Potential Use of *Galium verum* Essential Oil for Antibacterial Properties in Gelatin Based Hydrogels Prepared by Microwave Irradiation Technique

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Galium verum essential oil (EO) loaded gelatin hydrogel was prepared by microwave-assisted polymerization method. FT-IR analysis indicated no chemical interaction between the hydrogel matrix and EO. Good swelling behavior, increased thermo- and mechanical- properties was attributed to the synergistic effects of the secondary structure of gelatin after gelation and intermolecular hydrogen-bonding interactions between the hydrogel matrix and EO ingredient's. Antibacterial activity was investigated by the agar diffusion method.

Keywords:Polymer gels, Essential oil, Antibacterial activity,Tensile test

Hydrogels are suitable polymer materials for wound dressings, because it can absorb water up to thousand times of its dry polymer weight, it maintains wet environment, it provides cool sensation which reduces pain, bacteria entrapment and bacterial penetration [1]. Development of wound dressings and devices, which encourage the different steps of healing and optimize healing conditions, is a subject of great scientific and commercial interest. In an ideal condition, along to be non-antigenic and biocompatible, a good wound dressing should create and keep a moist environment, absorb wound fluids and exudates from the surface of wound, prevent both wound desiccation and maceration and, finally, protect lesion from infections [2,3].The incorporation of plant essential oils (EO) into wound dressing biofilms represents a new interest and a long term friendly alternative regarding human tissue. Many EO, such as thyme oil, lavender [4], cinnamon and lemongrass [5], peppermint and Tea tree [6], rosemary, chamomile blue, eucalyptus, citronella and cedarwood [7] and few others have been demonstrated to have a wide spectrum of antimicrobial activity. For most of these oil extracts have also been reported to have antifungal, anti-termite, anti-inflammatory or therapeutic effects for some skin disorders and not only. Essential oils have been used to combat infections and to control epidemic multi-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* [8].

Essential oils, introduced in the polymeric matrix significantly affect the hydrogel properties [9]. Due to their hydrophobicity, tend to minimizetheir interactions with the hydrogel hydrophilic phase, and this leads to poor dispersion and eventual phase separation either in solution or in the final dried film [6]. The EO influenceon the hydrogel stability and properties depends on the different structural factors, such as the kind of matrix, the composition and amount of oil added and the interactions with the polymeric matrix [10,11]. Generally the oil phase reduces the hydrophylic nature of the matrix, but there are different results obtained for different kind of oils and hydrogel matrices. Ahmad et al.[11], obtained a different effect in gelatin based films loaded with lemon and bergamot essential oil: for the same concentration of EO, the hydrogel with bergamot oil has a lower water retention capacity compared with those containing lemon EO. The decreasing swelling capacity of EO loaded hydrogels is explained by the interactions between the EO compounds and the hydrophilic parts of the polymer, promoting an overall increase in the hydrophobic nature of the matrix. Similar effect was observed by Shen et al. [7], in the case of biodegradable chitosan films containing citronella and cedarwood oil. The moisture uptake of the films decreased from 41.1% to 38.8% as the concentration of the EO increased from 10% to 20%.

Swelling behavior of hydrogels plays an essential role in biomedical applications and is mainly related to the network elasticity, the presence of hydrophilic groups (-OH, -COOH, -$CONH_2$, -SO_3H) in polymer chains, the extent of crosslinking**,** and porosity of the polymer[12].There are results suggesting that the addition of EOs in matrices based on natural polymers like gelatin, alginate, starch and chitosan plasticized the matrix by increasing the elongation at break and reducing the tensile strength (TS) [7,10,13-17]. According to these results, through EO incorporation a more elastic structure is formed and chain-relaxation ability of the polymeric network also significantly increased [18]. Other results described different effect of incorporation of EOs into the polymeric network, like the decrease of the elasticity and the increase of the TS [5,6,19]. Therefore, probably due to the differences in reactivity in binding or interacting with the polymer network, different EOs affected differently the mechanical properties of the films [7].

