TOMAS BATA UNIVERSITY IN ZLIN
FACULTY OF TECHNOLOGY
Polymer Centre

Doctoral Thesis

Hydrogels for biomedical applications

Hydrogely pro použití v biomedicíně

Amarjargal Saarai

July 2012
Zlín, Czech Republic

Doctoral study programme:	P 2808 Chemistry and Materials Technology
Course:	2808V006 Technology of Macromolecular Compounds
Supervisor:	Prof. Ing. Petr Sáha, CSc.
Consultant:	Doc. Ing. Věra Kašpárková, CSc. Ing. Tomáš Sedláček, PhD.

CONTENT

ACKNOWLEDGEMENT

First of all I would like to express my deepest gratitude to my supervisor, Prof. Petr Saha, for giving me the opportunity to pursue my studies at Polymer Centre, and for his constant support and encouragement within the study period.

I am deeply thankful to my consultant, Assoc. Prof. Vera Kasparkova, for her fruitful guidance, efforts and patience to read and revise my drafts carefully, and give me valuable remarks, all of which contributed to the finalization of my thesis.

I would also thank Dr. Tomas Sedlacek for his encouragement, creative suggestions, motivation and insightful comments.

My sincere thanks also go to Assoc. Prof. Nabanita Saha for supervising my research in its early stages and contribution to the patent included in this thesis.

I am grateful to all the members of the Polymer Centre and the University Institute for creating a helpful and friendly working environment, especially to Professor Takeshi Kitano, Dr. Alena Kalendova, Mr. Michal Machovsky and Mr. Pavel Bazant for their valuable technical support.

And last but not least, I am overwhelmed with gratitude to my family for their everlasting support, patience and understanding.

ABSTRACT

Owing to its unique properties, hydrogels are rapidly growing group of materials employed in biomedical and pharmaceutical applications. Because of their biocompatibility, biodegradability, hydrophilicity, excellent swelling behaviour and absence of toxicity, biopolymer-based hydrogels are considered as promising wound healing and covering materials.

The presented work is dealing with preparation of hydrogels from combinations of polyelectrolyte biopolymers with opposite charges. Among various biopolymers, protein-polysaccharide combinations are reported as the most promising pairs for hydrogel formation which were also used in this work. Nevertheless, it should be stressed that hydrogels on the basis of these biopolymers prepared solely by physical crosslinking induced by secondary physical forces do not exhibit sufficient mechanical strength after water or wound exudates absorption. To overcome this disadvantage, several modifications, such as chemical crosslinking of the hydrogel forming polymers, have been investigated and applied. On the other hand, improvement of the mechanical strength of hydrogels by crosslinking results in a reduction of their ability to absorb wound exudates. Compromise between the swelling properties and hydrogels mechanical strength is thus of critical importance with respect to their successful application.

In the thesis, development and characterization of physicochemical properties of Sodium alginate/Gelatine hydrogels, arising through either physical or chemical crosslinking is presented. The work is divided into two main chapters. The first part informs on the current status of the knowledge related to hydrogels, their properties, preparation and characterization. It describes hydrogel classification, methods for their preparation, including chemical and physical crosslinking, theoretical background for the swelling behaviour and viscoelastic properties and provides also information on their practical application. In the second part of this work, results obtained during the doctoral work are reported in the form of three papers and one patent. Contents of the papers and patent are provided in short abstracts and discussion modules related to the particular state of the solved problem. At the end of the thesis, the full-texts of the papers and patent are enclosed.

Key words: Sodium Alginate, Gelatine, Hydrogels, Crosslinking, Wound Dressing

ABSTRAKT

Díky svým jedinečným vlastnostem jsou hydrogely rychle rostoucí skupinou materiálů používanou v biomedicínských a farmaceutických aplikacích. Vzhledem k jejich biokompatibilitě, biodegradabilitě, hydrofilitě, schopnostem pohlcovat kapaliny a absenci toxicity jsou biopolymerní hydrogely považovány rovněž za materiály vhodné pro podporu hojení ran.

Představená práce se zabývá přípravou hydrogelů z biopolymerních polyelektrolytů s nesoucích opačný náboj. Za jedny z nejslibnějších materiálů lze v tomto ohledu považovat kombinace proteinů a polysacharidů, které byly použity v této disertaci. Je však třeba zdůraznit, že hydrogely připravené z uvedených biopolymerů fyzikálním síťováním pomocí sekundárních sil nevykazují při kontaktu s vodou nebo exsudátem dostatečnou mechanickou pevnost. Tento nedostatek je možno odstranit prostřednictvím různých modifikací, například chemickým síťováním polymerů, ze kterých je hydrogel připraven. Zlepšení mechanické pevnosti hydrogelů síťováním však vede ke snížení jejich schopnosti absorbovat eksudát. S ohledem na úspěšnou aplikaci hydrogelů má tedy zásadní význam nalezení optimálního složení, které představuje kompromis mezi jejich absorpční schopností a mechanickou pevností.

Disertační práce představuje aktivity spojené s vývojem a charakterizací fyzikálně-chemických vlastností hydrogelů složených z alginátu sodného a želatiny, které byly připraveny pomocí fyzikálního nebo chemického síťování. Práce je rozdělena do dvou hlavních kapitol. První část informuje o aktuálním stavu znalostí týkajících se hydrogelů, o jejich vlastnostech, přípravě a charakterizaci. Popisuje rovněž klasifikaci hydrogelů, metody jejich přípravy, včetně chemického a fyzikálního síťování, bobtnání, viskoelastických vlastností a poskytuje informace o jejich použití v praxi. V druhé části práce jsou formou tří publikací a jednoho patentu souhrnně prezentovány výsledky získané v průběhu doktorského studia. Obsahy dokumentů jsou představeny v krátkých abstraktech a diskusních modulech týkajících se daného řešeného problému. V závěru práce jsou přiloženy plné texty článků a patentu.

Klíčová slova: Alginát sodný, želatina, hydrogely, síťování, krytí ran

LIST OF PAPERS

The following papers and patents have resulted from the doctoral research and are available in full-text at the end of this dissertation:

Publication I:

AMARJARGAL SAARAI, TOMAS SEDLACEK, VERA KASPARKOVA, TAKESHI KITANO, PETR SAHA, On the Characterization of Sodium Alginate/ Gelatine-Based Hydrogels for Wound Dressing, *Journal of Applied Polymer Science*, 2012, 2012, vol. 126, 79-88.

Publication II:

AMARJARGAL SAARAI, VERA KASPARKOVA, TOMAS SEDLACEK, PETR SAHA, On the Development and Characterization of Crosslinked Sodium Alginate/ Gelatine-Based Hydrogels, *Journal of the Mechanical Behavior of Biomedical Materials*, under review

Publication III:

AMARJARGAL SAARAI, TOMAS SEDLACEK, VERA KASPARKOVA, On the Characterization of Genipin Crosslinked Sodium Alginate/Gelatine Hydrogels for Wound Dressings, *Journal of the Mechanical Behavior of Biomedical Materials*, prepared for publication

Patent I:

SAHA NABANITA, SAHA TOMAS, AMARJARGAL SAARAI, Dry Substance of Hydrogel to Cover Wounds and Process for Preparing Thereof, *CZ patent 302380*, date of the patent 09.03.2011

Patent II:

SAHA NABANITA, SAHA TOMAS, AMARJARGAL SAARAI, Dry Material of Hydrogel for Wound Dressing and its Method of Preparation, *International Patent Publication Number WO 2011/100935 A1*, *International Application Number: PCT/CZ/2011/000017,* date of application 25. 8. 2011

LIST OF SYMBOLS AND ACRONYMS

ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
$CaCl_2$	Calcium chloride
DMA	Dynamic mechanical analysis
f	Elastic force
G’	Storage module
G’’	Loss module
ΔG_{el}	Elastic free energy
ΔG_{ion}	Ionic free energy
ΔG_{mix}	Mixing free energy
ΔG_{total}	Total free Gibbs energy
L	Length
$\bar{M}_C$	Molecular weight
PVA	Poly (vinyl alcohol)
SA	Sodium Alginate
SD	Swelling degree
SEM	Scanning Electron Microscopy
S	Entropy

T	Temperature
t	Time
tanδ	Loss factor, tan delta
Tg	Glass transition temperature
U	Internal energy
V	Volume
χ	Polymer-solvent interaction parameter
ω	Frequency

FIGURES AND TABLES

THEORETICAL BACKGROUND

1. Introduction to hydrogels

Hydrogels are three-dimensional hydrophilic polymer networks capable of swelling to equilibrium in the presence of excess water or biological fluids [1-3]. When equilibrated in aqueous medium, they reach their final hydrated network structure given by balance of swelling and elastic forces. The hydrophilicity of the network is connected with occurrence hydrophilic groups such as hydroxyl (-OH), carboxyl (-COOH), amidic (-CONH-), primary amidic ($-CONH_2$) or sulphonic ($-SO_3H$) in polymer chains. Moreover, it is also possible to produce hydrogels containing a portion of hydrophobic part [2, 4, 5] by blending or copolymerizing hydrophilic and hydrophobic polymers.

Hydrogels can be prepared in various forms including solid moulded forms, pressed powder matrices, microparticles, coatings, membranes or sheets as well as encapsulated solids [6]. Due to the unique properties of hydrogels, described below, they have recently received considerable attention within the field of biomedical application:

- the high water or biological fluid content makes them compatible with most living tissues;
- soft elastomeric nature provides a minimal mechanical/ frictional irritation to the surrounding living cells and tissue;
- low interfacial tension contributes to a reduction in protein adsorption and hence biofouling and cell adhesion;
- their swelling capacity results in high permeability for low molecular weight drug molecules and metabolites [7, 8, 9].

Hydrogels are widely used in bio-applications and play an essential role in modern strategies to cure malfunctions and injuries of living systems. Their structure is determined by crosslinks between polymer chains formed via physical interactions including H-bonding and hydrophobic forces, and various chemical bonds. Hydrogels based on natural and synthetic polymers, as well as on their combinations, have been investigated for biomedical applications including drug delivery systems [10, 11], tissue engineering [7], wound dressings [12], and healing/ repairing and regeneration of a wide variety of tissues and organs.

Application of hydrogels as wound dressing for treatment of skin wounds and burns was encouraged by the concepts of Winter (1962) and Hinman and Maibach

(1963), which stated that a moist wound environment can improve epidermal healing [13, 14]. The main benefit of hydrogels is their ability to create an optimal moist environment for wound healing, to provide moisturing of the dry wounds and to assure moisture absorption from exudating wounds [13]. Beside this, hydrogels offer many other advantages for wound healing such as easy application [14, 15], transparency [16, 17], good adhesion [18, 19], oxygen permeability [20], the ability to promote analgesia by cooling the skin [14, 21] and autolytic debridement [22]. Currently, numerous hydrogels have been developed for both dry and exuding wounds, such as pressure ulcers, skin tears, surgical wounds and burns [23].

In recent years, smart hydrogels responding to a wide range of stimuli, including temperature, pressure, pH, gases, liquids and biological indicators, have attracted great interest both in science and technology, since they offer new opportunities for medical and pharmaceutical applications in the drug delivery, articular cartilage, biomaterial scaffold, corneal replacement and tissue engineering as well as wound dressing [24-27].

1.1 Hydrogels classification

Hydrogels can be classified according to their source of origin, ionic charge, preparation method, nature of crosslinks and biodegradability, as it is shown in Figure 1.

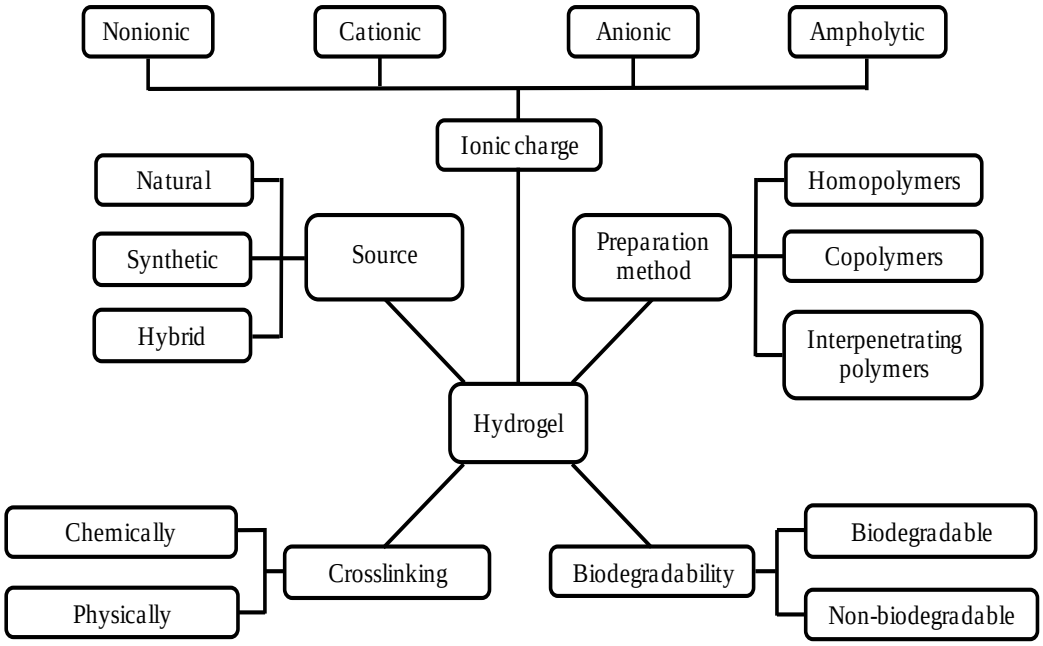


Fig. 1. Classification of hydrogel

Both natural (agarose, alginate, chitosan, collagen, fibrin, gelatine and hyaluronic acid etc.) and synthetic polymers (poly(ethylene oxide), poly(l-hydroxyethylene), poly(acrylic acid) etc.) can be used for hydrogel forming [3-5]. Thus, according to their composition, they can be classified into synthetic, natural or hybrid hydrogels. Whilst natural hydrogels have been widely used for biomedical applications due to their non-toxicity, biocompatibility and biodegradability, they also have some limitations such as poor mechanical properties, which need to be suppressed by suitable modifications, for example by mixing with synthetic polymers.

Another possibility to classify hydrogels refers to way of their crosslinking. Then they can be classified as chemically and physically crosslinked systems [6, 10]. Via chemical crosslinking, covalent bonds are formed while through physical crosslinking non-covalent interactions, such as hydrophobic and ionic interactions, are established. Even if chemical crosslinkers employed for hydrogel preparation offer better mechanical properties, they are often toxic and their residues must be completely removed before biomedical application [12, 24].

Regarding to ionic strength, hydrogels may be classified as non-ionic and ionic (anionic, cationic, ampholytic). The non-ionic hydrogels can include for example polyacrylamide, poly(vinyl alcohol) and poly(N-vinyl pyrrolidone) whilst poly(N,N-dimethylacrylamide co-acrylamide) or gelatine can be given as examples of the polymers suitable for ionic hydrogels.

Furthermore, depending on the method of preparation, homopolymer, copolymer and interpenetrating hydrogels can be mentioned [11, 26]. Homopolymer hydrogels are networks of one type of hydrophilic monomer unit, while copolymer hydrogels are formed by different comonomer units and interpenetrating hydrogels are formed by mixtures of various homopolymers [28].

Finally, classification essential for the biomedical applications can be performed based on the biodegradability; biodegradable or non-biodegradable hydrogels can be then specified. Here it can be highlighted that devices made of biodegradable materials offer essential advantage for bio-applications, as they do not require additional surgery intervention for their removal [27].

1.2 Preparation of hydrogels

Hydrogels are crosslinked networks usually formed by hydrophilic polymers. This implies that appropriate crosslinks are presented in order to avoid dissolution of the hydrophilic polymer chain in aqueous solution. Only such structures, in-

duced by either chemical or physical crosslinks (Fig.2), ensure specific properties inevitable for biomedical applications [10, 28, 29], as for example a suitable mechanical strength.

The crosslinking may take place in two environments:

- in vitro during the preparation of a hydrogel;
- in vivo (in situ) after application in a precise location of the human body [3].

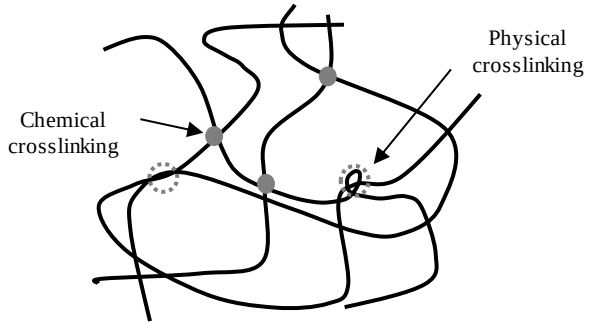


Fig.2. Sketch of chemical and physical crosslinking [3, 28]

To formulate a crosslinked network from polymer molecules, the polymers have to possess chemically active functional groups. Therefore, polymers with carboxyl, amine or hydroxyl groups can be explicit as suitable examples for easily crosslinked materials acceptable for hydrogel formation [8, 17, 30].

1.2.1 Chemical crosslinking

Chemical crosslinking generally yields more stable hydrogels with better mechanical properties compared to physical one. Chemical crosslinking can be achieved for instance by radical polymerization of suitable functionalities presented in polymer, chemical reaction of complementary groups, photopolymerization or enzymes [31].

Free radical polymerization, widely used method for bio-applications, can be performed by several ways. For example, vinyl-bearing macromers are polymerized forming hydrogels with the help of redox or thermal initiators or photopolymerization using UV light [8, 29]. While high initiator concentration leads to reduced crosslinking time and enhanced mechanical properties, it is important to consider the initiator concentration due to possible cytotoxic effects and reduction of swelling. The advantage of photo-initiation is a fast crosslinking rate, however the disadvantage of the method is that cells exposed to a high-intensity UV irradia-

tion for prolonged time may have an adverse effect on cellular metabolic activity [1].

As noted earlier, the solubility properties of water-soluble polymers are governed by the presence of functional groups [1, 31]. Covalent linkages between polymers chains are established by the reaction of functional groups having complementary reactivity. Typical reactions are Schiff base formation [32], Michael type additions [33], peptide ligation [34] as well as click chemistry [35]. Among them Schiff base formation between an aldehyde and an amino group is the most widely used technique. Glutaraldehyde crosslinked gelatine hydrogel, graphically presented in Fig. 3, can be mentioned as an example here [36].

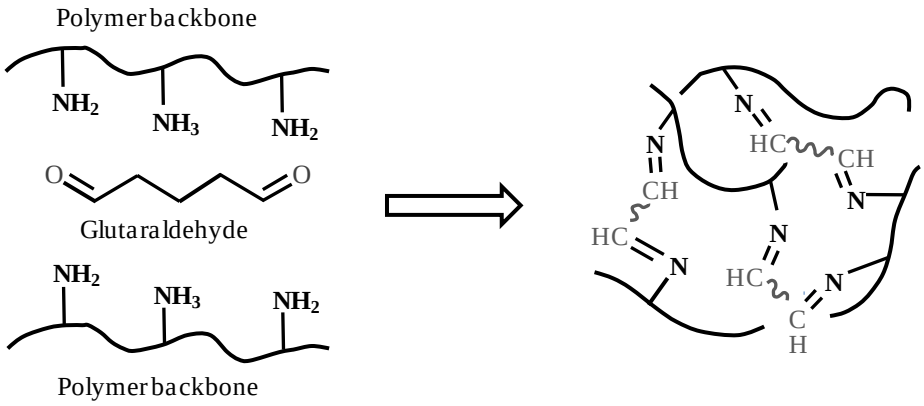


Fig.3. Preparation of hydrogels via Schiff base formation [8] employing aldehyde/amine containing polymers

Nevertheless, it should be kept in mind that disadvantage of glutaraldehyde utilization consists in its toxicity even at low concentrations, possibility of leaching out into the body during matrix degradation, and resulting inhibition of cell growth [8]. Therefore, hydrogels prepared via glutaraldehyde crosslinking need to be thoroughly extracted with a view to remove any traces of unreacted crosslinker before use in bio-applications, and extracts have to be carefully checked for glutaraldehyde residues.

Enzymes often exhibit a high degree of substrate specificity, potentially avoiding side reactions during crosslinking [9]. With this advantage, it is possible to control and predict the gelation kinetics, thus control overall crosslinking rate. Tianhong Chen et al. [37] compared the ability of transglutaminase and tyrosinase

to catalyze the hydrogel formation. In their work, gel formation was catalyzed and initiated by adding of the enzymes to solutions of gelatine and blends containing gelatine and chitosan [30, 36]. Results of these works showed that tyrosinase-catalyzed gelatine–chitosan gels were considerably weaker compared to transglutaminase-catalyzed gels. The advantage of the enzymatic method consists in the gel crosslinking under mild conditions without the need of low-molecular weight compounds utilization, radiation, or the prior grafting of crosslinkable functionality [29]. Since the gelation kinetics can be well controlled, the enzyme based systems are proper for in situ gelling systems [12, 37].

1.2.2 Physical crosslinking

Compared to chemical crosslinking, physical one offers the advantage of the generally mild reaction conditions, since no reactive groups, crosslinking agents, initiators or photo irradiation are required [8, 23]. Depending on the nature of gelling system, the junctions can be molecular entanglements, ordered crystalline regions, phase separated micro-domains and secondary forces including ionic, hydrogen bonding or hydrophobic forces (see examples in Fig. 4) [1, 38]. Nevertheless, the main drawback of physically crosslinking hydrogels is their relative instability, and possible rapid and unpredictable disintegration [38].

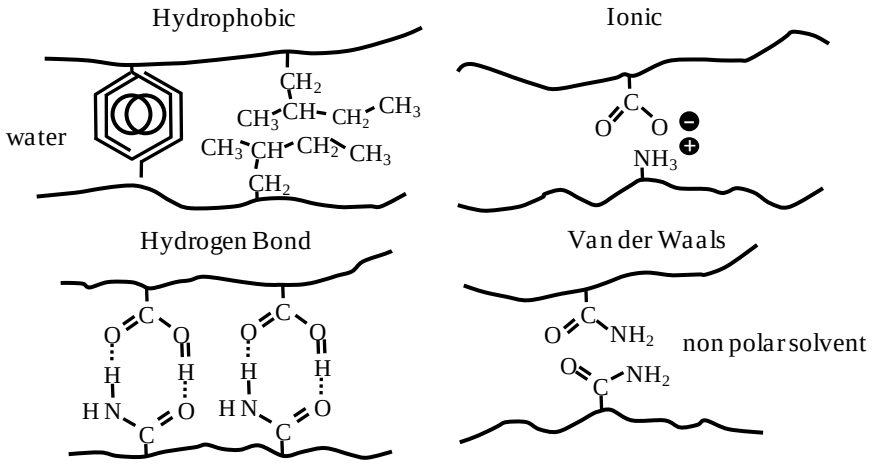


Fig.4. Interactions of specific functional groups in the formation of physically crosslinked gels [38]

Hydrophobic interactions hydrogels

Polymers with hydrophobic domains can crosslink in aqueous environments via reverse thermal gelation (sol-gel transition). Polymers (or oligomers) with such

gelation properties are referred to as gelators and are typically moderately hydrophobic [38-40]. The gelation occurs when the hydrophobic segment is coupled to the hydrophilic polymer segment of an amphiphilic polymer. These polymers are usually water soluble at low temperatures. As the temperature is increased, the hydrophobic domains aggregate to minimize the hydrophobic surface area, reducing the amount of structured water surrounding the hydrophobic domains and maximizing the solvent entropy [9, 41]. The temperature at which gelation occurs depends on the concentration of the polymer, the length of the hydrophobic block and the chemical structure of the polymer [38].

Ionic interaction hydrogels

Hydrogels involving ionic reactions are formed when a polyelectrolyte is combined with a multivalent ion of opposite charge. When polyelectrolytes of opposite charges are mixed, they may form gels or precipitate depending on their concentration, the ionic strength and pH of the solution. Both naturally occurring and synthetic polyelectrolytes have been ionically crosslinked [27, 40, 42]. For instance, alginate is capable of forming ionically crosslinked hydrogels by divalent calcium ions at room temperature and under physiological conditions, which can be then used for wound dressings [32], encapsulation of enzymes/ cells or the release of proteins [43, 44]. In this case, the crosslinking is achieved by the ionical interaction between calcium ions and the carboxyl groups of the blocks of guluronic acid residues of two neighbouring alginate chains, resulting in formation of three-dimensional network (see Fig. 5) [45].

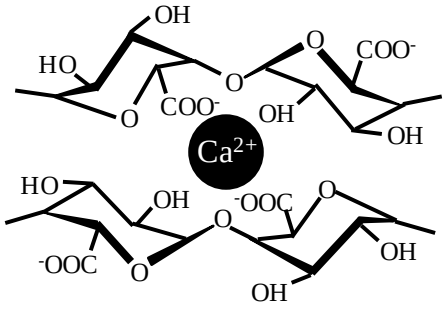


Fig.5. Three-dimensional structure of $CaCl_2$ crosslinked alginate chains [31, 46, 47]

Hydrogen bonded hydrogels

Hydrogen bonded hydrogels formed by mixing of two or more natural polymers can display rheological synergism. In such cases they are often applied as injectable hydrogels for drug release [38]. Their viscoelastic properties are more gel-

like than those of the individual polymers due to the extensive hydrogen bonding interactions. For example, blends of gelatine-agar, starch-carboxymethyl cellulose, and hyaluronic acid-methylcellulose form physically crosslinked gel-like structures with excellent biocompatibility [38, 48]. However, these hydrogen bonded networks can dilute and disperse over a few hours in vivo due to an influx of water, which restricts their use to relatively short-acting drug release systems [39].

Regarding aforementioned facts, it can be noted that a combination of physical and chemical crosslinking offers the possibility of obtaining materials with improved physical and mechanical properties without compromising biocompatibility [8].

1.3 Properties of hydrogels

As hydrogel structural and functional properties are comparable to many of the soft tissues in the human body, they have found numerous applications in biomedical field [49]. Nevertheless, in such cases utilized hydrogels have to possess a combination of favourable properties such as biodegradability, biocompatibility, absorption capacity, swelling, permeability, surface smoothness, optical clarity as well as mechanical strength [18, 19, 50].

These properties of hydrogels, for an intended application, can be tailored by selecting proper starting materials and processing techniques resulting in final hydrogel network structure. Exact characterization of this structure is quite complicated due to occurrence of different types of possible networks including regular, irregular, loosely or highly crosslinked network types [6, 10, 24, 51]. Therefore, an ideal network (usually a Gaussian network) of chains is usually assumed for the purpose of the hydrogel network structure characterization, as it is indicated in Fig. 6 [3, 28].

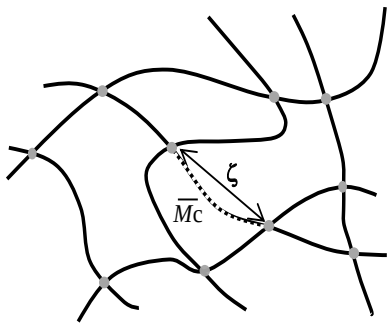


Fig.6. Ideal Gaussian network [3, 28]

According to literature review, the most important parameters used for characterization of the hydrogels network structure are the polymer volume fraction in the swollen state, the molecular weight of the polymer chain between two neighbouring crosslinking points $\overline{M}_C$, and the corresponding length or mesh size, ζ, [5, 41, 52-55]. In addition, one of the other important properties of an elastic polymer network is degree of crosslinking, i.e., the number density of junctions or crosslinks joining the chain segments into a network structure which gives rise to elastic properties [56-58].

The polymer volume fraction in the swollen state is a measure of the amount of fluid imbibed and retained by the hydrogel. The molecular weight between two consecutive crosslinks, which can be either chemical or physical in nature, is a measure of the degree of crosslinking of the polymer [24, 52, 53]. It should be noted that due to the random nature of polymers only an average values of $\overline{M}_C$ can be calculated. The correlation length or mesh size between two adjacent crosslinks, ζ, provides a measure of the space available between the macromolecular chains [6, 24, 41, 52-54]. These parameters can be determined experimentally, while the equilibrium swelling and rubber elasticity theories can serve as a theoretical background for correlation between these parameters and hydrogel properties [28, 53].

1.3.1 Swelling behaviour

In practice, hydrogels are usually described by their degree of swelling. The swelling capacity of a hydrogel can be determined by the amount of space inside the hydrogel network available to accommodate water and aqueous liquids. Absorption in hydrogels is then influenced by many factors, including network parameters, for example crosslinking density, nature of the solution, hydrogel structure (porous or poreless), and preparation techniques. Among them, the crosslinking density is the most important factor usable for determination of the swelling characteristics of a given hydrogel [8, 59].

The swelling behaviour can be seen as a two step process consisting of diffusion followed by relaxation [1]. In case of lower crosslinking density, both processes take place, while in case of highly crosslinked hydrogels, the relaxation mechanism potentially changes toward a single diffusion process as polymer chain movement is limited by the high crosslink density (see Fig. 7) [9].

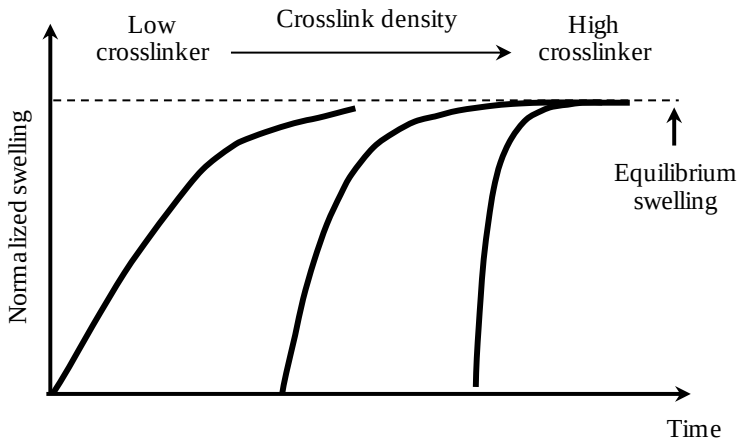


Fig.7. Swelling curves of polymers with different crosslinking density [1]

Infinite solubility of hydrogels is prevented by elastic forces, which originate from the network crosslinking [8, 41]. Expansion of hydrogel network is induced by swelling force which is given by:

- polymer-solvent interactions
- electrostatic interactions
- osmosis [1, 24].

When equilibrated in aqueous medium, the hydrogels reach their final hydrated network structure, which brings into balance swelling and elastic forces, as it is depicted in Figure 8. Therefore, hydrogels with different swelling capacities can be obtained by modulating the contribution of individual forces [1, 60, 61].

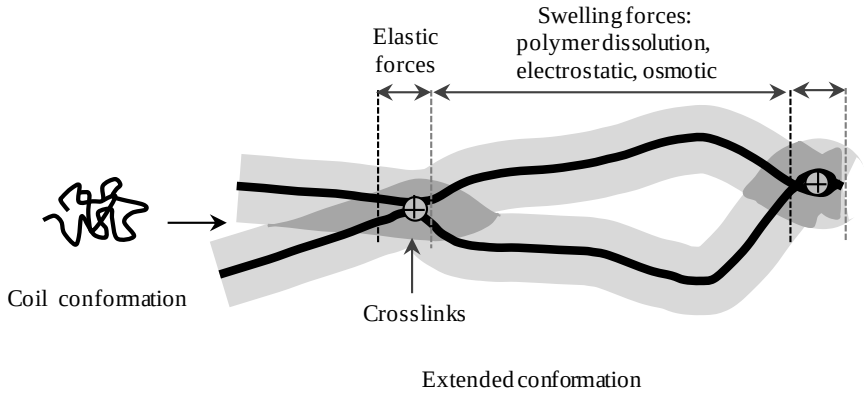


Fig.8. Elastic and swelling forces in hydrated hydrogel [1, 41]

The theoretical description of the swelling of the hydrogels at equilibrium is based on the minimization of Gibbs free energy of the gel [41]. According to the Flory-Rehner theory [62-64] when polymer network, free of ionic moieties, is in contact with an aqueous solution or a biological fluid, it starts to swell due to the thermodynamic compatibility of the polymer chains and water. The swelling force is counterbalanced by the elastic force induced by crosslinks of the network. At equilibrium, these two forces are equal and the Gibbs free energy can be used to describe this situation [3, 53]:

$$\Delta G_{total} = \Delta G_{el} + \Delta G_{mix} \quad (1)$$

In equation (1), total ΔG_{total} is the change of total free Gibbs energy in hydrogel, ΔG_{el} is the change of free energy contributed by elastic force of the hydrogel (polymer) chains and ΔG_{mix} is the change of free energy of mixing, expressing compatibility of the polymer with the molecules of the surrounding fluid. This compatibility is usually expressed through the polymer-solvent interaction parameter, χ [65].

In a case of ionic hydrogel placed in a swelling agent, there are three contributions to the total Gibbs free energy of the system; namely elastic (ΔG_{el}), mixing (ΔG_{mix}), and ionic free energies (ΔG_{ion}), as given in equation (2) [60, 64, 66].

$$\Delta G_{total} = \Delta G_{el} + \Delta G_{mix} + \Delta G_{ion} \quad (2)$$

Non-ionic hydrogels swell in aqueous medium solely due to polymer-water interactions while in case of the ionic hydrogels, swelling is dependent on the pH of the aqueous medium, which determines the degree of dissociation of the ionic chains. Cationic hydrogels display superior swelling in acidic media since their chain dissociation is favoured at low pHs [1, 9]. Similarly, anionic hydrogels dissociate more in higher pH media, hence, displaying superior swelling in neutral to basic solutions [1, 41].

Ampholytic hydrogels possess both positive and negative charges that are balanced at a certain pH, their iso-electric point. A change in pH can change the overall ionic (cationic or anionic) character of this type of hydrogel. For example, ampholytic gelatine (type B) dissolves in water less due to its cationic nature compare to an acidic medium [12, 32].

Besides non-ionic and ionic hydrogels, the hydrophobically modified hydrogels containing a hydrophilic backbone with pendant hydrophobic groups can be also employed [10, 50]. In an aqueous solution, the balance between the hydrophilic and hydrophobic interactions changes with temperature. Therefore, depending on the nature of these groups, hydrophobic association occurs at a specific temperature resulting then in gelation (for details see Fig. 9) [1].

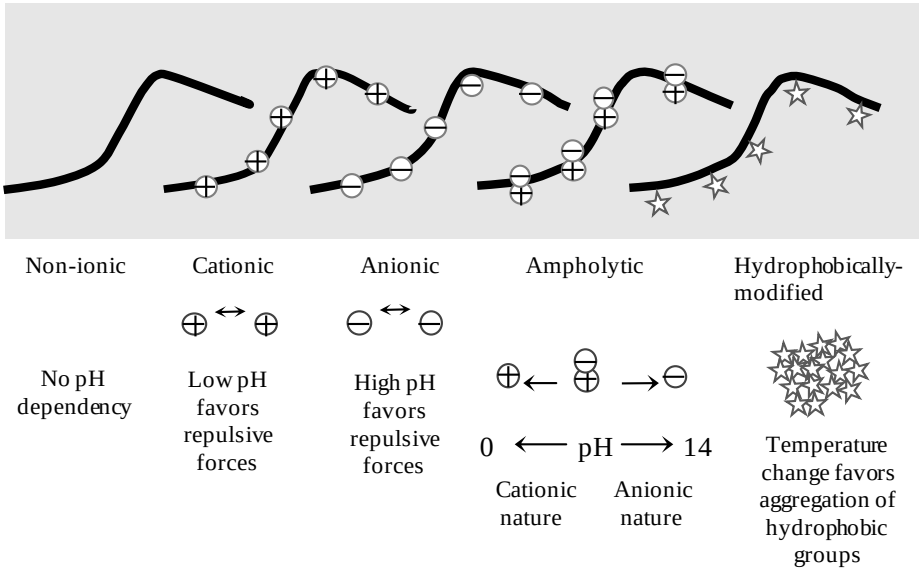


Fig. 9. Behaviour of different hydrogels structures [1]

1.3.2 Mechanical properties

Besides swelling properties, good mechanical strength and elasticity is important for hydrogels intended for bio-applications. However, in most cases, hydrogels have weaker mechanical strength and poorer elasticity, missing the sophisticated complexity of native tissue. The mechanical strength of the hydrogel can be improved by increasing either the crosslinking density or the concentration of the precursors [60, 67, 68]. On the other hand, it could result in a concomitant reduction of the ability to swell and absorb wound exudate [16, 49]. Therefore, a compromise between the hydrophilicity and sufficient mechanical strength of hydrogels is critical for their potential application as wound dressing materials.

The theories of rubber elasticity and viscoelasticity can be used for understanding of the mechanical behaviour of hydrogels. These theories are based on time-independent and time-dependent recovery of the network structure, respectively [68]. Beside these theories dealing with network behaviour, there is variety of methods for the mechanical analysis of hydrogels, including elonga-

tion/compression analysis [3, 68, 69], dynamic mechanical analysis (DMA) [68, 70] and oscillatory rheometry [3].

Viscoelasticity

Quantitative information on the viscoelastic properties of hydrogels can be obtained by dynamic mechanical analysis. This is frequently used method, measuring the response of a sample when it is deformed under periodic oscillation, stress or strain, as it is illustrated in Figure 10 [51, 58, 68].

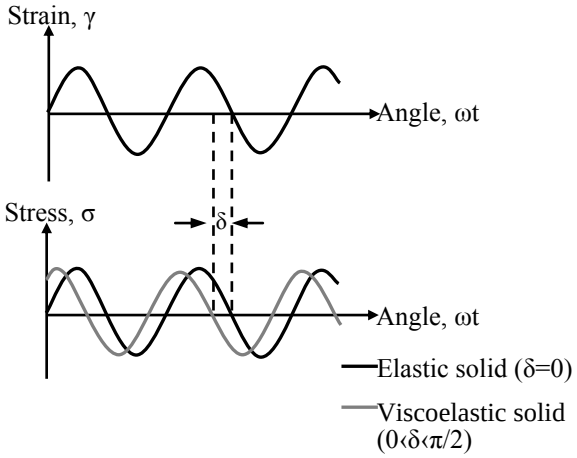


Fig.10. Typical stress/strain response for different materials during oscillatory measurements [58, 68]

In the dynamic mode of testing, if the strain is a complex oscillatory function of time with maximum amplitude, γ_m, and frequency, ω, then complex strain, γ^*, can be defined by equation (3).

$$\gamma^* = \gamma_m \exp(i\omega t) \tag{3}$$

where, γ_m, is the maximum shear strain amplitude, ω, stands for the oscillation frequency and, t, is the time.

Correspondingly, the measured response in terms of shear stress, the complex stress, σ^*, is defined:

$$\sigma^* = \sigma_m \exp(i\omega t + \delta) \tag{4}$$

A standard expression for sinusoidal tests is the complex dynamic modulus, G^*, defined as the ratio of the complex stress, σ^*, to the applied complex strain, γ^*:

$$G^* = \frac{\sigma^*}{\gamma^*} \tag{5}$$

Equation (5) then can be rewritten:

$$G^* = \frac{\sigma_m}{\gamma_m}\exp(i\delta) = \frac{\sigma_m}{\gamma_m}\cos\delta + i\frac{\sigma_m}{\lambda_m}\sin\delta = G^{/} + iG^{//} \tag{6}$$

where, $G^{/}$, is referred to as the storage modulus defining the energy stored due to the applied strain, $G^{//}$, is the loss modulus and determines energy of dissipation. From these expressions, the tangent of the phase angle can be expressed:

$$\tan\delta = \frac{G^{//}}{G^{/}} \tag{7}$$

where $\tan\delta$, the loss factor or damping, is a measure of the ratio of the energy dissipated as heat to the maximum energy stored in the material during one cycle of oscillation [58, 68-70]. While the phase angle is zero for an elastic solid, it is equal to $\pi/2$ for a viscous liquid [58, 70].

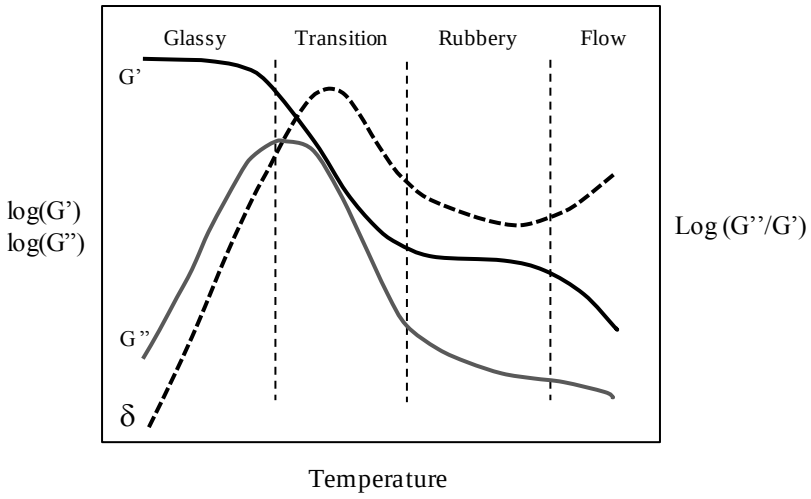


Fig.11. General behaviour of the $G^{/}, G^{//}$ and $\tan\delta$ as a function of temperature [71]

Measurements of viscoelastic properties of hydrogels is influenced by several parameters including frequency, temperature, dynamic strain rate, static pre-load, time effects such as creep and relaxation [71]. Among them, frequency and temperature effects are most important.

