TOMAS BATA UNIVERSITY IN ZLIN
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BIOARTIFICIAL POLYMERIC MATERIALS WITH A LATENT APPLICATION IN MEDICAL FIELD

Bioarteficiální polymerní materiály s latentním využitím v oblasti zdravotnictví

Doctoral Thesis

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ABSTRACT

The presented doctoral thesis is dedicated to the preparation and characterisation of bioartificial polymeric materials with latent medical application. Besides of the progress that polymer science has reached, including polymers in medical field, there are still significant unsolved problems related to this topic. One of those problems which are indeed one of the most complicated issues in medicine is fibrous adhesion. This phenomenon appears as a consequence of a surgery and it might generate further inconveniences. Although polymers have been used in this matter, an alternative or complementary treatment could contribute in the development of new techniques which may attenuate, reduce or even eliminate the mentioned problem.

Blending polymers is a valuable method for obtaining materials with superior performance and better properties than the individual components. Furthermore, a polymer blend with two different surfaces represents an interesting approach for differentiating tissues and therefore, for reducing the fibrous adhesion. For the mentioned reasons, this thesis contains a broad description of biomaterials and their uses as a frame for understanding the characteristics that bioartificial polymeric materials need to fulfil. Simultaneously, collagen, poly(vinyl alcohol) and poly(vinyl pyrrolidone) are deeply described and special attention is paid to blends of these kind of materials in the medical field.

Three approaches about preparation of bioartificial polymeric materials are developed within this thesis. In the first one, poly(vinyl alcohol) as a biodegradable and biocompatible polymer is dissolved in ethylene glycol and the solution is subjected to microwave irradiation. The process is monitored by UV-VIS and FTIR spectroscopy and as a result, the treatment does not cause significant changes in the polymer and degradation can be considered as negligible with regard to polymer processing. Moreover, SEC confirms that no variations in poly(vinyl

alcohol) molar mass and neither chain cleavage nor crosslinking reactions are observed.

In the second one, poly(vinyl alcohol) and poly(vinyl pyrrolidone) are blended and the obtained films are crosslinked and plasticised with the further intention of being used as bio-materials with latent medical application. The obtained films are characterised by differential scanning calorimetry (DSC), mechanical properties, swelling and solubility behaviour. The polymer blend exhibits an appropriate performance in the studied parameters and as a consequence, the obtained films could be suitable for use as medium or long term implants.

Finally and as a remarkable result, a double-sided bioartificial polymeric material is obtained and it is characterised by different instrumental methods. The material exhibits higher water resistance and mechanical properties than the raw polymers. The characterisation indicates that the combination of crosslinker and plasticiser agents do not affect negatively the performance of the bioartificial film in the range of physiological relevant frequencies at normal human temperature which might indicate that films can be suitable candidate for medical applications.

Key words: Bio-artificial polymeric materials, poly(vinyl alcohol), poly(vinyl pyrrolidone), collagen, mechanical properties, thermal properties.

ABSTRAKT

Předložená doktorská disertační práce je věnována přípravě a charakterizaci bioarteficiálního polymerního materiálu s latentní medicínskou aplikací. Navzdory velkému pokroku, kterého dosáhla věda o polymerech, včetně polymerů v oblasti medicíny, stale v této oblasti zůstávají významné nevyřešené problémy. Jedním z těchto problémů, který je vskutku jeden z nejkomplikovanějších, jsou srůsty. Ten to jev vzniká jako pooperační následek a může způsobovat mnoho dalších potíží. Ačkoliv se polymery již v této věci zkoušely, alternativní nebo doplňující léčba by mohla přispět k rozvoji nových technik, které by zeslabily, omezily nebo zcela odstranily tento problém.

Míchání polymerů je cenná metoda získávání materiálů s lepší funkcí a vlastnostmi než mají jednotlivé složky samostatně. Dále, polymerní směs se dvěma různými povrchy představuje zajímavý přístup pro separaci tkání, čímž by se zamezilo vzniku srůstů. Z uvedených důvodů tato disertace obsahuje široký popis biomateriálů a jejich použití jako rámec pro porozumění vlastnostem, které musí biomateriál splňovat. Současně jsou detailně popsány kolagen, poly(vinylalkohol) a poly(vinylpyrrolidon), přičemž je věnována speciální pozornost směsím těchto druhů materiálů v oblasti medicíny.

V této práci byly rozvinuty tři přístupy k přípravě bioarteficiálních polymerních materiálů. Za prvé, poly(vinylalkohol), jako biodegradabilní a biokompatibilní polymer, byl rozpuštěn v ethylenglykolu a vzniklý roztok byl vystaven mikrovlnnému záření. Proces byl sledován pomocí UV-VIS a FT-IR spektroskopií. Bylo zjištěno, že tato expozice nezpůsobuje významné změny polymeru a že jeho degradace může být považována za zanedbatelnou z hlediska pozdějšího zpracování polymeru. Navíc, SEC potvrdila, že v polymeru se neodehrávají žádné změny molární hmotnosti, ani štěpení řetězců, ani síťování se nepozorovalo.

Za druhé, poly(vinylalkohol) a poly(vinylpyrrolidon) byly zamíchány a získané filmy nesíťovány a změkčeny se záměrem dále je využít jako biomateriál s latentní medicínskou aplikací. Získané filmy byly charakterizovány diferenciální skenovaní kalorimetrií (DSC), byly zkoumány mechanické vlastnosti, bobtnání a rozpustnost. Polymerní směs vykazuje vhodné vlastnosti ve smyslu studovaných parametrů a v důsledku toho lze považovat připravený materiál za případně vhodný pro použití jako středně či dlouhodobý implantát.

Konečně, jako třetí významný výsledek, byl připraven dvojstranný bioarteficiální materiál, který byl charakterizován různými přístrojovými metodami. Tento materiál má větší odolnost vůči vodě a má lepší mechanické vlastnosti, než výchozí polymery. Provedená charakterizace ukazuje, že kombinace síťovacího činidla a změkčovadla neovlivňuje negativně funkci bioarteficiální fólie v rozsahu fyziologicky významných frekvencí při normální teplotě lidského těla, což naznačuje případnou aplikaci v medicíně.

Klíčová Slova: Bio-arteficiální polymerní materiál, poly(vinylalkohol), poly(vinylpyrrolidon), kolagen, mechanické vlastnosti, tepelné vlastnosti.

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ABBREVIATIONS AND SYMBOLS

COLL	Collagen
PVA	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
DAS	4,4'-diazido-2,2'-stilbenedisulfonic acid disodium salt tetrahydrate
EG	Ethylene glycol
GA	Glutaraldehyde
LA	Lactic Acid
UV	Ultra violet
FTIR	Fourier transform infrared spectroscopy
SEC	Size-Exclusion Chromatography
DSC	Differential Scanning Calorimetry
DMA	Dynamic Mechanical Analysis
SEM	Scanning Electron Microscopy
ECM	Extracellular matrix
MWI	Microwave Irradiation
T_g	Glass transition temperature
T_m	Melting temperature
E	Young Modulus
σ	Stress at break
ε	Elongation at break
E'	Storage Modulus

PUBLICATION OUTPUT AND AUTHOR´S CONTRIBUTION

The following papers published in peer-reviewed journals have resulted from this doctoral research and they are available in full-text at the end of this dissertation as the framing papers of the present doctoral thesis.

Publication I:

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Publication II: **Preparation and characterization of poly(vinyl alcohol)-poly(vinyl pyrrolidone) blends for medical applications**

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Publication III: **Preparation and characterisation of a new double-sided bio-artificial material prepared by casting of poly(vinyl alcohol) on collagen**

Andrés Bernal, Radka Balkova, Ivo Kuritka and Petr Saha

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For publication I and II, the authors have designed and developed the research project, followed by the preparation and analysis of the obtained materials. The first author has written the first draft of the paper and finally all authors worked on the text correcting it and improving it.

For publication III, 50 % of the experimental part was done at Tomas Bata University in Zlin by Bernal and Kuritka, and the other 50 % was done by Dr. Balkova in Brno University of Technology. The first draft was elaborated together by Bernal and Balkova, and then the manuscript was corrected and improved by all the authors.

INTRODUCTION

In the last decades, biomaterial science has gone through an extraordinary development, and one of the reasons is because it is associated with other disciplines such as biology, chemistry, engineering and medicine. These fields have achieved materials for being used during medical treatment or in the restoration of organs and tissues, and although metals, ceramics, composites and polymers are included within biomaterials, the later deserve a special attention as a consequence of their mechanical properties, and because it is possible to synthesise them with biodegradable or bioresorbable properties [1].

Since interaction with biological system is involved, a negative body reaction which includes inflammatory response, strong infection or even death could be present [2]. Nevertheless, the combination of natural and synthetic polymers might contribute to attenuate or reduce these effects. For that reason, bioartificial polymeric materials have been proposed as new materials and they should usefully combine the biocompatibility of the biological component with the physical and mechanical properties of the synthetic one [3]. Thus, this thesis is focused on the development and characterisation of a new bioartificial polymer which could be used as a matrix for tissue regeneration or reparation. The bioartificial polymer might signify an advance in medical treatment against fibrous adhesion or other kind of problems related to surgeries or invasive procedures.

The thesis is organised into 3 main parts. The first one deals with the theoretical background of polymers, with special attention to polymers intended for medical use and particularly to collagen (COLL), poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA). The second part includes the conclusions of the research work, as well as the main contribution to science according to the Ph.D. studies. Finally, participation in academic and scientific events appears as well as the papers which were prepared during the doctoral programme.

1. THEORETICAL BACKGROUND

1.1 Polymers as biomaterials

Although there had been several attempts to define biomaterials and the scope of biomaterials science, just in 1987 some consistency was achieved by the Consensus Conference on Definitions in Biomaterials Science of the European Society for Biomaterials. It is derived from a considered and debated definition which was discussed in further events and some modifications emerged in order to reduce the meaning related to the biomedical material concept. In this matter, a biomaterial was defined as a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine [4].

The use of this kind of materials has grown very fast in the last decades as a result of the concurrence of several disciplines including chemistry, chemical engineering, materials science, mechanics, surface science, bioengineering, biology, and medicine, with considerable input from ethicists, government-regulated standards organizations, and entrepreneurs [5]. Additionally, biomaterials encompass many fields of medicine and their repercussion in the human quality life cannot be reduced in a number of patients with a better quality life, or in the development of the science. The effect of biomaterials is enormous and specifically, polymers are used by tens of millions of people annually and hundreds of thousands of lives are expected to be saved each year [6].

Certainly, its scope is incalculable. Sutures, screws or even a transplantation of a whole organ among others (table 1) are an appetiser of its magnitude [7]. No wonder that nowadays the biomaterial field has deeply permeated the medical

industry and it was estimated that in the year 2000, their cost just in the USA was 9 billions of dollars which is an indicative of its transcendence in economy [8].

Table 1. Use of Biomaterials

Problem area	*Examples*
Replacement of diseased or damaged part	Artificial hip joint, kidney dialysis machine
Assist in healing	Sutures, bone plates, and screws
Improve function	Cardiac pacemaker, intraocular lens
Correct functional abnormality	Cardiac pacemaker
Aid to diagnosis	Probes and catheters
Aid to treatment	Catheters, drains
Correct cosmetic problem	Augmentation mammoplasty, chin augmentation

Biomaterials can be divided into four major classes: polymers, metals, ceramics, and natural materials [8]. The former have found relevance in diverse biomedical fields, including tissue engineering, implantation of medical devices, artificial organs, prostheses, ophthalmology, dentistry and bone repairing among others [9]. They have been used as a temporary scaffold, a temporary barrier, and a drug delivery system as well [1]. The main advantages of the polymeric biomaterials compared to metal or ceramic ones are ease of manufacturability to produce various shapes (latex, film, sheet, fibres, etc.), ease of secondary processability, reasonable cost, and availability with desired mechanical and physical properties. The required properties of polymeric biomaterials are similar to other biomaterials,

that is, biocompatibility, sterilisability, adequate mechanical and physical properties [7].

In connexion with biopolymers, and depending on their behaviour after an implant or when in contact with biological fluids, polymers can be classified as non-degradable or biodegradable. A polymer susceptible to degradation by biological activity, with degradation accompanied by a lowering of its molar mass is considered biodegradable. Therefore, non-degradable polymers cannot undergo this process. The use of biodegradable polymers for fabrication of biomedical implants offers at least two advantages: the first one is the elimination of the need of a second surgery to remove the implanted prosthesis after the healing of the tissues, and the second one is the possibility of triggering and guiding the tissue regeneration via material degradation [1]. Functional groups, properly located on a polymer as well as their structure, are usually responsible for biocompatibility and/or biodegradability, and may impart either therapeutic or toxic characteristics. Cell and protein binding reactions and growth may strongly be affected by functional groups of an implanted polymer. In addition, cell and protein binding reactions and growth of the attached cells can be effectively manipulated by appropriate functionalisation of the surface of an implant [9].

Currently, polymeric biomaterials can be divided into two basic categories: synthetic and biological. The list of synthetic polymers used in medicine includes polyvinyl chloride, polyethylene, polypropylene, polymethylmetacrylate, and polystyrene among others [7]. The biological ones consist namely of polypeptides, polysaccharides, nucleic acids, polyesters, hydroxyapatites and their composites [1]. They perform a diverse set of functions in their native setting. In many cases, the matrices and scaffolds would ideally be made of biodegradable polymers whose properties closely resemble those of the extracellular matrix (ECM), a soft, tough, and elastomeric proteinaceous network that provides mechanical stability

and structural integrity to tissues and organs [10]. It is important to point out that collagen as a biological polymer is essential in the ECM and its use in biomedical application is broadly referenced. Moreover, it is regarded by many as an ideal scaffold or matrix for tissue engineering as it is the major protein component of the ECM, providing support to connective tissues such as skin, tendons, bones, cartilage, blood vessels, and ligaments [11-13]. For all of these reasons, it is significant to consider the importance of natural and synthetic polymers in the medical field and particularly, the role that collagen, PVP and PVA play on it.

1.2 Natural Polymers

Polymers can be classified depending on their origin. A natural source produces natural polymers. Many of them can be found in biological system and are called biopolymers, e.g. nucleic acids [14]. The study and utilisation of natural polymers is an ancient science, and typical examples, such as paper, silk, skin and bone artefacts can be found in museums around the world. These natural polymers perform a diverse set of functions in their native setting. For example, polysaccharides function in membranes and intracellular communication, and proteins function as structural materials and catalysts. Nature can also provide an impressive array of polymers that can be used in fibres, adhesives, coatings, gels, foams, films, thermoplastics, and thermo-sets resins [15], and almost all of them have medical application. Additionally, natural polymers can be classified into several groups, such as organic and inorganic systems, proteins, fibrous proteins, phosphorous proteins, polysaccharides, natural hydrocarbon resins and lignin. Nevertheless proteins deserve a special attention in this thesis, because one of the goals in this work is to combine successfully collagen, the most abundant protein in animal kingdom, with synthetic polymers and therefore to extend the knowledge in the field of materials for medical application.

1.2.1 Proteins

Proteins are occurring in all parts of cells and in a great variety, ranging in size from relatively small peptides to huge polymers. Furthermore, they exhibit enormous diversity of biological function, representing the molecular instruments through which genetic information is expressed [16]. However, to reach this organisation level it is necessary to go back to the basic units of the proteins: amino acids. Two amino acid molecules are joined through a peptide bond (Fig. 1). Although hydrolysis of the peptide bond is an exergonic reaction, it occurs slowly because of its high activation energy. As a result, the peptide bonds in proteins are highly stable in most intracellular conditions [16].

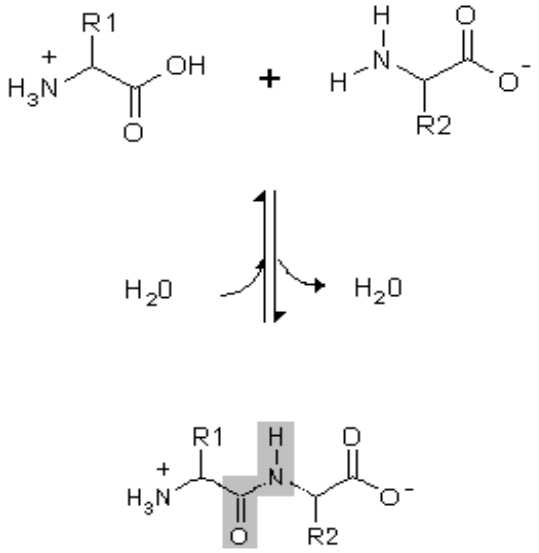

Fig. 1. Formation of peptide bond by condensation

It is well known that proteins have four different organisation levels or structures (Fig. 2). The primary structure is related to the sequence of amino acids; the secondary refers to particularly stable arrangements of amino acid residues, giving rise to recurring structural patterns. Segments of polypeptides often fold locally into α-helices and β-pleated sheets. The tertiary structure describes all aspects of

the three-dimensional folding of a polypeptide. Finally, quaternary refers to the regular association of two or more polypeptide chains to form a complex.

At this point it is necessary to indicate that proteins regulate the functions of a cell, which are related to the levels of their structures. In fact, due to these arrangements, proteins can be classified into fibrous and globular. The two groups differ functionally since the structures that provide support, shape, and external protection to vertebrates are made of fibrous proteins, whereas most enzymes and regulatory proteins are globular ones [16]. One of the most important and well known fibrous proteins is collagen, which will be treated thoroughly in the next part.

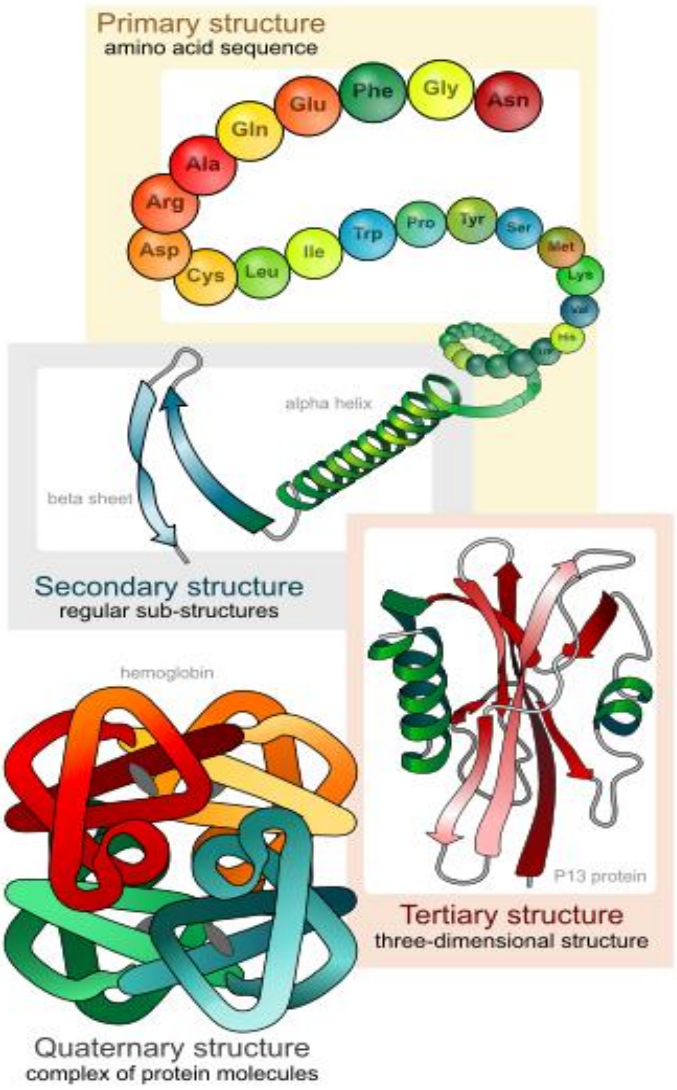

Fig. 2. Protein Structures. (This image is from the public domain)

Collagen

Collagen is a fibrous protein which forms connective tissue in mammals, and approximately 25 % of the total amount of polypeptides in their bodies is made by this molecule [17]. In fact, the most abundant proteins in ECM are members of the collagen family [18], including sponges, invertebrates, and vertebrates.

Collagen is synthesised from the 20 common amino acids, yet it is unique in terms of its amino acid composition, repeating sequence pattern, high degree of post-translational modification, and characteristic intermolecular crosslinks [19]. There are so far, 26 genetically distinct collagen types. Despite the differences among them, all share in common a triple helical structure composed of three polypeptides consisting of Glycine-X-Y repeats, where X is any amino acid, and Y is frequently proline or hydroxyproline (Fig. 3). Each chain is a left-handed helix and, the three chains wind around each other in a right-handed super-helix [20].

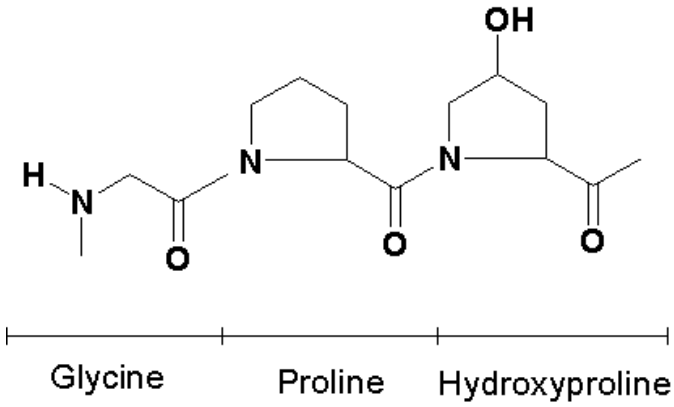

Fig. 3. Collagen Triple Unit

Based on the structure and supra-molecular organisation, those 26 kinds of collagen can be grouped into fibril-forming collagens, fibril-associated collagens, network-forming collagens, anchoring fibrils, trans-membrane collagens, basement membrane collagens, and others with unique functions. The most abundant and

widespread family of collagens with about 90 % of the total collagen is represented by the fibril-forming collagens, including Type I.

The biosynthesis of collagen (Fig. 4) consists of transcription and translation, post-translational modifications, secretion and, extracellular processing and modification [18]. After the transcription of the pro-collagen genes and processing of the pre-mRNAs, the α-chains are synthesised on the ribosome of the endoplasmic reticulum. The signal peptides at the amino-terminal ends of the chains are removed by a signal peptidase after translocation across the membrane of the rough endoplasmic reticulum.

A large number of post-translational modifications are involved in collagen biosynthesis. Proline and lysine residues in the Y position are hydroxylated to 4-hydroxyproline and hydroxylysine, and some of the prolines in the X position are hydroxylated to 3-hydroxyproline. Galactosyl moieties can be attached to some hydroxylysine residues by hydroxylysyl galatosyltransferase, and glucose can be attached to some of the galactosyl hydroxylysine residues by galactocyl hydroxylysyl glucosyltransferase. In addition to the lysines in the triple-helical region, the lysines in the short telopeptides can also be glycosylated [22].

After the folding of the three polypeptide chains, the pro-collagen molecule is transported to the Golgi complex, where phosphorylation of some serine residues and sulfation of tyrosine residues in the pro-peptides of Type I and III collagen take place. Finally, the pro-collagens are secreted out of the cell, where the extracellular processing takes place. Type I collagen are cleaved off enzymatically by specific endo-proteinases, after the pro-collagen molecule has entered in the extracellular space. Cleavage of the carboxy-terminal pro-peptide is required for the initiation of fibril formation. During this process, crosslinking is essential for the tensile strength of tissue, since it increases the resistance of the collagen fibres against proteolysis.

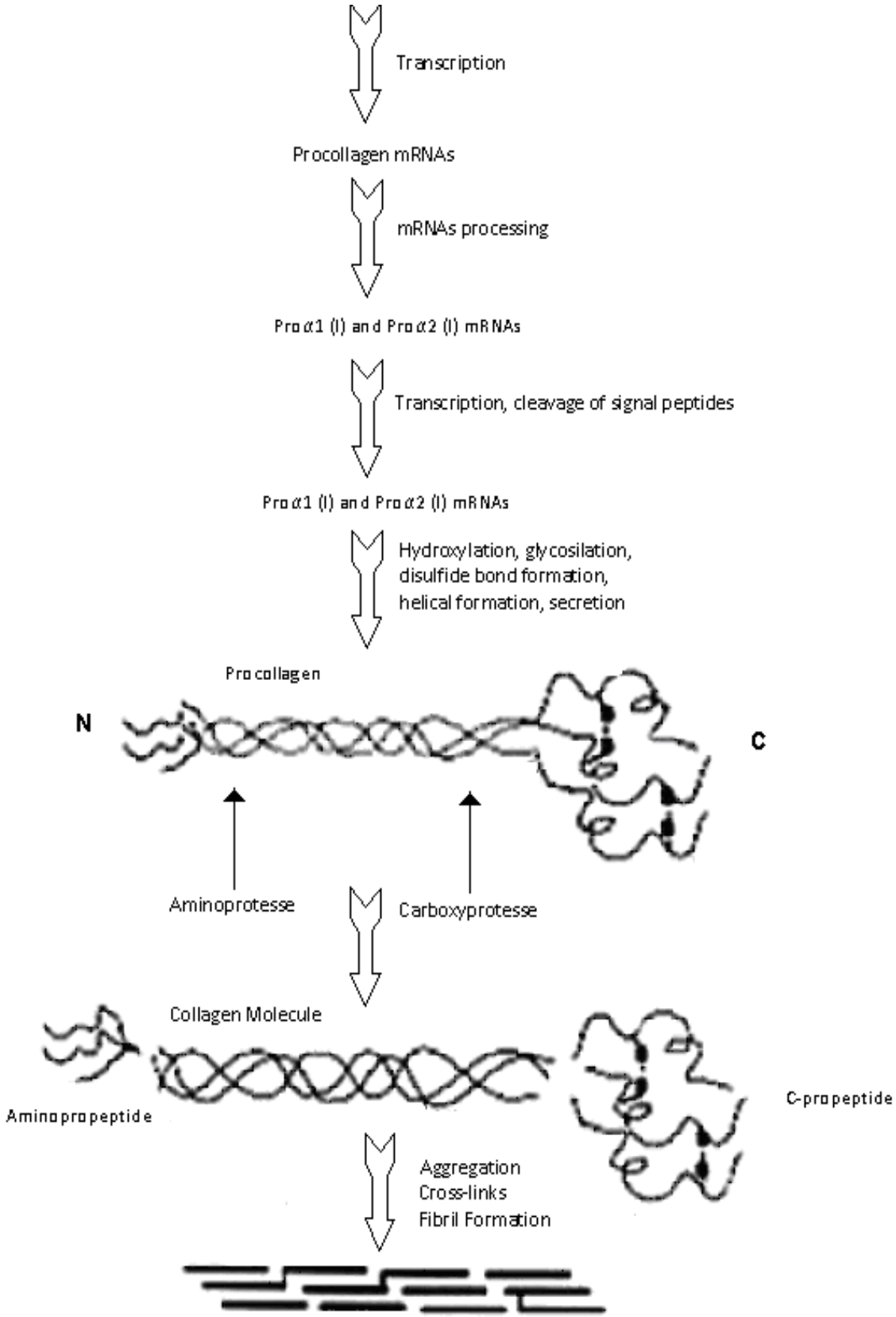

Fig. 4. Representation of Collagen Biosynthesis (Adapted from [21]).

The formation of specific covalent crosslinks among collagen molecules stabilises collagen fibrils in tissues. Lysyl oxidase initiates collagen crosslinking by catalysing the formation of lysyl- and hydroxylysyl-derived aldehydes, at specific residues in the telo-peptides. These aldehydes then undergo a series of condensation reactions with adjacent lysyl residues from the telo-peptide or with specific lysine residues from the triple helical domain to provide the initial crosslinks (Fig. 5) [13, 19, 21-22].

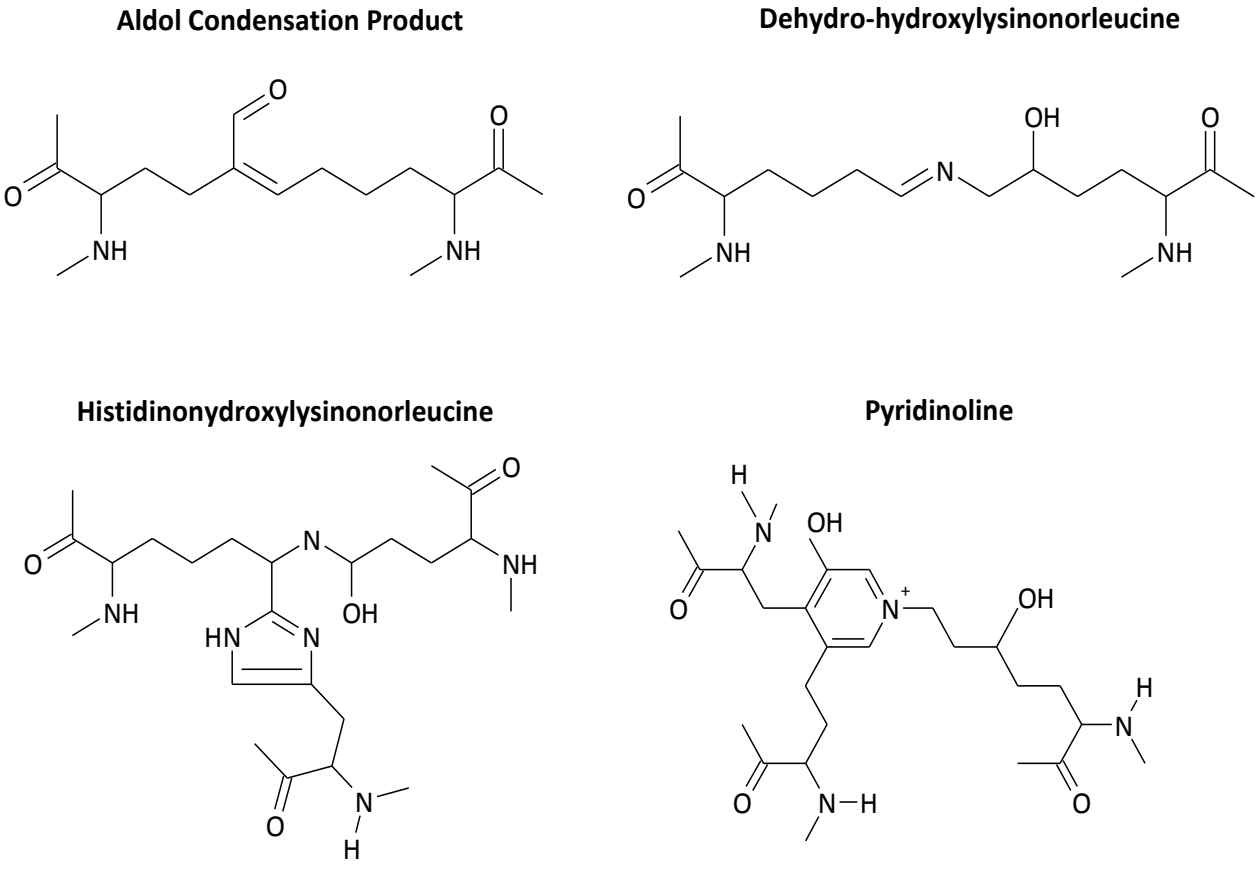

Fig. 5. The chemical structures of some collagen crosslinks (Adapted from [19]).

