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ADVANCED PACKAGING FOR FOOD AND PHARMACEUTICAL APPLICATIONS BASED ON WATER-SOLUBLE POLYMER

DOCTORAL THESIS

Program:	P2901 Chemistry and food technology
Course:	2901V013 Food technology
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ABSTRACT

The presented thesis summarizes the current state of the art of the polymeric materials used for preparation of advanced packaging. The essential part of the thesis is dedicated to microencapsulation, the method of special packaging preparation that finds application in various fields, such as food and pharmaceutical industries, agriculture and/or biotechnology. The experimental part of the thesis is focused on detailed description of the interactions between water-soluble poly (vinyl alcohol) (PVA) and lactic acid (LA), which is a relatively promising compound from both economical and ecological point of view. Concretely, the influence of PVA hydrolysis degree and LA concentration on the resulting properties of the cast polymer films are the subjects of the systematic study. The effect of the factors mentioned above on the ability to form 3D polymer network by using dialdehyde is described subsequently. The knowledge obtained in this study was applied in the preparation process of the microcapsules based on lipophylic core containing β-carotene-shell material based on water-soluble polymer by the method of simple coacervation. The prepared systems were characterized and the effects of the above-mentioned parameters on the quality and stability of the microcapsules under various conditions were evaluated. The results reveal a positive effect of the high hydrolysis degree as well as LA presence on the encapsulation efficiency, thermal stability at temperature 70 °C, low pH and some of the organic solvents of the microcapsules.

Keywords: water-soluble polymer, cast film, microencapsulation, coacervation, poly(vinyl alcohol), crosslinking, glutaraldehyde, lactic acid (LA) and β-carotene, stability

ABSTRAKT

Předkládaná práce přináší přehled do problematiky použití polymerních materiálů pro přípravu pokročilých obalů. Podstatná část této práce se věnuje mikroenkapsulaci, jako techniky využívané pro speciální obalové aplikace pro různé aplikace včetně potravinářských výrob, farmacie, zemědělství či biotechnologie. Experimentální část se zaměřuje na detailní popis interakcí vodorozpustného polyvinylalkoholu (PVA) s kyselinou mléčnou (LA), jakožto perspektivní látkou z hlediska ekonomického i ekologického. Konkrétně je zde sledován vliv stupně hydrolýzy PVA a koncentrace LA na výsledné vlastnosti odlévaných polymerních filmů. Následně je popsán vliv výše zmíněných faktorů na schopnost tvorby polymerních 3D struktur pomocí dialdehydického síťovadla. Získané parametry byly aplikovány jako výchozí poznatky pro přípravu mikrokapslí na bázi lipofilního jádra obsahujícího β-karoten-obal na bázi vodorozpustného polymeru pomocí metody jednoduché koacervace. Připravené systémy byly charakterizovány a zároveň byl vyhodnocen vliv výše zmiňovaných faktorů na kvalitu a stabilitu mikrokapsle v různých podmínkách. Výsledky ukazují pozitivní vliv vysokého stupně hydrolýzy a přítomnosti LA na vlastnosti připravených mikročástic z hlediska efektivity enkapsulace a odolnosti vůči teplotám nad 70 °C, nízkému pH i některým organickým rozpouštědlům.

Klíčová slova: vodorozpustný polymer, odlévaný film, mikroenkapsulace, koacervace, polyvinylalkohol, síťování, glutaraldehyd, kyselina mléčná, β-karoten, stabilita

INTRODUCTION

Packaging can be defined as a technology of enclosing products for carrying from producer to user which is involved in protection, preservation, containment, convenience, compliance and confidence. Among these products, food and pharmaceuticals hold a place of special importance due to their principal chemical instability. On the other hand, since they are consumed to maintain life, safety aspect is a critical dimension of their packaging requirements. In general, packaging materials authorized for use in contact with food are acceptable for pharmaceuticals. Food and pharmaceuticals may undergo loss in quality due to failure of the package and product package interaction. The package failure may be caused by the loss of properties (e.g. structural and mechanical strength, integrity) and improper use or selection of packaging materials [1-3]. Generally, the materials used for food and pharmaceutical packaging consist of a variety of materials such as polymers, glass, metals, papers and board, or combination thereof. Among them, polymers are the most versatile materials whose properties and functionalities can be easily manipulated. Due to these advantages, polymers offer a wide range of properties from soft gels to extremely strong fibres that can fulfil all the packaging requirements.

Food and pharmaceutical packaging have been traditionally defined as a passive barrier to delay the adverse effect of the environment on the contained product. In other words, the main key for these traditional materials in contact with foods is to be as inert as possible, i.e., there should be a minimum of interaction between product and packaging [4]. Thus, most of the research was focused on the adverse effect of such interactions, but more recently there has been growing interest in development of packaging materials that interact deliberately with the environment and with the product, playing an active role in reservation such as active packaging as well as intelligent packaging. The active

systems aim shelf life extension of the product by keeping or improving its quality, while the purpose of intelligent systems is to give an indication and to monitor the freshness of the product.

To use the package as a delivery system is a new generation of active packaging that can release active compounds (e.g. antimicrobials, antioxidants, enzymes, flavours, nutraceuticals and drug) at different controlled rates [5]. This can be achieved by microencapsulation defined as a process by which tiny particles of solid, liquid, or gaseous active ingredients are packed in a continuous polymeric material with the purpose of protection, controlled release and compatibility of the core materials. These microcapsules may be ranged between 0.2 and 5.000 μm in size and can be produced by using numerous techniques including coacervation (phase separation), spray drying, and surface polymerization [6].

Convenient properties of microencapsulation make it technologically important and very attractive for many applications. This technique offers very promising applications in a wide array of biotechnology, biomedical field, micro/ nanotechnology and food industries such as controlling of the release of active agents (e.g. drugs, vitamins, and food supplements), converting liquids into solids separating reactive compounds, providing environmental protection (e.g. heat, humidity and pressure), improved material handling properties (e.g. toxic materials).

1. THEORETICAL PART

1.1 POLYMERS IN FOOD AND PHARMACEUTICAL PACKAGING

Polymers have the potential to impact many aspects of food and pharmaceutical systems. Both natural and synthetic polymers can be used in food (as packaging materials and in the construction of product processing plant and equipment) and pharmaceutical industries (as excipients, drug delivery systems, bandage, suture or packaging).

The use of polymers as packaging materials has increased enormously during the past decades due to their light weight, flexibility, transparency, availability in a wide range of packaging structures, shapes, and designs which influence products cost effectively and conveniently. For instance, thermoplastics have found widespread use for packaging because of their biological inertness and hydrophobicity. Polymers for food and pharmaceutical packaging can exist not only in the form of containers, container components and flexible packaging but they are also edible films, coatings and laminations [7, 8]. The major groups of polymers used in food/pharmaceutical packaging applications (along two scales: their sources and degradability) are shown in Figure 1.

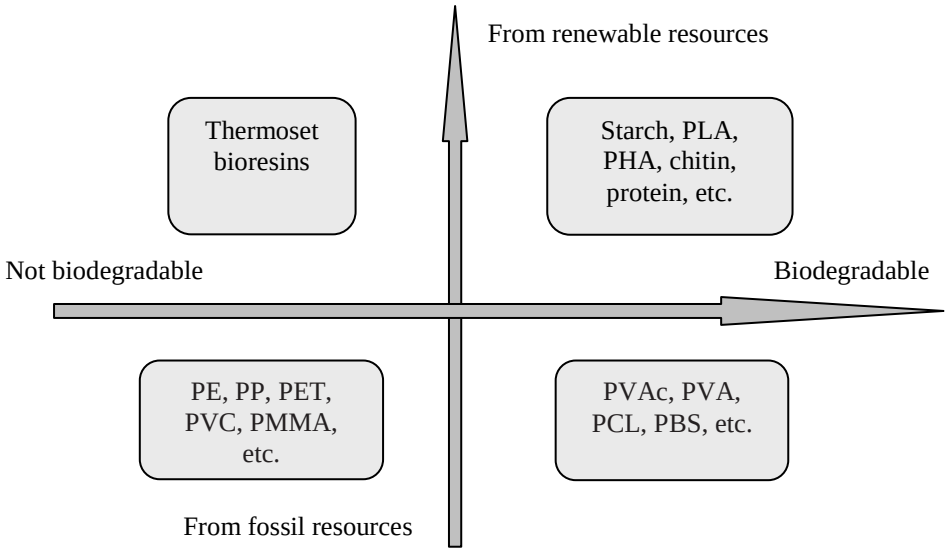


Figure1: Polymers used in food/ pharmaceutical packaging

Up to now, polymers corresponding to non-biodegradable polymers from fossil resources, have been increasingly used as food/ pharmaceutical packaging materials due to the large availability at relatively low cost and good mechanical performance (e.g. tensile and tear strength, good barrier to gases, anhydride and aroma compounds, heat sealability) [1, 9, 10]. However, recently their use has to be limited because they are not biodegradable and pose serious ecological problems. Polyvinyl acetate (PVAc), ethylene vinyl alcohol (EVON), poly(vinyl) alcohol (PVA) and water soluble polymers placed on biodegradable polymers from fossil resources, are commonly used for packaging applications. For example, EVON films provide excellent oxygen barriers in dry applications, and co-extruded biaxially oriented films containing this are widely applied for processed meat, cheese as well as some dry food packaging. The top left quarter of the figure comprises thermosetting bioderived resins that typically do not feature in packaging applications, while the top right quarter corresponding to "biodegradable" polymers from "renewable sources" includes the polymers with the most challenging applications in food/pharmaceutical packaging [11].

In recent years smart polymers (stimuli - responsive polymers) that can respond to a wide range of stimuli, including temperature, pressure, pH, gases, liquids and biological indicators, have attracted great interest both in science and technology. They offer new opportunities for food industry in the separation, analyses, selective removal of undesirable components and food/ pharmaceutical packaging. For instance, smart polymeric materials that are sensitive to the partial pressure of different gases provide optimum "breathability" to a pack extending the shelf life of food products such as fresh salads and cut vegetables. They have also very promising applications in the biomedical field as delivery systems of therapeutic agents, tissue engineering scaffolds, cell culture supports, bioseparation devices, sensors or actuators systems [12-14].

1.1.1 Key properties of polymeric materials used in packaging

The properties to be considered in relation to food/ pharmaceutical packaging materials may include gas and water vapour permeability, sealing capability, thermoforming properties, resistance (towards water, grease, UV light, etc.), transparency, anti-fogging capacity, printability, availability and, of course, costs [1, 8, 11]. Moreover, biodegradability of packaging materials must also be taken into account.

Barrier properties

All plastics are to some degree permeable to gases (O_2, CO_2, N_2, ethylene, etc.), water vapour, aromas and light compounds in comparison with glass and metal packaging materials. Owing to this, the interaction between food/ pharmaceutical and packaging, moisture and aroma loss or uptake, reaction with oxygen as well as the growth of aerobic microorganisms can occur and it would seriously reduce their quality and shelf life [8, 15, 16]. Therefore, a good barrier plastic must be taken into account in the packaging to protect them from transmission of the above mentioned compounds and to sustain their freshness and overall quality during storage. Thus, within the last few decades, many important works have been done on barrier polymers. Consequently, several high barrier polymers such as polyvinyl alcohol (PVA), EVON copolymers, polyacrylonitrile (PAN), polyvinylidene chloride (PVdC), polyethylene naphthalate (PEN), oriented polypropylene (OPP) and polyamide 6 (PA6) have been developed.

Figure 2 schematically shows the current barrier property requirements that packaging and encapsulating materials have to fulfil for a variety of products.

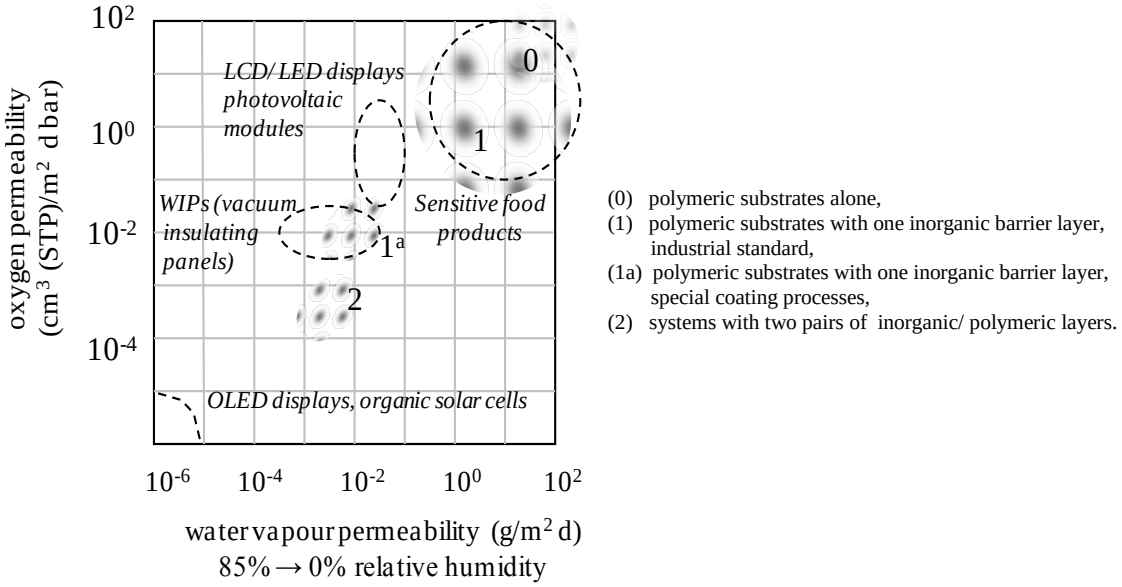


Figure 2: Barrier properties as required for different product sectors (dotted circles) and performance of the following encapsulation/ packaging materials (shaded areas) [17]

As mentioned before, polymers can provide an attractive balance of properties such as flexibility, transparency, toughness, low weight, and ease of processing. However, the permeabilities of favourably priced commodity polymers (for food packaging) and also more expensive specialty polymers (for encapsulation of technical devices) to water vapour, oxygen, and other substances are far too high for most applications. Figure 3 presents oxygen and water vapour permeabilities of commodity polymers that are currently used for food/ pharmaceutical packaging applications. It should be mentioned that gas barrier properties of hydrophilic polymers are considerably dependent on the humidity. For instance, excellent gas barrier properties of dry PVA significantly decreased in the presence of moisture [8].

Moreover, water and oxygen permeabilities have essential implications in the consideration of polymer films for food/ pharmaceutical packaging and many polymers such as PP, PVC, PET, polyolefins (PE) have been used for this purpose [1, 8,11, 18].

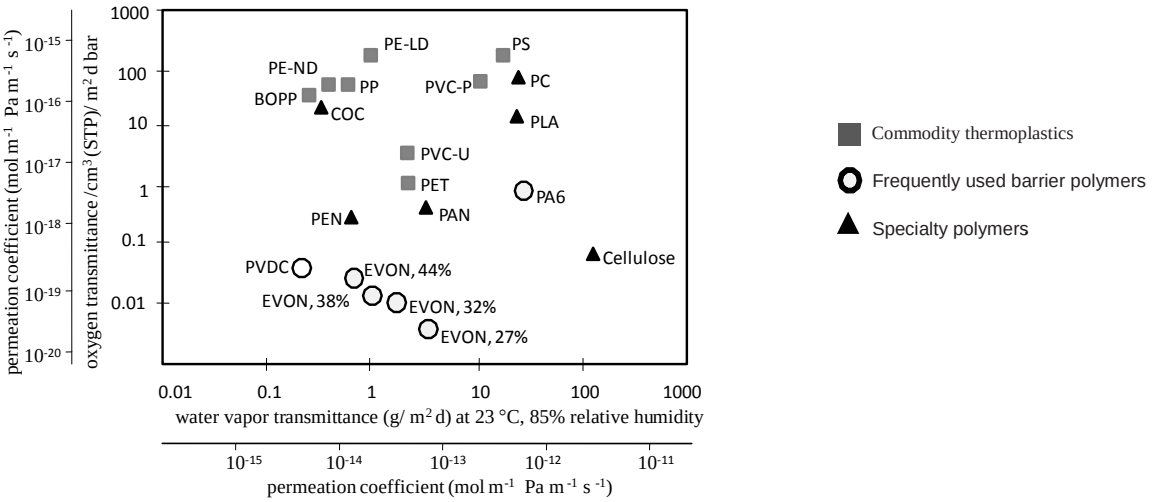


Figure 3: Transmittance (i.e. permeability normalized to 100 μm material thickness) for oxygen and water vapour, for typical packaging polymers, at 23°C. Additionalscales are shown for permeation coefficient in SI units [17]

As evidenced in the literature, to use multi-layers of different films performs the promising approach to obtain high barrier films with required properties, in particular for food packaging [19].

