dynamic changes of a tear resistance in relation to the drying time and water content were investigated by testing samples after 30 min, 90 min and 24 h of the drying procedure. In addition, formulations described in a patent (Pat. no. US 8685445, Hassan et al. 2014), consisting of gelatin, Aquacoat®CPD, glycerol and triethyl citrate and alkalized to pH 7-8 with sodium hydroxide were prepared and tested. Gelatin to Aquacoat solids weight ratio was 8:1 or 3:1 (8:1A and 3:1A formulations). For the purpose of the mechanical tests, reference films, consisting of gelatin (26.2 %), glycerol (12.5 %) and water, were prepared (Gel films).

### *3.3. Measurement of disintegration time*

Disintegration tests were performed by shaking the films in flasks containing the following fluids: pharmacopoeial Simulated Gastric Fluid (SGF; with and without pepsin), citrate buffer pH 3.0, phosphate buffers pH 3.0 - 6.8, Fasted State Simulated Gastric Fluid (FaSSGF pH 1.6), Fasted State Simulated Intestinal Fluid (FaSSIF pH 6.5), Fed State Simulated Intestinal Fluid (FeSSIF pH 5.0). The film (samples 10 mm x 10 mm) was placed in 50 ml of the fluid at 37 °C±0.5 °C and subjected to shaking 150 rpm (linear movement with 18 mm stroke length). Endpoint of disintegration test was determined visually by observation in 5 min intervals. In case of absence of visible signs of disintegration the test was stopped after 180 min.

### *3.4. Mechanical properties*

The measurements of tear resistance (TR) and elongation at break (%EAB) were performed in an uniaxial tensile test with TA.XT Plus texture analyzer (Stable Micro Systems, Godalming, UK) (Fig. 4). Samples of the 3:1 G-CAP films and reference GEL films were investigated. The films dried for 30 min, 90 min or 24 h were tested by placing samples (10 mm x 50 mm) between two tensile grips in a distance of 30 mm and measuring the extension parameters. The thickness of the investigated samples was 250 µm ± 20 µm. Water content was measured with WPS 210 S Moisture Analyzer (Radwag, Radom, Poland) as a weight loss after drying.

### *3.5. Microscopy*

Structure of the films was examined by an optical microscopy (Stereoscopic microscope Nikon Eclipse 50i, Tokyo, Japan). Images were captured (40x and 100x magnification) with visible light and UV light (330-380 nm wavelength).

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Conflicts of interest: None declared.

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