Taking into account the consecrated antimicrobial properties of EOs, we have focused our attention on the influence of EOs on the hydrogel properties respectively

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on the influence of hydrogel composition on the antibacterial activity of the essential oil, since different types of essential oils can interact differently with different matrices.

The purpose of this work is to study the bacterial inhibition activity of *Gallium verum* EO in different cross-linked compositions. To investigate the influence of films composition on the essential oil antibacterial properties respectively to investigate the effect of essential oil on the films mechanical and thermal properties, the formulated materials was characterized by means of FT-IR spectroscopy, swelling tests, bacterial resistance tests, tensile tests and calorimetric measurements. The behavior of prepared materials was discussed in correlation with the structural properties.

Experimental part

Materials

Pharmaceutical grade pig skin gelatin (type A, gel strength 300) powder, glycerol (≥ 99.5%) and salicylic acid (SA) were purchased from Sigma-Aldrich.Glucose (≥ 99.9%) purchased from S.C. HIPOCRATE 2000 SRL was used for the preparation of the 10% glucose cross-linking solution.*Galium verum* essential oil was obtained by Stejarul Biological Research Centre, Piatra Neamþ, Romania, according to the methodindicated in the Romanian Pharmacopoeia, the X edition (RF X). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were obtained from Microbiology Laboratory (Faculty of Biology and Geology of Babes-Bolyai University, Cluj-Napoca, Romania).

Preparation of hydrogel

Cross-linked films of pure gelatin were prepared by microwave-assisted polymerization method from a mixture of solid and liquid phase, with composition presented in table 1, mixed in a ratio of 1/3.60 (w/v) . The cross-linking of the films was realized with 10% glucose solution respective with 0.02% salicylic acid solution by irradiation in a household microwave system at 480 W for different time periods following 5 second heating and 1-2 second mixing steps, to prepare four different films:salicylic acid cross-linked blank sample (B_{SA}), glucose cross-linked blank sample (B_G), essential oil loaded film cross-linked with salicylic acid (A_{SA-EO}) respectively with glucose (A_{G-EO}), whose composition is reported in table 1. Once the solution becomes clear *Galium verum* EO was incorporated in the hydrogel matrix without the use of emulsifying agents. The mixture was poured into polystyrene Petri dishes and air dried until constant weight. The dried films were wrapped in wax paper and kept at temperatures between 3-5ºC.

Characterization

Fourier Transmission Infra-Red Spectroscopy (FT-IR)

The composition and structure of the cross-linked gelatin films were confirmed by IR ATR spectra registered on Perkin-Elmer FTIR model equipped with ATR accessory (PIKE MIRacle™), with diamond crystal plate. The resolution was 4 cm^{-1} in the range of 4000 – 400 cm^{-1}. Films analyzed were 0.12 mm thick, previously dried at ambient temperature until constant weight.

Swelling studies

The swelling property study was done by immersing a 2x2 cm disk samples with known weight of each dried formulations in two different swelling solution: water and *p*H 10.0 buffer solution at room temperature. The swollen samples was gently blotted and weighed at predetermined time. The degree of swelling (SW) for each disk sample at time *t* was calculated using equation (1):

$$SW = \frac{Wt - Wi}{Wi} \times 100\ (\%) \qquad (1)$$

where: Wt is the weight of the swollen samples at any given time and Wi is the weight of the dry sample.

The test was performed on three specimens and the average mean of results were reported.

Bacterial resistance test

The antibacterial properties of the prepared formulations were evaluated using the agar diffusion test method. Before analyzing, each sample were sterilized using butane gas flame. The microorganisms used in this experiment are *Staphylococcus aureus* and *Escherichia coli*.About 1µL of colony forming units of the bacterial cell were spread on agar-agar plates and the small disks of gelatin based hydrogels (2x2 cm) were placed on them aseptically. After incubation at 28ºC for 24h after prior incubation at 37ºC for 1h the inhibition zone was measured as the area around the disc where bacteria would not survive as they were susceptible to the antibacterial agent that diffused from the sample to the surrounding medium.