As can been seen in the Fig. 11, the viscoelastic materials behave differently in various phases over the broad temperature ranges referred to as the "Glassy", "Transition", "Rubbery", and "Flow Regions" [68, 71, 72]. In the glassy region the polymer chains are stiff in nature. The transition region exists when the materials are crossing from the glassy to the rubbery region. In this region, the viscoelastic material goes through the most rapid change in stiffness (relaxed state), possesses the highest damping characteristics and G'' increases as the temperature arises [68, 71]. The glass transition temperature of a material, T_g, is commonly defined as the peak of the loss factor curve. In the rubbery region, the material reaches a lower plateau in stiffness and loss factor [71, 72]. As the polymeric material is heated beyond the rubbery region, its viscosity, it means resistance to flow, steadily decreases. Finally, in the liquid flow region G' shows a sharper reduction because of the onset of viscous flow in the polymer [68].

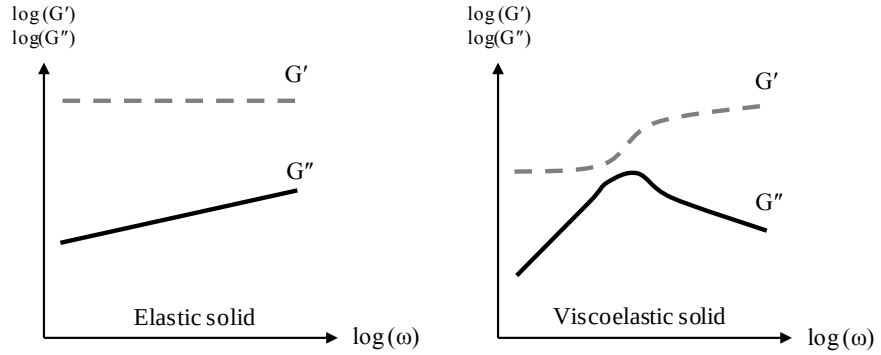


Fig.12. Typical behaviour of the G' and G'' of the elastic solid and viscoelastic as a function of frequency [58]

The behaviour of the storage modulus, G', and loss modulus, G'' of a model hydrogel as a function of frequency, ω, is shown in Fig. 12. The elastic response of the hydrogel is characterized by a storage modulus, which is frequency independent and a loss modulus, which decreases with reducing frequency [70]. On the other hand, a viscoelastic solid shows a plateau with a constant G' in the low frequency region and G'' is frequency dependent [58].

Rubber elasticity

Most of hydrogels in their swollen state are considered to be a rubber, which means that they are crosslinked networks with rather large free volume allowing them to respond to external stresses with a rapid rearrangement of the stretched polymer segments. When a hydrogel is in the rubbery region, its mechanical be-

haviour is dependent mainly on the network structure [3, 58]. However, at low temperature, these hydrogels can lose their rubber elasticity and show viscoelastic behaviour.

To derive a relationship between the network characteristics of hydrogel and the mechanical stress-strain behaviour, classical and statistical thermodynamics as well as phenomenological approaches have been used and an equation of state for rubber elasticity was found to be valid and worthwhile [68]. From classical thermodynamics, the equation of state for rubber elasticity may be expressed as the sum of the internal energy and the entropy of extensions [51, 58, 68].

$$f = \left(\frac{\partial U}{\partial L}\right)_{T.V} + T\left(\frac{\partial f}{\partial T}\right)_{L.V} \tag{8}$$

where f is the elastic force of the elastomeric polymer in response to a tensile force, U is the internal energy, T , is the temperature, and L and V are the length and volume of the sample, respectively. For elastomeric polymers, an increase in length brings about a decrease in entropy because of changes in the end-to-end distances of the network chains. The elastic force, f, and entropy, S, are related through the Maxwell equation:

$$-\left(\frac{\partial S}{\partial L}\right)_{T.V} = \left(\frac{\partial f}{\partial T}\right)_{L.V} \tag{9}$$

Stress-strain analysis of the energetic and entropic contributions to the elastic force indicated that entropy accounts for more than 90 % of the stress [3]. For this reason, the rubber elasticity entropic model is a reasonable approximation for hydrogels [58, 71].

2. Hydrogels in wound dressings

Wounds can be defined as defects or breaks in the tissue, most often in the skin, resulting from physical or thermal damage and can be classified as acute or chronic. The acute ones are usually tissue injuries caused by mechanical and chemical stress that heal completely with minimal scaring within 8-12 weeks. Another category of acute wounds includes burns that arise for example from electrical and thermal sources [22, 73]. Compared to acute wounds, chronic ones take longer time to heal. Among chronic wounds diabetic foot ulcers, venous and arterial leg ulcers as well as decubitus ulcers can be mentioned [22].

Skin wounds can also be classified according to the number of skin layers that are affected. Superficial wounds are the damage of the epidermis alone, while partial thickness wounds are the damage of the epidermis and deeper layers containing blood vessels, hair follicles and sweat glands. Full thickness wounds are defined as the damage of fat layer or deeper tissue as well [73].

The healing of wounds results from a number of overlapping stages, including inflammation, migration, proliferation, and maturation [22, 74, 75]. In the first stage a rapid achievement of a sterile environment occurs. This is followed by migration involving transport of growth factors into the exudates and promoting movements of epithelial cells, fibroblasts and keratinocytes to the injured area for damaged tissue reparation. The next stage, proliferation, consists of wound closure and restoration of the epithelial cells. The main function of maturation is to slowly organize the closed wound matrix and increase its strength and elasticity [22, 75]. Based on the types of wounds and models of healings, numerous wound dressing materials including films, foams, hydrocolloids, semi-permeable adhesive films as well as hydrogels, were developed [76].

The appropriate dressing materials must meet a number of requirements [12, 13, 16, 20, 22]. They have to

- be capable of maintaining high humidity at the wound-dressing interface whilst removing, through adsorption, excess wound exudate and associated toxic compounds;
- permit the exchange of gases whilst maintaining an impermeable layer to microorganisms so preventing secondary infections;
- provide thermal insulation; 20
- be biocompatible and not provoke any allergic reaction through their prolonged contact with tissue;

- show minimal adhesion to the surface of the wound so that the dressing can be removed without trauma;
- be physically strong even when wet;
- be produced in a sterile form;
- be easy disposable of at the end of use.

Among many dressings, special attention has been paid to hydrogels due to their unique properties that can fulfil the essential requirements of ideal wound cover material including immediate pain control, easy application, transparency allowing healing follow up, absorbing and preventing loss of body fluids, providing barrier against bacteria, oxygen permeability, controlling of drug dosage, promoting analgesia by cooling the skin and facilitating autolytic debridement [14, 21]. Furthermore, it is also known that hydrogels can promote fibroblast proliferation by reducing the fluid loss from the wound surface and protect the wound external noxae and help in maintaining a micro-climate for biosynthetic reactions on the wound surface necessary for cellular activities [30, 77].

Hydrogels, as basic materials for manufacturing of wound dressings were invented in 1989 by Rosiak et al [76]. Since then, there are a number of commercially available hydrogel wound dressings in a number of physical forms including granules, sheets, fibres as well as flakes [12, 17, 64]. It can be noted that hydrogels are useful for all stages of wound healing with the exception of infected or heavily exuding wounds. The wound healing efficacy of hydrogels can be improved by incorporation of drugs, growth factors and biologically active materials [78-80].

Wound dressing hydrogels can be prepared from either natural (e.g. alginate, hyaluronic acid, chitosan, collagen, fibrin, gelatine and cellulose) or synthetic polymers (poly(l-hydroxyethylene), poly(lactide-co-glycolide), poly(ethylene glycol) and poly(propylene fumarate)) [36, 64]. Natural based wound dressing hydrogels are considered as promising covering options because of their non-toxicity, biocompatibility, biodegradability but also hydrophilicity, and excellent swelling behaviour. While, they have a form of flexible and durable covering material permeable to water vapour and metabolites, they protect the wound against bacterial infection [12, 81]. Among the various natural polymers used for hydrogel preparation, gelatine and alginate are easily available in abundance and, therefore, are comparatively cheap.

In this work, gelatine and alginate have been utilized as main precursors for preparation of wound dressing hydrogels. Hence, a concise, relevant background on these two biopolymers is provided.

2.1 Alginate

Alginate is a water soluble, linear polysaccharide extracted from brown seaweed, composed of altering blocks of β -1,4-linked D-mannuronic acid (M) and α -1,4-linked L-glucuronic acid (G) [43, 82-85]. The important feature of alginates is their ability to form gels by electrostatic interaction between the carboxylic moieties on the G blocks of L-glucuronic acid and divalent cations, such as Ca^{+2}, Mg^{+2}, Ba^{+2} and Sr^{+2} (see Fig. 5) [86]. The composition sequence (M/G ratio), G-block length and molecular weight of polymer are critical factors affecting the physical properties of alginate and its resultant hydrogels [45, 87]. For example, mechanical properties of alginate hydrogels can be improved by increasing the length of G-block and molecular weight of polymer.

The alginate hydrogels are well known as biocompatible, degradable and non-toxic materials widely applied as carriers for drug delivery [88], hemostatic wound dressings [89, 90] and immuno-isolation systems for transplantation [46, 91]. The use of alginate-based hydrogels as wound dressings can be attributed to their ability to form strong, hydrophilic gels upon contact with moisture [22, 92, 93]. A number of reports have also suggested that certain alginate dressings can enhance wound healing by stimulating monocytes to produce elevated levels of cytokines, such as interleukin–6 and tumor necrosis factor–α [32, 94, 95]. Production of these cytokines at wound sites results in pro-inflammatory factors that are advantageous to wound healing [87]. Furthermore, it was observed that calcium ions released from alginate hydrogels crosslinked with $CaCl_2$ play a physiological role aiding the haemostasis during the first stage of wound healing [22]. Alginate wound dressing can be used on different types of wounds with medium to high amount of exudates such as leg ulcers, burns, pressure ulcers and surgical wounds [95].

2.2 Gelatine

Another natural polymer that can be applied for hydrogels preparation is gelatine. It can be defined as a water soluble, biodegradable polypeptide obtained either by acidic (type A gelatine) or alkaline hydrolysis (type B gelatine) of collagen derived from natural sources such as skins, bones, and connective tissues of animals [17, 36]. Type B has more carboxyl groups and a lower isoelectric point (IEP 4.8 ÷ 5.0) than type A (IEP 7.0 ÷ 9.0). For practical applications, both gelatine types can be combined in order to optimize desired characteristics of the final product, with type A imparting firmness and type B providing plasticity [12, 32, 96].

Gelatine offers a uniquely broad range of properties for a wide variety of applications in the medical and health care industry such as artificial organs and temporary scaffolds for damaged tissues [77]. Properties such as biodegradability, biocompatibility, proangiogenic and non-immunogenic characteristics, low level of cytotoxicity and haemostatic effect make gelatine also a suitable wound dressing material [37, 97]. In addition, gelatine based hydrogel has the potential to mimic the extracellular matrix, which may promote the tissue regeneration necessary for healing [32]. However, due to the high water content, mechanical properties of hydrogels based on pure gelatine often fail to fulfil the requirements for wound dressing, especially in terms of its mechanical properties [98, 99].

In order to overcome this problem, a number of approaches including crosslinking techniques have been employed in order to improve mechanical integrity of gelatine hydrogels [100]. The amino acid composition of gelatine provides options for multifunctional crosslinking in side chains via amino, carboxyl and hydroxyl groups that react with a wide variety of established crosslinkers such as carbodiimides, glutaraldehyde or genipin [36]. However, it should be kept in mind that chemical crosslinking involves additional difficulties connected to the removal of unreacted crosslinker that is usually toxic. Further methods such as photocrosslinking can also be applied after functionalization of gelatine chain with methacrylate or phenolic groups, although, the introduction of strongly physically interacting functional groups at the side chain might lead to the formation of a reversibly physically crosslinked network [101]. The mechanical properties of the crosslinked hydrogels significantly depend on gelatine content, Bloom index and the crosslinking density [36, 102].

A wide range of potential biomedical applications of gelatine have been documented in the literature. Crosslinked gelatine hydrogel has been investigated as a peripheral nerve guide conduit material [103], bone substitute [104], protein releasing matrix [26, 105, 106] as well as wound dressing [32, 98].

2.3 Combination of sodium alginate and gelatine

Single polymer hydrogels can seldom fully satisfy the requirements of efficient wound dressing materials due to their weak structure formed by limited interchain interactions. However, hydrogels formed by combinations of polyelectrolyte biomolecules with opposite charges offer distinct advantages arising from the strength of the multiple intermolecular associations involved [36, 107].

According to literature review, the most promising biopolymer pairs for hydrogel formation are protein-polysaccharide combinations. The presence of different polysaccharides in such hydrogels may increase viscosity, promote or inhibit gelation and enhance gel strength, while the presence of protein may be used for introduction of degradability, temperature induced phase transition and sensitivity to the presence of biologically active molecules [107]. From this point of view, a composite hydrogel matrix derived from sodium alginate (SA) and gelatine (G) could have the synergic beneficial aspects of both the polymers. For instance, the composite hydrogel can introduce the haemostatic effect of gelatine and the wound healing promoting ability of alginate [94]. Furthermore, Sakai et al. [122] observed that low cell-adhesiveness and poor support of cell proliferation of alginates can be enhanced by combination it with gelatine. Practical examples of SA/G products for biomedical application are presented in Table 1.

Physical crosslinking induced by electrostatic interaction between sodium alginate and gelatine is presented in Figure 13. The interaction occurs between the negatively charged carboxyl groups on the alginate and positively charged amino groups of arginine, lysine or histidine in gelatine [36].

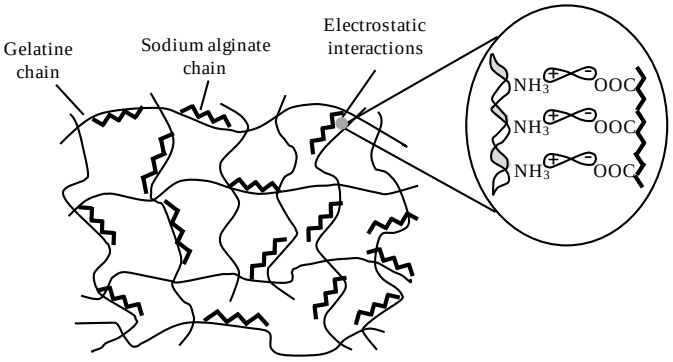


Fig. 13. Hydrogel formed by electrostatic interaction between sodium alginate and gelatine

Nevertheless, hydrogels on the basis of these biopolymers prepared by physical crosslinking induced by secondary physical forces including chain entanglement, ionic interaction, and van der Waals forces, do not exhibit sufficient mechanical strength after absorption of the wound exudates. They dissolve in the wound secretion at body temperature and thus cannot be completely removed from the wound [123]. To suppress this dissolution, several modifications, such as chemical crosslinking of either alginate or gelatine, have been investigated [122].

Several works have been performed to study formulation, characterization as well as application of crosslinked materials based on a combination of sodium alginate and gelatine [32, 95, 97, 99, 108, 109, 117-119, 124]. Z. Dong et al. [99] reported an approach using Ca^{+2} as crosslinking agent to prepare drug (ciprofloxacin hydrochloride) loaded film from alginate and gelatine by solvent casting method. It has been successfully applied for localized drug delivery in vivo or in vitro with controllable release rate. C. Xiao et al. [117] studied blend films from sodium alginate and gelatine and examined their characteristics stating that the strong intermolecular bonds and ionic interactions in the blend films resulted in the enhancement of their mechanical properties and thermal stability. B. Balakrishnan et al. [95] used borax to crosslink oxidized alginate and gelatine to develop in situ forming hydrogel wound dressings and obtained material with significantly improved fluid uptake and good tuneable degradation properties. Oxidized alginate/gelatine hydrogel was mainly investigated for wound dressing [32, 95, 122], cardiac tissue [125], cartilage [126] and bone tissue engineering [109]. Accordingly, sodium alginate/gelatine composite materials (e.g. films, hydrogels) demonstrated improved performance and favourable mechanical and swelling properties compared with single polymer materials.

3. Summary

The growth of comprehensive medical and pharmaceutical wound care has led to considerable attention directed towards the development of wound dressing materials. The ideal dressing material needs to ensure that the wound remains free of infection and moist with exudates but not macerated, while also fulfilling prerequisites concerning structure and biocompatibility. Among these materials, hydrogels based on polymeric and biopolymeric materials are considered as promising options because of their insoluble, swellable and hydrophilic properties. The survey given above shows that sodium alginate and gelatine can be ideal candidates for preparation of medical hydrogels, suitable for production of highly hydrophilic and biocompatible wound dressings.

Table. 1. Alginate/ Gelatine and their combinations with other natural or synthetic polymers for biomedical applications

Combinations	Form	Application	Method	Note	References
Oxidized alginate/ Gelatine	Hydrogel	Wound dressing; tissue engineering and drug delivery	Gelatine crosslinking with alginate dialdehyde in the presence of borax	Highly optimal for maintaining a moist environment conducive for wound healing good water absorptivity; optimal water vapour transmission rate; mild antiseptic properties; nontoxic; biodegradable; biocompatible	[32, 95, 108]
Marine gelatine/ Oxidized alginate	Hydrogel	Wound healing		Capable of modulating cellular behaviour through different cytokines, chemokines and growth factors production patterns	[97]
Oxidized alginate/ Gelatine/ tricalcium phosphate	Hydrogel	Bone tissue engineering; drug delivery; other biomedical applications		Controlled degradability; biocompatible; non-toxic	[109]
Alginate/Poly(γ-glutamic acid)	Hydrogel	Wound dressing; drug delivery	Alginate crosslinking with calcium poly(γ-glutamate)/ solvent casting	Ability to accelerate hemostasis; good fluid retention; non-cytotoxicity; transparent; high sensitive to pH	[110]
Poly(vinyl alcohol)/ Sodium alginate	Hydrogel	Wound dressing	Freeze–thawing method	Swellable, flexible and elastic compared to that with only PVA	[111]
Poly(vinyl alcohol)/ Gelatine	Hydrogel	Wound dressing and drug delivery	Esterification of poly(vinyl alcohol) with gelatine	Hemocompatible; moisture retentive sufficient strength; super absorbent	[112, 113]
Chitosan/ Gelatine	Hydrogel	Drug delivery; sell encapsulation; tissue engineering	Neutralizing chitosan/gelatine solutions with a small amount of $NaHCO_3$ to retain the formulation in aqueous solution at physiologi-	Biocompatible, biodegradable, and adhesive to human tissue	[114]

			cal pH.		
Oxidized hyaluronan/ Gelatine	Hydrogel	Tissue engineering	Gelatine crosslinking with oxidized hyaluronan	Excellent biocompatible and biodegradable	[115]
Chitosan-Gelatine	Film	Wound healing	Solvent casting	Improved wound healing property than chitosan film alone	[116]
Alginate/ Gelatine	Film	Controlled drug delivery	Ca^{+2} crosslinking/ solvent casting	Good compatibility between alginate and gelatine as well as between the matrix and the drug; sensitive to pH and ionic strength	[99]
Alginate/ Gelatine	Film	Biomedical applications	Solvent casting	Improved mechanical properties and thermal stability	[117]
Alginate/ Gelatine	Fibers	Wound dressing	Spinning	Good miscibility between alginate and gelatine; improved water-retention properties compared to pure alginate fiber	[118]
Alginate/ Gelatine; gelatine/hyaluronate; chitosan/hyaluronate	Sponge	Wound dressing	Freeze-drying/ crosslinking with 1-ethyl-(3-3-imethylaminopropyl) carbodiimide hydrochloride	Increased water uptake; excellent wound healing	[119]
Chitosan/ Gelatine	Sponge	Wound dressing	Freezing	Safe reliability; good antibacterial property; excellent wound healing	[120]
Alginate/ Chitosan	Beads	Drug release	Extrusion method	Potentially useful for controlling the drug release characteristics	[121]

AIMS OF THE DOCTORAL STUDY

The main objectives of this doctoral study are formulation, preparation and characterization of biopolymer based hydrogels aimed at the production of highly hydrophilic and biocompatible wound dressings. The following goals are pursued within the framework of this thesis:

- Development of sodium alginate/gelatine (SA/G) hydrogels, their characterization and critical evaluation of physico-chemical properties influencing their application in wound dressing;
- Selection of suitable crosslinking agent for SA/G hydrogels via testing of various crosslinkers including calcium chloride ($CaCl_2$), glutaraldehyde or genipin;
- Examination of morphology and verification of miscibility and blend homogeneity between SA and G with the help of scanning electron microscopy;
- Investigation of the chemical composition of the hydrogels by means of Fourier transform infrared spectroscopy;
- Testing of swelling behaviour as a function of various factors including composition of the hydrogels, swelling time and environment;
- Evaluation of viscoelastic properties as a function of hydrogel composition in terms of SA/G ratio as well as swelling time, performed by measurement of an oscillatory shear rheological analysis and dynamic mechanical analysis;
- Finding the optimal composition of SA/G hydrogels with the viscoelastic response fully comparable with that of human skin, showing simultaneously very good absorption properties.

SUMMARY OF THE PAPERS

The key findings of the doctoral thesis are highlighted as summaries of each of the three papers and patent.

Paper I was focused on the preparation and characterization of physically crosslinked SA/G hydrogels with various concentrations of sodium alginate and gelatine to obtain highly hydrophilic and biocompatible wound dressing material.

The effects of SA/ G ratio (30/70 to 70/30) on the morphology and chemical structure were examined via SEM and ATIR-FTIR, respectively. Swelling properties were determined gravimetrically and viscoelastic properties of prepared hydrogels were monitored by using an oscillatory shear rheological analysis.

It was found that the samples with SA as the matrix had a droplet-like morphology with the droplets formed of minor G phase, while samples with G as the matrix rendered a fibrous morphology of imbedded SA phase. As to hydrogel chemical structure, significant changes in the shape and intensity of FTIR absorption frequencies of either SA or G were observed and attributed to the presence of intermolecular interactions including hydrogen-bonding and electrostatic attractions between SA and G chains/molecules.

According to swelling test using water as a swelling medium, the absorption ability of the hydrogels was promoted with the increase of the G content, due to the enhanced hydrophilicity, which is more favorable for the diffusion of water molecules into the hydrogels. As to water uptake, the maximum water content expressed as the equilibrium swelling degree was significantly dependent on SA/G ration laying in the range of 270 to 1100 %. This indicated that SA/G hydrogels can easily prevent a wound from accumulation of fluid by absorbing the exudates.

The viscoelastic properties of SA/G hydrogels notably depended on their composition and were related to the swelling behaviour of hydrogels. The increase in viscoelastic module was observed when the content of SA rose, probably due to the increased number of SA chains that produce a dense network.SA/G ratio in terms of a compromise between appropriate viscoelastic properties and absorption abilities was found to be SA/G 50/50 showing the viscoelastic response fully comparable to that of human skin while simultaneously retaining very good absorption properties.

Based on the results it was however concluded, that SA/G hydrogels prepared by physical crosslinking induced only by secondary physical forces have a loose network structure and insufficient mechanical strength after the wound exudates absorption.

Paper II was concentrated on the chemical crosslinking of SA/G hydrogels with two well-known crosslinking agents, calcium chloride (Ca^{2+}) and glutaraldehyde (GTA).The main attention was paid to finding an optimal balance between the mechanical strength and water uptake of crosslinked SA/G hydrogels, with a different ratio between SA and G, by the optimization of their composition and structure. The physicochemical properties of the resultant hydrogels were systematically investigated through SEM, FTIR, DMA and a swelling test. The hydrogel composition of SA/G was widened and ranged from 20/80 to 80/20. Testing of crosslinking agent performance resulted in finding of their optimum concentration of 0.2 % for GTA and 2.5 % for Ca^{2+}) and optimum crosslinking time (60 min for GTA and 5 min for Ca^{2+})

Most of the Ca^{2+} crosslinked SA/G hydrogels revealed an interconnected morphology with small aggregate structures whereas GTA crosslinked ones indicated different morphologies depending on G content. In particular, the morphologies underwent a change from compact to an island like structure with increasing G content in the hydrogels. The SA/G-50/50 hydrogel samples crosslinked with either Ca^{2+} or GTA showed a smooth and homogeneous morphology, suggesting the good component miscibility and blend homogeneity.

FTIR spectra of the Ca^{2+} crosslinked SA/G hydrogels revealed a small shift in symmetric stretching carboxyl groups, indicating an ionic binding between the Ca^{2+} ions and the SA. Increasing the G content in hydrogels crosslinked with GTA significantly changed the shapes of the amide I and II bands in the FTIR spectra, thus confirming the G – GTA crosslink formation.

Swelling test showed that the equilibrium swelling was slightly higher for the Ca^{2+} crosslinked hydrogels compared to the GTA crosslinked ones. Furthermore, the equilibrium swelling in the Ca^{2+} crosslinked hydrogels noticeably decreased with an increasing SA content; whereas in the case of GTA crosslinked hydrogels, equilibrium swelling declined as a G content rose.

DMA results revealed the enhanced viscoelastic properties and improved thermal stability of the crosslinked hydrogels when compared with non-crosslinked materials.

Among the tested hydrogels, the sample with composition of SA/G 50/50 showed high miscibility, homogeneity, and provided a good balance of swelling and viscoelastic properties. However, due to the known toxicity of GTA, the Ca^{2+} crosslinked hydrogels are more appropriate for biomedical applications.

Paper III was aimed on the development of naturally derived SA/G hydrogels crosslinked with genipin (GP). The main aim of the GP application was to overcome the cytotoxic effects of the GTA crosslinking.

According to FTIR analyses, spectra of SA/G hydrogels crosslinked with GP showed minor variations with respect to those of uncrosslinked samples, with

only moderately increase of the relative intensity of the amide I and II bands. These findings were attributed to the formation of heterocyclic compound of GP linked to the G and also the formation of the secondary amide group as a result of the reaction between G and GP.

The degree of swelling was found to be higher than 200 % in all samples, confirming that the SA/G-GP hydrogels can be considered as an efficient wound dressing with a high absorption capacity. It was also found that with increasing GP concentration, the swelling degree markedly reduced and the thermal stability enhanced under physiological conditions (PBS buffer, pH 7.4 at 37°C). DMA analysis revealed that the SA/G hydrogels with GP, could form relatively strong elastic gels which showed satisfactory viscoelastic properties. Since GP shows low toxicity, crosslinked hydrogels could be a promising candidate for biomedical applications.

CZ patent 302380 and **WO 2011/100935 A1 international patent application** were directed towards improvement of mechanical properties of physically crosslinked hydrogels described in Paper I. The invention covers the hydrogel composition containing G and SA in weight ratios of 30/70to 70/30, together with polyethylene glycol, glycerine, nanofibres of polyvinyl alcohol in the amount of 10 – 40 % (w/w), sodium chloride and sea buckthorn oil. Among the individual hydrogel components, G helps regeneration of damaged tissues, SA absorbs fluids and acts as antimicrobial agent, polyethylenglykol acts as a substitute of damaged skin and glycerine as a humectant. Nanofibres of polyvinyl alcohol create a fibre matrix and improve mechanical strength of the product. Presence of sea buckthorn oil promotes a healing process and also helps to diminish subsequent scars of healed wounds.

A part of the invention describes also method for hydrogel preparation. The procedure starts with preparation of initial aqueous polymeric solution of G at 75 – 85°C followed by addition of further components such as SA, polyethylene glycol, glycerine and respectively sea buckthorn oil under stirring. Then polyvinyl alcohol nanofibres are added and finally, the viscous mass is poured into acrylic dishes where it is kept at room temperature of 20 – 25°C until the whole volume of water is removed and the final dry material of hydrogel is achieved.

Hydrogel is delivered in a dry form which, according to the invention, enables its' long term storage ability. This is advantageous mainly due to absence of loss of water during storage and, moreover, in the dry substance the germ proliferation is extremely reduced. One more important advantage of "dry" hydrogels is their high absorption capacity during the healing process.

CLOSING REMARKS

The presented doctoral thesis is focused on the preparation and characterization of hydrogels suitable for wound dressings. As a material, combination of sodium alginate and gelatine blended in different ratios was used. Hydrogels were crosslinked either through weak physical forces or using chemical crosslinking agents. Based on the experiments performed, the following conclusions can be drawn:

- Physically crosslinked SA/G hydrogels were successfully prepared and characterized. Within the tested composition, hydrogels showed excellent swelling properties in water at room temperature. However, the weak physical crosslinking was the reason for insufficient strength of the formed gel and the hydrogels gradually disintegrated under swelling at 37 °C (physiological temperature) which, led to deterioration of their mechanical properties.

- The above given drawback related to rapid hydrogel disintegration was reduced by incorporation of polyvinylalcohol nanofibers in the SA/G matrix at the amount of 10 to 40 wt. %. The nanofiber role is primary seen in the formation of a supporting matrix for otherwise soft and gell-like polymers resulting in improvement of mechanical strength of the swelled hydrogels and can be considered as one possible option for improvement of the formulation.

- Hydrogels with improved properties were formulated by chemical crosslinking of SA/G hydrogels performed either withCa^{+2}, which selectively crosslinks SA chains or glutaraldehyde applicable for crosslinking of gelatine (Paper II). Physicochemical characterization proved differences between the crosslinked and uncrosslinked SA/G hydrogels which depended on the concentration and type of crosslinking agents, crosslinking time and the composition of the hydrogels. The resulting hydrogels showed significantly improved viscoelastic properties and thermal stability. Swelling was tested in physiologically relevant conditions, in phosphate buffered saline with pH 7.4 at 37 °C and revealed the equilibrium swelling of the Ca^{2+} crosslinked hydrogels slightly higher compared to GTA crosslinked hydrogels of the corresponding composition.

- Hydrogels composed of SA/G crosslinked with genipin provided fully naturally based and biocompatible product. It was also demonstrated that the choice of the crosslinking agent in the preparation of the hydrogels plays a very important role in providing and maintaining favourable swelling, mechanical properties and good biocompatibility.

- Characterization of physically and chemically crosslinked hydrogels using SEM, swelling tests and rheology evidenced that equal ration of both constituents in matrix can be considered as a preferred hydrogel composition. Samples with SA/G 50/50 provided compromise between satisfactorily mechanical properties of hydrogels and their absorption abilities.SEM analyses, swelling tests and rheological characterization proved that equal ration between the constituents afforded samples with high miscibility, homogeneity, desired viscoelastic properties and absorption abilities.

CONTRIBUTIONS TO THE SCIENCE AND PRACTICE

The major contribution of this doctoral thesis comes from the research findings that extend knowledge on the following items:

- The development of biopolymer based hydrogels for biomedical applications and, finding of suitable crosslinking agents and optimizing their concentration that provide stable and biocompatible product.
- The preparation of smart, advanced materials based on the combination of sodium alginate and gelatine, showing the swelling behaviour dependent on pH of swelling medium.
- The optimization of hydrogel composition and structure promoting and maintaining the adequate moist environment, required elasticity and mechanical strength and comfort usage.
- The finding of promising candidates for medical dressings applicable for wound healing and management.

REFERENCES

1. OMIDIAN, H.; PARK, K. Introduction to Hydrogels, In Biomedical Applications of Hydrogels Handbook; OTTENBRITE, R.; PARK, K.; OKANO, T., Eds.; Springer: New York, 2010; p. 1-17.
2. ILIA, G. Phosphorus containing hydrogels. Polym. Adv. Technol. 2009, vol. 20, p. 707–722.
3. ZAVAN, B.; CORTIVO, R.; ABATANGELO, G. Hydrogels and Tissue Engineering, In Hydrogels, Biological properties and Applications; BARBUCCI, R., Ed.; Springer-Verlag: Milan, 2009; p.1-20
4. GANJI, F.; FARAHANI, E. V. Hydrogels in Controlled Drug Delivery Systems. Iran. Polym. J. 2009, vol. 18, p. 63-88.
5. COVIELLO, T.; MATRICARDI, P.; MARIANECCI, C.; ALHAIQUE, F. Polysaccharide hydrogels for modified release formulations. J. Control. Release, 2007, vol. 119, p. 5-24.
6. HOFFMAN, A. S. Hydrogels for biomedical applications. Adv. Drug. Deliver. Rev. 2002, vol. 43, p. 3–12
7. BAROLI, B. Hydrogels for Tissue Engineering and Delivery of Tissue-Inducing Substances. J. Pharm. Sci. 2007, vol. 96, p. 2197-2223.
8. JIN, R.; DIJKSTRA, P. J. Hydrogels for Tissue Engineering Applications, In Biomedical Applications of Hydrogels Handbook; Ottenbrite, R.; Park, K.; Okano, T., Eds.; Springer: New York, 2010; p. 203-227
9. Wilson, A. M.; Justin, G.; Guiseppi-Elie, A. Electroconductive Hydrogels, In Biomedical Applications of Hydrogels Handbook; OTTENBRITE, R.; PARK, K.; OKANO, T., Eds.; Springer: New York, 2010; p. 319-339.
10. PEPPAS, N. A. Hydrogels, In Biomaterials science: an introduction to materials in medicine; RATNER, B. D.; HOFFMAN, A. S.; SCHOEN, F. J.; LEMONS, J. E., Eds.; Academic Press: California, 1996; p. 60-64.
11. LIN, C. C.; METTERS, A. T. Hydrogels in controlled release formulations: Network design and mathematical modelling. Adv. Drug. Deliver. Rev. 2006, vol. 58, p. 1379–1408.
12. JONES, A.; VAUGHAN, D. Hydrogel dressing in the management of a variety of wound types: a review. J. Orthop. Nurs. 2005, vol. 9, p. 51-60.
13. PURNA, S. K.; MARY, B. Collagen based dressings - a review. Burns. 2000, vol. 26, p. 54-62.
14. KORTING, H. C.; SCHULLMANN, C.; WHITE, R. J. Management of minor acute cutaneous wounds: importance of wound healing in a moist environment. J. Eur. Acad. Dermatol. Venereol. 2010, vol. 25, p. 130-137.
15. LLOYD, L. L.; KENNEDY, J. F.; METHACANON, P.; PATERSON, M.; KNILL, C. J. Carbohydrate polymers as wound management aids. J.Carbohydr. Polym. 1998, vol. 37, p. 315-322.

16. PAL, K.; BANTHIA, A. K.; MAJUMDAR, D. K. J. Preparation of Transparent Starch Based Hydrogel Membrane with Potential Application as Wound Dressing. Trends Biomater. Artif. Organs. 2006, vol. 20, p. 59-67.
17. ZAMAN, H. U.; ISLAM, J. M. M.; KHAN, M. A.; KHAN, R. A. Physico-mechanical properties of wound dressing material and its biomedical application. J. Mech. Behav. Biomed. Mater. 2011, vol. 4, p. 1369-1375.
18. YOSHII, F.; ZHANSHAN, Y.; ISOBE, K.; SHIOZAKI, K.; MAKUNCHI, K. Electron beam crosslinked PEO and PEO/PVA hydrogelsfor wound dressing. Radiat. Phys. Chem. 1999, vol. 55, p. 133–138.
19. WANG, M.; XU, L.; HU, H.; ZHAI, M.; PENG, J.; NHO, Y et al. Radiation synthesis of PVP/CMC hydrogels as wound dressing. J. Nucl. Instr. Meth. Phys. Res. B. 2007, vol. 265, p. 385–389.
20. THOMAS, S. Wound dressing, In The epidermis in wound healing; ROVEE, D. T.; MAIBACH, H. I., Eds.; CRC Press: Washington, DC, 2004; p. 215-245.
21. HAMID, Z. A. A.; BLENCOWE, A.; OZCELIK, B.; PALMER, J. A.; STEVENS, G. W. et al. Epoxy-amine synthesised hydrogel scaffolds for soft-tissue engineering. Biomaterials. 2010, vol. 31, p. 6454-6467.
22. BOATENG, J. S.; MATTHEWS, K. H.; STEVENS, H. N. E.; ECCLESTON, G. M. Wound Healing Dressings and Drug Delivery Systems: A Review. J. Pharm. Sci. 2008, vol. 97, p. 2892-2923.
23. CZECH, Z.; ZEEGER, K. Crosslinked hydrogels and their use as wound dressing. U. S. Patent, 5135755, 1992.
24. TEBMAR, J.; BRANDL, F.; GOPFERICH, A. Hydrogels for tissue engineering, In Fundamentals of tissue engineering and regenerative medicine; MEYER, U.; MEYER, Th.; HANDSCHEL, J.; WIESMANN, H. P., Eds.; Springer-Verlag: Berlin, 2009; Chapter 7, p. 495-517.
25. KIKUCHI, A.; OKANO, T. Hydrogels: In Polymeric Drug Delivery Systems; KWON, G. K., Ed.; Taylor & Francis: New York, 2005; p. 129-195.
26. JILIE, K.; LI, M. Smart hydrogels, In Smart polymers: applications in biotechnology and biomedicine; GALAEV, I.; MATTIASSON, B., Eds.; CRC Press: Boca Raton, 2008; p. 247-269.
27. ZHANG, X.; WU, D.; CHU, C. C. Synthesis and characterization of partially biodegradable, temperature and pH sensitive Dex–MA/PNIPAAm hydrogels. Biomaterials. 2004, vol. 25, p. 4719–4730.
28. PEPPAS, N. A. Hydrogels, In Biomedical engineering desk reference; RATHER, B. D.; BANKMAN, I., Eds.; Elsevier Inc: Oxford, 2009; p. 188-195.
29. HENNINK, W. E.; NOSTRUM, C. F. V. Novel crosslinking methods to design hydrogels. Adv. Drug. Deliver. Rev. 2002, vol. 54, p. 13–36.
30. PAL, K.; BANTHIA, A. K.; MAJUMDAR, D. K. Polymer hydrogels: Characterization and Biomedical Applications –A mini review. Des. Monomers Polym. 2009, vol. 12, p. 197-220.

31. GULREZ, S. K. H.; AL-ASSAF, S.; PHILLIPS, G. O. Hydrogels: Methods of Preparation, Characterisation and Applications, In Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications; CARPI, A., Ed.; In Tech: Rijeka, 2011; p. 117-150.
32. BALAKRISHNAN, B.; MOHANTY, M.; UMACHANKAR, P. R.; JAYAKRISHNAN, A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatine. Biomaterials. 2005, vol. 26, p. 6335–6342.
33. TORTORA, M.; CAVALIERI, F.; CHIESSI, E.; PARADOSSI, G. Michael-Type Addition Reactions for the In Situ Formation of Poly(vinyl alcohol)-Based Hydrogels. Biomacromolecules. 2007, vol. 8, p. 209-214.
34. JUNG, J. P.; JONES, J. L.; CRONIER, C. A.; COLLIER, J. H. Modulating the mechanical properties of self-assembled peptide hydrogels via native chemical ligation. Biomaterials. 2008, vol. 29, p. 2143-2151.
35. KOSCHELLA, A.; HARTLIEB, M.; HEINZE, TH. A "click-chemistry" approach to cellulose-based hydrogels. Carbohyd. Polym. 2011, vol. 86, p. 154– 161.
36. FARRIS, S.; SCHAICH, K. M.; LIU, L. S.; PIERGIOVANNI, L.; YAM, K. L. Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: a review. Trends Food Sci. Technol. 2009, vol. 20, p. 316-332.
37. CHEN, T.; EMBREE, H. D.; BROWN, E. M.; TAYLOR, M. M.; PAYNE, G. F. Enzyme-catalyzed gel formation of gelatine and chitosan: potential for in situ applications. Biomaterials. 2003, vol. 24, p. 2831–2841.
38. GARCHA, L.; AGUILAR, M. R.; ROMAN, J. S. Biodegradable Hydrogels for Controlled Drug Release, In Biomedical Applications of Hydrogels Handbook; OTTENBRITE, R.; PARK, K.; OKANO, T., Eds.; Springer: New York, 2010; p. 147-156.
39. TODD, R. H.; DANIEL, S. K. Hydrogels in drug delivery: Progress and challenges. Polym. 2008, vol. 49, p. 1993-2007.
40. FRUTOS, G.; CABANILLAS, A.; PARIS, R.; GARRIDO, I. Q. A novel controlled drug delivery system based on pH-responsive hydrogels included in soft gelatine capsules. ActaBiomaterialia. 2010, vol. 6, p. 4650–4656.
41. GANJI, F.; FARAHANI, S. V.; FARAHANI, E. V. Theoretical description of hydrogels swelling: A review. J. Iran. Polym, 2010, vol. 19, p. 375-398.
42. GRIESHABER, S. E.; JHA, A. K.; FARRAN, A. J. E.; JIA, A. X. Biomaterials technologies for tissue engineering, In Biomaterials for tissue engineering applications; BURDICK, J. A.; MAUCK, R. L (Eds.).; Springer-Verlag: Germany, 2011; Chapter 2, p. 9-47.