According to literature review, edible films and coatings can act as barrier to control the transfer of moisture, gases, lipids and aroma compounds. For example, numerous proteins (e.g. collagen, gelatin, casein, whey proteins, and corn zein and soy protein) used to produce films are generally excellent barriers to the transport of gases but moderate barriers to the transport of moisture [20]. Their water vapour barrier ability is limited by the inherent hydrophilic nature of proteins. Water vapour permeability (WVP) can be directly related to the quantity of (-ON) group in the molecule [21] and to the environmental conditions [22]. In general, a high relative humidity (90% RH) and low (−30 °C) storage temperature improve WVP. For example, increasing the relative humidity gradient at a constant temperature increased transfer of moisture through films based on hydroxypropyl methylcellulose, stearic and palmitic acids.

Main parameters commonly used in film mass transport are permeability, diffusivity and solubility. From Fick's (*which states that the amount of gas*

passing perpendicularly through unit surface against unit times is proportional to the concentration gradient) and Henri's *(which states that the amount of gas dissolved in a given mass (of plastic) is directly proportional to the partial pressure applied by the gas)* laws permeability is directly related to diffusivity and solubility of the gas or water vapour in the polymer film. Their relationships can be described according to the following equation [23].

$$P = DS \qquad (1)$$

where, P is permeability (mol·m/N·s); D is diffusivity ($m^2 \cdot s^{-1}$) and S is solubility (mol/N·m), respectively.

Mechanical properties

Mechanical properties as well as barrier properties are important for packaging materials. Among the many mechanical properties of plastic materials, tensile properties (e.g. yield strength, tensile strength, modulus of elasticity [Young's modulus], and elongation at break) are the most commonly considered and offer an indication of expected film integrity under conditions of stress that would occur during processing, handling and storage.

The maximum yield strength is a very important property of the packaging films defined by the maximum tensile stress and it gives information on the maximum allowable load before plastic deformation occurs.

Tensile strength is the ultimate strength which is the maximum stress applied at the point at which the packaging film breaks, while strain expresses the maximum change in the length of the film before breaking. Elastic modulus is defined by the ratio of applied stress to the corresponding strain in the region of linear elastic deformation and can be regarded as an index of stiffness and rigidity of a packaging film.

It should be emphasized that there are many factors that can affect mechanical properties, such as additives, plasticizers and environment (e.g. temperature and relative humidity). For instance, as the content of plasticizer increases, tensile strength decreases and elongation increases. In addition, humidity acts as a plasticizer, increasing elongation and decreasing strength, while as the temperature increases, Young's modulus and ultimate tensile strength and elongation at break decreases. Molecular weight also influences on the mechanical properties that, as molecular weight increases, the strength of the film tends to increase [11, 24, 25].

Biodegradability

Biodegradable packaging materials can be defined as materials derived from renewable sources [26]. They may be classified into three main categories depending on their origin and production (Figure 4).

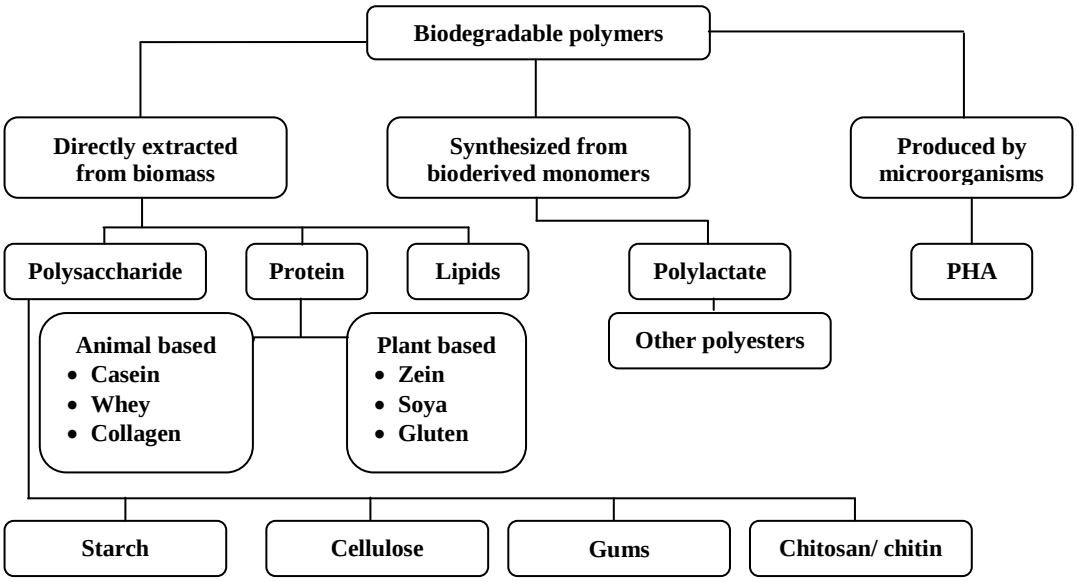


Figure 4: Schematic presentation of biobased polymers based on their origin and method of production

Biodegradable polymers, either synthetic or natural, can be used for food/ pharmaceutical packaging including edible films, coating and controlled delivery systems and have promising application in these area. For example, numerous drug delivery systems have been based on proteins (e.g. collagen and gelatin) and polysaccharides (e.g. starch and chitosan) because of their biocompatibility and biodegradability [27]. However, non-biodegradable polymers such as polyethylene terephthalate (PET) and polystyrene (PS) are still widely used for such purposes. The main problems related to renewable biopolymers are performance, processing and cost. Among them, performance and processing are more involved with polymers extracted directly from biomass, such as cellulose, starch and proteins. In contrast, polymers belonging to synthesized from bioderived monomers and produced by microorganisms generally perform very well and are easily processed using standard plastics techniques, but they tend to be expensive [28].

1.1.2 Interactions between plastic packaging materials and products

Most of the food and pharmaceutical products can interact to some degree with their packaging. The term “interaction“ encompasses the sum of all mass transport between food, its packaging and the environment which can change the composition, quality and physical-chemical characteristics of the food and packaging. Generally, food/pharmaceutical and plastic packaging interactions can be divided into three groups according to the direction of the mass transport [2].

- Migration
- Absorption/scalping
- Permeation

In the migration, plastic packaging components transfer into food, while in the scalping, food components transfer to the packaging. Permeation is referred to the transport of components through the package in both directions. The migration has become main concern in the selection and use of materials for food and pharmaceutical packaging due to the possible effect upon human health. The main mechanism of the migration of substances such as additives and monomers (e.g. plasticizers, antioxidants, light and thermal stabilizers, antistatic agents as well as slip additives) is the diffusion described by Fick's law [29]. Monomers are reactive substances with respect to living organisms, thus potentially toxic. Vinyl chloride monomer can be mentioned as an example of this and its levels in PVC packaging materials are strongly controlled [30]. Figure 5 shows possible interactions between food, polymer film and the environment as well as their adverse consequences. The degree of the interactions depends on the contact area between product and packaging material, contact time, food and pharmaceutical composition, concentration of migrant, storage temperature, polymer morphology and polarity of polymeric packaging materials [31].

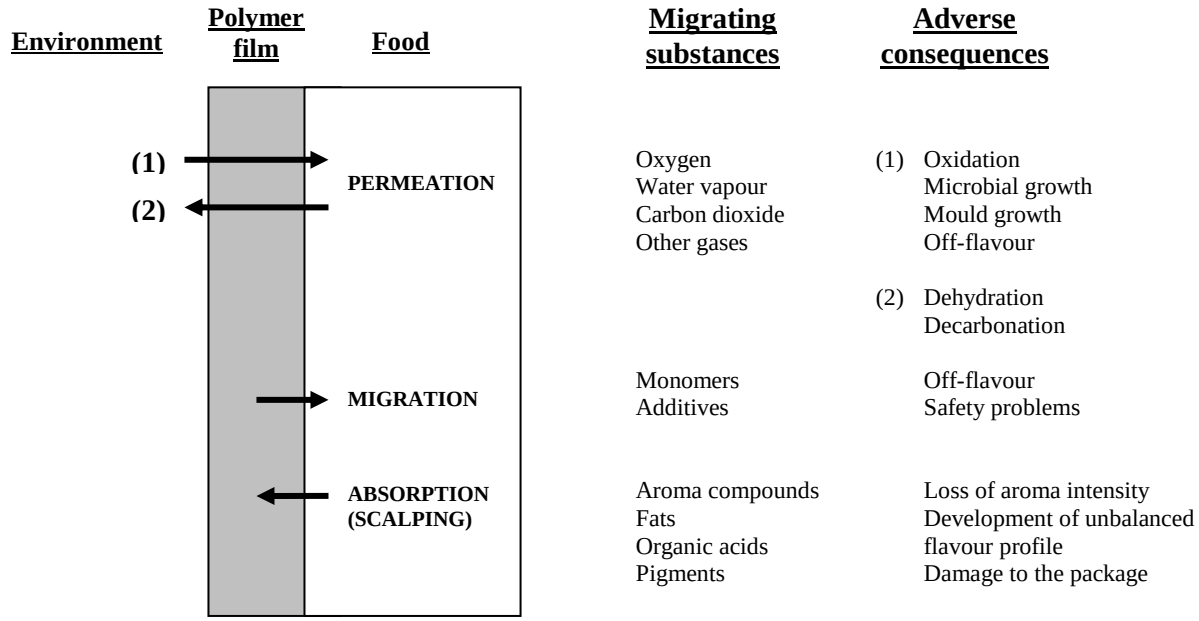


Figure 5: Possible interactions between food, polymer film and the environment [30].

Previously, mass transfer of polymer packaging systems was mostly studied focusing on its adverse effect, but recently there has been growing interest in the interaction among product, packaging materials and environment in a positive way to get an active role in preservation which would not be practical for competitive materials such as glass and metal. Examples of this are using the materials as a delivery system to release active compounds such as antimicrobials, antioxidants and enzymes and using selective permeable films to control the permeation of gases and water vapour as well as selective absorption of undesirable aromas.

1.1.3 Plastic packaging technologies

There are many plastic packaging innovations in food/ pharmaceutical industry including nanotechnology, active packaging, bioactive packaging, modified atmosphere packaging, intelligent packaging as well as edible films and coatings. The scientific literature contains numerous reports on the applications of these packaging concepts. Examples from different methods are given here.

Active Packaging (AP)

Nowadays, AP, in which the product, packaging and the environment interact *intentionally* to extend shelf life, is intensively applied for food and pharmaceutical packaging. It is related to the incorporation of active agents such as scavengers, antioxidants, antimicrobial agents, odour removers into plastic packaging material or onto its surface, in multilayer structures or in particular elements associated with the packaging (labels, sachets etc.). Their active functions may include scavenging oxygen, carbon dioxide or ethylene, controlling microbial growth and moisture migration, absorbing odour taints, releasing ethanol, preservatives (antimicrobial and antioxidant compounds *etc.*)

and flavour/ odour as well as maintaining temperature control [32-35]. In addition, *controlled release packaging* (CRP) that can release active compounds at different controlled rates to increase the quality and safety of a wide range of foods during extended storage, is one of the most challenging [5]. Many CRPs, such as controlled release of drug delivery and antioxidant and antimicrobial agents, have been used in food and pharmaceutical packaging.

Modified Atmosphere Packaging (MAP)

MAP can be defined as the enclosure of food products in a high barrier film in which the gaseous environment has been changed or modified to control respiration rates, reduce microbiological growth, or retard enzymatic spoilage with the intent of extending shelf-life. Oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2) are the main gases used in MAP and the choice of gas is absolutely depended on their properties and the food being packed. For example, MAP with gas combination of 70-80% O_2 to ensure the red oxymyoglobin colour and 20-30% CO_2 to inhibit microbial growth is mainly applied for fresh red meats [33]. In some cases, additional gases are used in combination with the above mentioned gases such as carbon monoxide (CO), sulfur dioxide (SO_2) to inhibit or control the growth of bacteria and moulds [36].

Equilibrium Modified Atmosphere Packaging consisting of the lowered level of O_2 and heightened level of CO_2 is the most commonly used MAP technology. This kind of package slows down the normal respiration of the product (e.g. fruits and vegetables) and extends their shelf life.

Intelligent Packaging (IP)

AP is more responsible for taking some action as mentioned above, while IP is responsible for sensing and providing information about the function and properties of packaged food such as pack integrity tamper evidence, food safety

and quality. The intelligent devices (e.g. time-temperature indicators, gas sensing dyes, microbial growth indicators, physical shock indicators and numerous tamper proofs) can be integrated in package materials or attached to the inside or outside of a package [4, 19, 37].

1.2 Bioactive packaging (BP)

1.2.1 Migratory and non-migratory bioactive packaging

Bioactive packaging refers to a packaging material that has been modified by bioactive components. Generally, it can be classified into two main groups (Figure 6) according to their action [4].

- Non-migratory bioactive packaging (NMBP)
- Migratory bioactive packaging (MBP)

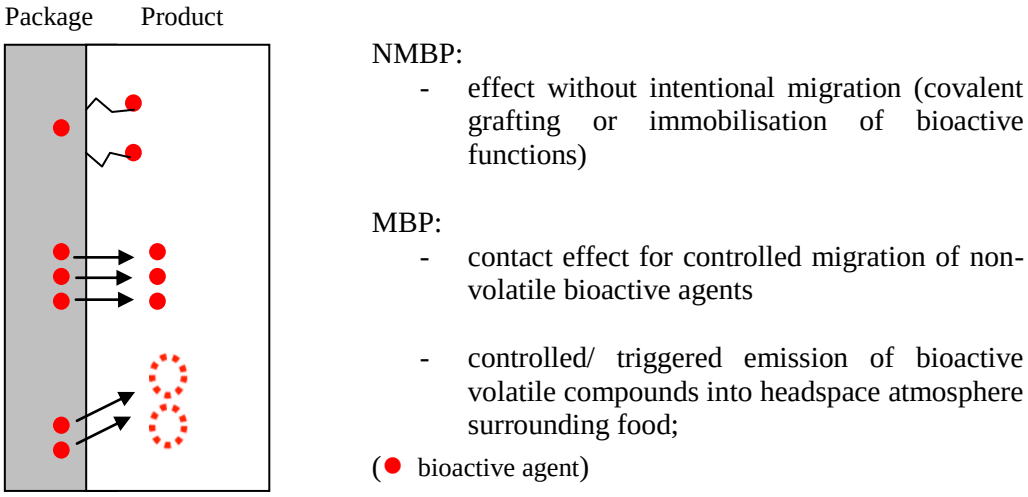


Figure 6: Scheme of two different types of bioactive food contact materials classified as a function of intentional or unintentional migrations

NMBP possess biological activity without the active components migrating out of the polymer into the packaged goods while MBP releases its bioactive component into the surrounding medium.

Bioactive compounds that catalyze or elicit a specific response whiten a given biological systems, can either be natural or synthetic [34]. Enzyme,

peptide, polysaccharide, phospholipid analog, antibody, polyethylene glycol, oligonucleotide and antimicrobial agent are the most common types of bioactive compounds intended for a variety of applications including food and pharmaceutical packaging and equipment as well as biomedical devices.

NMBP can be formed by immobilizing (through covalent attachment) of bioactive molecules to the polymer backbone or may result from an inherent bioactive effect of the polymer structure, as with chitosan [38].

There are three major methods of immobilizing a bioactive compound to a polymeric surface (Figure 7).

- Adsorption via electrostatic interactions
- Ligand-receptor pairing
- Covalent attachment

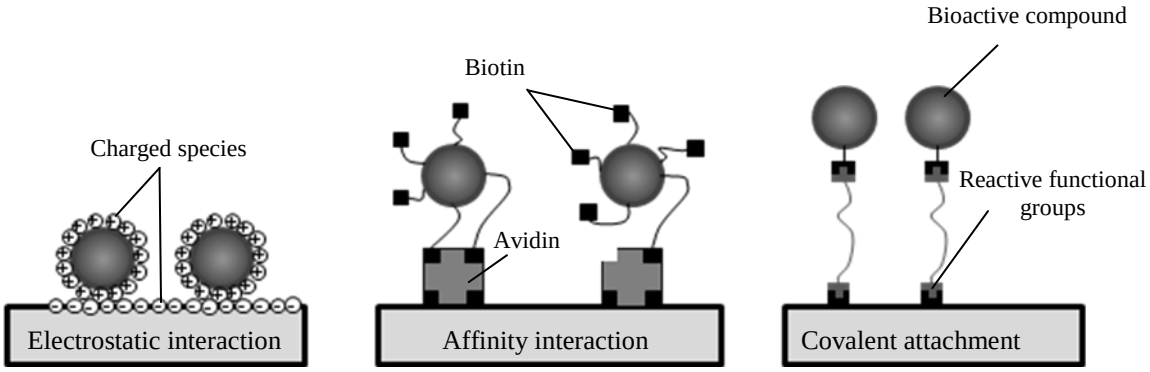


Figure 7: Mechanisms of immobilization [39]

Non-covalent adsorption is sometimes desirable, as in certain drug delivery applications and regenerable antimicrobial textiles. The biotin–avidin interaction is the strongest reported non-covalent bond, with an unbinding force of up to 250pN. It is attractive in surface bioconjugations because of the number of biotinylated reagents available. Among these methods, covalent immobilizations offer several advantages by providing the most stable bond between the compound and the functionalized polymer surface. In the case of active food packaging applications, a covalent linkage ensures that the bioactive compound

will not migrate to the food and thus may offer the regulatory advantage of not requiring approval as a food additive [39].