Mechanical properties

The mechanical properties of the prepared materials were determined by standard tensile tests. The measurements were carried out at temperature of 18ºC and humidity of 50%. Stress-strain curves of 50 x 10 mm, 0.12 mm thick strip-shaped films were recorded using an Instron 3366 (10 kN) tensile test machine, with a constant crosshead speed of 50 mm/min. The load was measured with a 10 kN load with an accuracy of 0.5% of the indicated load. The Young's Modulus *E* (MPa), the tensile strength*ó* (MPa)and the elongation at break for each composition were tested on three specimens, the reported data are the mean values of three measurements.

Formulation	Solid phase (% w/w)		Liquid phase (% v/v)			
	Gelatin	Glycerol	Salicylic acid solution 0.02%	Glucose solution 10%	*Galium verum* essential oil	*Water*
B_{SA}	55.50	44.50	25	0	0	75
B_G	55.50	44.50	0	25	0	75
A_{SA-EO}	55.50	44.50	24.99	0	0.02	74.99
A_{G-EO}	55.50	44.50	0	24.99	0.02	74.99

Table 1 SAMPLES COMPOSITION

Differential Scanning Calorimetry (DSC)

Calorimetric measurements of the presented hydrogel formulations were performedusing a 823eDSC (Mettler Toledo). The temperature range studied was 25°C to 150°C with a heating rate of 2°C min^{-1}.DSC thermograms were used to investigate the melting temperature (T_m, °C), the melting enthalpy (ΔH_m J g^{-1}), the degradation ($T_{onset,d}$ and $T_{max.d}$, °C) and gelling (T_g, °C) temperatures.

Results and discussions

Fourier Transmission Infra-Red Spectroscopy (FT-IR)

Since gelatin cross-linking occurs through either hydrogen bonding (physical cross-linking) or covalent bonding (chemical cross-linking) involving the $–NH_2$ and –COOH groups and cross-linking agents, it is necessary to establish the different reactions taking place. The structure and the bonding of the hydrogel samples were investigated by FT-IR spectroscopy.

Figure 1 shows the FT-IR spectra of the glucose respectively SA cross-linked blank (B_G and B_{SA}) and *Galium verum* EO loaded (A_{G-EO} and A_{SA-EO}) hydrogel samples.

As we can see in figure 1, the gelatin based hydrogel samples were characterized by amide I (1640-1644 cm^{-1}) [20-24], amide II (1549-1553 cm^{-1}) [25,26] and amide III (1236-1241 cm^{-1}) [27] absorption bands, characteristic for collagen in random coil protein (gelatin) which proved that the gelatin retained its random coil structure (the hydrolysate form of collagen) even after the gelling process [28]. The FT-IR spectra of the *Galiu verum*EO loaded samples (A_{G-EO} and A_{SA-EO}) regardless the type of the used cross-linking agent, related with the peptide bonds of gelatin have no changes comparing with the spectra of blank samples (B_G and B_{SA}). This result demonstrated that *Galium verum* EO did not destroy the secondary structure of gelatin. The FT-IR spectrum of the *Galium verum* loaded hydrogels showed no new bands formed which means that there are no chemical interactions happened between the hydrogel matrix and EO. The main modification due to the interactions between EO and hydrogel matrix is visible in the region of 1034 - 1644 cm^{-1} regarding the intensities of the characteristic absorption bands, in the case of SA cross-linked samples (fig. 1.b).For comparing the intensities, the absorption peak at about 1644 cm^{-1} was considered as internal standard, due to the stability and inactive nature of C=O groups of gelatin molecule in the process of hydrogel preparation. Increased intensity of the peak at 1034 cm^{-1} due to the streching vibration of hydroxyl groups of glycerol[22], in the spectrum of EO loaded SA cross-linked sample (A_{SA-EO}) reported to the blank sample (B_{SA})can be explained either by the presence of -OH phenolic groups of the EO, or it may be due to the more intense vibration of –OH groups of glycerol in the presence of *Galium verum* EO, suggesting that the essential oil physically interact with SA. These interactions, basically through hydrogen bonds, releases the –OH glycerine groups, which were initially physically bounded to SA, so that the –OH glycerine groups can vibrate with a higher intensity (A_{SA-EO}) compared to the B_{SA}sample.Itis also observed, that in the case of hydrogel samples cross-linked with glucose, there is no differences in the wave number or intensities of the above discussed absorption bands. Those two observations conclude that the reaction between *Galium verum* EO and SA cross-linked hydrogel sample takes place throughthe functional groups of SA and the phenolic group of the EO. This interaction, although it occurs in the case of glucose cross-linked samples, the density of the connections is much smaller or the probability of an interaction is lower probably due to the steric impediments caused by the glucose structure.