43. YOO, I.; SEONG, G. H.; CHANG, H.; PARK, J. K. Encapsulation of lactobacillus casei, cells in liquid-core alginate capsules, for lactic acid production. Enzym. Microb. Tech. 1996, vol. 19, p. 426-433.
44. GOMBOTZ, W. R.; WEE, S. F. Protein release from alginate matrices. Adv. Drug Deliver. Rev.1998, vol. 31, p. 267–285.
45. LEE, K. Y.; MOONEY, D. Alginate: Properties and biomedical applications. J. Prog. Polym. Sci. 2011.
46. MIRSHFIEY, A.; REHM, B. H. A. Alginate and Its Comonomer Mannuronic Acid: Medical Relevance as Drugs, In Alginates: Biology and Applications; REHM, B. H. A., Ed.; Springer: Berlin Heidelberg, 2009; p. 229-260.
47. SCHACHT, E. H. Polymer chemistry and hydrogel systems. J. Phys.: Conf. Ser 3, 2004, p. 22-28.
48. ROY, S.; PAL, K.; ANIS, A.; PRAMANIK, K.; PRABHAKAR, K. Polymers in Mucoadhesive Drug Delivery System: A Brief Note. Design. Monomer. Polym. 2009, vol. 12, p. 483-495.
49. ZHANG, C.; AUNG, A.; LIAO, L.; VARGHESE, S. A novel single precursor-based biodegradable hydrogel with enhanced mechanical properties. Soft Matter. 2009, vol. 5, p. 3831–3834.
50. GIBAS, I.; JANIK, H. Review: Synthetic polymer hydrogels for biomedical applications. Chem. Chem. Technol. 2010, vol. 4, p. 297-304.
51. BORZACCHIELLO, A.; AMBROSIO, L. Structure property relationships in hydrogels, In Hydrogels, Biological properties and Applications; BARBUCCI, R., Ed.; Springer-Verlag: Milan, 2009; p. 9-21.
52. PEPPAS, N. A.; BURES, P.; LEOBANDUNG, W.; ICHIKAWA, H. Hydrogels in pharmaceutical formulations. J. Pharm. Biopharm. 2000, vol. 50, p. 27-46.
53. PEPPAS, N. A.; HILT, J. Z.; KHADEMHOSSEINI, A.; LANGER, R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. Adv. Mater. 2006, vol. 18, p. 1345–1360.
54. CAYKARA, T.; BOZKAYA, U.; KANTOGLU, O. Network Structure and Swelling Behaviour of Poly(acrylamide/crotonic acid) Hydrogels in Aqueous Salt Solutions. J. Polym. Sci: Part B: Polym. Phys. 2003, vol. 41, p. 1656–1664.
55. PEPPAS, N. A.; HUANG, Y.; TORRES-LUGO, M.; WARD, J. H.; ZHANG, J. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annu. Rev. Biomed. Eng. 2000, vol. 2, p. 9–29.
56. DE SMEDT, S. C.; LAUWERS, A.; DEMEESTER, J.; STEENBERGEN, M. J. V.; HENNINK, W. E.; ROEFS, S. P. Characterization of the network structure of dextran glycidyl methacrylate hydrogels by studying the rheological and swelling behaviour. J. Macromolecules, 1995, vol. 28, p. 5082-5088.

57. MARK, J. E.; ERMAN, B. Fundamentals In rubberlike elasticity, Cambridge University Press: Cambridge, 2007: chapter 2, p. 3-93.
58. WEISS, P.; FATIMI, A.; GUICHEUX, J.; VINATIER, C. Hydrogels for Cartilage Tissue Engineering, In Biomedical Applications of Hydrogels Handbook; OTTENBRITE, R.; PARK, K.; OKANO, T., Eds.; Springer: New York, 2010; p. 247-268.
59. WEBER, L. M.; LOPEZ, C. G.; ANSETH, K. S. The effects of PEG hydrogel crosslinking density on protein diffusion and encapsulated islet survival and function. J. Biomed. Mater. Res. A. 2009, vol. 90, p. 720–729.
60. OMIDIAN, H.; HASHEMI, S. A.; ASKARI, F.; NAFISI, S. Swelling and crosslink density measurements for hydrogels. Iran. J. Polym. Sci. Technol. 1994, vol. 3, p. 115-119.
61. SUUBERG, E. M.; OTAKE, Y.; LANGNER, M. J.; LEUNG, K. T.; MILOSAVJEVIC, I. Coal macro molecular network structure analysis: Solvent swelling thermodynamics and its implications. Energ. Fuel. 1994, vol. 8, p. 1247-1262.
62. AYAKARA, T. C.; REK, C. O. Z.; LU, O. M. K.; VEN, O. G. Equilibrium Swelling Behaviour of pH- and Temperature- Sensitive Poly(N-vinyl 2-pyrrolidone-g-citric acid) Polyelectrolyte Hydrogels. J. Polym. Sci: Part B: Polym. Phys. 2000, vol. 38, p. 2063–2071.
63. LOWMAN, A. M.; PEPPAS, N. A. Hydrogels, In Encyclopedia of controlled drug delivery; MATHIOWITZ, E., Wiley: New York, 1999; p. 397-418.
64. SLAUGHTER, B. V.; KHURSHID, S. S.; FISHER, O. Z.; KHADEMHOSSEINI, A.; PEPPAS, N. A. Hydrogels in Regenerative Medicine. Adv. Mater. 2009, vol. 21, p. 3307–3329.
65. ORWOLL, R. A.; ARNOLD, P. A. Polymer–Solvent Interaction Parameter χ, In Physical Properties of Polymers Handbook; MARK, J. E., Ed.; AIP Press: New York, 1996; p. 177-196.
66. BAJWA, R. S.; AL-HALHOULI, M.; DAME, G.; URBAN, G. A. New Impedance Measurements Based Microsystems Setup for Characterizing Biomaterial Hydrogel. Int. J. Environ. Eng. 2011, vol. 2, p. 158-164.
67. PACIOS, I. E.; PIEROLA, I. F. Discrimination of the roles of crosslinking density and morphology in the swelling behaviour of crosslinked polymers: poly(N-vinylimidazole) hydrogels. J. Appl. Polym. Sci. 2009, vol. 112, p. 1579-1586.
68. ANSETH, K. S.; BOWMAN, C. N.; BRANNON-PEPPAS, L. Mechanical properties of hydrogels and their experimental determination. Biomaterials. 1996, vol. 17, 1647-1657.
69. JONES, D. S. Dynamic mechanical analysis of polymeric systems of pharmaceutical and biomedical significance. Int. J. Pharm. 1999, vol. 179, p. 167-178.

70. MEYVIS, T. K. L.; STUBBE, B. G.; VAN STEENBERGEN, V.M. J.; HENNIK, W. E.; DE SMEDT, S. C.; DEMEESTER, J. A comparison between the use of dynamic mechanical analysis and oscillatory shear rheometry for the characterization of hydrogels. J. Pharm. 2002, vol. 244, p. 163-168.
71. MACIOCE, P. Viscoelastic damping 101. J. Mech. Eng. 2008, p. 9-12.
72. SPERLING, L. H. Introduction to physical polymer science; John Wiley & Sons: New Jersey, 2006; Chapter 8, p. 355-370.
73. PERCIVAL, N. J. Classification of wounds and their management, Surgery: Oxford, 2002, vol. 20, p. 114–117.
74. GARLICK, J, A. Human skin equivalent models of epidermal wound healing in the Epidermis in Wound Healing, In The epidermis in wound healing; ROVEE, D. T.; MAIBACH, H. I., Eds.; CRC Press: Washington, DC, 2004; p. 3-22.
75. BRADDOCK, M.; CAMPBELL, C. J.; ZUDER, D. Current therapies for wound healing: electrical stimulation, biological therapeutics, and the potential for gene therapy. Int. J. Dermatol.1999, vol. 38, p. 808–817.
76. KOKABI, M.; SIIROUSAZAR, M.; HASSAN, Z. M. PVA-clay nanocompo-site hydrogels for wound dressing. Polym. J. 2007, vol. 43, p. 773–781.
77. SULEA, D.; GHIZA, M. V.; MICUTZ, M.; ALBU, M.G.; BRAZDARU, L.; STAICU, T. et al. Characterization and in vitro release of chlorhexidine digluconate comprised in type I collagen hydrogels. Rev. Roum. Chi. 2010, vol. 55(9), p. 543-551.
78. SAHA, N.; SAHA, T.; SAARAI, A. Dry hydrogel substances for wound covering, Patent filed at Czech patent office (UPV CR), File number PV 2010-22300.
79. AIELLO, A.; FATTORUSSO, E.; MENNA, M.; VITALONE, R.; SCHRODER, H.; MULLER, W. E. G. Mumijo Traditional Medicine: Fossil Deposits from Antarctica (Chemical Composition and Beneficial Bioactivity). Evid. Based Complement. Alternat. Med. 2008, p. 1-8.
80. ESHGHI, F.; HOSSEINIMEHR, S. J.; RAHMANI, N.; KHANDEMLOO, M.; NOROZI, M. S.; HOJATI, O. Effects of Aloe vera cream on posthemorrhoidectomy pain and wound healing: results of a randomized, blind, placebo-control study. J. Altern. Complement. Med. 2010, vol. 16, p. 647-650.
81. SAARAI, A.; KASPARKOVA, V.; SEDLACEK, T.; SAHA, P. Comparative Study of Crosslinked Sodium Alginate/Gelatine Hydrogels for Wound Dressing. Proceeding of the 4th WSEAS International Conference on Engineering Mechanics, Structures, Engineering Geology, Corfu Island, Greece, July 14-16, 2011; Nikos Mastorakis., Eds.; WSEAS Press: 2011.
82. KUO, C. K.; MA, P. X. Maintaining dimensions and mechanical properties of ionicallycrosslinked alginate hydrogel scaffolds in vitro. J. Biomed. Mater. Res. 2007, vol. 84, p. 899-907.

83. JEJURIKAR, A.; LAWRIE, G.; MARTIN, D.; GRONDAHL, L. A novel strategy for preparing mechanically robust ionically cross-linked alginate hydrogels. Biomed. Mater. 2011, vol. 6, 025010.
84. DONATI, I.; PAOLETTI, S. Material Properties of Alginates, In Alginates: Biology and Applications; REHM, B. H. A., Ed., Springer: Berlin Heidelberg, 2009; p. 1-45.
85. DRAGET, K. I.; TAYLOR, C. Chemical, physical and biological properties of alginates and their biomedical implications. Food Hydrocolloids. 2011, vol. 25, p. 251–256.
86. KUEN, Y. L.; DAVID, J. M. Hydrogels for Tissue Engineering. Chem. Rev. 2001, vol. 7, p. 1969-1879.
87. MURAKAMI, K.; AOKI, H.; NAKAMURA, S.; NAKAMURA, S. I; TAKIKAWA, M.; HANZAWA, M. et al. Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. Biomaterials. 2010, vol. 31, p. 83–90.
88. AYALA, G. G.; MALINCONICO, M.; LAURIENZO, P. Marine derived polysaccharides for biomedical applications: chemical modification approaches. Molecules. 2008, vol. 13, p. 2069-2106.
89. LAURIENZO, P. Marine polysaccharides in pharmaceutical applications: an overview. Mar. Drugs. 2010, vol. 8, p. 2435-2465.
90. QIN, Y. The gel swelling properties of alginate fibers and their applications in wound management. Polym. Adv. Technol. 2008, vol. 19, p. 6–14.
91. JEANIE, L. D.; DAVID, J. M. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003, vol. 24, p. 4337–4351.
92. KONG, H. J.; LEE, K. Y.; MOONEY, D. Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration. J. Polym. J. 2002, vol. 43, p. 6239–6246.
93. GEORGE, M.; ABRAHAM, T. E. Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan — a review. J. Control. Release. 2006, vol. 114, p. 1–14.
94. THOMAS, A.; HARDING, K. G.; MOORE, K. Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-a. Biomaterials. 2000, vol. 21, p. 1797-1802.
95. BALAKRISHNAN, B.; JAYAKRISHNAN, A. Self cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. Biomaterials. 2005, vol. 26, p. 3940–3951.
96. VEIS, A. The molecular characterization of gelatine, In the macromolecular chemistry of gelatine; HORECKER, B; KAPLAN, N. O; SCHERAGA, H. A., Eds., Academic Press: New York, 1964; p. 49-123.
97. ZENG, Q.; CHEN, W. The functional behaviour of a macrophage/fibroblast co-culture model derived from normal and diabetic mice

with a marine gelatine oxidized alginate hydrogel. Biomaterials. 2010, vol. 31, p. 5772-5781.
98. GREEN, R. A.; BAEK, S.; POOLE-WARREN, L. A.; MARTENS, P. J. Conducting polymer-hydrogels for medical electrode applications. Sci. Technol. Adv. Mater. 2010, vol. 11, 014107.
99. DONG, Z.; WANG, Q.; DU, Y. Alginate/gelatine blend films and their properties for drug controlled release. J. Membrane Sci. 2006, vol. 280, p. 37–44.
100. YOUNG, S.; WONG, M.; TABATA, Y.; MIKOS, A. G. Gelatine as a delivery vehicle for the controlled release of bioactive molecules. J. Control. Release. 2005, vol. 109, p. 256– 274.
101. YAO, C. H.; LIU, B. S.; CHANG, C. J.; HSU, S. H.; CHEN, Y. S. Preparation of networks of gelatine and genipin as degradable biomaterials. Mater. Chem. Phys. 2004, vol. 83, p. 204–208.
102. SCHRIEBER, R.; GAREIS, H. From collagen to gelatine, In Gelatine Handbook; Wiley: New York, 2007, p. 45-117.
103. CHEN, Y. S.; CHANG, J. Y.; CHENG, C.Y.; TSAI, F. J.; YAO, C. H.; LIU, B. S. An in vivo evaluation of a biodegradable genipin-cross-linked gelatine peripheral nerve guide conduit material. Biomaterials. 2005, vol. 26, p. 3911–3918.
104. CHEN, K. Y.; SHYU, P. C.; CHEN, Y. S.; YAO, C. H. Novel Bone Substitute Composed of Oligomeric Proanthocyanidins-Crosslinked Gelatine and Tricalcium Phosphate. Macromol. Biosci. 2008, vol. 8, p. 942–950.
105. TABATA, Y.; YAMAMOTO, M.; IKADA, Y. Biodegradable hydrogels for bone regeneration through growth factor release. Pure Appl. Chem. 1998, vol. 70, p. 1277-1282.
106. TABATA, Y.; IKADA, Y. Protein release from gelatine matrices. Adv. Drug Deliver. Rev.1998, vol. 31, p. 287–301.
107. YIN, W.; SU, R.; QI, W.; HE, Z. A casein-polysaccharide hybrid hydrogel cross-linked by transglutaminase for drug delivery. J. Mater. Sci. 2011, p. 1-11.
108. BALAKRISHNAN, B.; MOHANTY, M.; FERNANDEZ, A. C.; MOHANAN, P. V.; JAYAKRISHNAN, A. Evaluation of the effect of incorporation of dibutyryl cyclic adenosine monophosphate in an in situ-forming hydrogel wound dressing based on oxidized alginate and gelatine. Biomaterials, 2006, vol. 27, p. 1355-1361.
109. CAI, K.; ZHANG, J.; DENG, L.; YANG, Y.; HU, Y.; CHEN, C. et al. Physical and Biological Properties of a Novel Hydrogel Composite Based on Oxidized Alginate, Gelatine and Tricalcium Phosphate for Bone Tissue Engineering. Biomaterials, 2007, vol. 9, p. 1082-1088.
110. HUANG, M. H.; YANG, M. C. Swelling and biocompatibility of sodium alginate/poly(g-glutamic acid) hydrogels. Polym. Adv. Technol. 2010, vol. 21, p. 561–567.

111. KIM, J. O.; PARK, J. K.; KIM, J. H.; JIN, S. G.; YONG, C. S.; LI, D. X. et al. Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. Int. J. Pharm. 2008, vol. 359, p. 79–86.
112. PAL, K.; BANTHIA, A. K.; MAJUMDAR, D. K. Biomedical evaluation of polyvinyl alcohol–gelatine esterified hydrogel for wound dressing. J. Mater Sci: Mater. Med. 2007, vol. 18, p. 1889–1894.
113. PAWDE, S. M.; DESHMUKH, K. Characterization of Polyvinyl Alcohol/Gelatine Blend Hydrogel Films for Biomedical Applications, J. Appl. Polym. Sci. 2008, vol. 109, p. 3431–3437.
114. CHANG, Y.; XIAO, L.; TANG, Q. Preparation and characterization of a novel thermosensitive hydrogel based on chitosan and gelatine blends. J. Appl. Polym. Sci. 2009, vol. 113, p. 400–407.
115. WENG, L.; PAN, H.; CHEN, W. Self-crosslinkable hydrogels composed of partially oxidized hyaluronan and gelatine: In vitro and in vivo responses, J. Biomed. Mater. Res. Part A, 2007, vol. 85A, p. 352-365.
116. HIMA BINDU, T. V. L.; VIDYAVATHI, M.; KAVITHA, K.; SASTRY, T. P.; SURESH KUMAR, R.V. Preparation and Evaluation of Chitosan-Gelatine Composite Films for Wound Healing Activity. Trends Biomater. Artif. Organs, 2010, vol. 24, p. 123-130.
117. XIAO, C.; LIU, H.; ZHANG, L. Blend films from sodium alginate and gelatine solutions. J. Macromol. Sci.-Pure Appl. Chem. 2001, vol. 38, p. 317-328.
118. FAN, L.; DU, Y.; HUANG, R.; WANG, Q.; WANG, X.; ZHANG, L. Preparation and Characterization of Alginate/Gelatine Blend Fibers. J. Appl. Polym. Sci. 2005, vol. 96, p. 1625–1629.
119. CHOI, Y. S.; LEE, S. B.; HONG, S. R.; LEE, Y. M.; SONG, K, W.; PARK, M. H. Studies on gelatine-based sponges. Part III: A comparative study of cross-linked gelatine/alginate, gelatine/hyaluronate and chitosan/hyaluronate sponges and their application as a wound dressing in full-thickness skin defect of rat. J. Mater. Sci: Mater. Med. 2001, vol. 12, p. 67-73.
120. DENG, C. M.; HE, L. Z.; ZHAO, M.; YANG, D.; LIU, Y. Biological properties of the chitosan-gelatine sponge wound dressing. Carbohydr. Polymer. 2007, vol. 69, p. 583–589.
121. WONG, T. W.; CHAN, L. W.; KHO, S. B.; HENG, P. W. S. Design of controlled-release solid dosage forms of alginate and chitosan using microwave. J. Control. Release, 2002, vol. 84, p. 99–114.
122. SAKAI, S.; YAMAGUCHI, S.; TAKEI, T.; KAWAKAMI, K. Oxidized Alginate-Cross-Linked Alginate/Gelatine Hydrogel Fibers for Fabricating Tubular Constructs with Layered Smooth Muscle Cells and Endothelial Cells in Collagen Gels. Biomacromolecules. 2008, vol. 9, p. 2036–2041. 35

123. DAVIS, P. J. et al. Wound dressings comprising hydrated hydrogels and enzymes U. S. Patent 0183701, July 22, 2010.
124. ROY, A.; BAJPAI, J.; BAJPAI, A. K. Development of calcium alginate-gelatine based microspheres for controlled release of endosulfan as a model pesticide. Indian J. Chem. Technol. 2009, vol. 16, p. 388-395.
125. SHIMIZU, T.; YAMATO, M.; KIKUCHI, A.; OKANO, T. Cell sheet engineering for myocardial tissue reconstruction. Heart Fail. Rev. 2003, vol. 24, p. 2309-2317.
126. LEDDY, H. A.; AWAD, H. A.; GUILAK, F. Molecular Diffusion in Tissue-Engineered Cartilage Constructs: Effects of Scaffold Material, Time, and Culture Conditions. J. Biomed. Mater. Res.: B Appl. Biomater. 2004, vol. 70, p. 397-406.

LIST OF PUBLICATIONS

Manuscripts overview:

1. SAHA, N.; SAARAI, A.; ROY, N.; KITANO, T.; SAHA, P.: Polymeric Biomaterial Based Hydrogels for Biomedical Applications. *Journal of Biomaterials and Nanobiotechnology, 2011, 2, 85-90.*
2. SAARAI, A.; SEDLACEK, T.; KASPARKOVA, V.; KITANO, T.; SAHA, P.: On the Characterization of Sodium Alginate/ Gelatine-Based Hydrogels for Wound Dressing. *Journal of Applied Polymer Science, 2012, vol. 126, 79-88.*
3. SAARAI, A.; KASPARKOVA, V.; SEDLACEK, T.; SAHA, P.: On the Development and Characterization of Crosslinked Sodium Alginate/ Gelatine-Based Hydrogels. *Journal of the Mechanical Behavior of Biomedical Materials, under review*
4. SAARAI, A.; SEDLACEK, T.; KASPARKOVA, V.: On the Characterization of Genipin Crosslinked Sodium Alginate/ Gelatine Hydrogels for Wound Dressings. *Journal of the Mechanical Behavior of Biomedical Materials, prepared for publication*

Applied research outputs:

5. SAHA, N.; SAHA, T.; SAARAI, A. Dry Material of Hydrogels for Wound Dressing and its Method of Preparation, *Utility model 20931 U1, 31.05.2010*
6. SAHA, N.; SAHA, T.; SAARAI, A. Dry Substance of Hydrogel to Cover Wounds and Process for Preparing Thereof, *CZ patent 302380, date of the patent 09.03.2011*
7. SAHA, N.; SAHA, T.; SAARAI, A. Dry Material of Hydrogels for Wound Dressing and its Method of Preparation, *International Patent Publication Number WO 2011/100935 A1, International Application Number: PCT/CZ/2011/000017*

Conference contributions:

8. SAHA, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Sea buckthorn Oil Incorporated Medicated Hydrogel Based on Gelatin - Sodium Alginate. *In SPE European Medical Polymers Conference, Belfast, United Kingdom, 2008.*
9. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Swelling Behavior and Viscoelastic Properties of a New Hydrogel Based on Gelatin-Sodium Alginate. *Fifth International Conference on Polymer Modification, Degradation and Stabilization, Liege, Belgium, 2008*
10. SAHA, N.; ROY, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Polymeric Biomaterials Promise for Healthcare Application. International Conference on Hi-Tech Materials (ICHTM 09) 11-13 February, 2009, IIT, Kharagpur, India
11. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Viscoelastic Behavior of Bio based Antimicrobial Hydrogel for Medical Applications, 25th Annual Meeting of the Polymer Processing Society, Goa, India, March 1- 5, 2009
12. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Natural Resource Based medicated Hydrogel for Health care, Frontiers in Polymer Science, International Symposium, Mainz, Germany, 7-9 June, 2009
13. STLOUKAL, P.; ROY, N.; SAARAI. A: Preparation of submicroparticles based on biodegradable co-polyester. International Conference on Plastko 2010, April 13-14.2010, Zlin, Czech Republic.
14. SAHA, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Swellable dressing materials for wound healing. 26th Annual Meeting of the Polymer Processing Society, Banff, Canada, July 4- 5, 2010
15. SAARAI, A.; KASPARKOVA, V.; SEDLACEK, T.; SAHA, P: Comparative study of crosslinked sodium alginate/gelatin hydrogels for wound dressing. Proceeding of the 4th WSEAS International Conference on Engineering Mechanics, Structures, Engineering Geology, Corfu Island, Greece, July 14-16. 2011

PUBLICATION I

On the Characterisation of Sodium Alginate/Gelatine-Based Hydrogels for Wound Dressing

Amarjargal Saarai, Tomas Sedlacek,Vera Kasparkova, Takeshi Kitano, Petr Saha

Journal of Applied Polymer Science, 2012, 2012, vol. 126, 79-88.

On the Characterization of Sodium Alginate/ Gelatine-Based Hydrogels for Wound Dressing

Amarjargal Saarai,[1,2] Tomas Sedlacek,[1,2] Vera Kasparkova,[2,3] Takeshi Kitano,[1,2] Petr Saha[1,2]

[1]*Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Nad Ovcirnou 3685, Zlin 760 01, Czech Republic*
[2]*Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, nam. T. G. Masaryka 275, Zlin 760 01, Czech Republic*
[3]*Department of Fat, Surfactant and Cosmetics Technology, Faculty of Technology, Tomas Bata University in Zlin, nam. T.G. Masaryka 5555, Zlin 760 01, Czech Republic*

Received 26 October 2011; accepted 26 November 2011
DOI 10.1002/app.36590
Published online 24 February 2012 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: In this study, sodium alginate/gelatine (SA/G) hydrogels were prepared to obtain wound dressing with good, moist, healing, and biocompatibility properties. The physicochemical properties of hydrogels were evaluated by scanning electron microscopy, Fourier transform infrared spectroscopy, and a swelling test. Dynamic viscoelastic properties including the storage, loss moduli, G' and G'', and loss angle, tan delta of both freshly prepared and swelled gels were examined in oscillatory experiments. Its results revealed that tested SA/G hydrogels exhibit highly elastic behavior similar to the viscoelastic response of human skin. Based on the performed analysis, it could be suggested that the SA/G hydrogel is a potential wound dressing material providing and maintaining the adequate moist environment required to prevent scab formation and the dehydration of the wound bed. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 126: E79–E88, 2012

Key words: sodium alginate; gelatine; hydrogels; wound dressing; swelling behavior; viscoelastic properties

INTRODUCTION

The expansion of comprehensive medical and pharmaceutical wound care has led to considerable attention being placed on the development of wound dressing materials. The ideal dressing material needs to ensure that the wound remains free of infection and moist with exudates but not macerated, while also fulfilling prerequisites concerning structure and biocompatibility. In addition, it should permit the exchange of gases, maintain an impermeable layer to microorganisms so as to prevent secondary infection, not inflame any allergic reaction through prolonged contact with tissue, and it should be removed without trauma and pain. Furthermore, the dressing must be physically strong even when wet, and easy to dispose of when removed.[1–8] Currently, there are numerous wound dressing materials with different functions (e.g., antibacterial, absorbent, adherence, and debridement) and physical forms (e.g., film, foam, gel) as well as materials (e.g. carrageenan, alginate, collagen). Among these materials, hydrogels based on polymeric and biopolymeric materials forming a three-dimensional network, are considered as promising options because of their insoluble, swellable, and hydrophilic properties. Moreover, they can be fabricated to be flexible, durable, and permeable to water vapour and metabolites while also safely covering the wound to prevent bacterial infection. Due to these advantages, hydrogels possess most of the desirable characteristics of an ideal dressing.

Alginates belong to a group of the extensively studied and commonly used gel-forming agents, composed of rather stiff linear polysaccharides. They are obtained by extraction from seaweed, where they occur naturally as mixed calcium and sodium salts of alginic acid consisting mainly of residues of β-1,4-linked D-mannuronic acid and α-1,4-linked L-glucuronic acid. On absorbing moisture, gluronic acid forms firmer gels while mannuronate acid is able to form soft, flexible gels.[8–10] The use of alginate-based hydrogels as dressing can be attributed to their ability to form gels on contact with moisture. Their high moisture absorption occurs via the formation of a strong hydrophilic gel, which limits wound secretions and minimizes bacterial contamination.[4,11] Sodium alginate (SA) used as a wound dressing

Correspondence to: A. Saarai (amarjargal_sa@yahoo.com).
Contract grant sponsor: Ministry of Education, Youth and Sports of the Czech Republic; contract grant number: MSM 70088352101.
Contract grant sponsor: Ministry of Industry and Trade of the Czech Republic; contract grant number: project 2A-1TP1/126.

meets the requirements of nontoxicity and a high level of exudation absorbency.[12,13]

Gelatine (G), another natural polymer, is a collagen-derived connective tissue protein with unique gelation properties attributed to a physical crosslinking of the triple-helix conformation of native collagen.[14–17] It can be obtained either from acidic (Type A gelatine) or alkaline hydrolysis (Type B gelatine) collagen. Type B has more carboxyl groups and a lower isoelectric point (IEP-4.8-5.0) than Type A (IEP-7.0-9.0).[17–19] Gelatine has been used in a wide variety of wound dressings because of its high water absorption and ability to activate microphages and haemostasis in bleeding wounds.[20,21] The main feature of gelatine is its ability to form a thermally reversible network in aqueous media.[17,22] The structural network in gelatine-based hydrogels is a combination of elastic, triple-helix conformation, and a viscous chain, disorder of polypeptide fragment, units.[14,17,23]

The preparation and use of hydrogels based on a combination of either SA or gelatine with other natural or synthetic polymers (agar/SA,[24] chitosan/SA,[25] SA/poly(γ-glutamic acid),[26] polyvinyl alcohol/SA,[11] chitosan/gelatine,[27] polyvinyl alcohol/gelatine,[20] oxidized hyaluronan/gelatine,[28] gelatine/hyaluronate[29] etc.) as wound dressings is being widely reported, while it is referred that these hydrogels posses a good biocompatibility and fulfil the required quality criteria for wound dressing material. However, the combination of SA and gelatine (G) within the list of commercial products of hydrogels for health care applications, especially for wound treatment or wound protection was studied sporadically.[29,30]

The interest in hydrogels is also motivated by their soft consistency derived from their high water content and their tissue-like behavior caused by their specific viscoelastic performance.[31] These viscoelastic properties are strictly related to the internal structure of hydrogels in terms of, e.g., crosslinking density, chain length, degree of swelling, and molecule stiffness of the used polymer. Optimized viscoelastic properties, such as storage (G') and loss modulus (G''), investigated using dynamic mechanical analysis (DMA) and rheometric analysis,[32] are of concern during hydrogel properties development and help to meet the requirements for its absorption efficacy, elasticity as well as painless removal.[33,34]

However, so far there are only a few reports concerning DMA and/or rheometric analyses of wound dressing hydrogels. The use of these methods for investigation of hydrogel viscoelastic behavior is often complicated by compositional and swelling changes[35–37] as well as structural defects in the gel network.[38,39] Meyvis et al.[32] compared the use of DMA (multi-strain and single strain) and oscillatory shear rheometry for the characterization of pharmaceutical hydrogels. They reported that the choice of method for the mechanical characterization of hydrogels depends on the accuracy, speed of measurement, and the amount of sample available. The best agreement was found between the "multi- strain" DMA and rheometer results. In their review, Anseth et al.,[37] thoroughly resolved the mechanical properties of polymer-based hydrogels using tensile and DMA tests in dependence on monomers used, polymerization conditions and effect of degree of swelling. They reported that the mechanical properties are highly dependent on polymer structure, especially the crosslinking density and the degree of swelling.

For this study, "SA/G hydrogels" with various concentrations of SA and gelatine were prepared with the aim to obtain and characterize blends of SA and G as a reference system suitable for highly hydrophilic and biocompatible wound dressings and other medical applications. Desirable properties of SA and gelatine were the motivation behind the choice of these natural materials and were prioritized over the fact that physically crosslinked gelatine-based hydrogels are known to dissolve as they are immersed in physiological buffer saline or water at intended service temperature (above 35°C). Blended hydrogels having increase insolubility will be objective of future work utilizing crosslinking of either G, or SA via glutaraldehyde, and $CaCl_2$, respectively.

The viscoelastic properties of SA/G hydrogels were studied using an oscillatory shear rheological analysis and correlated to the various compositions of the hydrogels. Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and a swelling and viscoelastic technique were utilized for the study of the prepared materials.

MATERIALS AND METHODS

Materials

SA, gelatine (Type B, 250 bloom), glycerol, and sodium chloride (NaCl) (analytical grade) were obtained from Lachema (Czech Republic). Polyethylene glycol (PEG, MW: 3000 g/mol) was purchased from Sigma (Czech Republic). Reverse osmosis purified water was used for the hydrogel preparation and swelling measurements.

Preparation of SA/G hydrogels

The "SA/G hydrogels" were prepared using various concentrations of SA and G while keeping the amount of other components (PEG, glycerol, NaCI and water) constant. An aqueous gelatine solution of (15–30% w/w) was prepared by dissolving polymer

TABLE I
Composition of the SA/G Hydrogels

Hydrogel composition SA/G (g)	70/30	60/40	50/50	40/60	30/70
Gelatine (G)	3	4	5	6	7
Sodium alginate (SA)	7	6	5	4	3
Poly ethylene glycol (PEG)	2	2	2	2	2
Glycerol	2	2	2	2	2
Sodium chloride (NaCl)	0.2	0.2	0.2	0.2	0.2
Water	20	20	20	20	20

granules in water at 80°C under continuous stirring (300 rpm) until a homogeneous solution was obtained. After dissolving G, relevant portions of SA, PEG, NaCl and glycerol (see Table I) were added. Then, the stirring rate was reduced to 100 rpm and continued at 80°C for another 5 min until a viscous hydrogel was formed. The prepared hydrogel was poured into circular moulds (diameter 25 mm) with a thickness of 2 mm and cooled down (samples denoted "A"). Finally, the samples were dried (samples denoted "B") at room temperature for 72 h. For purpose of comparison, reference samples composed of pure G and pure SA were prepared using the identical procedure.

Scanning electron microscopy

The cross-sectional morphologies of the SA/G hydrogels were examined by SEM at an accelerating voltage of 20 kV (VEGA\\LMU, TESCAN, Czech Republic). Cross-sectional specimens were prepared by fracturing of B samples in liquid nitrogen to examine miscibility and morphology of the blended, dried systems preceding swelling phase. Before observation, the specimens were coated with a thin layer of gold under a vacuum. SEM images were analyzed using an image analysis VEGA Software.

Swelling behavior

The SA/G hydrogels were immersed in distilled water (pH 7.0) at room temperature (23 ± 2°C). The degree of swelling was determined gravimetrically. The swelled samples (denoted C) were taken from the water at selected time intervals (1, 2, 3, 4, 8, 12 h), wiped with tissue paper, weighed and placed in water again. The percentage of swelling and water content were calculated using the following equation:

$$\text{Swelling}\,\% = \frac{(W_S - W_D)}{W_D} \times 100 \quad (1)$$

$$\text{Water content}\,\% = \frac{(W_S - W_D)}{W_S} \times 100 \quad (2)$$

where W_S and W_D are the weights of the swollen and dry samples, respectively.

Viscoelastic properties

The viscoelastic properties of the SA/G hydrogels were measured by rotational rheometer (Rheometrics ARES Rheometer, TA Instruments) using a parallel-plate 25 mm in diameter. All measurements were performed in the linear viscoelastic regime with a strain of 1%. The measurement went through frequency scanning at 33°C (surface temperature of human skin[40]) in the frequency range of 0.1–100 rad/s. The storage modulus (G'), loss modulus (G''), complex viscosity (η^*), and tan delta ($\delta = G''/G'$) of the samples were recorded as a function of frequency. Each measurement was performed at least twice, on two different disk specimens from the same hydrogel sample.

Fourier transform infrared spectroscopy

The chemical composition of the samples B was investigated by FTIR spectroscopy. Attenuated total reflectance/Fourier transform infrared (ATR–FTIR) spectroscopy was conducted with a FTIR instrument (Nicolet 320, Nicolet Instrument Corporation) using a Zn-Se crystal and the software package OMNIC over the range of 4000–1000 cm^{-1}. A resolution of 2 cm^{-1} was maintained in all cases.

RESULTS AND DISCUSSION

Morphology of SA/G hydrogels

The effects of SA and G contents on the morphology of dried SA/G hydrogels (sample B) are shown in SEM micrographs in Figure 1. The morphologies of SA/G 100/0 and SA/G 0/100 are depicted in Figure 1(a,b), respectively. From the figures, it is seen that the pure SA sample shows a particle-like, aggregate structure while the pure G sample exhibits uniform, relatively homogenous morphology.

The morphologies of SA/G hydrogel materials of various compositions (70/30, 60/40, 50/50, 40/60, and 30/70) are shown in Figure 1(c–g), respectively. By comparison, it is obvious that the morphologies of the pure samples differ significantly from the binary blends. The morphologies of the SA/G-70/30, 60/40, and 50/50 samples reveal structures having

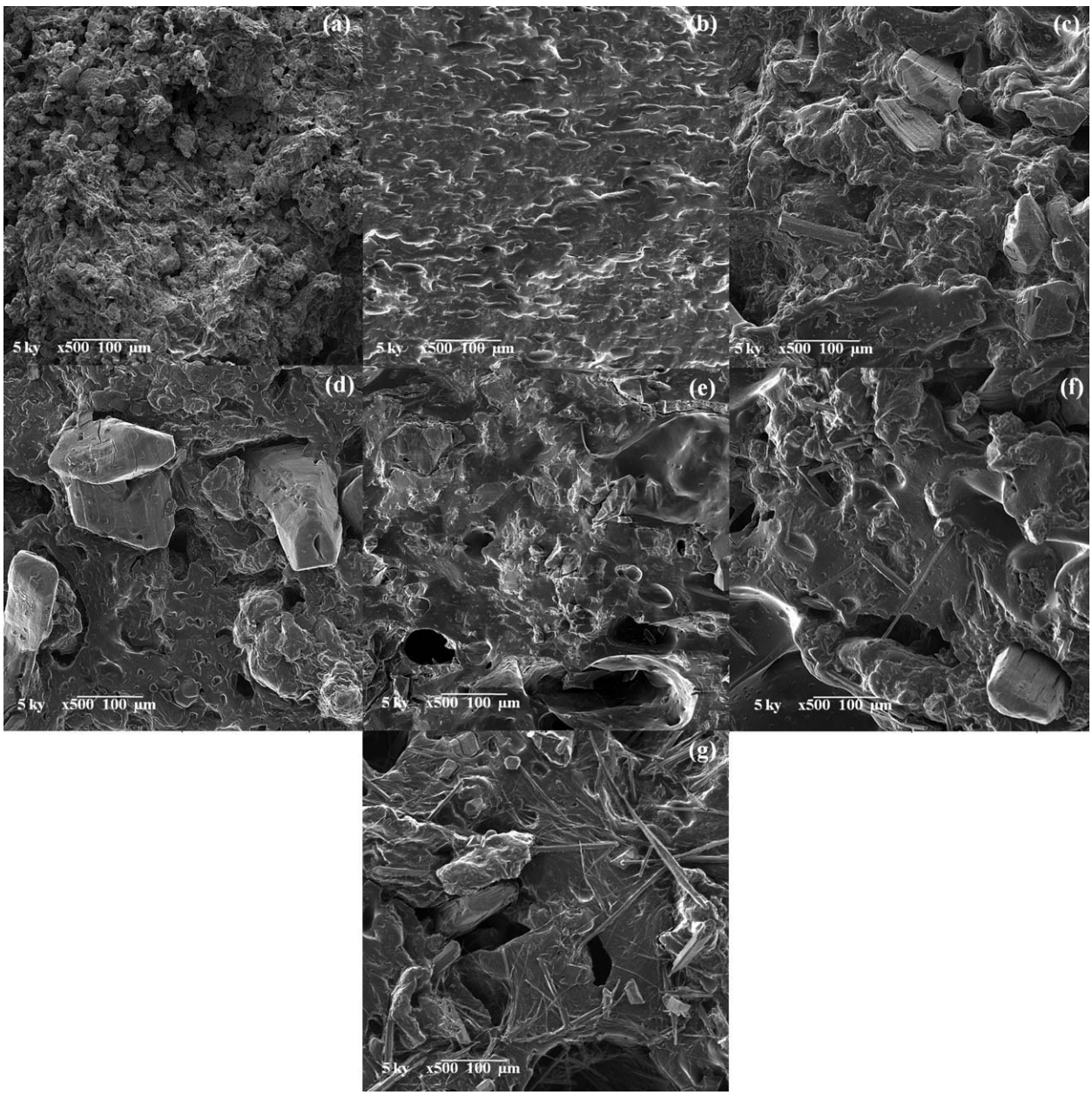


Figure 1 SEM micrographs of dried hydrogels: (a) SA/G-100/0, (b) SA/G-0/100, (c) SA/G-70/30, (d) SA/G-60/40, (e) SA/G-50/50, (f) SA/G-40/60, and (g) SA/G-30/70.

uniform SA as a matrix and G as dispersed phase [Fig. 1(c–e)]. On the other hand, samples with a prevailing amount of G, SA/G 40/60 and 30/70, have G as the matrix and SA as the dispersed phase [Fig. 1(f,g)]. It should be noted that for the samples with SA as the matrix, a droplet-like morphology of minor G phase is observed, while samples with G as the matrix render a fibrous morphology of SA phase.

Swelling behavior of G/SA hydrogels

The degree of swelling indicates the ability of the dressing to absorb the wound fluid and exudates. The swelling behavior of SA/G hydrogels as a function of time is shown in Figure 2. As can be seen, all samples demonstrate a rapid increase in swelling degree within the first 4 h, and this swelling continues to increase slowly for up to 12 h (Table II). However, within the tested time interval, only the samples with lower G content reach the equilibrium swelling. It is furthermore obvious that swelling degree decreases with an increase of SA content in the hydrogels, which can be attributed to the structure of the SA matrix, with pores of small sizes hindering the diffusion of water molecules into the hydrogel structure. A similar behavior for SA-based hydrogels has been reported by Bajpai et al.[41] and Anmika

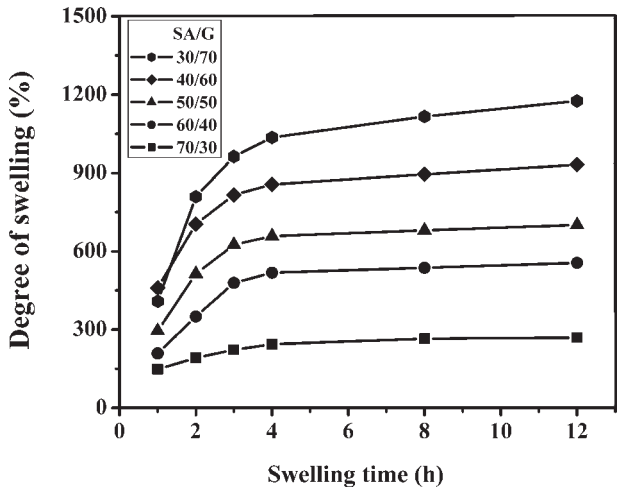


Figure 2 Effect of composition and time on the degree of swelling.

et al.[42] The increase in the swelling degree of hydrogel samples with high G content refers to the strong hydrophilicity of G molecules, which is reportedly due to the presence of an ionizable NH_2^- and COO^- functional group that can increase the volume between polymeric chains and the swelling capacity of the hydrogel by hydrostatic repulsion.[41,43]

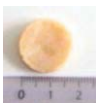
A maximum swelling degree of SA/G hydrogels at 1176% was observed in the composition SA/G 30/70. Even such a level of swelling is not a final value and would be slightly higher when approaching equilibrium state. Analogous swelling behaviors were observed for the commercial hydrogel dressings Geliperm® (Geistilich, Switzerland) and Vigilon® (Bard, Crawley, UK).[21,30] As to water uptake, the maximum water content of all swelled hydrogels (Samples C) was in the range of 74–94% (Table II), indicating that the SA/G hydrogels could fulfil the requirement of an ideal wound dressing[1–3,21,44] and prevent a wound from accumulation of fluid by absorbing the exudates.