Once intra and intermolecular crosslink have occurred, collagen presents remarkable features that make it a valuable material for being used in the medical field. The primary reason for the usefulness of collagen in biomedical application is that collagen forms fibres with extra strength and stability through its self-aggregation and crosslinking [12].

Collagen in medical use

Collagen has gained widespread clinical and consumer acceptance, being seen as a safe material with properties that can be adapted to meet a range of different clinical applications [19], such as biodegradability, low immunogenicity and possibilities for large scale isolation [18]. Indeed, the use of collagen in the medical device industry is a consequence of its availability in commercial quantities, ability to trigger blood coagulation and platelet aggregation, stimulation of chemotaxis of connective tissue and inflammatory cells, and capability to support cell attachment and growth [17].

Unfortunately in its natural state, collagen cannot be processed by injection moulding or conventional extrusion techniques. Therefore, the processing of collagen into films, sponges, beads, fibres and tubes involves modifications of three basic processes, i.e. casting, freeze drying and, extrusion. Typically, dispersed or solubilised collagen is prepared at a concentration of about 1 % (w/v). The material is allowed to air-dry overnight at room temperature and the resulting film has a thickness of about 100 μm. On the other hand, extrusion requires substantial material modification of collagen hydrolysate with plasticisers and other additives. Collagen films and membranes have been used for immobilisation of biological materials, such as factor XIII from blood, for guided tissue regeneration, filling of tooth extraction sites, as haemodialysis membranes, retinal reattachment, as a dural substitute, nerve regeneration, repair of the tympanic membrane, cartilage, meniscus and bone repair, control of local bleeding, repair of liver injuries, and as a protective barrier during brain surgery and wound repair [17].

Collagen type I is used as a source for atelocollagen. The presence of telopeptide at both nitrogen- and carbon-terminals confers the antigenicity on this polymer. The atelocollagen obtained by pepsin treatment is low in immunogenicity because

it is free from telopeptides, and it is used clinically for a wide range of purposes, including wound-healing, vessel prosthesis and also as a bone cartilage substitute and haemostatic agent [23]. Under physiological conditions, atelocollagen collects to form a fibre-like natural collagen. This means that atelocollagen administered into the living body is not dissolved immediately but exists for a long time, which is advantageous to a sustained release carrier. It has been confirmed that after realising of a drug, atelocollagen is eliminated by a process of degradation and absorption similar to the metabolism of endogenous collagen [24].

Due to atelocollagen is soluble in acid medium, films can be obtained by casting, although others methods have been used for obtaining it in different configurations with different purposes as well [25-27]. Furthermore, atellocollagen has been utilised in polymer blends, drug delivery systems, polymer grafting, tissue engineering, among others [24, 28].

As it can be seen, there is a broad applicability of collagen in the biomedical field. However, the high cost of pure type I of collagen, variability of isolated collagen, hydrophilic behaviour which leads to swelling and more rapid release, variability in enzymatic degradation rate as compared with hydrolytic degradation, and complex handling properties, are some of the disadvantages of collagen as a biomaterial [12]. Table 2 shows the advantages and disadvantages of collagen for medical uses.

Despite of these disadvantages, collagen is definitely an excellent material in medical use. Moreover, collagen has been used in blends with other synthetic polymers and there are reports about the thermal degradation of the blends, how to characterise collagen-PVP and collagen-PVA, the effect of UV irradiation on the surface, surface properties and the interaction among functional groups (crosslinking), [29-38].

Table 2. Advantages and disadvantages of collagen as a biomaterial

Advantages
Available in abundance and easily purified from living organisms (constitutes more than 30% of vertebrate tissues)
Non-antigenic
Biodegradable and bioreabsorbable
Non-toxic and biocompatible
Synergic with bioactive components
Biological plastic due to high tensile strength and minimal expressibility
Haemostatic — promotes blood coagulation
Formulated in a number of different forms
Biodegradability can be regulated by cross-linking
Easily modifiable to produce materials as desired by utilizing its functional groups
Compatible with synthetic polymers
Disadvantages
High cost of pure type I collagen
Variability of isolated collagen (e.g. crosslink density, fibre size, trace impurities, etc.)
Hydrophilicity which leads to swelling and more rapid release
Variability in enzymatic degradation rate as compared with hydrolytic degradation
Complex handling properties
Side effects, such as bovine spongiform encephalopathy (BSF) and mineralization

1.3 Synthetic Polymers

Polymer products synthesised in laboratories and in industry represent a set of individual chemical compounds which can differ in their degree of polymerisation, tacticity, number of branching and the lengths that connect their polymer chains, as well as in other characteristics that describe the configuration of the macromolecule. Their number is practically infinite (they represent the largest class of biomaterials currently) and many types are used in the biomedical field [8, 39].

The spectrum of applications includes but are not limited to coatings on devices (e.g., to improve blood compatibility), devices (e.g., implantable drug delivery systems, artificial heart), implants (e.g., bone pins and screws, articulating surface in artificial joints), catheters and dialysis tubing, vascular graft, membranes for oxygenation and detoxification, substrate for potential applications in nerve regeneration, plasma expanders, haemoglobin substitutes, reconstructive or plastic surgery, gene therapy among others. Injectable drug delivery systems and tissue engineering, which have emerged in the past two decades, constitute some of the recent applications for synthetic polymers [40-44].

For specific biomedical applications an ideal polymer and its derivatives would be non-toxic, non-immunogenic, non-haemolytic, biodegradable, and do not exhibit inflammatory response. In addition, the biomaterial must not interfere with wound healing or induce fibrosis or a foreign body response [45]. In order to satisfy these characteristics some criteria have to be taken in consideration. For instance, mechanical properties and the degradation rates require matching with the needs for the application. However, in nearly every case, these materials were adopted from other areas of science and technology without substantial redesign for medical use. Although these materials helped usher in new medical treatments, critical problems in biocompatibility, mechanical properties, degradation and numerous other areas remain [6].

Synthetic biodegradable polymers in general offer greater advantages over natural materials in that they can be tailored to give a wider range of properties and have more predictable lot-to-lot uniformity than materials from natural sources. A more reliable source of raw materials is obtained with synthetic polymers that are free of concerns of immunogenicity as well [41, 46].

In spite of the amount of synthetic polymers that are used in medical application, PVP and PVA were chosen for this research and the reasons will be explained in the next part.

1.3.1. Poly(vinyl pyrrolidone)

PVP (fig. 6) is a water-soluble polymer which being highly biocompatible is often included in pharmaceutical and cosmetic formulations [47-48]. As a consequence of its biocompatibility, low toxicity, film forming and adhesive characteristics, unusual complexing ability, relatively inert behaviour towards salts and acids, and its resistance to thermal degradation in solution, it has an extraordinary commercial success. Under normal conditions, PVP is stable as a solid and in solution. In strong acid solution, PVP is unusually stable, with no changes in appearance or viscosity for two months at 24 °C in 15 % HCl [49]. For all of those reasons, PVP is used in many biomedical applications such as controlled drug-release technology, electrochemical devices, as an effective and interesting tissue engineering matrix, as a main component of temporary skin covers, wound dressings, for the preparation of synthetic plasmas (substitute of plasma blood), for creations of hydrogels or thromboresistant hydrophilic gels, as a factor giving higher biological activity of bioartificial polymeric materials and in processes for increase the hydrophilic character of blended polymeric materials. Furthermore, because of its outstanding absorption, it is very useful in pharmacy and medicine [29-30, 50-53].

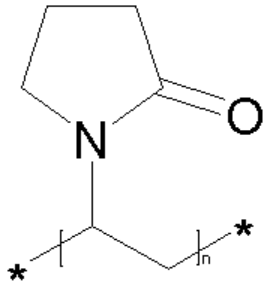

Fig. 6. Poly (vinyl pyrrolidone) Structure

Soluble PVP was first used during World War II as a blood-plasma substitute. Although it has excellent properties for this purpose, it has no longer been used for decades. Today, soluble PVP is one of the most versatile and widely used pharmaceutical auxiliaries [54], being suitable for large number of other uses. However, issues concerned with the rigid but fragile nature of PVP and its lack of sturdiness have resulted in processing difficulties [55]. Because of the absence of reactive groups in its chemical structure generally is hard to crosslink this polymer, although 4,4'-diazido-2,2'-stilbenedisulfonic acid disodium salt tetrahydrate (DAS) has been used [56]. Another way to improve or modify the mechanical properties of PVP is blending it with other polymers such as PVA [53, 57] chitosan, [58-59] or even collagen [29-30, 32-33]. The combination of these polymers, exhibits a significant range of properties suitable for biomedical applications which is an important characteristic for developing of this work.

1.3.2. Poly(vinyl alcohol)

PVA (fig. 7) is a water soluble polymer which is used in industry because of its high capability of water absorption [60], and is one of the world's largest volumes synthetic resin produced due to its excellent chemical resistance, physical properties, biocompatibility, and completes biodegradability indeed [61]. PVA has unique features such as excellent film-forming property and non-toxicity. Since PVA is water soluble, films are easily prepared by a casting evaporation technique from

aqueous polymer solutions, thus avoiding the use of organic solvents. The resultant films are clear, homogeneous and resistant to tear [62].

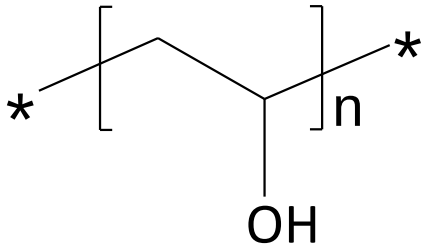

Fig. 7. Poly (vinyl alcohol) Structure

As a promising biomaterial, diverse researches have been focused on the application of PVA in biomedical and pharmaceutical fields. High mechanical strength, rubber-like elasticity, low-protein adsorption, high water content, and no adhesion to surrounding tissues make PVA gels a potential material for soft contact lenses, soft tissue replacements, articular cartilage, inter-vertebrate disc nuclei, trans-catheter arterial embolisation agent, artificial skin, and vocal cord [31, 63-66]. The high content of hydroxyl groups provides PVA and PVA-based materials with other properties suitable for biomedical applications (e.g. hydrophilic, nontoxic, non-carcinogenic, non-immunogenic, and inert in body fluids). It can be mentioned that PVA has been found relevance as a part of controlled drug delivery systems, dialysis membrane, wound dressing, artificial cartilage, and tissue engineering scaffold [64], as well as it can be included artificial pancreas, synthetic vitreous body, artificial skin, and cardiovascular device because of easy preparation, excellent chemical resistance, and physical properties [50]. PVA has served as well in suture material for tight tying, artificial tendons, artificial ligaments, and reinforcing fibres for biocomposite materials, in the synthesis of membranes for use as artificial pancreas material [67].

PVA has been used in blends and composites with natural polymers since its hydrophilic and filming character allows for some degree of compatibility with functional natural polymeric materials. Cast films of PVA combined with natural polymers such as collagen have been investigated for possible medical purposes. Applications of PVA together with PVP are reported as well [31, 68]. The biodegradability and water solubility of PVA ensure its easy degradation and elimination after use [69].

Although PVA has good mechanical properties in the dry state, the high hydrophilicity limits its scope of applications in wet state, that is, living environments [64]. PVA has poor stability in water because of its highly hydrophilic character. Therefore, to overcome this problem PVA should be treated by copolymerization, grafting, crosslinking, and blending in order to reduce the solubility and the hydrophilic character [50].

The simplest and the most commonly employed crosslinking reaction involving chemical crosslinking of PVA with glutaraldehyde (GA) in presence of acidic conditions has been covered extensively in many research reports. It has been reported that chemical crosslinking of PVA can be used as an effective way of producing pharmaceutically safe and useful products (hydrogels, sludge, foam, sponge etc.) for drug delivery [70].

GA is an important reagent in the biomedical field, and it has been used extensively as an agent for fixation of cells, for immobilising enzymes, and for crosslinking proteins and polysaccharides. Compared to other aldehydes, which are less efficient in generating chemically, biologically, and thermally stable crosslinks, GA is able to react relatively rapidly with the functional groups present, resulting in a tightly crosslinked network, containing inter- and/or intramolecular crosslinks. After the chemical modification of PVA by GA in the presence of hydrochloric acid,

the resulting gel can contain the crosslinker molecules as crosslinking and grafted moieties [71-73].

1.4. Polymer Blends

In the past decades, blends have been intensively investigated due to the need to satisfy specific sectors of the polymer industry. Moreover, polymer blends show superior performance in relation to the individual components and, as a result, the range of applications has grown continuously for this class of materials [74].

A mixture of polymers could provide some specific features for materials with medical applications such as antimicrobial properties and better compatibility, not to mention that its production is less-time consuming, easier and cheaper than to develop new polymer on monomer with similar properties.

From the macroscopical point of view, homogeneous mixture of two or more different species of polymers is considered a blend. However, in most of the cases, blends are homogeneous on scales larger than several times the wavelengths of visible light, the constituents are separable by physical means, and no account is taken of the miscibility or immiscibility of the constituent macromolecules [75]. There are many variations of polymer blends, from simple binary mixtures to combinations of block copolymers and homo-polymers, interpenetrating networks, reactive compatibilised systems, molecular composites, impact modified polymers, emulsion blends, engineering polymer blends and countless other systems. The characteristics of a polymer blend are highly dependant upon the method of preparation, e.g. simple mixtures of polymer powders with heating to allow for diffusion controlled mixing, solvent mixing and mechanical melt mixing including high shear intensity mixing [76]. Mainly, due to the very small entropy of mixing and usually positive heats of mixing, two polymers will be immiscible unless some strong interactions such as hydrogen bonding [77], ionic and dipole, π-electrons

and charge-transfer complexes appears. For this reason, it is important to define two concepts: miscibility and compatibility. The former is the capability of a mixture to form a single phase over certain ranges of temperature, pressure, and composition. The later is the potential of the individual component substances in either an immiscible polymer blend or a polymer composite to exhibit interfacial adhesion [78].

Blends of synthetic and natural polymers represent a new class of materials with better mechanical properties and biocompatibility than those of the single components [79]. In this matter, bioartificial polymers can be mentioned because they perform all the previous requirements and they appear as a novel material with latent medical applications.

1.5. Bioartificial polymeric materials

Bioartificial polymeric materials were designed with the purpose of producing materials with enhanced properties with respect to the single components. During the last two decades synthetic and natural polymers have been used separately as potential biomaterials. The success of synthetic polymers relies mainly on their wide range of mechanical properties, transformation processes that allow a variety of different shapes to be easily obtained, and low production costs. Natural polymers present good biocompatibility, but their mechanical properties are often poor, the necessity of preserving biological properties complicates their processability, and their production or recovery costs are very high. In general, the biocompatibility of a material is determined by the interactions at a molecular level between the material and the constituents of the living system. The basic philosophy of bioartificial polymeric materials is to smooth the interactions between synthetic and living systems by creating a two-component material, inside which changes at the molecular level have already occurred (as a result of the

interactions between the synthetic and the biological component) before coming into contact with the living tissue. Such a material, with pre-established molecular interactions, should behave better macroscopically than a fully synthetic material, with regard to the biological response of the host [80].

In order to overcome the poor biological performance of synthetic polymers and to enhance the mechanical characteristics of biopolymers, bioartificial polymers have been introduced. These materials based on blends of both synthetic and natural polymers could be usefully employed as biomaterials or as low-environmental impact materials. They should usefully combine the biocompatibility of the biological component with the physical and mechanical properties of the synthetic component.

1.5.1. Bioartificial films and bi-layers

Films formed by blending of two or more polymers usually result in modified physical and mechanical properties compared to films made of the initial components. In addition, since synthetic polymers are easily obtained and have low production cost, the blending of natural and synthetic polymers may improve the cost-performance ratio of the resulting films [81].

One approach to enhancing the biocompatibility of an implant material is to exploit the normal interaction of cells with their ECM molecules, which are often natural polymers by combining natural macromolecules such as collagen with water-soluble synthetic polymers. Such materials have been used for different biological applications. These include biodegradable, leak proof membranes and purification of proteins.

Bioartificial polymeric materials can be cast as films and a variety of potential applications have been targeted for these materials. These include dialysis membranes, wound dressing, artificial skin, cardiovascular devices and nerve guide

channels and as implantable devices to release biologically active substances in a controlled manner. A further potential use may be found in orthopaedic applications, for example, bone graft substitutes [82-83].

On the other hand, with the further intention to obtain a film with two different surfaces, a bi-layer is an optimal structure which fulfils the requirements for bioartificial polymeric materials. In a bi-layer, interfacial adhesion has to be present between the two components. Actually, collagen may form different types of hydrogen bonds with PVP: between carbonyl group of PVP and hydroxyl group of collagen, and between the hydrogen from the peptide bond of collagen and carbonyl group of PVP [30].

One of the biggest advantages in bi-layer systems is that they present different surfaces properties on each side. It means that the relation among them has at least two ways of interaction. This is a promising opportunity in the biomedical field. In fact, the use of physical barriers and substances that reduce the inflammatory response is a useful strategy in order to prevent or to reduce the adhesion within body cavities. Fibrous adhesion formation within body cavities is a major clinical problem. After abdominal surgery peritoneal adhesions develop in nearly all patients and represent the most common cause of small-bowel obstruction, secondary female infertility, and pain [84]. As a consequence, collagen, PVP, and PVA show good compatibility and miscibility, and due to their wide use in medical application, they were chosen for this research. At this point it is necessary to claim that the interface in the bi-layers structure is in fact, a blend, and they could be prepared easily by solvent evaporation technique.

2. AIMS OF THE WORK

This work contributes to the field of biomedical polymers. According to the experience that has been gained during the doctoral studies, the Individual Study Programme Curriculum, the literature review, and the current work, it could be claimed that a bi-layer structure would serve as a matrix for tissue regeneration or reparation. If a perforative or a peritoneal adhesion appear, a sheet of bi-layered flexible material might be surgically inserted into the body, repairing defects between two different body compartments or cavities, thus creating a functional interface between two organs or tissues, wherever separation and two-side functional membrane is needed in living organism. For all that, collagen-PVA bi-layer systems could represent an important advance in medical treatment against those problems. Moreover, bi-layer structures could be considered as a general approach to be used in diverse medical treatments or proposes. As a consequence of all the aspects mentioned until now, this dissertation will be dedicated:

To investigate MWI as a safe source of heating on the preparation of PVA solutions and determine the degradation by some instrumental methods of polymers (Paper I)

To prepare and to characterise PVA/PVP blends using specific additives in order to obtain suitable films with prospective medical application (Paper II)

To prepare and characterise a bi-layer PVA-collagen film suitable for medical applications (Paper III)

3. METHODOLOGY

3.1. Materials

Poly(vinyl alcohol) (Mw ~ 47,000 g mol^{-1}) with a polymerisation degree of 1,000 and 98 % hydrolysis, poly(vinyl pyrrolidone) (Mw ~ 40,000 g mol^{-1}), 4,4'-diazido-2,2'-stilbenedisulfonic acid disodium salt tetrahydrate of analytical grade, ethylene glycol at 99 %, and a 50 % water solution of glutaraldehyde were provided by Sigma Aldrich, The Czech Republic. Lactic acid (analytical grade) (LA) was produced by Lachema, The Czech Republic, hydrochloric acid and acetic acid (analytical grade) were supplied by Penta, The Czech Republic. Atelocollagen emulsion (1.43 wt%) from bovine Achilles tendon with pH 3.5 was supplied by Vipo, Slovakia. They were used without further purification.

3.2. Sample Preparation

Specific details for sample preparation can be found in the experimental sections which are included within the papers as well as the description of the used equipments and specifications of the storage conditions of the samples.

3.3. Characterisation

Several instrumental methods for polymers characterisation were used during this research. Fig. 8 shows a representative diagram which includes the techniques that were chosen according to the needs of the work. Although all of the methods are well-know in polymer science, it is important to point out some specific information in the frame of the present document. With the purpose to identify the differences on both sides of the bi-layer structure, therefore distinguish them, and make a prediction about future uses, it was necessary to analyse the

information related to the surface. Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscope on the other hand, were used in order to obtain surface and cross-section images and to evaluate the morphology in the bi-layers.

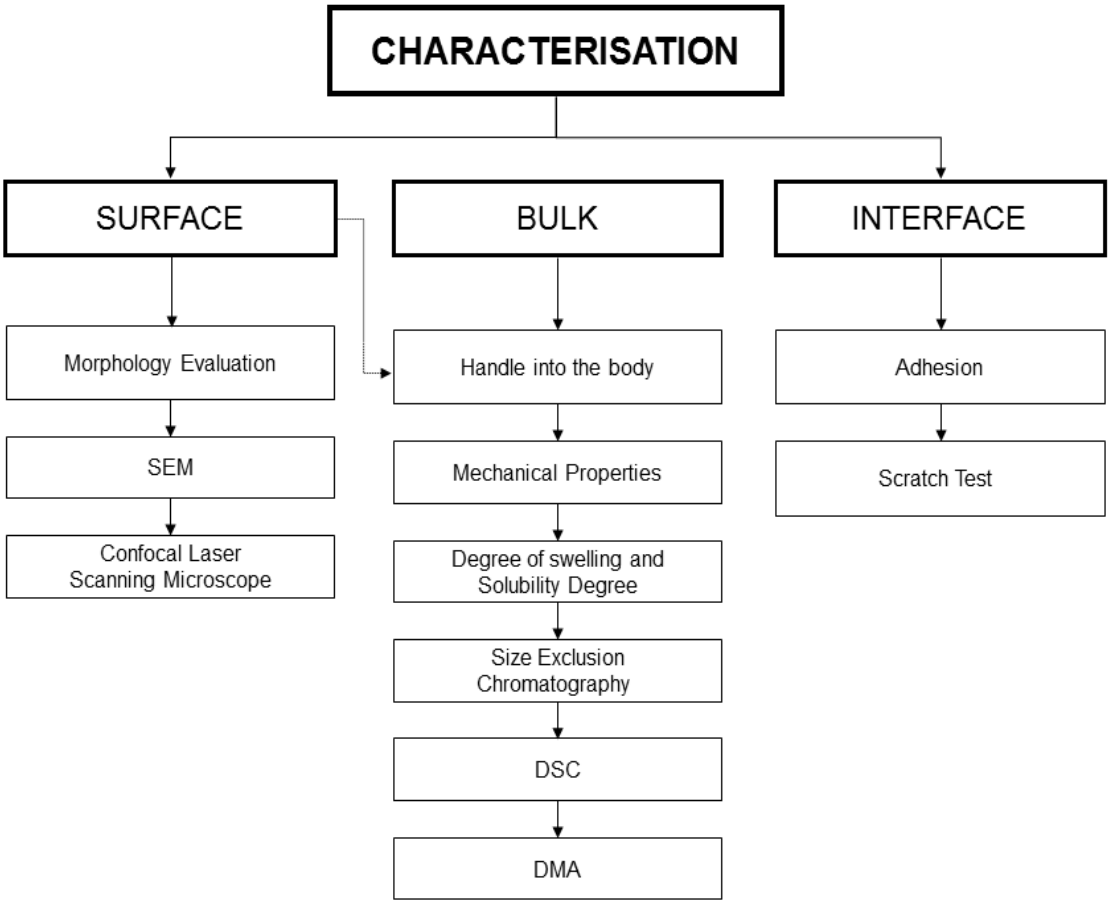

Fig. 8. Instrumental methods used for characterisation

For a specific performance, each biomaterial and device needs to fulfil some requirements including some mechanical properties. These requirements were evaluated as according to ISO 527-3, ISO 527-2 and ISO 6383-1.

4. FINDINGS SYNOPSIS

This doctoral thesis is focused on the preparation and characterisation of bioartificial polymeric materials with latent medical applications and it consists of three original papers which were produced as a result of the investigative process. Samples with the perspective for further implants were obtained by casting method and films with adequate water solubility and mechanical properties were achieved. Other characteristics were evaluated according to specific requirements for the characterisation process.

The first part of the work consisted of the development of experience on polymer processing techniques and instrumental methods for characterisation of polymers. In this matter, ***the first paper*** dealt with the study of degradation of PVA which was dissolved in ethylene glycol (EG) and underwent to microwave irradiation (MWI). The effect of the MWI was evaluated on samples which were taken at certain periods of time (from 4 min to 60 min) under controlled temperature. Ultra violet spectra (UV-VIS), Fourier Transform Infrared spectrometry (FTIR) and Size Exclusion Chromatography (SEC) were used as characterisation techniques and as a result, a small effect, mainly dehydration was determined. The collected information suggested that the samples experienced loss of hydroxyl groups with formation of unsaturated conjugated bonds. The UV-VIS spectra (Fig. 9) showed strong absorption at 330 nm which was assigned to oligo-conjugated unsaturated structures and it could be an indicative that more conjugated bonds in the sample were produced during the MWI as a result of dehydration. The biggest change in absorbance spectrum during MW treatment was manifested after 4 min as the steep increase of the signal. The spectral band loss its structure and most probably a mixture of oligo- or polyene-carbonyl electron system was manifested. The spectra broadening testified the creation of low concentrated defects in form of conjugated double bond structures by

dehydration during first minutes of MWI although their propagation was stabilised after reaching maximum with prolonged time of irradiation. On the contrary, the defects remained delocalised over few C=C or C=O bonds increased slowly with irradiation time, thus a degradation mechanism preferring their formation before consecutive polyene generation is concluded.

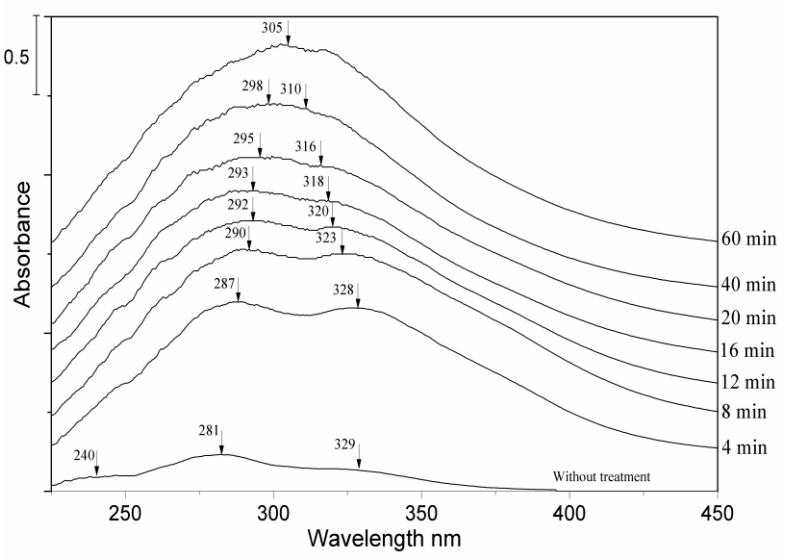

Fig. 9. UV- VIS Spectra for PVA during the treatment.

In more detail, the time dependence of absorbance intensity during MWI (Fig. 10) indicated clearly that conjugated double bond structures were formed by dehydration during first minutes of the treatment although their successive growth was stabilized after reaching maximum within 8 min, and the absorbance at the wavelength 360 and 380 nm remained nearly constant after 20 min. A plausible explanation is that the degradation begins with dehydration and subsequent carbonyl group formation due to a rearrangement and continues by consecutive dehydrations, followed first by conjugated double bond system propagation and then by its stabilization. FTIR, on the other hand, did not show absorption bands for acetate group at 1700 cm^{-1}, which reinforced the idea that even if MWI heated the samples, there is almost not thermal degradation during the process.

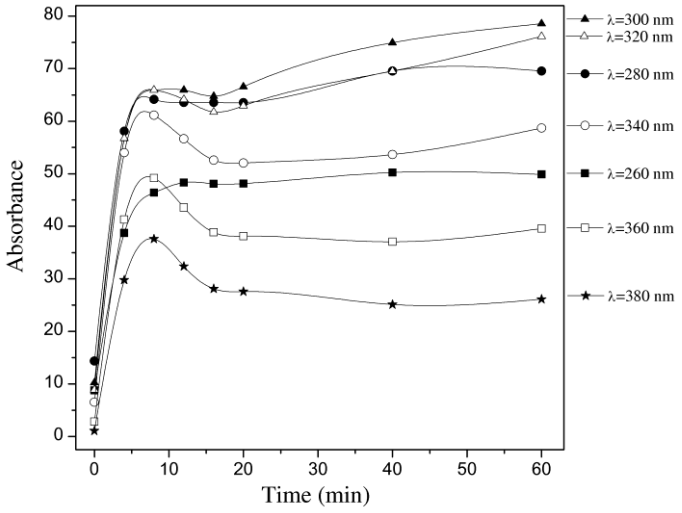

Fig. 10. Time dependence of absorbance intensity during MWI on PVA.

SEC indicated that MWI did not produce any important change on PVA molar mass, no crosslinking reactions occurred and degradation could be considered as negligible (Table 3). Compared with starting material, weight average molar mass M_w of studied samples remained almost unchanged up to 20 min treatment. Furthermore, MWI can be considered as a suitable and safe source of heating for dissolving PVA.