All these changes led to an assumption that intermolecular interactions and molecular compatibility between the *Galium verum* EO ingredient's functional groups and –OH and $–NH_2$ groups in the hydrogel matrix could exist.

Swelling studies

Gelatin is an amphoteric polymer which can reacts as acid or base because of the presence of carboxyl and amino side chain groups. The isoelectric point (pI) of gelatin is 4.9. At this point, the numbers of positive and negative charges are equal; therefore the total charge of the network is zero. The hydrogel network will collapsed due to the electrical attractions between opposite charges. By changing the *p*H of medium in order to increase the degree of ionization, the hydrogels swelled significantly. Below pI, the gelatin network carries a net positive charge yielding a cationic gel. Above pI, the network was negatively charged forming an anionic gel.

Swelling is an important step to understanding the structure and stability of hydrogels and is affected by several factors, such as: matrix composition, network density and type of interactions, swelling of materials, ionic strength, *p*H, temperature etc.

Since the prepared cross-linked gelatin hydrogels are candidate materials for wound dressing, transdermal delivery systems, the swelling behavior, evaluated from their water uptake values, were studied in neutral and bazic *p*H environments. The results are shown in figures 2 and 3.

Even if, generally EOs, due to their hydrophobic nature, tend to minimize their interactions with any hydrophilic phase [5,7,14], according to the data presented in figures 2 and 3 the tested *Galium verum* EO increased the swelling degree of cross-linked gelatin hydrogels (A_{G-EO} and A_{SA-EO})in all the tested *p*H environment, compared to blank samples (B_G and B_{SA}). Increased water holding capacity of EO loaded samples suggest the formation of a homogeneous phase, since water molecules should diffuse mainly through the continuous polymer phase [5]. Similar results were

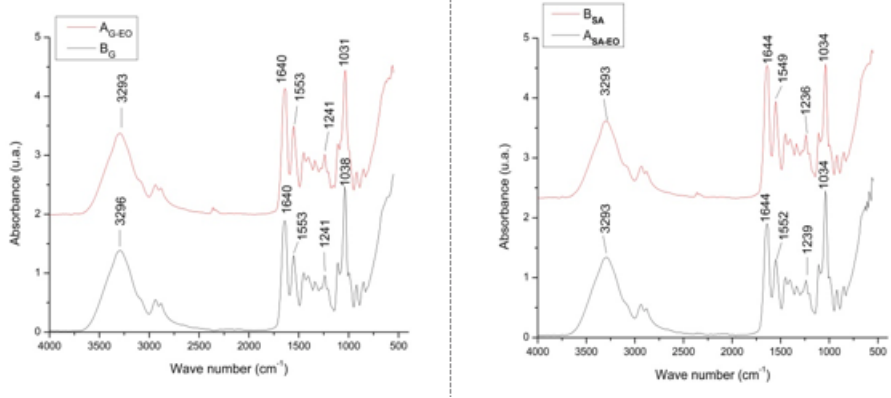

Fig. 1. IR spectra of the a) glucose (B_G and A_{G-EO}) and b) salicylic acid (B_{SA} and A_{SA-EO}) cross-linked hydrogel samples.