MBP releases its bioactive agents (non-volatile) by diffusion through the polymer matrix (packaging). Therefore, the diffusion coefficient (diffusivity), D represents a kinetic property of the polymer-permeant system. Figure 8 shows the activated diffusion process through the packaging polymer films.

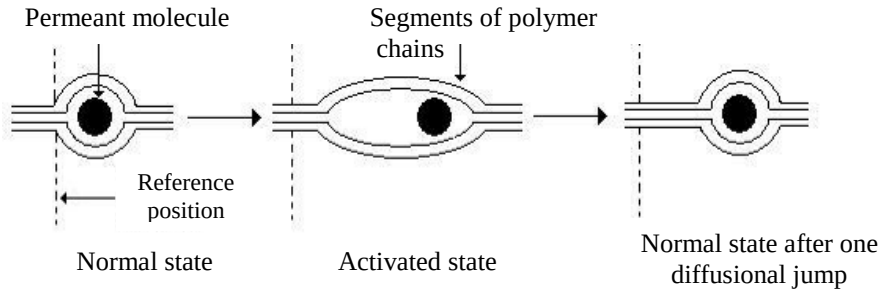


Figure 8: The activation process for diffusion [17]

Activated diffusion is described as the opening of a void space among a series of segments of polymer chain due to oscillations of the segments. Then, this active state is followed by translational motion of the permeant within the void space before the segments return to their normal state. Both active and normal states are long-lived, as compared with the translational motion of the permeant. Factors affecting the structure of a polymer have a direct effect on segmental mobility, and therefore, influence its mass transport properties [17, 40]. The diffusion can be described by Fick's first and second law of diffusion written as equations (2) and (3) for one-dimensional diffusion [23, 29, 40].

$$J = -D\left(\frac{\partial C}{\partial \chi}\right) \qquad (2)$$

$$\left(\frac{\partial C}{\partial \tau}\right) = -D\left(\frac{\partial^2 C}{\partial \chi^2}\right) \qquad (3)$$

where, J is the diffusive mass transfer rate of permeant per unit area, C is the concentration of permeant, τ is time, x is the length and D is the diffusion coefficient.

The mass transfer of a volatile bioactive agent in the packaging system is balanced more dynamically. Their release rate from the packaging system is considerably dependent on the volatility relating to the chemical interaction between the volatile agent and the packaging materials [32, 34].

1.2.2 Antimicrobial packaging

The quest for hygienic living conditions leads to a great attention to developments of non-toxic anti-infective packaging materials using a variety of antimicrobial substances. Antimicrobial packaging is the most promising bioactive packaging that controls conditions either inside the food or in the package headspace actively and responsively [41, 42]. It must extend the lag phase and reduce the log phase of microorganisms in order to extend shelf life and to maintain product quality and safety. Depending on an intended use, antibacterial packaging can be imparted by several ways [41-45].

- ***Addition of sachets or pads containing volatile antimicrobial agents into packages:*** Oxygen and moisture absorbers as well as ethanol vapour generators are the main types of sachets used commercially. Oxygen-scavenging packaging possesses indirect antimicrobial properties against aerobic micro-organisms, particularly moulds by reducing headspace oxygen in the package. Moisture absorbers reduce water activity and also indirectly affect microbial growth on the food [42]. Both of them are used especially in bakery, pasta and meat packaging to prevent oxidation and water condensation. In the case of ethanol generators, they are more developed in bakery packaging (MAP) due to their antifungal activity.

- ***Incorporation of volatile and non-volatile antimicrobial agents into polymers:*** It has been commercially applied in drug and pesticide delivery, household goods, textiles, surgical implants and other biomedical devices. Recently, there has been growing interest in incorporation of antimicrobials into packaging for food applications [42]. Antimicrobials can be incorporated into packaging polymers in the melt or by solvent compounding. Thermal polymer processing methods like extrusion and injection moulding require thermally stable antimicrobials (e.g. silver substituted zeolites), while in the case of solvent compounding, both the antimicrobial and the polymer need to be soluble in the same solvent. If the incorporated antimicrobial agents are non-volatile, packaging materials must contact the surface of the food so that the antimicrobial agents can diffuse to the surface. The diffusion of antimicrobials from packaging material has been the subject of several research papers [46-50]. If the incorporated antimicrobial agents are volatile (e.g. chlorine dioxide, sulphur dioxide, carbon dioxide and allyl isothiocyanate), packaging materials do not need to contact the surface of the food [41].

- ***Coating or adsorbing antimicrobials onto polymer surfaces:*** Coating is the most suitable method to place the specific antimicrobial agent in a controlled manner without subjecting it to high temperature or shearing forces. It can also serve as a carrier for antimicrobial compounds in order to maintain high concentrations of preservatives on the surface of products (food). There are many studies focusing on packaging materials with non-volatile (e.g. nisin [51], silver nanoparticles [52] and volatile like essential oil [53], antimicrobial agents.

- ***Immobilization of antimicrobials to polymers by ion or covalent linkages:*** This system does not release antimicrobial agents but suppresses the growth of microorganisms at the contact surface. Immobilization of the antimicrobial agents to polymers by ionic or covalent bonding can be achieved when both antimicrobial agent and the polymer have functional groups.

Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. Examples of polymers used for food packaging applications that have functional groups are ethylene vinyl acetate (EVA), ethylene methyl acrylate (EMA), nylon and polystyrene (PS).

- ***Use of polymers that are inherently antimicrobial:*** Cationic polymers such as chitosan and poly-L-lysine are inherently antimicrobial and have been used in films and coatings. These polymers interact with negative charges on the cell membrane and cause the leakage of their intracellular components [42, 54].

Currently, novel processing techniques have lead to resurgence of research interest in the design and processing of antimicrobial packaging materials. These latest developments have set the stage for their use in promising technologies that include various plastic or biopolymer based antimicrobial active packaging films and containers for food and pharmaceutical products. Microcapsules that can deliver antimicrobial agents from plastic films or edible coatings can be mentioned as an example of these (Figure 9) [55].

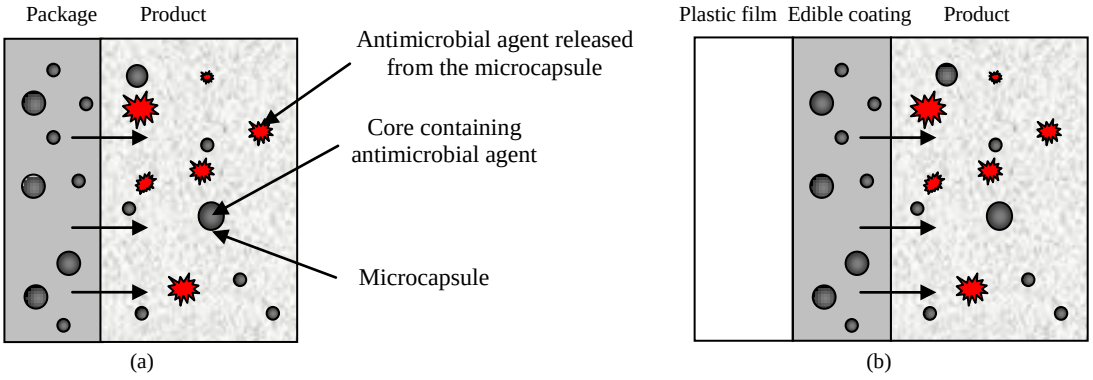


Figure 9: Migration of antimicrobial agent from (a) plastic film and (b) edible coating

There are some studies describing microencapsulating actives such as antibacterial and antifungal agents into a variety of polymeric food packaging materials including PE, PP, PVC, polyester and PVdC [55].

Antimicrobial agents

Antibacterial agents can be defined as natural or synthetic compounds that prevent or inhibit the growth and division of bacteria. A number of antimicrobial agents (e.g. organic acids, acid salts and anhydrides, para benzoic acids, alcohol, bacteriocins, fatty acids and their esters, chelating agents, enzymes, metals, antioxidants, antibiotic, fungicides, sanitizing gas, sanitizers, polysaccharide, phenolics, plant volatiles, probiotics) have been used for antimicrobial food packaging [43].

Among them, organic acids are the most commonly used chemical antimicrobials because of their efficacy and cost effectiveness. For instance, benzoic acid is used in the preservation of pharmaceutical products while salicylic acid is suitable for the topical treatment of fungal infections of the skin. Generally, acids display significant antimicrobial activity only when they are present in their undissociated state (Figure 10). In particular, undissociated acid molecules are able to pass through bacterial cell wall, dissociate there, thus reducing intracellular pH of bacteria. In this respect, the energy intensive process caused by the efflux of excess protons (H^+) exhaust the cell metabolism and, in consequence, bacterial cell death occurs. A carboxylic acid dissociates according to the following scheme.

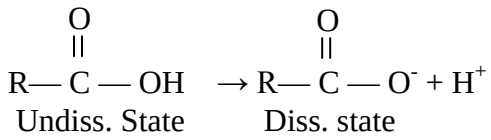


Figure 10: The general scheme for acid dissociation

The availability of the undissociated form characterized by the pKa value indicating the pH value at which 50% of the acid is present in the undissociated state, should be taken into account to use acids as microbicides (Table 1).

Table 1: pKa values of acid compounds used as microbicides

Acid or esters	pKa
Propionic acid	4.8
Sorbic acid	4.8
Acetic acid	4.7
Lactic acid	3.8
Benzoic acid	4.2
Salicylic acid	3.0
Dehydroacetic acid	5.4
Sulphurous acid	1.8, 6.9
Methyl- *p*- hydroxybenzoic acid	8.5
Propyl- *p*- hydroxybenzoic acid	8.1

In their undissociated state the acids may also alter the membrane permeability of the microbial cell and interfere with many enzymatic processes in the cell leading to nutrient transport inhibition [56-58].

Lactic acid (LA)

LA, a multifunctional chemical derived from renewable resources like sugars and whey, is widely used in numerous applications in food, pharmaceutical, textile and leather as well as polymer industries. It occurs naturally in two optical isomers, D(–) and L(+)-lactic acids (Figure 11).

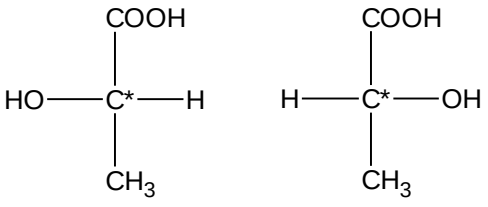


Figure 11: Lactic acid forms

Among them, the L (+) form of lactic acid is used for food and drug industry, because the human body is only adapted to assimilate this form [59].

It is manufactured either by microbial fermentations, e.g. by Lactobacilli or by chemical synthesis. However, biotechnological fermentation has received significant importance due to renewable resources, low energy requirements and high purity. Lactic acid lowers the pH to levels unfavourable for the growth of spoilage. Also, its combination with other acids such as acetic [60], benzoic or sorbic acid is more effective against yeasts and moulds. Moreover, lactic acid has the ability of to inhibit specifically mycotoxin formation [57, 61].

Besides, it should be emphasized that, one the most promising applications of lactic acid is its use for biodegradable and biocompatible lactate polymers, such as polylactic acid (PLA) [62-64].

1.3 MICROENCAPSULATION

Recently, there has been growing interest in the application of active packaging that can release active compounds (e.g. antimicrobials, antioxidants, enzymes, flavours, nutraceuticals and drugs) at different controlled rates. This can be achieved by microencapsulation that enclosing micron- sized particles of solids, droplets of liquids or gases in an inert shell with the purpose of protection, controlled release and compatibility of the core materials [6, 28, 65-68].

Controlled release is the most common application of microcapsules which means that the core materials (e.g. drugs, enzymes and vitamins) are encapsulated for delivery form textiles at a specific time, rate, or situation. This system plays an important role as drug delivery system aiming at improved bioavailability of conventional drugs and it has also revolutionized the food industry.

Microencapsulation with protective purpose prevents unstable or reactive materials against external influences such as oxidation, alkalinity, acidity,

moisture, polluting gases and unwanted interactions between active ingredients and other components in the system.

Compatibility is connected with the mixing of incompatible products, the conversion of liquids into powders (e.g. microencapsulation of essential oils) to prevent clumping and improve compounding combining the properties of different types of materials.

Microencapsulation has been widely used in many industrial areas, varying from agriculture to pharmaceutics and food for a number of reasons. The main reasons can be summarized as follows [6, 69-73]:

- Protection of unstable, sensitive materials from their environment (e.g. oxidation barrier for beta-carotene)
- Better processability (e.g. improving solubility, dispersibility, flowability)
- Controlling the release of encapsulated ingredients (e.g. gradual release of flavours during microwaving, leavening agents in baking and citric acid release during sausage manufacture)
- Masking undesirable flavours (e.g. taste masking of potassium chloride for nutritional supplements)
- Handling liquids as solids
- Controlled and targeted drug delivery
- Safe and convenient handling of toxic materials and
- Enzyme and microorganism immobilization.

At present, many different techniques are used for microencapsulation of food ingredients including flavouring agents, colorants, enzymes, microorganisms, essential oils, amino acids, vitamins, minerals or sweeteners and drugs [74, 75]. However, microencapsulated products in the pharmaceutical and food industries should comply with all relevant laws and regulatory requirements such as the FDA (Food and Drug Administration) and WHO (World Health Organization).

1.3.1 Microcapsules

Encapsulated particles are called “microcapsules” and may be ranged between 0.2 and 5.000 μm in size. Microcapsule structure can be divided into the core (the active agent, the internal phase, the filling) and the shell materials (the wall, the coating, the carrier, the encapsulant, the membrane) and may have regular or irregular shapes. They can be classified as mononuclear, polynuclear or matrix type depending on its morphology (Figure 12) [6, 76].

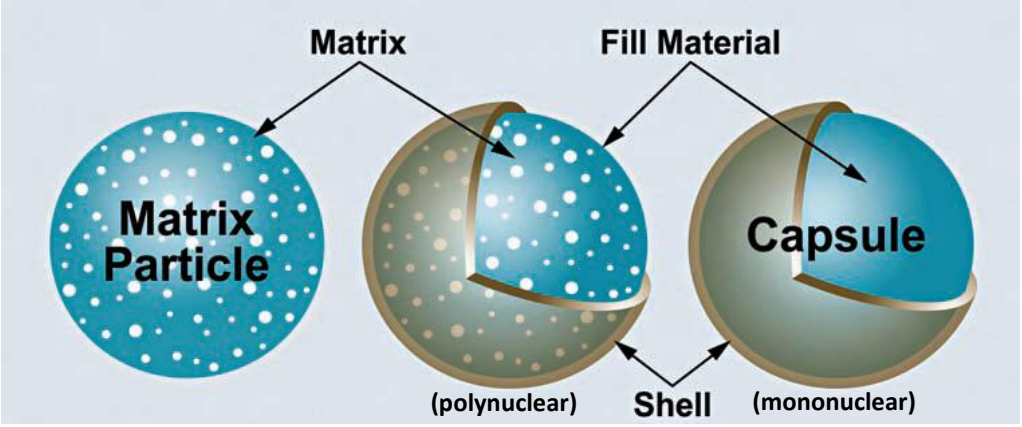


Figure 12: Morphology of microcapsules

In matrix encapsulation, the core material is distributed homogeneously into the shell. Mononuclear (core-shell) microcapsules contain the shell around the core, whereas polynuclear capsules have many cores enclosed within the shell.

The core may exist in solid, liquid or gaseous form, while the shell is a continuous, porous or nonporous polymeric layer.

It should be noted that the efficiency of microencapsulation depends on compatibility of the core with the shell. Also, to make smaller microcapsules with thinner walls is the most challenging, suited to the current needs and matches the features of nanotechnology [77].