Table 3. M_w, M_n, and polydispersity index for PVA samples treated with MWI

Time of treatment (min)	M_w (g mol^{-1})	M_n (g mol^{-1})	$P = M_w/M_n$
0	38,500	11,000	3.5
4	38,300	10,300	3.7
8	37,700	10,300	3.7
12	38,000	10,400	3.7
16	38,800	10,100	3.7
20	38,500	10,200	3.8
40	36,100	9,400	3.8
60	34,400	9,300	3.7

As the second approach to reach the aims of the doctoral studies, the research was centred on the production of bioartificial polymeric material. For that reason, blends of PVA and PVP were prepared. Films were obtained and DAS, GA and LA were used as crosslinker and plasticiser agents. ***The second paper*** included the characterisation in terms of degree of swelling, solubility degree, mechanical properties and DSC. Samples of pristine material were tested as well as samples with single or combination of the aforementioned additives. The casting method, as a simple polymer production technique was chosen for obtaining PVP/PVA films as versatile candidates for medical applications. Fig. 11 shows the thermograms for the studied samples and it is notable that LA reduced the crystallinity of the samples affecting the glass transition temperature (T_g) and the melting temperature (T_m) due to the influence of LA on the hydrogen bonding strength among PVA chains. GA, on the other side, diminished the hydrophilicity causing a reduction of free hydroxyl groups and, as a consequence solubility, swelling degree and mechanical properties were modified. The addition of PVP to PVA evidenced a reduction of T_g, which implied that PVP plasticised PVA probably as a result of PVA/PVP bonding, which disrupted the crystalline phase of PVA. The crystalline regions of PVA were more accessible to PVP and therefore, the PVA/PVP interactions were readily formed. The presence of DAS, even if this agent did not crosslink effectively PVP, reduced the mobility and fewer active points for interacting with the PVA chains were available. As a consequence, the crystallinity region of PVA was not affected at the same level and a higher T_m was manifested. Moreover, it was established that the T_m for PVA depends on the PVP content which is obviously related to the decrease of the PVA crystallinity in the blend. The presence of additives in the blend did not change the polymer compatibility.

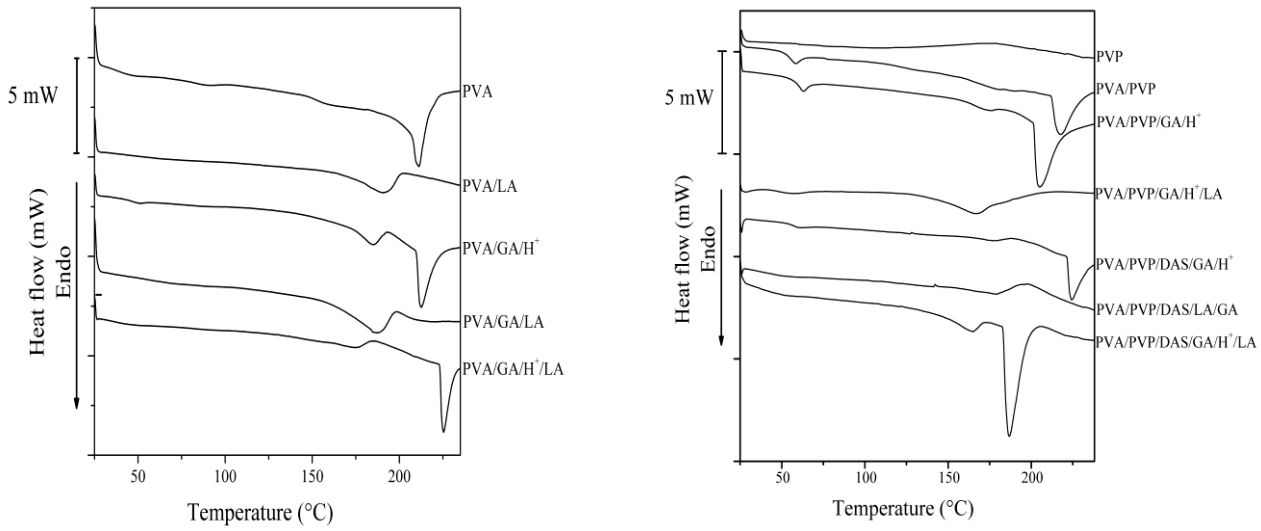

Fig. 11. DSC thermograms for PVA (left) and PVP (right) and its blends.

The mechanical properties were studied (Table 4) and it was found that LA in PVA and PVA/PVP blends reduces considerably the Young's Modulus (*E*) and at the same time, the elongation at break (*ε*) was noticeably increased due to plasticiser effect. It was corroborated that GA in acid media crosslinked PVA, whereas it did not react with PVP.

Table 4. Mechanical properties for PVP and PVA blends

Sample	Thickness (mm)	Young's Modulus (MPa)	Tensile Strength (MPa)	Elongation at break (%)
PVA	0.236 ± 0.017	1100 ± 170	14 ± 3	38 ± 7
PVA/LA	0.274 ± 0.018	229 ± 18	21 ± 2	205 ± 11
PVA/GA/H^+	0.120	360 ± 60	8.5 ± 1.1	32 ± 5
PVA/GA/LA	0.274 ± 0.057	240 ± 20	22 ± 3	195 ± 18
PVA/GA/H^+/LA	0.286 ± 0.064	140 ± 15	26 ± 5	224 ± 8
PVA/PVP	0.270 ± 0.028	2460 ± 140	21 ± 4	5 ± 1
PVA/PVP/GA/H^+	0.226 ± 0.006	2420 ± 100	16 ± 3	4.6 ± 0.2
PVA/PVP/GA/H^+/LA	0.244 ± 0.013	460 ± 70	12.9 ± 1.8	99 ± 8
PVA/PVP/DAS/GA/H^+	0.276 ± 0.011	1580 ± 80	14 ± 2	32 ± 14
PVA/PVP/DAS/LA/GA	0.300 ± 0.025	770 ± 70	11 ± 4	79 ± 19
PVA/PVP/DAS/GA/H^+/LA	0.286 ± 0.008	745± 158	18 ± 2	58 ± 27

Although DAS did not crosslink effectively PVP due to the low molecular weight of the polymer, its presence did not negatively affect the blend regarding to the examined characteristics. PVA/PVP blends were miscible and/or compatible and the explanation could be found in the formation of hydrogen bonding between hydroxyl groups of PVA and carbonyl groups of PVP, idea that was supported by the fact that PVP increases dramatically the *E* of PVA. Although LA did not react with PVP, the blend with PVA had higher ε and lower *E*. Finally, it was established that PVP/PVA blends could be a versatile candidate for medical applications and it was possible to produce films with reasonable mechanical properties and resistant to water solubility for being used as a medium or long term implants.

The aim of the ***third paper*** was the production of a bi-layer film prepared by casting of PVA on collagen. Dynamic Mechanical Analysis (DMA), DSC, tensile test, tear resistance, scratch test and FTIR were used in order to get information about how the bi-layer behaves in a broad temperature ranges on one side, and on the other, how the single components were affected by plasticisers and crosslinker agents. Evidence about LA reducing crystallinity on PVA was founded as well as its function as grafter of hydroxyl groups which consequently affected T_g and T_m. GA crosslinked PVA although it was not reacted with collagen and separated phases were identified.

DMA evidenced that films presented elastic behaviour at all frequencies and temperatures which were examined. However, the trends for PVA blends indicated that the increase in frequencies produced a slight rise on T_g and the storage modulus (*E'*) decreased with the increases of temperature due to increase of chain mobility, promoting less resistance for rearrangement of molecules. On the basis of the requirements for biomaterials, the bi-layer PVA-collagen showed appropriate

mechanical performance, mechanical durability, and physical properties in order to be used at 37 °C, the normal human temperature.

The tear resistance of the bi-layer was the lowest due to brittle character of COLL blends revealed by tensile test. The surface of torn surfaces was smooth without any deformations visible under used microscopic magnification as can be seen in Fig. 12.

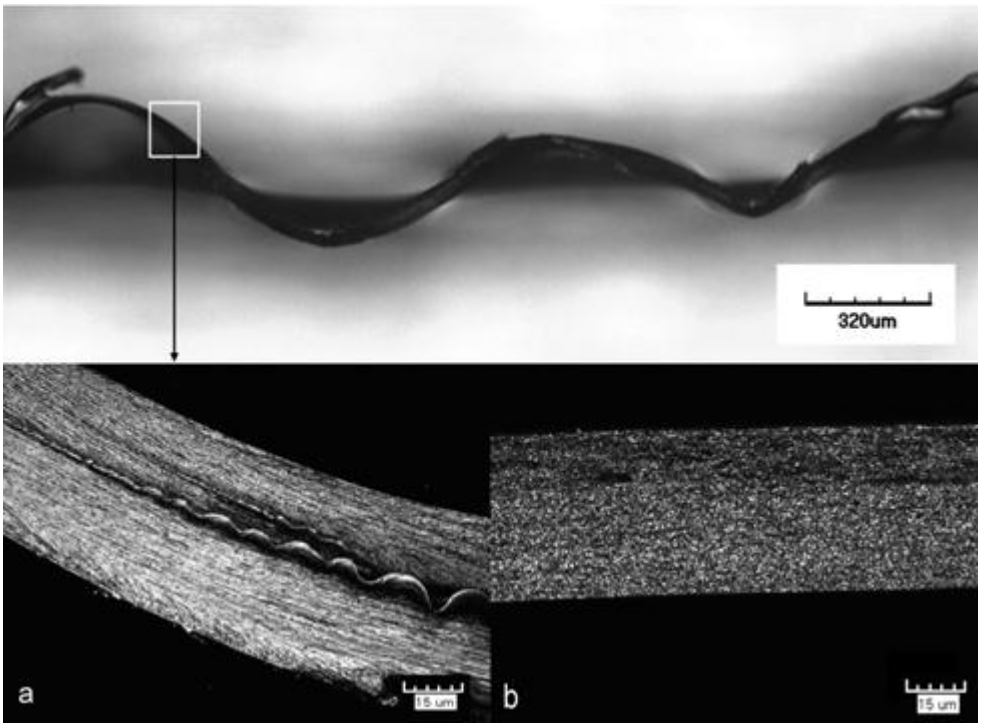

Fig. 12. Torn surfaces of (a) PVA10-LA and (b) BAP-COLL-PVA

The FTIR spectra of the bilayer are shown in Fig. 13. As can be seen, COLL spectrum presents a set of overlapping strong bands above 3000 cm^{-1}, which were associated to N-H and O-H stretching in various local hydrogen bonding environments. The bands at 1647 cm^{-1} and 1543 cm^{-1} represent amide I and II, respectively, and the band at 1428 cm^{-1} was assigned to –OH stretching. The spectra of both sides of the bilayer differ in the region 2000–1500 cm^{-1}. One side revealed pronounced amide I and II bands while the other one did not show these bands, which means that one side consists of COLL and the other one of PVA.

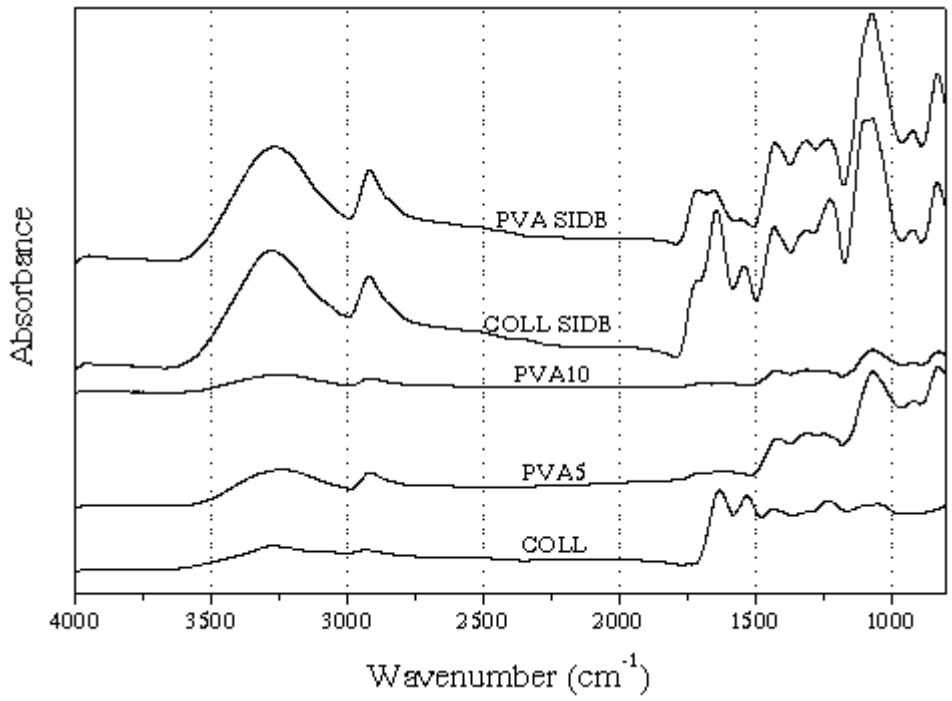

Fig. 13. FTIR-ATR spectra for bilayer and single components of the blend

As the final conclusion, the new bioartificial material (bi-layer) exhibited viscoelastic features useful for being used in contact with living organism and it revealed properties suitable for prostpective applications in medicine compared to neat synthetic and natural biocompatible polymers.

5. CLOSING REMARKS

5.1. Conclusions

This thesis has described a conceptual framework for polymers in medical applications and among them, collagen as a natural polymer, and PVP and PVA as synthetics ones have deserved special attention. These polymers have been used in different therapeutic fields due to their remarkable features. However, problems related to fibrous adhesion are not totally solved, and this work could be an interesting approach to extend the knowledge in this matter, and it might serve as starting point in new matrices for tissue regeneration with a prospective for better healing of abdominal surgeries.

As significant conclusions, it bears mentioning that PVA dissolved in EG does not undergo significant thermal degradation caused by MWI. It brings important benefits in order to obtain appropriate solutions or precursors for PVA films due to even if PVA is water soluble, the dissolution time is relatively long, and the risk of degradation is present. Moreover, the reached temperature is not high enough for causing crosslink or side reactions on the sample which mean that is possible to claim that MWI is an adequate source of heating for PVA solutions.

The poor mechanical properties of PVP could be overcome by the blended with PVA. In fact, PVP/PVA blends are versatile candidates for medical applications and films have been obtained by casting method. LA, GA and DAS influence the degree of swelling and control the solubility which is a good combination of features for being considered as a prospective material for medium or long term implants in medicine. In addition, LA could bring some additional benefits due to its antibacterial properties and biodegradability.

LA is a noteworthy plasticiser for collagen as well. Its incorporation to the neat material affects the mechanical properties, and as a result, films with better

manoeuvrability can be obtained. On the other hand, GA is an effective crosslinker agent for collagen and PVA but it does not react with PVP which makes it a useful and selective compound for PVA/PVP blends. In the same matter, DAS neither crosslinks PVA nor bring negative consequences from the mechanical point of view to the blends. The combination of LA and GA on the neat materials causes an intermediate effect. This effect produces a material with better properties towards prospective applications in medicine than the single components.

The collagen-PVA bi-layer presented viscoelastic features which perhaps make it useful for being used in contact with living organism. Moreover, in the range of normal human temperature, and for the physiological relevant frequencies, the samples exhibit values in which they do not experiment important damage or mechanical changes.

5.2. Contribution

This research contributes to the strengthening and expanding of the existing potential areas of polymers in medical application. As important aspects in the scientific field can be mentioned that:

- ✓ Microwave irradiation can be considered as a useful approach for dissolving PVA and no degradation takes place during the process.
- ✓ LA, GA and DAS are an attractive combination for plasticising and crosslink PVA/PVP blends or bi-layered material.
- ✓ The poor processability of collagen and PVP could overcome blending these polymers or using GA, LA and DAS. Moreover, the combination of these additives are attractive for reducing the high solubility of PVA which bring additional benefits in order to obtain materials for a medium or long term uses into the biological systems.

As a summary, these results could be considered as a step on the development of materials which are easy to obtain, with adequate mechanical properties, with appropriate surfaces properties which could be used as a matrix for tissue regeneration and with the latent possibility to avoid or reduce the abdominal adhesion.

5.3. Future Prospects

The use of bi-layers within biomedical field is practically nonexistent and there are just few reports in the scientific literature. Hence, it is necessary to motivate the work around these versatile materials, and to collect information in order to estimate their uses in the medical field. In this matter, it would be motivating that *in-vitro* experiment focused on cytotoxicity and proliferation using hepatocytes and skin cells or eventually endothelial cells, and *in-vivo* experiments in association with a team of surgeons will be carried out with the aim to identify if the bi-layer structure is able to differentiate tissues and therefore to discover if the cell attachment is regulated by different mechanism and rates. As a consequence to tackle the problem through scientific research pertinent studies and publications might appear and it would be possible to estimate the promising applicability and usefulness that bi-layers might have, strengthening theoretical framework in polymers in medical field.

Important challenge to achieve consists of getting a material with similar physical and mechanical properties than ECM. In such circumstances, the risk of reaction or rejection by the body will be reduced. Different additives could be used and films that have been obtained so far could be improved. Different natural and synthetic polymers could be used for this purpose.

REFERENCES

[1]. FAMBRI, L., MIGLIARESI, C., KESENCI, K., PISKIN, E. Biodegradable polymers. In BARBUCCI, R. *Integrated biomaterial science.*1st ed. New York: Springer, 2002. 1037 p. ISBN 978-0306466786

[2]. IKADA, Y., TSUJI, H. Biodegradable polyesters for medical and ecological applications. *Macromol. Rapid Commun*. 2000, vol. 21, no. 3, p. 117-132.

[3]. GIUSTI, P., LAZZERI, L., DE PETRIS, S., PALLA, M., CASCONE, M.G. Collagen-based new bioartificial polymeric materials. *Biomaterials.* 1994, vol. 15, no. 15, p. 1229-1233.

[4]. WILLIAMS, D. On the nature of biomaterials. *Biomaterials*. 2009, vol. 30, no. 30, p. 5897-5909.

[5]. RATNER, B., BRYANT, S. Biomaterials: where we have been and where we are going. *Annu. Rev. Biomed. Eng*. 2004, vol. 6, p. 41–75.

[6]. LANGER, R., TIRRELL, D. Designing materials for biology and medicine. *Nature*. 2004, vol. 428, no. 6982, p. 487-492.

[7]. WONG, J.Y., BRONZINO, J.D. *Biomaterials*. 1st ed. Boca Raton: CRC Press, 2007. 277 p. ISBN 978-0-84-93-7888-1

[8]. RATNER, B.D., HOFFMAN, A.S., SCHOEN, F.J., LEMONS J.E. *Biomaterials science: an introduction to materials in medicine.* 2nd ed. San Diego: Elsevier Academic Press, 2004. p. 851. ISBN-13 978-0-12-582463-7

[9]. GRODZINSKI, J.J. Biomedical application of functional polymers. *React. Funct. Polym*. 1999, vol. 39, no. 2, p. 99-138.

[10]. MALAFAYA, P., SILVA, G., REIS, RUI. Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug Delivery Rev*., 2007, vol. 59, no. 4-5, p. 207-233.

[11]. PO FOO, C., KAPLAN, D.L. Genetic engineering of fibrous proteins: spider dragline silk and collagen. *Adv. Drug Delivery Rev.,* 2002, vol. 54, no. 8, p. 1131-1143.

[12]. LEE, C., SINGLA, A., LEE, Y. Biomedical applications of collagen. *Int. J. Pharm.* 2001, vol. 221, no. 1-2, p. 1-22.

[13]. FRIESS, W. Collagen – Biomaterial for drug delivery. *Eur. J. Pharm. Biopharm.* 1998, vol. 45, no. 2, p. 113-136.

[14]. BANSAL, R.K. *A Text book of organic chemistry*. 4th ed. New Delhi: New Age International Publishers, 2003. 861 p. ISBN 81-224-1459-1

[15]. LONG, Y. *Biodegradable polymer blends and composites from renewable resources.* 1st ed. New Jersey: John Wiley & Sons, Inc., 2009. 487 p. ISBN 978-0-470-14683-5

[16]. LEHNINGER, A., NELSON, D., COX, M. *Principles of biochemistry.* 4th ed. W.H. Freeman, 2004. 1110 p. ISBN 978-0716743392

[17]. SILVER, F., GARG, A. Collagen: characterization, processing and medical applications. In DOMB, A., KOST, J., WISERMAN, D. *Handbook of Biodegradable Polymers. Part of the Book Series. Drug Targeting and Delivery.* Amsterdam: Harwood Academic Publishers, 1997, vol. 7, p. 319-346.

[18]. GELSE, K., PÖSCHL, E., AIGNER, T. Collagen-structure, function, and biosynthesis. *Adv. Drug Delivery Rev.* 2003, vol. 55, no. 12, p. 1531-1546.

[19]. BRODSKY, B., WERKMEISTER, J., RAMSHAW, J. Collagen and gelatins. In STEINÜCHEL, A., MARCHESSAULT, R. *Biopolymers for medical and pharmaceutical applications*. Weiheim: Wiley-vch, 2005, vol. 2, p. 791-826.

[20]. SATO, K., YOMOGIDA, K., WADA, T., YORIHUZI, T., NISHIMUNE, Y., HOSOKAWA, N., NAGATA, K. Type XXVI collagen, a new member of the collagen family, is specifically expressed in the testis and ovary. *J. Biol. Chem.* 2002, vol. 277, no. 40, p. 37678-37684.

[21]. NIYIBIZI, C., WANG, S. MI, Z., ROBBINS, P. Gene therapy approaches for osteogenesis imperfecta. *Gene Therapy.* 2004, vol. 11, no. 4, p. 408-416.

[22]. BODE, M. Characterization of type I and type III collagens in human tissues. *Thesis*. University of Oulu, Oulu, 2000, 76 p. ISBN 951-42-5553-4

[23]. MINAKUCHI, Y., TAKESHITA, F., KOSAKA, N., SASAKY, H., YAMAMOTO, Y., KOUNO, M., HONMA, K., NAGAHARA, S., HANAI, LK, SANO, A., KATO, T., TERADA,

M., OCHIYA, T. Atelocollagen-mediated synthetic small interfering RNA delivery for effective gene silencing in vitro and in vivo. *Nucleic Acids Res.* 2004, vol. 32, no. 13, p. e-109

[24]. SANO, A., MAEDA, M., NAGAHARA, S., OCHIYA, T., HONMA, K., ITOH, H., MIYATA, T., FUJIOKA, K. Atelocollagen for protein and gene delivery. *Adv. Drug Delivery Rev*. 2003, vol. 55, no. 12, p. 1651-1677.

[25]. MATTHEW, H. Polymers for tissue engineering scaffolds. In DUMITRIU, S. *Polymeric Biomaterials.* 2nd ed. Boca Raton: CRC Press, 2001, vol. 8, p. 167-180.

[26]. ROUSSEAU, C.F., GAGNIEU, C.H. In vitro cytocompatibility of porcine type I atelocollagen crosslinked by oxidized glycogen. *Biomaterials.* 2002, vol. 23, no. 6, p. 1503-1510.

[27]. TAKESHITA, F., MINAKUCHI, Y., NAGAHARA, S., HONMA, K., SASAKI, H., HIRAI, K., TERATANI, T., NAMATAME, N., YAMAMOTO, Y., HANAI, K., KATO, T., SANO, A., OCHIYA, T., VERMA, I.M. Efficient delivery of small interfering RNA to bone-metastatic tumors by using atelocollagen in vivo. *Proc. Nat. Acad. Sci. U.S.A*. 2005, vol. 102, no. 34, p. 12177-12182.

[28]. TANAKA, Y., YAMAOKA, H., NISHIZAWA, S., NAGATA, S., OGASAWARA, T., ASAWA, Y., FUJIHARA, Y., TAKATO, T., HOSHI, K. The optimization of porous polymeric scaffolds for chondrocyte/atelocollagen based tissue-engineered cartilage. *Biomaterials.* 2010, vol. 31, no. 16, p. 4506-4516

[29]. SIONKOWSKA, A., PLANECKA, A., KOZLOWSKA, J., SKOPINSKA-WISNIEWSKA, J. Collagen Fibril Formation in Poly(vinyl alcohol) and poly (vinyl pyrrolidone) films. *J. Mol. Liq.* 2009, vol. 144, no. 1-2, p. 71-74.

[30]. SIONKOWSKA, A. Interaction of collagen and poly(vinyl pyrrolidone) in blends. *Eur. Polym. J.* 2003, vol. 39, no. 11, p. 2135-2140.

[31]. ALEXY, P., BAKOŠ, D., HANZELOVÁ, S., KUKOLÍKOVÁ, L., KUPEC, J., CHARVÁTOVÁ, P., CHIELLINI, E., CINELLI, P. Poly(vinyl alcohol) collagen hydrolysate thermoplastic blends: I. Experimental design optimisation and biodegradation behaviour. *Polym. Test.* 2003, vol. 22, no. 7, p. 801-809.

[32]. SIONKOWSKA, A., WISNIEWSKI, M., KACZMAREK, H., SKOPINSKA, J., CHEVALLIER, P., MANTOVANI, D., LAZARE, S., TOKAREV, V. The Influence of UV

irradiation on surface composition of collagen/PVP blended films. *Appl. Surf. Sci.* 2006, vol. 253, no. 4, p. 1970-1977.

[33]. SIONKOWSKA, A., KACZMAREK, H., WIŚNIEWSKI, M., KOWALONEK, J., SKOPINSKA, J. Surface characteristics of UV-irradiated collagen/PVP blended films. *Surf. Sci.* 2004, vol. 566-568 part 1, p. 608-612.

[34]. SIONKOWSKA. A., KOZLOWSKA. J., PLANECKA, A., SKOPINSKA-WIŚNIEWSKA, J. Collagen fibrils in UV irradiated poly(vinyl pyrrolidone) films. *Appl. Surf. Sci.* 2008, vol. 255, no. 5, part 1, p. 2030-2039.

[35]. SIONKOWSKA, A., KOZLOWSKA, J., PLANECKA, A., SKOPINSKA-WISNIEWSKA, J. Phothochemical stability of poly(vinyl pyrrolidone) in the presence of collagen. *Polym. Degrad. Stab.* 2008, vol. 93, no. 12, p. 2127-2132.

[36]. ALEXY, P. BAKOŠ, D., CRKONOVÁ, G., KRAMÁROVÁ, Z., HOFFMAN, J., JULINOVÁ, M., CHIELLINI, E., CINELLI, P. Poly(vinyl alcohol) collagen hydrolysate thermoplastic blends: II. Water penetration and biodegradability of melt extruded films. *Polym. Test.* 2003, vol. 22, no. 7, p. 811-818.

[37]. ALEXI, P., BAKOŠ, D., CRKONOVÁ, G., KOLOMAZNIK, K., KRŠIAK, M. Blends of Polyvinyl alcohol with collagen hydrolysate: Thermal degradation and processing properties. *Macromol. Symp.* 2001, vol. 170, no. 1, p. 41-49.

[38]. SCOTCHFORD, C.A., CASCONE, M.G., DOWNES, S., GIUSTI, P. Osteoblast Response to Collagen-PVA Bioartificial Polymers in vitro: the Effects of Cross-linking Method and Collagen Content. *Biomaterials*. 1998, vol. 19, no. 1-3, p. 1-11.

[39]. KUCHANOV, S. Principles of quantitative description of the chemical structure of synthetic polymers. *Adv. Polym. Sci.* 2000, vol. 152, p. 157-201.

[40]. SHASTRI, V.P. Non-degradable biocompatible polymers in medicine: past, present and future. *Current Pharmaceutical Biotechnology.* 2003, vol. 4, no. 5, p. 331-337.

[41]. LU, Y., CHEN, S.C. Micro and nano-fabrication of biodegradable polymers for drug delivery. *Adv. Drug Delivery Rev*. 2004, vol. 56, no. 11, p. 1621-1633.

[42]. SGOURAS, D., DUNCAN, R. Methods for the evaluation of biocompatibility of soluble synthetic polymers which have potential for biomedical use: 1 – Use of the tetrazolium-based colorimetric assay (MTT) as a preliminary screen for

evaluation for in vitro cytotoxicity. *Journal of material science: materials in medicine*. 1990, vol. 1, no. 2, p. 61-68.

[43]. MIKOS, A., SARAKINOS, G., LEITE, S., VACANT, J., LANGER, R. Laminated three-dimensional biodegradable foams for use in tissue engineering. *Biomaterials.* 1993, vol. 14, no. 5, p. 323-330.

[44]. NAIR, L.S., LAURENCIN, C.T. Biodegradable polymers as biomaterials. *Prog. Polym. Sci*. 2007, vol. 32, 2007, p.762-798.

[45]. HIN, T.S. *Engineering materials for biomedical application*. 1st ed. Singapure: World Scientific Publishing, 2004. 352 p. ISBN. 981-256-061-0

[46]. MIDDLETON, J., TIPTON, A. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials*. 2000, vol. 21, no. 23, p. 2335-2346.

[47]. D´ERRICO, G., DE LELLIS, M., MANGLAPLA, G., TEDESCHI, A., ORTONA, O., FUSCO, S. BORZACCHIELLO, A., AMBROSIO, L. Structural and mechanical properties of UV-photo-cross-linked poly(*N*-vinyl-2-pyrrolidone) hydrogels. *Biomacromolecules.* 2008, vol. 9, no. 1, p. 231–240.

[48]. JONES, S., MARTIN, G., ROYALL, P. BROWN, M. Biocompatible Polymer Blends: effects of physical processing on the molecular interaction of poly(vinyl alcohol) and poly(vinyl pyrrolidone). *J. Appl. Polym. Sci.* 2005, vol. 98, no. 5, p. 2290–2299.

[49]. ROBINSON, B.V., SULLIVAN, F.M., BORZELLECA, J.F., SCHWARTZ, S.L. *PVP. A critical review of the kinetics and toxicology of polyvinylpyrrolidone (povidone).* 1st ed. Chelsea: Lewis Publisher Incorporation, 1990. 209 p. ISBN 0-87371-288-9

[50]. ABD EL-MOHDY, H.L. GHANEM, S. Biodegradability, antimicrobial activity and properties of PVA/PVP hydrogels prepared by *γ*-irradiation. *J. Polym. Res.* 2009, vol. 16, no. 1, p. 1-10.

[51]. PARK, K.R., NHO, Y.C. Synthesis of PVA/PVP hydrogels having two-layer by radiation and their physical properties. *Radiat. Phys. Chem.* 2003, vol. 67, no. 3-4, p. 361-365.

[52]. JIAO, Y. LIU, Z. DING, S., LI, L., ZHOU, C. Preparation of biodegradable crosslinking agents and application in PVP hydrogel. *J. Appl. Polym. Sci.* 2006, vol. 101, no. 3, p. 1515-1521.

[53]. SEDLARIK, V., SAHA, N., KURITKA, I., SAHA, P. Characterization of polymeric biocomposite based on poly(vinyl alcohol) and poly(vinyl pyrrolidone). *Polym. Compos.* 2006, vol. 27, no. 2, p. 147-152.

[54]. BÜHLER, V. *Polyvinylpyrrolidone. Excipients for pharmaceuticals.* Povidone, crospovidone and copovidone. 1st ed. Berlin: Springer, 2005. 254 p. ISBN 3-540-23412-8

[55]. YEH, J., CHEN, C., HUANG, K., NIEN, Y., CHEN, J., HUANG, P. Synthesis, characterization, and application of PVP/chitosan blended polymers. *J. Appl. Polym. Sci.* 2006, vol. 101, no. 2, p. 885-891.