Viscoelastic properties of G/SA hydrogels

The angular frequency dependence of the storage modulus (G') and loss modulus (G'') for SA/G hydrogels is depicted in Figure 3. From Figure 3(a) it can be seen that the G' of all hydrogels is almost one order of magnitude higher than G'' over the whole angular frequency range and both the G' and G'' of all samples slightly increase with angular frequency. In addition, an increase in G' and G'' values is observed when the SA concentration rises, probably due to the increased number of SA chains that produce a dense network.[41,45] Obviously, after 8 h swelling, the G' and G'' of all samples drop dramatically because of the presence of water, which increases mobility of the gel network and decreases stiffness [Fig. 3(b)]. It should be noted that the described viscoelastic behavior of swelled SA/G hydrogels is in agreement with the viscoelastic response of human skin reported in the literature.[46,47]

In Figure 4(a), the tan δ of samples A is plotted against angular frequency. At first, a plateau is observed until 5 rad/s, then an increasing trend, due to the enhanced influence of the viscous part, attended by a more significant change in G'' compare with G', begins. The angular frequency dependency of the tan δ values for swelled samples C is shown in Figure 4(b). Over the whole frequency range tan δ gently increases, along with a slightly steeper increase of G'' with the frequency than that of G' [see Fig. 4(a)]. Interestingly, the SA/G 70/30

TABLE II
Physical Characteristics of the SA/G Hydrogel Samples [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hydrogel composition SA/G	70/30	60/40	50/50	40/60	30/70	Image of hydrogels
Samples after preparation A (before drying), $n = 3$						
Weight (g)	0.735 ± 0.064	0.729 ± 0.051	0.894 ± 0.117	0.858 ± 0.161	0.876 ± 0.094	
Diameter (mm)	25.0 ± 0.00	25.0 ± 0.00	25.0 ± 0.00	25.0 ± 0.00	25.0 ± 0.00	
Thickness (mm)	1.94 ± 0.23	1.74 ± 0.18	1.99 ± 0.21	1.70 ± 0.33	1.76 ± 0.25	
Moisture content (%)	49.2 ± 0.52	46.9 ± 0.76	49.4 ± 0.17	49.1 ± 1.24	52.9 ± 0.86	
Dried samples B (after drying), $n = 3$						
Weight (g)	0.373 ± 0.032	0.386 ± 0.027	0.452 ± 0.059	0.452 ± 0.082	0.412 ± 0.046	
Diameter (mm)	19.82 ± 0.40	20.25 ± 0.54	20.71 ± 0.26	19.96 ± 0.14	20.92 ± 0.56	
Thickness (mm)	1.54 ± 0.19	1.23 ± 0.12	1.39 ± 0.19	1.19 ± 0.22	1.02 ± 0.16	
Swelled samples C (12 h after swelling), $n = 3$						
Weight (g)	1.387 ± 0.201	2.285 ± 0.47	2.295 ± 0.64	3.804 ± 0.81	3.981 ± 0.92	
Diameter (mm)	31 ± 2.14	38 ± 3.69	38.583 ± 3.6	42.21 ± 2.49	43.42 ± 3.87	
Thickness (mm)	2.29 ± 0.31	2.48 ± 0.198	2.55 ± 0.32	2.83 ± 0.49	2.90 ± 0.42	
Water content (%)	72.54 ± 4.68	81.86 ± 6.53	84.03 ± 3.96	87.97 ± 3.46	90.1 ± 3.74	

All measurements were done in triplicate.

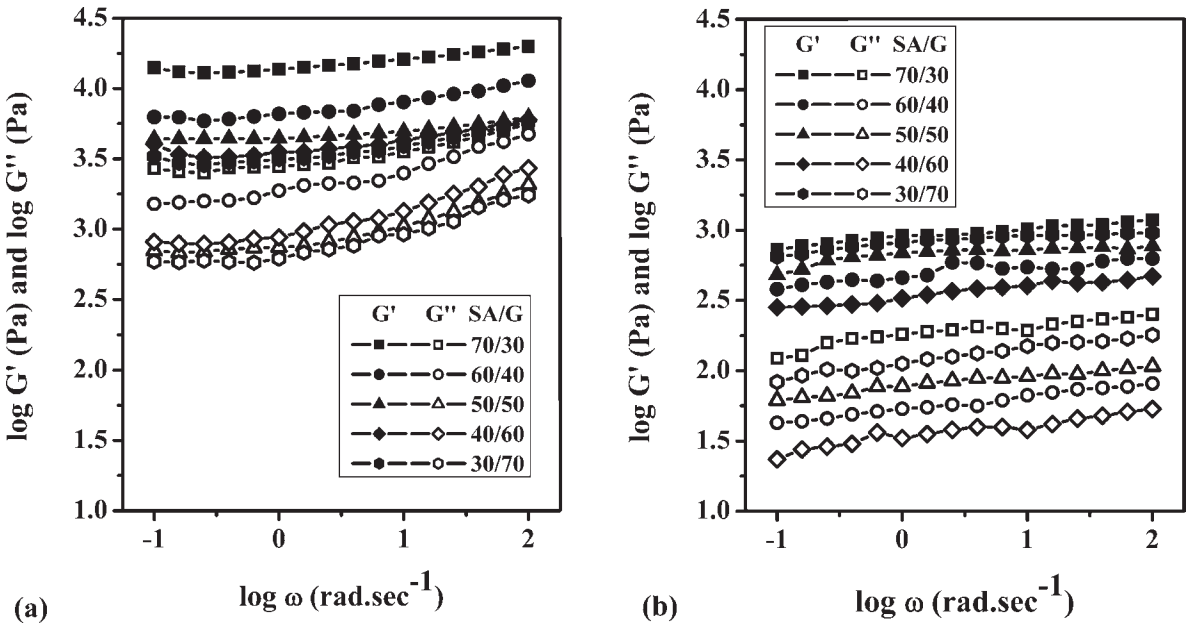


Figure 3 Storage, G', and Loss, G'', moduli of freshly prepared samples A (a) and 8 h swelled samples C (b).

and 30/70 samples are slightly more elastic than the other samples, most probably due to the different morphologies of the prepared hydrogels.

In Figure 5, the dependence of G' and G'' on the swelling time of the hydrogels with different ratios of SA/G at the angular frequency ω of 0.39 rad s^{-1} is shown. The viscoelastic moduli gradually decrease for up to 4 h swelling time. After this time period elapses, changes of G' and G'' are not so prominent. This observation complies with the swelling behavior depicted in Figure 2 describing the effect of composition and time on the degree of swelling.

The G' and G'' of samples A and C with different compositions, recorded at the angular frequency of 0.39, 3.9 and 39 rad s^{-1}, are depicted in Figure 6. Comparing samples A and C, it is obvious that after 8 h of swelling both G' and G'' drop notably; interestingly G'' undergoes a more significant change. Furthermore, as can be seen from Figure 6(a), both G' and G'' of samples A decrease with the increasing contents of G. The swelled samples C, contrary to the freshly prepared samples A, do not follow the same decreasing tendency of the storage and loss moduli with G content increase [Fig. 6(b)]. The maximum values of moduli for samples C were found in the compositions SA/G 70/30 and 30/70. The viscoelastic properties of these hydrogels are influenced by stiff molecules of SA representing either the

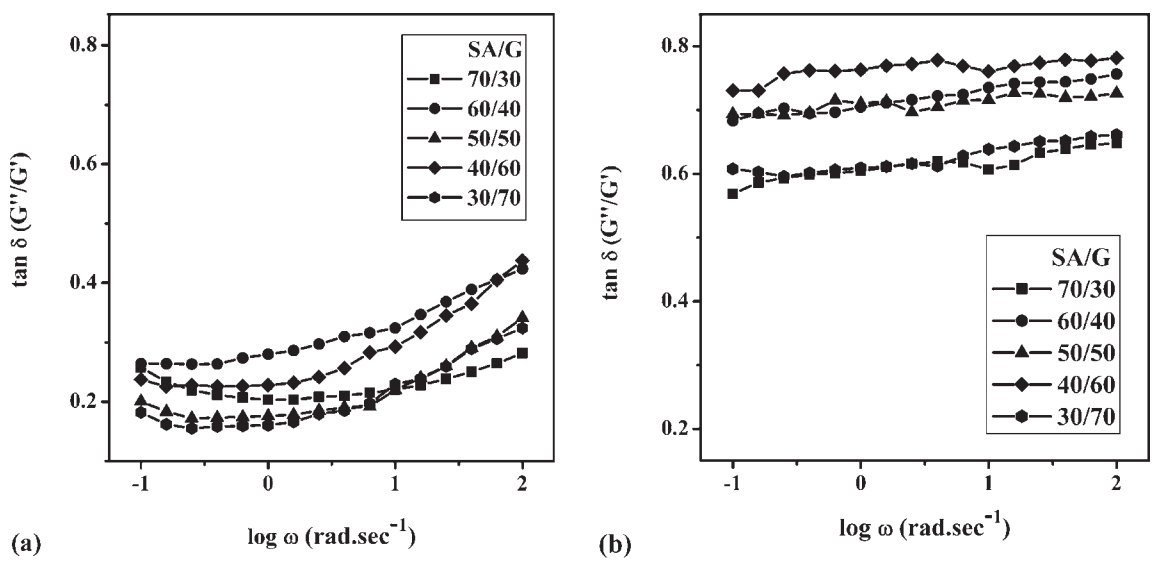


Figure 4 Effect of angular frequency on tan δ of samples A (a) and C (b).

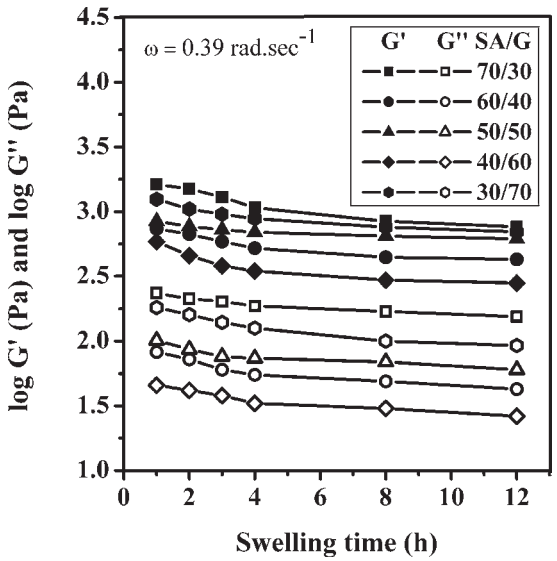


Figure 5 Effect of swelling time on storage modulus, G', and loss modulus, G'', of SA/G hydrogels.

continuous phase (SA/G 70/30) or needle-like dispersed particles (SA/G 30/70) in hydrogel structure. The local minimum of G' and G'' was observed for hydrogel compositions of SA/G 60/40 and 40/60. The authors believe that such behavior could be caused by phase separation phenomenon. Using SEM, it was determined that the morphology of 60/40 and 40/60 compositions induced specific porosity levels in a continuous phase matrix (SA/G 60/40) or in discontinuous phase particles (SA/G 40/60). Interestingly, viscoelastic moduli of G/SA 50/50 are higher than those of 40/60 and 60/40 at measured angular frequencies. This can be a consequence of attraction forces between the charged ions leading to intensive interactions by the balanced anionic carboxylate groups (—COO^-) of SA and the cationic protonated amino groups (NH_3^+) of gelatine.[48]

Figure 7 presents the relation between complex viscosity, η*, and angular frequency for samples C with various SA/G compositions and shows that η* decreases linearly with angular frequency. Furthermore, decreased η* of samples C have a similar trend as observed for G' and G'' [Fig. 6(b)].

The storage and loss moduli of samples C as a function of the swelling degree are represented in Figure 8(a,b). As can be seen in Figure 8(a), the highest values of G' are observed for the hydrogel of SA/G 70/30 composition. Notably, for this hydrogel the smallest change of swelling degree (200–300%) was connected with the most significant decline of storage modulus. On the other side, only a minor G' change over the widest region of swelling degree (400–1200%) was determined for the hydrogel with the opposite SA/G ratio of 30/70. The changes of swelling degree as well as changes of G' ($\Delta G''$) observed for samples with the composition of SA/G 60/40, 50/50, and 40/60 lie between the above mentioned SA/G 30/70 and 70/30 hydrogels. Furthermore, from Figure 8(a) it is clear that while the highest G' of unswelled samples was found for SA/G 70/30, the highest G' values of fully swelled hydrogels were recorded simultaneously for SA/G 70/30 and SA/G 30/70 samples.

Values of G'' for all studied samples are almost an order of magnitude lower than those of G' over the entire range of swelling [see Fig. 8(b)]. The results also reveal that the levels of G'' follow the trend described for G'. However, the magnitude of G'' changes ($\Delta G''$) is independent of hydrogel

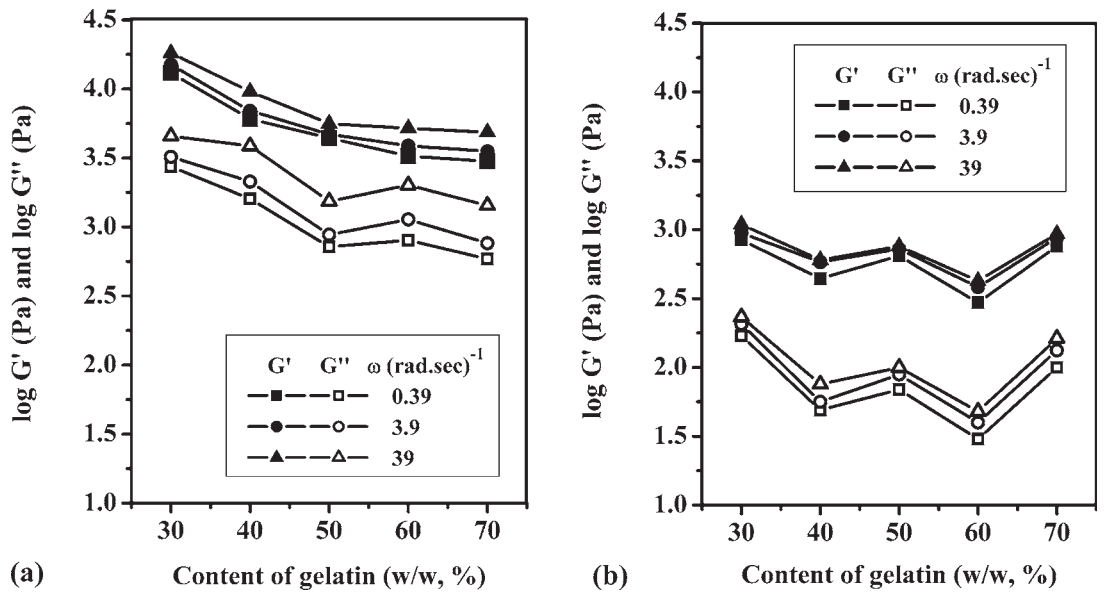


Figure 6 The effect of composition on storage modulus, G', and loss modulus, G'', of samples A (a) and samples C (b).

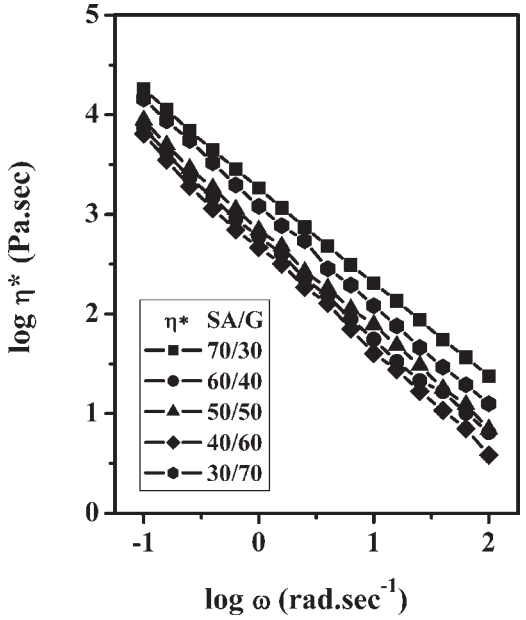


Figure 7 Effect of angular frequency on complex viscosity of samples C.

composition, thus differing significantly from the behavior of G' and indicating predominant elastic response within the course of hydrogel swelling.

The described results, namely the values of elastic (G') and viscous (G'') moduli and the swelling degrees of the prepared samples, indicate that the viscoelastic properties of SA/G hydrogels are appropriate for wound dressing.[49,50]

FTIR characterization of SA/G hydrogels

The FTIR spectra of G/SA hydrogels are shown in Figure 9. The FTIR spectrum of SA/G-100/0 indicates the characteristic absorption peaks observed at 3274 cm^{-1} typical for hydroxyl stretching and a peak at 1637 cm^{-1} which corresponds to a stretch of C=O. Peaks at 1521, 1458, 1408, and 1349 cm^{-1} in the SA spectrum indicate the anti-symmetric stretch and symmetric stretch of $-COO^-$ in associated carboxylic acid salt.[51,52] Two other interactions in the C—O stretch of C—OH groups can be found at 1030, 1080 cm^{-1} and the peak at 1248 cm^{-1} corresponds to the anti-symmetric stretching of C—O—C.

The FTIR spectrum of SA/G 0/100 exhibits a peak at 3309 cm^{-1} due to the N—H stretching in secondary amides, the C=O stretching at 1631 cm^{-1} for the amide I, which is characteristic of the coil structure of gelatine,[22] as well as N—H deformation at 1550 cm^{-1} for amide II.[51] Most of the remaining peaks, at 1040, 1079, 1317, 1346, 1404, and 1453 cm^{-1} can correspond to the stretching of C—O bonds. Furthermore, on close inspection of the lower wave number region of the spectrum, two bands are observed at 2877 and 2940 cm^{-1}, due to aliphatic symmetric and C—H stretching, respectively (The response of C—H bonds is presented by the absorption peaks at 2940 and 2877 cm^{-1}). These results are in agreement with the observation reported by Pawde et al.[20]

The spectra of SA/G hydrogels show peaks at 3265, 1631, 1453, 1408, 1334, 1081, 1028, and 993 cm^{-1}, which indicate O—H stretch, the COO^- (asymmetric), COO^- (symmetric), C—O, C—O—H, respectively, exhibiting strong intermolecular hydrogen bonding.[51,52] Particularly, the intensity of some peaks decreases with increasing of SA (e.g., 1234 cm^{-1}). The adsorption peak, thanks to the stretching of C—O—C ethers coupled with the 1241 and 1119 cm^{-1} of G, shifts to 1234 cm^{-1}. Moreover, the gelatine band related to amides II 1551 cm^{-1} weakens and almost disappears in the SA/G 70/30 sample. The band of SA/G 30/70 at 3291 cm^{-1} shifts to a

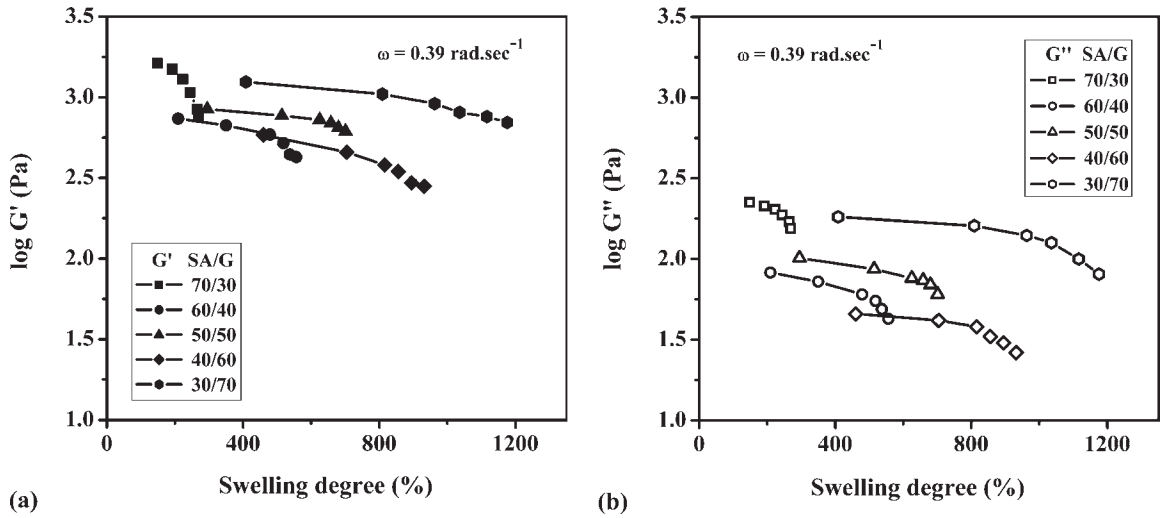


Figure 8 Relation between viscoelastic properties (G' and G'') at angular frequency of 0.39 rad s^{-1} and degree of swelling (%) for samples C.

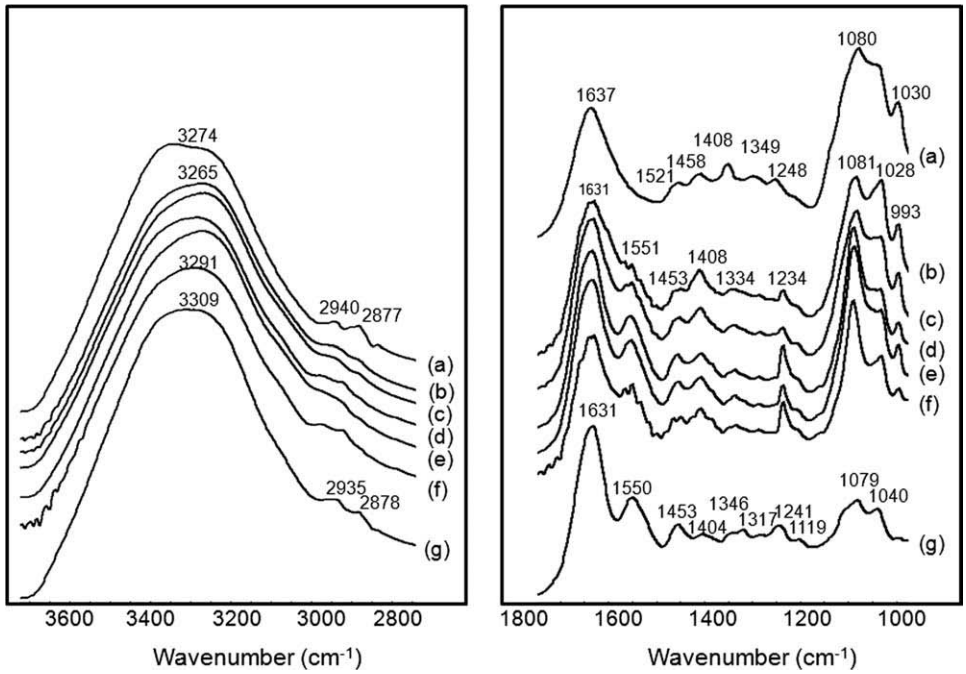


Figure 9 FTIR spectra for: (a) SA/G-100/0, (b) SA/G-70/30, (c) SA/G-60/40, (d) SA/G-50/50, (e) SA/G-40/60, (f) SA/G-30/70, and (g) SA/G-0/100.

lower wavenumber (3265 cm^{-1}) with an increasing concentration of SA which reflects increased hydrogen bonding between SA and G.

CONCLUSIONS

In this work, SA/G hydrogels based on SA and gelatine (G) are introduced as potential wound dressing materials. To evaluate the performance of SA/G hydrogels for wound dressing applications, the morphology, swelling behavior and viscoelastic properties of hydrogels with various ratios of SA and G have been investigated. The results demonstrate that SA/G ratio remarkably influences the structure and morphology of hydrogels. In particular, it is obvious that with a decrease in SA content, the respective hydrogel morphology changes from particle-like to fibrous-like. It can also be observed that the hydrogel morphology closely correlates with the swelling degree, which decreases with an increase in SA content. Results from the determination of water content and swelling behavior show that hydrogels exhibit a high capability to absorb fluid, making them promising materials for exudative wound dressings. The viscoelastic properties of SA/G hydrogels notably depend on their composition and are related to the swelling behavior of hydrogels. The viscoelastic properties of SA/G hydrogels decreased, with an increased swelling degree due to water absorption into the hydrogel structure. The optimum SA/G ratio in terms of a compromise between viscoelastic properties and absorption abilities was found to be SA/G 50/50. The hydrogel with this composition shows the viscoelastic response fully comparable with that of human skin while simultaneously retaining very good absorption properties.

This article was created with support of Operational Program Research and Development for Innovations co-funded by the European Regional Development Fund (ERDF) and national budget of Czech Republic, within the framework of project Centre of Polymer Systems (reg. number: CZ.1.05/2.1.00/03.0111).

References

1. Annie, J.; David V. J Orthop Nurs 2005, 9, 51.
2. Lloyd, L. L.; Kennedy, J. F.; Methacanon, P.; Paterson, M.; Knill, C. J. J Carbohydr Polym 1998, 37, 315.
3. Stephen, T. In The Epidermis in Wound Healing; Rovee, D. T.; Maibach, H. I., Eds.; CRC Press: Washington, DC, 2004, Chapter 12.
4. Boateng, J. S.; Matthews, K. H.; Stevens, H. N.; Gillian. M. Eccleston. J Pharm Sci 2008, 97, 2892.
5. Smith, T. J.; Kennedy, J. E.; Higginbotham, C. L. J Mater Sci Mater Med 2009, 20, 1193.
6. Northern Health and Social Services Board, NHSSB wound management manual, United Kingdom, 2005.
7. Smith, T. J.; Kennedy, J. E.; Higginbotham, C. L. J Mech Behav Biomed Mater 2009, 2, 264.
8. Donati, I.; Paoletti, S. In Alginates: Biology and Applications; Rehm, B. H. A. Ed.; Springer-Verlag: Berlin Heidelberg, 2009, Chapter 1.
9. Immirzi, B.; Santagata, G.; Vox, G.; Schettini, E. J Biosys Eng 2009, 102, 461.

10. Coviello, T.; Matricardi, P.; Marianecci, C.; Alhaique, F. J Control Release 2007, 14, 5.
11. Jong, O. K.; Jung, K. P.; Jeong, H. K.; Sung, G. J.; Chul, S. Y.; Dong, X. L. J Pharm 2008, 359, 79.
12. Thomas, A.; Harding, K. G.; Moore, K. J Biomater 2000, 21, 1797.
13. Paul, W.; Sharma, C. P. In Encyclopedia of Surface and Colloid Science, Marcel Dekker: New York, 2004.
14. Farris, S.; Schaich, K. M.; Liu, L. S.; Piergiovanni, L.; Yam, K. L. J Trends Food Sci Technol 2009, 20, 316.
15. Ioannis S. Arvanitoyannis. In Protein-Based Films and Coatings, Gennadios, A., Ed.; CRC Press: Florida, 2000, Chapter 11.
16. Sperling, L. H. In Introduction to Physical Polymer Science, Sperling, L. H., Ed.; John Wiley & Sons: Canada, 2006, Chapter 9.
17. Schrieber, R.; Gareis, H. Gelatine Handbook; Wiley-VCH: Germany, 2007.
18. Peng, H. T.; Martineau, L.; Shek, P. N. J Mater Sci Mater Med 2008, 19, 997.
19. Zhang, Z. K.; Li, G. Y.; Shi, B. J Soc Leather Technol Chem 2006, 90, 23.
20. Pawde, S. M.; Deshmukh, K. J Polym Sci 2008, 109, 3431.
21. Yoo, H. J.; Kim, H. D. J Pharm 2007, 359, 326.
22. Yakimets, I.; Paes, S. S.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Mitchell, J. R. J Biomacromol 2007, 8, 1710.
23. Slaughter, B. V.; Khurshid, S.; Fisher, O. Z.; Khademhosseini, A.; Peppas, N. A. J Adv Mater 2009, 21, 3307.
24. Cole, S.; Garbe, M. Alginate Hydrogel foam Wound Dressing, US Patent 4,948,575, 1990.
25. Murakami, K.; Aoki, H.; Nakamura, S.; Nakamura, S.; Nakamura, S.; Takikawa, M.; Hanzawa, M.; Kishimoto, S.; Hattori, H.; Tanaka, Y.; Kiyosawa, T.; Sato, Y.; Ishihara, M. J Biomater 2010, 31, 83.
26. Mei, H. H.; Ming, C. Y. J Polym Adv Technol 2009, 4, 1.
27. Chun, M. D.; Lan, Z. H.; Ming, Z.; Dan, Y.; Yi, L. J Carbohydr Polym 2007, 69, 583.
28. Weng, L.; Pan, H.; Chen, W. J Biomed Mater Res A 2007, 2, 352.
29. Choi, Y. S.; Lee, S. B.; Hong, S. R.; Lee, Y. M.; Song, K. W.; Park, M. H. J Mater Med 2001, 12, 67.
30. Balakrishnana, B.; Mohantyb, M.; Umashankarc, P. R.; Jayakrishnana, A. J Biomater 2005, 26, 6335.
31. Mallika, S. K. Imaging Viscoelasticity in Hydropolymers and Breast Tissue Ultrasound, PhD Thesis, University of California, Davis, 2006.
32. Meyvis, T. K. L.; Stubbe, B. G.; Van Steenbergen, M. J.; Hennik, W. E.; De Smedt, S. C. J. Demeester, J. J Pharm 2002, 244, 163.
33. Matthews, K. H.; Auffret, A. D.; Humphrey, M. J.; Stevens, H. N. E.; Eccleston, G. M. J Pharm 2005, 289, 51.
34. Razzak, M. T.; Erizal, Z.; Dewi, S. P.; Lely, H.; Taty, E.; Sukirno, C. J Radiat Phys Chem 1999, 55, 153.
35. Stadler, R.; Gronski, W. J Colloid Polym Sci 1983, 261, 215.
36. Cote, G. L.; Gant, R. M.; Melissa, G.; Yaping, A. H. Self-Cleaning Membrane for Implantable Biosensors: Dynamic mechanical analysis (DMA) and tensile test, US Pat. 20,100,056,894, 2010.
37. Anseth, K. S.; Bowman, C. N.; Brannon-Peppas, L. J Biomater 1996, 17, 1647.
38. Otegui, J.; Fernandez, E.; Retama, J. R.; Cabarcos, E. L.; Mijiangos, C.; Lopez, D. J Polym Eng Sci 2009, 49, 964.
39. Lutz, P. J. J Polym Bull 2007, 58, 161.
40. Denda, M.; Sokable, T.; Fukumi-Tominaga, T.; Tominaga, M. J Invest Dermatol 2007, 127, 654.
41. Bajpai, J.; Mishra, S.; Bajpai, A. K. J Appl Polym Sci 2007, 106, 961.
42. Anmika, R.; Bajpai, J.; Baijai, A. K. J Chem Technol 2009, 16, 388.
43. Agustin, M. R.; Fausto, B. B.; Juan, C.; Sanchez, D.; Alejandro, G. A. J Polym Bull 2009, 62, 539.
44. Mehrdad, K.; Mohammad, S.; Zuhair, M. H. J Eur Polym 2007, 42, 773.
45. Barbara, I.; Gabriella, S.; Giuliano, V.; Evelia, S. J Biosys Eng 2009, 102, 461.
46. Holt, B.; Tripathi, A.; Mordan, J. J Biomech 2008, 28, 2689.
47. Parul, N.; Connie, K. S.; Charles, W. P. J Biomech 2005, 3, 313.
48. Zhanfeng, D.; Qun, W.; Yumin, D. J Membr Sci 2006, 280, 37.
49. Simonida, L. T.; Maja, M. M.; Sava, N. D.; Jovanka, M. F.; Suljovrujic, E. H. J Radiat Phys Chem 2009, 1, 1.
50. Ademar, B. L.; Sizue, O. R.; Sonia, M. M. J Radiat Phys Chem 2002, 63, 543.
51. Lambert, B.; Shurvell, H. F.; Lightner, D.; Cooks, R. G. Organic Structural Spectroscopy; Wiley-VCH: New York, 2007.
52. Barbara, H. S. Infrared Spectroscopy: Fundamentals and applications; Wiley & Sons: England, 2004, Chapter 5.

PUBLICATION II

On the Development and Characterisation of Crosslinked Sodium Alginate/Gelatine-Based Hydrogels

Amarjargal Saarai, Vera Kasparkova, Tomas Sedlacek, Petr Saha

Journal of the Mechanical Behavior of Biomedical Materials,

Under review

On the Characterisation of Sodium Alginate/Gelatin-Based Hydrogels for Wound Dressing

Amarjargal Saarai[1], Tomas Sedlacek[1], Vera Kasparkova[2], Nabanita Saha[1], Takeshi Kitano[1], Petr Saha[1]

1) Centre of Polymer Systems, Polymer Centre, Tomas Bata University in Zlin, nam. T.G. Masaryka 5555, 760 01 Zlin, Czech Republic

2) Centre of Polymer Systems, Department of Fat, Surfactant and Cosmetics Technology, Tomas Bata University in Zlin, nam. T.G. Masaryka 5555, 760 01 Zlin, Czech Republic

The name and contact of each contributing author:

Amarjargal Saarai *E-mail address*: amarjargal_sa@yahoo.com, *phone: + 420- 57603 3027, fax: +420 57 603 1444* (Corresponding author)

Tomas Sedlacek *E-mail address*: sedlacek@ft.utb.cz

Vera Kasparkova *E-mail address*: vkasparkova@ft.utb.cz

Nabanita Saha *E-mail address*: nabanita@ft.utb.cz

Takeshi Kitano *E-mail address:* t_kitano297@yahoo.com

Petr Saha *E-mail address:* saha@utb.cz

ABSTRACT: In this study, sodium alginate/gelatin (SA/G) hydrogels were prepared in order to obtain wound dressing with good, moist, healing and biocompatibility properties. The physicochemical properties of hydrogels were evaluated by scanning electron microscopy, Fourier transform infrared spectroscopy and a swelling test. Dynamic viscoelastic properties including the storage and loss moduli, G' and G'', were examined in oscillatory experiments for both freshly prepared and swelled gels. The rheological characterization indicated that SA/G hydrogels exhibit highly elastic behavior similar to the viscoelastic response of human skin. The obtained results suggest that the SA/G hydrogel is a potential wound dressing material providing and maintaining the adequate moist environment required to prevent scab formation and the dehydration of the wound bed.

KEYWORDS: sodium alginate; gelatin; hydrogels; wound dressing; swelling behavior; viscoelastic properties

INTRODUCTION The expansion of comprehensive medical and pharmaceutical wound care has led to considerable attention being placed on the development of wound dressing materials. The ideal dressing material needs to ensure that the wound remains free of infection and moist with exudates but not macerated, while also fulfilling prerequisites concerning structure and biocompatibility. In addition, it should permit the exchange of gases, maintain an impermeable layer to microorganisms so as to prevent secondary infection, not inflame any allergic reaction through prolonged contact with tissue, and it should be removed without trauma and pain. Furthermore, the dressing must be physically strong even when wet, and easy to dispose of when removed[1-8]. Currently, there are numerous wound dressing materials with different functions (e.g., antibacterial, absorbent, adherence, debridement) and physical forms (e.g., film, foam, gel) as well as materials (e.g. carrageenan, alginate, collagen). Among these materials, hydrogels based on polymeric and biopolymeric materials forming a three-dimensional network, are considered as promising options because of their insoluble, swellable and hydrophilic properties. Moreover, they can be fabricated to be flexible, durable, and permeable to water vapor and metabolites while also safely covering the wound to prevent bacterial infection. Due to these advantages, hydrogels possess most of the desirable characteristics of an ideal dressing.

Alginates belong to a group of the commonly used gel-forming agents that have been extensively studied. They are composed of rather stiff linear polysaccharides and are obtained by extraction from seaweed, where they occur naturally as mixed calcium and sodium salts of alginic acid. Alginic acid is a linear copolymer consisting mainly of residues of β -1,4-linked D-mannuronic acid and α -1,4-linked L-glucuronic acid. Upon absorbing moisture, gluronic acid forms firmer gels while mannuronate acid is able to form soft, flexible gels[8-10]. The use of alginate-based hydrogels as dressing can be attributed to their ability to form gels upon contact with moisture.

Their high moisture absorption occurs via the formation of a strong hydrophilic gel, which limits wound secretions and minimizes bacterial contamination[4, 11]. Sodium alginate (SA) used as a wound dressing meets the requirements of nontoxicity and a high level of exudation absorbency[12-13].

Gelatin (G), another natural polymer, is a collagen-derived connective tissue protein with unique gelation properties attributed to a physical crosslinking of the triple-helix conformation of native collagen[14-17]. It can be obtained either from acidic (Type A gelatin) or alkaline hydrolysis (Type B gelatin) collagen. Type B has more carboxyl groups and a lower isoelectric point (IEP- 4.8-5.0) than Type A (IEP- 7.0-9.0)[17-19]. Gelatin has been used in a wide variety of wound dressings because of its high water absorption and ability to activate microphages and haemostasis in bleeding wounds[20, 21]. The main feature of gelatin is its ability to form a thermally reversible network in aqueous media[17, 22]. The structural network in gelatin-based hydrogels is a combination of an elastic, triple-helix conformation and a viscous chain disorder of polypeptide fragment units[14, 17, 23].

The preparation and use of hydrogels based on a combination of either sodium alginate or gelatin with other natural or synthetic polymers (agar/ sodium alginate[24], chitosan/ sodium alginate[25], sodium alginate/ poly(γ-glutamic acid)[26], polyvinyl alcohol/ sodium alginate[11], chitosan/ gelatin[27], polyvinyl alcohol/ gelatin[20], oxidized hyaluronan/ gelatin[28], gelatin/ hyaluronate[29] etc.) as wound dressings has already been reported. The publications revealed that these hydrogels posses a good biocompatibility and fulfill the required quality criteria for wound dressing material. However, the combination of sodium alginate (SA) and gelatin (G) has not yet been reported within the list of commercial products of hydrogels for health care applications, especially for wound treatment or wound protection.

The interest in hydrogels is also motivated by their soft consistency derived from their high water content and their tissue-like behavior caused by their specific viscoelastic performance[30]. These viscoelastic properties are strictly related to the internal structure of hydrogels in terms of, e.g., cross-linking density, chain length, degree of swelling and molecule stiffness of the used polymer. Optimized viscoelastic properties, such as storage (G') and loss modulus (G''), investigated using dynamic mechanical analysis (DMA) and rheometric analysis[31], are of concern during hydrogel properties development and help to meet the requirements for its absorption efficacy, elasticity as well as painless removal[32, 33].

However, so far there are only a few reports concerning DMA and/or rheometric analyses of wound dressing hydrogels. The use of these methods for investigation of hydrogel viscoelastic behaviour is often complicated by compositional and swelling changes[34-36] as well as structural defects in the gel network[37, 38]. Meyvis et al.[31] compared the use of DMA (multi-strain and single strain) and oscillatory shear rheometry for the characterization of pharmaceutical hydrogels. They reported that the choice of method for the mechanical characterization of hydrogels depends on the accuracy, speed of measurement, and the amount of sample available. The best agreement was found between the "multi- strain" DMA and rheometer results. In their review, Anseth et al.[36] thoroughly resolved the mechanical properties of polymer-based hydrogels using tensile and DMA tests in dependence on commonomer composition, polymerization conditions and effect of degree of swelling. They reported that the mechanical properties are highly dependent on polymer structure, especially the crosslinking density and the degree of swelling.

For this study, "SA/G hydrogels" with various concentrations of sodium alginate and gelatin were prepared with the aim to obtain and characterize wound dressings suitable for medical applications. Desirable properties of sodium alginate and gelatin were the motivation behind the

choice of these natural materials and the production of highly hydrophilic and biocompatible wound dressings. The ability of both sodium alginate and gelatin to form gel by physical crosslinking was employed with advantage. The viscoelastic properties of SA/G hydrogels were studied using an oscillatory shear rheological analysis and correlated to the various compositions of the hydrogels. Scanning electron microscopy, Fourier transform infrared spectroscopy, and a swelling and viscoelastic technique were utilized for the study of the prepared materials.

EXPERIMENTAL

Materials

Sodium alginate, gelatin (Type B, 250 bloom), glycerol, and sodium chloride (NaCl) (analytical grade) were obtained from Lachema (Czech Republic). Polyethylene glycol (PEG, MW: 3000 g/mol) was purchased from Sigma (Czech Republic). Reverse osmosis purified water was used for the hydrogel preparation and swelling measurements.