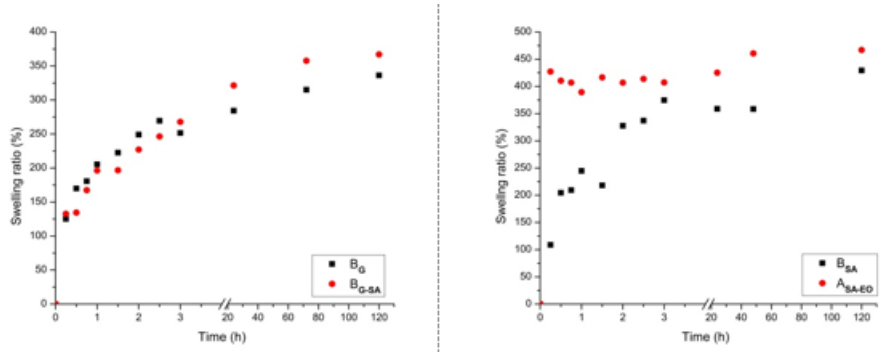

Fig. 2. Swelling studies of the a) glucose (B_G and A_{G-EO}) and b) salicylic acid (B_{SA} and A_{SA-EO}) cross-linked hydrogels in neutral *p*H environment.

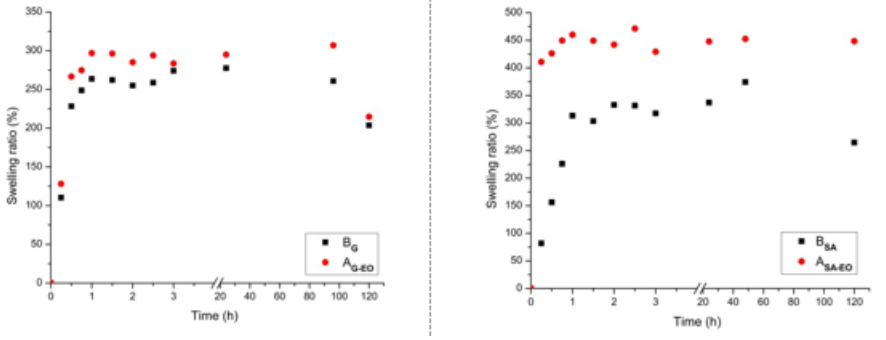

Fig. 3. Swelling studies of the a) glucose (B_G and A_{G-EO}) and b) salicylic acid (B_{SA} and A_{SA-EO}) cross-linked hydrogels in basic *p*H environment.

observed by Rocha-Garcia et al. [29], in the case of gelatin/poly(ethylene glycol) hydrogels for controlled release of tramadol. The increased value of the maximum swelling capacity with about 30% after physical cross-linking with citric acid was explained by the formation of a flexible network through the intermolecular hydrogen bonds. The same swelling response was observed for the *Galium verum* EO loaded samples, indicating a more flexible polymeric network that allows the diffusion of water molecules. The higher stability and swelling degree of the *Galium verum* EO loaded samples can be attributed to the formation of a secondary network through the intermolecular hydrogen bonds between the functional groups of EO and the hydrogel matrix, which increase the stability of the hydrogel, generating the effect of a physically cross-linked network [18].

In the case of SA cross-linked samples the maximum swelling degree increased from 350 to 450% after EO incorporationand the stability until the beginning of the degradation process increased with 58.3 % (from 50h to 120h) in basic *p*H environment. In all *p*H environment the SA cross-linked *Galium verum* EO loaded samples has higher swelling degree (450% in basic *p*H and 470% in neutral *p*H environment) compared to that of glucose cross-linked samples (320% in basic *p*H and 370% in neutral *p*H environment).