[56]. LU, J., NGUYEN, Q., ZHOU, J., PING, Z. Poly(vinyl alcohol)/poly(vinyl pyrrolidone) interpenetrating polymer network: synthesis and pervaporation properties. *J. Appl. Polym. Sci.* 2003, vol. 89, no. 10, p. 2808–2814.

[57]. LIANG, G., ZHANG, K., FENG, R. Miscibility, thermal stability and retention of PVP for crossliinked PVA/PVP blends. *Chin. J. Polym. Sci.* 1994, vol. 12, no. 3, p. 201-209.

[58]. ANJALI DEVI, D., SMITHA, B., SRIDHAR, S., AMINABHAVI, T.M. Novel crosslinked chitosan/poly(vinylpyrrolidone) blend membranes for dehydrating tetrahydrofuran by the pervaporation technique. *J. Membr. Sci.* 2006, vol. 280, no. 1-2, p. 45-53.

[59]. HAAF, F., SANNER, A., STRAUB, F. Polymers of N-vinylpyrrolidone: synthesis, characterization and uses. *Polym. J.* 1985, vol. 17, no. 1, p. 143-152.

[60]. KACZMARE, H., PODGÓRSKI, A. The effect of UV-irradiation on poly(vinyl alcohol) composites with montmorillonite. *J. Photochem. Photobiol., A.* 2007, vol. 191, no. 2-3, p. 209-215.

[61]. DING, B., KIM, H.Y., LEE, S.C., SHAO, C.L., LEE, D.R., PARK, S.J., KWAG, G.B., CHOI, K.J. Preparation and characterization of a nanoscale poly(vinyl alcohol) fiber aggregate produced by an electrospinning method. *J. Polym. Sci., Part B: Polym. Phys.* 2002, vol. 40, no. 13, p. 1261–1268.

[62]. AL-SABAGH, A.M., ABDEEN, Z. Preparation and characterization of hydrogel based on poly(vinyl alcohol) cross-linked by different cross-linkers used to dry organic solvents. *j. Polym. Environ.* 2010, vol. 18, no. 4, p. 576-583.

[63]. EL-ZAHER, N.A., OSIRIS, W.G. Thermal and structural properties of poly(vinyl alcohol) doped with hydroxypropyl cellulose. *J. Appl. Polym. Sci.* 2005, vol. 96, no. 5, p. 1914-1923.

[64]. ZHOU, W., GOU, B., LIU, M., LIAO, R., RABIE, A., JIA, D. Poly(vinyl alcohol)/halloysite nanotubes bionanocomposite films: properties and in vitro osteoblasts and fibroblasts response. *Journal of Biomedical Materials Research Part A.* 2010, vol. 93A, no. 4, p. 1574-1587.

[65]. CHAN, L., HAO, J., HENG, P. Evaluation of permeability and mechanical properties of composite polyvinyl alcohol films. *Chem. Pharm. Bull.* 1999, vol. 47, no. 10, p. 1412-1416.

[66]. JIANG, Y., SCHADLICH, A., AMADO, E., WEIS, C., ODERMATT, E., MADER, K., KRESSLER, J. In-vivo studies on intraperitoneally administrated poly(vinyl alcohol). *J. Biomed. Mater. Res. B. Appl. Biomater.* 2010, vol. 93, no. 1, p. 275-284.

[67]. GOUGH, J., SCOTCHFORD, C., DOWNES, S. Cytotoxicity of glutaraldehyde crosslinked collagen/poly(vinyl alcohol) films is by the mechanism of apoptosis. *J. Biomed. Mater. Res.* 2002, vol. 61, no. 1, p. 121-130.

[68]. SEDLARIK, V., SAHA, N., KURITKA, I., SAHA, P. Preparation and characterization of poly(vinyl alcohol)/lactic acid compounded polymeric films. *Int. J. Polym. Anal. Charact.* 2006, vol. 11, no. 4, p. 253-270.

[69]. PARK, J.S., PARK, J.W., RUCKENSTEIN, E. A dynamic mechanical and thermal analysis of unplasticized and plasticized poly(vinyl alcohol)/methylcellulose blends. *J. Appl. Polym. Sci.* 2001, vol. 80, no. 10, p. 1825–1834.

[70]. PATEL, A., VAVIA, P. Evaluation of synthesized crosslinked polyvinyl alcohol as potential disintegrant. *J. Pharm. Pharmaceut. Sci.* 2010, vol. 13, no. 2, p. 114-127.

[71]. PARK, J.S., PARK, J.W., RUCKENSTEIN, E. On the viscoelastic properties of poly(vinyl alcohol) and chemically crosslinked poly(vinyl alcohol). *J. Appl. Polym. Sci.* 2001, vol. 82, no. 7, p. 1816–1823.

[72]. YEOM, C.K., LEE, K.H. Pervaporation separation of water-acetic acid mixtures through poly(vinyl alcohol) membranes crosslinked with glutaraldehyde. *J. Membr. Sci.* 1996, vol. 109, no. 2, p. 257-265.

[73]. FIGUEIREDO, K., ALVES, T., BORGES, C. Poly(vinyl alcohol) films crosslinked by glutaraldehyde under mild conditions. *J. Appl. Polym. Sci.* 2009, vol. 111, no. 6, p. 3074–3080.

[74]. MANO, V., SCARPELLI, M., BARBANI, N., GIUSTI, P. Binary blends based on poly(n-isopropylacrylamide): miscibility studies with PVA, PVP, and PAA. *J. Appl. Polym. Sci.* 2004, vol. 92, no. 2, p. 743-748.

[75]. IUPAC. *Compendium of Chemical Terminology* [online]. 2nd ed (the "Gold Book"). Oxford: Blackwell Scientific Publications, 2006. Available on World Wide Web http://goldbook.iupac.org/P04736.html.

[76]. ROBENSON, L. *Polymer Blends: A Comprehensive Review.* 1st ed. Munich: Hanser, 2007. 461 p. ISBN 978-1-56990-408.

[77]. SPERLING, L.H. *Introduction to Physical Polymer Science.* 4th ed. New Jersey: John Wiley & Sons, Inc., 2006. 845 p. ISBN 978-0-471-70606-9

[78]. WORK, W.J., HORIE, K., HESS, M., STEPTO, R.F. Definitions of terms related to polymer blends, composites, and multiphase polymeric materials. (IUPAC Recommendations 2004). *Pure Appl. Chem*. 2004, vol. 76, no. 11, p. 1985-2007.

[79]. MARSANO, E., VICINI, S., SKOPINSKA, J., WISNIEWSKY, M., SIONKOWSKA, A. Chitosan and poly(vinyl pyrrolidone): compatibility and miscibility of blends. *Macromol. Symp.* 2004, vol. 218, no. 1, p. 251-260.

[80]. CASCONE, M. Dynamic-Mechanical properties of bioartificial polymeric materials. *Polym. Int.* 1997, vol. 43, no. 1, p. 55–69.

[81]. LI, J., ZIVANOVIC, S., DAVIDSON, P.M., KIT, K. Characterization and comparison of chitosan/PVP and chitosan/PEO blend films. *Carbohydr. Polym.* 2010, vol. 79, no. 3, p. 786-791.

[82]. SCOTCHFORD, C.A., CASCONE, M.G., DOWNES, S., GIUSTI, P. Osteoblast response to collagen-PVA bioartificial polymers in vitro: the effects of cross-linking method and collagen content. *Biomaterials*. 1998, vol. 19, no. 1-3, p. 1-11.

[83]. AHMAD, S., HASAN, N., ABID, C.K.V., MAZUMDAR, N. Preparation and characterization of films based on crosslinked blends of gum acacia, polyvinylalcohol, and polyvinylpyrrolidone-Iodine complex. *J. Appl. Polym. Sci.* 2008, vol. 109, no. 2, p. 775-781.

[84]. CERVANTES, C., OLAYA, E., TESTAS, M., GARCÍA-LÓPEZ, N., COSTE, G., ARRELLIN, G., LUNA, ADRIÁN, KRÖTZSCH, E. Collagen-PVP, a collagen synthesis modulator, decreases intraperitoneal adhesions. *Journal of Surgical Research.* 2003, vol. 110, no. 1, p. 207-210.

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BERNAL, Andrés; KURITKA, Ivo and SAHA, Petr. Preparation and characterisation of poly(vinyl alcohol)-poly(vinyl pyrrolidone) blend: A biomaterial with latent medical applications. Accepted in Journal of Applied Polymer Science.

BERNAL, Andrés; BALKOVA, Radka; KURITKA, Ivo and SAHA, Petr. Preparation and characterisation of a new double-sided bio-artificial material prepared by casting of poly(vinyl alcohol) on collagen. Accepted in Polymer Bulletin.

BERNAL, Andrés; KURITKA, Ivo and SAHA, Petr. Characterisation of a cross-linked material with medical purposes: Poly(vinyl alcohol)-poly(vinyl pyrrolidone) blend. Plastko 2012. Zlin, Czech Republic. ISBN 978-80-7454-137-7

BERNAL, Andrés; KURITKA, Ivo and SAHA, Petr. Poly(vinyl alcohol)-poly(vinyl pyrrolidone) blends: Preparation and characterization for a prospective medical application. 4th WSEAS International Conference on Material Science. 2011**.** Catania, Sicily, Italy. ISBN 9778-1-61804-047-3

BERNAL, Andrés; KURITA, Ivo and BALKOVA, Radka. Natural-Synthetic bi-layer structure: A new alternative for tissue Regeneration. Plastko 2010. Zlín, Czech Republic. ISBN 978-80-7318-909-9

BERNAL, Andrés. Identifying and overcoming misconceptions in the teaching-learning process of the stoichiometry structuring concept. (Original title in spanish: Identificación y superación de érrores conceptuales en la enseñanza y aprendizaje del concepto estructurante estequiometría). 1st ed. Bogotá: Kimpres, LTDA. 2009. 140 p. ISBN 978-958-97936-7-1

BERNAL, Andrés. Chemistry test (Original title in spanish: Prueba de Química). In: MOYA, J. The most 139 complicated questions in the state exam. Questions and statistical solutions (Las 139 preguntas más difíciles del ICFES. Respuestas y Soluciones Estadísticas. Tecnología Educativa LTDA. 2006. p. 114-128. ISBN 958336463-0

BERNAL, Andrés. The development of integral formation dimensions and its assessment. (Original title in spanish: El desarrollo de las dimensiones de la formación integral y su evaluación). *Memory Sociedade Brasileira de Química/ 27ª Reuniáo anual, The Chemistry Latin American Congress XXVI* Salvador, Bahia. Brasil, 2004.

BERNAL, Andrés. The development of integral formation dimensions and its assessment (Original title in Spanish: El desarrollo de las dimensiones de la formación integral y su evaluación). 2004, master degree thesis.

BERNAL, Andrés. The development of integral formation dimensions and its assessment (Original title in spanish: El desarrollo de las dimensiones de la formación integral y su evaluación. S*cience Teachers Formation Congress*. *Journal Tecne, Episteme y Didaxis TEΔ,* Science Teachers Formation Congress. 2003. No. Extra, p.108-110

BERNAL, Andrés. Synthesis, characterisation and mechanic-quantum study of oxa[1.1.1]propellane (Original title in Spanish: Síntesis, caracterización y estudio mecano-cuántico de oxa [1.1.1] propelano. 1999, under degree thesis.

Awards

VII Distinguish Teacher Award. Jesuit Colombian School Association. 2008

APPENDIX

This appendix holds the full text version of three framing publications where the ready may find further information of each research along with experimental details, results, discussion and the corresponding references.

PAPER I

The effect of microwave irradiation on poly(vinyl alcohol) dissolved in ethylene glycol

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Journal of Applied Polymer Science

The Effect of Microwave Irradiation on Poly(vinyl alcohol) Dissolved in Ethylene Glycol

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ABSTRACT: Poly(vinyl alcohol) (PVA) dissolved in ethylene glycol is subjected to microwave (MW) irradiation for 1 h to determine possible degradation. Fourier transform infrared spectroscopy results show that MW treatment produces a minor effect on the solutions. Ultraviolet–visible spectroscopy suggests that PVA could undergoes loss of hydroxyl groups followed by formation of unsaturated conjugated bonds although the extent of degradation is limited, whereas size exclusion chromatography indicates that MW irradiation do not cause significant changes in PVA molar mass and neither chain cleavage nor crosslinking reactions are observed. Hence, polymer degradation induced by MWs in solution can be considered as negligible for prospective applications. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Nowadays, material science is interested in production of biodegradable, biocompatible, and workable polymers with a broad range of properties closely related to their advanced uses as multifunctional materials. One of those materials, widely known is poly(vinyl alcohol) (PVA). This polymer is used in adhesives, cosmetics, textile and pharmaceutical industry, paints, and even as a colloid protector in emulsion polymerization.[1] Numerous advantageous properties of PVA have lead to its broad practical applications due to its chemical resistance, favorable physical properties, and complete biodegradability.[2] Furthermore, it is water soluble and has broad industrial application as a result of its high capability of water absorption.[3]

During shelf life, polymers can be degraded by numerous ways including action of chemical substances, mechanical forces, and/or radiation. IUPAC has defined polymeric degradation as chemical changes in a polymeric material that usually result in undesirable changes in the in-use properties of the material.[4] In most of the cases, degradation is accompanied by worsening of physicochemical properties, such as a decrease in molar mass, whereas in some circumstances, degradation also includes changes in chemical structure of the backbone or elimination of polymer side groups. It can also be accompanied by crosslinking. Frequently, degradation results in the loss of, or deterioration in, useful properties of the material.[4] This concept is valid for this study, in which degradation caused by microwave (MW) irradiation is examined. Thermal degradation of PVA on the other hand, has been investigated in more detail than other degradation ways. Thermogravimetry, thermal analysis Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry techniques have been used for this purpose.[5] The mechanism of thermal degradation of PVA comprises two steps. The first one involves elimination reactions, whereas the second is dominated by chain scission and cyclization. Additionally, highly hydrolyzed PVA shows a better thermal stability than PVA with a low degree of hydrolysis.[6] Degradation of PVA bulk material has been studied by several authors,[1,2,5,6] and it was concluded that the elimination of hydroxyl side groups is present and considerable amounts of isolated and conjugated polyenes in the degradation residue and small amounts of carbonyl groups could appear. Moreover, Ultraviolet–visible (UV–vis) spectroscopy has shown that the unsaturated double bonds produced by elimination do not lead to the formation of noticeable amounts of conjugation.[5]

In two related studies,[7,8] solid PVA films were heated by MW irradiation, and it was found that irradiation heating at moderate temperature (100–150°C) tends to cause polymer

dehydration with crosslinking formation through ether bridges, whereas heating to higher temperatures or by conventional heating cause formation of double bonds. Thus, the crosslinking is ascribed specifically to nonthermal MW effect. On the other side, another type of irradiation, for example, UV, does not cause crosslinking even after 10 h of treatment.[3] Although most of the studies have been carried out on bulk material, for particular applications, for example, electro spinning and casting PVA in form of solution in a suitable solvent is required.[9–11] The solutions are sometimes heated or even boiled to enhance the dissolution rate, and to overcome the relatively poor solubility of highly hydrolyzed PVA. The use of advantages of MW ovens is a good choice for polymer synthesis or for polymer-analog reactions.[12] There are also numerous reports describing that many slow or "difficult to run" reactions were suddenly made fast and easy by MW treatment.[13–15] Although MW application is an attractive alternative to convective heating, there are not enough reports about the resulting properties of PVA solutions after MW treatment, even though it is obvious that absorption of a quantum of MW radiation cannot directly induce chemical reactions, as its energy is too low to break chemical bonds.[16] Albeit MW was proposed to be used for accelerating chemical reactions by their efficient heating of the reactants, some difficulties could appear such as the reflection and absorption of MW by reactants, which does not allow a uniform MW heating.[17] The ability of a specific substance to convert electromagnetic energy into a heat at a given frequency and temperature is determined by the so-called loss factor tan δ. A reaction medium with a high tan δ value is required for efficient absorption and, consequently, for rapid heating. As ethylene glycol (EG) exhibits a high value of tan δ (1350 at 2.45 GHz),[18] it was chosen as the solvent in this study. Indeed, EG boils at 196–198°C and its use can provide homogeneous MW heating of the solution to relatively high temperature.

The objective of this study was to identify a possible degradation of PVA dissolved in EG when the solution was subjected to MW irradiation, and in case that degradation occurs, to investigate its mechanism. During the MW treatment, the process was monitored by UV–vis, FTIR spectrometry, and size exclusion chromatography (SEC).

EXPERIMENTAL

Materials and Sample Preparation

PVA ($M_w \sim 47{,}000$ g mol^{-1}), polymerization degree of 1000, 98% hydrolysis, and EG (99%) were supplied by Fluka (Sigma-Aldrich, Prague, The Czech Republic) and used without further purification.

PVA solution 5% w/w in EG was prepared at 120°C in a beaker covered by aluminium foil and it was stirred and heated for 1.5 h using a Heidolph MR Hei-Standard magnetic stirrer with heating (Heidolph Instruments GmbH., Schwabach, Germany). The solution was prepared by conventional heating because PVA could undergo crosslinking in solid state caused by MW irradiation at relatively low temperature (100–150°C) through ether bridges,[7,8] whereas conventional heating do not produce such effects. The prepared polymer solution was irradiated for 60 min by MWs in an open vessel reflux system. A CWR-Tech MW domestic oven (M7017P-M, UK) with a standard frequency of 2.45 GHz and power of 700 W was modified toward this purpose and equipped with an external cooler to reduce the risk of explosion. Moreover, the temperature of the system was controlled by using a noncontact infrared digital thermometer GIM 1840 (Greisinger Electronic GmbH., Regenstauf, Germany) immediately whenever the cavity was opened. The temperature was always close to the boiling point of EG. Samples were taken at 4, 8, 12, 16, 20, 40, and 60 min and subsequently, the polymer solutions were diluted in water in 1 : 2 w/w ratios and they were allowed to cool to the laboratory conditions and used to obtain UV–vis spectra. For the same purpose, a blank sample of EG was subjected to MW irradiation for 1 h and no changes were detected by UV–vis absorption spectra. For FTIR analysis, the solutions were cast on polished silicon wafer substrate and dried in an oven at 28°C for 1 week. The obtained films had a thickness of about 200 μm.

Analytical Techniques

UV–vis spectra were taken with an AvaSpec-2048 spectrometer (Avantes, Eerbeek, The Netherlands). The apparatus for FTIR was a Scimitar FTS2000 (Digilab, Cambridge, MA, USA), and all FTIR absorption spectra were recorded by transmission mode at the resolution of 2 cm^{-1} and plotted in absorbance scale. SEC analyses were performed using a setup consisting of a Waters 600E pump, a Waters 2414 differential refractometer (Waters GmbH, Eschborn, Germany), in-line degasser (Watrex, Prague, Czech Republic), and a Rheodyne 7725 injector. Analyses were carried out with a TSK GMPWXL column (7.8 × 300 mm^2) (Tosoh Bioscience, Tokyo, Japan) at 30°C with a 100 μL injection loop. Aqueous 0.1M solution of $NaNO_3$ with 15% acetonitrile and flow rate of 0.8 mL/min was used as a mobile phase. The column was calibrated using narrow molecular weight pullulan standards (Polymer Laboratories Ltd., Shropshire, UK) with molar masses ranging from 677 to 788,000 g mol^{-1}. Data processing was fulfiled with Cirrus Multi Detector Software (Polymer Laboratories Ltd., Shropshire, UK). Sample concentration of about 1.5 mg mL^{-1} was used. Conventional calibration, relative to pullulan standards, was applied and weight average molar mass M_w, number average molar mass M_n, and polydispersity index $P = M_w/M_n$ of the tested samples were determined. For SEC analyses, the samples were accurately weighed and dissolved in the mobile phase for $\sim$ 10 h at room temperature followed by dissolution at 70°C for 30 min under shaking. Before measurements, the samples were filtered through a 0.45 μm Chromafil PP/PET filter (Millipore, Billerica, MA, USA).

RESULTS AND DISCUSSION

UV–vis Analysis

The presence of carbonyl groups in PVA arises from the production of the polymer. PVA is obtained by hydrolysis of poly(vinyl acetate), thus, the residual acetate groups resulting of incomplete hydrolysis process are always present as well as a small amount of degradation products as inevitable impurities caused by processing of the polymer. UV–vis spectra of the virgin and the irradiated PVA confirm the presence of residual carbonyl groups in all the samples as can be seen in Figure 1, where a time series of absorption spectra is plotted (graphs are vertically

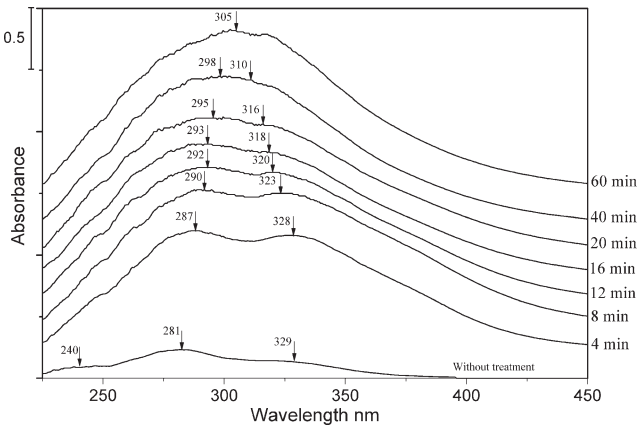

Figure 1. UV Spectra for PVA during the treatment.

shifted for better clarity). The low intensive band due to electronic transition related to carbonyl group appeared at a wavelength (λ) of about 278 nm.[3] A second band at 330 nm due to carbonyl functionality was observed as well.[19] It has been also reported that the band at 280 nm is attributable to diene ketone —$(CH{=}CH)_2$—CO— and a shoulder or a peak of lower intensity at 330 nm to triene ketone groups —$(CH{=}CH)_3$—CO—.[20] The absorption peak centered at 240 nm can be interpreted as a manifestation of the monoene ketone. Hence, the degradation products are inevitably present to a small extent even in the raw material, possibly coming from its production and shelf ageing. The respective absorption bands are manifested in the spectrum of untreated material. On the other hand, no absorbance was present for the virgin material in visible light region. The biggest change in absorbance spectrum during MW treatment was manifested after 4 min as the steep increase of the signal. Then, the change in the shape of the spectra proceeded slowly, the two maximum at initial λ values of 281 and 329 nm merged smoothly into one broad band with a maximum at 305 nm within 60 min. A pale yellow discoloration of the solution was observed in later stages of irradiation which is attributable to the absorption peak shoulder exceeding wavelength 400 nm. The spectral band loss its structure and most probably a mixture of oligo- or polyene or polyene-carbonyl electron systems was manifested. It was described in the literature for solid PVA that the conjugated unsaturated system successively extends over more and more double bonds as the polymer undergoes to the thermal degradation. The absorption peak intervals in the same kind of transition are about 30–40 nm for small number of double bonds in conjugation, which means that for $n = 4$ and 5 in —$(CH{=}CH)_n$—CO— maximum at 360–380 an 390–420 nm can be expected.[21] These values were observed for analogous polyenals CH_3—$(CH{=}CH)_n$—COH (where $n = 2$–5) in solutions as well.[22] Similar features are observable also for sequences of —$(CH{=}CH)_n$— without being in conjugation with —CO— moiety in degraded PVA films, although the maximum in $\pi \rightarrow \pi^*$ absorption are shifted toward to shorter wavelengths by 40–60 nm and resolved absorption bands are observable.[21] Discoloration, yellowish, red, and finally brown color appearance of the sample are generally experienced in the previously cited literature with the progress of solid sample conventional heating degradation due to polyene or polyene-carbonyls formation. On the other hand, UV–vis spectra of bulk PVA irradiated with MW were described showing a strong absorption in the range 250–400 nm and the growth of absorption was observed to be more pronounced at the wavelength 330 nm for thermal heating and at 280 nm for MW heating, which were assigned to different polyconjugated unsaturated structures created by thermal degradation, but the extent of discoloration at higher wavelengths was suppressed for MW and the films become yellow and not brown. Crosslinking of the chains of PVA by ether (C—O—C) bridges was observed as the prevailing degradation mechanism in this case, in which interpretation requires dehydration via intermolecular reaction of hydroxyl groups eclipsing the intramolecular one due to a specific MW effect on polar groups.[7,8]

The time dependence of absorbance intensity during MW irradiation of PVA solution is shown in Figure 2 for selected wavelengths. It is clearly seen that conjugated double bond structures were formed by dehydration during first minutes of MW irradiation although their successive growth was stabilized after reaching maximum within 8 min, and the absorbance at the wavelength 360 and 380 nm remained nearly constant after 20 min, which means that the extent of conjugation did not exceed the length of four or five double bonds significantly. Similarly, the defects manifested by absorption at 260 nm were saturated after 20 min as well. The absorbance in the wavelength region around the maximum at 300 nm initially followed the same trend but after the 16th minute the course changed and it progressed again. A plausible explanation is that the degradation begins with dehydration and subsequent carbonyl group formation due to a rearrangement and continues by consecutive dehydrations, followed by conjugated double bond system propagation. According to all previously discussed literature, this is typical degradation pattern for PVA in solid state, which results into the intensive discoloration due to formation of polyene structures extended over multiple mer units. The behavior of PVA observed in Figure 2 testifies the presence of another mechanism which affects the process in opposite way, and thus, limits the polyene propagation. The defects grow up to critical

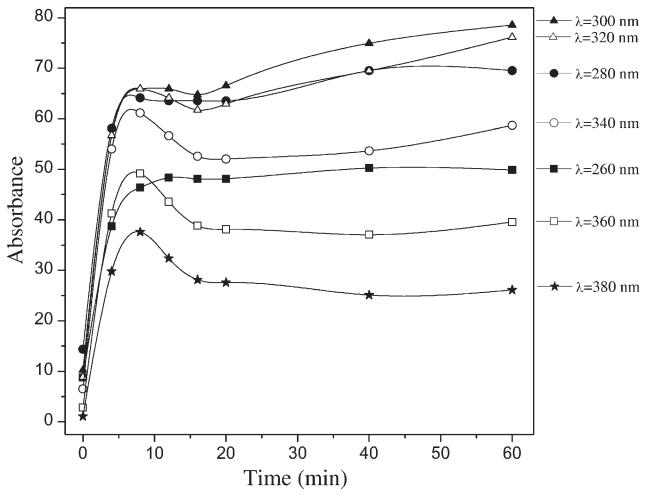

Figure 2. Time dependence of absorbance intensity during MW irradiation of PVA solution.

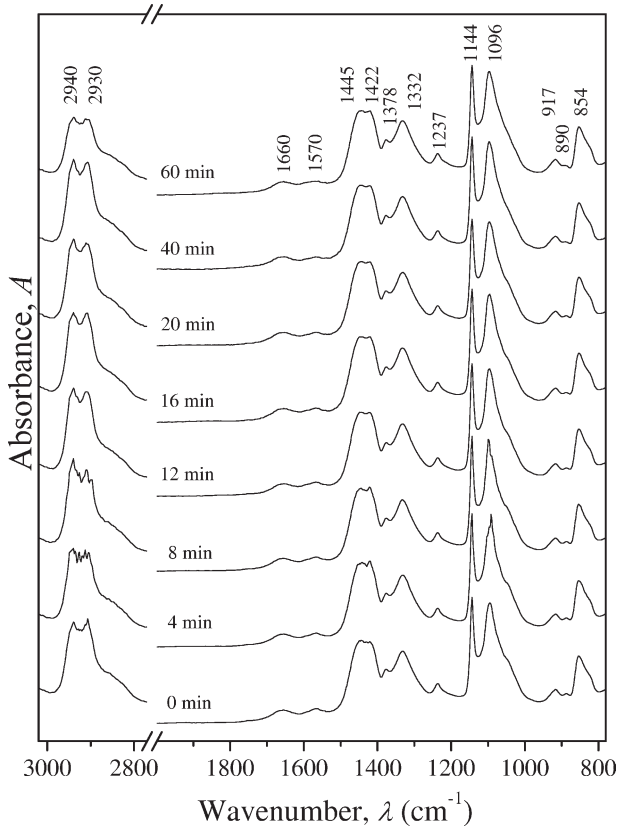

Figure 3. FTIR Spectra for PVA during the treatment.

size of approximately five double bonds in conjugation with C=O group and then, dissociate or regenerate back to defects which remain delocalized over few C=C or C=O bonds.

FTIR Analysis

The FTIR spectrum for virgin PVA has been elucidated in different studies.[5,20,23] In this work, the technique was used to monitor the possible degradation during MW irradiation and the most significant spectral regions of MW treated samples are shown in Figure 3. There is a strong broad absorption band centered at 3336 cm^{-1} without any fine structure (not included in the graph for the sake of better presentation of the lower wavenumber region), which is characteristic for OH group. The bands at 2940 and 2930 cm^{-1} are clearly associated with saturated C—H stretching, whereas the bands at 1445 and 1422 cm^{-1} are related to —CH_2— bending.[24] The spectra do not show even weak bands for carbonyl group over 1700 cm^{-1}, indicating that only small amount of acetate groups, below the detection limit, can be present in the polymer chain as the used PVA is highly hydrolyzed. Two peaks at 1660 and 1570 cm^{-1} can be attributed to conjugated diones or single carbonyls in a conjugation with C=C double bonds in solid state, where peaks over 1700 cm^{-1} are not manifested.[23,25–27] Bands in this region can be ascribed to conjugated C=C systems as well. Both interpretations are in agreement with observed UV–vis spectra; however, these bands do not vary with the time of MW irradiation which supports the idea that thermal degradation either does not occur or only small amount of carbonyl groups is formed or the degradation proceeds without isolated carbonyl formation. In either of aforementioned cases, the degree of degradation is very low as these features can be observed by the means of UV–vis spectrometry only which is due to its higher sensitivity than that of FTIR. The peak at 1144 cm^{-1} is connected with C—O stretching modes and a strong dependency of its intensity on crystallinity degree of the solid PVA material was observed.[28,29] Other authors[7,8] connect this band with C—O—C in ether bridges and crosslinking of PVA is deduced from increased in absorbance at this wavenumber. The bands at 1378 and 1332 cm^{-1} can be attributed to combination frequencies of CH and OH.[30] A strong peak at 1060–1030 cm^{-1} is assigned for stretching C—O in C—O—H group,[7] but the band is shifted at 1096 cm^{-1} due to interaction with unsaturated bonds.[24] The band related to CH_2 rocking at 917 cm^{-1} and a peak at 854 cm^{-1}, which are associated to C—O stretching are manifested in the spectra as well.[29] FTIR spectra do not show any evidence of PVA degradation. The above discussed bands are present in the spectra of all the samples irrespective of treatment time. Neither increase in typical carbonyl absorption region (about 1700 cm^{-1}) nor increase of absorption in C—O—C region was observed (1144 cm^{-1}). With respect to the UV analysis, the degree of degradation is very low, below the detection limit of the FTIR spectrometer, that is, less than 1% of changed hydroxyl groups and hence mer units.