Preparation of SA/G hydrogels

The "SA/G hydrogels" were prepared using various concentrations of SA and G while keeping the amount of other components (PEG, glycerol, NaCI and water) constant. An aqueous gelatin solution of (15-30% w/w) was prepared by dissolving polymer granules in water at 80 °C under continuous stirring (300 rpm) until a homogeneous solution was obtained. After dissolving G, relevant portions of SA, PEG, NaCl and glycerol (see TABLE 1) were added. Then, the stirring rate was reduced to 100 rpm and continued at 80 °C for another 5 minutes until a viscous hydrogel was formed. The prepared hydrogel was poured into circular molds (diameter 25 mm) with a thickness of 2 mm and cooled down (samples denoted "A"). Finally, the samples were dried (samples denoted "B") at room temperature for 72 hours. For purpose of comparison, reference samples composed of pure G and pure SA were prepared using the identical procedure.

Characterization of SA/G hydrogels

Scanning Electron Microscopy

The cross-sectional morphologies of the SA/G hydrogels were examined by scanning electron microscopy (SEM) at an accelerating voltage of 20 kV (VEGA\\LMU, TESCAN, Czech

Republic). Cross-sectional samples were prepared by fracturing in liquid nitrogen. Prior to observation, the samples were coated with a thin layer of gold under a vacuum. SEM images were analyzed using an image analysis VEGA Software.

Fourier Transform Infrared Spectroscopy

The chemical composition of the samples B was investigated by Fourier transform infrared spectroscopy. Attenuated total reflectance/ Fourier transform infrared (ATR-FTIR) spectroscopy was conducted with a FTIR instrument (Nicolet 320, Nicolet Instrument Corporation, USA.) using a Zn-Se crystal and the software package OMNIC over the range of 4000-1000 cm^{-1}. A resolution of 2 cm^{-1} was maintained in all cases.

Swelling Behavior

The SA/G hydrogels were immersed in distilled water (pH 7.0) at room temperature. The degree of swelling was determined gravimetrically. The swelled samples (denoted C) were taken from the water at selected time intervals (1, 2, 3, 4, 8, 12 hours), wiped with tissue paper, weighed and placed in water again. The percentage of swelling and water content were calculated using the following equation:

$$Swelling\ \% = \frac{(W_s - W_D)}{W_D} \times 100; \quad (1)$$

$$Water\ content\ \% = \frac{(W_s - W_D)}{W_s} \times 100; \quad (2)$$

where w_S and w_D are the weights of the swollen and dry samples, respectively.

Viscoelastic properties

The viscoelastic properties of the SA/G hydrogels were measured by rotational rheometer (Rheometrics ARES Rheometer, TA Instruments, USA) using a parallel-plate 25 mm in

diameter. All measurements were performed in the linear viscoelastic regime with a strain of 1%. The measurement went through frequency scanning at 33 ^{o}C (surface temperature of human skin[39]) in the frequency range of 0.1-100 rad/s. The storage modulus (G′), loss modulus (G”), complex viscosity (η*), and tan delta (δ= G”/G’) of the samples were recorded as a function of frequency. Each measurement was performed at least twice, on two different disk specimens from the same hydrogel sample.

RESULTS AND DISCUSSION

Morphology of SA/G hydrogels

The effects of SA and G contents on the morphology of dried SA/G hydrogels (sample B) are shown in SEM micrographs in Figure 1. The morphologies of SA/G 100/0 and SA/G 0/100 are depicted in Figure 1(a) and (b), respectively. From the figures it is seen that the pure SA sample shows a particle-like, aggregate structure while the pure G sample exhibits uniform, relatively homogenous morphology.

The morphologies of SA/G hydrogel materials of various compositions (70/30, 60/40, 50/50, 40/60 and 30/70) are shown in Figure 1(c)–(g), respectively. By comparison, it is obvious that the morphologies of the pure samples differ significantly from the binary blends. The morphologies of the SA/G-70/30, 60/40 and 50/50 samples reveal structures having uniform SA as a matrix and G as dispersed phase (Figure 1(c)–(e)). On the other hand, samples with a prevailing amount of G, SA/G 40/60 and 30/70, have G as the matrix and SA as the dispersed phase (Figure 1(f), (g)). It should be noted that for the samples with SA as the matrix, a droplet-like morphology of minor G phase is observed, while samples with G as the matrix render a fibrous morphology of SA phase.

FTIR Characterization of SA/G hydrogels

The FTIR spectra of G/SA hydrogels are shown in Figure 2. The FTIR spectrum of SA/G-100/0 indicates the characteristic absorption peaks observed at 3274 cm^{-1} typical for hydroxyl stretching and a peak at 1637 cm^{-1} which corresponds to a stretch of C=O. Peaks at 1521, 1458, 1408 and 1349 cm^{-1} in the SA spectrum indicate the antisymmetric stretch and symmetric stretch

of – COO^{-} in associated carboxylic acid salt[40, 41]. Two other interactions in the C–O stretch of C–OH groups can be found at 1030, 1080 cm^{-1} and the peak at 1248 cm^{-1} corresponds to the antisymmetric stretching of C–O–C.

The FTIR spectrum of SA/G 0/100 exhibits a peak at 3309 cm^{-1} due to the N–H stretching in secondary amides, the C=O stretching at 1631 cm^{-1} for the amide I, which is characteristic of the coil structure of gelatin,[22] as well as N–H deformation at 1550 cm^{-1} for amide II[40]. Most of the remaining peaks, at 1040, 1079, 1317, 1346, 1404, and 1453 cm^{-1} can correspond to the stretching of C–O bonds. Furthermore, on close inspection of the lower wave number region of the spectrum, two bands are observed at 2877 and 2940 cm^{-1}, due to aliphatic symmetric and C–H stretching, respectively. (The response of C–H bonds is presented by the absorption peaks at 2940 and 2877 cm^{-1}). These results are in agreement with the observation reported by Pawde et al.[20].

The spectra of SA/G hydrogels show peaks at 3265, 1631, 1453, 1408, 1334, 1081, 1028 and 993 cm^{-1}, which indicate O–H stretch, the COO^{-} (asymmetric), COO^{-} (symmetric), C–O, C–O–H respectively, exhibiting strong intermolecular hydrogen bonding[40, 41]. Particularly, the intensity of some peaks decreases with increasing of SA (e.g.1234 cm^{-1}). The adsorption peak, thanks to the stretching of C–O–C ethers coupled with the 1241 and 1119 cm^{-1} of G, shifts to 1234 cm^{-1}. Moreover, the gelatin band related to amides II 1551 cm^{-1} weakens and almost disappears in the SA/G 70/30 sample. The band of SA/G 30/70 at 3291 cm^{-1} shifts to a lower wavenumber (3265 cm^{-1}) with an increasing concentration of SA which reflects increased hydrogen bonding between SA and G.

Swelling behavior of G/SA hydrogels

The degree of swelling indicates the ability of the dressing to absorb the wound fluid and exudates. The swelling behaviour of SA/G hydrogels as a function of time is shown in Figure 3. As can be seen, all samples demonstrate a rapid increase in swelling degree within the first 4 hours, and this swelling continues to increase slowly for up to 12 hours (TABLE 2). However, within the *tested time* interval, only the samples with lower G content reach the equilibrium swelling. It is furthermore obvious that swelling degree decreases with an increase of SA content in the hydrogels, which can be attributed to the structure of the SA matrix, with pores of small sizes hindering the diffusion of water molecules into the hydrogel structure. A similar behavior for SA-based hydrogels has been reported by Bajpai et al.[42] and Anamika et al.[43]. The increase in the swelling degree of hydrogel samples with high G content refers to the strong hydrophilicity of G molecules, which is reportedly due to the presence of an ionizable $NH_2^-$and COO^- functional group[44] that can increase the volume between polymeric chains and the swelling capacity of the hydrogel by hydrostatic repulsion[42, 44].

A maximum swelling degree of SA/G hydrogels at 1176 % was observed in the composition SA/G 30/70. Even such a level of swelling is not a final value and would be slightly higher when approaching equilibrium state. Analogous swelling behaviours were observed for the commercial hydrogel dressings Geliperm® (Geistilich, Switzerland) and Vigilon® (Bard, Crawley, UK)[21, 45]. As to water uptake, the maximum water content of all swelled hydrogels (samples C) was in the range of 74÷94 % (TABLE 2), indicating that the SA/G hydrogels could fulfill the requirement of an ideal wound dressing[1-3, 21, 46] and prevent a wound from accumulation of fluid by absorbing the exudates.

Viscoelastic properties of G/SA hydrogels

The angular frequency dependence of the storage modulus (G') and loss modulus (G") for SA/G hydrogels is depicted in Figure 4. From Figure 4(a) it can be seen that the G' of all hydrogels is almost one order of magnitude higher than G" over the whole angular frequency range and both the G' and G" of all samples slightly increase with angular frequency. In addition, an increase in G' and G" values is observed when the SA concentration rises, probably due to the increased number of SA chains that produce a dense network[42, 48]. Obviously, after 8 hours swelling, the G' and G" of all samples drop dramatically because of the presence of water, which increases mobility of the gel network and decreases stiffness (Figure 4(b)). It should be noted that the described viscoelastic behavior of swelled SA/G hydrogels is in agreement with the viscoelastic response of human skin reported in the literature[44, 49].

In Figure 5(a), the tan δ of samples A is plotted against angular frequency. At first, a plateau is observed until 5 rad/s, then an increasing trend, due to the enhanced influence of the viscous part, attended by a more significant change in G" compare to G', begins. The angular frequency dependency of the tan δ values for swelled samples C is shown in Figure 5(b). Over the whole frequency range tan δ gently increases, along with a slightly steeper increase of G" with the frequency than that of G' (see Fig. 5(a)). Interestingly, the SA/G 70/30 and 30/70 samples are slightly more elastic than the other samples, most probably due to the different morphologies of the prepared hydrogels.

In Figure 6, the dependence of G' and G" on the swelling time of the hydrogels with different ratios of SA/G at the angular frequency ω of 0.39 rad.s^{-1} is shown. The viscoelastic moduli gradually decrease for up to 4 hours swelling time. After this time period elapses, changes of G'

and G" are not so prominent. This observation complies with the swelling behavior depicted in Figure 3 describing the effect of composition and time on the degree of swelling.

The G' and G'' of samples A and C with different compositions, recorded at the angular frequency of 0.39, 3.9 and 39 rad.s^{-1}, are depicted in Figure 7. Comparing samples A and C, it is obvious that after eight hours of swelling both G' and G" drop notably; interestingly G" undergoes a more significant change. Furthermore, as can be seen from Figure 7(a), both G' and G" of samples A decrease with the increasing contents of G. The swelled samples C, contrary to the freshly prepared samples A, does not follow the same decreasing tendency of the storage and loss moduli with G content increase (Fig. 7(b)). The maximum values of moduli for samples C were found in the compositions SA/G 70/30 and 30/70. The viscoelastic properties of these hydrogels are influenced by stiff molecules of SA representing either the continuous phase (SA/G 70/30) or needle-like dispersed particles (SA/G 30/70) in hydrogel structure. The local minimum of G' and G" was observed for hydrogel compositions of SA/G 60/40 and 40/60. The authors believe that such behaviour could be caused by phase separation phenomenon. Using SEM, it was determined that the morphology of 60/40 and 40/60 compositions induced specific porosity levels in a continuous phase matrix (SA/G 60/40) or in discontinuous phase particles (SA/G 40/60). Interestingly, viscoelastic moduli of G/SA 50/50 are higher than those of 40/60 and 60/40 at measured angular frequencies. This can be a consequence of attraction forces between the charged ions leading to intensive interactions by the balanced anionic carboxylate groups ($-COO^{-}$) of SA and the cationic protonated amino groups (NH_3^{+}) of gelatin[50].

Figure 8 presents the relation between complex viscosity, η^*, and angular frequency for samples C with various SA/G compositions and shows that η^* decreases linearly with angular frequency.

Furthermore, decreased η* of samples C have a similar trend as observed for G' and G" (Figure 7(b)).

The storage and loss moduli of samples C as a function of the swelling degree are represented in Figure 9(a), (b). As can be seen in Figure 9(a), the highest values of G' are observed for the hydrogel of SA/G 70/30 composition. Notably, for this hydrogel the smallest change of swelling degree (200-300%) was connected with the most significant decline of storage modulus. On the other side, only a minor G' change over the widest region of swelling degree (400-1200%) was determined for the hydrogel with the opposite SA/G ratio of 30/70. The changes of swelling degree as well as changes of G' (ΔG") observed for samples with the composition of SA/G 60/40, 50/50 and 40/60 lie between the above mentioned SA/G 30/70 and 70/30 hydrogels. Furthermore, from Figure 9(a) it is clear that while the highest G' of unswelled samples was found for SA/G 70/30, the highest G' values of fully swelled hydrogels were recorded simultaneously for SA/G 70/30 and SA/G 30/70 samples.

Values of G" for all studied samples are almost an order of magnitude lower than those of G' over the entire range of swelling (see Fig 9(b)). The results also reveal that the levels of G" follow the trend described for G'. However, the magnitude of G" changes (ΔG") is independent of hydrogel composition, thus differing significantly from the behavior of G' and indicating predominant elastic response within the course of hydrogel swelling.

The described results, namely the values of elastic (G') and viscous (G") moduli and the swelling degrees of the prepared samples, indicate that the viscoelastic properties of SA/G hydrogels are appropriate for wound dressing[51, 52].

CONCLUSIONS

In this work, SA/G hydrogels based on sodium alginate (SA) and gelatin (G) are introduced as potential wound dressing materials. In order to evaluate the performance of SA/G hydrogels for wound dressing applications, the morphology, swelling behavior and viscoelastic properties of hydrogels with various ratios of SA and G have been investigated. The results demonstrate that SA/G ratio remarkably influences the structure and morphology of hydrogels. In particular, it is obvious that with a decrease in SA content, the respective hydrogel morphology changes from particle-like to fibrous-like. It can also be observed that the hydrogel morphology closely correlates with the swelling degree, which decreases with an increase in SA content. Results from the determination of water content and swelling behavior show that hydrogels exhibit a high capability to absorb fluid, making them promising materials for exudative wound dressings. The viscoelastic properties of SA/G hydrogels notably depend on their composition and are related to the swelling behavior of hydrogels. The viscoelastic properties of SA/G hydrogels decreased, with an increased swelling degree due to water absorption into the hydrogel structure. The optimum SA/G ratio in terms of a compromise between viscoelastic properties and absorption abilities was found to be SA/G 50/50. The hydrogel with this composition shows the viscoelastic response fully comparable to that of human skin while simultaneously retaining very good absorption properties.

AKCNOWLEDGEMENT

This article was created with support of Operational Program Research and Development for Innovations co-funded by the European Regional Development Fund (ERDF) and national budget of Czech Republic, within the framework of project Centre of Polymer Systems (reg. number: CZ.1.05/2.1.00/03.0111). The authors are thankful to the Ministry of Education,

Youth and Sports of the Czech Republic (MSM 70088352101) and the Ministry of Industry and Trade of the Czech Republic (project 2A-1TP1/126) for the financial support provided.

REFERENCES

1. Jones, A; Vaughan, D. *J. Orthop. Nurs.* **2005**, 9, 51-60.

2. Lloyd L.L.; Kennedy, J.F.; Methacanon, P.; Paterson, M.; Knill C.J. *J. Carbohyd. Polym.* **1998**, 37, 315-322.

3. Rovee, D. T.; Howard I. Maibach In The epidermis in wound healing; Stephen Thomas., Eds.; CRC Press: Washington, DC, **2004**; Vol. 4, pp. 215-245.

4. Boateng, J. S.; Matthews, K. H.; Stevens, H. N.; Gillian. M. Eccleston. *J. Pharma Sci.* **2008**, 97, 2892-2923.

5. Smith, T. J.; Kennedy, J. E.; Higginbotham, C. L. *J. Mater. Sci.: Mater. Med.* **2009**, 20, 1193-1199.

6. Northern Health; Social Services Board. *NHSSB wound management manual*, Antrim, United Kingdom. **2005**, 10-21.

7. Smith, T. J.; Kennedy, J. E.; Higginbotham, C. L. *J.Mecn. Behav of Biomed. Mater.* **2009**, 2, 264-271.

8. Rehm, B. H. A. Alginates: Biology and Applications; Ivan Donati, Sergio Paoleti., Eds.; Springer-Verlag: Berlin Heidelberg, **2009**; vol. 13, Chapter 1, pp 1-55.

9. Immirzi, B.; Santagata, G.; Vox, G.; Schettini, E. *J. Biosys. Eng.* **2009**, DOI: 10.1016/j.

10. Coviello, T.; Matricardi, P.; Marianecci, C.; Alhaique, F. *J. Control. Rel.* **2007**, 14, 5-24.

11. Jong, O. K.; Jung, K. P.; Jeong, H. K.; Sung, G. J.; Chul, S. Y.; Dong, X. L. *J. Pharma.* **2008**, 359, 79-86.

12. Thomas, A; Harding, K. G.; Moore, K. *J. Biomater*. **2000**, 21, 1797-1802.

13. Chandra, W. P.; Sharma, P. In: Polysaccharides: Biomedical Applications; Ponisseril, S.; Arthur, H. Eds.; CRC. Taylor Fracis: Florida, **2006**; Vol. 7, pp 5056-5068.

14. Farris, S.; Schaich, K. M.; Liu, L. S.; Piergiovanni, L.; Yam, K. L. *J. Trend. Food Sci. Techn.* ***2009***, 20, 316-332.

15. Arvanitoyannis, I. S. In Formation and Properties of Collagen and Gelatin films and Coatings, Aristippos Gennadios, Eds,; CRC Press LLC: Florida, **2000**; Vol. 11, pp. 275-304.

16. Sperling, L. H. Ed.; Introduction to Physical Polymer Science: Gelation and Network Formation.; John Wiley & Sons, Inc.: Canada , **2006**; Vol. 9, pp. 473-501 :

17. Schrieber, R.; Gareis, H. *Gelatine Handbook*. Eds., Wiley: Eberbach, Germany, **2007**; Vol. 6, Chapter 2, pp 48-117.

18. Peng, H. T.; Martineau, L.; Shek, P. N. *J. Mater Sci: Mater Med*. **2008**, 19, 997-1007.

19. Zhang, Z.; Guoying, L; Shi, B. *J. Society of Leath. Techn. Chem*. **2006**, 90, 23-28.

20. Pawde, S. M.; Deshmukh, K. *Appl. Polym. Sci.* **2008**, 109, 3431-3437.

21. Yoo, H. J.; Kim, H. D. *J. Pharma*. **2007**, 359 326-332.

22. Yakimets, I.; Paes, S. S.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Mitchell, J. R. *J. Biomacromolec*. **2007**, 8, 1710-1722.

23. Slaughter, B.V.; Khurshid, S. S.; Fisher, O. Z.; Khademhosseini, A.; Peppas, N. A. *J. Adv. Mater*. **2009**, 21, 3307-3329.

24. Cole, S. (Garbe, M. co). U.S. Patent 4,948,575, August 14, 1990.

25. Murakami, K.; Aoki, H.; Nakamura, S.; Nakamura, S.; Takikawa, M. *J. Biomaterials.* **2010**, 31, 83-90.

26. Mei, H. H.; Ming, C. Y. *J. Polym. Adv. Technol*. **2009**, 4, 1-7.

27. Chun, M. D.; Lan, Z. H.; Ming, Z; Dan, Y.; Yi Liu. *J. Carbohyd. Polym.* **2007**, 69, 583-589.

28. Weng, L.; Pan, H.; Chen, W.; *J Biomed: Mater. Res. A.* **2007**, 2, 352-365.

29. Choi, Y. S.; Lee, S. B.; Hong, S. R.; Lee, Y. M.; Song, K. W.; Park, M. H. *J. Mater. in Med.* **2001**, 12, 67-73.

30. Mallika, S. K. Imaging viscoelasticity in hydropolymers and breast tissue ultrasound. PhD Thesis, University of Califorina, Davis, Fall 2006.

31. Meyvis, T. K. L.; Stubbe, B. G.; Van Steenbergen, M. J.; Hennik, W. E.; De Smedt, S. C.; Demeester, J. *J. Pharma*. **2002**, 244, 163-168.

32. Matthews, K. H.; Auffret, A. D.; Humphrey, M. J.; Stevens. H. E.; Eccleston G. M.; *J. Pharma*. 2005, 289, 51-62.

33. Razzak, M. T.; Erizal, Z.; Dewi, S.P.; Lely, H.; Taty, E.; Sukirno C. *J. Radiation. Phys. Chem*. **1999**, 55, 153-165.

34. Stadler, R.; Gronski, W. *J. Col. Polym. Sci*. **1983**, 261, 215-223.

35. Cote, G. L. (Gant, R. M.; Melissa, G.; Yaping, A, H. co). U.S. Patent 2, 010, 005, 6894, March 4, 2010.

36. Anseth, K. S.; Bowman, C. N.; Peppas, L. B. *J. Biomater*. **1996**, 17, 1647-1657.

37. Otegui, J.; Fernandez, E.; Retama, J. R.; Cabarcos, E. L.; Mijiangos, C.; Lopez, D. *J. Polym. Engineer. Sci.* **2009**, 49, 964-970.

38. Lutz, P. J. *J. Polym. Bullet*. **2007**, 58, 161-171.

39. Denda, M.; Sokable, T.; Tominaga, T. F.; Tominaga, M. *J. Invest. Dermat*. **2007**, 127, 654-659.

40. Lambert, B.; Shurvell, H.F.; Lightner, D.; Cooks, R. G. *Organic Structural Spectroscopy*. Ed., Wiley: New York, **2007**, Chapter 8, pp .158-202.

41. Barbara, H. S. Infrared Spectroscopy: Fundamentals and applications. Ed., Wiley: Atrium, England, **2004**; Chapter 4, pp 71-93.

42. Bajpai, J.; Mishra, D.; Bajpai, A.K. *J. App. Polym. Sci*. **2007**, 106, 961-972.

43. Anamika. R.; Bajpai, J.; Baijai, K. *J. Chem. Techn*. **2009**, 16, 388-395.

44. Agustin, M. R.; Fausto, B. B.; Juan, C.; Sanchez, D.; Alejandro, G. A. *J. Polym. Bullet*. **2009**, 62, 539-548.

45. Biji. B.; Mohantyb, M.; Umashankarc, P.R.; Jayakrishnana, A. *J. Biomater*. **2005**, 26, 6335-6342.

46. Mehrdad, K.; Mohammad, S.; Zuhair, M. H. *J. European Polym*. **2007**, 42, 773-781.

47. Holt, B.; Tripathi, A.; Mordan, J. *J.Biomech*. **2008**, 28, 2689-2695.

48. Barbara, I.; Gabriella, S.; Giuliano, V.; Evelia. S. *J. Biosys. Eng*. **2009**, 102, 461-472.

49. Parul, N.; Connie, K. S.; Charles, W. P. *J.Biomech*. **2005**, 3, 313-319.

50. Zhanfeng, D.; Qun, W; Yumin, D. *J. Memb. Sci*. **2006**, 280, 37-44.

51. Simonida, L. T.; Maja M. M.; Sava. N. D.; Jovanka M. F.; Suljovrujic, E. H. *J. Rad. Phys. Chem*. **2009**, 1, 1-7.

52. Ademar, B. L.; Sizue, O. R.; Sonia, M. M. *J. Rad. Phys. Chem.* **2002**, 63, 543-546.

TABLES CAPTION:

TABLE 1 Composition of the SA/G hydrogels

TABLE 2 Description of the SA/G hydrogel samples

FIGURES CAPTION:

FIGURE 1 SEM micrographs of dried hydrogels: (a) SA/G-100/0, (b) SA/G-0/100, (c) SA/G-70/30, (d) SA/G-60/40, (e) SA/G-50/50, (f) SA/G-40/60, (g) SA/G-30/70.

FIGURE 2 FTIR spectra for: (a) SA/G-100/0, (b) SA/G -70/30, (c) SA/G -60/40, (d) SA/G – 50/50, (e) SA/G - 40/60, (f) SA/G -30/70, (g) SA/G -0/100.

FIGURE 3 Effect of composition and time on the degree of swelling.

FIGURE 4 Storage (G') and Loss (G'') moduli of freshly prepared samples A (a) and 8 hours swelled samples C (b).

FIGURE 5 Effect of angular frequency on tan δ of samples A (a) and C (b).

FIGURE 6 Effect of swelling time on storage modulus (G') and loss modulus (G'') of SA/G hydrogels.

FIGURE 7 The effect of composition on storage modulus (*G'*) and loss modulus (*G''*) of samples A (a) and samples C (b).

FIGURE 8 Effect of angular frequency on complex viscosity of samples C.

FIGURE 9 Relation between viscoelastic properties (G' and G'') at angular frequency of 0.39 rad.s^{-1} and degree of swelling (%) for samples C.

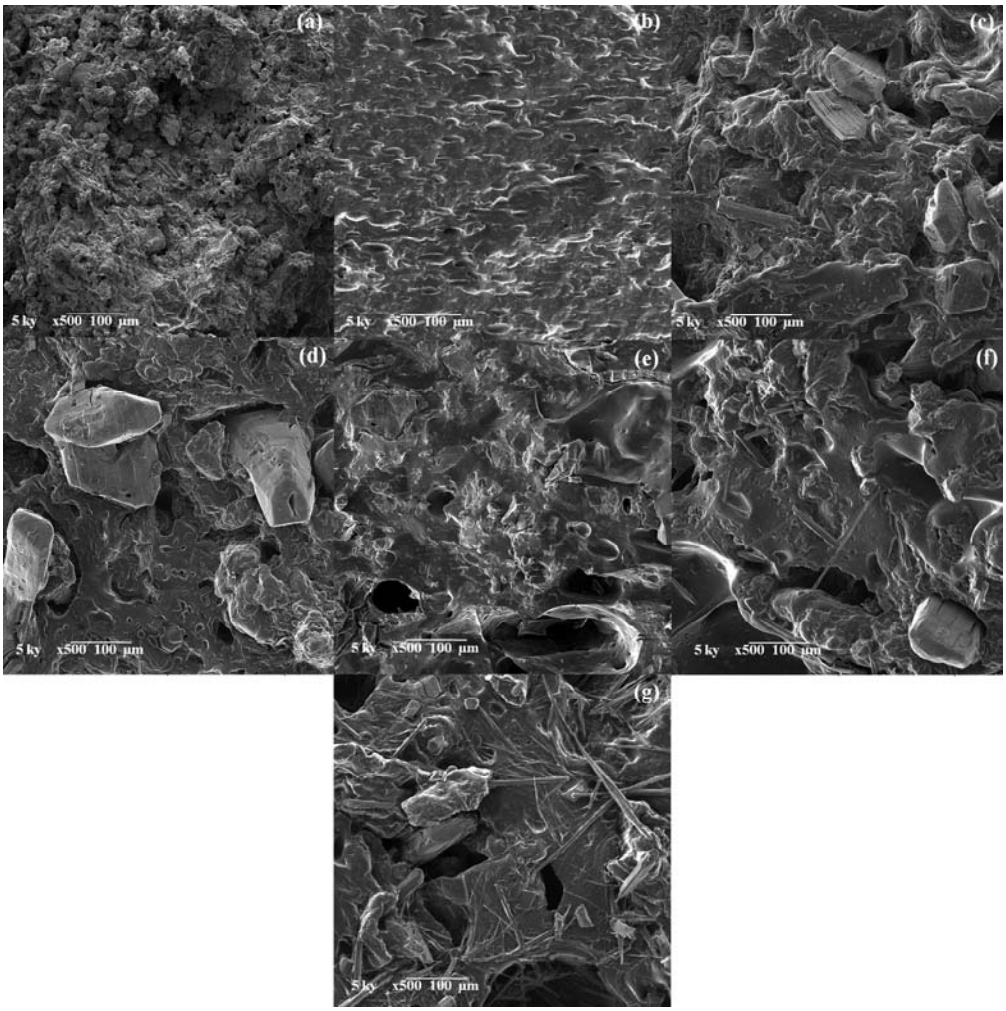
TABLE 1 Composition of the SA/G hydrogels

Hydrogel composition SA/G	70/30	60/40	50/50	40/60	30/70
Gelatin (G) [g]	3	4	5	6	7
Sodium alginate (SA) [g]	7	6	5	4	3
Poly Ethylene Glycol (PEG) [g]	2	2	2	2	2
Glycerol [g]	2	2	2	2	2
Sodium Chloride (NaCl) [g]	0.2	0.2	0.2	0.2	0.2
Water [g]	20	20	20	20	20

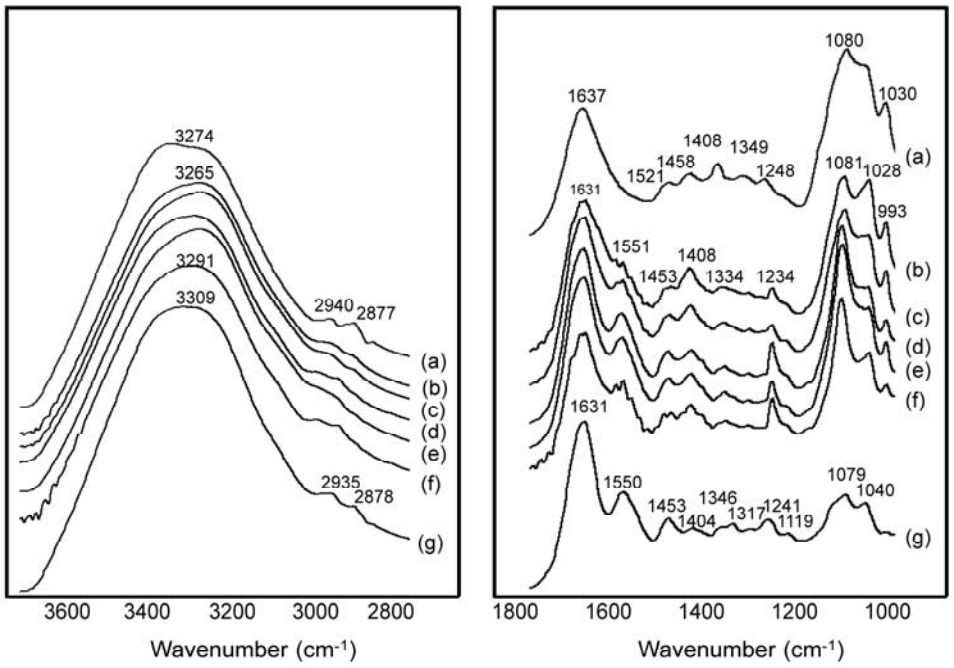
TABLE 2 Physical characteristics of the SA/G hydrogel samples

Hydrogel composition SA/G	70/30	60/40	50/50	40/60	30/70	Image of hydrogels
Samples after preparation A (before drying), n = 3						
Weight (g)	0.735±0.064	0.729±0.051	0.894±0.117	0.858±0.161	0.876±0.094	
Diameter (mm)	25.0±0.00	25.0±0.00	25.0±0.00	25.0±0.00	25.0±0.00	
Thickness (mm)	1.94±0.23	1.74±0.18	1.99±0.21	1.70±0.33	1.76±0.25	
Moisture content (%)	49.2±0.52	46.9±0.76	49.4±0.17	49.1±1.24	52.9±0.86	
Dried samples B (after drying), n = 3						
Weight (g)	0.373±0.032	0.386±0.027	0.452±0.059	0.452±0.082	0.412±0.046	
Diameter (mm)	19.82±0.40	20.25±0.54	20.71±0.26	19.96±0.14	20.92±0.56	
Thickness (mm)	1.54±0.19	1.23±0.12	1.39±0.19	1.19±0.22	1.02±0.16	
Swelled samples C (12 h after swelling), n = 3						
Weight (g)	1.387±0.201	2.285±0.47	2.295±0.64	3.804±0.81	3.981±0.92	
Diameter (mm)	31±2.14	38±3.69	38.583±3.6	42.21±2.49	43.42±3.87	
Thickness (mm)	2.29±0.31	2.48±0.198	2.55±0.32	2.83±0.49	2.90±0.42	
Water content (%)	72.54±4.68	81.86±6.53	84.03±3.96	87.97±3.46	90.1±3.74	

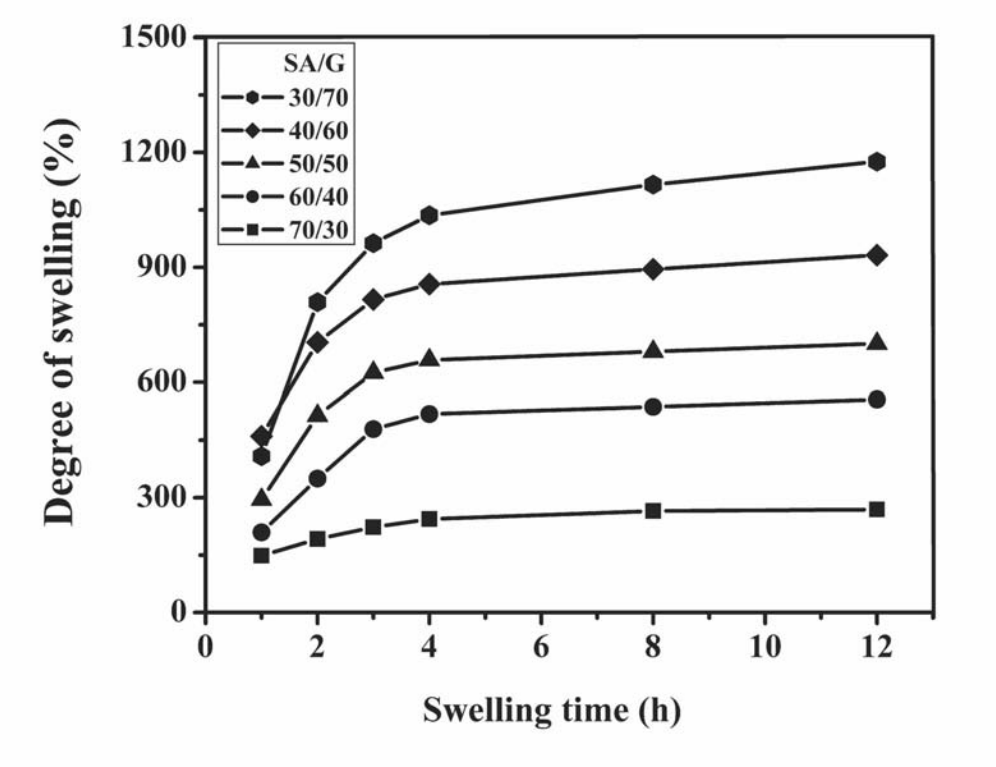
**All measurements were done in triplicate.*



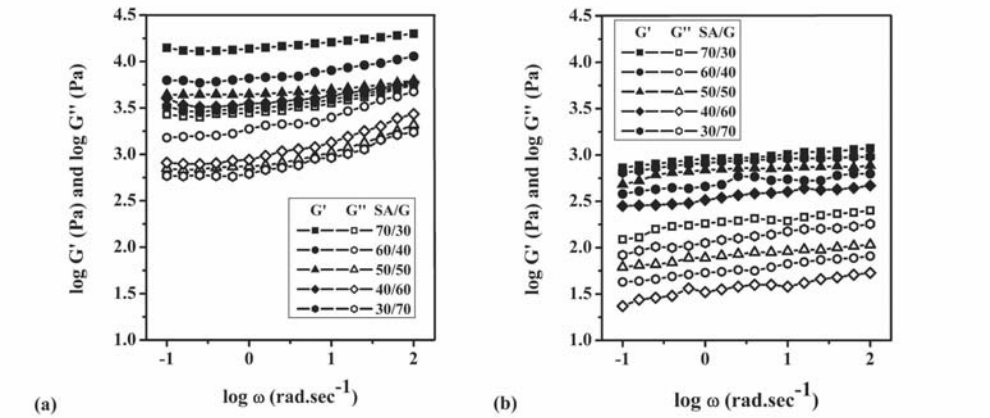
SEM micrographs of dried hydrogels: (a) SA/G-100/0, (b) SA/G-0/100, (c) SA/G-70/30, (d) SA/G-60/40, (e) SA/G-50/50, (f) SA/G-40/60, (g) SA/G-30/70
153x153mm (600 x 600 DPI)



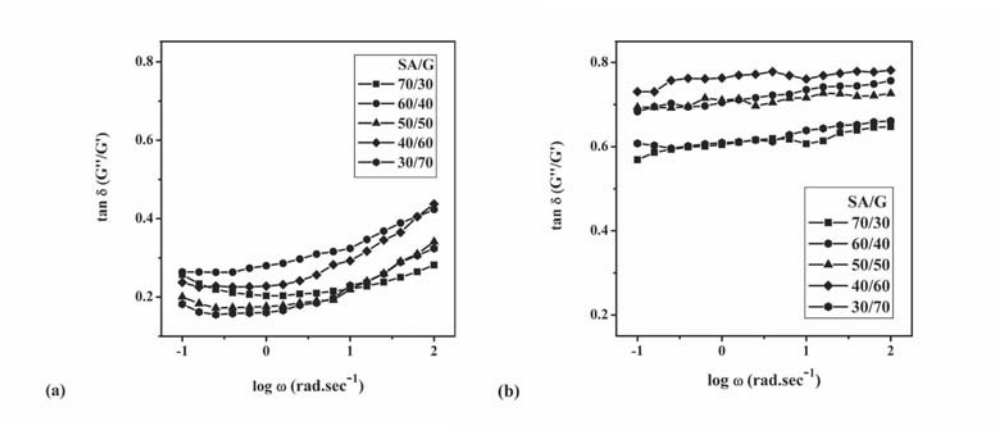
FTIR spectra for: (a) SA/G-100/0, (b) SA/G -70/30, (c) SA/G -60/40, (d) SA/G – 50/50, (e) SA/G - 40/60, (f) SA/G -30/70, (g) SA/G -0/100
301x210mm (600 x 600 DPI)



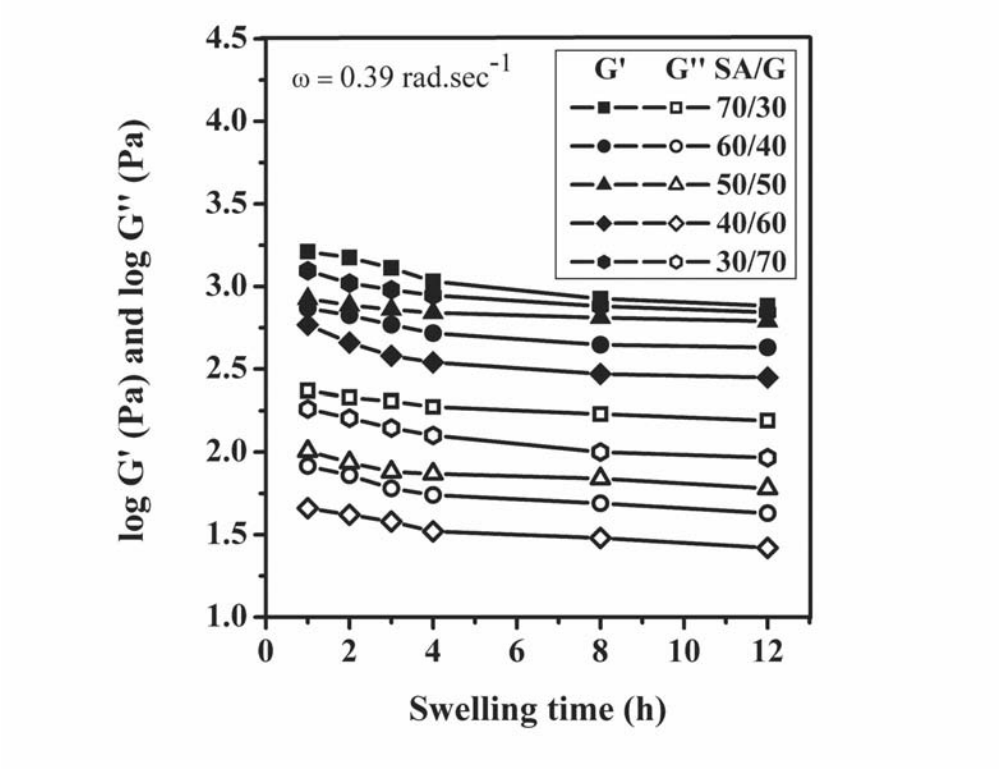
Effect of composition and time on the degree of swelling
89x69mm (600 x 600 DPI)



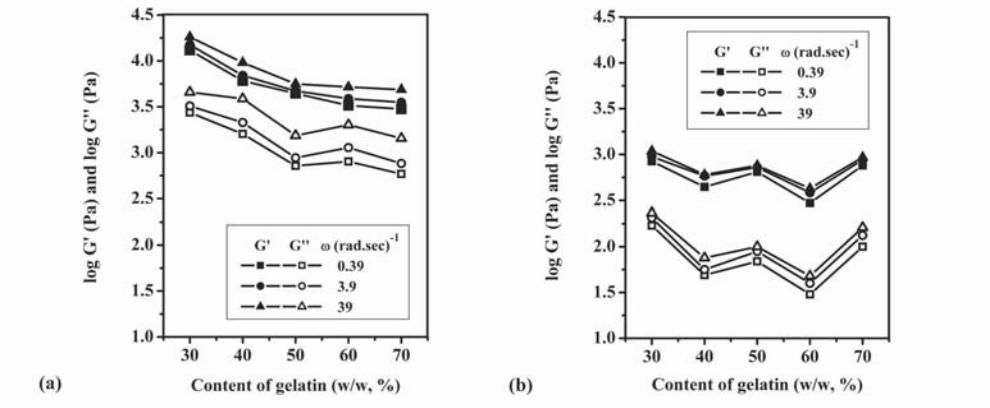
Storage (G') and Loss (G'') moduli of freshly prepared samples A (a) and 8 hours swelled samples C (b)
84x35mm (600 x 600 DPI)



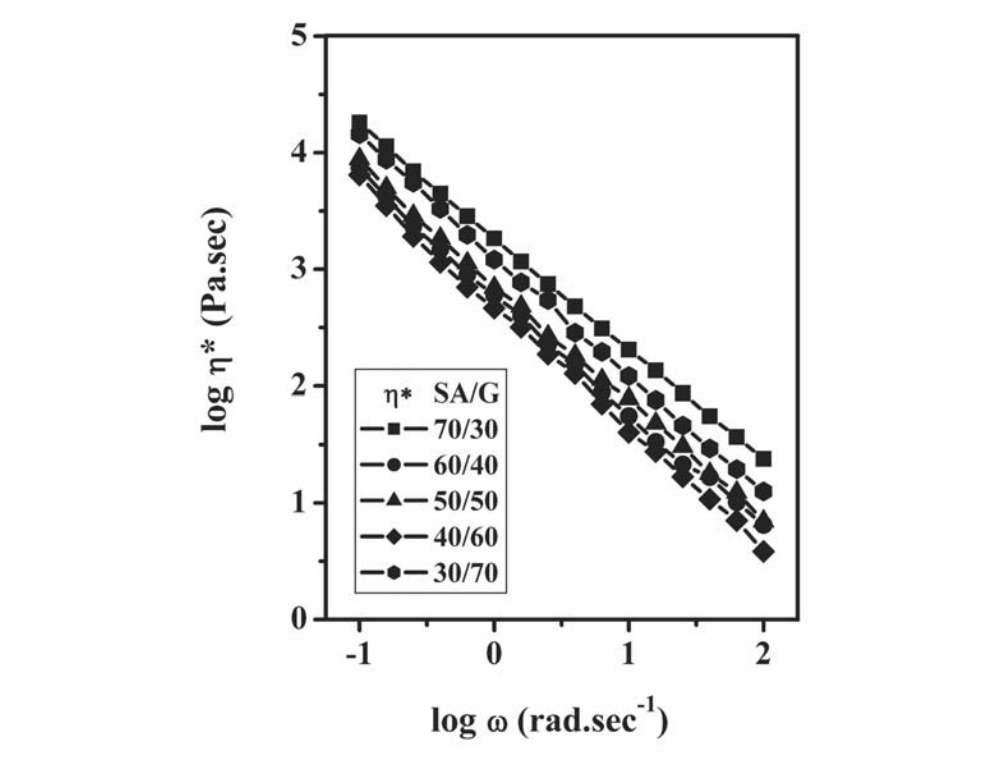
Effect of angular frequency on tan δ of samples A (a) and C (b)
87x38mm (600 x 600 DPI)



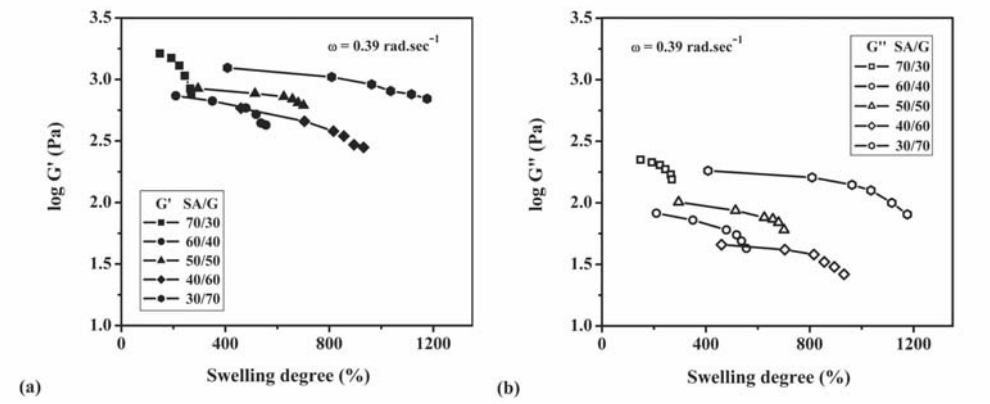
Effect of swelling time on storage modulus (G') and loss modulus (G'') of SA/G hydrogels
89x69mm (600 x 600 DPI)



The effect of composition on storage modulus (G') and loss modulus (G'') of samples A (a) and samples C (b)
89x37mm (600 x 600 DPI)



Effect of angular frequency on complex viscosity of samples C
89x69mm (600 x 600 DPI)



Relation between viscoelastic properties (G′ and G′′) at angular frequency of 0.39 rad.s-1 and degree of swelling (%) for samples C
89x36mm (600 x 600 DPI)

PUBLICATION III

On the Characterisation of Genipin Crosslinked Sodium Alginate/Gelatine-Based Hydrogels for Wound Dressing

Amarjargal Saarai, Tomas Sedlacek, Vera Kasparkova

Journal of the Mechanical Behavior of Biomedical Materials,

Prepared for publication

On the Characterization of Genipin Crosslinked Sodium Alginate/Gelatine Hydrogels for Wound Dressing

A. Saarai [1,2], V. Kasparkova [1,3], T. Sedlacek [1,2]

1) Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Nad Ovcirnou 3685, 760 01 Zlin, Czech Republic

2) Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, nam. T. G. Masaryka 275, 760 01 Zlin, Czech Republic

3) Department of Fat, Surfactant and Cosmetics Technology, Faculty of Technology, Tomas Bata University in Zlin, nam. T.G. Masaryka 5555, 760 01 Zlin, Czech Republic

Key words: sodium alginate; gelatine; hydrogels; genipin; swelling; viscoelastic properties;

Abstract

In an attempt to overcome the cytotoxicity problem of the chemically crosslinked hydrogels, genipin (GP) was used for development of a naturally crosslinked wound dressings based on sodium alginate (SA) and gelatine (G). The prepared hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy, swelling tests, and dynamic mechanical analysis (DMA). FTIR spectra of SA/G-GP hydrogels revealed an increase in amide I and II absorbancies indicating the formation of heterocyclic compound of GP linked to the G and also the formation of the secondary amide group as a result of the reaction between G and GP. With increasing GP concentration, the swelling degree was markedly reduced and the thermal stability enhanced. DMA analysis revealed that the SA/G hydrogels with GP, could form relatively consistent elastic gels with appropriate viscoelastic properties and swelling behaviour. Since GP is assigned to have low toxicity, prepared hydrogels appear to be a promising candidate for biomedical applications, such as wound dressings.