1.3.2 Microencapsulation techniques

Numerous techniques of encapsulation of various core materials have been developed; each technique has its unique mechanism of microcapsule formation and application.

The microencapsulation techniques most widely used can be divided into two groups [6, 68] depending on physical/ chemical properties of both the core and the coating material, on size of microcapsule desired, release mechanisms and applications. They are listed in Table 1.

Table 2: Different techniques of microencapsulation.

Chemical process	Physical process	
	Physico-chemical	Physico-mechanical
• Suspension,dispersion and emulsion polymerization • Polycondensation	• Coacervation • Layer-by-layer • Sol-gel encapsulation • Supercritical CO- assisted microencapsulation	• Spray- drying • Multiple nozzlespraying • Fluid-bed coating • Centrifugal techniques • Vacuum encapsulation • Electrostatic encapsulation

Chemical methods:

Chemical methods include emulsion, suspension, precipitation or dispersion polymerization and interfacial polycondensation. This type of encapsulation involves polymerization during the process of preparing the microcapsules.

Physical method:

Physical methods are subdivided into two groups named physico-chemical and physico-mechanical as shown in Table 2. These methods involve the controlled precipitation of a polymeric solution wherein physical changes

usually occur. A detailed description of these methods can be found in the literature cited.

Since coacervation has been used as the method of encapsulation in this thesis a brief description of its basic principles is given here.

Coacervation can be defined as colloidal polymer aggregation process brought by partial desolvation of fully solvated macromolecules. It is classified into simple and complex coacervations that differ in their phase separation mechanisms. In the first case, phase separation is encouraged by addition of alcohol or salt (salting out), change in temperature or pH, while during complex coacervation an oppositely charged polymer is added to the polymer solution leading to the formation of a coacervate phase via anion–cation interactions [6, 65, 69, 77-79]. This method is efficient and can produce microcapsules with a broad range of sizes.

Generally, coacervation process consists of three steps that are carried out under continuous agitation as shown schematically in Figure 13.

- Phase separation of the primary polymer solution produces a three-phase system consisting of a solid or liquid phase made up of the core materials (e.g. drug, vitamin), a polymer-rich (coacervate) and a polymer-lean liquid phase. This occurs when macromolecules have an increased tendency to interact with one another, which may be caused by a reduction in their ability to interact with the solvent (simple coacervation) or by an ionic interaction between oppositely charged macromolecules (complex coacervation) [80].

- The polymer-rich phase deposits as microdroplets on the interface of the dispersed core materials. The microdroplets then start to spread leading to fusion into a membrane. Continued deposition of the coating is promoted by a reduction in the total free interfacial energy of the system brought

about by a decrease of the coating material surface area during coalescence of the liquid polymer droplets.

- The polymer membrane is hardened through thermal, desolvation, or chemical methods

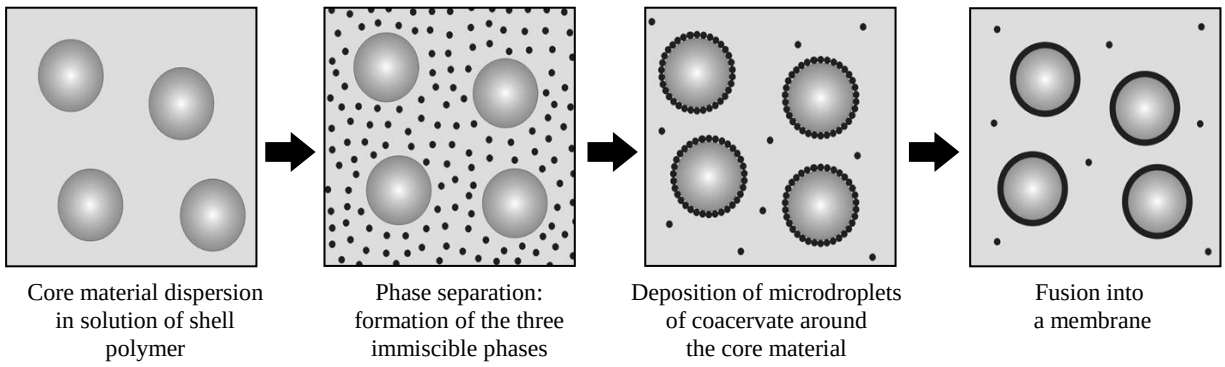


Figure 13: Schematic representation of microencapsulation by coacervation [43].

This process is suitable for encapsulation of both water-soluble and water-insoluble drugs. However, the coacervation process is mainly used to encapsulate water-soluble drugs like peptides, proteins, and vaccines. In particular, the core material must be compatible with the shell (recipient polymer) as noted before and must be insoluble (or scarcely soluble) in the coacervation medium [81].

Coacervation also may be subdivided into aqueous (hydrophilic coating and water-insoluble core) and nonaqueous phase separation (hydrophobic coating and water soluble or immiscible core) techniques. The former method has been used to encapsulate oils. In this case, emulsification is the first major step of the microencapsulation process. An emulsion can be defined as the dispersion and stabilization of one liquid within another liquid in which it is immiscible [82]. The emulsion formation determines the resulting particle size in the final process of encapsulation. The most common emulsion type is oil-in-water (O/W). However, multiple emulsions such as oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W) are commonly used [83].

1.3.3 Release mechanisms

Main functions of microcapsules can be represented by

- Keeping and protecting the core material during storage
- Releasing the core material at the right time and at a controlled release rate to improve the effectiveness of the active agents.

The most important parameters affecting the release of an active ingredient from a reservoir system are the capsule size, the morphological and molecular characteristics of the wall and solubility of the core in the release medium as well as degradation rate of polymers (wall). A variety of release mechanisms have been already described and proposed for microcapsules [6, 27, 65, 69].

Fracturation:

This is caused by breakage of the shell under mechanical stress (e.g. pressure, shearing, friction) (Figure 14. a) and by swelling of the core materials (Figure 14. b). In the latter case, the shell must allow the diffusion of solvent into the core. Encapsulated dried products will swell when they come into contact with a suitable solvent. The core can also swell as a result of changes in osmotic pressure within the capsules and incorporation of a swelling agent into the core or by an electromagnetic method using discharge or magnetic force.

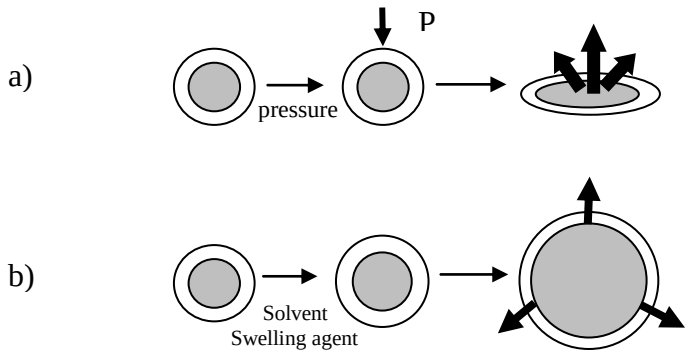


Figure 14: Release mechanisms of microcapsules:
a) mechanical stress, b) swelling and dissolution

Biodegradation:

Release from microcapsules can be accomplished by biodegradation mechanisms of the shell as a result of enzymatic breakdown. For example, gelatin coatings may be degraded by the action of gelatinase (Figure 15).

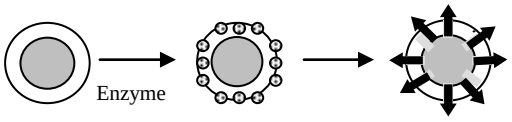


Figure 15: Release mechanism of microcapsules by enzymatic degradation

Dissolution and melting:

The integrity of microcapsules can be destroyed by dissolution in an appropriate solvent or by thermal effect. Water soluble coatings can be easily dissolved away from the core by increasing the moisture in the systems. Fat capsule can be mentioned as an example of thermal release. The coating melts and releases the core in the environment such as it occurs during baking [6, 69]. For instance, sodium bicarbonate is a baking ingredient that reacts with food acids to produce leavening agents, which give baked goods their volume and lightness of texture. To delay and control the leavening process, the sodium bicarbonate is encapsulated in a fat, which is solid at room temperature but melts at a temperature of about 50°C [82].

Diffusion:

Diffusion can be defined as mass transformation from regions of high concentration to regions of low concentration as a result of random molecular motions (Figure 16). The rate of mass transfer is proportional to the concentration gradient [23, 34]. As mentioned before, diffusional release can be described by Fick's first and second law of diffusion [77].

Finally, special emphasis is laid on the mechanical stability of the shell materials (in particular in the wet state) that can influence drug-release mechanisms (Figure. 16).

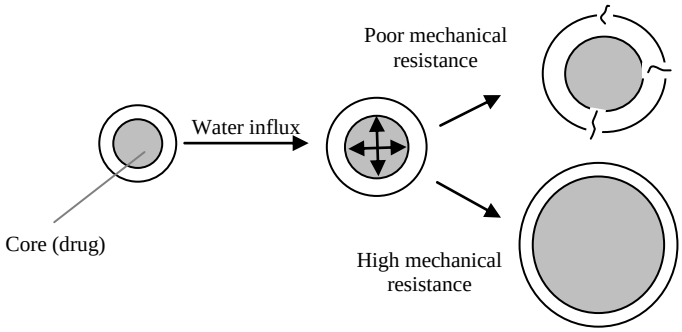


Figure 16: Schematic representation of the drug-release mechanisms depending on mechanical stability on the film coating

In case of poorly mechanically stable film coatings, drug release occurs through cracks created after a certain lag time, whereas in case of mechanically stable film coating, the release is generally controlled by diffusion through the intact polymeric networks [78].

Controlled release kinetics

Since microcapsules have been investigated for controlled release of active agents its releasing kinetics should be considered. Controlled release kinetics can be described by a variety of patterns including first and zero-order kinetics. Zero-order release is the simplest profile where the release rate remains constant until the package no longer contains an active compound while in the case of first-order release kinetics, the release rate is proportional to the mass of active compound contained within the package. The rate declines exponentially with time in first-order release, approaching a release rate of zero as the package approaches emptiness. A comparison of these release kinetics is shown in Figure 17.

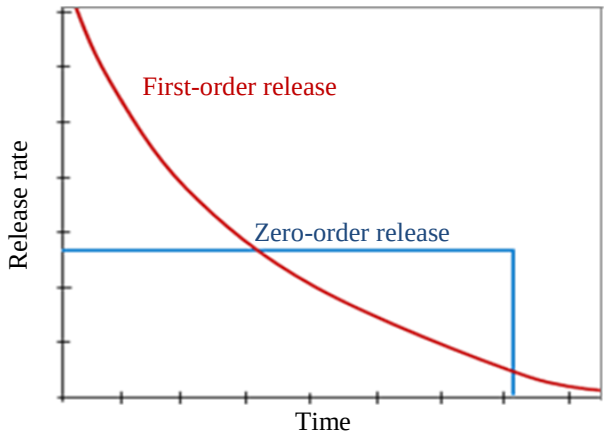


Figure 17: Zero order and first order release patterns from packaging containing the same initial concentration of active ingredient [84]

As can been seen from the figure, zero-order kinetics typically result in lower peak concentrations and more extended release than is the case with first order kinetics. These release kinetics can be expressed as follows.

Zero-order release: $$k = -\frac{dA}{dt} \quad (4)$$

First-order release: $$k[C] = -\frac{dA}{dt} \quad (5)$$

where $-dA/dt$ is the change in the concentration of active ingredient over time, k is the rate constant and $[C]$ is the active's concentration [84].

1.3.4 Coating materials for microencapsulation

The microcapsule core material can be coated by using a wide variety of materials including natural (e.g. polysaccharides alginate, agarose, chitosan as well as proteins such as gelatine) and synthetic polymers (e.g. polyacrylates, polyurethane and its less-toxic derivates and polyethers). They can either be used alone or in combination in order to achieve the desired functionality.

Coating material should have good rheological properties at its high concentration, ease of manipulation during the process of encapsulation, can produce a stable emulsion or dispersion with the active ingredient and does not

react or degrade the active material during processing and storage [76]. Other important feature to be taken into account is the core material to be coated and the required size, strength, solubility and release mechanism of the microcapsules. Besides these, the material properties of the coating like permeability, resistance to encountered conditions (e.g. shear, temperature, pH, light and enzyme) during processing and gastrointestinal tract transit should be considered.

The most common wall materials include gelatine and polyvinyl alcohol, soluble starch and gum Arabic [85-90].

Polyvinyl alcohol (PVA)

Poly (vinyl alcohol) (PVA) is a synthetic, water soluble polymer produced by polymerization of vinyl acetate (PVAc). Owing to its biodegradability, biocompatibility, excellent chemical resistance, film-forming, emulsifying and adhesive properties, PVA can be used in a wide range of industrial, commercial, medical, food and pharmaceutical applications such as controlled release systems, film formation and packaging [91, 92].

There are various methods of converting PVAc to PVA including transesterification (Figure 18), aminolysis (Figure 19) and hydrolysis (Figure 20) [15].

Transesterification:

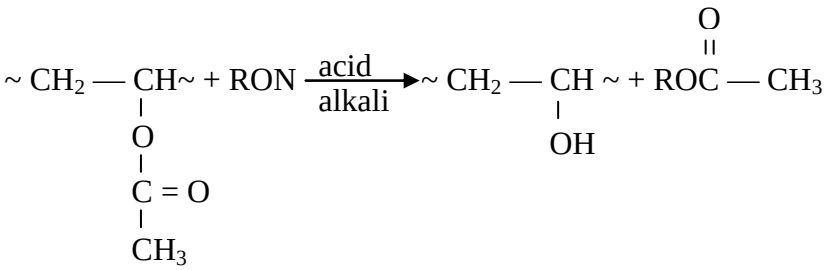


Figure 18: PVA produced by transesterification

Aminolysis:

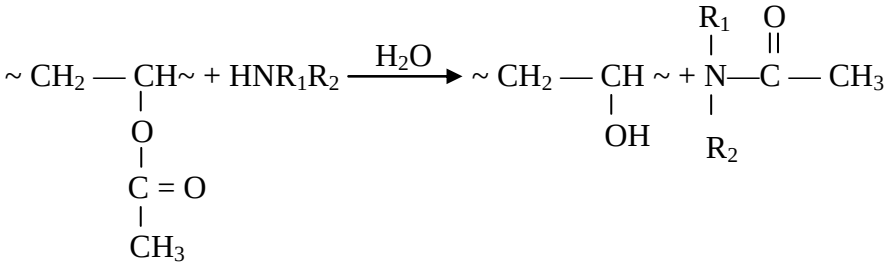


Figure 19: PVA produced by aminolysis

Hydrolysis:

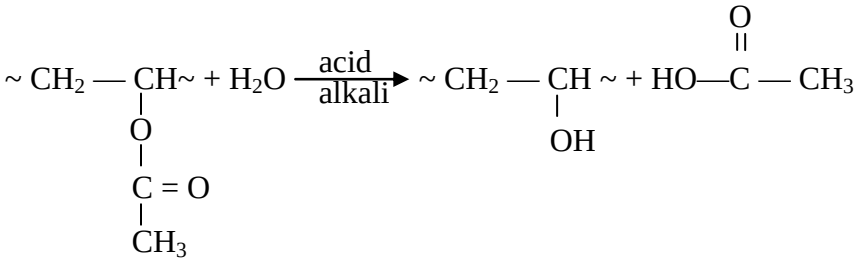


Figure 20: PVA produced by hydrolysis [15]

By varying the catalyst concentration, reaction temperature and time, it is possible to adjust the residual acetyl group content. PVA is classified into two groups (Figure 21), namely fully hydrolyzed and partially hydrolyzed [93, 94]. The distribution of the acetyl group in partially hydrolyzed PVA is dependent on the reaction conditions.

(A) (B)

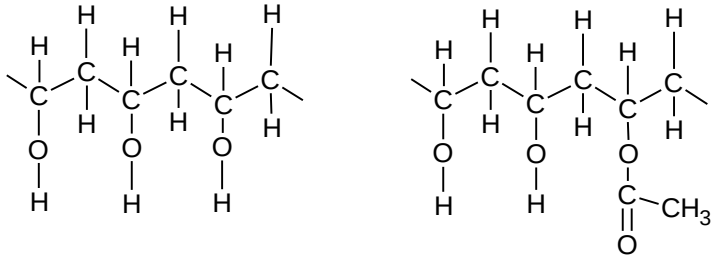


Figure 21: Structure of PVA: (A) fully hydrolyzed, (B) partially hydrolyzed

An acetyl group in the polymer has an overall effect on its chemical properties, solubility and the crystallizability of PVA [94, 95]. For example, fully hydrolyzed PVA shows a higher tensile strength and greater Young's modulus than partially hydrolyzed PVA, but is less resistant to elongation and tearing. It has also been noted that PVA grades with high degrees of hydrolysis have low solubility in water [96].