SEC Analysis

PVA belongs to polymers whose composition plays important role in SEC analysis, when aqueous system is used. Although comprehensive studies describing chromatographic behaviors of PVA in aqueous solutions exist, determination of molar mass and molar mass distribution of this polymer are not a simple issue.[31,32] Thanks to various degree of hydrolysis, PVA can exhibit varying extent of hydrophobicity (caused by presence of vinyl acetate group) and hydrophilicity (caused by presence of vinyl alcohol group). Attention has to be, hence, paid to careful selection of columns and mobile phase to restrict nonsize exclusion effects during chromatographic separation. To eliminate these effects and suppress enthalpic interactions that might compete with size exclusion process, analyses were performed in aqueous mobile phase consisting of 0.01*M* $NaNO_3$ and 15% (v/v) acetonitrile. Changes of M_w, M_n, and polydispersity index P as a function of increased time of MW treatment are summarized in Table I. From the results, it is obvious that MW irradiation influenced PVA molar mass only to minor extent. Compared with starting material, weight average molar mass M_w of studied samples remained almost unchanged up to 20 min treatment. Molar mass reduction first occurred after 40 min of irradiation (decrease from 38,500 g mol^{-1} to 36,100 g mol^{-1}) and continued further after 60 min of irradiation (M_w 34,400 g mol^{-1}). Comparison of distribution curves recorded for the samples treated for 0, 12, and 60 min, depicted in Figure 4, corroborates the above given conclusions and clearly illustrates slight, but notable shift of entire polymer distribution to lower molar mass region. In addition to molar mass lowering, small but distinct peak with molar mass higher that 5×10^5 g mol^{-1} (labeled with arrow) was observed after a careful inspection of "zoomed" chromatogram (Figure 5). This peak was detected for

Table I. Values of M_w, M_n, Polydispersity Index P Determined for PVA Samples Treated with Microwaves

Time of treatment (min)	M_w (g mol^{-1})	M_n (g mol^{-1})	$P = M_w/M_n$
0	38,500	11,000	3.5
4	38,300	10,300	3.7
8	37,700	10,300	3.7
12	38,000	10,400	3.7
16	38,800	10,100	3.7
20	38,500	10,200	3.8
40	36,100	9400	3.8
60	34,400	9300	3.7

all samples irrespective treatment time and indicates the presence of structures with higher molar mass than that of the main polymer. The presence of similar peak on PVA chromatogram was also reported by Lacík et al.[31] and it was attributed to the formation of aggregates.

CONCLUSION

The aim of this work was to investigate the stability of PVA solution in EG under MW irradiation. The results have shown that MW treatment produces only minor changes of PVA. According to FTIR study, there are no evidences of significant presence of possible polymer degradation products, which can be considered as a manifestation of thermal stability under the given conditions. UV–vis spectra broadening testifies creation of low concentrated defects in form of conjugated double bond structures by dehydration during first minutes of MW irradiation although their propagation is stabilized after reaching maximum with prolonged time of irradiation. On the contrary, the defects remaining delocalized over few C=C or C=O bonds increase slowly with irradiation time, thus a degradation mechanism preferring their formation before consecutive polyene generation is concluded. In next, according to SEC study, MW heating does not cause crosslinking or other pronounced changes of molar mass namely due to chain cleavage of the polymer sample either. As a consequence, MW treatment within the investigated time scale can be recommended as an appropriate technique for heating of PVA solutions in EG because the results have shown that no significant changes have occurred during that part of the treatment. MW irradiation for 20 min did not cause any reasonable deterioration of the molar mass distribution. Moreover, the time span could be extended up to 60 min with the risk of negligible degradation, if needed. The MW-assisted heating of PVA in EG solution can, hence, bring benefits in shortening time and saving money consumed for the polymer processing.

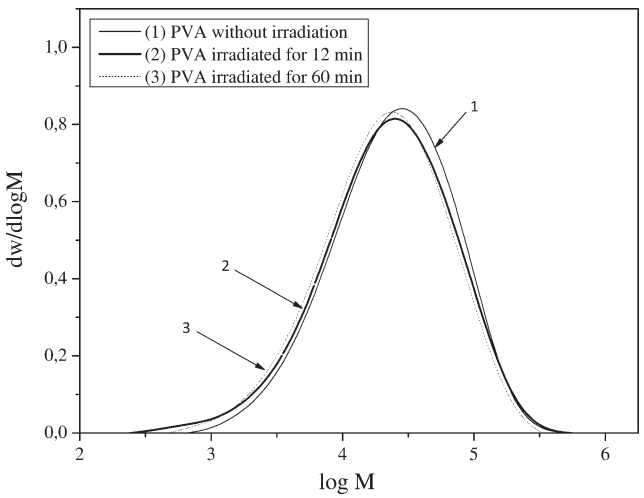

Figure 4. Differential distribution curves obtained for (1) starting PVA material, (2) sample irradiated for 12 min, and (3) sample irradiated for and 60 min.

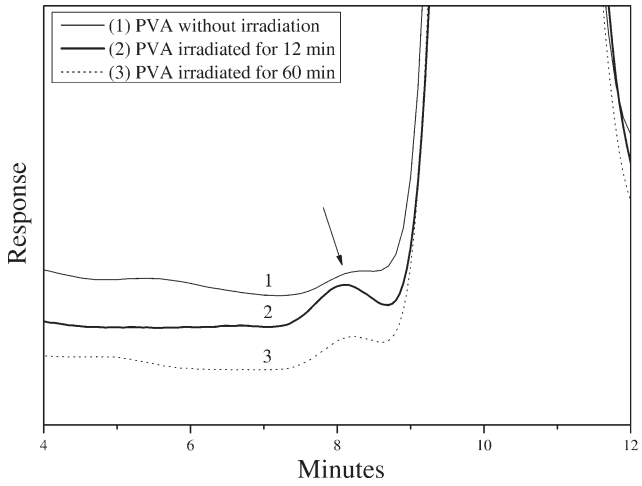

Figure 5. Elution curves recorded for (1) PVA starting material, (2) sample irradiated for 12 min, and (3) sample irradiated for 60 min.

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REFERENCES

1. Barrera, J.; Rodríguez, J.; Perilla, J.; Algecira, N. *Ing. Investig.* **2007,** *27*, 100.
2. El-Zaher, N.; Osiris, W. *J. Appl. Polym. Sci.* **2005,** *96*, 1914.
3. Kaczmare, H.; Podgorski, A. *J. Photochem. Photobiol. A.* **2007,** *191*, 209.
4. IUPAC. In Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"); McNaught, A. D.; Wilkinson, A., Eds.; Blackwell Scientific Publications: Oxford, **1997;** XML online corrected version: http://goldbook.iupac.org (2006-) ISBN 0-9678550-9-8.

5. Holland, B.; Hay, J. *Polymer.* **2001,** *42*, 6775.
6. Peng, Z.; Kong, L. *Polym. Degrad. Stab.* **2007,** *92*, 1061.
7. Petrova, N.; Evtushenko, A.; Chikhacheva, I.; Zubov, V.; Kubrakova, I. *Russ. J. Appl. Chem.* **2005,** *78*, 1158.
8. Alekseeva, N.; Evtushenko, A.; Chikhacheva, I.; Zubov, V.; Kubrakova, I. *Mendeleev. Commun.* **2005,** *14*, 170.
9. Lin, T.; Fang, J.; Wang, H.; Cheng, T.; Wang, X. *Nanotechnology* **2006,** *17*, 3718.
10. Ali, M.; Saion, E.; Yahya, N.; Kassim, A.; Dahlan, K.; Hashim, S. *J. Eng. Sci. Technol.* **2007,** *2*, 111.
11. Abd El-Kader, K.; Abdel Hamied, S. *J. Appl. Polym. Sci.* **2002,** *86*, 1219.
12. Chikhacheva, I.; Zubov, V.; Puolokainen, M. *Russ. J. Gen. Chem.* **2011,** *81*, 545.
13. Manjula, M.; Lokanatha, K.; Raj, J.; Manjula, C.; Siddaramaiah; Ranganathaiah, C. *J. Polym. Res.* **2010,** *17*, 89.
14. Gedye, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Rousell, J. *Tetrahedron. Lett.* **1986,** *27*, 279.
15. Adachi, K.; Iwamura, T.; Chujo, Y. *Polym. Bull.* **2005,** *55*, 309.
16. De la Hoz, A.; Diaz-Ortiz, A.; Moreno, A. *Chem. Soc. Rev.* **2005,** *34*, 164.
17. Huang, K.; Lin, Z.; Yang, X. *Prog. Electromagn. Res.* **2004,** *49*, 273.
18. Kappe, C. *Angew. Chem. Int. Ed.* **2004,** *43*, 6250.
19. Jayasekara, R.; Harding, I.; Bowater, I.; Christie, G.; Lonergan, G. *Polym. Test.* **2004,** *23*, 17.
20. Finch C.A. Polyvinyl Alcohol Developments; John Wiley & Sons: Great Britain, **1992.**
21. Maruyama, K.; Akahoshi, H.; Kobayashi, M.; Tanizaky, Y. *Bull. Chem. Soc. Jpn.* **1985,** *58*, 2923.
22. Das, P.; Becker, R. *J. Phys. Chem.* **1982,** *86*, 921.
23. Mansur, M.; Sadahira, C.; Souza, A.; Mansur, A. *Mater. Sci. Eng. C.* **2008,** *28*, 539.
24. Lambert, J.; Shurvell, H.; Lightner, D.; Graham, R. Organic Structural Spectroscopy; Prentice Hall: USA, **2001.**
25. Gren, E.; Grinvalde, K.; Vanag, G. *J. Appl. Spectrosc.* **1967,** *6*, 146.
26. Dabrowsky, J.; Tencer, M. *Bull. Chem. Soc. Jpn.* **1976,** *49*, 981.
27. Tayyari, S.; Zahedi-Tabrizi, M.; Laleh, S.; Moosavi-Tekyeh, Z.; Rahemi, H.; Wang, Y. *J. Mol. Struct.* **2007,** *827*, 176.
28. Wong, S.; Altinkaya, S.; Mallapragada, S. *J. Polym. Sci. Part B: Polym. Phys.* **2007,** *45*, 930.
29. Bhat, N.; Nate, M.; Kurup, M.; Bambole, V.; Sabharwal, S. *Nucl. Instrum. Meth. B.* **2005,** *237*, 585.
30. Hemanta-Kumar, G.; Lakshmana-Rao, J.; Gopal, N.; Narasimhulu, K.; Chakradhar, R.; Varada-Rajulu, A. *Polymer.* **2004,** *45*, 5407.
31. Lacík, I.; Snauková, R.; Simková, B.; Hanzelová, S.; Alexy, P. *Macromol. Symp.* **2003,** *197*, 101.
32. Nagy, D. J. In Handbook of Size Exclusion Chromatography and Related Technologies: Size Exclusion Chromatography of Polyvinyl Alcohol and Polyvinyl Acetate, Chi-San, Wu., Eds.; Marcel Dekker Inc.: New York, **2004;** pp 267–289.

PAPER II

Preparation and characterization of poly(vinyl alcohol)-poly(vinyl pyrrolidone) blends for medical applications

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Preparation and Characterization of Poly(vinyl alcohol)-poly(vinyl pyrrolidone) Blend: A Biomaterial with Latent Medical Applications

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ABSTRACT: Aqueous solutions of poly(vinyl alcohol) and poly(vinyl pyrrolidone) are blended and films are produced by casting method with the further intention of being used as bio-materials with latent medical application. Glutaraldehyde, 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium salt tetra-hydrate are used as crosslinker agents, whereas lactic acid is the plasticizer in the blend. The obtained films are characterized by differential scanning calorimetry (DSC), mechanical properties, swelling and solubility behavior. DSC measurements show that the blends exhibit a single glass transition temperature indicating that they are miscible, even in the presence of the plasticizer and crosslinker agents. By the combination of all mentioned additives, a relevant enhancement of the swelling is observed, accompanied by a stabilization of the solubility during the tested time. Finally, mechanical properties show an appropriate performance in the studied parameters. As a consequence, the obtained films could be suitable for use as medium or long-term implants. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: biomaterials; mechanical properties; thermal properties; poly(vinyl alcohol); poly(vinyl pyrrolidone)

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INTRODUCTION

Polymers are widely acknowledged as being some of the most versatile materials due to their broad physical and mechanical properties. This allows them to be used for different proposes, including medical applications such as tissue engineering, implants, artificial organs, prostheses, ophthalmology, dentistry, bone repair,[1] or as a temporary scaffold, a temporary barrier, and a drug delivery system.[2]

Among synthetic polymers, both poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) have been intensively studied as biomaterials. Researches have found for the former that it is very useful in pharmacy and medicine due to its outstanding absorption and complex abilities.[3] Despite the fact that the human body is not able to degrade PVP, this polymer can be gradually excreted into the urine without concentrating in the kidneys,[4] when the molar mass does not exceed 30,000 g mol^{-1}, which is an interesting attribute for biomedical uses. Numerous attractive features have been reported for PVA, such as high hydrophilicity, recognized biodegradability, biocompatibility, and good processability on film formation.[5] Both polymers are water soluble,[6] an important property for processing, although this characteristic could be a disadvantage when being used as a long-term implant. Blends, on the other hand, might represent an appropriate solution for material design. They have been investigated in order to satisfy the needs of specific sectors within the polymer industry. Generally, they show superior performance in relation to the individual components and, as a result, the range of applications grows continuously for this class of materials.[7] The combination of PVA and PVP in blends has emerged as a new tool for preparation of biomaterials,[8] and there are abundant amount of reports which describe the multifunctional utilities for these kind of blends.[9–12] As a consequence of the very small entropy of mixing and usually positive heats of mixing, any pair of polymers will be immiscible unless some strong interactions such as hydrogen bonding, ionic and dipole, π electrons and charge-transfer complexes appear.[13] In the case of PVA and PVP blends, the ring of pyrrolidone contains a proton accepting carbonyl group, while PVA has hydroxyl groups and therefore, hydrogen bonding is expected among them. Moreover, the use of modifiers can change the properties of PVA/PVP blends in many aspects. In fact, the water solubility of single components could be modified by blending PVA/PVP or by the use of some additives even if it is

not common.[14] In this context, glutaraldehyde (GA) has been chosen as a crosslinker agent for PVA because it forms an excellent polymer network in which the mechanical and swelling properties can be controlled. These crosslinking reactions can be conducted under mild conditions because of the lack of ability for crosslinking PVP.[15–17] For PVP, a common crosslinker is 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium salt tetrahydrate (DAS) which is not indeed an effective PVA crosslinker.[15] Hence, the possibility to independently control the crosslinking mechanism for both polymers is considered as the main reason for the selection of the agents. By crosslinking, a reduction of hydrophilicity can be utilized as a valuable alternative to improve the water resistance of the blend for specific medical uses. Mechanical test, swelling, and solubility degree are determined in this study since biomedical polymers must be used in the biological environment. Water adsorption can influence the dimensional stability and mechanical properties of the prosthetic element and, moreover, water by itself can be a powerful degrading agent. It is evident that a careful characterization of the bulk properties is fundamental for determining the structure–properties correlation.[18]

The aim of this work was to prepare and characterize PVA/PVP blends using specific additives in order to obtain suitable films with prospective medical application. Information about mechanical properties, degree of swelling, solubility degree, and thermal properties were obtained in this research. On the basis of the requirements for biomaterials, PVA/PVP blend with LA, GA, and DAS, has showed appropriate mechanical performance and water resistance. As a result of those characteristics, the material may be considered as a suitable candidate for further investigation as a long or medium-term implant in the medical field.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) (PVA, M_w = 47,000 g mol^{-1}) with a polymerization degree of 1000 and 98% hydrolysis, poly(vinyl pyrrolidone) (PVP, M_w = 40,000 g mol^{-1}), 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium salt tetrahydrate (DAS) of analytical grade and a 50% water solution of glutaraldehyde (GA) were provided by Sigma Aldrich, The Czech Republic. Lactic acid (analytical grade) (LA) was produced by Lachema, The Czech Republic, hydrochloric acid, and acetic acid (analytical grade) were supplied by Penta, The Czech Republic. They were used without further purification.

Sample Preparation

PVA and PVP Films. A PVA solution at 5 wt % was prepared by dissolving the polymer in distilled water at 80°C for 12 h under continuous magnetic stirring. Once, the solution was obtained, GA at 0.25 wt % related to the total amount of polymer was added as crosslinker agent and the solution was heated up to 80°C during 20 min as a crucial step in the crosslinking mechanism. A second set of samples were prepared with GA and hydrochloric acid (indicated as H^+ hereafter) at 1.2 wt % was added. Finally, PVA solution and LA at 15 wt % related to the total amount of the polymer was blended and stirred for 15 min. The solutions were cast on polyethylene substrates and allowed to dry at 35°C for 4 days in air circulating oven. Films with a thickness of about 200 μm were obtained. Table I summarizes, designs, and describes each sample.

PVP was dissolved by adding slowly the polymer to water at room temperature, always under vigorous magnetic stirring. Simultaneously, in distilled water a 4 wt % aqueous solution of DAS was prepared in a dark room and it was added to the polymer solution. After removal of the bubbles, the blend was cast on polyethylene substrates and it was allowed to dry at 35°C for 4 days in air circulating oven. Films with a thickness of about 200 μm were obtained. Once samples with DAS were dried, they were irradiated for 5 min by a home made instrument with four Sylvania black-light F8W/T5/BL350 lamps. The samples were stored in polyethylene bags and kept on a dark place at laboratory conditions, i.e., temperature 21–23°C and relative humidity 40–60%.

PVA/PVP Blends. Blends of 1 : 1 wt/wt ratio of PVA and PVP solutions were blended under magnetic stirring using the same additives as for the single components. Blends with DAS were always kept in dark room. All the samples were allowed to dry for four days at 35°C.

Characterization

Differential Scanning Calorimetry. Calorimetric measurements were carried out in a DSC 1 calorimeter, Mettler Toledo (Greifensee, Zurich, Switzerland), under nitrogen flowing at a rate 30 mL min^{-1}. The specimens were pressed in unsealed aluminum pans. Heating cycle was performed in order to obtain glass transition temperature (T_g) and melting temperature (T_m). The samples were cooled down by air at an exponentially decreasing rate. The heating of the cycle was performed from 25 to 240°C at a rate of 20°C/min. The T_g was determined as the midpoint temperature by standard extrapolation of the linear part of DSC curves using Mettler-Toledo Stare software and the T_m as the maximum value of the melting peak. The relative crystallinity (X_c) was estimated from the endothermic area using eq. (1):

$$X_c = \Delta H_f / \Delta H_f^0, \quad (1)$$

where ΔH_f is the measured enthalpy of fusion from DSC thermograms and ΔH_f^0 is the enthalpy of fusion for 100% crystalline PVA (138.6 J g^{-1}).[19]

Degree of Swelling and Solubility Degree. As PVA and PVP are water soluble polymers, gravimetric method was used to calculate the degree of swelling and the solubility of the films. Squares of 1.5 cm^2 were cut and dried at 60°C until constant weight (W_1). After that, they were immersed into 5 mL of distilled water at 37°C (normal human temperature) at different time intervals (1, 3, 5, 10, 20, and 30 min). Subsequently, the samples were taken out from the water and the surface moisture was carefully removed by paper napkin. They were weighted again (W_2). Finally, samples were allowed to dry until constant weight at 60°C and weighted once more (W_3). The degree of swelling (DS) and the solubility of the film (SF) were calculated according to the eqs. (2) and (3), respectively. Five replicate tests were done for each sample and these values were undergone to Dixon test for identification and rejection of outlier data with 95 % of

Table I. Prepared Films

No.	Name	Description
1	PVA	PVA at 5 wt %
2	PVA/LA	PVA at 5 wt % and LA at 15 wt % related to the total amount of polymer
3	PVA/GA/H^+	PVA at 5 wt %, GA at 0.25 wt % related to the total amount of polymer and 50 μL of hydrochloric acid
4	PVA/GA/LA	PVA at 5 wt %, GA at 0.25 wt % and LA at 15 wt % both related to the total amount of polymer
5	PVA/GA/H^+/LA	PVA at 5 wt %, GA at 0.25 wt %, LA at 15 wt % both related to the total amount of polymer and 50 μL of hydrochloric acid
6	PVP	PVP at 5 wt %
7	PVA/PVP/GA/H^+	PVP at 5 wt %, PVA at 5 wt %, GA at 0.25 wt % related to total amount of polymer and 50 μL of hydrochloric acid
8	PVA/PVP/GA/H^+/LA	PVA at 5 wt %, PVP at 5 wt %, GA at 0.25 wt %, LA at 15 wt % both related to the total amount of polymer and 50 μL of hydrochloric acid
9	PVA/PVP/DAS/GA/H^+	PVA at 5 wt %, PVP at 5 wt %, GA at 0.25 wt %, DAS at 4 wt % both related to total amount of polymer and 50 μL of hydrochloric acid
10	PVA/PVP/DAS/LA/GA	PVA at 5 wt %, PVP at 5 wt %, DAS at 4 wt %, LA at 15 wt % and GA at 0.25 wt % all of them related to total amount of polymer
11	PVA/PVP/DAS/GA/H^+/LA	PVA at 5 wt %, PVP at 5 wt %, DAS at 4 wt %, LA at 15 wt % and GA at 0.25 wt % all of them related to total amount of polymer and 50 μL of hydrochloric acid

confidence and no data was rejected. For all the measurements, an analytical balance with the accuracy 0.0001 g was used. (Denver Instrument SI-64, Goettingen, Germany).

$$\mathrm{DS}(\%) = \frac{W_2 - W_3}{W_3} \times 100 \quad (2)$$

$$\mathrm{SF}(\%) = \frac{W_1 - W_3}{W_1} \times 100 \quad (3)$$

Lactic Acid Leaching Analysis. From the leaching media of the samples containing LA, one aliquot of 1 mL was collected and diluted until 10 mL in distilled water. The total acidity was obtained by titration with a total acidity minititrator and pH meter for water analysis (Hanna Instrument HI84430, Woonsocket, Rhode Island).

Mechanical Properties. Mechanical properties of PVA and PVP blends, including Young Modulus (E), stress at break (σ), and elongation at break (ε) were tested on five specimens per sample. Rectangular test specimen specified in ISO 527-3[20] with a length of 100 mm, width of 10 mm and thickness of about 200 μm were used. The experiment was carried out using an Instron-type tensile testing machine (Testometric M350-5CT, Lincoln Close, Rochdale, England) and the rate was 50 mm/min. The test conditions are specified in ISO 291.[21] The thickness of the samples was measured by a micrometer with the accuracy of 0.01 mm and specimens were kept in polyethylene bags, in a dark room and at controlled temperature and relative humidity.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry

The thermograms for PVA blends are depicted in Figure 1 and the obtained thermal parameters are shown in Table II. The graphs were vertically shifted for better presentation. The characteristic melting temperature (T_m) for PVA at 211°C and the glass transition temperature (T_g) at 80°C were clearly manifested in the thermogram. Nevertheless, the thermal relaxations for PVA include the α-relaxation temperature (T_α) which is observed in the thermogram as a small exothermic effect at 100°C associated with conformational changes caused by either thermal motion or by the action of an external field without rupture of the chemical bonds.[22,23]

PVA/LA presented a noticeable reduction in the crystallinity related to the neat PVA by about 30% due to the influence of LA on the hydrogen bonding strength among PVA chains. As a result, a lesser amount of lattices were present, causing a decrease of 21°C in T_m. Furthermore, the increasing of PVA chain mobility was a consequence of the plasticizing effect, which was proven by a significant decrease in the recorded T_g

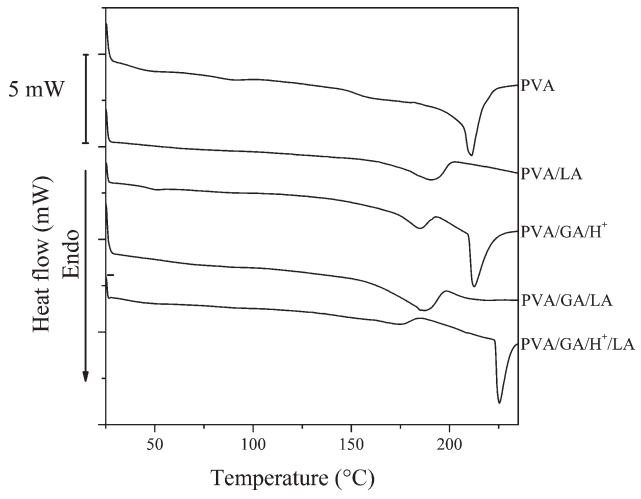

Figure 1. DSC thermograms for PVA and its blends.

Table II. Thermal Parameters of PVA and PVA/PVP Blends

Sample	Temperature °C		Enthalpy	Crystallinity
	T_g	T_m	ΔH_f (mJ g^{-1})	%
PVA	80	211	49	36
PVA/LA	55	190	35	25
PVA/GA/H$^+$	46/82	185/213	25/63	16/46
PVA/GA/LA	60	186	26	19
PVA/GA/H$^+$/LA	40/140	175/224	9/53	7/38
PVP	110	-	-	-
PVA/PVP	51	218	42	29
PVA/PVP/GA/H$^+$	51	174/205	78	57
PVA/PVP/GA/H$^+$/LA	48	167	51	37
PVA/PVP/DAS/GA/H$^+$	54	178/224	5/64	4/46
PVA/PVP/DAS/LA/GA	51	178	18	13
PVA/PVP/DAS/GA/H$^+$/LA	39	165/186	8/55	5/40

from 80°C to 55°C.[24] LA could be at least partially grafted causing disruption or attenuation of hydrogen bonding between parallel PVA chains, as well as disturbing the regularity of chain stacking by the presence of randomly distributed lactide groups pendant to the polymer chain. The α-relaxations in PVA did not manifest in this sample because LA modified the crystalline regions in PVA. Consequently, the polymer chains presented a higher degree of mobility or there were not significant restricted movements, as well as the changes in the crystalline structure affected the orientation of crystals, morphology, density, and perfection packing.[25]

The thermogram for PVA/GA/H$^+$ exhibited two endothermic peaks, which were attributed to the morphological effects caused by GA/H$^+$ into the matrix.[26] The peak centered at 213°C is related to T_m and practically did not change in comparison to the T_m for PVA, due to the relatively low GA concentration. The effect of crosslinker molecules on the crystal structure and the degree of crystallinity is manifested as the second peak related to the melting of the other minor crystalline phase at 185°C. PVA/GA/LA presented notably lower T_m and T_g than PVA, but just slightly different from PVA/LA. For that reason, it was possible to deduce that the stronger action is owed to the plasticizing effect of LA, which is in agreement with the swelling and solubility results as will be showed further in this text.

Two phenomena were identified in the PVA/GA/H$^+$/LA samples. In the first one, the T_m was sparingly higher than the same transition for pure PVA and PVA/GA/H$^+$. This might mean that the extra consumed energy was needed to overcome the intermolecular forces, which held the polymer molecules together, not as a crosslinked, but perhaps as a grafted material. The second phenomenon was that the matrix was partially crosslinked by GA/H$^+$, with the consequent reduction of the number of hydroxyl groups and hydrogen bonding interaction.[27] On the other hand, the increase of T_g could have occurred due to the presence of big bulky pendant groups.[28] Also, if mono-functional reaction is present,[26,29] the free volume is bigger, the chains of the matrix have more freedom, and a lower T_g can be expected. The combination of crosslinking and the presence of pendant groups influences the crystallinity and it will be virtually lost with the extent of crosslinking.[30]

The DSC thermograms for the PVA/PVP blends are shown in Figure 2 and their thermal transitions are exhibited in Table II. As interesting characteristic was that, of all the studied samples, just one T_g was exhibited. This might be a signal that the blends were miscible, albeit, it could indicate that the blends were only compatible.[15,31–33] The PVP themogram showed a very broad step at 110°C, which can be attributed to water evaporation, although it could indicate the T_g. This value is in concordance with some works,[34,35] while values of T_g for PVP ranging from 54 to 175°C were found in other sources; this may be attributed to the large influence of adsorbed moisture due to the hygroscopic nature of the material,[36] or to the differences in molecular weight.[37,38]

The addition of PVP to PVA evidenced a reduction of T_g, which implied that PVP plasticized PVA probably as a result of PVA/

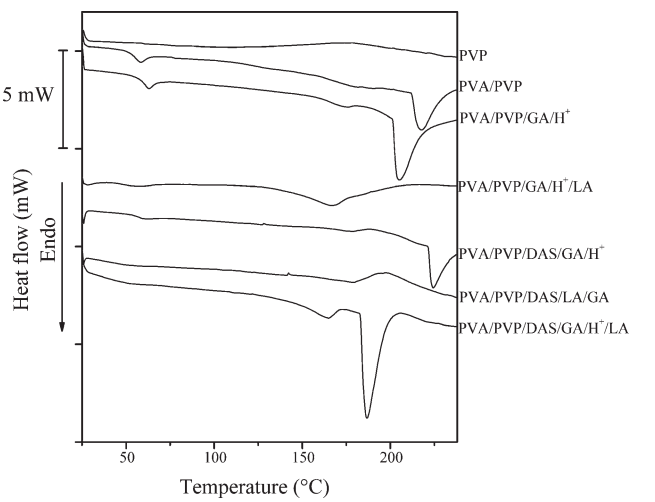

Figure 2. DSC thermograms for PVP and its blends.