Considering the behavior of EO loaded hydrogels, in the case of A_{G-EO} sample composition, the polymeric matrix started to break up after 90 h of immersion due to the hydrolysis processes comparing with the A_{SA-EO} sample, when the degradation process started after 120 h of immersion due to the breaking of the intermolecular bounds inside the polymeric matrix. This behavior of EO loaded hydrogel samples suggests the higher stability of SA cross-linked samples compared to that of glucose cross-linked samples due to the interactions between SA and *Galium verum* EO, also supported by the results of the FT-IR spectroscopy analysis.

Bacterial resistance test

The prepared samples were examined for their bacterial resistance (fig. 4). The unexpected deterioration in the anti-bacterial properties of *Galium verum* EO as a result of cross-linking with SA(table 2) could be due to the interaction between the EO and salicylic acid molecules, as suggested by the results of the FT-IR spectroscopy analysis and swelling tests.

Unexpected deterioration in the anti-bacterial properties of salicylic acid was reported also by Kantouch et al. [27] and explained by the interactions between the polymeric matrix molecules and the acid molecules.

The antibacterial efficiency of different EO is affected by the nature and structural characteristics of the matrix in which the EO is dispersed [30]. According to other studies that evaluated the inhibitory effect of different type of EOs incorporated in different polymeric matrices, the lower inhibitory effect of incorporated EOs compared with the activity of pure EOs may be due to a slower rate diffusion of phenolic compounds into the agar medium as a result of the internal cross-linking, or it may be due to the possible interactions between the hydroxyl groups of phenolic compounds and functional groups of the polymeric chains [31].

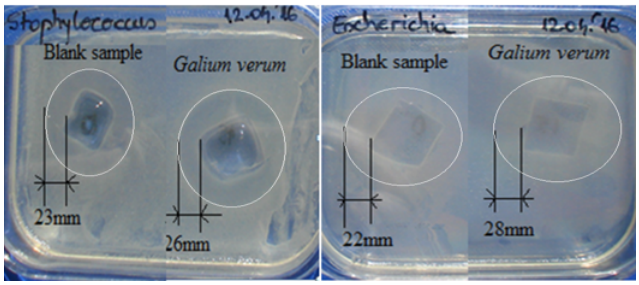

Fig.4. Sensitivity determination of the bacteria a) *Staphylococcus aureus* and b) *Escherichia coli* for the glucose cross-linked biodegradable films without (Blank sample) and with *Galium verum* essential oil

It is clear that the bacterial resistance of the *Galium verum* EO loaded samples depends on the composition and structure of the polymer matrix (table 2). Salicylic acid molecule has the ability to bind the molecule of EO resulting with the inhibition of the bactericidal activity.

Liakos et al. [6], reported a concentration dependent antibacterial activity of peppermint, cinnamon, lavender, Tea tree and lemongrass oils encapsulated in sodium

alginate films. The hydrogel samples with the highest EO concentration of 66% presented a maximum inhibition toward *E. coli*bacteria , considering the diameter of the inhibition zone of 2 mm (peppermint, lavender and Tea tree oil), 12 mm (cinnamon oil) and 3 mm (lemongrass oil).

Table2

EFFICIENCY OF *GALIUM VERUM* ESSENTIAL OIL AGAINST THE GROWTH OF THE TESTED MICROORGANISMS AS A FUNCTION OF CROSS-LINKING WITH GLUCOSE RESPECTIVELY WITH SALICYLIC ACID

Sample	Diameter of inhibition zone (mm)	
	Satphylococcus aureus	Escherichia coli
B_G	23	22
A_{G-EO}	26	28
B_{SA}	25	20
A_{SA-EO}	17	18

Based on the diameter of the inhibition zone, the bacterial killing ability of the SA cross - linked sample (A_{SA-EO}) is comparable to that of silver nanoparticles (19.8 mm for *S. aureus* and 21.6 mm for *E. coli*) [32-34] and zinc oxide nanoparticles (15-28 mm for *S. aureus* and 12-20 mm for *E. coli*) [35], widely used antibacterial agents in cosmetics and pharmaceutics.