1. Introduction

In recent years there has been a growing interest in the use of natural polymers, including sodium alginate (SA) [1-6] and gelatine (G) [7-11], for the development of hydrogels for biomedical applications. In the case of uncrosslinked SA/G hydrogels, the main limitation arises from their rapid dissolution in aqueous media near 37 °C, as the formed SA/G matrix is stabilized only by the electrostatic interactions between carboxyl groups of SA and amino groups of G [13-15]. The improved thermostability and mechanical properties of the hydrogels can be achieved through chemical or physical crosslinking [13, 16-21]. However, for chemical crosslinkers such as formaldehyde, glutaraldehyde and carbodiimides, problems can occur related to cytotoxic effects of unreacted agents that may impair the biocompatibility of the hydrogel [17, 22-26]. To avoid these drawbacks, physical crosslinking is preferred for the preparation of biocompatible hydrogel, since neither reactive groups nor crosslinking agents are required. Nevertheless, it is difficult to obtain the appropriate strength of crosslinked network solely by physical bonds. It is, therefore, desirable to exploit a suitable chemical crosslinking agent that can form stable and biocompatible hydrogels, acceptable for example as wound dressing, without cytotoxic reaction.

It has been reported that a naturally occurring crosslinking agent, genipin (GP), obtained from the fruits of *Gardinia Jasminoides Ellis* [16, 22, 23, 27-29], can fulfil requirements of biocompatibility, as it is about 5000–10,000 times less cytotoxic than glutaraldehyde [16, 30-32]. Therefore, it has increasingly been employed for crosslinking of G [11, 16-18, 23-24, 33-35], particularly for biomedical applications. According to Sung et al. [36], Liang et al. [37] and Butler et al. [38], the reaction between G and GP is a two-step process comprising two distinct reactions. The first reaction occurs when the GP molecules undergo a nucleophilic attack by the primary amines of G, resulting in the heterocyclic linking of GP to the G amine. This reaction is followed by the second one, in which the ester group on GP undergoes a nucleophilic substitution. The resultant covalent crosslinks between the primary residues leave minimal residual toxicity [31, 32, 39-41]. Liang et al. [24], investigating the structure of GP or carbodiimide- crosslinked gelatine hydrogels using gel permeation chromatography, found that besides intramolecular and short-range intermolecular crosslinks, additional long-range intermolecular crosslinks could be introduced into GP crosslinked gelatine hydrogel. The cytotoxicity of this hydrogel was found to be lower than that of carbodiimide crosslinked

material. Bigi et al. [17] reported that gelatine films crosslinked by GP show mechanical, thermal and swelling properties comparable to those crosslinked with glutaraldehyde (GTA) simultaneously presenting lower cytotoxicity. Chang et al. [23] used GP for G hydrogel crosslinking with intention to develop a wound dressing membrane and they found that the tissue inflammation observed after hydrogel application was less severe and the healing rate was notably faster than those observed for GTA crosslinked dressing. The authors emphasized that the biocompatibility of the GP crosslinked dressing was superior compared to GTA crosslinked one. These encouraging results and biologically advantageous properties of GP based hydrogels were motivation for using this naturally occurring crosslinking agent to prepare SA/G based hydrogels for wound dressings.

The purpose of the study was to develop and characterize naturally derived SA/G hydrogels crosslinked by GP. The main attention was paid to the optimal hydrogels composition for the production of biocompatible wound dressing with satisfactory swelling and viscoelastic properties. Obtained hydrogels were thoroughly characterized using scanning electron microscopy, Fourier transform infrared spectroscopy, swelling tests, and dynamic mechanical analysis.

2. Materials and methods

2.1. Materials

Low viscosity sodium alginate (W2011502) with a high content of mannuronic acid (molar ratio of mannuronic acid to guluronic acid (M/G) of 1.56) and a molecular weight of around 40000 g/mol was purchased from Sigma-Aldrich (Czech Republic). Gelatine (Type B, bovine skin, bloom 225 with a molecular weight of 142000 g/mol), polyethylene glycol (PEG), NaCl, glutaraldehyde (25% aqueous solution) were obtained from Sigma-Aldrich (Czech Republic). Genipin (molecular weight 226.23 g/mol, 98% by HPLC) was purchased from Sigma-Aldrich (USA). Glycerol, $CaCl_2$, Na_2HPO_4 and NaH_2PO_4, NaOH were supplied by Lachema (Czech Republic). The reverse osmosis purified water was used for the hydrogel preparation.

2.2. Preparation of SA/G-GP hydrogels

Gelatine was dissolved in water at temperature of 50 °C under continuous stirring at 100 rpm to obtain 10 wt% solutions. The pH of aqueous gelatine solution was adjusted to 7.5 with 0.1 N NaOH at 37 °C and filtered. Then, GP was added to the G solutions to obtain G-GP reaction mixtures with two different weight percentages of GP, 0.5 and 1. The G-GP mixture was kept at 35 °C under continuous stirring at 150 rpm till the crosslinking reaction was finished, resulting in the change or the originally light-yellow G solution colour to dark-blue gel with enhanced viscosity. Whereas the G-GP mixture with 1.0 wt% of GP afforded a consistent dark-blue gel within 3 hours, the lower G amount required a longer reaction time of 5 hours.

SA/G-GP blends with different weight ratios of SA/G: 0/100, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20 and 100/0 were prepared by addition of filtered 10% aqueous SA solution to reacted G-GP and the stirring rate was reduced to 100 rpm and continued at 35 °C for another 30 min. Further, the SA/G-GP blends were added with 0.1 wt% of PEG and glycerol under vigorous stirring which continued for 10÷20 min at 35 °C. Obtained homogeneous gel was poured into circular moulds and cooled down to room temperature. The fresh samples were taken out of the moulds and dried at room temperature for 72 h. Finally, dried samples were washed several times in deionized water to remove GP residues and dried again. SA/G samples with 0.5 and 1.0 GP contents were coded as: SA/G-0.5GP and SA/G-1.0GP, respectively.

2.3. Characterization of SA/G-GP hydrogels

2.3.1. Scanning Electron Microscopy

The cross-sectional morphologies of the SA/G-GP hydrogels were examined by scanning electron microscopy (SEM) at an accelerating voltage of 20 kV (VEGA\\LMU, TESCAN, Czech Republic). Cross-sectional samples were prepared by fracturing in liquid nitrogen. Prior to observation, the samples were coated with a thin layer of gold under vacuum.

2.3.2. Fourier Transform Infrared Spectroscopy

The chemical composition of the samples was verified by Fourier transform infrared spectroscopy. Attenuated total reflectance/Fourier transform infrared spectroscopy (ATR-FTIR) was conducted with a FTIR instrument (Nicolet 320, Nicolet Instrument Corporation, USA) using a Zn-Se crystal and software package OMNIC over the range of 4000-1000 cm^{-1}. A resolution of 2 cm^{-1} was maintained in all cases.

2.3.3. Swelling Behaviour

The swelling degree (SD) was determined gravimetrically, as follows. The SA/G-GP hydrogels were immersed in Phosphate buffered saline (PBS) with pH 7.4 at 37 °C. The swelled samples were taken from the PBS at selected time intervals of 0.5, 1.5, 3, 5, 8, 12 and 24 hours, wiped with tissue paper, weighted and placed in PBS again. The SD, in percentage, was calculated using Equation (1):

$$SD = \frac{(W_S - W_D)}{W_D} \cdot 100\ [\%]\ ; \qquad (1)$$

where W_S and W_D are the weights of the swollen and dry samples, respectively.

2.3.4. Dynamic Mechanical Analysis

Visco-elastic properties of the SA/G-GP hydrogels were determined with the help of dynamic mechanical analyzer (Mettler Toledo, model DMA/SDTA 861^e, Switzerland) using a double shear sandwich fixture. Applied force amplitude of 0.02 N and the displacement amplitude of 10 μm assured the linear viscoelastic regime during all experimental conditions. Firstly, the frequency sweep test was carried within frequency range of 0.1-200 Hz at 37 °C. Consequently, the storage modulus, G' , loss modulus, G'' and *tan δ* (*tan δ = G''/G'*) of swelled samples were recorded as a function of temperature over the range of +5 °C to +40 °C at a frequency of 10 Hz and a heating rate of 3 °C/min.

3. Results and Discussion

3.1. Morphology of SA/G-GP hydrogels

SEM analysis, performed on the cross-sections of dried SA/G hydrogels in order to evaluate the effect of the content of both polymers and GP on samples morphologies, of SA/G-0.5GP and SA/G-1.0GP are depicted in Figure 1 and 2, respectively.

SEM images clearly indicate significant changes in the morphology of the studied hydrogels in dependence on their composition. Compared to uncrosslinked blends [12], the crosslinked ones are morphed into more dense structures. The homogeneous inter-connected network structure of G matrix with SA noticed as fibrous layers is observed (see Fig. 1(a) and 2 (a)) for samples with high G content (80%). Moreover, comparing Fig. 2 b) to e) and g), it is obvious that increasing SA content in the hydrogels is connected with further changes in their structure, namely transition from discontinuous fibrous to more uniform morphology. Another example of the morphology change is found for SA/G-70/30 hydrogel crosslinked with 1.0GP having characteristics droplet-like morphology (Fig. 2 f). As it is clear from Figs. 1 (e), (f) and 2 (g), coarse-grained internal structure is observed in samples with SA contents above 40% (0.5GP) and 80 (1.0GP). In this connection, it is also noteworthy that the viscoelastic properties of these samples (SA/G-0.5GP-60/40, 70/30 and SA/G-1.0GP-80/20) could not be determined due to the phase separation of individual hydrogel component. Finally, it should be noted that for hydrogels with SA content of 50% (Fig. 1(d) and 2 (d)), the observed samples itemize smooth and homogenous cross-sectional surface morphology, irrespective of GP content, which is characteristic for good miscibility of the blend.

3.2. FTIR of the SA/G-GP hydrogels

FTIR spectra of uncrosslinked SA/G blends together with SA/G-0/100 and SA/G-100/0 samples are presented in Figure 3 (a). SA/G-0/100 are characterized by amino bands at 1241 and 1544 cm^{-1}, a strong carbonyl peak at 1637 cm^{-1} and a broad peak of primary amine (N-H stretching) at 3305 cm^{-1}. The spectrum of SA/G-100/0 has peaks at 1030 and 1089 cm^{-1} assigned to the typical of SA saccharide structure [42-44], carboxyl group peaks at 1602 and 1408 cm^{-1}, and a typical hydroxyl peak (O-H) at around 3349 cm^{-1}. The shift of hydroxyl, carbonyl, and amino bands in the spectra of these uncrosslinked SA/G hydrogels confirms that G and SA formed hydrogen bonding (COOH-HO, NH_2-HO) and electrostatic attraction (NH_2^+ or NH_3^+-

COO^-) in prepared blends [44-46]. This is in accord with previous studies which have revealed the peak shifts, differences in peak shapes and the appearance of new bands dependent on changes in the ratio of SA and G [12].

When SA/G hydrogels are submitted to crosslinking with GP, conformational changes can occur as a result of structural rearrangement of chains forming covalent bonds. Increasing content of GP from 0.5 to 1.0 wt% did not produce any changes in the vibrational spectra of prepared hydrogels. Hence only the FTIR spectra of SA/G-1.0GP samples with the same ratio of SA/G as in the case of uncrosslinked hydrogel are depicted in Fig. 3 (b) , together with GP used for crosslinking. In the figure it can be seen that new peaks does not appear after the reaction with GP, which corresponds to observations published in [11, 18, 47]. The structural changes related to crosslinking could be revealed by differences in amides I (C-O stretching), II (N-H deformation), and III (C-N stretching and N-H deformation) in the spectra, compared to SA/G blends without crosslinking [12]. It can be observed that spectra of crosslinked SA/G-GP samples are attended with only minor variations with respect to their composition [33,47] and the relative intensity of the amide I and II bands is moderately increased, at almost similar wave numbers as it is observed for uncrosslinked hydrogels. In addition, the relative intensity of these bands grows as the G content in the SA/G-GP hydrogels is arisen, which can be attributed to the formation of heterocyclic compound of GP linked to the gelatine [38]. Furthermore, the apparent increase in the intensity of the amide I band also indicates the formation of the secondary amide group formed by the reaction between the amino groups of G and ester groups of the GP [33, 47]. Moreover, overlapping N-H and O-H stretching bands results in a shift towards lower wavenumbers and a decrease in the relative intensity (Fig. 3(b)), probably due to the conformational changes of G after reaction with GP [30, 46, 48]. The other peaks related to the C-H asymmetric and symmetric stretching (2937 cm^{-1}, 2871 cm^{-1}), amide III (1407cm^{-1}) and C-H and C-O stretching (around 1090 and 1030 cm^{-1}) observed on the spectra of all samples remain unchanged.

3.3. Swelling behaviour of SA/G-GP hydrogels

The swelling behaviour of SA/G-GP hydrogels with different ratios of SA/G and crosslinker concentrations expressed as a function of the swelling time is depicted in Fig. 4. It can be seen that the SA/G-0.5GP samples (Fig. 4(a)) except of 70/30 and 60/40, have similar swelling

tendency; the swelling ratio increases gradually during the first 3 hours, then its increase continues slowly up to 8 hours and finally its slight decline begins. SA/G-0.5GP 70/30 and 60/40 samples shows sharp increase in the swelling within the first 1.5 and 3 hours, respectively, followed by sample dissolution, indicating presence of insufficiently crosslinked network structure related to low G content. Furthermore, it is obvious that the increase of both G and GP content induces a decrease of swelling degree (compare Fig. 4(a) and (b). On the other hand, with increasing content of SA, increased swelling is observed resulting even in disintegration of the SA/G-0.5GP hydrogels with 50, 60 and 70% SA content in PBS with pH 7.4, due to a significant increase in the carboxylic group content [49, 50] and imperfectly crosslinked G. It can be seen in Fig. 4 that equilibrium swelling of SA/G-1.0GP hydrogels is lower than that of SA/G-0.5GP hydrogels. This may be explained by the fact that higher concentration of GP results in extended crosslinking of the G restricting the mobility of the polymer chains hindering thus moisture uptake and increasing the gel strength.

As can be seen in Fig. 4(b), SA/G-1.0GP 70/30 sample exhibits significantly higher swelling, compared to other SA/G-1.0GP hydrogels, that may be attributed to the relatively long distance between crosslinking points and weak network strength. In other words, lower content of G leads to a lower level of crosslinking in connection with higher polymer chains mobility.

Furthermore, it should be noted that the swelling behaviour of SA/G-1.0GP hydrogels is similar to the swelling of hydrogels crosslinked with GTA evaluated in a previous study [12], whereas the swelling of fewer crosslinked SA/G-0.5GP samples is higher. Definitely, swelling degree found to be higher than 200% for all tested samples indicating that prepared SA/G-GP hydrogels can be considered as an efficient wound dressing with a high absorption capacity.

3.4. Viscoelastic properties of SA/G-GP hydrogels

The frequency dependence of the viscoelastic properties of SA/G hydrogels crosslinked with GP in a swollen state is shown in Fig. 5. As can be seen from the figure, the values of both moduli *G'* and *G''* of all samples, except of SA/G-1.0GP-70/30, show a negligible frequency dependence, which demonstrates that the hydrogels have a mechanically stable network. As it is clear from Fig. 5(a) the level of *G'* of SA/G-0.5GP hydrogels is, found to be approximately one order magnitude higher than the *G''* within the experimental frequency range, indicating that the elastic behaviour of the sample predominates over its viscous one. Arise in the GP concentration

from 0.5 to 1.0 wt% is reflected in more than one and a half order of magnitude increase of the *G'* compared to *G''*, which is likely due to a higher crosslinking density (Fig. (5b)). The viscoelastic behaviour of SA/G-1.0GP-70/30 sample differ significantly from the remaining samples. For this sample *G'* increases gradually with raising frequency of deformation (0.1 and 200 Hz), whereas the values of *G''* is almost frequency independent. The weaker network stability of this sample at low frequencies (0.1÷1Hz) could be attributed to relatively low crosslink density resulting also in a high equilibrium swelling of matrix (see Fig. 4(b)). This corresponds to dissolution of the sample after 12 hours of swelling. Furthermore, it should be noted that decline in G content in tested GP crosslinked hydrogels is connected with decrease of *G'* due to the number reduction of gelatine chains serving as elastically active segments.

The viscoelastic behaviour of hydrogels can also be described using the $tan\delta$ dependence on frequency, as it is depicted in Fig. 6. As can be seen from Fig. 6(a), the *tan* δ curve of all the SA/G-0.5GP samples are nearly frequency independent, and close to each other in a narrow range ($tan\delta$ =0.6 to 0.7). This behaviour indicates dominant viscoelastic relaxations of the formed networks. As it is clear from Fig. 6(b), $tan\delta$ of all SA/G-1.0GP gels except of 70/30, decreases gradually with increasing frequency and a more elastic behaviour ($tan\delta$ =0.5 to 0.6) is observed in the high frequency range (10÷200 Hz). On the other hand, values of $tan\delta$ for SA/G-1.0GP 70/30 are significantly higher than the others in the entire frequency range tested showing a loose network structure even if higher GP concentration is used.

The temperature dependent dynamic viscoelastic properties of GP crosslinked SA/G hydrogels in the equilibrium swelling state is presented in Fig. 7. It is obvious that *G'* is higher than *G''* in all tested samples confirming that materials behave as solid-like gels. Besides, both *G'* and *G''* of all samples are almost unchanged within the whole temperature range. Only in case of SA/G-1.0GP-20/80 the *G'* and *G''* slight decrease with increasing temperature is presented. It can be also highlighted that an increase in GP concentration promotes enhancement in *G'*.

Temperature dependencies of *tan* δ of all tested hydrogels are depicted in Fig. 8. As can be seen from the figure, *tan* δ values for SA/G-0.5GP hydrogels are significantly higher compared to hydrogels where higher concentration of crosslinking agent are used. Exception here is SA/G-1.0GP-70/30 sample showing insufficient crosslinking density resulting in the highest *tan* δ

value observed. Moreover, it can be noted that *tan* δ is relatively insensitive to temperature changes, except of SA/G-1.0GP-70/30 (Fig. 8 (b)).

Finally it should be mentioned that the viscoelastic properties of GP crosslinked SA/G hydrogels are quite close to those of GTA crosslinked hydrogels evaluated in a previous study [12].

4. Conclusions

The study was focused on the development and characterization of hydrogels based entirely on natural, biocompatible materials. For this purpose sodium alginate/gelatine matrix crosslinked with genipin was employed as a promising candidate for biomedical applications. The prepared hydrogels were characterized by FTIR spectroscopy, SEM, swelling test as well as by DMA. The SEM images confirmed that the morphologies of the hydrogels were dependent on their composition in terms of SA, G, and GP content. It was proved that GP influence was more pronounced for samples with higher SA content. The performed FTIR analysis revealed that GP as a crosslinker induced the formation of heterocyclic amines and ester bonds between G and GP molecules, which influenced the swelling and viscoelastic properties of the prepared hydrogels. The swelling degree of tested samples was found between 250÷500%, which confirmed that SA/G-GP hydrogels possess a high absorption capacity. According to the DMA results, the elasticity of swelled hydrogels increased with increasing content of G and GP. SEM photomicrographs proved that GP crosslinked hydrogels, with an equal ratio of both components SA/G-1.0GP 50/50, posses high miscibility, homogeneity and provide good balance of swelling and dynamic viscoelastic properties.

The results substantiated that the GP crosslinked SA/G hydrogels are promising materials with favourable swelling, mechanical properties and anticipated good biocompatibility, which can find application in moist wound dressings.

Acknowledgements

This article was created with the support of Operational Program Research and Development for Innovations, co-funded by the European Regional Development Fund (ERDF) and the national budget of the Czech Republic, within the framework of the project Centre of Polymer Systems (reg. number: CZ.1.05/2.1.00/03.0111).

References

1. Sikareepaisan, P., Ruktanonchai, U., Supaphol, P. Preparation and characterization of asiaticoside-loaded alginate films and their potential for use as effectual wound dressings. Carbohyd. Polym. 2011, 83, 1457-1469.
2. Laurienzo, P. Marine Polysaccharides in Pharmaceutical Applications: An overview. Mar. Drugs, 2010, 8, 2435-2465.
3. Panouille, M., Larreta-Garde, V. Gelation behaviour of gelatine and alginate mixtures. Food Hydrocolloid. 2009, 23, 1074-1080.
4. Kuo, C.K., Ma, P.X. Maintaining dimensions and mechanical properties of ionically crosslinked alginate hydrogel scaffolds in vitro. J. Biomed. Mater. Res. 2008, 84, 899-907.
5. Balakrishnan, B., Mohanty, M., Umashankar, P.R., Jayakrishnan. A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatine, Biomaterials, 2005, 26, 6335-6342.
6. Bubenikova, S., Stancu, I.C., Kalinovska, L., Schacht, E., Lippens, E., Declercq, H. et al., Chemoselective crosslinking of alginate with thiol-terminated peptides for tissue engineering applications, Carbohyd. Polym. 2012, 88, 1239-1250.
7. Picard, J., Doumeche, B., Panouille, M., Larreta-Garde, V. Gelatine-Polysaccharide mixed biogels: enzyme-catalyzed dynamics and IPNs, Macromol. Symp. 2010, 337-344.
8. Curcio, M., Spizzirri, U. G., Iemma, F., Puoci, F., Cirillo., Parisi, O. et al., Grafted thermo-responsive gelatine microspheres as delivery systems in triggered drug release. Eur. J. Pharm. Biopharm. 2010, 76, 48-55.
9. Xiao, W., He, J., Nicol, J.W., Wang, L., Hustson, C.B., Wang, B. et al., Synthesis and characterization of photocrosslinkable gelatine and silk fibroin interpenetrating polymer network hydrogels. Acta Biomaterial, 2011, 7, 2384-2393.
10. Balaji, S., Kumar, R., Sripriya, R., Kakkar, P., Ramesh, D.V., Reddy, P. N. et al., Preparation and comparative characterization of keratin-chitosan and keratin-gelatine composite scaffolds for tissue engineering applications. Mater. Sci. Eng. 2012, 32, 975-982.
11. Tonda-Turo, C., Gentile, P., Saracino, S., Chiono, V., Nandagiri, V.K., Muzio, G. et al., Comparative analysis of gelatine scaffolds crosslinked by genipin and silane coupling agent. J. Biol. Macromol. 2011, 49, 700-706.
12. Saarai, A., Sedlacek, T., Kasparkova, V., Kitano, T., Saha, P. On the characterization of sodium alginate/gelatine based hydrogels for wound dressing. J. Appl. Polym. Sci. 2012.
13. Michon, C., Cuveleier, G., Relkin, P., Launay. Influence of thermal history on the stability of gelatine gels. J. Biol. Macromol. 1997, 20, 259-264.
14. Hwang, C.M., Sant, S., Masaeli, M., Kachouie, N.N., Zamanian, B., Lee, S.H. et al., Fabrication of three dimensional porous cell-laden hydrogel for tissue engineering. Biofabrication. 2010, 2, 1-12.
15. Xiao, C., Liu, H., Zhang, L. Blend films form sodium alginate and gelatine solutions. J. Macromol. Sci. Pure Appl. Chem. 2001, 317-328.
16. Sung, H.W., Huang, D.M., Chang, W.H., Huang, R.N, Hsu, J.C. Evaluation of gelatine hydrogel crosslinked with various crosslinking agents as bioadhesives: In vitro study. J. Biomed. Mater. Res. 1999, 46, 520-530.

17. Bigi, A., Cojazzi, G., Panzavolta, S., Roveri, N., Rubini. Stabilization of gelatine films by crosslinking with genipin. Biomaterials, 2002, 23, 4827-4832.
18. Liu, B.S., Yao, C.H., Wang, W., Lee, S., Lo, C.C., Liu, C.K. et al., Effect of drying method on the characteristics of genipin crosslinked gelatine films. J. Med. Biol. Eng. 2008, 29, 29-3818.
19. Dranca, I., Vyazovkin, S. Thermal stability of gelatine gels: Effect of preparation conditions on the activation energy barrier to melting. Polymer, 2009, 50, 4859-4867.
20. Farris, S., Schaich, K.M., Liu, L.S., Cooke, P.H., Piergiovanni, L., Yam, K.L. Gelatine-pectin composite films from polyion-complex hydrogels. Food Hydrocolloid. 2011, 25, 61-70.
21. Mishra, R. K., Majeed, A.B.A., Banthia, A.K. Development and characterization of pectin/gelatine hydrogel membranes for wound dressing. J. Plast. Technol. 2011, 15, 82-95.
22. Sung. H.W., Liang. I.L., Chen, C.N., Huaung, R.N., Liang, H.F. Stability of a biological tissue fixed with a naturally occurring crosslinking agent (genipin). J. Biomed. Mater. Res. 2001, 55, 538-546.
23. Chang, W.H., Chang, Y., Sung, H.W. A genipin crosslinked gelatine membrane as wound-dressing material: in vitro and in vivo studies. J. Biomater. Sci. Polym. Ed. 2003, 5, 481-495.
24. Liang, H.C., Chang, W.H., Liang, .H.F., Lee, M.H., Sung, H.W. Crosslinking structures of gelatine hydrogels crosslinked with genipin or a water-soluble carbodiimide. J. Appl. Polym. Sci. 2004, 91, 4017-4026.
25.Lai, J.Y. Biocompatibility of chemically crosslinked gelatine hydrogels for ophthalmic use. J. Mater. Sci: Mater. Med. 2010, 21, 1899-1911.
26. Mi. S., Khutoryanskiy, V.V., Jones, R.R., Zhu, X., Hamley, I.W., Connon, C.J. Photochemical crosslinking of plastically compressed collagen gel produces an optimal scaffold for corneal tissue engineering. J. Biomed. Mater. Res. Part A. 2011, 99, 1-8.
27. Tsai, T.R., Tseng, T.Y., Chen, C.F., Tsai, T.H. Identification and determination of geniposide contained in gardenia jasminoides and in two preparations of mixed traditional Chinese medicines. J. Chromatogr. 2002, 961, 83-88.
28. Sung, H.W., Chang, Y., Liang, I.L., Chang, W.H., Chen, Y.C. Fixation of biological tissues with a naturally occurring crosslinking agent: Fixation rate and effects of pH, temperature, and initial fixative concentration. J. Biomed. Mater. Res. 2000, 52, 77-87.
29. Sung. H.W., Chang, Y., Liang, H.C., Liang, H.F., Lee, M.H., Hsu, C.K. A naturally occurring crosslinking agent extracted from gardenia fruit and its applications in tissue engineering, in: Hwang, H.C., Woo, L.Y. (Eds.), Biomedical Engineering. Kluwer Academic/Plenum Publishers, New York, 2003. 437-445.
30. Mi, F.L., Shyu, S.S., Peng, C.K. Characterization of ring-opening polymerization of genipin and pH dependent crosslinking reactions between chitosan and genipin. J. Polym. Sci: Part A: Polym. Chem. 2005, 43, 1985-2000.
31. Nishi, C., Nakajima, N., Ikada, Y., In vitro evaluation of cytotoxicity of diepoxy compounds used for biomaterial modification. J. Biomed. Mater. Res. 1995, 29, 829-834.

32. Sung, H.W., Huang, R.N., Huang, L.L., Tsai, C.C. In vitro evaluation of cytotoxicity of a naturally occurring crosslinking reagent for biological tissue fixation. J. Biomater. Sci. Polym. Ed. 1999, 10, 63-78.
33. Chiono, V., Pulieri, E., Ciardelli G, Ahluwalia, A., Giusti, P. Genipin crosslinked chitosan/gelatine blends for biomedical applications. J. Mater. Sci. Mater. Med. 2008, 19, 889-898.
34. Taylor, M.M., Bumanlag, L.P., Marmer, W.N., Brown, E.M., Potential application for genipin modified gelatine in leather processing. J. Am. Leather Chem. As. 2008, 104, 79-91.
35. Touyama, R., Inoue, K., Takeda, Y., Yatsuzuka, M., Ikumoto, T., Moritome, N. et al., Studies on the blue pigments produced from genipin and methylamine. II. On the formation mechanisms of brownish-red intermediates leading to the blue pigment formation. Chem. Pharm. Bull. 1994, 42, 1571-1578.
36. Sung, H.W, Chang, W.H., Ma, C.Y., Lee, M.H. Crosslinking of biological tissues using genipin and/or carbodiimide. J. Biomed. Mater. Res. 2003, 64, 427-438.
37. Liang, H.C., Chang, W.H., Lin, K.J., Sung, H.W. Genipin crosslinked gelatine microsphere as a drug carrier for intramuscular administration: In vitro and in vivo studies. J. Biomed. Mater. Res. 2003, 65, 271-282.
38. Butler, M.F., Ng, Y.F., Pudney, P.D.A. Mechanism and kinetics of the crosslinking reacton between biopplymers containing primary amine groups and genipin. J. Polym. Sci. Part A: Polym. Chem. 2003, 41, 3941-3953.
39. Huang, L.L., Sung, H.W., Tsai, C.C., Huang, D.M. Biocompatibility study of a biological tissue fixed with a naturally occurring crosslinking reagent. J. Biomed. Mater. Res. 1998, 42, 568-576.
40. Tsai, C.C., Huang, R.N., Sung, H.W., Liang, H.C. In vitro evaluation of the genotoxicity of a naturally occurring crosslinking agent (genipin) for biologic tissue fixation. J. Biomed. Mater. Res. 2000, 52, 58-65.
41. Haag, J., Baiguera, S., Jungebluth, P., Barale, D., Gaudio, C.D., Castiglione, F. Biomechanical and angiogenic properties of tissue engineered rat trachea using genipin crosslinked decellularized tissue. Biomaterials, 2012, 33, 780-789.
42. Sartori, C., Finch, D.S., Ralph, B. Determinaton of the cation content of alginate thin films by FTi.r. spectroscopy. Polymer, 1997, 38, 43-51.
43. S.G. Kim, G.T. Lim, J. Jegal, K.H. Lee. Pervaporation separation of MTBE (methyl tert-butyl ether) and methanol mixtures through polyion complex composite membranes consisting of sodiumalginate/chitosan. J. Membr. Sci., 174 (2000), pp. 1–15
44. Li, Y., Jia, H., Cheng, Q., Pan, F., Jiang, Z. Sodium alginate-gelatine polyelectrolyte complex membranes with both high water vapor permeance and high permselectivity. J. Membr. Sci., 2011, 375, 304-312.
45. Xiao, C., Liu, H., Lu, Y., Zhang, L., Blend films from sodium alginate and gelatine solutions. J. Macromol. Sci.-Pure Appl. Chem. 2001, 38, 317-328.

46. Moura, M.J., Faneca, H., Lima, M.P., Gil, M.H., Figueiredo, M.M. In situ forming chitosan hydrogels prepared via ionic/covalent co-crossliniking. Biomacromolecules, 2011, 12, 3275-3284.
47. Mi, F.L. Synthesis and characterization of a novel chitosan-gelatine bioconjugate with fluorescence emission. Biomacromolecules, 2005, 6, 975-987.
48. Silva, S.S., Motta, A., Rodrigues, M.T., Pinheiro, A.F.M., Gomes, M.E., Mano, J.F. et al., Novel genipin crosslinked chitosan/silk fibroin sponges for cartilage engineering strategies. Biomacromolecules, 2008, 9, 2764-2774.
49. Chen, S.C., Wu, Y.C., Mi, F.L., Lin, Y.H., Yu, L.C., Sung, H.W. A novel pH sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate crosslinked by genipin for protein drug delivery. J. Control. Release. 2004, 96, 285-300.
50. Mi, F.L., Liang, H.F., Lin, Y.S., Yang, T.F., Sung, H.W. pH-sensitive behavior of two component hydrogels composed of N, O carboxymethyl chitosan and alginate. J. Biomater. Sci. Polym. Ed. 2005, 16, 1333-1345.