Mechanical properties, chemical resistance and solubility of PVA are also dependent on the juxtaposition of the syndiotactic and isotactic chain sequences, that of the 1,3- and 1,2- glycol arrangements and keto- or aldehyde groups [94].

PVA film is permeable to moisture, but a high barrier to all types of gases, because of a high intermolecular cohesion or high tendency to crystallize. However, its gas barrier rapidly diminishes as the hydrolysis is decreased.

Also, viscosity, tensile strength, adhesive strength, water and solvent resistance as well as dispersing power increase with increasing molecular weight. However, flexibility, water sensibility and ease of solvation are increased with decreasing molecular weight. On the other hand, water resistance, tensile strength and solvent resistance are increased with the increasing degree of hydrolysis, whilst flexibility, dispersing power and water sensitivity are increased with the decreasing degree of hydrolysis. The melting point (T_m) and glass transition temperature (T_g) depend on the content and distribution of the acetyl groups and tacticity of PVA. Further, PVA has the ability to reduce the surface tension of water and fully hydrolyzed PVA forms more viscous solutions than partially hydrolyzed PVA [96].

The literature search suggests that polyvinyl alcohol can be used for microencapsulation as a wall forming material, provided that it coacervates upon a change of a phase separation caused by pH, temperature, solvent concentration and electrolyte concentration. There are many reports based on the phase behaviour of PVA aqueous solutions as well as the permeation characteristics of PVA films. Partially hydrolyzed PVA solutions exhibit a lower critical solution

temperature behaviour separating into two phases upon increasing the temperature of the aqueous solution. Many inorganic compounds including salts are capable of inducing phase separation of PVA aqueous solution [78, 97]. A number of studies describing the uses of PVA in controlled release application have been already reported [97-101].

AIMS OF WORK

The aim of the presented thesis is to describe the interactions between water-soluble poly(vinyl alcohol) (PVA) and lactic acid (LA). The main attention is paid to the investigation of the PVA hydrolysis degree effect on the relations between polymer and modifier.

The crosslinkability of such material with a dialdehyde and subsequent characterization of the prepared 3D networks is the goal for the next step. This study will be conducted on the cast films as a model system.

The knowledge obtained from the studies of PVA films is supposed to be transferred into the technology of microcapsules preparation by using simple coacervation technique. The crucial attention must be paid to optimizing of the crosslinker concentration due to toxic properties of the dialdehyde compounds.

Stability and release kinetics of the selected model compound (β-carotene) will be studied as a function of time, pH, temperature and their combination. The morphology of the microcapsules will be visualized by microscopic techniques. Finally, the influence of LA modification of the encapsulating PVA matrix will be described.

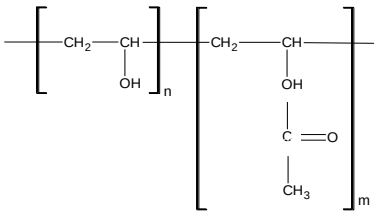
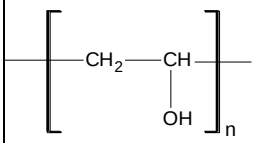
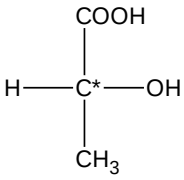
2. EXPERIMENTAL PART

2.1 MATERIALS AND SAMPLE PREPARATION

Materials used for the preparation of (PVA/LA) compounded polymeric films

Three types of poly(vinyl alcohol) (PVA) films with various hydrolysis degree (HD) were used in this study. Their characteristics are shown in Table 3 [94, 95] as well as information about L-lactic acid, which was used for modification of PVA.

Table 3: The main materials used in this work

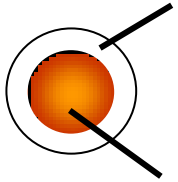
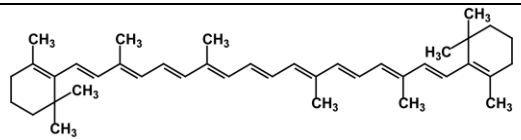
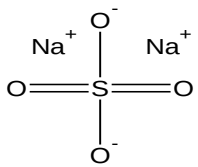
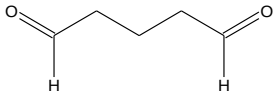
Commercial name	Polyvinyl alcohol	Mowiol 8-88	Mowiol 6-98	L-lactic acid
Designation	PVA 80	PVA 8-88	PVA 6-98	LA
Formula				
Molecular weight (g/mol)	9.000-10.000*	67.000*	47.000*	90.08
Source	Fluka, Germany			Penta, Czech Rep.

*M_w

Table 4 introduces the component used for preparation of microcapsules. Other compounds utilized for stability studies are dimethylformamide (DMF), tetrahydrofuran (THF), toluene, acetone and chloroform, methanol, acetic acid, sulphuric acid, hydrochloric acid were purchased from Penta, Czech Republic. All the chemicals were used as received without further purification.

Materials used for PVA/BCAR microcapsule preparation

Table 4: The main materials used in microencapsulation

Role	Material	Formula	Properties
 **Shell**	PVA 8-88 PVA 6-98	(see Table 3)	Beta carotene (BCAR) is a highly pigmented, fat-soluble carotenoid possessing antioxidant properties. It is a precursor of vitamin A that serves numerous biological functions including its ability to increase memory, reduce the risk of cardiovascular disease as well as to growth and repair of body tissues. However, carotenoids are oxidized by light and heat during food processing due to the presence of conjugated double bonds in their molecules [102,103].
Core	L-Lactic acid	LA was used for the modification of PVA	
	BCAR*		
Coacervating agent	Sodium sulphate		It is a stable compound which does not decompose and react with oxidising or reducing agents at normal temperature. Further, sodium sulphate is the most soluble compound in water at 32.4ºC (49.7g/100g) which is the main cause of its usage in coacervation [104].
Crosslinking agent	Glutaraldehyde (GAD)		GAD, a five carbon dialdehyde, is a highly reactive compound with many applications in different areas. It is used as a *crosslinker*, fixative, binding agent and preservative as well as for sterilization of hospital equipment [57, 105].

**dissolved in Silicone oil*

Preparation of polymer films

PVA films modified with various concentrations of lactic acid were prepared using solvent casting technique where the solvent (water) is allowed to evaporate slowly from a polymer solution under controlled conditions.

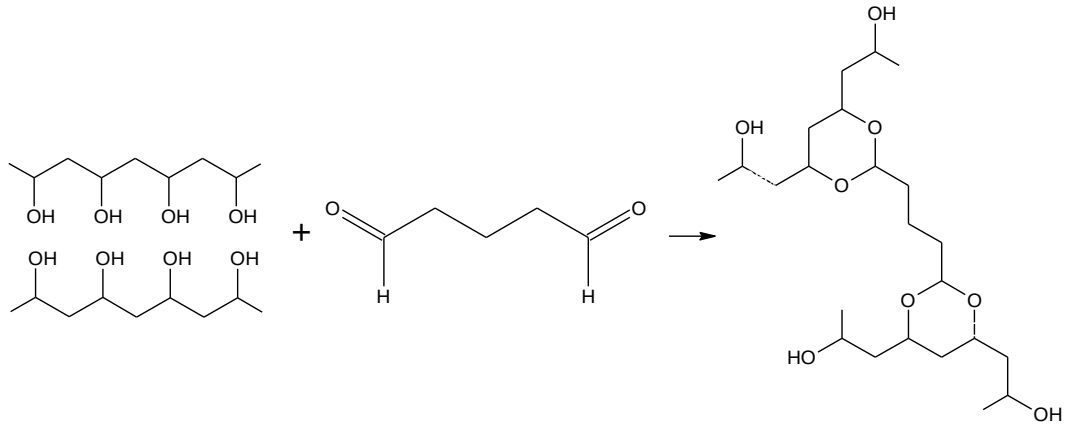
The film formation of PVA is encouraged by high intermolecular cohesion forces that can form a bond between the polymer molecules when the solution cast on a surface. Also, the polymer material coalesces and coalescence of an adjacent polymer molecule layer occurs through diffusion. When water evaporates, the viscosity of the solution increases and polymer chains are allowed to align in close proximity to each other. When there is adequate cohesive attraction between the molecules and sufficient diffusion as well as complete evaporation of water, polymer chains align themselves to form films [106]. However, the drying temperature and relative humidity that can determine the drying rate of cast solutions can affect the film structure and properties. In general, rapid drying of cast solutions limits the development of intermolecular associations within the film structure as solvent removal restricts the mobility of the molecular chains [107].

In order to prepare PVA/LA films by using solvent casting evaporation technique, PVA was dissolved in distilled water (10 wt% water solution) at 70°C for 30 minutes under continuous stirring. Then the relevant portion of LA (0, 5, 10, 15, 20, 25 and 30 wt. % related to PVA mass) was added to the solution and stirring of this mixture continued for another 15 minutes. After that, the resulting film-forming solution was cast into an acrylic mould and dried at 35°C for 48 hours in a temperature-controlled incubator. All obtained films were stored inside closed polyethylene bags to avoid moisture absorption and the thickness of the films were about 100-150 μm.

Preparation of PVA/BCAR microcapsules

Formation of BCAR loaded PVA microcapsules were prepared by simple coacervation method that include the three main steps, namely, emulsion of the core into the polymer solution, addition of phase inducer and crosslinking of the coacervated membrane.

In this process colloidal polymer aggregates formed upon the separation of an homogeneous aqueous polymer solution, are deposited onto the surface of dispersed liquid droplets, thus, resulting in the production of reservoir type of microcapsules. The separation can be induced by the addition of a strongly hydrophilic substance to form polymer-rich and -poor phases. The crosslinking of the polymer capsule is achieved by the addition of crosslinking solution into the reactor [78] that can be expressed as shown in Figure 22.



PVA GAD Crosslinked PVA

Figure 22: PVA and GAD crosslinking mechanism [112].

It should be noted that the size distribution of the final microcapsules is highly influenced by agitation quality (e. g., agitation rate and type) as well as the physicochemical properties of the dispersed and continuous phases.

In our case, 0.25 (wt. %) of an aqueous solution of PVA was taken into a reaction vessel at 30 ºC. Then, silicone oil containing BCAR was added under

high agitation (900rpm) to form an emulsion. The temperature of the vessel was then raised to 40 ºC. Coacervation was brought by gradual addition of aqueous sodium sulphate solution (20wt. %) for 50 min. After keeping this temperature for another 30 min, the temperature of the vessel was reduced to about 5 ºC. The crosslinking of the polymer capsule was carried out by addition of GAD solution consisting of methanol (16.67%), acetic (5%) and sulphuric acid (0.17%) as well as glutaraldehyde (4.08 %). After that, the temperature was raised to 40 ºC and the crosslinking reaction occurred for 3-4h. Finally, the vessel was cooled to room temperature and the obtained microcapsules were filtered, washed with 0.3% Tween solution as well as kept in sodium sulphate solution (0.1%). A schematic description of the various steps for PVA/BCAR microcapsule preparation is given in Figure 23.

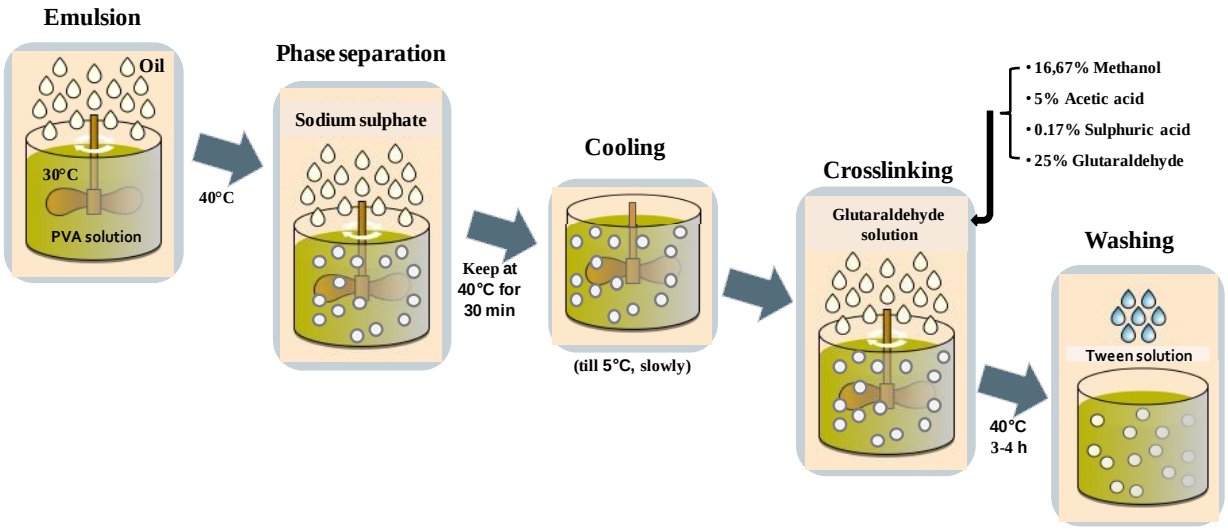


Figure 23: Microcapsule preparation by simple coacervation using PVA

2.2. CHARACTERIZATION METHODS

2.2.1 Determination of water content

Water content in polymeric films of pure PVA and PVA modified with LA was determined gravimetrically. The initial weight (W_i) of samples was determined before drying. Then the samples were dried at 60°C in vacuum oven up to constant weight (W_d). The water content (W) was then calculated by Equation (6).

$$W(\%) = \frac{(W_i - W_d)}{W_i} \times 100 \qquad (6)$$

2.2.2 Degree of swelling and solubility of the films

PVA and PVA/LA films were cut into square pieces of 1.5 cm^2 and dried until constant weight was reached. Each piece was immersed in distilled water at room temperature (25°C). The specimens were removed after predetermined time intervals (1-6 min). Then surface moisture was carefully removed by paper napkin and the weight of the films was measured. The degree of swelling (DS) was calculated as follows:

$$DS(\%) = \frac{(W_S - W_d)}{W_d} \times 100 \qquad (7)$$

where, W_S is the weight of the films after soaking process.

The swelled films were dried again until reaching a constant weight (W_a) at 60°C, then the solubility (S) was calculated according the Equation 8 [108]:

$$S(\%) = \frac{(W_d - W_a)}{W_d} \times 100 \qquad (8)$$

2.2.3 Mechanical test

Static tensile measurements

Tensile properties are performed by using tensile testing machine that is designed to elongate samples at a constant rate. As a specific load is applied, the known cross-sectional area of the sample is converted to stress (σ). By measuring the initial length of the sample and its incremental increase with each new load, strain (ε) is also recorded. Upon completing the process of adding tensile load and measuring each new length, all necessary data for a standard σ/ ε curve are generated [108].

Mechanical properties of PVA and PVA /LA films were carried out by using Instron 8871 at 25°C and 40% relative humidity. The initial length of samples was 50 mm, with a width of 10 mm and a thickness of about 100-150 mm. The speed of the moving clamp was 50 $mm \cdot min^{-1}$. The specimens were conditioned at 50% RH for one week to reach equilibrium before further investigation. At least eight specimens were tested in each case. All specimens were conditioned in the temperature-humidity controlled chamber at 25 °C and 50 % RH for 5 days before tensile testing experiment.

2.2.4 Thermal analysis

Differential scanning calorimetric (DSC) study

DSC is a thermo analytical technique for measuring the energy required to maintain zero temperature difference between an investigated sample and a reference material. In this method, the two specimens are subjected to identical temperature conditions in an environment which is heated or cooled at a controlled rate. Consequently, DSC curves that express phase transitions, such as melting, glass transitions are plotted as a function of time or temperature at a constant rate of heating [108].

PVA and PVA/LA films were analyzed by NETZSCH DSC 200 F3 differential scanning calorimeter (DSC) calibrated for temperature and heat flow using indium, for the assessment of the thermal properties. The samples (~ 10 mg) sealed in aluminum pans, were heated from -20°C to 160°C at heating rate of 20 °C·min^{-1}, which was followed by holding samples at 160°C for 15 min to avoid moisture influence (first heating scan) and then cooled to -20 °C. After keeping this temperature for 1 min, the second heating scan was run from -20 to 250. The value of T_g was determined from the second heating cycle at the midpoint stepwise increase of the specific heat associated with glass transition.

2.2.5 Spectroscopic analysis

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy provides information about the chemical bonding or molecular structure of materials either organic or inorganic. This technique is based on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to that molecule. During FTIR analysis, a spot on the specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks [108].