Considering the presented data, the A_{SA-EO} sample has an increased hydrostability and enhanced antibacterial property compared with that of other studied essential oils, so we decided to choose this matrix for further analysis, starting from the idea that cross-linking with SA allows the removal of glucose from the hydrogel matrix. In this way we obtain a biomaterial destined for diabetic or polysaccharide allergic patients to.

Mechanical properties

Table 3 shows the influence of *Glium verum* EO incorporation on the mechanical properties of the SA cross-linked gelatin film. The addition of *Galium verum* EO significantly affected the mechanical properties of the hydrogel.

Table 3

EFFECT OF THE *GALIUM VERUM* ESSENTIAL OIL ON THE MECHANICAL PROPERTIES OF THE SALICYLIC ACID CROSSLINKED GELATIN HYDROGELS

Sample	Tensile strength $-\sigma$ (MPa)	Elongation at breakε	Young's Modulus-E (MPa)
B_{SA}	2.76	1.57	175.19
A_{SA-EO}	3.86	2.29	168.44

The TS of the EO loaded sample increased from 2.76 to 3.86 MPa when the EO was added. Our findings suggest that the elongation at break significantly increased after the EO incorporation in the hydrogel matrix. The increased elasticity of the EO loaded sample can be explained by the partial replacement of stronger polymer-polymer interactions by weaker polymer-oil interactions, which may reduce the cohesion of the polymer network forces and increase the mobility of the polymer chain resulting in the increasing of the elasticity and elongation at break. Similar effect was observed by Wu et al. [14], regarding the increase of elongation at break of gelatin based hydrogels with about 24% after cinnamon EO incorporation. Even if the most studies [7,13-15], reported the increase of elongation at break after EO incorporation, Acosta et al. [5] and Liakos et al. [6] have described different effects of the incorporation of EOs into polymeric matrices. They found that the addition of EOs like cinnamon and oregano respectively peppermint and lemongrass, in films based on gelatine-starch and alginate hydrogels caused a decrease in the elongation at break and tensile strength of the films. The decreased TS of the EO loaded hydrogel samples may be due to the interactions that may occur between EO and polymer functional groups. The recombination action of the lipid layer and polymer jointly hinder the hydrogel bonding cross-linking of the polymer molecules, thus the force stabilizing the hydrogel network is destroyed and results in the decrease in TS [36,37]. Sanches-Gonzales et al. [10,31], Kechichian et al. [16], also found that there was a decrease in the TS after the incorporation of EOs like cinnamon and clove into starch films. Our results regarding the variation of TS after the EO incorporation are in correlation with the results of Bonilla et al. [19], they found an increase of TS in starch-chitosan films loaded with basil EO. The increased TS of *Galium verum* EO loaded gelatin hydrogels may be explained by the results of the FT-IR spectroscopy results. We have suggested that the EO physically interact with the SA moleculebut it does not replace the gelatin-gelatin protein bounds that stabilize the macromolecule. The hydrogen bonds between the EO functional groups and SA acts like a physical cross-linking network stabilizing the hydrogel and increasing the elasticity of the films. The increased TS of the EO loaded sample (A_{SA-EO}) also confirmed again the formation of a homogenous structure, without discontinuities, which imparts a highly fragile nature to the matrix.