PVP bonding, which disrupted the crystalline phase of PVA. The crystalline regions of PVA were more accessible to PVP and therefore, the PVA/PVP interactions were readily formed.[6] The film showed a small endothermic transition at about 51°C ascribed to T_g resulting from micro-Brownian motion of the main chain backbone. This decrease in comparison with PVA indicated that PVP was miscible with PVA in the amorphous phases.[39] The endothermic peak centered at 218°C corresponded to T_m.[40]

The PVA/PVP/GA/H^+ thermogram showed a small endothermic transition at 51°C assigned to T_g. This value was lower than the T_g exhibited by PVA/GA/H^+ and the explanation is founded in the plasticizing effect of PVP in the sample. The presence of two endothermic peaks at 174 and 205°C resembling the shape and proportion of the two peaks recorded for the melting of PVA/GA/H^+ testified for similar effect of GA. However, both T_m values were lower due to PVPs effect on semicrystalline PVA component.[41] By comparing PVA/PVP/GA/H^+ and PVA/PVP/GA/H^+/LA thermograms, significant differences appeared including that PVA/PVP/GA/H^+/LA presented only one T_m centered at 167°C. PVP reduced the endothermic curve of PVA, the peak become broader and it was shifted to lower temperatures by the combined action of PVP in the PVA crystallinity region[42,43] and LA as indicated above. The slight decrease in T_g was a consequence of the compensation of the plasticizing effect attributed to LA by the crosslinking action proportioned by GA/H^+. A broad exothermic region presented at 100°C can be ascribed to α-relaxations as evidence of a wide range of crystallite sizes and morphologies, which were affected by the additives in terms of intermolecular and intramolecular forces causing a "multi-phase" matrix.[23]

The thermograms for PVA/PVP/GA/H^+ and PVA/PVP/DAS/GA/H^+ presented minor differences, although an increase in T_m in the sample with DAS was the most significant. Even though DAS did not crosslink PVP, its presence reduced the mobility and fewer active points for interacting with the PVA chains were available. As a consequence, the crystallinity region of PVA was not affected at the same level and a higher T_m was manifested. Moreover, it has been established that the T_m for PVA depends on the PVP content which is obviously related to the decrease of the PVA crystallinity in the blend.[31,33,44] PVA/PVP/DAS/GA/H^+/LA exhibited a lower T_m because LA affected the crystalline region of PVA and plasticized the sample.

Finally, samples with DAS showed color changes before and after irradiation. If a bi-molecular reaction of DAS occurs, the efficiency of crosslinking decreases and a bronze color appears.[15] However, the thermal properties of photo-irradiated samples did not show relevant changes, which can be interpreted as DAS did not crosslink efficiently PVP or significantly affecting the crystallinity of PVA, and most importantly, DAS did not change the polymer compatibility.

Degree of Swelling

PVA Samples. As can be seen in Figure 3, during the first 10 min PVA/LA exhibited a higher degree of swelling than PVA. After that period, the swelling values for PVA/LA were slightly lower than for PVA but within the error bars. In both cases, the highest achieved degree of swelling was limited by the dissolution of the samples. A higher initial rate of swelling observed for PVA/LA can be interpreted as a consequence of the plasticizing effect of LA, which can be explained by the water molecules initially diffusing through the amorphous region of the polymer and attaching themselves to the hydroxyl side groups, disrupting inter- and intramolecular hydrogen bonding, and thereby swelling the polymer.[45,46] Hence, hydrophilic additives increase the swelling rate due to an increase of the free volume in polymer. For semicrystalline polymers such as PVA, a network of amorphous chains is formed with the crystallites acting as junction points and it may therefore be deduced that an increase in the initial rate of swelling is a consequence of the decrease of crystallinity,[24] as well as the rate of relaxation of the amorphous regions.[47,48]

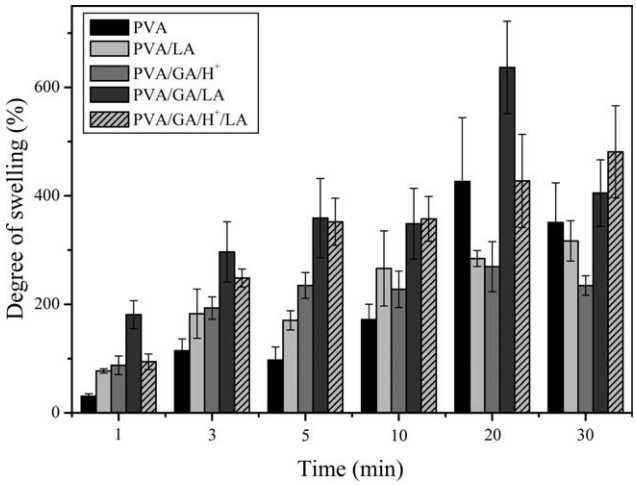

Figure 3. Degree of swelling for PVA and its blends

The combination of GA/H^+ and PVA crosslinked the polymer and thus, there were less free hydroxyl groups which reduce the hydrophilicity were expected. A decrease in the hydroxyl groups after the crosslinking reaction significantly reduced the affinity of the polymer for water leading to a reduction in the swelling ratio.[49] However, the concentration of GA/H^+ was relatively low in the prepared materials. Therefore, there was no decrease in the hydrophilicity, including PVA/GA/H^+ sample, which indeed showed a higher swelling degree than pure PVA in the first part of the experiment. For a low GA content sparse crosslinking was not able to promote a suitable dense network to totally prevent its solubility in water and the film had a high swelling value.[50] Another contribution to the initial swelling rate can be attributed to the increased disorder of the amorphous polymer matrix and the presence of hydrophilic components of the crosslinking system which could promote the diffusion of water, solvation, and unfolding of polymer chains. After that, the equilibrium swelling–deswelling was reached and the value of solubility was stabilized after 5 min of the process, when all soluble components were leached out from the specimen. In the case of pure PVA material, the swelling process passes continuously into dissolution of the polymer. On the other hand, crosslinking process causes a physical barrier limiting the disassociation of the macromolecules and the process ends in the stage of a swollen gel.

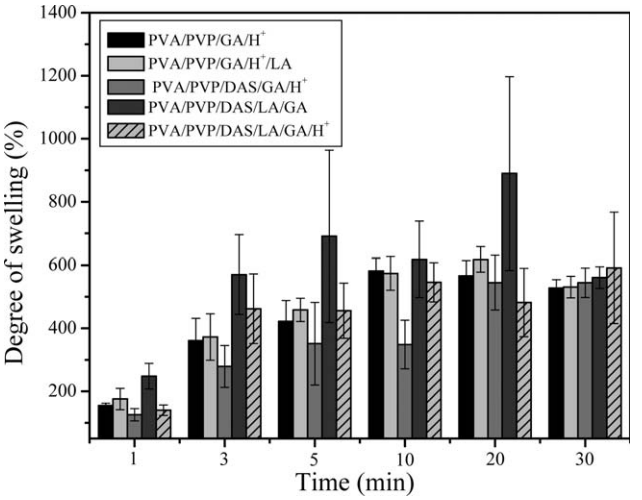

Figure 4. Degree of swelling for PVP/PVA and its blends

As it was observed in Figure 3, PVA/GA/LA material swelled to a greater extent than PVA/GA/H^+. This difference can be explained by the prevailing plasticizing effect of LA over its poor catalytic efficiency in the crosslinking of PVA by GA when compared with H^+. The comparison between PVA/GA/H^+ and PVA/GA/H^+LA showed that the former presented much lower values during the entire treatment which could corroborate that the plasticizing effect of LA was predominant in the system.

PVA/PVP Samples. The degree of swelling for PVA/PVP films in Figure 4 was higher than those of the analogous PVA films in Figure 3. This fact can be caused by the reduction of crystallinity in the blend due to the crystalline segments of the PVA chain having less chain solvation and greater stability than amorphous regions in a PVA network as a consequence of the interruption of PVA crystal formation by bulky pyrrolidone rings.[51] Furthermore, PVP as an amorphous polymer has a higher affinity for water than PVA, which makes it to swell to a greater extent.[10] The swelling of crosslinked PVP includes a very fast absorption as a consequence of hydrophilicity and capillarity, which are the basis for the notable higher values in comparison to PVA. The second step includes a typical diffusion mechanism and finally, in the third step, a minor increase in the water content occur based on very slow network relaxation.[52] Therefore, the addition of PVP promotes the swelling of PVA/PVP based blends significantly. In fact, PVA/PVP dissolved readily and it was not even possible to handle and take out as a single piece from water without dropping the slim. Indeed, the PVP/DAS specimens presented several inconveniences for determination of the swelling and solubility degree as a consequence of the quick dissolution process and the lack of strength to be manipulated in contact with water. These factors make the film unsuitable for medical applications. As a result, only crosslinked samples are discussed here. A fundamental relationship exists between the swelling of a crosslinked polymer in a solvent and the nature of the polymer and solvent. The additives can change and/or disturb the hydrogen-bonded structure of water and the molecular association of the water-soluble polymer in aqueous media, as well as the swelling behavior of the crosslinked PVP chains.[53] As an example, the values for PVA/PVP/GA/H^+ were around two times higher than PVA/GA/H^+ in all the tested times, which indicates that the swelling for PVA/PVP blends depend on the presence of PVP. On the contrary, a different phenomenon was detected for samples with DAS that were irradiated by ultraviolet light. Figure 4 depicts the degree of swelling for all PVA/PVP samples, although important attention is paid to PVA/PVP/GA/H^+ and PVA/PVP/DAS/GA/H^+. The photo-irradiated sample exhibited a lower degree of swelling in comparison to samples that did not undergo this process. The results could suggest that the DAS reaction with functional groups of PVP diminished the contribution of PVP to swelling in comparison to that in the GA crosslinked samples only. On the other hand, molecular chain length of PVP was relatively short, and a low degree or no crosslinked PVP structures of samples were obtained, therefore films were not compact enough to resist the dissolution of water, as discussed in the next section and supported by the literature as well.[54] Similar tendency was observed in PVA/PVP/GA/H^+/LA and PVA/PVP/DAS/GA/H^+/LA, which reinforces the idea that DAS did not effectively crosslink the samples.[15] As a significant result, PVA/PVP/DAS/LA/GA exhibited the highest degree of swelling for all studied samples, which is a signal of the predominant influence of LA in the blend, as well as the limited effectiveness of DAS for crosslinking PVP in the studied system. Nevertheless, PVA/PVP/DAS/LA/GA/H^+ revealed a combination of the efficiency of GA/H^+ as crosslinker of PVA and the plasticizing effect of LA, which is considered as an interesting behavior since this sample swelled to a relatively high level, but it properly resisted the dissolution during this time.

Solubility Degree

The initial statement which should be mentioned is that the solubility of PVA depends on the degree of hydrolysis, the molecular weight, and the tendency to form hydrogen bonding in aqueous solutions. In this matter, any alteration of those factors will have repercussions on the solubility of the film. Figure 5 shows the solubility degree for PVA samples and the first observed result was that LA increased the solubility of PVA film markedly during the tested period which can be attributed to the plasticizing effect caused by changes in the free volume of PVA.[55] Moreover, LA can contribute to the initial fast weight loss of the sample by its leaching. The addition of GA/H^+ to PVA presented a slight, but evident diminution on the solubility of the film in comparison to the neat polymer film during the first part of the experiment, followed by a marked decrease at the end of the tested time, which is a strong crosslinking signal. The explanation is related to the number of chains that were joined to the matrix because GA was able to promote a suitable network to prevent solubility in water, and the reaction on both edges of the GA molecule reduced the free volume of the material, which improved the water resistance of the films.[50] These kind of crosslinked polymers form gels which have the ability to absorb a solvent and swell, still keeping their three-dimensional structure which might help them become a possible candidate for long-term implantations.[56] As a noteworthy corresponding result, a difference between PVA/GA/H^+ and PVA/GA/LA was

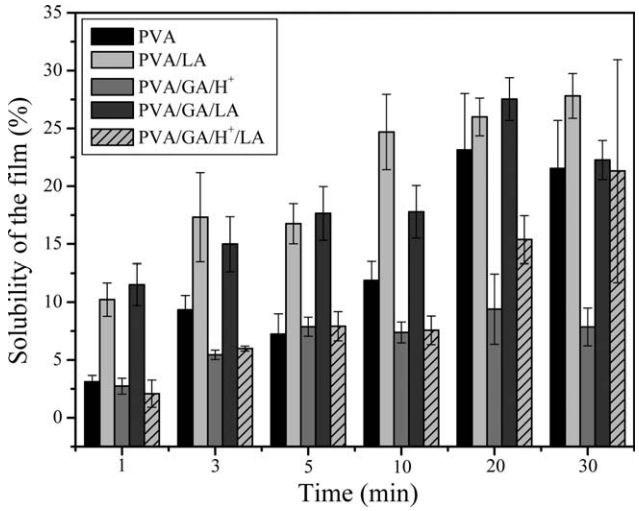

Figure 5. Solubility degree for PVA and its blends

observed and the films obtained by the combination of GA/LA exhibited a noticeably higher solubility than those with GA/H^+ which means that LA in contact with GA activated the crosslinking process to a much lower level than hydrochloric acid, and LA just plasticized the sample. Moreover, for PVA/GA/H^+/LA, every additive brought its own effects. GA/H^+ reduced the amount of hydroxyl side groups in PVA and is responsible for crosslinking, whereas LA increased the free volume of the matrix. As a consequence, the sample exhibited lower solubility than PVA/GA/LA and higher than PVA/GA/H^+.

The ease in which PVA and PVP is blended is attributed to hydrogen bonding which may take place between the proton-accepting carbonyl moiety in pyrrolidone rings and the hydroxyl side groups of PVA. Hydrogen bonding is also responsible for solubility of both PVA and PVP in water.[14,48] PVP is more hydrophilic than PVA, therefore its presence in the blends caused higher solubility values in comparison with the blends formed by PVA. The solubility degree for PVA/PVP samples is depicted in Figure 6 and it can deduced that in all the samples in which LA was present, the values were notably higher in comparison with samples where LA was absent. It was manifested once more, that the plasticizing effect of LA was preponderant in the system and dominated the dissolving mechanism. GA/H^+, on the other hand, was able to proportionate water resistance to the blend, and thus, the solubility showed lower values for PVA/PVP/GA/H^+ and PVA/PVP/DAS/GA/H^+.

By comparing the solubility of PVA/PVP/GA/H^+ and PVA/PVP/DAS/GA/H^+ there were not significant differences between them which lead to the conclusion that DAS did not crosslink efficiently to PVP. The same effect on solubility was observed for the couple formed by PVA/PVP/GA/H^+/LA and PVA/PVP/DAS/LA/GA/H^+. An explanation can be found in the low molecular weight of the PVP used in the studied materials, which caused the relative inefficiency of the DAS crosslinking process.[54] However, it cannot be excluded that the concentration of DAS was relatively high, and its decomposition rate was high as well. Furthermore, a higher probability of bimolecular coupling lead to a large dinitrilene concentration. Another adverse effect of the relatively high amount of DAS in the presence of inactive PVA virtually diluting PVP, could be intrachain crosslinking. In both ways, the solubility is not reduced, and even increased, because of a loss of interchain interactions.[15]

Lactic Acid leaching Analysis. The leaching of the samples with LA was analyzed and pH was obtained. For all the samples and intervals, the pH decreased slightly from 3.60 in the first minute to 3.10 after 30 min. This reduction in pH could indicate that the LA molecules, not strongly linked with the polymer, were delivered to the system within the first 2 min after the film was immersed in water. As a consequence of the changes in pH were not significantly exceeding physiologically values, the presence of LA will not negatively influence the tissues considering indeed, that it is well know that LA exhibits antibacterial properties. This characteristic also makes the films suitable for utilization in medical applications.[55]

Mechanical Properties

Young Modulus (E), stress at break (σ), and elongation at break (ε) were chosen for evaluation of the influence of LA, GA, and DAS on the mechanical properties of the obtained films. The results are reported in Table III, although it must to be mentioned that PVP and PVP/DAS were excluded here because the materials were too brittle and it was not possible to obtain suitable specimens for the test. It was found that LA in PVA and PVA/PVP blends considerably reduced the Young's Modulus and at the same time, the tensile strength and the elongation at break was noticeably increased. The reason can be founded in the plasticizer effect of LA.[55] On the contrary, PVA/GA/H^+ exhibited reduced mechanical properties as a result of crosslinking and because the concentration of GA was too low in comparison to LA and it did not cause softening of the material. However, if LA is accompanied by GA or GA/H^+, the elongation was higher as a consequence of the plasticizing effect. The expectable effect of an increase due to chain bonding by

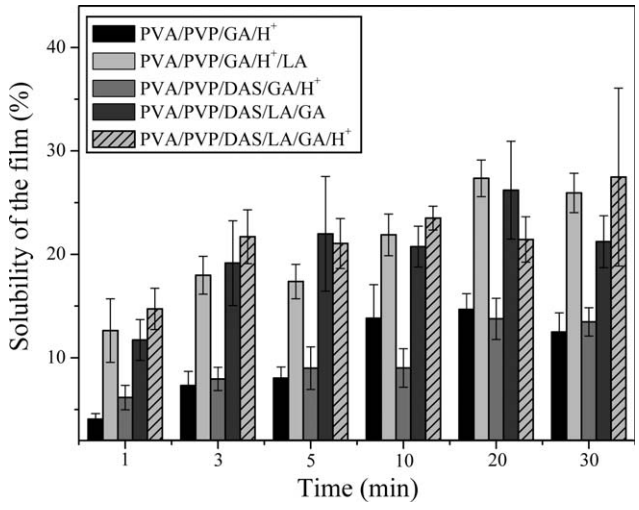

Figure 6. Solubility degree for PVA/PVP and its blends.

Table III. Mechanical Properties for PVP and PVA Blends

Sample	Thickness (mm)	Young's modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
PVA	0.236 ± 0.017	1100 ± 170	14 ± 3	38 ± 7
PVA/LA	0.274 ± 0.018	229 ± 18	21 ± 2	205 ± 11
PVA/GA/H^+	0.120	360 ± 60	8.5 ± 1.1	32 ± 5
PVA/GA/LA	0.274 ± 0.057	240 ± 20	22 ± 3	195 ± 18
PVA/GA/H^+/LA	0.286 ± 0.064	140 ± 15	26 ± 5	224 ± 8
PVA/PVP	0.270 ± 0.028	2460 ± 140	21 ± 4	5 ± 1
PVA/PVP/GA/H^+	0.226 ± 0.006	2420 ± 100	16 ± 3	4.6 ± 0.2
PVA/PVP/GA/H^+/LA	0.244 ± 0.013	460 ± 70	12.9 ± 1.8	99 ± 8
PVA/PVP/DAS/GA/H^+	0.276 ± 0.011	1580 ± 80	14 ± 2	32 ± 14
PVA/PVP/DAS/LA/GA	0.300 ± 0.025	770 ± 70	11 ± 4	79 ± 19
PVA/PVP/DAS/GA/H^+/LA	0.286 ± 0.008	745± 158	18 ± 2	58 ± 27

crosslinking is hidden within the overlap of error intervals for both materials, PVA/LA and PVA/GA/H^+/LA. It is important to point out that PVP dramatically increased the Young's modulus of PVA which could reinforce the idea that interactions between carbonyl and hydroxyl groups were present and caused brittleness of the prepared films. Furthermore, the low elongation at break is a result of the poor mechanical features of PVP, even if it is blended with PVA. Besides, LA does not influence PVP by chain grafting; its presence in the blend with PVA improves the film's elongation, which could confirm that LA contribution is due to the creation of hydrogen bonds.

It has been shown crosslinking PVA with DAS was not effective.[15] For that reason, the influence of DAS is focused on PVP. Mechanical properties of the PVA/PVP blends are governed by PVP, therefore it can be expected that DAS reduces the Young's Modulus and the tensile strength but increases the elongation. The DAS/GA mixture must specifically crosslink the two polymer components into intermingled PVA and PVP networks.[15] However, due to the low molecular weight of PVP, DAS did not crosslink the polymer, although it affected the mechanical properties. As a result, PVP/PVA/DAS/GA/H^+ had a higher Young's Modulus, lower tensile strength, and less elongation than PVP/PVA/DAS/GA/H^+/LA, because in this case, the concentration of LA was high enough for plasticizing the blend.

CONCLUSIONS

The casting method, as a simple polymer production technique was chosen for obtaining PVP/PVA films as versatile candidates for medical applications. GA/H^+ crosslinked effectively PVA whereas LA plasticized this polymer as well as the blend formed by PVA/PVP. Although DAS did not crosslink PVP due to the low molecular weight of the polymer, its presence did not negatively affect the blend regarding to the examined characteristics. Compared with raw materials, the crosslinked PVA/PVP films exhibited superior performance in terms of the properties which were analyzed by DSC, degree of swelling, solubility degree, and mechanical properties, as is explained as followed. DSC thermograms showed just one T_g for PVA/PVP samples which means that the films were miscible even with the use of LA, GA and DAS. The reported modifications of T_g and T_m suggested that the additives used did not negatively affect the thermal behavior within the studied temperature range. The crosslinked PVA/PVP films swelled to a greater extent than PVA, showing significantly higher values for water resistance. These characteristics provide the material with the ability to properly resist to biological systems and it could be considered as an interesting approach for a prospective material used in medicine. In addition, the chosen combination of the additives GA/H^+/LA/DAS seemed to fulfill the crosslinking and plasticizing needs for the PVA/PVP blends, representing an additional advantage. Furthermore, the additives used are commonly blended in polymers for medical applications and it is expected that LA would provide some supplementary benefits to the blend, due to its antibacterial properties. For all of the given reasons, the crosslinked PVA/PVP films should undergo further analysis in order to determine its applicability in medicine as a prospective material for medium or long-term implants.

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REFERENCES

1. Jagur-Grodzinski, J. *React. Funct. Polym.* **1999,** *39*, 99. DOI: 10.1016/S1381–5148(98)00054–6.
2. Fambri, L.; Migliaresi, C.; Kesenci, K.; Piskin, E. In Integrated Biomaterial Science; Barbucci, R., Ed.;Kluwer Academic Publishers:New York, **2002,** p 119–187.
3. Sionkowska, A.; Wisniewski, M.; Kaczmarek, H.; Skopinska, J.; Chevallier, P.; Mantovani, D.; Lazare, S.; Tokarev, V. *Appl. Surf. Sci.* **2006,** *253*, 1970. DOI:10.1016/j.apsusc.2006.03.048.

4. Shibata, H.; Nakagawa, S.; Tsutsumi, Y. *Molecules* **2005,** *10*, 162. DOI:10.3390/10010162.
5. Lai, G.; Du, Z.; Li, G. *Korea-Aust. Rheol. J.* **2007,** *19*, 81.
6. Jones, S. A.; Martin, G. P.; Royall, P. G.; Brown, M. B. *J. Appl. Polym. Sci.* **2005,** *98*, 2290. DOI:10.1002/app.22390.
7. Mano, V.; Scarpelli, M.; Barbani, N.; Giusti, P. *J. Appl. Polym. Sci.* **2004,** *92*, 743. DOI:10.1002/app.20027.
8. Barozzi, A.; Ganzarolli de Oliveira, M. *Biomaterials.* **2004,** *25*, 3773. DOI:10.1016/j.biomaterials.2003.10.035.
9. Yu, H.; Xu, X.; Chen, X.; Lu, T.; Zhang, P; Jing, X. *J. Appl. Polym. Sci.* **2007,** *103*, 125. DOI 10.1002/app.24835.
10. Lakouraj, M.; Tajbakhsh, M.; Mokhtary, M. *Iran. Polym. J.* **2005,** *14*, 1022.
11. Ahmad, S. I.; Hasan, N.; Abid, Z.; Mazumdar, N. *J. Appl. Polym. Sci.* **2008,** *109*, 775. DOI 10.1002/app.28140.
12. Nho, Y.; Park, K. *J. Appl. Polym. Sci.* **2002,** *85*, 1787. DOI: 10.1002/app.10812.
13. Sperling, L. H. Introduction to Physical Polymer Science; Wiley: New Jersey, **2006;** Chapter 13, p 687.
14. Sedlarik, V.; Saha, N.; Kuritka, I.; Saha, P. *Polym. Compos.* **2006,** *27*, 147. DOI:10.1002/pc.20197.
15. Lu, J.; Nguyen, Q.; Zhou, J.; Ping, Z.-H. *J. Appl. Polym. Sci.* **2003,** *89*, 2808. DOI:10.1002/app.12486.
16. De Azevedo, W. M.; De Souza, J. M.; De Melo, J. V. *Synthetic. Met.* **1999,** *100*, 241. DOI:10.1016/S0379–6779(98)01481–7.
17. Zhang, Y.; Zhu, P. C.; Edgren, D. *J. Polym. Res.* **2010,** *17*, 725.
18. Marconi, W.; Piozzi, A. In Integrated Biomaterial Science; Barbucci, R., Ed.; Kluwer Academic Publishers: New York, **2002;** p 25.
19. Kim, G.-M.; Asran, A.; Michler, G.; Simon, P.; Kim, J.-S. *Bioinspir. Biomim.* **2008,** *3*, 1. DOI:10.1088/1748–3182/3/4/046003.
20. EN ISO 527:Plastics. Determination of tensile properties, **1995.**
21. EN ISO 291:Plastics. Standard atmospheres for conditioning and testing, **1997.**
22. Bhajantri, R. F.; Ravindrachary, V.; Harisha, A.; Crasta, V.; Nayak, S. P.; Poojary, B. *Polymer* **2006,** *47*, 3591. DOI: 10.1016/j.polymer.2006.03.054.
23. Liang, S.; Yang, J.; Zhang, Z.; Bai, Y. *J. Appl. Polym. Sci.* **2011,** *122*, 813. DOI: 10.1002/app.34060.
24. Onyari, J.; Huang, S. *J. Appl. Polym. Sci.* **2009,** *113*, 2053.
25. El-Khodary, A. *Physica B: Condens. Matter.* **2009,** *404*, 1287.
26. Park, J. S.; Park, J. W.; Ruckenstein, E. *J. Appl. Polym. Sci.* **2001,** *82*, 1816.
27. Krumova, M.; López, D.; Benavente, R.; Mijangos, C.; Pereña, J. M. *Polymer.* **2000,** *41*, 9265.
28. Park, Y.; Lee, K. *J. Appl. Polym. Sci.* **2001,** *80*, 1785.
29. Yeom, C. K.; Lee, K. H. *J. Membr. Sci.* **1996,** *109*, 257.
30. Hari, P. R.; Sreenivasan, K. *J. Appl. Polym. Sci.* **2001,** *82*, 143.
31. Abd El-Mohdy, H. L.; Ghanem, S. *J. Polym. Res.* **2009,** *16*, 1.
32. Liang, G.; Zhan, K.; Feng, R. *Chin. J. Polym. Sci.* **1994,** *12*, 202.
33. Lewandowska, K. *Eur. Polym. J.* **2005,** *41*, 55.
34. Feldstein, M.; Shandryuk, G. A.; Kuptsov, S. A.; Plate, N. A. *Polymer* **2000,** *41*, 5327.
35. Ruan, L. P.; Yu, B. Y.; Fu, G. M.; Zhu, D. *J. Pharm. Biomed. Anal.* **2005,** *38*, 457.
36. Caykara, T.; Demirci, S.; Kantoglu, O. *Polym-Plast. Technol.* **2007,** *46*, 737.
37. Buera, M.; Levi, G.; Karel, M. *Biotechnol. Progr.* **1992,** *8*, 144.
38. Broman, E.; Khoo, C.; Taylor, L. S. *Int. J. Pharm.* **2001,** *222*, 139.
39. Feng, H.; Feng, Z.; Shen, L. *Polymer* **1993,** *34*, 2516.
40. Abdelrazek, E. M.; Elashmawi, I. S.; El-khodary, A.; Yassin, A. *Curr. Appl. Phys.* **2010,** *2*, 607.
41. Gonzalez, G. A.; Heinamaki, J.; Mirza, S.; Antikainen, O.; Iraizos, A.; Suzarte, A.; Yliruusi, J. *Eur. J. Pharm. Biopharm.* **2005,** *59*, 343.
42. Kim, S.; Park, S.; Kim, I.; Lee, Y.; Kim, S. *J. Appl. Polym. Sci.* **2002,** *86*, 1844.
43. Kim, S.; Park, S.; Kim, I.; Chung, T.; Kim, H. *J. Appl. Polym. Sci.* **2003,** *90*, 881.
44. Li, L.; Chang, C.; Weng, L. *Polymer* **1998,** *39*, 2355.
45. Hodge, R. M. *Polymer* **1996,** *37*, 1371.
46. Chan, L.; Hao, J.; Heng, P. *Chem. Pharm. Bull.* **1999,** *47*, 1412.
47. Tanigami, T.; Yano, K.; Yamaura, K.; Matswzawa, S. *Polymer* **1995,** *36*, 2941.
48. Cassu, S.; Felisberti, M. *Polymer* **1997,** *38*, 3907.
49. Mansur, H.; Sadahira, C.; Souza, A.; Mansur, A. *Mater. Sci. Eng. C.* **2008,** *28*, 539.
50. Figueiredo, K.; Alves, T.; Borges, C. *J. Appl. Polym.* **2009,** *111*, 3074.
51. Tomas, J.; Lowman, A.; Marcolongo, M. *J. Biomed. Mater. Res. A.* **2003,** *67*, 1329.
52. Miranda, L.; Lugao, A.; Machado, L.; Ramanathan, L. *Radiat. Phys. Chem.* **1999,** *55*, 709.
53. Can, H.; Kavlak, S.; Kirci, B.; Guner, A. *Adv. Polym. Tech.* **2003,** *22*, 252.
54. Li, Z.; Li, Y.; Qian, X.-F.; Yin, J.; Zhu, Z.-K. *Appl. Surf. Sci.* **2005,** *250*, 109.
55. Sedlarik, V.; Saha, N.; Kuritka, I.; Saha, P. *Int. J. Polym. Anal. Ch.* **2006,** *11*, 253.
56. Li, J.; Suo, J.; Deng, R. *J. Reinf. Plast. Compos.* **2010,** *29*, 618. DOI:10.1177/0731684408100696.

PAPER III

Preparation and characterisation of a new double-sided bio-artificial material prepared by casting of poly(vinyl alcohol) on collagen

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ORIGINAL PAPER

Preparation and characterisation of a new double-sided bio-artificial material prepared by casting of poly(vinyl alcohol) on collagen

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Abstract A new double-sided bio-artificial polymer material prepared by casting of poly(vinyl alcohol) (PVA) on collagen (COLL) was obtained. The single components were blended with lactic acid and glutaraldehyde as plasticiser and cross-linker agents, respectively, to change and characterise structure of both the polymers. Differential scanning calorimetry, dynamic mechanical analysis, tensile test, tear resistance test, scratch test and Fourier transform infrared spectroscopy were chosen to characterise all the prepared materials. The results showed that the additives led to the decrease of glass transition temperature, melting temperature and crystallinity with respect to raw materials. The new bio-artificial material revealed tough behaviour with yield stress, with less by half tensile strength compared to neat materials and with the strain of PVA (>100 %). Both PVA and COLL blends and the new bio-artificial material exhibited viscoelastic features useful for being used in contact with living organism.