FTIR spectroscopy analysis was carried out to evaluate the physico-chemical structure of the films. Attenuated total reflectance (ATR)-FTIR spectroscopy was conducted on powder and thin film by NICOLET 320 FTIR, equipped with ATR accessory utilizing a Zn-Se crystal and software package "OMNIC" over the range of 4000-650 cm^{-1} at room temperature. Uniform resolution of 2 cm^{-1} was maintained in all cases. The differential spectra were obtained by subtraction of polymeric mixtures spectra and pure PVA film.

2.2.6 Studies of antimicrobial properties

Antibacterial activity of the PVA and PVA/LA films against both (G+) and (G-) bacterial strains were evaluated herein by two methods, namely, agar diffusion test as well as dilution and spread plate technique.

Disk diffusion test

The effect of an antimicrobial agent against bacterial grown in culture can be measured by the agar diffusion test. A circular sample imparted by a specified amount of antimicrobial agents was placed on the surface of an agar plate whose surface has already been inoculated with a suspension of the test microorganism. Plates were incubated under optimum conditions for the test microorganism for 24 hours. Following incubation, plates were examined for zones of no growth indicated by halos around the sample (Figure 24).

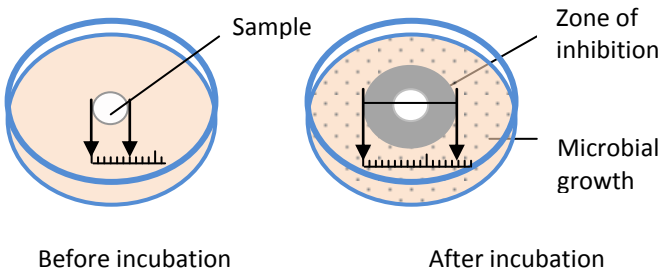


Figure 24: Determination of the "zone of inhibition" by the disk diffusion method

The antimicrobial agent diffuses through the agar, resulting in a concentration gradient that is inversely proportional to the distance from the disk. Degree of inhibition indicated by a zone of no growth around the disk is dependent on the rate of diffusion of the compound and cell. Therefore, the antimicrobial evaluated should not be highly hydrophobic because the compound will not diffuse and little or no inhibition will be detected.

The susceptibility of the test microorganism is related to inhibition zone size in millimetres. Microorganisms are termed susceptible when the zone is >30-35

mm in diameter, intermediate with a zone of 20- 30 mm, or resistant with a zone of <15- 20 mm [61].

In our case the test was performed in a Petri dish with a complete agar medium inoculated by the test organisms (approximately 10^8 colony forming unit (CFU) per millilitre) and a round specimen (8mm in diameter) was placed in the middle of this surface. After 24 hours inhibition at 37°C, the dimension of inhibition, which appeared on the surface, was measured in five directions and average values were calculated.

Dilution and spread plate technique

Dilutions and spread plating is used to measure of the concentration of viable cells present in a microbial culture containing a certain amount of sample with antibacterial agent to be tested [109, 110].

The samples of polymeric films (approx. 0.5g) were placed into test tube containing 9 ml of broth and 1 ml of bacterial culture. After 2 hours incubation at 37°C, the initial dilution was made by transferring 1 ml of mixture to 9 ml of distilled water and shaken vigorously and then several dilutions were repeated. From these diluted solutions the surviving bacteria were counted by the spread plate method. In particular, 100 µl of solution was transferred to nutrient agar and inoculated at 37°C for 24 hours.

Then the number CFU was counted (Figure 25) and the effectiveness of the PVA/LA antibacterial activity (EAA) was evaluated by using the following equation.

$$EAA(\%) = \frac{(N_0 - N_S)}{N_0} \times 100 \qquad (9)$$

where, N_0 and N_s represent number of CFU for blank and the sample containing antibacterial agent, respectively [111, 112].

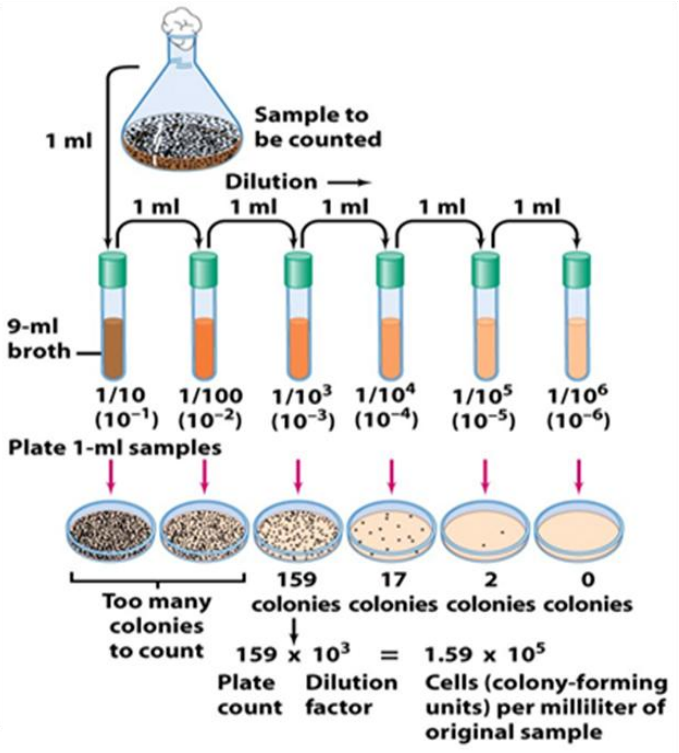


Figure 25: Quantitative Plating Procedure

2.2.7 Characterization of the microcapsules

Encapsulation efficiency

Encapsulation efficiency is an expression of the amount of oil incorporated into the microcapsule. The efficiency (%), oil content (%) and oil loading (%) were calculated by using the following formulae.

$$Encapsulation\ efficiency\ (\%) = \frac{w_1}{w_2} \times 100 \qquad (10)$$

$$Oil\ content\ (\%) = \frac{w_1}{w} \times 100 \qquad (11)$$

$$Oil\ loading\ (\%) = \frac{w_2}{w_3} \times 100 \qquad (12)$$

where, w is weight of microcapsules, w_1 is actual amount of oil encapsulated in a known amount of microcapsules, w_2 is amount of oil introduced in the same

amount of microcapsules and w_3 is total amount of polymer used including crosslinker. Each measurement was performed in triplicate [55, 70].

Particle size measurements

The polarized light microscope is designed to observe and photograph specimens that are visible primarily due to their optically anisotropic character [113]. Thus, images of microcapsules were obtained with the optical microscope. Also, the main size of the microcapsules was determined from the average for more than 500 particles measured using the optical microscope (Olympus- CX31) connected with a digital compact camera (Olympus-CD-350).

To assess the effects of the various process parameters on the microcapsule size distribution, the measured diameter size was characterized by evaluating number average diameter d_n and weight average diameter d_w as well as the Sauter mean diameter d_z [78].

$$d_n = \frac{\sum f_i d_i}{\sum f_i} \qquad (13)$$

$$d_w = \frac{\sum f_i d_i^2}{\sum f_i d_i} \qquad (14)$$

$$d_z = \frac{\sum f_i d_i^3}{\sum f_i d_i^2} \qquad (15)$$

where, f_i is the number of measured microcapsules having diameter d_i [μm].

Scanning electron microscopy study

Microcapsule morphology is evaluated by scanning electron microscopy (SEM). It is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that

contain information about the sample's properties including surface topography, composition. It should be noted that all samples must also be of an appropriate size to fit in the specimen chamber [108].

In our work, the microcapsules were observed under a scanning electron microscope (Scanning electron microscopy (TESCAN VEGA II LMU, Czech Republic, equipped with thermo-emission cathode). They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film under reduced pressure.

In vitro release studies

The releasing test is evaluated using UV spectrophotometry that determines the absorption of UV light (180-820 nm) by a sample. According to Beer- Lambert law, absorbance is proportional to concentration of investigated sample and expressed as the following.

$$A = \varepsilon \lambda c \qquad (16)$$

where, A is absorbance of the sample, ε is the molar absorption coefficient (absorptivity), λ is wavelength and c is concentration of the sample, respectively.

Thus, if the path length and the molar absorptivity are known and the absorbance is measured, the concentration of the studied sample can be deduced [114].

In this work, known quantities of microcapsules were placed directly into measuring flasks containing known volume of phosphate buffer (pH 7.4), hydrochloric acid (pH 2) or distilled water, respectively, as the dissolution medium. The flasks were tightly closed and kept at 37 ºC under continuous shaking (100 rpm). In predetermined time intervals, 10 ml of the dissolution medium is collected, filtered. The volume of the medium which was taken away was compensated by the fresh one. To extract the hydrophobic compound from

the release medium 10 ml of chloroform was added. The mixture was shaken and left for 24 hours subsequently before further investigation. Finally, the chloroform phase was assayed spectrophotometrically (Heλios Gamma) at 462 and 485 nm. A calibration A vs. c dependence was used for quantitative analysis.

Stability of the microcapsules

To evaluate the chemical stability, the microcapsules were incubated in hydrochloric acid (HCI, pH 2), carbonate buffer (pH 9), physiological solution (0.9 wt % NaCI water solution), phosphate buffered saline (PBS, pH 7.2) and distilled water for certain time interval.

Also, to examine the wall resistance to the different chemical solvent conditions, the PVA/BCAR and PVA/LA/BCAR microcapsules were sequentially incubated with the chloroform, DMF, THF, toluene and acetone.

Besides these, to explore the thermal stability, the microcapsule suspension was incubated at different temperature interval from 37°C to 100 °C for 24 h.

3. RESULTS AND DISCUSSION

The following chapter is divided into four parts according to the specification of the research work done within assigning of the presented Ph.D. thesis. The first of the subchapters (3.1) is dealing with the effect of lactic acid on PVA matrixes with various hydrolysis degrees on the mechanical and thermal properties, structural characteristics, solubility, crosslinkability and antibacterial activity against both G+ and G- bacterial strains. The crosslinkability of PVA/LA based materials is the object of the subchapter 3.2. Third subchapter (3.3) is focused on the effect of PVA hydrolysis degree and crosslinking agent concentration on β-carotene (BCAR) encapsulation efficiency and stability of the microcapsules. Finally, the influence of lactic acid on BCAR containing microcapsules formation and their subsequent stability are presented in subchapter 3.4.

Each subchapter corresponds to the information and experimental setup already presented in previous chapter 2. Thus, this information will not be further presented here.

All the data shown in the following part of this thesis are under preparation for both patent application submission and publication in scientific periodicals.

3.1 Polyvinyl alcohol/lactic acid (PVA/LA) compounded polymeric films: effect of PVA hydrolysis degree on resulting properties

This study follows up the work by Sedlarik *et al.* already published in 2006. This study was focused on characterization of PVA/lactic acid compounded polymeric films. The results showed excellent plasticization effect of LA in PVA films, which was supported by significantly decreased moisture sensitivity of the modified polymeric films [92]. These results are encouraging for further research in this field since it has been known that various hydrolysis degree of PVA can be crucial for material properties of the resulting systems. The high importance is also expected in case of modifications of polymer matrixes in encapsulation technologies. The prepared polymeric films were characterized on mechanical, thermal and antibacterial properties. In addition, the effect of LA modification on the solubility and crosslinkability of the films was tested.

Tensile properties

The LA concentration dependencies of Young's modulus, E, for various types of PVA are shown in Figure 26. In case of the pure PVA film the highest E can be noticed for PVA 6-98 (over 2 GPa); i.e. PVA with the higher hydrolysis degree (HD). It can be also observed that E decreases with the increasing amount of residual acetyl moieties on the PVA carbon backbone chain. The modification of all used PVA types leads to significant reduction of E values already at 5 wt. % LA (721 MPa). The trends of all the depicted dependences have exponential like course. In addition, it is obvious that LA works as a plasticizer compound here as it has various plasticizing effects on the studies of PVA matrixes. The fully hydrolyzed PVA (PVA 6-98) seems to be less affected by the LA present in comparison with partially hydrolyzed one.

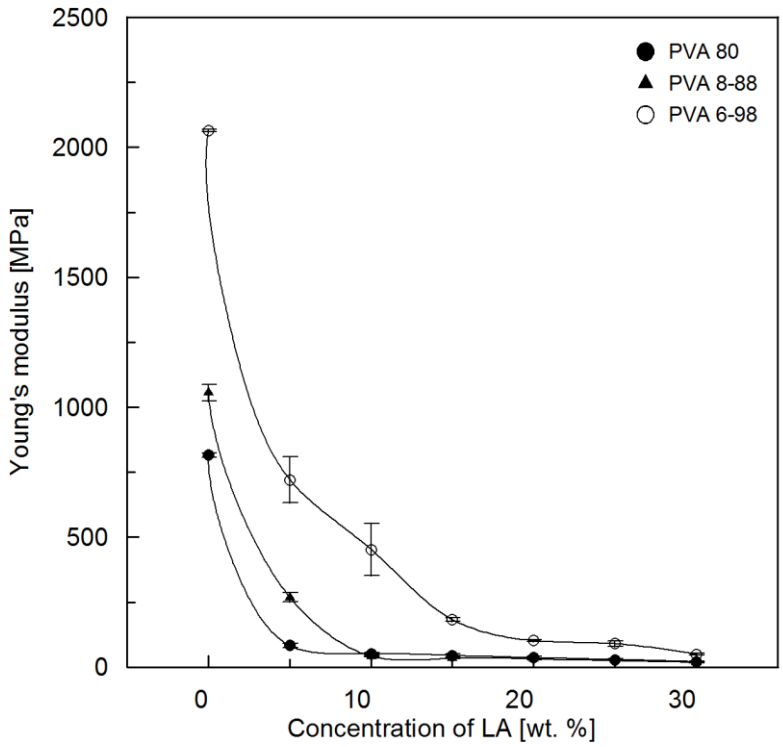


Figure 26: Young's modulus as a function of LA content in PVA films

Tensile stress versus LA concentration is shown in Figure 27. The results show more or less similar trend as it has been presented above (Figure 26). The higher HD of the PVA leads to the higher tensile strength. The tensile stress reduction phenomenon is gradual in all cases. The addition of 30 wt. % of LA leads to more than 70 % reduction of tensile properties in case of all three PVA matrixes.

The effect of LA presence on tensile strain of the prepared films based on PVAs with various HD is shown in Figure 28. The maximal elongation of the samples was observed for the films made of PVA 8-88 and PVA 6-98. The PVA film which was prepared by using PVA with 80 mol. % hydrolysis of the acetyl groups has significantly lower tensile strain value (almost one third). The modifications with LA lead to interesting results. While PVAs with 88 mol. % and higher (PVA 8-88 and PVA 6-98) prove increasing elasticity with the increasing content of LA, PVA 80 shows opposite reaction; i.e. reduction of tensile strain.