Differential Scanning Calorimetry (DSC)

DSC thermograms (fig. 5) show that all gelatin based samples, with or without *Galium verum* EO, produces one broad endothermic peak (T_m) at approximately 54°C, associated with the helix-to-coil transition of gelatin due to the breakage of hydrogen bonds [38]. T_m is an indicator of thermostability and generally the higher the value of T_m, the more thermodynamically stable is the macromolecule. Similar results were obtained for chitosan films containing citronella and cedarwood EOs [7]. With the increase of EO concentration no difference was observed in the endothermic peak. This peak tended to shift to lower temperatures just for a higher EO content (30%) and can be explained by the changes in the film's intermolecular interactions leading to the decrease of the crystallinity of the polymeric network. Stoica et al. [39] recorded lower T_m by about 2-5°C for the cinnamon EO loaded chitosan films compared with the blank sample. The value of T_m increased after increasing the concentration of EO. It can be concluded that the EO can have a different effect on the thermal stability of the hydrogels, depending on the type and the concentration of the oil.

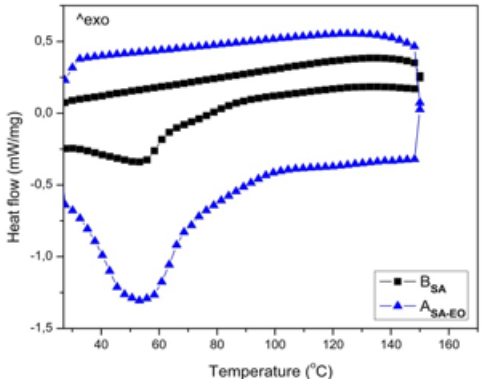

Fig. 5. DSC thermograms for salicylic acid cross-linked blank (B_{SA}) and *Galium verum* essential oil loaded (A_{SA-EO}) samples

Sample	T_m(°C)	ΔH_m (J/g)	$T_{onset\ d}$(°C)	$T_{max.d}$ (°C)	T_g (°C)
B_{SA}	54.0	25.3	86.5	95.5	32.5
A_{SA-EO}	54.0	44.9	86.6	96.7	31.1

Table4
DSC DATA FOR SALICYLIC ACID CROSS-LINKED BLANK (B_{SA}) AND *GALIUM* VERUM ESSENTIAL OIL LOADED SAMPLES (A_{SA-EO})

According to the data presented in table 4, the exothermic temperature of the EO containing sample ($T_{max.d}$ = 96.2°C) were higher than the blank films ($T_{max.d}$ = 95.5°C). Also the DH_m increased with about 19.6 J/g after EO incorporation, suggesting the increase in the crystallinity. This clearly showed that EO contributed to an improvement in the thermal stability of the SA cross-linked geatin hydrogel.

Conclusions

Two gelatin based *Galium verum* essential oil loaded hydrogel samples were prepared: glucose and salicylic acid cross-linked - by microwave assisted polymerization method. Structural properties of hydrogel samples were analysed by IR spectroscopy.The results of FT-IR spectroscopysuggests that *Galium verum* EO interacts differently with the polymeric matrix depending on the matrix structure and composition. Due to these different interactions, the essential oilcan develop a different influence on the mechanical and thermal properties of the hydrogel, but at the same time can negatively affect the bactericidal activity of the *Galium verum* EO.The results also indicated good dispersion and well interactions between gelatin and *Galium verum* essential oil, which imparted stability to the entire polymeric system. This behavior supports the idea that essential oils can interact differently with different polymeric matrices. The fact that *Galium verum* essential oil is fixed to the polymeric matrix by hydrogen bonds enables it to act both as bioactive substance that can be released in a controlled way and physically cross-linking agent. The supplementary cross-linking of the gelatin-salicylic acid system with *Galium verum* essential oil increases the swelling degree, the tensile strength and the elasticity of the hydrogel sample. It was important to notice that even if the bactericidal effect of the essential oil decreased in the salicylic acid cross-linked system, it is still comparable or higher than that of other used essential oils or antibacterial agents. Therefore, it was concluded the stabilizing effect of *Galium verum* essential oil on the gelatin-salicylic acid polymer matrix.

The new obtained material could be used as a controlled delivery device, being an elastic and resistant material with added value having therapeutic and bioactive properties of both salicylic acid and *Galium verum* essential oil.

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