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Introduction

Polymers have been in the centre of interest in almost all fields of science, industry and medicine due to their broad physical and mechanical properties which make them an interesting material for being used in contact with living organisms. However, due to a negative body reaction against artificial objects inserting into a body, causing a strong infection or even death, the scientific community have devoted the attention to find biocompatible materials that do not evoke any significant foreign-body reaction [1].

Currently, biomaterials can be divided into two basic categories: biological and synthetic. The biological ones consist of polypeptides, polysaccharides, nucleic acids, polyesters, hydroxyapatites and their composites [2]. The list of synthetic polymers used in medicine includes polyvinyl chloride, polyethylene (PE), polypropylene, polymethylmethacrylate and polystyrene among others [3]. One of the most studied natural polymers is collagen (COLL), which is widely used for its excellent biocompatibility and slight immunogenic reactions [4]. From synthetic polymers, poly(vinyl alcohol) (PVA) has been intensively investigated because of its attractive features such as high hydrophilicity, recognised biodegradability and biocompatibility [5].

Both COLL and PVA have been used for medical applications separately. The former is used for immobilisation of biological materials, such as factor XIII from blood, for guided tissue regeneration, as a filler of tooth extraction sites, in haemodialysis membranes, as retinal reattachment, as a dural substitute, nerve regeneration, for reparation of tympanic membranes, cartilage, meniscus and bones, as a control of local bleeding, for repairing of liver injuries, as a protective barrier during brain surgery or wound repair [6]. The latter is used as a matrix for immobilisation [7] and for haemodialysis membranes [8]. Furthermore, it was reported in many studies that blending of PVA and COLL led to significant improvements in many of their individual properties [9–18].

It is important to point out that the use of biodegradable polymers in biomedical field offers two important advantages among many others. The first one is elimination of the second surgery to remove the implanted prosthesis after the healing of tissues and the second one is the possibility of triggering and guiding tissue regeneration via degradation of the used material [19]. There is evidence that COLL and PVA can be blended with further intention instead of being used only as homogeneous biomaterials. This kind of material is called bio-artificial polymer and can be obtained in form of polymer blends, composites or a combination of both [20]. Originally, these new materials were conceived to overcome poor biological performance of the most of the synthetic polymers and to enhance mechanical characteristics of biopolymers in order to be employed as biomaterials or as low-environmental impact materials [21]. Up to now, bio-artificial materials based on COLL have shown positive results in a wide variety of treatments [5, 6, 9, 11,

16–18, 22–24]. However, a double-sided bio-artificial polymeric material consisting of COLL and PVA has not been reported yet and unique properties done by each side can be expected and thus, being attractive for usage in medicine. This article presents the first statement about double-sided bio-material, produced by sequential casting of PVA on COLL. Chemical composition and mechanical properties of single and double films were evaluated by differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA), tensile test, tear resistance test, scratch test and Fourier transform infrared (FTIR) spectroscopy.

Experimental

Materials

Atelocollagen emulsion (1.43 wt %) from bovine Achilles tendon with pH 3.5 was supplied by Vipo, Slovakia. PVA (Mw $\sim$ 47,000 g mol^{-1}) with polymerization degree of 1,000 and 98 % hydrolysis was supplied by Fluka as well as 50 % water solution of glutaraldehyde (GA). Lactic acid (LA, analytical grade) was produced by Lachema, the Czech Republic, and acetic acid (analytical grade) was supplied by Penta, the Czech Republic. They were used without further purification.

Sample preparation

As the main goal of this study is the preparation of the double-sided bio-artificial polymer, single and double layer preparation is described as follows.

Single layer

Atelocollagen was dissolved in 0.17 N acetic acid for 1 h at 80 °C under magnetic stirring to obtain 1 wt % solution. To prepare thin films, atelocollagen solution was poured on glass slides and polystyrene Petri dishes. Thin films of PVA were produced in the same way by casting 5 wt % (PVA5) and 10 wt % (PVA10) aqueous solutions obtained by dissolving PVA in distilled water at 80 °C for 12 h under continuous magnetic stirring. Apart from neat materials, blends of PVA and atelocollagen (COLL) with LA and GA were produced. PVA5, PVA10 and COLL solutions were after the addition of LA, GA and LA–GA stirred for other 20 min at 80 °C and they were cast on glass slides and polystyrene Petri dishes. GA was added in amount of 0.25 wt % and LA of 15 wt % related to total amount of polymer. Finally, all the blends were allowed to dry in an air-circulating oven at 35 °C for 4 days.

Bio-artificial film

The bio-artificial film was produced step by step in this way: a single layer of COLL was obtained and dried, and the PVA5 solution was cast on it. Both the polymers were blended with GA and LA. The system was allowed to dry under the same

conditions as for single layer films. Designation of all prepared specimen summarises Table 1.

Measurement

Differential scanning calorimetry

Calorimetric measurements were performed on calorimeter DSC 2920, TA Instruments under nitrogen flowing at a rate 70 ml min^{-1}. The specimens were pressed-in unsealed aluminium pans. Two cycles of heating were performed. The first one was done in order to dry the samples and to annul previous thermal and mechanical history. The second one was performed to obtain glass transition temperature (T_g) and melting temperature (T_m) of all films. The samples were cooled down by air at an exponentially decreasing rate. The heating of both the cycles was performed from 40 to 240 °C at a heating rate of 10 °C/min. The T_g was determined as the midpoint temperature by standard extrapolation of the linear part of DSC curves and the T_m as the maximum value of the melting peak. The specimens were air-stored at laboratory conditions in PE bags.

Dynamic mechanical analysis

DMA measurements were performed to determine dynamic modulus (E') and T_g using DMA/SDTA 861e (Mettler Toledo, Switzerland) instrument. Rectangular test specimen based on PVA with a length of 8.4 mm, width of 1.95 mm and thickness of about 200 μm were heated from −20 to 120 °C at a rate 3 °C/min under frequency varied from 1 to 10 Hz in a tensile mode. The samples were subjected to oscillating tensile force and the displacement amplitude was 30 μm in the linear

Table 1 The list of prepared films

No.	Name	Description
1	COLL	A COLL film from 1 wt% water solution
2	COLL–LA	A COLL film with LA (15 wt% related COLL)
3	COLL–GA–LA	A COLL film with LA and GA
4	PVA5	A PVA film form 5 wt% water solution
5	PVA5–LA	PVA5 film with LA (15 wt% related to PVA5)
6	PVA5–GA	PVA5 film with GA (0.25 wt% related to PVA5)
7	PVA5–LA–GA	PVA5 with GA and LA
8	PVA10	A PVA film from 10 wt% water solution
9	PVA10–LA	PVA10 film with LA (15 wt% related to PVA10)
10	PVA10–GA	PVA10 film with GA (0.25% related to PVA10)
11	PVA10–LA–GA	PVA10 film with LA and GA
12	BAP–COLL–PVA	Bio-artificial polymer. The first layer consists of COLL with GA and LA, the second one consists of PVA5 with GA and LA

viscoelastic region. The T_g values were estimated from the maximum of tan δ curve. Before testing, the samples were conditioned in a desiccator for 48 h.

Mechanical testing

The trouser tear method, specified in ISO 6383-1, was used for characterisation of polymer films. The method determines the tear resistance of plastic film or sheet <1-mm thick, tested under defined conditions. A rectangular test specimen having a longitudinal slit extending over its half length is subjected to a tensile test on the "trouser legs" formed by the slit. The average force required to tear the specimen completely along its length is used to calculate the tear resistance in N/mm. They were tested dimensionally third- and fifth-samples with respect to ISO 6383-1; the lengths were 50 and 30 mm, the widths were 25 and 10 mm, respectively. The test was performed using universal testing machine ZWICK Z010 at a rate 200 mm/min. The initial distance between grips was 50 and 30 mm, respectively, for longer and shorter specimens. The samples were conditioned in a desiccator for 18 h before testing. The samples thickness was measured by a micrometre with the accuracy of 0.0005 mm. The tearing tests were performed under laboratory (lab) conditions.

The tensile test was performed on ZWICK Z010 machine at a rate 5 mm/min under preload of 0.1 N because of a little bit vertical buckling of samples after clamping. The specimens of type 5B specified in ISO 527-2 were tested. The specimen width was 2 mm, the initial distance between grips was 20 mm and the gauge length was 12 mm. The thickness of samples was measured by a micrometre with the accuracy of 0.0005 mm and by Confocal Laser Scanning Microscope LEXT OLS 3000, Olympus. The samples were conditioned in a desiccator for 2 days before testing. The tests were performed under lab conditions.

Scratch resistance

The scratch test was performed with a home made scratch tester developed at the Institute of Materials Chemistry at Brno University of Technology, which was described in [25]. The test was performed in order to determine how the films are resistant against scratching, how they behave during scratching (brittle, ductile) and what their adhesion to glass is. The specimens were conditioned in a desiccator for 2 weeks before scratching. The scratches were produced on specimen surfaces by moving a diamond tip with a radius of 50 μm in a horizontal way at a rate 1 mm/min under continuously increasing normal force. The scratch length was 15 mm and the distance between the scratches was 2 mm. Two or three scratches were produced under normal force increasing in the range 0–0.5 and 0–1.5 N on each sample; the scratches formed under force 0–3 N were produced on PVA5–LA–GA and BAP–COLL–PVA films. Normal and lateral (friction) forces were recorded as a function of the scratch length. The scratch profile was recorded by Confocal Laser Scanning Microscope LEXT OLS 3000 (Olympus) after the scratching.

FTIR spectroscopy

In order to identify the differences between both the sides of bio-artificial polymeric material, FITR spectroscopy analysis was carried out on NICOLET 320 FTIR device equipped with attenuated total reflectance (ATR) accessory utilising the Zn–Se crystal and software package OMNIC over the range of wave lengths from 4,000 to 600 cm^{-1} at room temperature under resolution 2 cm^{-1}. Single layers of neat polymers were tested as well.

Results and discussion

Although the main aim of this study was to get a double-sided bio-artificial polymer material (BAP–COLL–PVA), the influence of plasticiser and crosslinker agents on COLL and PVA was examined at first. The final BAP–COLL–PVA properties were studied with respect to the normal body temperature (37 °C).

DSC analysis

The first heating curves, which are not shown in this study, revealed an expressive and wide endotherm belonging to water release up to 170 °C because the specimens were stored under laboratory conditions in PE bags before DSC measurement instead of drying. The second heating calorimetric curves for PVA10, PVA5 and their blends depicted in Figs. 1 and 2, respectively, revealed glass transition region and melting behaviour. The evaluated T_g and T_m are summarised in Table 2. These thermograms predicates about smaller and lesser perfect crystallites and/or thinner lamellae of PVA5 compared to PVA10 (Fig. 2).

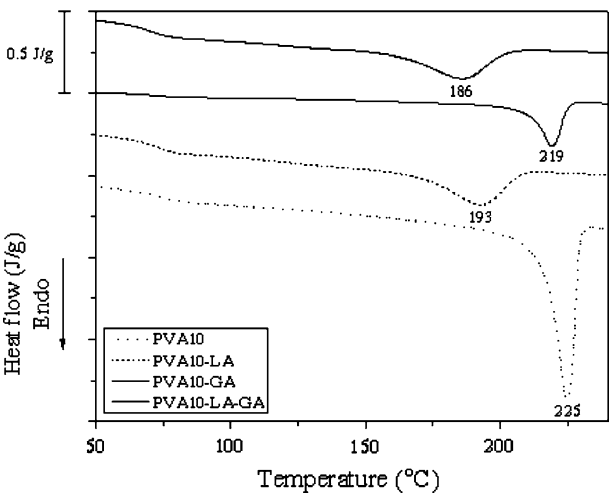

Fig. 1 DSC thermograms of PVA10 films; the second heating

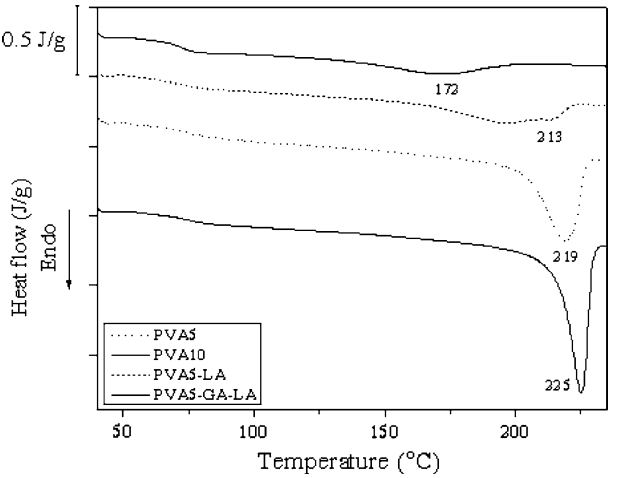

Fig. 2 DSC thermograms of PVA5 films; the second heating

Table 2 T_g and T_m measured by DSC and DMA

Sample	T_g by DSC (°C)	T_m by DSC (°C)	T_g by DMA (°C)			
			1 Hz	2 Hz	5 Hz	10 Hz
COLL	205	–	–	–	–	–
COLL–LA	154	–	–	–	–	–
COLL–GA–LA	130	–	–	–	–	–
PVA5	73	219	49	49	51	52
PVA5–LA	69	213	25	29	28	30
PVA5–GA	–	–	49	52	53	57
PVA5–LA–GA	73	172	29	30	31	32
PVA10	75	225	37	38	39	39
PVA10–LA	72	193	21	24	25	27
PVA10–GA	68	219	37	39	39	40
PVA10–LA–GA	69	186	20	21	22	22
BAP–COLL–PVA	77	199	–	–	–	–

The idea of LA incorporation to the PVA structure is supported by quite good compatibility of both the components due to their polarity, changes of free volume and consequent decreases of T_g of PVA [26]. LA is at least partially grafted on hydroxyl groups of PVA which, generally, causes disturbing or attenuation of hydrogen bonding between parallel PVA chains as well as disturbing of regularity of chain stacking by the presence of randomly distributed lactide groups pendant to polymer chains. LA decreased T_g, T_m and crystallinity both for PVA5 and for PVA10 acting as plasticiser. Smaller and much perfect PVA crystallites were formed in the presence of LA and this effect was higher for PVA5–LA for which

double endothermic peak was observed. It can reflect two sizes of crystallites and/or recrystallization during heating.

A small amount of GA caused 70 % decrease of PVA10 crystallinity, decrease of T_g and a very small decrease of T_m. These data point to a great increase of amorphous phase in PVA10. It was reported that monofunctional reaction could occur between GA and PVA if the content of GA is higher than 10 vol%, which leads to grafting and branching of PVA chains [27, 28]. But this was not the case of this study. The reason is the low concentration of GA (0.25 % related to the total amount of PVA), and the very low ratio of crosslinking agent to mer units being about 1:1000 and formation of very perfect crystallites (the shape of endotherm in Fig. 1), although in a small content because crystallinity decreased by more than three times with respect to neat PVA10.

The addition of both LA and GA into PVA5 and PVA10 led to the decrease of crystallinity by >50 % and the shape of DSC curves in Figs. 1 and 2 shows that plasticising effect of LA predominated. It can be also seen that more amorphous structure had the blend PVA5–LA–GA compared to PVA10–LA–GA for shallow and broad endothermic melting region.

The second heating DSC thermograph of the new bio-artificial polymer is depicted in Fig. 3. The DSC curve can be explained well only if DSC measurements of COLL and COLL-blended samples are known. An expressive glass transition region with the T_g of 205 °C is evident on DSC curve of COLL. But COLL must have been denatured before DSC measurement because no endothermic peak of COLL denaturation was revealed after the first heating, which should have occurred at about 200 °C [8]. The addition of LA to COLL decreased the T_g from 205 to 154 °C and the addition of LA–GA caused the decrease from 205 to 130 °C and thus, both LA and LA–GA revealed plasticising effect of COLL. Calorimetric behaviour emphasised the influence of PVA5–LA–GA blend in bio-artificial

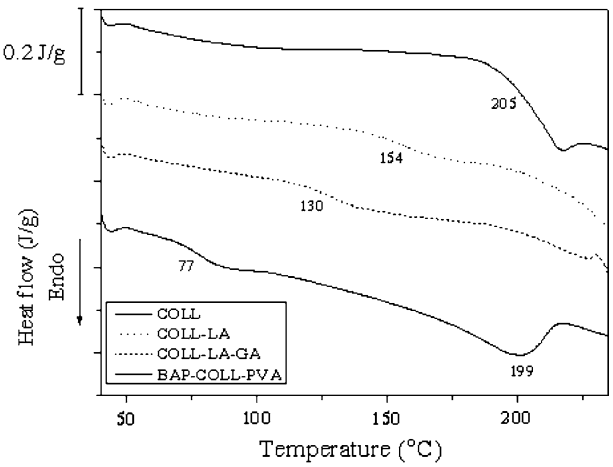

Fig. 3 DSC thermograms of COLL and its blends together with BAP–COLL–PVA; the second heating

polymer because glass transition at about 77 °C and melting peak at 199 °C was evaluated while contribution of COLL–LA–GA blend was hidden.

Dynamic mechanical analysis

DMA was used to determine storage modulus (E') and T_g as well as to investigate viscoelastic properties of the films as a function of temperature. On the basis of the requirements for biomaterials including mechanical performance and physical properties, the new bio-artificial polymer was analysed at 37 °C, the normal human temperature. The four frequencies that were chosen (1, 2, 5 and 10 Hz) were in the range of physiological relevant frequencies. These frequencies are considered to be from 0.298 to 75.39 rad/s [29], approximately from 0.050 to 12 Hz. For example, human stomach has recordable mechanical activity at a rate of about 3 cycles/min [30] (0.05 Hz), heart valves are used at 1.2 Hz, condoms at 2 Hz, plastic hip joints at 4 Hz and chewing or dental fillings at 10 Hz [31].

The temperature and frequency dependences of E' and tan δ of PVA10 and their blends are depicted in Figs. 4 and 5, respectively, to show the example how DMA curves looked like. The maximum of tan δ dependence represents T_g and the whole region corresponds to α relaxation of amorphous phase in which micro-Brownian motion of molecular chains become appreciable and E' decreases markedly due to increase of chain mobility, promoting less resistance for rearrangement of molecules.

For dry PVA, α relaxation appears at 80 °C, but as a consequence of water presence, this relaxation can shift to lower temperatures as it was manifested for PVA10 [27]. Tan δ values for PVA10 run from 0.28 to 0.37 at 37 °C, which can be interpreted that the film presented elastic behaviour at all tested frequencies. The difference in T_g values obtained by DMA and DSC are attributed to different conditions of measurement, especially heating rates and moisture state. The T_g values for PVA10 increased by only 2 °C (Fig. 4b) as a consequence of the changes in frequency from 1 to 10 Hz. Moreover, T_g for PVA10 blends changed as a result of the changes in the structure of materials (Fig. 5b). Similar behaviour for PVA5 and its blends were manifested although the absolute values of T_g were higher with respect to PVA10.

At 1 Hz, LA reduced the T_g by 16 °C for PVA10 due to plasticising effect. On the contrary, GA did not influence T_g although it is expected to act as a crosslinker agent. The addition of GA to PVA–LA has slight effect on T_g and E' was about two times higher in the whole temperature range as can be seen in Fig. 5a, which means higher stiffness. It is evident that GA did not change stiffness of PVA10 at 37 °C but higher stiffness was observed below 20 °C and over 60 °C. Moreover, the highest E' was measured for the blend with GA, PVA10–LA–GA, below 10 °C and over 60 °C. Plasticising effect of LA on PVA10 was reflected not only by the decrease of T_g but also by the decrease of E', which was for PVA10–LA and PVA10–LA–GA samples always lower in the temperature range 20–50 °C and by high activation energy of glass transition region (Table 3). The same results were obtained for corresponding PVA5 samples.

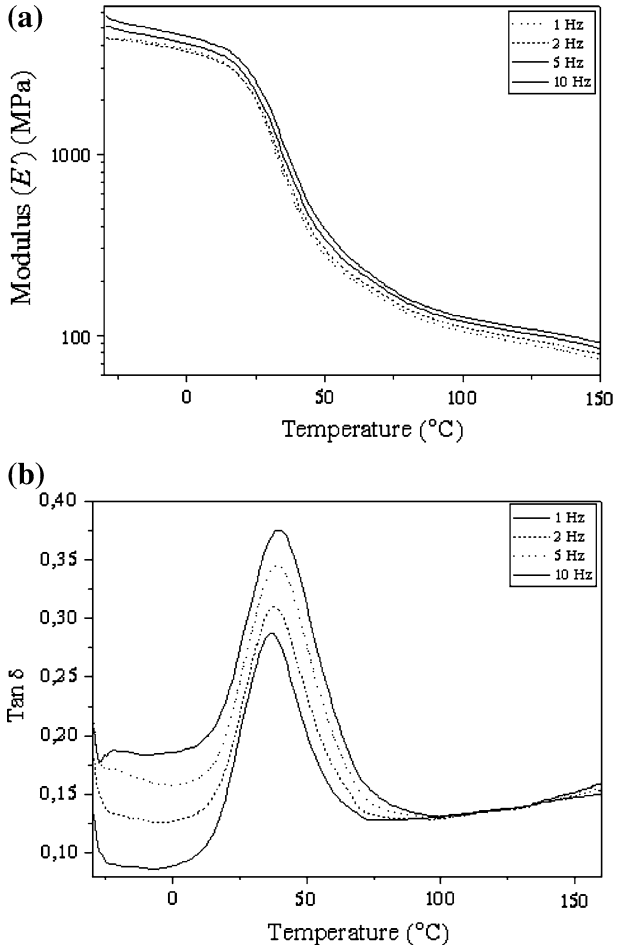

Fig. 4 DMA curves of PVA10 at different frequencies: **a** temperature dependence of storage modulus and **b** temperature dependence of tan δ

Storage modulus was for all measured samples always higher than loss modulus at all examined frequencies over the whole used temperature range, which adverted to elastic behaviour of all tested materials. Because of interest in viscoelastic properties of all PVA10 and PVA5 samples at normal human body temperature (37 °C), storage modules measured at 1 Hz is listed in Table 4 and only very slight effect of frequency was observed for E'.

The apparent activation energy of glass transition (E_a) was for PVA10 and PVA5 samples evaluated from Arrhenius plots shown in Fig. 6. It can be seen that E_a was for PVA5–GA and PVA10–GA samples nearly half compared to PVA5 and PVA10, respectively, confirming rigidity of samples with GA. Prevailing plasticising of LA in blends with GA was proved by nearly the same activation energy with respect to neat PVA.

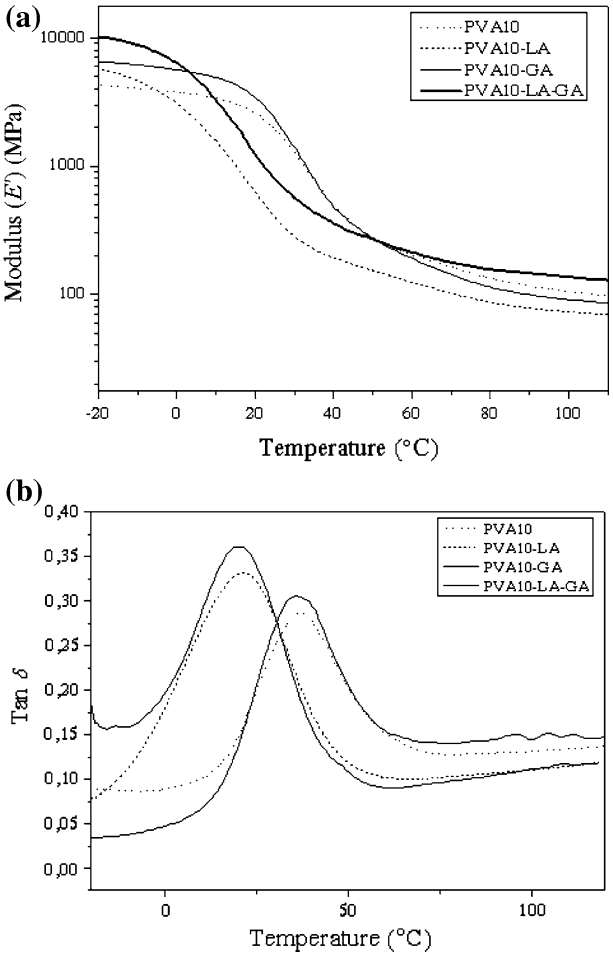

Fig. 5 DMA curves of PVA10 blends at 1 Hz: **a** temperature dependence of storage modulus and **b** temperature dependence of tan δ

Table 3 Apparent activation energy for PVA10 and their blends

Sample	Apparent activation energy—E_a (kJ mol^{-1})
PVA5	500 ± 110
PVA5–LA	410 ± 60
PVA5–GA	270 ± 60
PVA5–LA–GA	520 ± 30
PVA10	590 ± 120
PVA10–LA	340 ± 60
PVA10–GA	350 ± 150
PVA10–LA–GA	530 ± 160

Table 4 Storage modulus (E') for PVA5 and PVA10 blends at 1 Hz and at 37 °C

Sample	Storage modulus (E')
PVA5	220
PVA5–GA	40
PVA5–LA	9
PV5–LA–GA	198
PVA10	644
PVA10–GA	648
PVA10–LA	208
PVA10–LA–GA	399

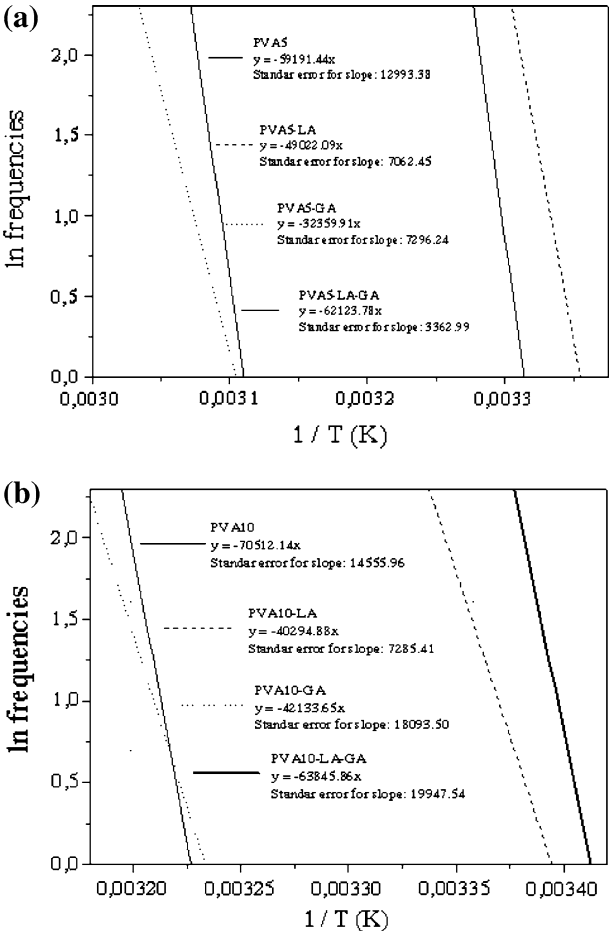

Fig. 6 Arrhenius plot for PVA5 (**a**) and PVA10 (**b**) and their blends

Mechanical testing: tensile test and tear resistance

Tensile test

Tensile properties were similar for PVA5 and PVA10 as can be seen in Fig. 7. The addition of LA caused ductile and tough materials without macroscopic visual yield point with strain at break of several hundred percent with respect to neat PVA. The recorded yield stress was 82 and 72 % lower for PVA–LA blends compared to the raw material (Fig. 8). On the contrary, the addition of GA to PVA10 decreased the strain at break but did not decrease yield stress, which made possible simpler tearing. The combination of GA–LA with PVA5 and PVA10 led to nearly the same mechanical behaviour of the blends with only LA which confirmed prevailing effect of LA against GA on PVA structure and properties but yet, stiffening effect of GA came forth due to a little bit higher conventional yield stress for blends with LA–GA

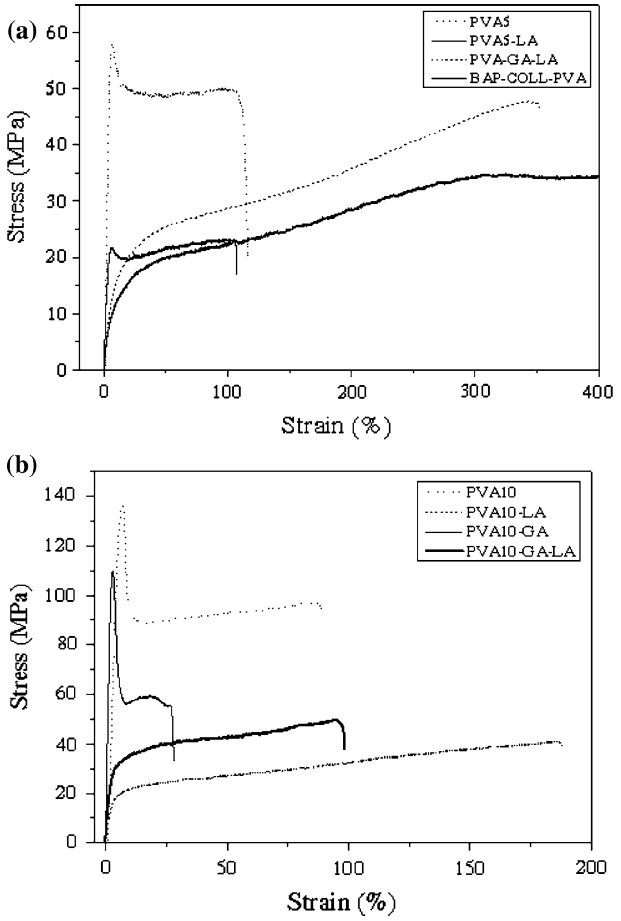

Fig. 7 Tensile test of **a** PVA5 samples together with BAP–COLL–PVA and **b** PVA10 samples

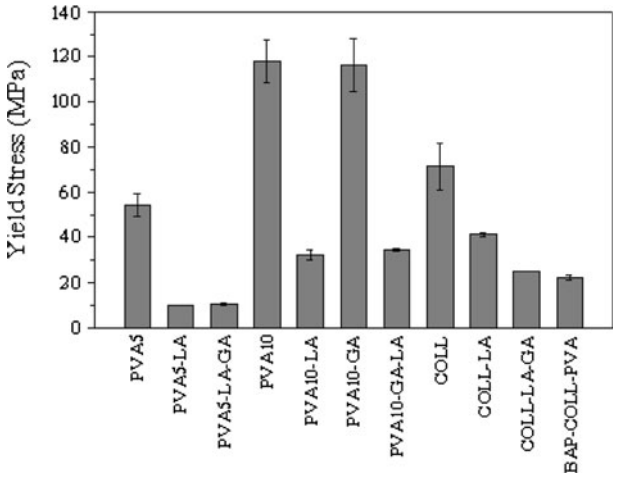

Fig. 8 Yield stress of COLL, PVA5, PVA10 and their blends together with BAP–COLL–PVA

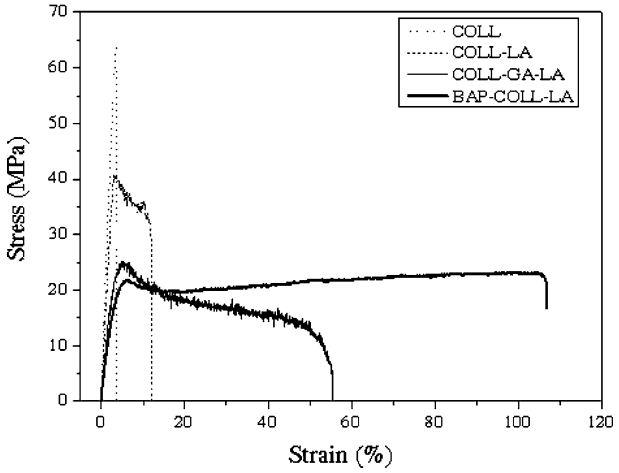

Fig. 9 Tensile test of COLL blends

compared to those with only LA (Fig. 7). This was proved also by DMA measurement.