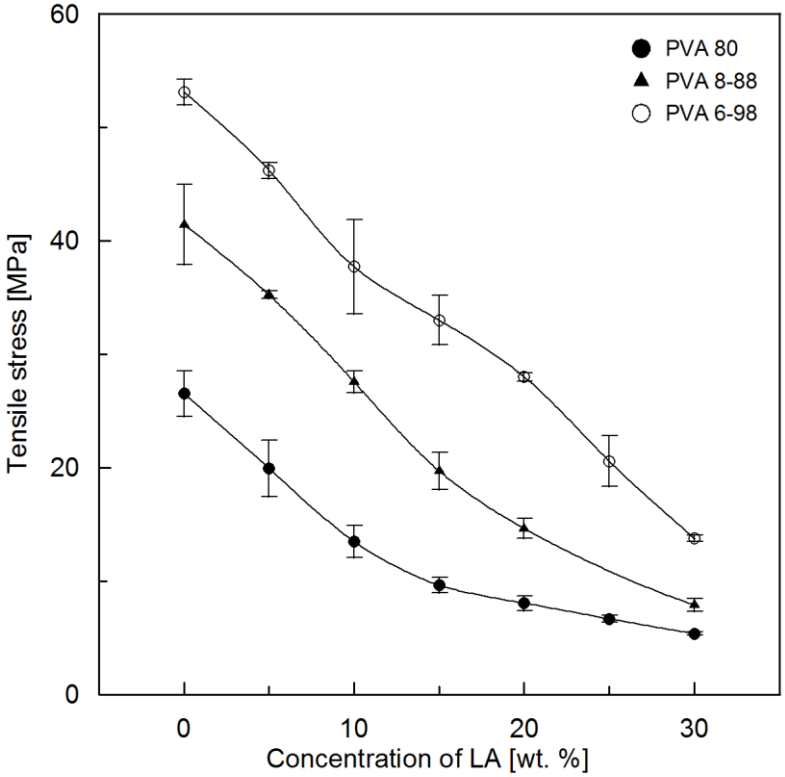


Figure 27: Tensile stress as a function of LA content in PVA films

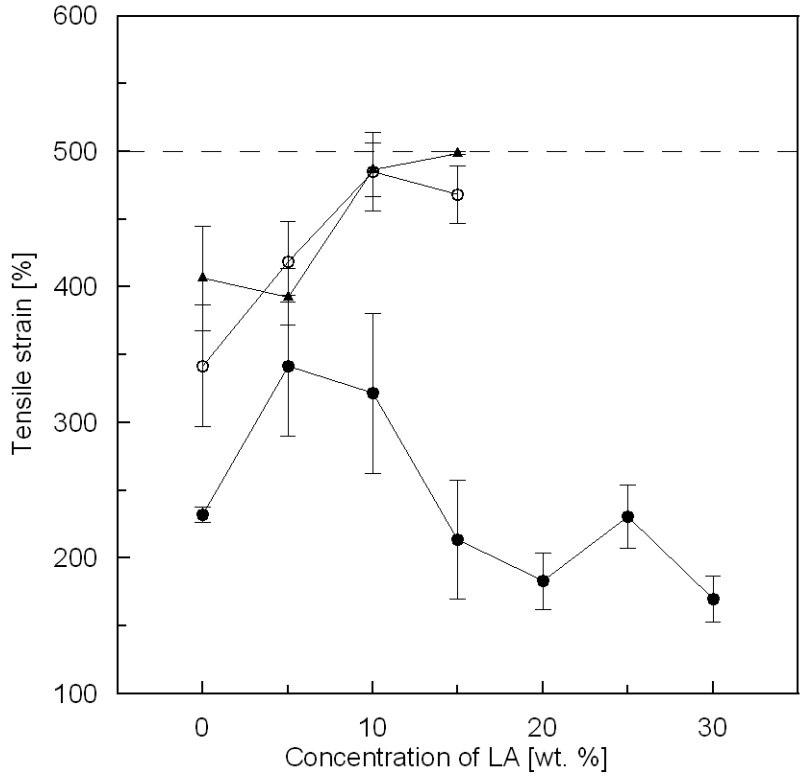


Figure 28: Tensile strain as a function of LA content in PVA films

The results presented in Figures 26-28 are in accordance with the already presented works. The higher HD of PVA is connected with higher regularity of the polymeric chains and consequently with higher crystallinity. It results into higher E and tensile strength values. Surprisingly, the amount of hydroxyl groups on the backbone chain has critical effect on interaction with LA as well. On the basis of the presented results could be assumed that hydroxyls present in the PVA structure may react with relevant part of the modifier (LA) as it was proposed by Sedlarik and Carlotti [92, 115]. The evidence of that phenomenon could be found in tensile properties testing results (especially Figure 28) where poor compatibility of PVA 80 and LA can be observed. On the other hand, increased presence of hydroxyl groups is more favourable for PVA-LA interactions. The result of this interaction leads to high maximal elongations, which were out of the range of the used tensile testing machine (more than 500 %). This limit is depicted as a dashed line in Figure 28.

It has been known that PVA based materials are extremely sensitive on atmospheric moisture. It means that conditioning factor plays an important role in the testing of mechanical properties. It has been already mentioned that all specimens for tensile testing were stored inside of a conditioning chamber at room temperature and 50 % RH for 5 days before measurements to bring the water content in the sample material into equilibrium. The gravimetric determination of the water content after such a conditioning was carried out on and its results can be seen as a function of LA concentration in Figure 29. It can be noticed that amount of water content present in the material after conditioning period is rising with increasing amount of LA for all three PVAs. While all pure PVAs keep their water content bellow 6 wt. %, the films with 30 wt. % LA show relatively significant differences in water content depending on HD of the individual PVA matrix. The lower value was found for PVA 80 following by fully hydrolyzed PVA 6-98 and the higher water content was observed for PVA 8-88 containing 30 wt. % of LA (above 17 wt. %). This

parameter should be considered for evaluation of PVA based materials mechanical properties. However, it was found that modifications of PVA with LA lead to decreasing conditioning sensitivity unlike glycerol where its leaching out has been reported elsewhere [62,116].

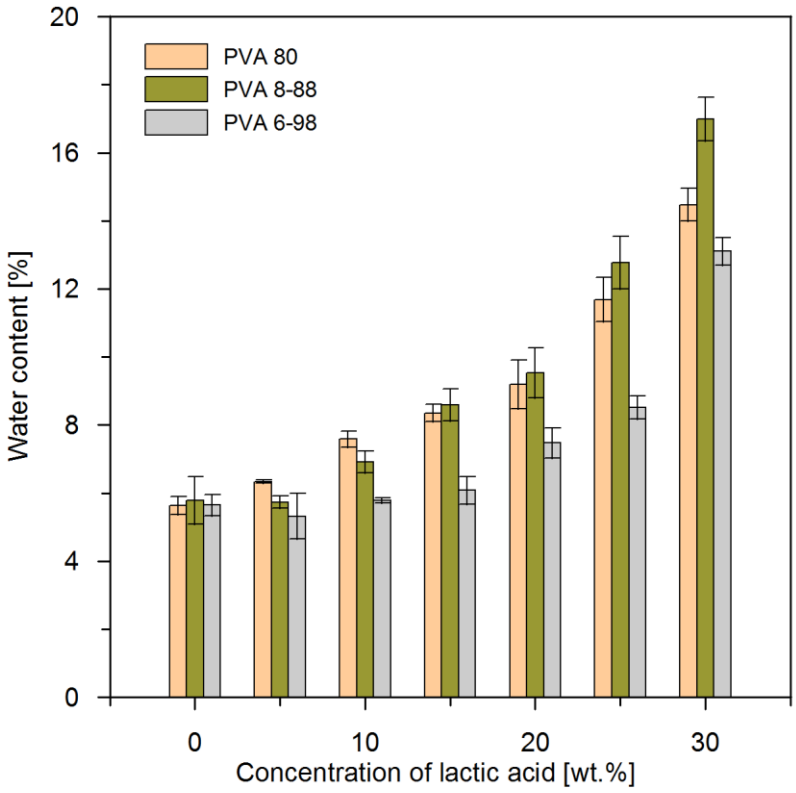


Figure 29: Gravimetrically determined water content of the PVA/LA films after conditioning procedure (50 % RH, room temperature, 5 days)

Thermal properties

In PVA/LA systems investigated in this study, LA seems to work partially as a plasticizer, increasing flexibility, workability and extensibility of the rigid plastic. The idea of LA incorporation in PVA structure is supported by quite good compatibility of both components due to their polarity and changes of free volume and consequent decreases of glass transition temperatures, T_g. The intensity of these changes is visible in Figure 30, where the measured T_g values are depicted as a function of LA concentration for various PVAs. It can be noticed that LA has significant plasticizing effect for all the tested types of PVA

in for the reduction of T_g values. It can be assumed that this fact is mostly connected with the LA part which is present in excess in the studied systems.

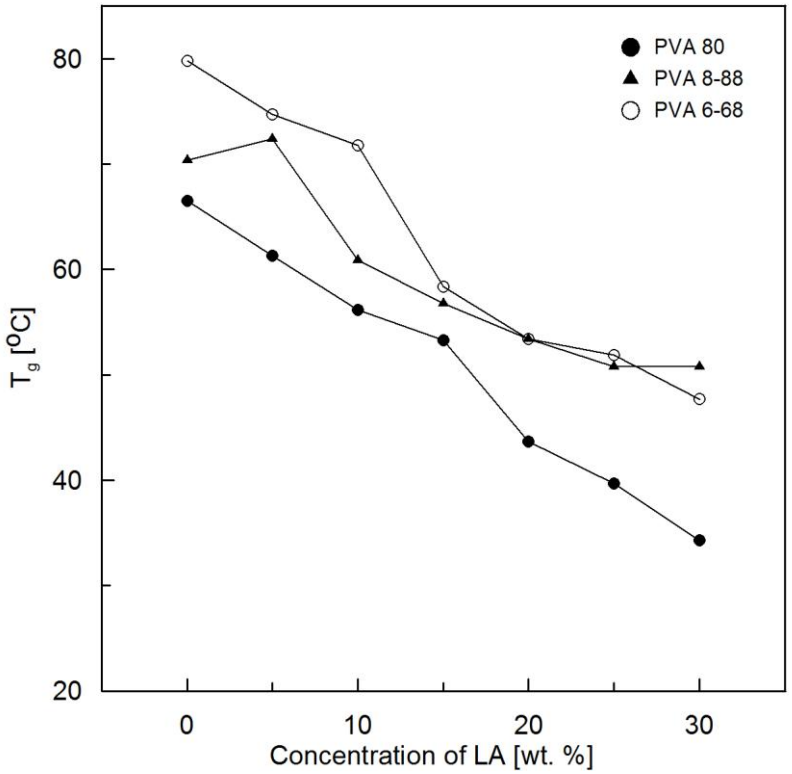


Figure 30: Glass transition temperature (T_g) as a function of LA concentration in the PVA based films

Another LA part (much smaller) may be involved in direct interaction with hydroxyl moieties of the matrix as revealed above. The proposed grafting (LA-OH) could influence the crystalline phase of the system and, consequently, the melting temperature, T_m. The effect of LA presence in the PVA films on the obtained values of T_m is shown in Figure 31. While partially hydrolyzed PVA 80 and PVA 8-88 based films are characteristic by a slight T_m reduction, fully hydrolyzed PVA 6-98 shows significant change of T_m from 229 °C for pure PVA 6-98 to 196 °C for the PVA 6-98/LA 30 wt. %. As described in literature, LA grafting on PVA chain through hydroxyl groups can be followed by reduction of T_m [117].

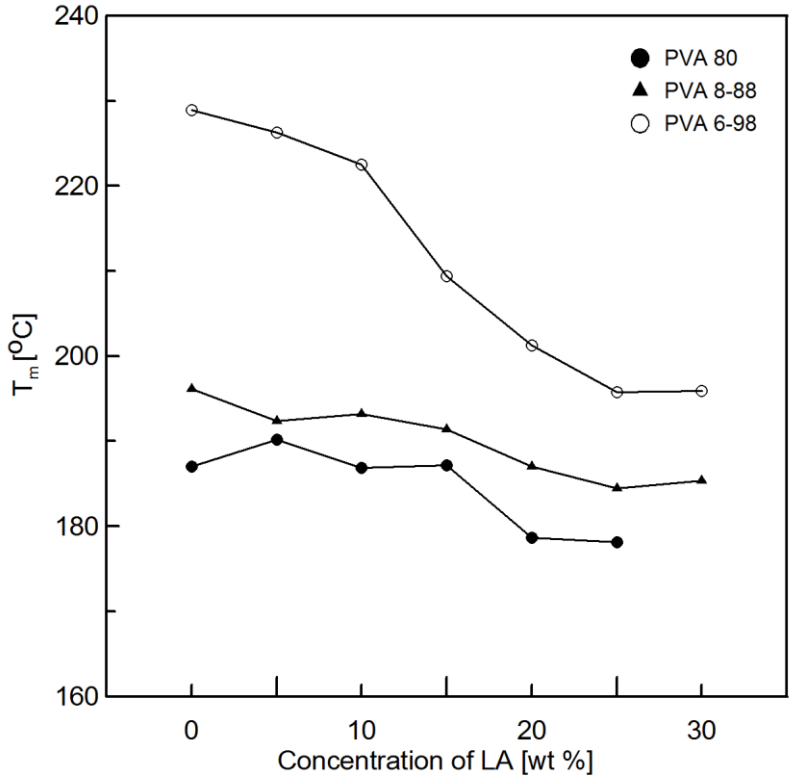


Figure 31: Melting temperature (T_m) as a function of LA concentration in the PVA based films

Physico-chemical properties of the films

To identify the physico-chemical structure of pure PVAs, LA and their mixtures FTIR spectroscopy analysis was carried out. The FTIR-ATR spectra of the pure components are shown in Figure 32. The FTIR spectrum of LA (Figure 32 (a)) shows characteristic absorption peaks at 3486 cm^{-1} (O-H groups), 1734 and 1726 cm^{-1} (C=O, aliphatic esters), 1232, 1130 and 1047 cm^{-1} (C-O groups). In fact, commercial LA in aqueous solution exists in the monomeric form; nevertheless, it can also consist of dimers and/or higher mers [117]. This could be connected with the appearance of an intensive peak at 1726 cm^{-1}.

The spectra of pure partially hydrolyzed PVA 80 (Figure 32 (b)), PVA 8-88 (Figure 32 (c)) and fully hydrolyzed PVA 6-98 (Figure 32 (d)) have several common peaks at 3270 cm^{-1} (OH groups, indicating presence of moisture), 2920 cm^{-1} (C-H stretching), 1413 cm^{-1} (OH groups) 1085 cm^{-1} (C-O stretching) and 823 cm^{-1} (C-H bending). On the contrary, typical peaks corresponding to the

hydrolysis degree can be found for PVA 6-98 at 1423 cm^{-1} (OH deformation) as well as for partially hydrolyzed PVA 80 and PAV 8-88 at 1730 cm^{-1} (C=O stretching) and 1241 cm^{-1} (C-O stretching).

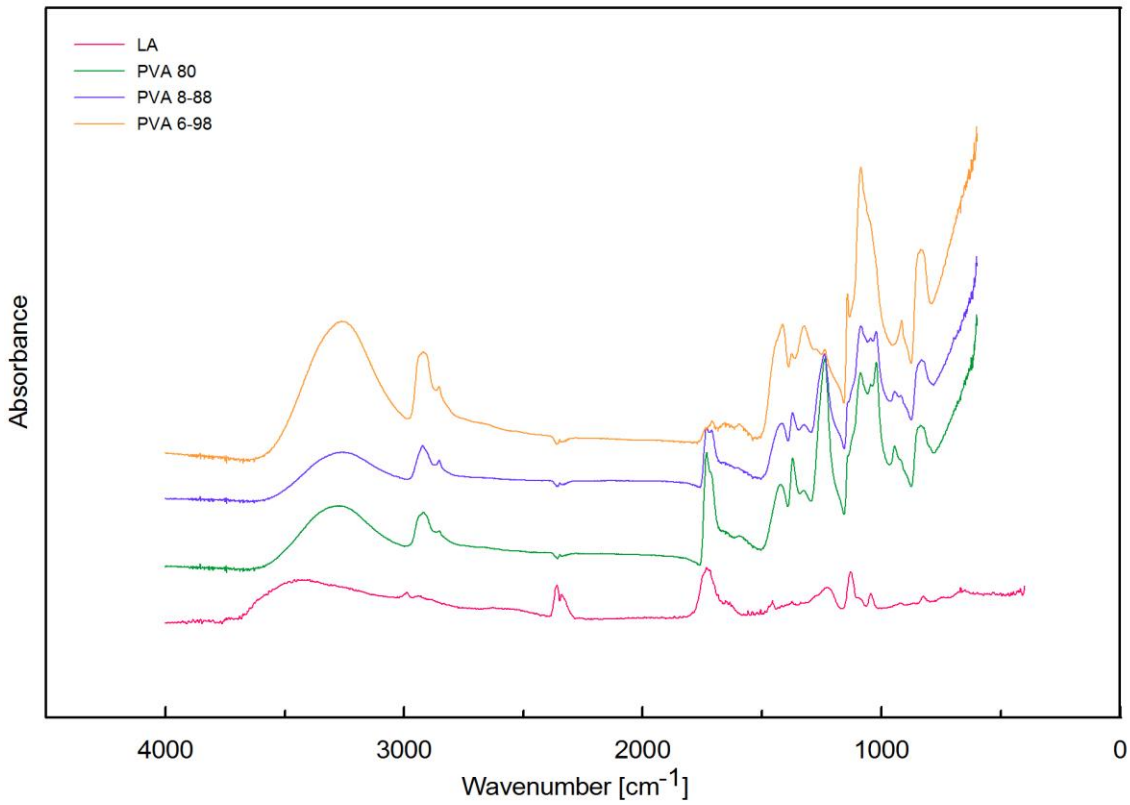


Figure 32: FTIR-ATR spectra of the pure components (a) LA, (b) PVA 80, (c) PVA 8-88 and (d) PVA 6-98

Figures 33-35 present FTIR-ATR spectra of the pure PVAs and their mixtures with LA. All spectra are characteristic by increasing intensities of the absorption peaks occurring at the wavenumbers characteristic for LA (i.e. 1726 cm^{-1} and in the interval from 1040 to 1240 cm^{-1}) with the increasing content of LA in systems (i.e. 1726 cm^{-1} and in the interval from 1040 to 1240 cm^{-1}). These intensities enhancements are marked by black arrows in the figures.