Figures Caption:

Figure 1. SEM micrographs of dried hydrogels crosslinked with 0.5 wt% GP : (a) SA/G 20/80, (b) SA/G 30/70, (c) SA/G 40/60, (d) SA/G 50/50, (e) SA/G 60/40, (f) SA/G 70/30

Figure 2. SEM micrographs of dried hydrogels crosslinked with 1.0 wt% GP : (a) SA/G 20/80, (b) SA/G 30/70, (c) SA/G 40/60, (d) SA/G 50/50, (e) SA/G 60/40, (f) SA/G 70/30, (g) SA/G 80/20

Figure 3. FTIR spectra of uncrosslinked SA/G hydrogels with SA/G-0/100 and SA/G-100/0 (a), and 1.0 wt%. GP crosslinked SA/G hydrogels with original GP (b)

Figure 4. Swelling degree of the SA/G hydrogels crosslinked with 0.5 wt%. (a) and 1.0 wt%. GP (b) in PBS (pH 7.4) at 37 °C as a function of swelling time

Figure 5. Storage (G') and Loss (G'') moduli of SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of frequency at 37 °C

Figure 6. Effect of angular frequency on tan δ of SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) at 37°C

Figure 7. Dynamic **v**iscoelastic properties of the SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of temperature at 10 Hz

Figure 8. Tan delta of the SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of temperature at 10 Hz

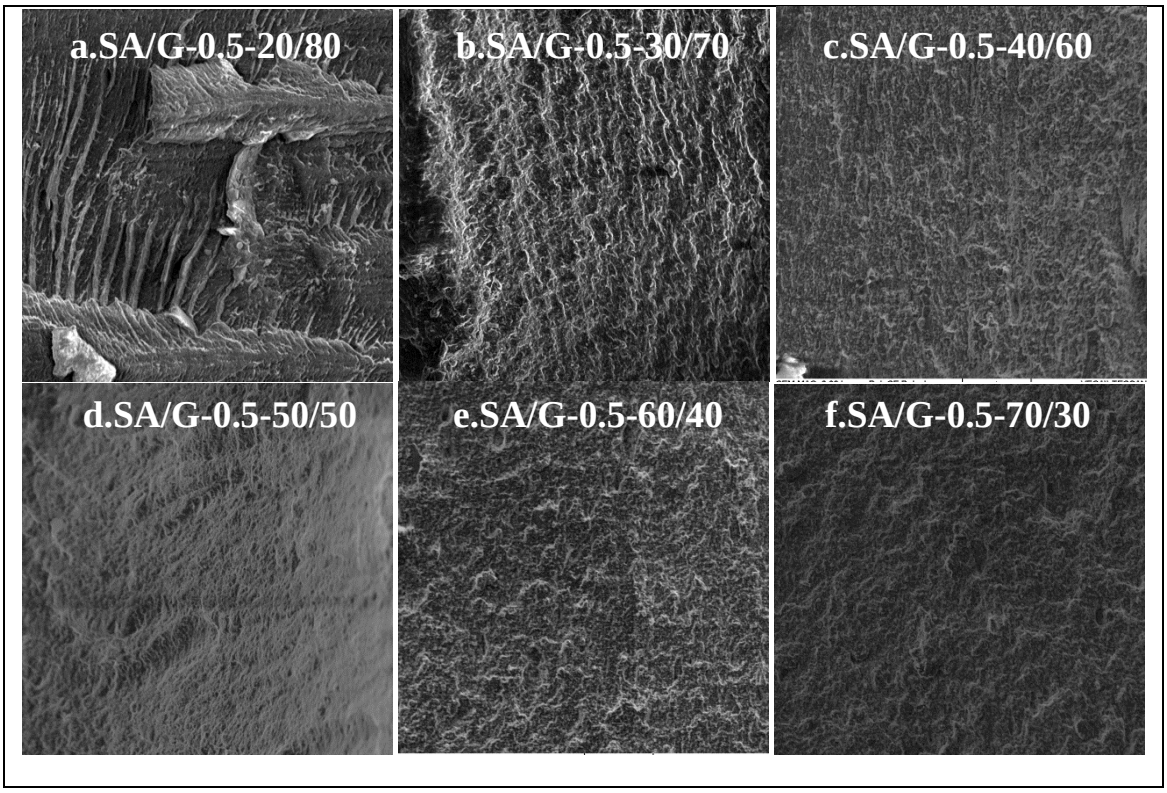


Figure 1. SEM micrographs of dried hydrogels crosslinked with 0.5 %wt GP : (a) SA/G 20/80, (b) SA/G 30/70, (c) SA/G 40/60, (d) SA/G 50/50, (e) SA/G 60/40, (f) SA/G 70/30

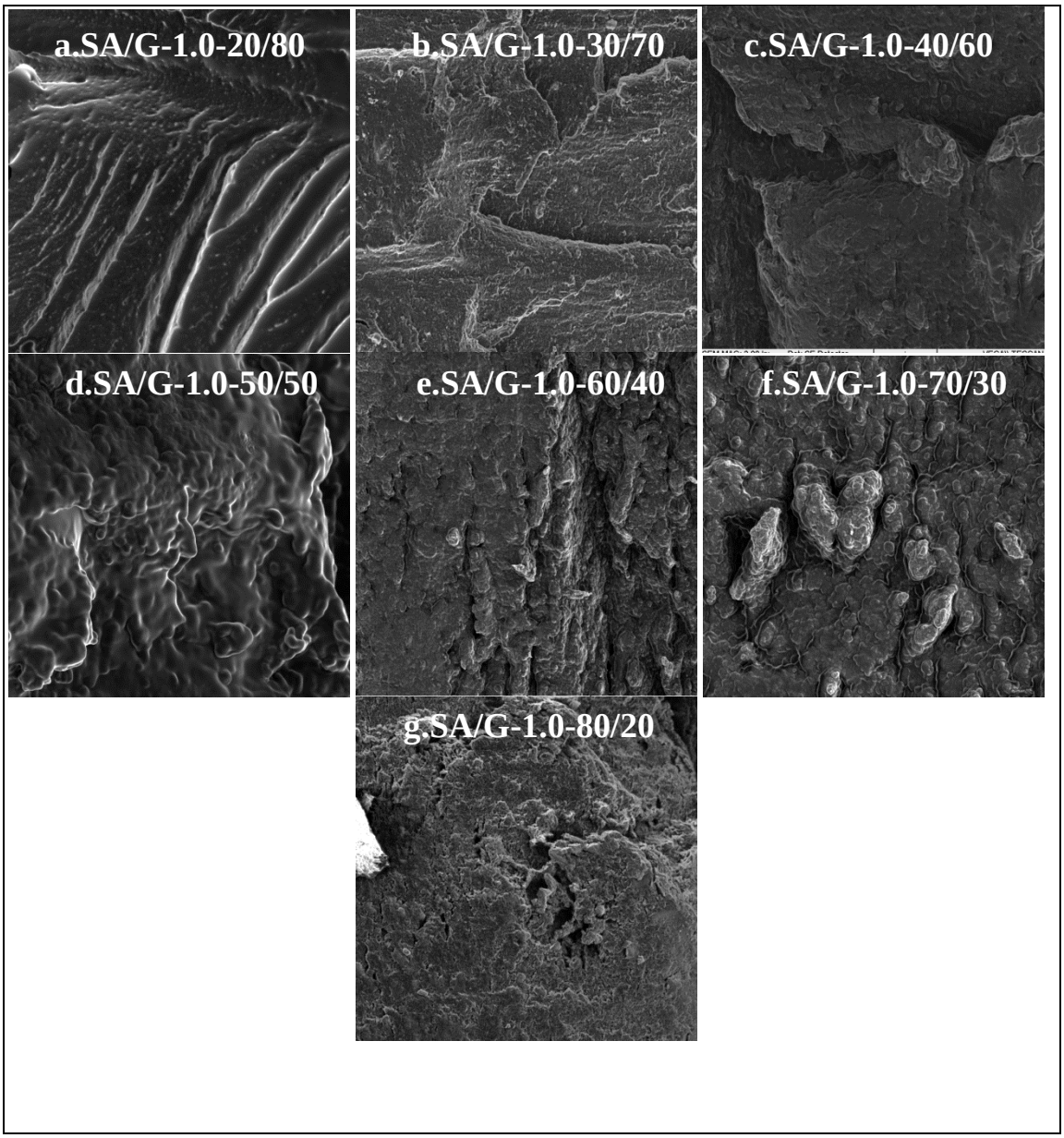
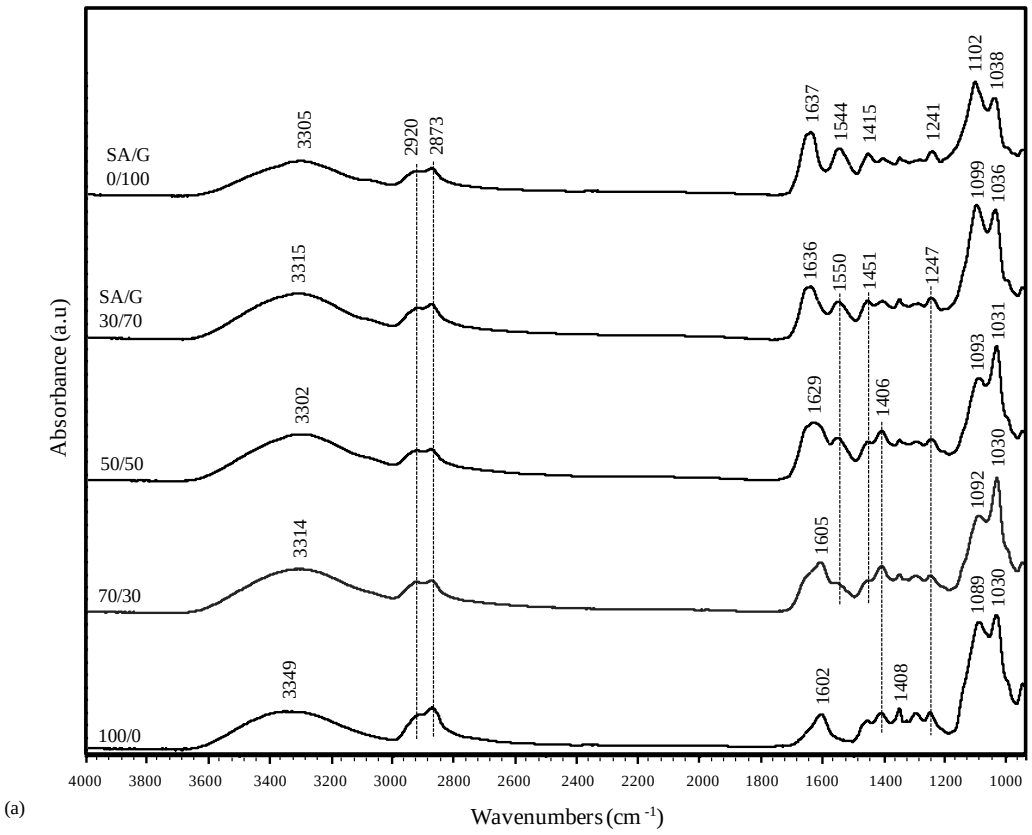
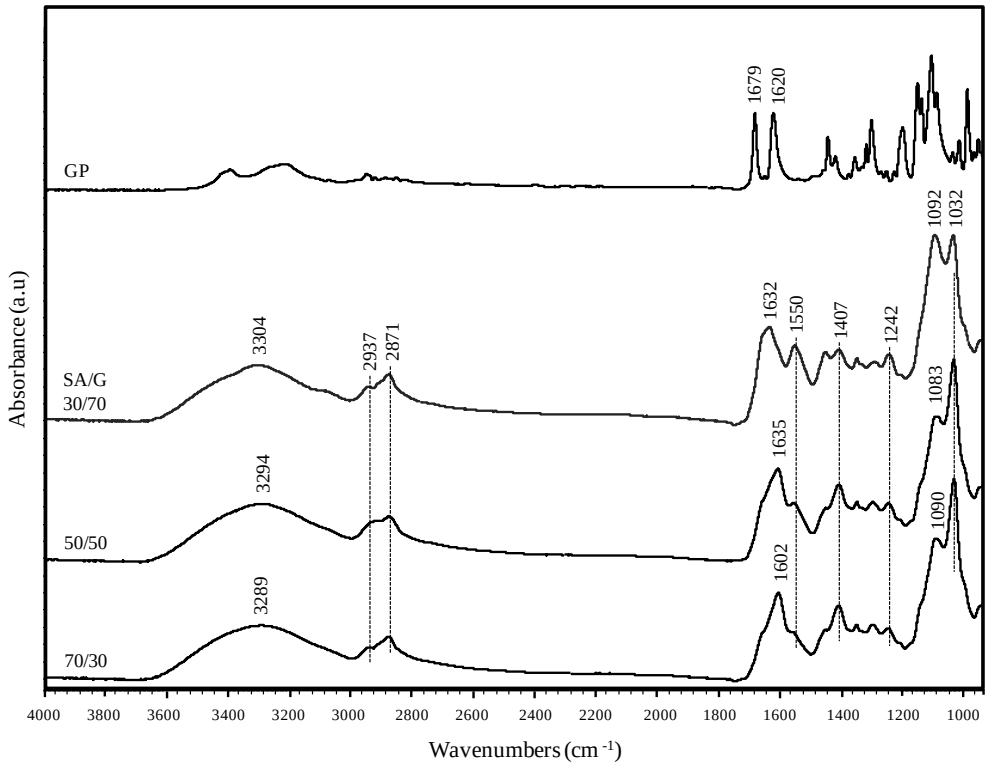


Figure 2. SEM micrographs of dried hydrogels crosslinked with 1.0 %wt GP : (a) SA/G 20/80, (b) SA/G 30/70, (c) SA/G 40/60, (d) SA/G 50/50, (e) SA/G 60/40, (f) SA/G 70/30, (g) SA/G 80/20

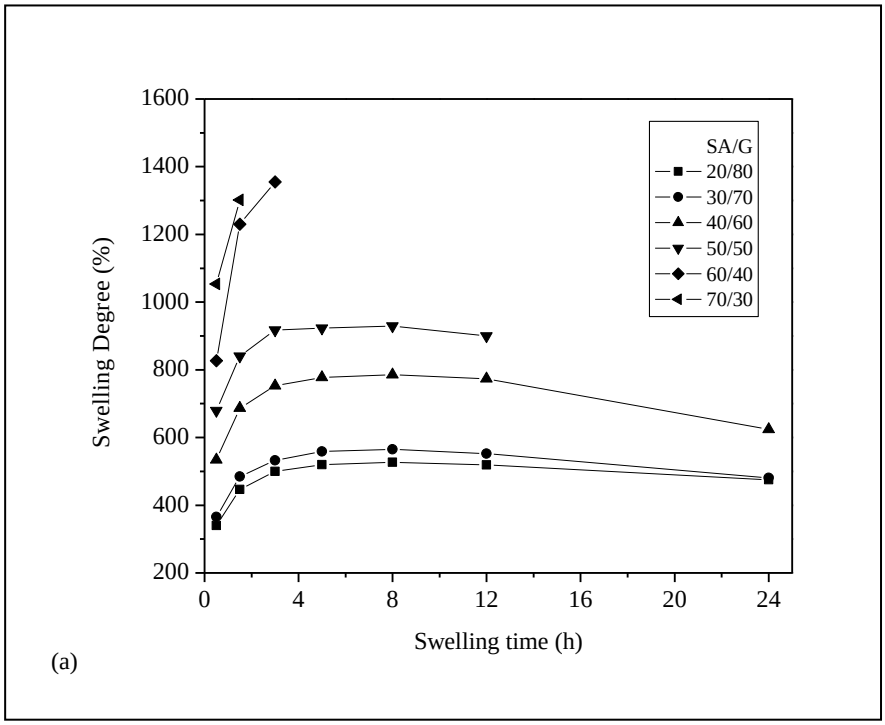


(a)



(b)

Fig. 3. FTIR spectra of uncrosslinked SA/G hydrogels with SA/G-0/100 and SA/G-100/0 (a), and 1.0 % wt. GP crosslinked SA/G hydrogels with original GP (b).



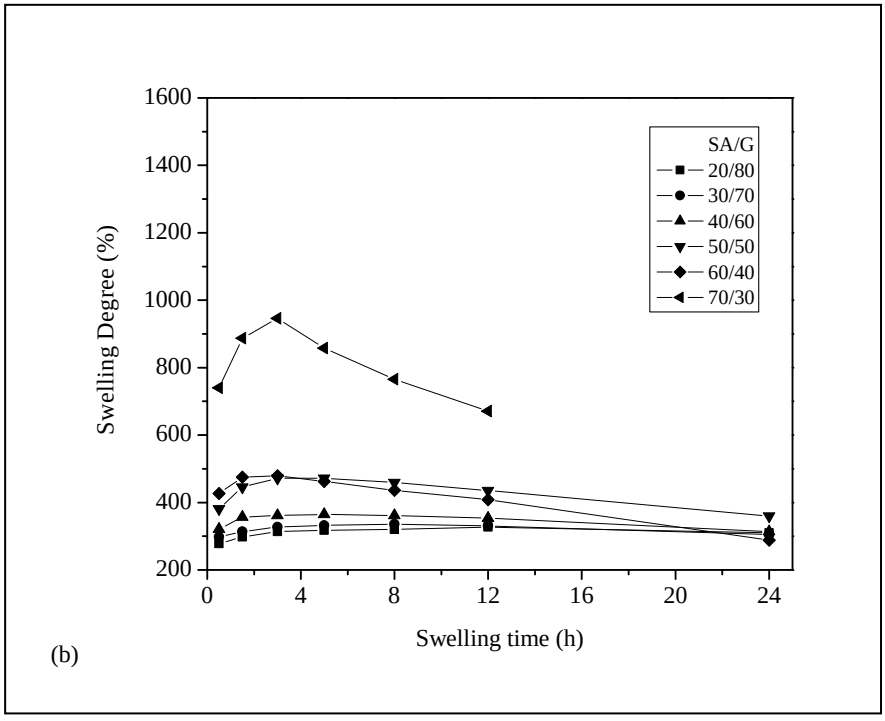


Fig. 4. Swelling degree of the SA/G hydrogels crosslinked with 0.5 % wt. (a) and 1.0 % wt. GP (b) in PBS (pH 7.4) at 37 °C as a function of swelling time.

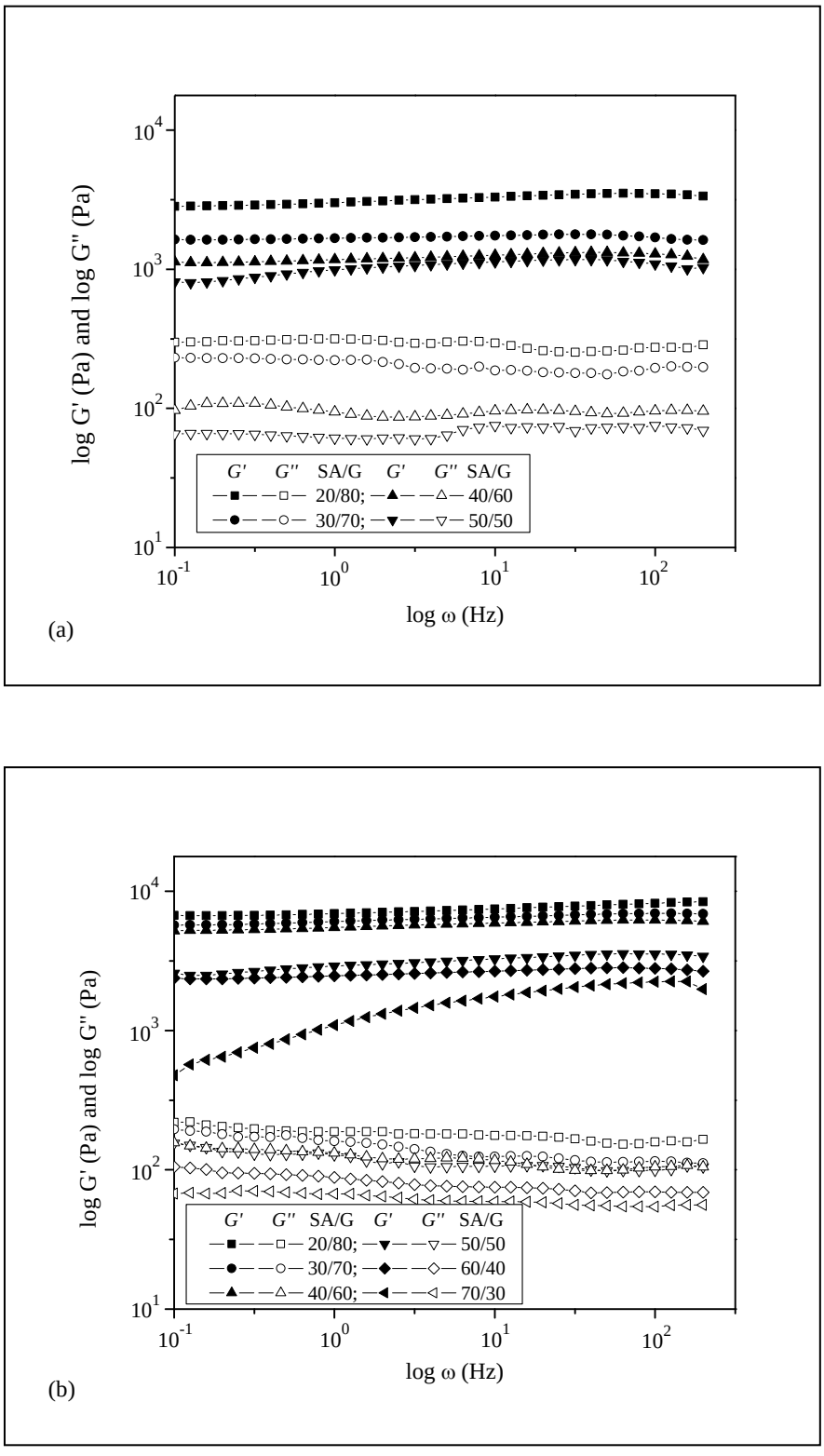
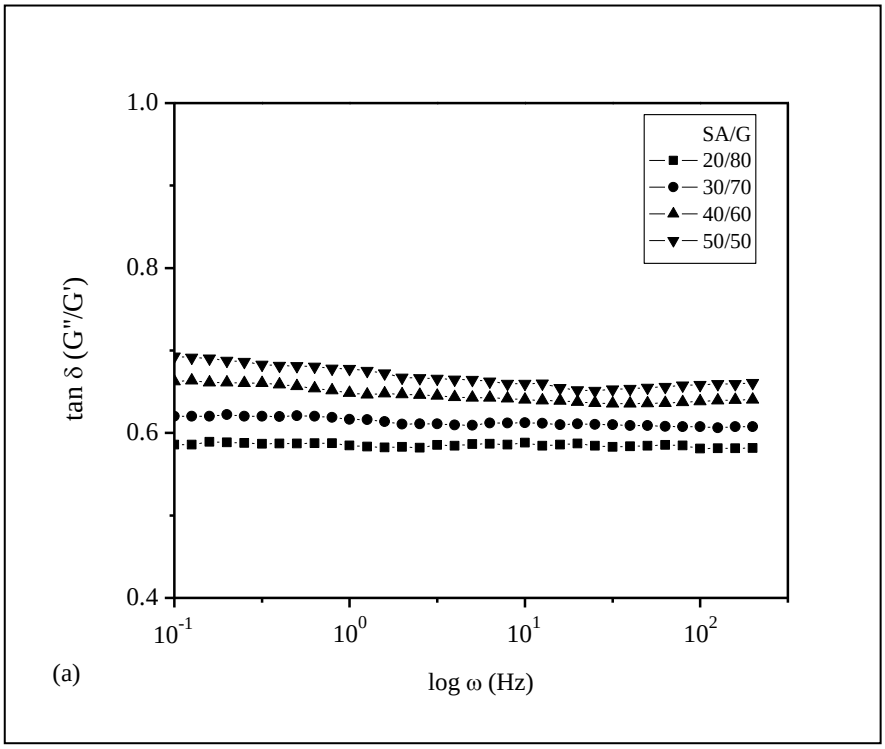


Fig. 5. Storage (G') and Loss (G'') moduli of SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of frequency at 37 °C



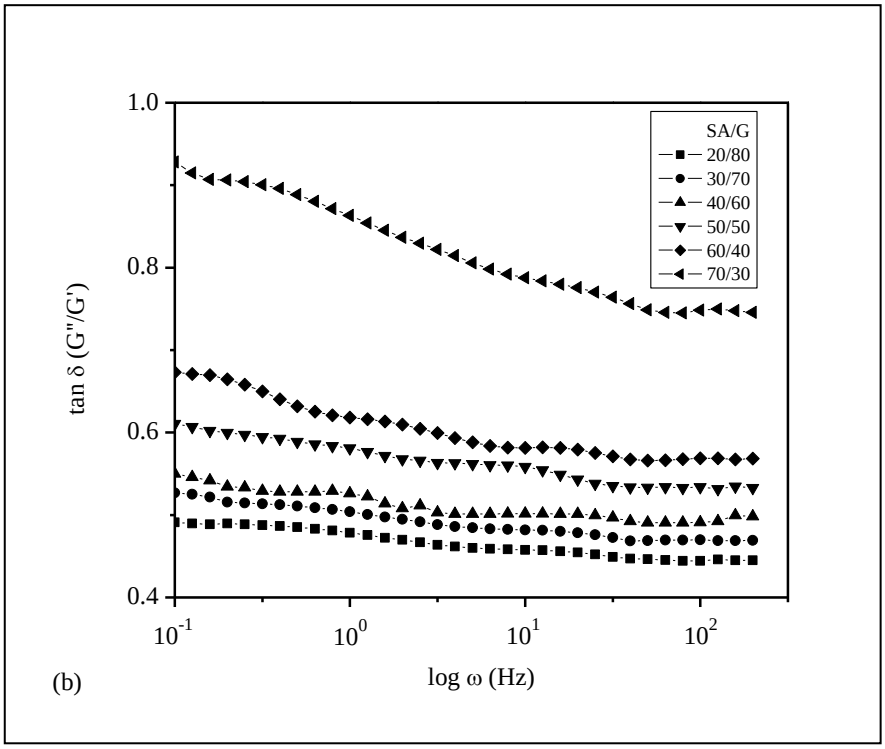


Fig. 6. Effect of angular frequency on tan δ of SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) at 37°C

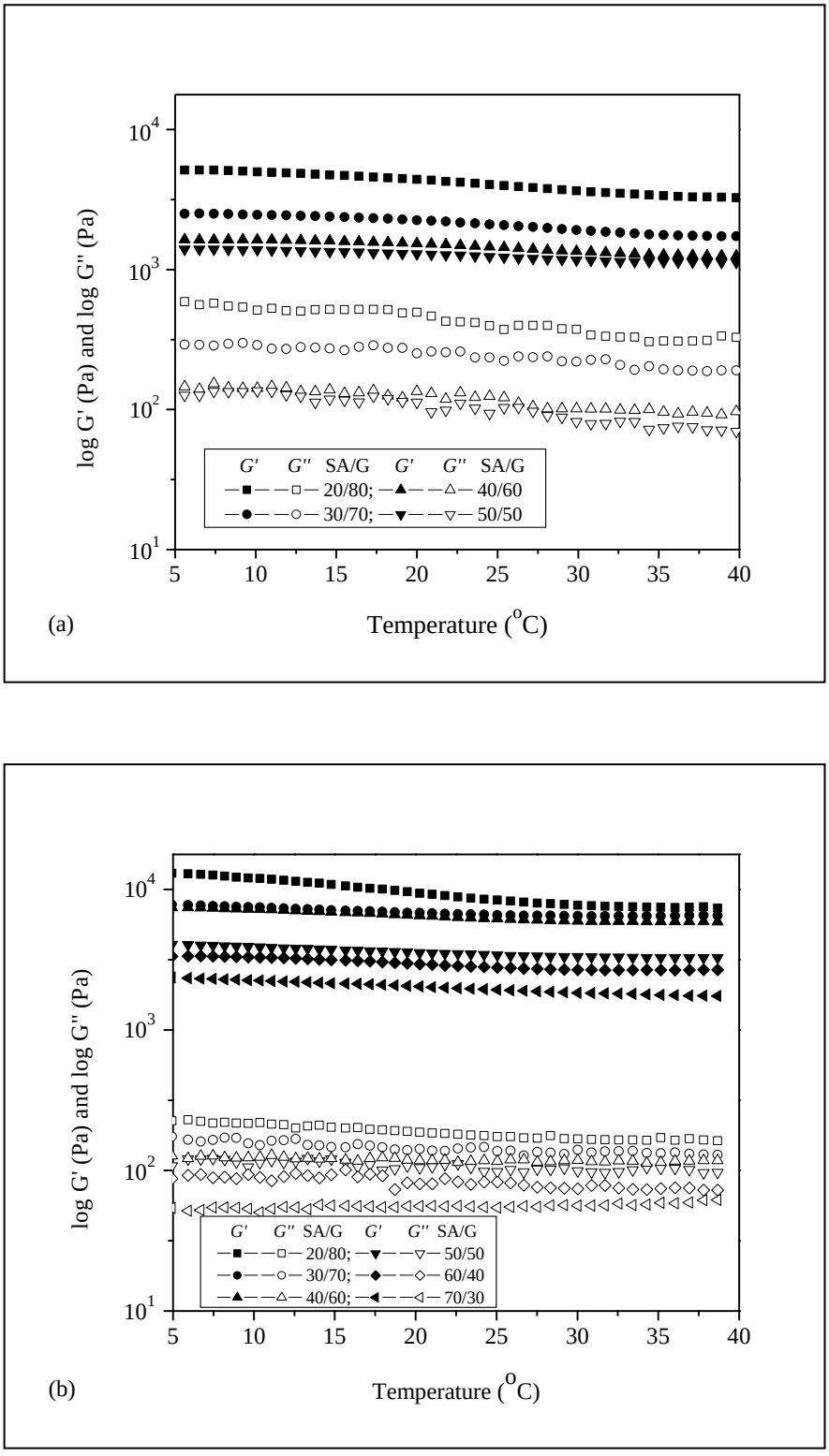


Fig. 7. Viscoelastic properties of the SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of temperature at 10 Hz

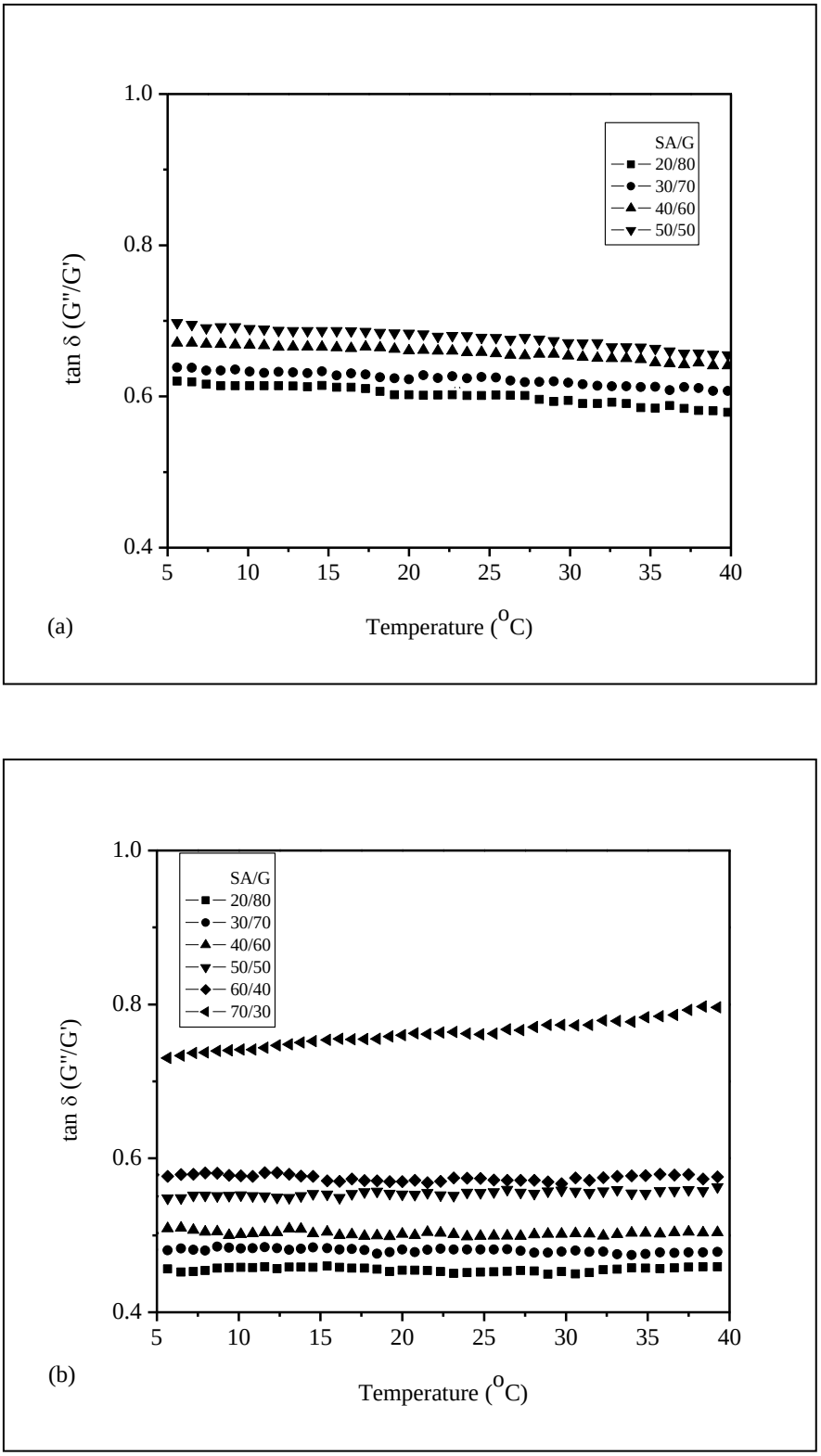


Fig. 8. Tan delta of the SA/G-0.5GP hydrogels (a) and SA/G-1.0GP hydrogels (b) as a function of temperature at 10 Hz

PATENT I

Dry Substance of Hydrogel to Cover Wounds and Process for Preparing Thereof

Saha Nabanita, Saha Tomas, Amarjargal Saarai

CZ patent 302380,

Date of the patent 09.03.2011

PATENTOVÝ SPIS

(19) ČESKÁ REPUBLIKA

ÚŘAD PRŮMYSLOVÉHO VLASTNICTVÍ

(21) Číslo přihlášky: **2010-126**
(22) Přihlášeno: **18.02.2010**
(40) Zveřejněno: **20.04.2011**
(Věstník č. 16/2011)
(47) Uděleno: **09.03.2011**
(24) Oznámení o udělení ve Věstníku: **20.04.2011**
(Věstník č. 16/2011)

(11) Číslo dokumentu:
302 380
(13) Druh dokumentu: **B6**
(51) Int. Cl.:

A61K 9/70	(2006.01)
A61K 47/42	(2006.01)
A61P 17/02	(2006.01)
A61K 47/36	(2006.01)
A61K 47/30	(2006.01)
A61K 47/10	(2006.01)
A61K 47/44	(2006.01)
A61K 36/18	(2006.01)

(56) Relevantní dokumenty:
WO 2008/010199; US 6753454.

(73) Majitel patentu:
Univerzita Tomáše Bati ve Zlíně, Zlín, CZ

(72) Původce:
Saha Nabanita doc. M.Sc. Ph.D., 721101, Midnapore (West) - West Bengal, IN
Sáha Tomáš Ing., Zlín - Mladcová, CZ
Saarai Amarjargal M.Sc. Ph.D., Darkhan City, MN

(74) Zástupce:
Ing. Dana Kreizlová, UTB ve Zlíně, nám. T. G. Masaryka 5555, Zlín, 76001

(54) Název vynálezu:

Suchá substance hydrogelu pro krytí ran a způsob její přípravy

(57) Anotace:
Suchá substance hydrogelu pro krytí ran vyrobená z přírodních a syntetických polymerů obsahuje želatinu a alginát sodný v hmotnostním poměru 3:7 až 7:3, přičemž tyto dvě složky dohromady tvoří 100 hm.d., a současně substance obsahuje 15 až 25 hm.d. polyethylénglykolu, 15 až 25 hm.d. glycerínu, 10 až 40 hm.d. polyvinylalkoholových nanovláken, 1 až 3 hm.d. chloridu sodného, případně další běžná aditiva. Způsob přípravy suché substance tohoto hydrogelu pro krytí ran spočívá v tom, že za stálého míchání při konstantní teplotě 75 až 85 °C se připraví výchozí polymerní roztok želatiny ve 180 až 220 hm.d. vody, po rozpuštění celého objemu želatiny se k výchozímu vodnému polymernímu roztoku želatiny přidají další složky - alginát sodný, polyethylénglykol, chlorid sodný a glycerín, případně rakytníkový olej, míchání této směsi pak pokračuje po dobu 5 až 6 min. při 250 až 350 ot/min., k výsledné viskózní hmotě hydrogelu se pak postupně přidá frakce polyvinylalkoholových nanovláken a rychlost míchání se sníží na 150 až 50 ot/min., nakonec se viskózní hmota za aseptických podmínek dávkuje do misek a podrobí zrání při pokojové teplotě 20 až 25 °C, během něhož se z viskózní hmoty zcela odstraní obsah vody a získá se finální suchá substance hydrogelu ve tvaru plochého tělíska.

CZ 302380 B6

Suchá substance hydrogelu pro krytí ran a způsob její přípravy

Oblast techniky

Vynález se týká suché substance hydrogelu pro krytí ran použitelného na živých tělech, zejména lidských. Předmětem vynálezu je rovněž způsob přípravy takové substance hydrogelu pro krytí ran.

Stav techniky

Dosud jsou známé různé hydrogely pro krytí ran ze syntetických a přírodních polymerů, stejně jako způsoby jejich přípravy. Jsou zaměřeny na získání produktu s nejlepšími mechanickými, botnacími a dalšími důležitými fyzikálními vlastnostmi, jako i s antibakteriálními a hojivými vlastnostmi.

Na hydrogely pro krytí ran je kladeno více různých požadavků. Tyto hydrogely by měly být měkké a příjemné zraněnému tělu a současně by měly odolávat manipulacím během přiložení a celé aplikace. Tyto požadavky jsou poněkud protichůdné a je obtížné jim v dostatečné míře vyhovět.

Jedna ze známých metod výroby hydrogelu pro krytí ran je uvedena v dokumentu WO 03/034900, který popisuje intradermální záplatu s propustným nosičem s nánosem gelu na bázi polyvinylpyrolidonu s určitou průměrnou molekulovou hmotností (nejlépe 900.000 až 1.500.000 Dalton) v množství opt. 15 až 20 % hm. Záplaty mohou obsahovat jednu nebo více přísad, jako je činidlo pro hojení ran, léčiva, modifikátory viskozity a zvlhčující přísady. Hydrogelový materiál je umístěn na podkladu vytvořeném z materiálu s dostačujícími mechanickými vlastnostmi a proto nejsou na hydrogel kladeny z tohoto hlediska žádné zvláštní požadavky.

Další postup spojení polymerního hydrogelu a substrátu je popsán v patentu US 5 480 717. Tento proces spojuje polymerní hydrogel se substrátem, aby byl získán hydrogelový laminát se značně zvýšenou odolností proti delaminaci. Podle tohoto patentu je jako syntetický polymer pro hydrogel preferován síťovaný polyvinylpyrolidon o určité molekulové hmotnosti (opt. 200.000 až 300.000 Dalton) v množství nejlépe 40 až 50 % hm. ve vodném roztoku. Síťování hydrogelu je důležité vzhledem k výsledné hodnotě adheze, ale nesouvisí s minimem mechanických vlastností, které jsou od materiálu vyžadovány, neboť tyto jsou zajištěny nosným substrátem. Je zde uvedeno, že pokud je molekulová hmotnost polyvinylpyrolidonu příliš vysoká, není možno získat roztok o dostatečně vysoké koncentraci polyvinylpyrolidonu a proto adheze k polymerním adhezívním vrstvám po ozáření není vyhovující. Navíc koncentrace polyvinylpyrolidonu předpokládané v tomto patentu jsou relativně vysoké, a to může vést k citelnému růstu ceny takových hydrogelů.

Polyvinylpyrolidon je vhodným syntetickým polymerem pro přípravu hydrogelů pro krytí ran, jak může být zřejmé z dalšího patentu US 4 871 490. Postup podle tohoto patentu je založen na lití vodného roztoku syntetického polymeru, například polyvinylpyrolidonu, přírodního polymeru, například agaru, a takzvaného změkčujícího činidla, například polyethylenglykolu do formy udělující tvar tomuto hydrogelu. Po ozáření je získán hydrogel pro krytí ran podle tohoto patentu. Na rozdíl od dříve uvedených hydrogelů je to samonosný materiál a jako takový vyžaduje určité mechanické vlastnosti. Komerční receptury obsahují kolem 7 % hm. polyvinylpyrolidonu a takové množství může být stále považováno za příliš vysoké vzhledem k ceně výrobku. Navíc tyto receptury jsou považovány za dobré pokud jde o krytí spálenin, zatímco jejich mechanické vlastnosti, chování při botnání a sušení poněkud zaostávají za současnými požadavky.

S cílem odstranění některých nevýhod již uvedených postupů a směsí přicházejí další metody a receptury, jako je směs podle patentu US 5 306 504. Toto řešení je založeno na síťovaném

polyvinylpyrolidonu, který je míchán s vodorozpustným multifunkčním amin obsahujícím polymerem. Polyvinylpyrolidon má kyselé skupiny otevírající cyklus, které mohou reagovat se zásaditými aminovými skupinami multifunkčního amin–obsahujícího polymeru za vzniku nerozpustné, ale ve vodě botnající síťované anfolytické soli. Příprava probíhá ve vodném prostředí s obsahem vody 40 až 80 % hm. Změkčovadlo – například polyethylenglykol – může být použito pro získání potřebné lepivosti. Změkčovadlo může zvyšovat lepivost, přitom však snižuje pevnost gelu.

Aby se dosáhlo účinnějšího průběhu procesu spojením dvou operací do jednoho kroku, byla vyvinuta technologie popsaná v patentu US 5 540 033, týkající se výroby sterilně baleného adhezívního hydrogelu. Směs obsahující radiačně síťovatelný polymer a současně inhibitor síťování je podle tohoto patentu obvyklým způsobem tvarována do požadovaného tvaru, následně je tvarovaná směs uzavřena do těsného obalu a vystavena dávce záření postačující k současnému síťování a sterilizaci směsi, čímž se získá finální hydrogelový výrobek. Polymery použité v této metodě jsou především polyethylenoxid, polyvinylpyrolidon a/nebo jejich směs (opt. 15 až 25 % hm. polyvinylpyrolidonu). Inhibitor síťování je s výhodou antioxidant, jako je kyselina askorbová. Pokud jde o další přísady, může být použito zvlhčující činidlo, jako je polyethylenglykol, aby se zlepšily fyzikální vlastnosti hydrogelu. Také může být přítomen urychlovač síťování, jako je ethylenglykoldimethakrylát. Touto metodou lze připravit hydrogel s nejméně 80% podílem gelu a absorpční kapacitou (měřeno podílem gelu) nejméně 5. Tato sofistikovaná směs umožňuje dosáhnout dalšího zlepšení vlastností hydrogelu a dovoluje provádět síťování a sterilizaci v jednom kroku. Nicméně obsah syntetického polymeru a počet aditiv může vést k vyšší ceně získaného produktu.

Želatinující systém podle patentu US 5 578 661 se skládá z vodné směsi nejméně tří polymerních komponent. První z nich je vodorozpustný polymer, například polyvinylpyrolidon, v množství 3 až 35 % hm. Tato složka může být míchána s polyethylenoxidem v hmotnostním poměru 10 : 1 až 25 : 1. Druhá polymerní složka je kyselé skupiny obsahující polymer a třetí složkou je polymer obsahující aminoskupiny, například heparin a agar. Směs může také zahrnovat přídavné složky jako baktericidní látky a antibiotika pro léčebné účinky a také zvlhčující přísady pro zvýšení rozpustnosti třetí nebo druhé složky ve směsi. Jako zvlhčující přísada je preferován polyethylenglykol. Pokud jde o obsah polyvinylpyrolidonu v příkladech, je zde uváděno kolem 10 % z celkové hmotnosti směsi. Želatinační poměr jako měřítko absorpční kapacity hydrogelu může překročit 5. Tento gelotvorný systém obsahuje velký podíl polymerních složek a množství dalších přísad a tak může být stejně jako předchozí systém poněkud nákladný.