The influence of the additives on tensile mechanical properties of COLL is depicted in Fig. 9. COLL revealed brittle tensile behaviour. The addition of LA and LA–GA led to the formation of tough materials with yield stress. The LA addition caused the decrease of tensile strength by 40 % with respect to COLL but the addition of LA together with GA acted in opposite way with respect to PVA due to further decrease of strength by another 40 % compared to COLL–LA. The decrease of strength was accompanied by gradual increase of strain at break which increased approximately three and sixteen times by addition of LA and LA–GA to COLL,

respectively. These data are in a good agreement with DSC data revealing the decrease of T_g.

Tensile tests of bio-artificial material revealed that the shape of its tensile curve was nearly the same as that of PVA5. It seems that yield stress is done by COLL blend but the strain, orientation and strain hardening by PVA5 blend. It is evident that it was obtained tough material with yield stress with less by half tensile strength compared to neat materials (PVA5 and COLL) and with the strain of PVA5 (>100 %).

Tear resistance

The typical tear force curves for PVA10 and their blends are presented in Fig. 10. The tear resistance was evaluated as the median of the force value in the plateau region. If the film thickness varied or the crack did not spread directly as in case of blends PVA10–LA and PVA–LA–GA, the plateau was not formed.

Tear resistance of PVA10 and PVA5 samples together with bio-artificial film is shown in Fig. 11. The addition of LA almost doubled tear resistance of PVA10 and high ductility of PVA10–LA was manifested by curled sides of torn surfaces as can be seen in Fig. 12a. It confirmed plasticising effect of LA and formation of tough material. Even very small content of GA increased brittleness of PVA10 because tear resistance decreased by about 25 % probably due to crosslinking of GA. Tear resistance of the blend PVA10–LA–GA confirmed predominant effect of LA against GA because it was 25 % higher than tear resistance of PVA10 but 25 % lower than PVA10–LA. The effect of additives on PVA5 was the same, although the absolute values of tear resistance differed for different structure.

Tear resistance of the bio-artificial film (which is not shown in the graph) was the lowest due to brittle character of COLL blends revealed by tensile test. The surface of torn surfaces was smooth without any deformations visible under used microscopic magnification as can be seen in Fig. 12b.

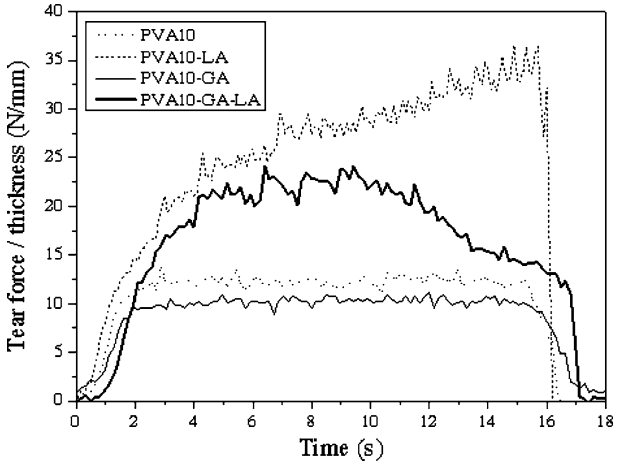

Fig. 10 Tear force curves of PVA10 and its blends

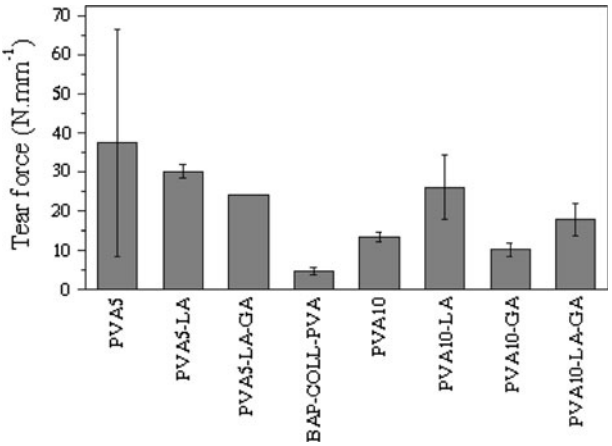

Fig. 11 Tear resistance of PVA5 and PVA10 specimens together with bio-artificial film

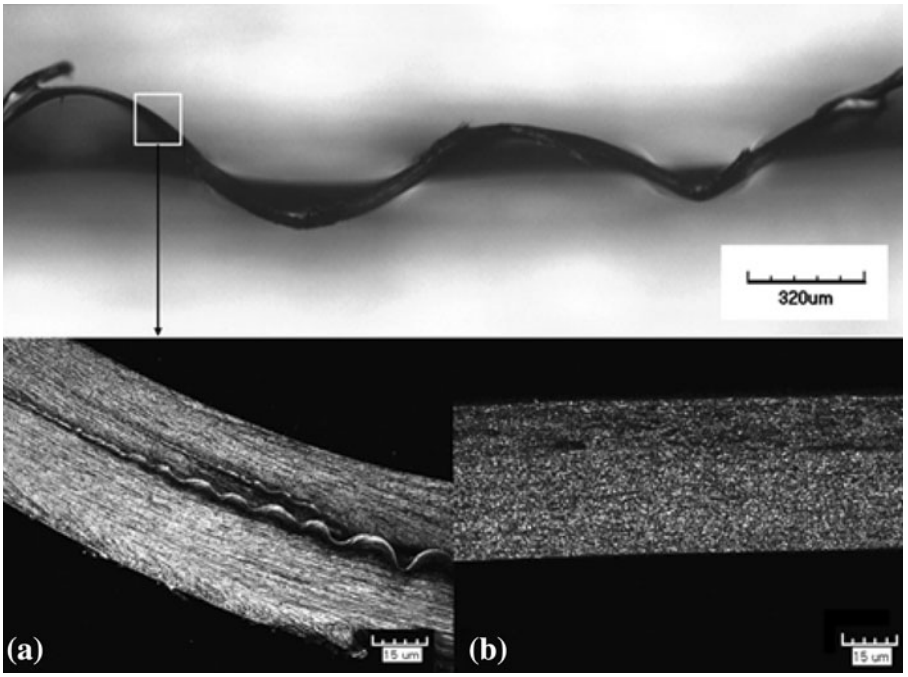

Fig. 12 Torn surfaces of **a** PVA10–LA and **b** BAP–COLL–PVA

Scratch resistance

First of all, it must be pointed out that it is not possible to perform the scratch test on thin films without adhesion to the substrate. The samples containing PVA10

Table 5 Description of the adhesion in the samples

Specimen	Adhesion to the glass slide before test evaluated by human eye (% of area)	Nominal force (N)	Failure to glass (mN)	Appearance of the scratch
PVA5	Good adhesion	0–0.5	No	Smooth
		0–1.5	No	
PVA5–LA	Poor adhesion along margins	0–0.5	No	Smooth
		0–1.5	No	
PVA5–LA–GA	Poor adhesion along margins	0–0.5	No	Smooth
		0–1.5	Yes	
PVA10	10–30 % without adhesion	0–0.5	No	Smooth
PVA10–LA	50 % without adhesion	0–0.5	No	Smooth
		0–1.5	No	
PVA10–LA–GA	10–40 % without adhesion	0–0.5	No	Smooth
		0–1.5	No	
COLL	Good adhesion	0–0.5	No	Wrinkled
		0–1.5	Yes	
COLL–LA	Good adhesion	0–0.5	No	Smooth
		0–1.5	Yes	
COLL–LA–GA	Good adhesion	0–0.5	No	Slightly wrinkled
		0–1.5	Yes	
BAP–COLL–PVA	Good adhesion	0–0.5	No	Very rough surface unsuitable for scratch test
		0–1.5	No	

revealed bad adhesion to glass slides before the scratch test easily visible by human eye and that is why most of the samples could not be tested. Quantification of adhesion of all tested films to glass slide is presented in Table 5. The worst adhesion, about 50 %, was observed for PVA10–LA films and PVA10 films were very easily peeled off the glass slide. The blends with PVA5 revealed better adhesion which was lost only near margins of glass slides for different inner structure. On the other hand, the best adhesion to glass was shown by COLL and its blends.

The existence of the scratch is a proof of plastic deformation and appearance and profile of the scratch reflects toughness and ductility of material. The scratches on all samples containing PVA10 and PVA5 did not show cracks or other failure and they looked smooth as is displayed in Fig. 13, for example PVA10–LA and PVA5–LA which point to plastic deformation as tensile tests revealed. Although PVA5 samples revealed better adhesion to glass before the test, it was lost during scratching but no connection to addition of additives or the used nominal force was observed. The appearance of loss of adhesion to glass for PVA5–LA–GA is depicted in Fig. 14a.

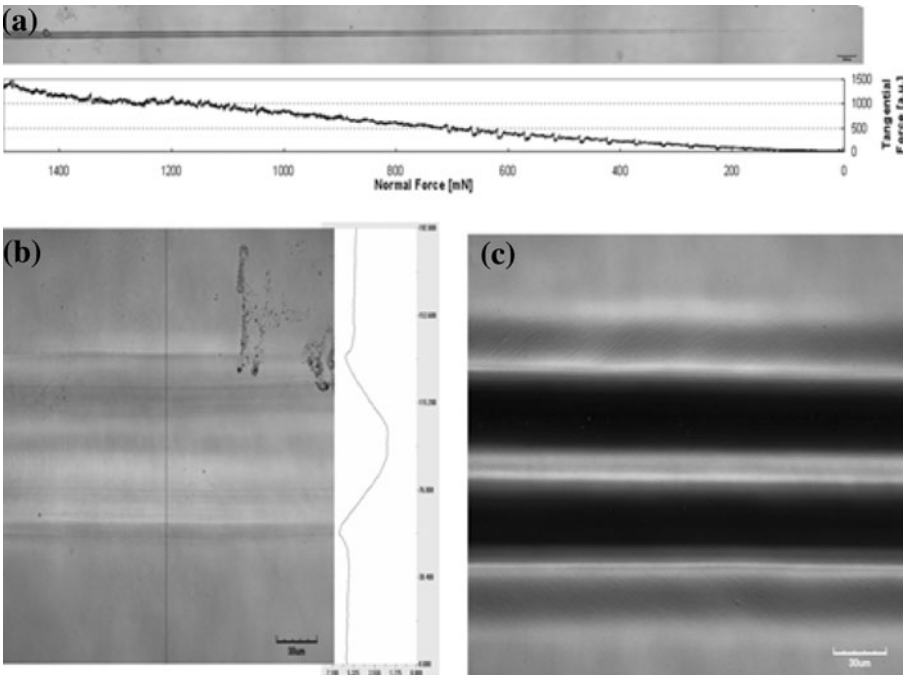

Fig. 13 Scratch image on **a** PVA10–LA film together with tangential force profile of scratching in normal force range 0–1.5 N (optical image; *scale bar* 320 μm), **b** detail of the **a** scratch image together with the height profile in a distance about 1.3 μm from the end of the scratch (CLSM image; *scale bar* 30 μm) and **c** CLSM image of scratch on PVA5–LA (*scale bar* 30 μm)

The scratches on COLL were not smooth but revealed fish-like appearance (Fig. 15a) because of its brittle character. The volume of cracks and the brittleness decreased with LA and LA–GA addition, respectively, as can be seen in Fig. 15b because tough materials were formed. The change of COLL structure was also accompanied by increasing loss of adhesion to glass along the scratch in the way LA and LA–GA. The image of the scratch and corresponding tangential force for COLL–LA–GA is shown in Fig. 14b. COLL and its blends were scratched through the film on glass slide using nominal force to 1.5 N because of brittle character together with PVA5–LA–GA (Table 5).

The bio-artificial films were not scratched through the films especially because of too high thickness and also because of high surface roughness as can be seen in Fig. 16a. The appearance of the scratch was without cracks but was as smooth as in case of PVA samples (Fig. 16b).

FTIR spectroscopy

The FTIR spectra of COLL, PVA5, PVA10 and both sides of BAF–COLL–PVA are shown in Fig. 17. As it was expected, PVA5 and PVA10 spectra differed only in the

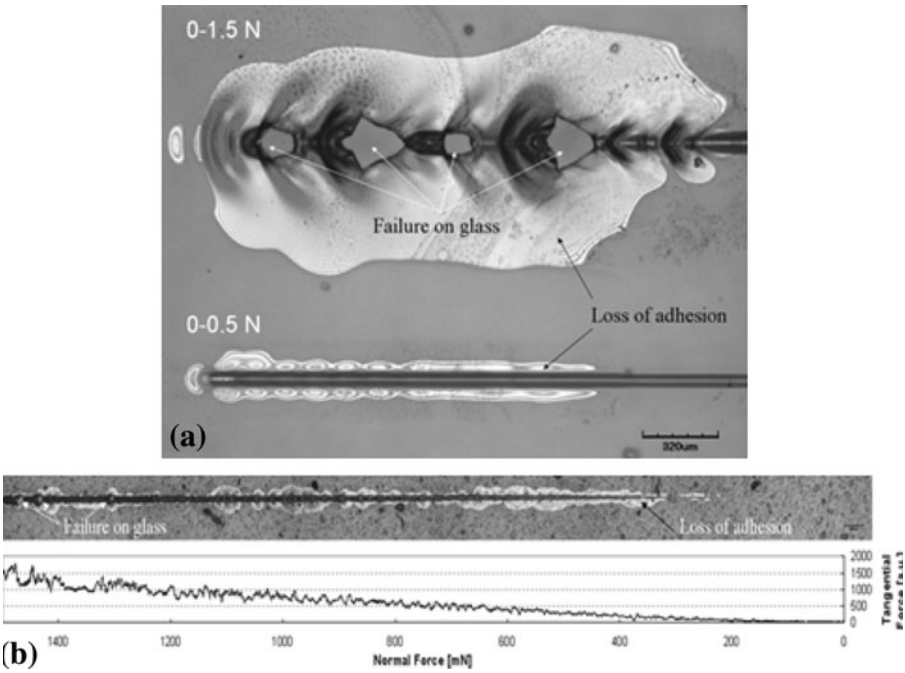

Fig. 14 Optical image of **a** the end of scratches on film PVA5–LA–GA performed in a force range 0–1.5 N (*upper*) and in the range 0–0.5 N (*bottom*); loss of adhesion between the film and glass slide is evident together with failure through the film on glass for the upper scratch, **b** the whole scratch on COLL–LA–GA with visible loss of adhesion along it and two spots of failure through the film together with tangential force profile performed under normal force range 0–1.5 N (*scale bar* 320 µm)

absorption intensity but the peaks were located almost at the same wavenumbers. Both the spectra exhibit a strong broad band centred at 3,245 cm^{-1}, belonging to OH groups. The band at 2,906 cm^{-1} presents saturated C–H stretching and the band at 1,428 cm^{-1} stretching of $–CH_2–$. The band at 1,700 cm^{-1} was assigned to acetate group. The strong peak at 1,068 cm^{-1} corresponds to C–O–H stretching and peak at 822 cm^{-1} to C–O stretching.

The COLL spectrum presents a set of overlapping strong bands above 3,000 cm^{-1}, which were associated to N–H and O–H stretching in various local hydrogen bonding environments. Low intensity band at 2,988 cm^{-1} was assigned to –CH stretching. The bands at 1,647 and 1543 cm^{-1} represent amide I and II, respectively, and the band at 1,428 cm^{-1} was assigned to –OH stretching. The band at 1,221 cm^{-1} was classified as N–H bending.

The spectra of both the sides of BAF–COLL–PVA differ in the region 2,000–1,500 cm^{-1}. One side revealed amide I and II bands while the other one did not show these bands, which means that one side consists of COLL and the other one of PVA (Fig. 17).

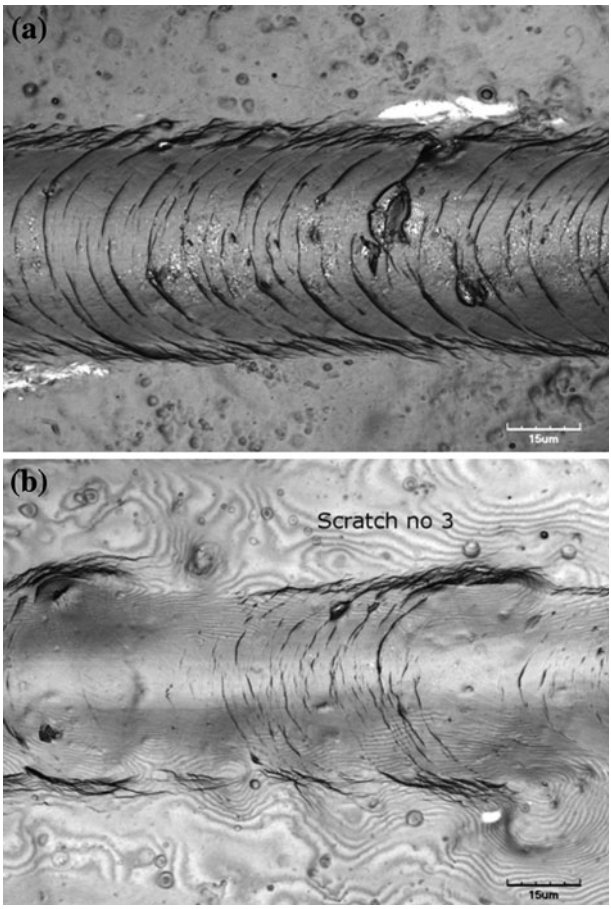

Fig. 15 CLSM images of scratches performed in a force range 0–0.5 N on **a** COLL film (1.3 mm from the scratch end) and on **b** COLL–LA–GA

Conclusion

The main goal of this research was to characterise new bio-artificial polymer material prepared from atelocollagen (COLL) and PVA, both blended with LA and GA. LA significantly plasticised both PVA and COLL. GA decreased crystallinity of PVA but viscoelastic and mechanical properties were very similar to those of neat PVA although tear resistance was better. It is supposed that inter crosslinking of small amount of GA occurred. The addition of both LA and GA revealed prevailing effect of LA in blends with PVA but plasticising effect of LA was multiplied by GA in COLL. The additives changed atelocollagen from brittle to tough material with yield stress while PVA changed from tough material with yield stress to tough material without yield stress with several times higher strain.

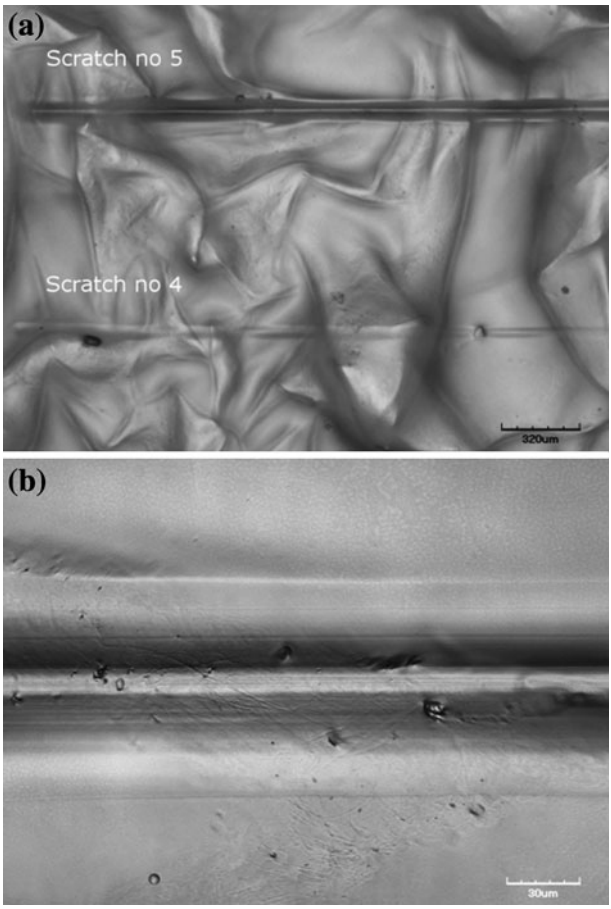

Fig. 16 Images of bio-artificial film: **a** optical image of the end of two scratches performed under normal force range 0–1.5 N (*upper*; *scale bar* 320 μm) and 0–0.5 N (*bottom*) and **b** detail of the upper scratch from **a** image (CLSM mode)

FTIR measurement proved that new bio-artificial polymer was double-sided without sharp interphase. It revealed the best tear resistance thanks to brittle character of COLL blend, half strength with respect to neat PVA and COLL and strain similar to neat PVA. It behaved like tough material with yield stress with good adhesion to glass. The latter was also good for COLL samples but not for PVA samples including the neat ones.

Both PVA and COLL blends and new bio-artificial material exhibited viscoelastic features useful for being used in contact with living organism. The bio-artificial material revealed properties suitable for prospective applications in medicine compared to neat synthetic and natural biocompatible polymers.

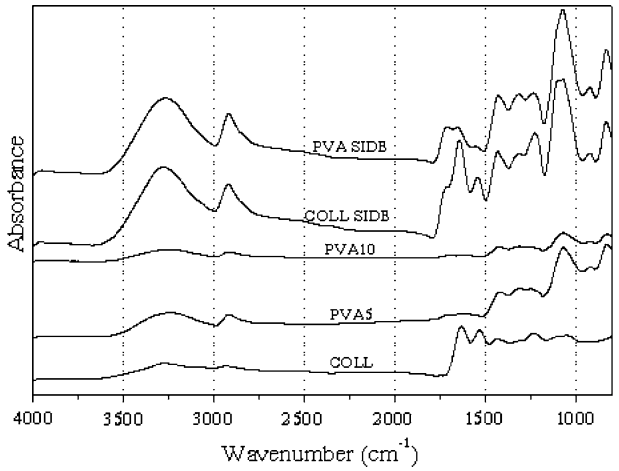

Fig. 17 FTIR–ATR spectra for COLL, PVA5, PVA10 and BAP–COLL–PVA

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References

1. Ikada Y, Tsuji H (2000) Biodegradable polyesters for medical and ecological applications. Macromol Rapid Commun 21:117–132. doi:10.1002/(SICI)1521-3927(20000201)21:3<117:AID-MARC117>3.0.CO;2-X
2. Barbucci R (2002) Integrated biomaterials science. Springer, New York
3. Wong JY, Bronzino JD (2007) Biomaterials. CRC Press, Boca Raton
4. Friess W (1998) Collagen—biomaterial for drug delivery. Eur J Pharm Biopharm 45:113–136. doi:10.1016/S0939-6411(98)00017-4
5. Lai G, Du Z, Li G (2007) The rheological behaviour of collagen dispersion/poly(vinyl alcohol) blends. Korea-Australia Rheology Journal 19:81–88
6. Silver F, Garg A (1997) Collagen: characterization, processing and medical applications. In: Domb A, Kost J, Wiserman D (eds) Handbook of biodegradable polymers. Part of the book series. Drug targeting and delivery, vol 4. Academic Publishers, Amsterdam, pp 319–346
7. Pourciel M, Launay J, Sant W, Conédéra V, Martinez A, Temple-Boyer P (2003) Development of photo-polymerisable polyvinyl alcohol for biotechnological applications. Sens Actuators B 94:330–336. doi:10.1016/S0925-4005(03)00463-5
8. Sarti B, Scandola M (1995) Viscoelastic and thermal properties of collagen/poly(vinyl alcohol) blends. Biomaterials 16:785–792. doi:10.1016/0142-9612(95)99641-X
9. Sionkowska A, Planecka A, Kozlowska J, Skopinska-Wisniewska J (2009) Collagen fibril formation in poly(vinyl alcohol) and poly(vinyl pyrrolidone) films. J Mol Liq 144:71–74. doi:10.1016/j.molliq.2008.09.007
10. Sionkowska A (2003) Interaction of collagen and poly(vinyl pyrrolidone) in blends. Eur Polym J 39:2135–2140. doi:10.1016/S0014-3057(03)00161-7

11. Alexy P, Bakos D, Hanzelova S, Kukolikova L, Kupec J, Charvatova P, Chiellini E, Cinelli P (2003) Poly(vinyl alcohol) collagen hydrolysate thermoplastic blends: I. Experimental design optimisation and biodegradation behaviour. Polym Test 22:801–809. doi:10.1016/S0142-9418(03)00016-3
12. Sionkowska A, Wisniewsky M, Kaczmarek H, Skopinska J, Chevallier P, Mantovani D, Lazare S, Tokarev V (2006) The influence of UV irradiation on surface composition of collagen/PVP blended films. Appl Surf Sci 253:1970–1977. doi:10.1016/j.apsusc.2006.03.048
13. Sionkowska A, Kaczmarek H, Wisniewski M, Kowalonek J, Skopinska J (2004) Surface characteristics of UV-irradiated collagen/PVP blended films. Surface Science 566–568 Part 1:608–612. doi:10.1016/j.susc.2004.05.114
14. Sionkowska A, Kozlowska J, Planecka A, Skopinska-Wisniewska J (2008) Collagen fibrils in UV irradiated poly(vinyl pyrrolidone) films. Appl Surf Sci 255:2030–2039. doi:10.1016/j.apsusc.2008.06.200
15. Sionkowska A, Kozlowska J, Planecka A, Skopinska-Wisniewska J (2008) Photochemical stability of poly(vinyl pyrrolidone) in the presence of collagen. Polym Degrad Stab 93:2127–2132. doi:10.1016/j.polymdegradstab.2008.08.010
16. Alexy P, Bakos D, Crkonova G, Kramarova Z, Hoffman J, Julinova M, Chiellini E, Cinelli P (2003) Poly(vinyl alcohol) collagen hydrolysate thermoplastic blends: II. Water penetration and biodegradability of melt extruded films. Polym Test 22:811–818. doi:10.1016/S0142-9418(03)00015-1
17. Alexi P, Bakos D, Crkonova G, Kolomaznik K, Krsiak M (2001) Blends of polyvinyl alcohol with collagen hydrolysate: thermal degradation and processing properties. Macromol Symp 170:41–49. doi:10.1002/1521-3900(200106)170:1<41:AID-MASY41>3.0.CO;2-B
18. Scotchford CA, Cascone MG, Downes S, Guisti P (1998) Osteoblast response to collagen–PVA bioartificial polymers in vitro: the effects of crosslinking method and collagen content. Biomaterials 19:1–11. doi:10.1016/S0142-9612(97)00236-6
19. Fambri L, Migliaresi C, Kesenci K, Piskin E (2002) Biodegradables polymers. In: Barbucci R (ed) Integrated biomaterial science. Kluwer Academic Publishers, New York, pp 119–187
20. Cascone M (1997) Dynamic–mechanical properties of bioartificial polymeric materials. Polym Int 43:55–69. doi:10.1002/(SICI)1097-0126(199705)43:1<55:AID-PI762>3.0.CO;2-#
21. Mano V, Ribeiro E, Silva M (2007) Bioartificial polymeric materials based on collagen and poly (*N*-isopropylacrylamide). Mater Res 10:165–170. doi:10.1590/S1516-14392007000200012
22. Sperling LH (2006) Introduction to physical polymer science. Wiley, Hoboken, NJ, USA
23. Kadler K, Holmes D, Trotter J, Chapman J (1996) Review article. Collagen fibril formation. Biochem J 316:1–11
24. Cervantes C, Olaya E, Testas M, Garcia-Lopez N, Coste G, Arrellin G, Luna A, Krotzsch E (2003) Collagen–PVP, a collagen synthesis modulator, decreases intraperitoneal adhesions. J Surg Res 110:207–210. doi:10.1016/S0022-4804(02)00099-9
25. Prikryl R, Salyk O, Kripal L, Cech V (2002) New scratch tester developed for plasma polymer characterization. Czechoslov J Phys 52:D824–D828
26. Sedlarik V, Saha N, Kuritka I, Saha P (2006) Preparation and characterization of poly(vinyl alcohol)/lactic acid compounded polymeric films. Int J Polym Anal Charact 11:253–270. doi:10.1080/10236660600750190
27. Park JS, Park JW, Ruckenstein E (2001) On the viscoelastic properties of poly(vinyl alcohol) and chemically crosslinked poly(vinyl alcohol). J Appl Polym Sci 82:1816–1823. doi:10.1002/app.2023
28. Yeom ChK, Lee KH (1996) Pervaporation separation of water–acetic acid mixtures through poly (vinyl alcohol) membranes crosslinked with glutaraldehyde. J Membr Sci 109:257–265. doi:10.1016/0376-7388(95)00196-4
29. Holt B, Tripathi A, Morgan J (2008) Viscoelastic response of human skin to low magnitude physiologically relevant shear. J Biomech 41:2689–2695. doi:10.1016/j.jbiomech.2008.06.008
30. Collard JM, Romagnoli R (2001) Human stomach has a recordable mechanical activity at a rate of about three cycles/minute. Eur J Surg 167:188–194. doi:10.1080/110241501750099357
31. Menard K (1999) Dynamic mechanical analysis: a practical introduction. CRC Press, Boca Raton, FL, USA