Po vzoru již uvedených hydrogelových systémů byl s cílem dalšího zlepšení fyzikálních vlastností získaných hydrogelových materiálů v aplikacích pro krytí ran, zvláště mechanických vlastností a chování při botnání a sušení, vyvinut materiál podle patentové přihlášky US 2008/0033064. Tento způsob přípravy hydrogelu pro krytí ran zahrnuje krok přípravy výchozího vodného roztoku obsahujícího nejméně 15 % syntetického polymeru síťovatelného zářením (vztaženo na hmotnost směsi), dále nejméně jedno zvlhčující činidlo, přírodní polymer a vodu, nalití tohoto vodného roztoku do formy pro tvarování, ponechání vodného roztoku ve formě ke zrání po dobu postačující k získání polotovaru o obsahu nejméně 35 % syntetického polymeru, dále odstranění takto tvarovaného polotovaru z formy a podrobení účinku záření, aby byl polotovar síťován a sterilizován. Vodný roztok obsahuje nejméně 15 % syntetického polymeru, který je radiačně síťovatelný. Tento hydrogelový systém, tak, jak je popsán, umožňuje dosažení ještě lepších vlastností finálního produktu – hydrogelu pro krytí ran – a navíc metoda výhodně spojuje dva kroky – síťování a sterilizaci. Nicméně účinnost tohoto systému – stejně jako předchozího – závisí na obsahu radiačně síťovatelného polymeru – polyvinylpyrolidonu. Aby byly splněny tyto požadavky závislé na obsahu polyvinylpyrolidonu, jsou tím zároveň nepříznivě ovlivněny jiné charakteristiky hydrogelu, které musí být proto dodatečně zlepšovány dodáním dalších složek do tohoto systému. Tím výsledná cena hydrogelu roste. To je hlavní nevýhoda dosavadních metod přípravy hydrogelů pro krytí ran, kterou však není možno překonat bez podstatné změny v sestavě těchto hydrogelů a v technologickém procesu.

S cílem odstranění výše uvedených nevýhod a nedostatků hydrogelů pro krytí ran známých ze stavu techniky a technologií pro jejich přípravu byl vyvinut hydrogel pro krytí ran podle užitného vzoru ČR 18770. Podstata tohoto technického řešení spočívá v tom, že hydrogel obsahuje polyvinylpyrolidon s molekulovou hmotností 30 až 50,000 Dalton, karboxymethylcelulózu nebo kolagen, agar, polyethylenglykol s molekulovou hmotností 200 až 20,000 Dalton, glycerín, případně obsahuje alespoň jedno antibakteriální a/nebo antiseptické činidlo, s výhodou kyselinu boritou, přičemž všechny uvedené složky dohromady představují do 10 hm. % hydrogelu a zbývající část do 100 hm. % tvoří voda.

Hydrogel pro krytí ran podle uvedeného užitného vzoru má s výhodou kruhový nebo čtvercový tvar, tloušťku 2 až 3 mm a plochu 500 až 6000 mm^2.

Výsledný produkt má obsah vlhkosti kolem 94 až 90 %. Úbytek hmotnosti ve finálním produktu (hydrogelu) oproti počáteční hmotnosti roztoku je kolem 10 až 30 hm. %. Pokud jde o hlavní polymery, je možné doplnit polyvinylpyrrolidon buď karboxymethylcelulózou, nebo kolagenem k dosažení víceméně podobného účinku.

Pokud jde o ostatní složky, agar působí jako želatinační činidlo, polyethylenglykol jako hojicí složka a glycerín představuje zvlhčující činidlo. Kyselina boritá, je-li použita, působí jako antiseptické a antibakteriální činidlo. Přítomnost kyseliny borité způsobuje, že hydrogel pro krytí ran odolává mikrobiálním infekcím na menších popáleninách a řezných poraněních kůže a kromě toho způsobuje chladivý pocit.

Značnou výhodou hydrogelu podle tohoto užitného vzoru je jeho příznivá cena. Tento hydrogel je ekologicky přátelský a snadno se skladuje a používá. Pokud jde o jeho uživatelské vlastnosti, jeho semitransparentní charakter umožňuje stálé monitorování hojicího procesu, což je velmi důležitá výhoda. Navíc tento hydrogel zlepšuje podmínky hojicího procesu díky svým absorpčním schopnostem a je nelepivý na pokožku.

Poslední z uvedených hydrogelů představuje velmi dobrou a úspěšnou alternativu v oblasti léčivých hydrogelů. Nicméně, tento i všechny dříve známé hydrogely pro medicínské aplikace jsou na trhu dostupné v mokré formě, s vysokým obsahem vody. Tato skutečnost je způsobena snahou dosáhnout nejvyššího uživatelského komfortu ohledně rychlosti užití; nicméně na druhé straně má tato skutečnost své citelné nevýhody.

Hlavní společnou nevýhodou všech takzvaných „mokrých hydrogelů" je skutečnost, že nemohou být skladovány po dlouhý čas vzhledem k možné ztrátě vody a/nebo možnému množení bakterií. Současně jsou „mokré hydrogely" velmi citlivé a náročné na dodržení sterilních aseptických podmínek během manipulace a skladování. Mokré hydrogely obsahují obvykle až 95 % hm. vody, což je prakticky rovnovážný stav, proto také nejsou schopné absorbovat větší množství tělních tekutin uvolněných během hojícího procesu, což je jejich značnou nevýhodou. Vysoký obsah vody u mokrých hydrogelů se u nich také promítá do značného zvýšení nákladů na dopravu a tím i do zvýšení finální ceny výrobku.

Podstata vynálezu

Uvedené nevýhody a nedostatky dosud známých hydrogelů pro krytí ran a způsobů jejich přípravy jsou do značné míry odstraněny u suché substance hydrogelu pro krytí ran podle vynálezu a způsobu výroby substance tohoto hydrogelu. Podstata vynálezu spočívá v tom, že suchá substance hydrogelu obsahuje želatinu a alginát sodný v hmotnostním poměru 3:7 až 7:3, přičemž tyto dvě složky společně tvoří 100 hmotnostních dílů (hm.d.) směsi a současně suchá substance obsahuje polyethylenglykol v množství 15 až 25 hm.d., glycerín v množství 15 až 25 hm.d., nanovlákna z polyvinylalkoholu v množství 10 až 40 hm.d., chlorid sodný v množství 1 až

3 hm.d., případně jiné obvyklé přísady. Suchá substance hydrogelu může dále obsahovat rakytníkový olej v množství až 15 hm.d. Tato suchá substance je tvořena plochým tělískem, s výhodou kruhového nebo oválného tvaru, o tloušťce 1 až 2 mm a ploše 400 až 200 000 mm.

Způsob přípravy suché substance hydrogelu podle vynálezu spočívá v tom, že kontinuálním mícháním želatiny ve 180 až 220 hm. d. vody při konstantní teplotě 75 až 85 °C se připraví výchozí vodní polymerní roztok želatiny, po rozpuštění celého objemu želatiny jsou k tomuto roztoku přidány ostatní složky směsi kromě nanovláken, načež se směs kontinuálně míchá po dobu 5 až 6 min. při 250 až 350 ot/min., čímž se vytvoří viskózní hmota hydrogelu, potom se postupně přidá frakce nanovláken z polyvinylalkoholu, načež se rychlost míchání viskózní hmoty sníží na 150 až 50 ot/min. a nakonec se viskózní hmota nalije do plochých misek, kde zraje při pokojové teplotě 20 až 25 °C, dokud se neodpaří veškerá voda, čímž se získá konečná suchá substance hydrogelu.

Želatina napomáhá regeneraci poraněné tkáně a absorbuje krev nebo uvolněné tělní tekutiny, alginát sodný zadržuje vodu a působí jako antimikrobiální činidlo. Polyethylenglykol působí jako náhrada poškozené kožní membrány, glycerín jako zvlhčující činidlo, chlorid sodný jako surfaktant. Nanovlákna z polyvinylalkoholu vytvářejí vláknitou matrici a zlepšují mechanickou pevnost vzniklého celku. Olej rakytníkový působí jako hojivý prostředek a také pomáhá zmenšit následné jizvy léčených ran.

Hlavní výhodou suché substance hydrogelu pro krytí ran podle vynálezu je skutečnost, že tato substance může být dlouhodobě skladována ve srovnání se známými „mokrými" hydrogely. Prodloužení životnosti je zde dáno dvěma hlavními důvody: nehrozí nebezpečí ztráty vody a navíc je v suché substanci velmi ztíženo množení bakterií.

Další důležitou výhodou suché substance hydrogelu pro krytí ran podle vynálezu je skutečnost, že její hmotnost je snížena pod 50 % (cca na 40 %) oproti mokrým hydrogelům. Výrobky podle vynálezu jsou lehké a tím jsou také nižší jejich náklady na dopravu.

Jinou důležitou výhodou „suchých" hydrogelů podle vynálezu je skutečnost, že mohou být před vlastním použitím připraveny s nižším stupněm nasycení vodu – pod rovnovážným bodem – a tak mohou následně absorbovat poměrně značné množství exudátů v průběhu hojicího procesu.

Popsané hydrogely podle vynálezu mohou obsahovat některá pomocná léčiva – vodorozpustné prostředky k ochraně rány, urychlení hojení a prevenci infekce. Výsledná mokrá matrice připravená před použitím je makroporézní a tak má dobrou propustnost pro kyslík. Vzhledem k přítomnosti polyvinylalkoholových nanovláken může být hydrogel podle vynálezu použit jako gázový polštářek nebo pěnový obvaz.

Příklady provedení vynálezu

Vynález je blíže objasněn pomocí následujících příkladů konkrétního provedení.

Příklad 1

Výchozí polymerní roztok, obsahující 40 hm.d. želatiny ve 190 hm.d. vody, byl připraven za stálého míchání při konstantní teplotě 75 °C. Jakmile byl rozpuštěn celý objem želatiny, byly přidány další složky: 60 hm.d. alginátu sodného, 16 hm.d. polyethylenglykolu, 1,5 hm.d. chloridu sodného a 16 hm.d. glycerinu. Míchání této směsi pak pokračovalo po dobu 5 min. při 250 ot/min. K výsledné viskózní hmotě hydrogelu byla pak postupně přidána frakce polyvinylalkoholových nanovláken v množství 15 hm.d. a rychlost míchání byla snížena na 60 ot/min. Nakonec byla viskózní hmota za aseptických podmínek dávkována do akrylových misek o kruhovém průměru

velikosti 25 mm ve tloušťce 2 mm. Pak byl hydrogel uvolněn z misek a sušen při pokojové teplotě 20 °C. Během procesu zrání byl z viskózní hmoty zcela odstraněn obsah vody a byla získána finální suchá substance hydrogelu ve tvaru plochého kruhového tělíska. Výsledný produkt světle žluté barvy měl tloušťku 1,4 mm a plochu 490 mm^2.

Pro použití tohoto produktu pro krytí ran na lidském nebo zvířecím těle je před aplikací třeba ponořit jej do sterilní destilované vody při pokojové teplotě v aseptickém prostředí.

Příklad 2

Výchozí polymerní roztok, obsahující 60 hm.d. želatiny ve 210 hm.d. vody, byl připraven za stálého míchání při konstantní teplotě 85 °C. Jakmile byl rozpuštěn celý objem želatiny, byly přidány další složky: 40 hm.d. alginátu sodného, 24 hm.d. polyethylenglykolu, 2,5 hm.d. chloridu sodného, 24 hm.d. glycerínu a 10 hm.d. rakytníkového oleje. Míchání této směsi pak pokračovalo po dobu 6 min. při 350 ot/min. K výsledné viskózní hmotě hydrogelu byla pak postupně přidána frakce polyvinylalkoholových nanovláken v množství 35 hm.d. a rychlost míchání byla snížena na 140 ot/min. Nakonec byla viskózní hmota za aseptických podmínek dávkována do akrylových misek stejně jako v příkladě 1. Pak byl hydrogel uvolněn z misek a sušen při pokojové teplotě 25 °C. Během procesu zrání byl z viskózní hmoty zcela odstraněn obsah vody a byla získána finální suchá substance hydrogelu ve tvaru plochého kruhového tělíska. Výsledný produkt oranžové barvy měl tloušťku 1,6 mm a plochu 490 mm^2.

Pro použití tohoto produktu pro krytí ran na lidském nebo zvířecím těle je před aplikací opět třeba ponořit jej do sterilní destilované vody při pokojové teplotě v aseptickém prostředí.

Průmyslová využitelnost

Hydrogel pro krytí ran podle vynálezu bude používán pro lékařské účely při ošetřování spálenin a velkých ran. Může být použit v chirurgii pro pooperační péči, kde umožní snadné a bezbolestné sledování hojicího procesu. Díky svým absorpčním schopnostem najde užití také ve speciálních aplikacích pro krytí pomalu se hojících nebo velmi poškozených tkání. Hydrogel pro krytí ran podle tohoto vynálezu může pomoci také v aktuálních krizových situacích pro rychlou první pomoc a ošetření zraněných osob. Možné jsou rovněž některé aplikace ve veterinárním lékařství.

PATENTOVÉ NÁROKY

1. Suchá substance hydrogelu pro krytí ran vyrobená z přírodních a syntetických polymerů, **vyznačující se tím**, že obsahuje želatinu a alginát sodný v hmotnostním poměru 3 : 7 až 7 : 3, přičemž tyto dvě složky dohromady tvoří 100 hm.d., a současně substance obsahuje 15 až 25 hm.d. polyethylenglykolu, 15 až 25 hm.d. glycerínu, 10 až 40 hm.d. polyvinylalkoholových nanovláken, 1 až 3 hm.d. chloridu sodného, případně další aditiva.

2. Suchá substance hydrogelu pro krytí ran podle nároku 1, **vyznačující se tím**, že obsahuje až 15 hm.d. rakytníkového oleje.

3. Suchá substance hydrogelu pro krytí ran podle nároku 1, **vyznačující se tím**, že je tvořena plochým tělískem, s výhodou kruhového nebo oválného tvaru, o tloušťce 1 až 2 mm a ploše 400 až 200 000 mm^2.

4. Způsob přípravy suché substance hydrogelu pro krytí ran podle nároku 1, případně 2 a/nebo 3, **vyznačující se tím**, že za stálého míchání při konstantní teplotě 75 až 85 °C se připraví výchozí polymerní roztok želatiny ve 180 až 220 hm.d. vody, po rozpuštění celého objemu želatiny se k výchozímu vodnému polymernímu roztoku želatiny přidají další složky – alginát sodný, polyethylenglykol, chlorid sodný a glycerín, případně rakytníkový olej, míchání této směsi pak pokračuje po dobu 5 až 6 min. při 250 až 350 ot/min, k výsledné viskózní hmotě hydrogelu se pak postupně přidá frakce polyvinylalkoholových nanovláken a rychlost míchání se sníží na 150 až 50 ot/min, nakonec se viskózní hmota za aseptických podmínek dávkuje do misek a podrobí zrání při pokojové teplotě 20 až 25 °C, během něhož se z viskózní hmoty zcela odstraní obsah vody a získá se finální suchá substance hydrogelu.

Konec dokumentu

PATENT II

Dry Material of Hydrogel for Wound Dressing and its Method of Preparation

Saha Nabanita, Saha Tomas, Amarjargal Saarai

International Patent Application WO 2011/100935 A1,

Publication date 25. 8. 2011

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 August 2011 (25.08.2011)

PCT

(10) International Publication Number
WO 2011/100935 A1

(51) International Patent Classification:
A61L 15/32 (2006.01)

(21) International Application Number:
PCT/CZ2011/000017

(22) International Filing Date:
15 February 2011 (15.02.2011)

(25) Filing Language: Czech

(26) Publication Language: English

(30) Priority Data:
PV 2010-126 18 February 2010 (18.02.2010) CZ

(71) Applicant *(for all designated States except US)*: **UNIVERZITA TOMASE BATI VE ZLINE** [CZ/CZ]; Nam. T. G. Masaryka 5555, 76001 Zlin (CZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SAHA, Nabanita** [IN/IN]; Sarat-Pali, Midnapore A1, West Bengal (IN). **SAHA, Tomas** [CZ/CZ]; Naves 4, Mladcova, 760 01 Zlin (CZ). **SAARAI, Amarjargal** [MN/MN]; Apartment 8-21, 15th Bag, Darkhan City (MN).

(74) Agent: **KREIZLOVA, Dana**; Univerzita Tomase Bati ve Zline, Univerzitni institut, nam. T.G. Masaryka 5555, 760 01 Zlin (CZ).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/100935 A1

(54) Title: DRY MATERIAL OF HYDROGEL FOR WOUND DRESSING AND ITS METHOD OF PREPARATION

(57) Abstract: The dry material of hydrogel substance for wound dressing made from synthetic and natural polymers contains gelatin and sodium alginate in the weight ratio 3:7 up to 7:3, whereby these two components create 100 wt. pts., and simultaneously contains 15-25 wt. pts of polyethylenglykol, 15-25 wt. pts of glycerine, 10-40 wt. pts of polyvinylalcohol nanofibres, 1 -3 wt. pts of natrium chloride, eventually further usual additives. A method of preparing the dry substance of hydrogel for wound dressing lies in t h a t initial aqueous polymeric solution of gelatin will be achieved during continuous stirring it in 180 - 220 wt. pts of water at the constant temperature 75 - 85°C, after dissolving whole volume of gelatin further components such as sodium alginate, polyethylene glycol, sodium chloride, glycerin and respectively sea buckthorn oil will be added to the initial aqueous polymeric solution of gelatin, whereupon stirring of this mixture will continue for 5 - 6 min at 250 - 350 rpm in order to a viscous hydrogel mass will be developed, then slowly a fraction of polyvinyl alcohol nanofibres will be added whereon the mixing speed of viscous mass will be reduced till 150 - 50 rpm, finally, the viscous mass will be poured into acrylic dishes of 25 mm diameter where it will be incubated at room temperature of 20 - 25°C until the whole volume of water is removed and the final dry material of hydrogel is achieved.

Dry material of hydrogel for wound dressing and its method of preparation

Field of Invention

The invention relates to dry substance of hydrogel for wound dressing used on bodies, in particular human bodies. The presented invention also relates to the method of preparation of such hydrogel for wound dressing.

Background Art

To date several hydrogels for wound dressing from synthetic and natural polymers as well as methods of their preparation are known. They are oriented to obtain a product with best mechanical, swelling and other important physical properties, as well as with antibacterial and wound-healing properties.

There are several more requirements imposed on hydrogels for wound dressing. They should be soft and friendly to wounded body and at the same time they should resist the manipulations during application and thereafter. These requirements are rather contradictory and it is difficult to meet them sufficiently.

One of the known methods for manufacturing hydrogel for wound dressing is described in the WO 03/034900 document which discloses an intradermal patch with a permeable backing coated with a gel based on polyvinylpyrrolidone with certain average molecular weight (pref. 900.000 – 1.500.000 Dalton) in an amount of pref. 15 – 20 % of weight. The patches may comprise one or more additional components such as wound-healing agent, medicines, viscosity enhancing agents and humectants. The hydrogel material is placed on a base made from a material with sufficient mechanical properties and therefore no extra mechanical requirements are imposed on the hydrogel.

Further process for adhering a polymeric hydrogel to a substrate is disclosed in US patent No. 5,480,717. This process is characterized by the adhering a polymeric hydrogel to a substrate in order to obtain a hydrogel laminate with greatly improved de-lamination resistance. According to this patent the preferred synthetic polymer for hydrogel is the

cross-linked polyvinylpyrrolidone of particular molecular weight (pref. 200.000 – 300.000 Dalton) in an amount of pref. 40 – 50 % of weight in aqueous solution. The cross-linking of hydrogel is important with regard to the result adhesion value but it is not connected with the minimum of mechanical properties required, because they are assured by the substrate. It is said herein that if the molecular weight of the polyvinylpyrrolidone is too high it is not possible to obtain the solution with a high enough polyvinylpyrrolidone concentration and therefore the adhesion to the polymeric adhesive layers after irradiation is not acceptable. Moreover, the concentrations of the polyvinylpyrrolidone supposed in this patent are relatively high and it can lead to extending costs for such hydrogels.

The polyvinylpyrrolidone is the appropriate synthetic polymer for the preparation of hydrogel for wound dressing, as can be seen from another US patent No. 4,871,490. The method of this patent is based on pouring the aqueous solution of synthetic polymer, e.g. polyvinylpyrrolidone, natural polymer, e.g. agar, and so-called plasticizing agent, e.g. polyethylene glycol, into a mould imparting a shape to the hydrogel. After irradiation the hydrogel for wound dressing is obtained according to this patent. Unlike the previous mentioned hydrogels this is a self-supporting material and as such it requires certain mechanical properties. The commercial formulas contain about 7 % of weight Polyvinylpyrrolidone and such amount can be seen still too high regarding the costs of the product. Moreover, these formulations are said to be good at burn dressings, whilst their mechanical, swelling and drying properties fall behind current requirements in some respect.

To eliminate some of the disadvantages of the before said methods and compositions, there are further methods or compositions being developed, such as the composition according to US patent No. 5,306,504. This solution is based on cross-linked polyvinylpyrrolidone, which is mixed with water-soluble multifunctional amine-containing polymer. Polyvinyl-pyrrolidone has ring opened pyrrolidone acid groups which can react with the basics amine groups of the multifunctional amine-containing polymer to form a water-insoluble, water-swellable, cross-linked ampholyte salt. The preparation takes place in an aqueous medium with the water content 40 – 80 % of

weight. A plasticizer - e.g. polyethylene glycol – can be used for tack development. The plasticizer may increase tack, however it decreases gel strength.

To achieve a more effective process cumulating two operations into one, a method described in US patent No. 5,540,033 regarding production of a sterile packaged adhesive hydrogel product was developed. The composition containing the polymer cross-linkable by irradiation and a cross-linking inhibitor is shaped into a desired shape by a common way, subsequently the shaped mixture is enclosed into a sealed package and subjected to dose of radiation sufficient to simultaneously cross-link and sterilize the mixture in order to obtain the final hydrogel product. Polymers used in this method are mainly polyethylene oxide, polyvinylpyrrolidone and/or mixture thereof (pref. 15 -25 % of weight of polvinylpyrrolidone). A cross-linking inhibitor is preferably an antioxidant as ascorbic acid. Regarding further additives a humectant such as polyethylene glycol can be used so as to improve the physical properties of the hydrogel. Also, cross-linking promoter as is ethylene glycol dimethacrylate can be present. This method can provide hydrogel with at least 80 % gel value and absorptive capacity (measured by the gel ratio) at least 5. This sophisticated composition reaches further improvement inthe properties of hydrogel and allows to cross-link and to sterilize the product in one step. However, the content of synthetic polymer and the number of additives can lead to higher costs of obtained products.

The gel-forming system according to US patent No. 5,578,661 comprises an aqueous mixture of at least three polymeric components. The first of them is a water-soluble polymer, e.g. polyvinylpyrrolidone, in an amount of 3 – 35 % of weight. This component can be mixed with polyethylene oxide in a weight ratio from 10: 1 up to 25: 1. The second polymeric component is an acid-containing polymer and the third polymeric component is an amino-containing polymer, e.g. heparin and agar. The composition can also include additional components as bactericides and antibiotics for the curative actions, and humectant for increasing the solubility of the third or second component in the mixture. Such humectant is preferably polyethylene glycol. Regarding the content of polyvinylpyrrolidone in examples there is presented the content of 10 % of weight of the mixture. The gel ratio as a measure of the absorption capacity of hydrogels is able to

exceed 5. This gel-forming system contains a great portion of polymeric components and manifold number of further components and so it can be rather expensive as well as the previous one.

Following the above mentioned hydrogel systems in order to further improve the physical properties of the obtained hydrogel materials in wound dressing, in particular the mechanical, swelling and drying properties, the material according to US patent application No. 2008/0033064 has been developed. This method of preparing hydrogel for wound dressing comprises the step of providing initial aqueous solution containing at least 15 % of synthetic polymer cross-linkable by irradiation (based on the weight of the mixture) at least one humectant, a natural polymer and water, pouring the initial aqueous solution into a mould for shaping, allowing the aqueous solution to mature in the mould during a period of time sufficient to obtain a semi-product having a content of at least 35 % of synthetic polymer, removing the semi-product thus shaped from the mould and subjecting the semi-product to irradiation in order to cross-link an sterilize the semi-product. The aqueous solution comprises at least 15 % of synthetic polymer, which is cross-linkable by irradiation. This hydrogel system as described allows to achieve even better properties of the final product – hydrogel for wound dressing and, moreover, the method couples advantageously two steps – cross-linking and sterilization. However, the efficiency of this system – as well as of the previous ones – depends on the content of the irradiation cross-linkable polymer – polyvinyl-pyrrolidone. In order to fulfil this requirement on the content of polyvinylpyrrolidone there are lowered the other characteristics of the hydrogel, which have to be thereafter improved by adding of further components to this system. Thus the result price of hydrogel increases. This is the main disadvantage of the methods of preparing the hydrogel for wound dressing known so far which however cannot be overrun without essential change in formation of these hydrogels and in the technological process.

So as to restrain the above mentioned disadvantages and insufficiencies of the hydrogel for wound dressing known so far in preparation technologies , the hydrogel for wound dressing according to CZ utility model 18770 has been developed. The principle of the related technical solution consists in that the hydrogel contains polyvinylpyrrolidone having

molecular weight of 30 -50,000 Dalton, carboxymethyl cellulose or collagen, agar, polyethylene glycol having molecular weight of 200 - 20,000 Dalton, glycerin, and respectively contains at least one antibacterial and/or antiseptic agent, advantageously boric acid, whereas all the mentioned components together gain 0 – 10 w/v % of hydrogel and the remaining part onto 100 w/v % amount of water.
Hydrogel for wound dressing according to the mentioned utility model has got advantageously a round or square shape, thickness of 2 – 3 mm and the area of 500 – 6000 mm^2.

The resultant product has about 94-90 % moisture content. The weight decrease in final product (hydrogel) from the initial weight of solution is about 10 - 30 %. Regarding the main polymers, it is possible to complement polyvinylpyrrolidone by using either carboxymethyl cellulose or collagen to achieve more or less similar performance.
Regarding the other components, agar acts as a gelling agent, polyethylene glycol performs as a healing component and glycerin represents humectant. Boric acid – if used - acts as antiseptic cum antibacterial agent. Presence of boric acid within hydrogel for wound dressing resists microbial infection on minor burns and cuts of skin besides providing cool feelings.

The considerable advantage of the hydrogel for wound dressing according to this utility model is its cost effectiveness. This hydrogel is eco-friendly and easy to store and use. Regarding its end-user qualities, this hydrogel for wound dressing due to its semitransparent character allows instant monitoring of healing process which is a very important advantage. Moreover, it improves the conditions of healing process thanks to its praiseworthy absorption capability. Besides, this hydrogel is not sticky on the skin.

The last mentioned hydrogel represents a very good and successful alternative in the field of healing hydrogels. However, this one as well as all the previously known hydrogels for medical use are delivered on the market in the wet form, with the great content of water. This fact is caused by the effort to reach the highest user comfort enabling the promptness of using; however, on the other hand, it has more considerable disadvantages.

The main common disadvantage of all so called "wet hydrogels" is the fact that they cannot be stored for a long time (loss of water, germs proliferation). Simultaneously the wet hydrogels are very sensitive and demanding so as to maintain the sterilized, germ free conditions during the handling and storage. Moreover, the wet hydrogels contain usually up to 95% water, which is almost state equilibrium, thus they are not able to absorb much exudates during the healing process, which is their considerable disadvantage. The high weight content of water by wet hydrogels leads to multiple increasing of their delivery costs and thus the increase of final price of product.

Nature of the invention

The stated disadvantages and inefficiencies of so far known hydrogels for wound dressing and the ways of their preparation have been to a large extent removed at the dry material of hydrogel for wound dressings as per the invention and the way of production of the substance of the hydrogel. The nature of the invention lies in the fact, that the hydrogel substance contains gelatin and Sodium alginate in weight ration of 3:7 – 7:3, where both these components represent 100 of wt. pts. of the mixture; parallel to this the dry substance also contains polyethylenglykol in the amount of 15-25 wt. pts., 15-25 wt. pts. of glycerine, nanofibres of polyvinyl alcohol in the amount of 10-40 wt. pts., sodium chloride in the amount of 1-3 wt. pts., eventually other usual ingredients. The dry substance of the hydrogel may further contain sea buckthorn oil in the amount of up to 15 wt. pts. This dry substance has a flat shape, pref. round of square, is usually 1-2mm thick and has area of 40-200 000 mm^2.

The preparation method of the dry hydrogel material according to the invention lies preparation of a base water polymer solution of gelatin by continuous stirring of gelatine in 180-220 wt. pts. of water at constant temperature between 75 and 85 Celsius degrees. After complete dissolving of the gelatine volume other components are being added (except for nanofibres) and the mixture is again continuously stirred for 5 to 6 minutes at the speed of 250-350 r.p.m., whereby a viscid substance is created. Afterwards fractions of nanofibres of polyvinyl alcohol are gradually added, and the speed of stirring is decreased to 150-50 r.p.m.; finally the viscid substance is poured to flat dishes and is left

to mature at room temperature of 20-25 Celsius degrees until all water has been evaporated and the final dry hydrogel substance is achieved.

Gelatine helps regeneration of damaged tissues and absorbs blood of detached body fluids, Sodium alginate holds back water and acts as antimicrobial agent. Polyethylenglykol acts as a substitute of damaged skin membrane, glycerine as a humectant agent, natrium chloride as surfactant. Nanofibres of polyvinyl alcohol create a fibre matrix and improve mechanical strength of the achieved whole. Sea buckthorn oil acts as a healing agent and also helps to diminish subsequent scarves of healed wounds.

The main advantage of the dry material of hydrogel for wound dressing according to this invention is its´ long term storage capacity/ability, in comparison to the known "wet" hydrogels. There are two main reasons for its prolonged longevity: there is no danger of loss of water and, moreover, in the dry substance the germ proliferation is extremely reduced.

Another important advantage of the dry material of hydrogel for wound dressing according to this invention is the fact that the weight of dry hydrogels is reduced under 50% (ca 40%) compared to wet hydrogels. The products of the invention are light and so their delivery costs are lowered, too.

One more important advantage of "dry" hydrogels as per the invention is the fact that they can be prepared before use with lower degree of saturation by water – under the equilibrium point – and so they can absorb subsequently absorb a lot of exudates during the healing process.

The described hydrogels according to this invention can contain some additional medicines – water soluble means for wound protection, healing acceleration and prevention of wound infection. The resulting wet matrix prepared before use is macroporous and has therefore good oxygen diffusion properties. Thanks to the presence of polyvinyl alcohol nanofibres, the hydrogel according to the invention can be used as gauze pad or sponge dressing for external use.

Examples

The invention is closely explained through the examples of specific implementation as follows.

Example 1

The initial polymer solution, containing 40 wt. pts of gelatin in 190 wt. pts of water was achieved through continuous stirring at the constant temperature of 75°C. As soon as the entire volume of gelatin has been dissolved, further components were added: sodium alginate 60 wt. pts, polyethylene glycol 16 wt. pts, sodium chloride 1,5 wt. pts, glycerin 16 wt. pts; stirring of this mixture continued for 5 min at 250 rpm. To the resulting viscous hydrogel mass then slowly a fraction of polyvinyl alcohol nanofibres in an amount of 15 wt. pts was added, the mixing speed of the viscous mass was reduced till 60 rpm. Finally, the viscous mass was dosed in aseptic environment to set of acrylic dishes with diameter of 25 ***mm*** and thickness 2 ***mm***. Afterwards the hydrogel was removed from the dishes and was left to dry at room temperature of 20°C. During the incubation process the whole volume of water was removed from the viscous mass and the final dry material of hydrogel has been achieved. The resulting product of pale yellow colour has got the thickness of 1.4 ***mm*** and area of 490 mm^2.

When using the product for wound dressing on human or animal body, it is necessary prior to its application to dip it in sterile distilled water at room temperature under aseptic environment.

Example 2

The initial polymeric solution, containing 60 wt. pts of gelatin in 210 wt. pts of water was achieved through continuous stirring at the constant temperature of 85°C. As soon as whole volume of gelatin has been dissolved further components were added: sodium alginate 40 wt. pts, polyethylene glycol 24 wt. pts, sodium chloride 2,5 wt. pts, glycerin 24 wt. pts; stirring of this mixture continued for 6 min at 350 rpm. To the resulting viscous hydrogel mass then slowly a fraction of polyvinyl alcohol nanofibres in an amount of 35 wt. pts was added, the mixing speed of the viscous mass was reduced to 140 rpm. Finally, the viscous mass was under aseptic environment dosed to a set of acrylic dishes with diameter 25 ***mm*** and thickness 2 ***mm*** where it incubated and dried at room temperature of 25°C. During the incubation process whole volume of water was removed from the viscous mass and the final dry substance of hydrogel has

been achieved. The resulting product of pale yellow colour has got the thickness of 1.6 ***mm*** and area of 490 mm^2.

When using the product for wound dressing on human or animal body, it is again necessary prior to its application to dip it in sterile distilled water at room temperature under aseptic environment.

Industrial Applicability

The hydrogel for wound dressing according to this invention will be used for medical purposes for treating burns and large wounds. It can be applied in the area of surgery in the postoperative care as it enables easy and painless monitoring of the healing process. Thanks to its absorption capability it will find use also in special purposes like covering of slowly healing or very damaged tissues. The hydrogel wound covering according to this invention can help also in crisis situations for prompt first-aid treatment of wounded persons. Applications in veterinary medicine are possible, too.

CLAIMS

1. Dry material of hydrogel for wound dressing made from natural and synthetic polymers, characterized by that it contains gelatin and sodium alginate in weight ratio 3:7 - 7:3, whereas this two parts together constitute 100 wt. pts and, simultaneously, the substance contains polyethylene glycol 15 - 25 wt. pts, glycerin 15 - 25 wt. pts, polyvinyl alcohol nanofibers 10 – 40 wt. pts, sodium chloride 1 – 3 wt. pts, eventually other usual additives.

2. Dry material of hydrogel for wound covering according to claim 1, characterized by that it contains sea-buckthorn oil up to 15 wt. pts.

3. Dry material of hydrogel for wound covering according to claim 1, characterized by that it has got the flat shape, preferably round or oval shape, the thickness 1 -2 mm and the area 400 to 200 000 mm^2.

4. A method of preparing the dry material of hydrogel for wound dressing according to claim 1, eventually claim 2 and/or 3, consisting in physical technique under control heating, characterized by that initial aqueous polymeric solution of gelatin will be achieved during continuous stirring it in 180 – 220 wt. pts of water at the constant temperature 75 – 85°C, after dissolving whole volume of gelatin further components such as sodium alginate, polyethylene glycol, sodium chloride, glycerin and respectively sea buckthorn oil will be added to the initial aqueous polymeric solution of gelatin, whereupon stirring of this mixture will continue for 5 – 6 min at 250 – 350 rpm in order to a viscous hydrogel mass will be developed, then slowly a fraction of polyvinyl alcohol nanofibers will be added whereon the mixing speed of viscous mass will be reduced till 150 – 50 rpm, finally, the viscous mass will be poured into acrylic dishes of 25 mm diameter where it will be incubated at room temperature of 20 – 25°C until the whole volume of water is removed and the final dry substance of hydrogel is achieved.

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2011/000017

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L15/32
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 135 755 A (CZECH ZBIGNIEW DR DIPL CHEM [DE] ET AL) 4 August 1992 (1992-08-04) column 1, line 6 - line 9 column 1, line 40 - line 49 column 2, line 4 - line 28 -----	1-4
A	WO 2006/028364 A1 (BROOCKEVILLE CORP N V [NL]; OLEJNIK ALICJA KLAUDIA [PL]) 16 March 2006 (2006-03-16) page 4, line 29 - page 5, line 25 page 7, line 18 - line 25 -----	1-4

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2011	01/08/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dudás, Eszter

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CZ2011/000017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5135755 A	04-08-1992	AT 126070 T	15-08-1995
		AU 621187 B2	05-03-1992
		AU 4876990 A	16-08-1990
		BG 60006 A3	15-06-1993
		BR 9000531 A	15-01-1991
		CA 2009475 A1	08-08-1990
		CS 9000593 A2	15-10-1991
		DD 292138 A5	25-07-1991
		DE 3903672 C1	01-02-1990
		DE 59009486 D1	14-09-1995
		DK 0382128 T3	09-08-1995
		EP 0382128 A2	16-08-1990
		ES 2078915 T3	01-01-1996
		FI 102725 B1	15-02-1999
		GR 3017878 T3	31-01-1996
		HU 53798 A2	28-12-1990
		IE 72218 B1	09-04-1997
		IL 93166 A	30-05-1994
		JP 2108232 C	06-11-1996
		JP 2265560 A	30-10-1990
		JP 8002368 B	17-01-1996
		NO 900581 A	09-08-1990
		PL 163801 B1	31-05-1994
		PT 93078 A	31-08-1990
		RO 105547 B1	25-09-1992
		RU 2086234 C1	10-08-1997
WO 2006028364 A1	16-03-2006	AT 465761 T	15-05-2010
		EP 1789102 A1	30-05-2007
		US 2008033064 A1	07-02-2008

CURRICULUM VITAE

Personal information:

Name/Surname:	AMARJARGAL SAARAI, MSc. Ph.D
Date of birth:	15 April 1969
Nationality:	Mongolian

Education:

- 1992 Ing. – Water Supply and Sewerage, Sanitary engineer, Mongolian University of Science and Technology (MUST), Ulaanbaatar, Mongolia
- 1997 MSc. – Water Supply and Sewerage, MUST, Ulaanbaatar, Mongolia
- 1999 PhD – in Sanitary engineering (Water and Waste Water Treatment), MUST, Ulaanbaatar, Mongolia
- 2007 – present Doctoral student (Technology of Macromolecular Substances), Polymer Centre, Faculty of Technology, Tomas Bata University (TBU) in Zlín, Czech Republic

Work experience:

- **1992-2006** Assistant lecturer-Assistant Professor, Department of Building Materials, Darkhan School of Technology, MUST, Darkhan, Mongolia

Membership of scientific bodies:

- Society of Plastics Engineers

Publications:

1. SAARAI, A.; SEDLACEK, T.; KASPARKOVA, V.; KITANO, T.; SAHA, P.: On the Characterization of Sodium Alginate/ Gelatine-Based

Hydrogels for Wound Dressing. *Journal of Applied Polymer Science, 2012, vol. 126, 79-88.*

2. SAARAI, A.; KASPARKOVA, V.; SEDLACEK, T.; SAHA, P.: On the Development and Characterization of Crosslinked Sodium Alginate/ Gelatine-Based Hydrogels. *Journal of the Mechanical Behavior of Biomedical Materials, under review*
3. SAARAI, A.; SEDLACEK, T.; KASPARKOVA, V.: On the Characterization of Genipin Crosslinked Sodium Alginate/ Gelatine Hydrogels for Wound Dressings. *Journal of the Mechanical Behavior of Biomedical Materials, prepared for publication*
4. SAHA, N.; SAARAI, A.; ROY, N.; KITANO, T.; SAHA, P.: Polymeric Biomaterial Based Hydrogels for Biomedical Applications. *Journal of Biomaterials and Nanobiotechnology, 2011, 2, 85-90.*

Patents:

1. SAHA, N.; SAHA, T.; SAARAI, A. Dry Material of Hydrogels for Wound Dressing and its Method of Preparation, *Utility model 20931 U1, 31.05.2010*
2. SAHA, N.; SAHA, T.; SAARAI, A. Dry substance of Hydrogel to Cover Wounds and Process for Preparing Thereof, *CZ patent 302380, date of the patent 09.03.2011*
3. SAHA, N.; SAHA, T.; SAARAI, A. Dry Material of Hydrogels for Wound Dressing and its Method of Preparation, *International Patent Publication Number WO 2011/100935 A1, International Application Number: PCT/CZ/2011/000017*

Conference contributions:

1. SAHA, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Sea Buckthorn Oil Incorporated Medicated Hydrogel Based on Gelatin - Sodium Alginate. *In*

SPE European Medical Polymers Conference, Belfast, United Kingdom, 2008.

2. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Swelling Behavior and Viscoelastic Properties of a New Hydrogel Based on Gelatin-Sodium Alginate. *Fifth International Conference on Polymer Modification, Degradation and Stabilization, Liege, Belgium, 2008*
3. SAHA, N.; ROY, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Polymeric Biomaterials Promise for Healthcare Application. *International Conference on Hi-Tech Materials (ICHTM 09) 11-13 February, 2009, IIT, Kharagpur, India*
4. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Viscoelastic Behavior of Bio based Antimicrobial Hydrogel for Medical Applications, *25th Annual Meeting of the Polymer Processing Society, Goa, India, March 1- 5, 2009*
5. SAARAI, A.; SAHA, N.; KITANO, T.; SÁHA, P: Natural Resource Based medicated Hydrogel for Health care, *Frontiers in Polymer Science, International Symposium, Mainz, Germany, 7-9 June, 2009*
6. STLOUKAL, P.; ROY, N.; SAARAI. A: Preparation of Submicroparticles Based on Biodegradable Co-polyester. *International Conference on Plastko 2010, April 13-14.2010, Zlin, Czech Republic.*
7. SAHA, N.; SAARAI, A.; KITANO, T.; SÁHA, P: Swellable Dressing Materials for Wound Healing. *26th Annual Meeting of the Polymer Processing Society, Banff, Canada, July 4- 5, 2010*
8. SAARAI, A.; KASPARKOVA, V.; SEDLACEK, T.; SAHA, P: Comparative Study of Crosslinked Sodium Alginate/Gelatin Hydrogels for Wound Dressing. *Proceeding of the 4th WSEAS International Conference on Engineering Mechanics, Structures, Engineering Geology, Corfu Island, Greece, July 14-16. 2011*