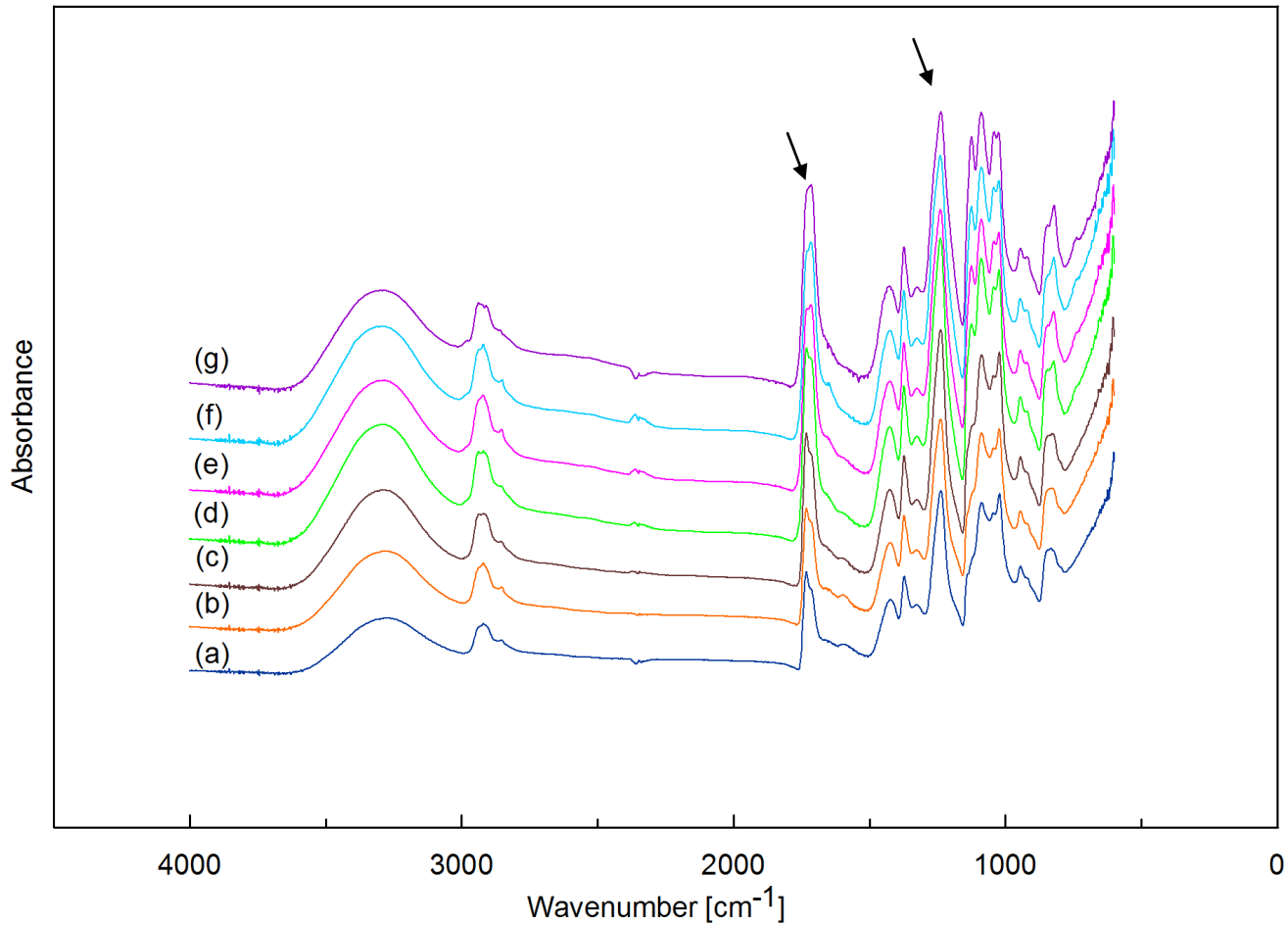


Figure 33: FTIR-ATR spectra of the polymer films based on pure PVA 80 (a) and PVA 80 modified with LA 5 wt. % (b), 10 wt. % (c), 15 wt. % (d), 20 wt. % (e), 25 wt. % (f) and 30 wt. % (g)

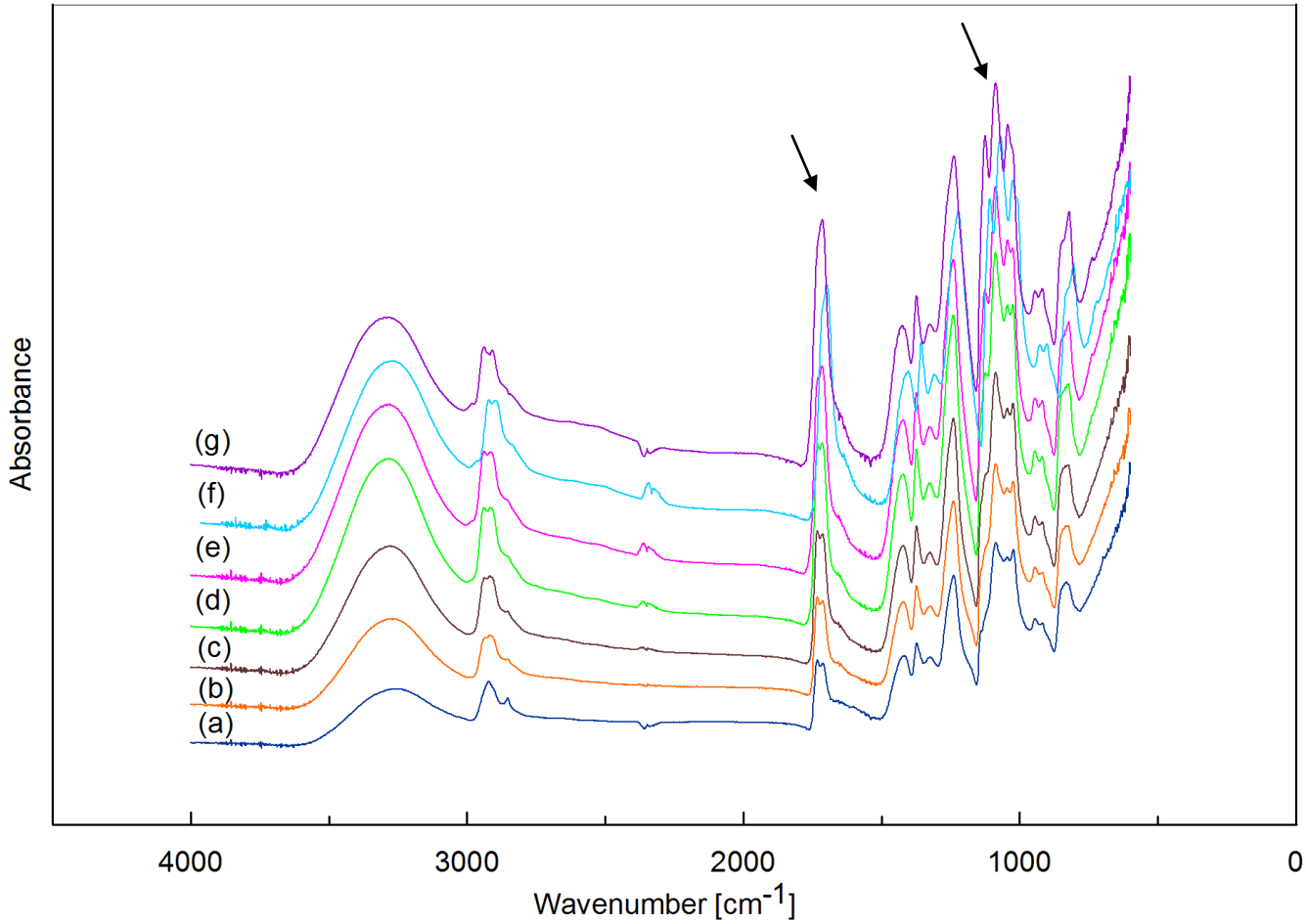


Figure 34: FTIR-ATR spectra of the polymer films based on pure PVA 8-88 (a) and PVA 8-88 modified with LA 5 wt. % (b), 10 wt. % (c), 15 wt. % (d), 20 wt. % (e), 25 wt. % (f) and 30 wt. % (g)

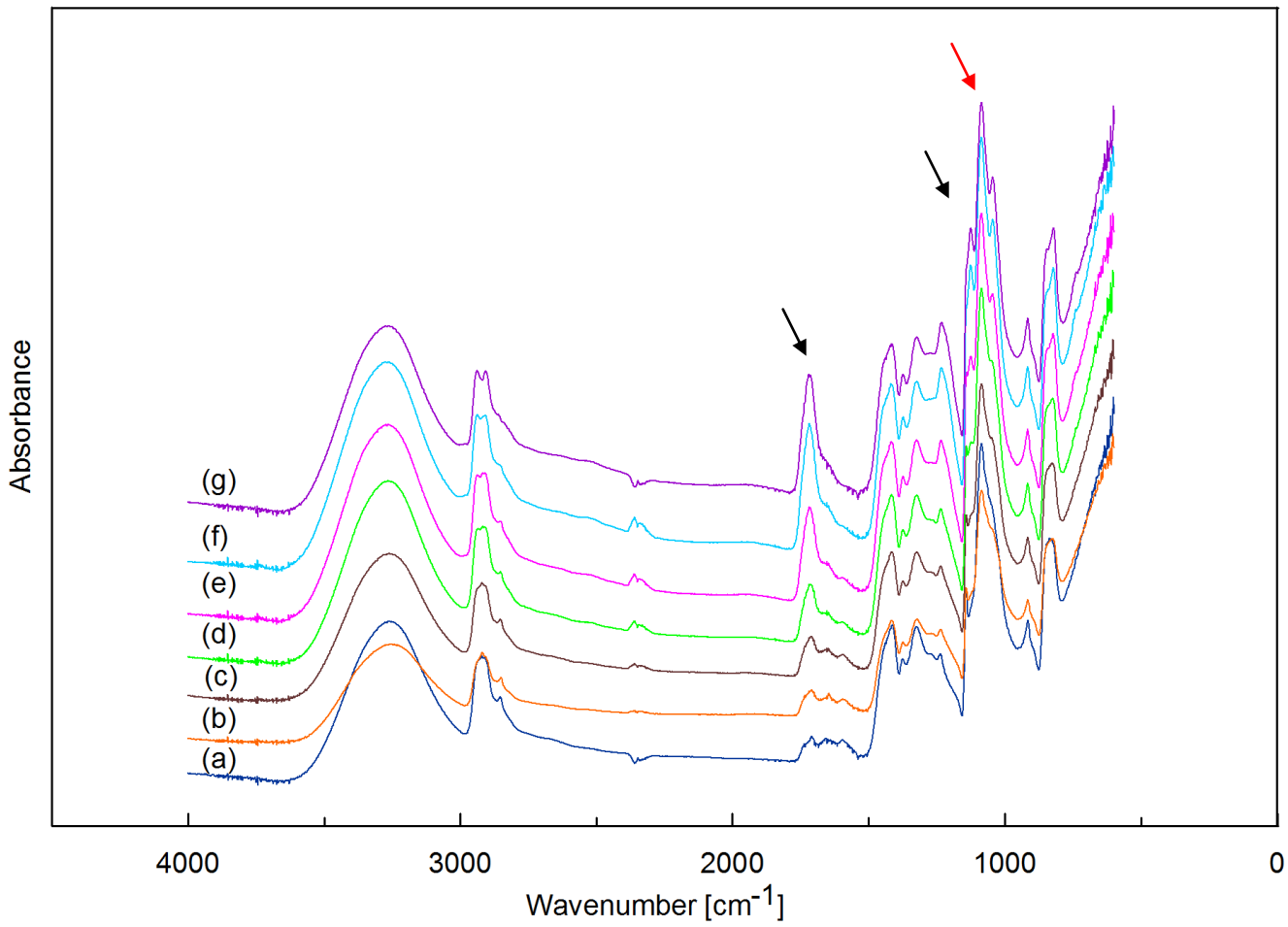


Figure 35: FTIR-ATR spectra of the polymer films based on pure PVA 6-98 (a) and PVA 6-98 modified with LA 5 wt. % (b), 10 wt. % (c), 15 wt. % (d), 20 wt. % (e), 25 wt. % (f) and 30 wt. % (g)

Relatively significant intensity increase of the absorbtion peak occurring at 1080 cm^{-1} (marked by a red arrow) was found in Figure 35. This peak could be an evidence of the noticeable esterification process, which could take place between hydroxyl groups of fully hydrolyzed PVA 6-98 and carboxyls of LA. This assumption corresponds to the results obtained from mechanical and thermal properties investigations shown above.

Water-swelling testing

The effect of LA modification of PVA with various HD on the water-interaction of the resulting films was studied gravimetrically. It has been known that hydrolysis degree (content of the residual acetyl moieties) has significant influence on water-solubility of the PVA based materials. It has been reported that the higher HD the lower water-solubility. It is in discrepancy with the expectation, which considers chemical structure of the material assuming that higher amount of hydroxyls, the higher solubility in water. The literature and practical information explain this phenomenon by polymer chain arrangement in the structure. Highly hydrolyzed PVA has significantly higher ability to crystallize. The crystalline structure is not favourable for dissolution subsequently.

The results obtained from the experimental work are in agreement with the text presented in the previous paragraph. Figures 36-38 show the degree of time dependence of swelling (calculated according to the Equation 7) as a function of LA concentration in the studied films based on PVA 80, PVA 8-88 and PVA 6-98, respectively. It should be mentioned that while partially hydrolyzed PVAs were possible to study in the range of seconds, fully hydrolyzed PVA kept its shape and uniformity for several hours after introduction of a specimen into a water bath. The data shown in the following Figures 36-38 represent average values from the at least three identical measurements. Standard deviation was bellow 20 % in all cases. Figure 36 describing swelling behaviour of the PVA

80/LA samples, shows negative values of swelling already after 15 s of the experiment. It reveals a strong solubility of the matrix. The modifications with LA even increases the dissolved specimen phase.

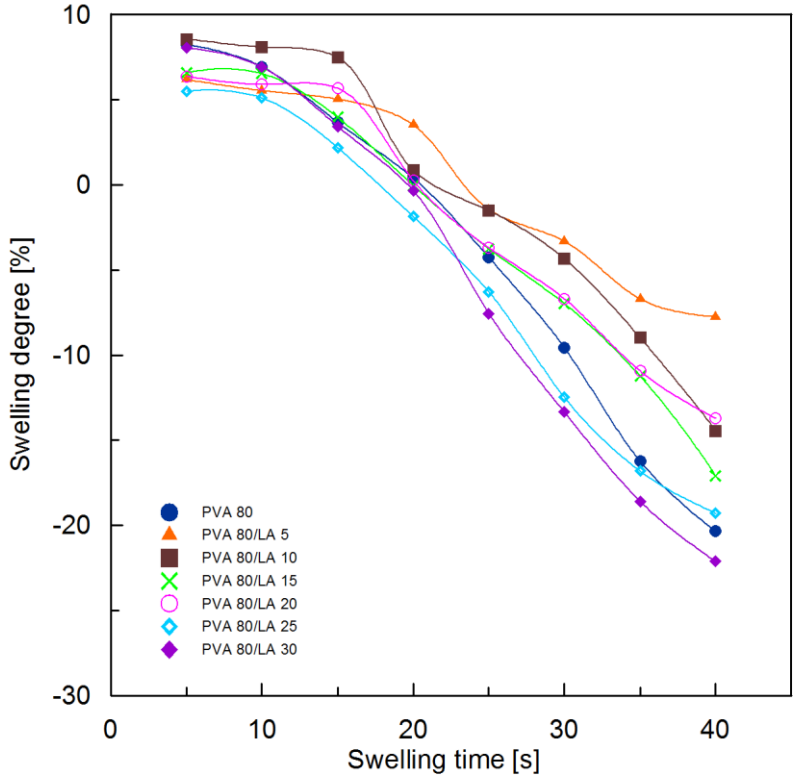


Figure 36: Water-swelling behavior of the PVA 80 based polymer films modified with LA

The 8 mol % in HD (difference in HD between PVA 80 and PVA 8-88) means relatively significant difference in solubility of the PVA films (Figure 37). The solubility, characterized by reduction of swelling degree occurs after more than 30 s in most of the cases. The influence of LA modification of sample-water interaction is not unambiguous since the results do not show a logical trend. It can be elucidated by rather low accuracy of the method used for swelling degree determination in this study despite often using of this methodology scientific works.

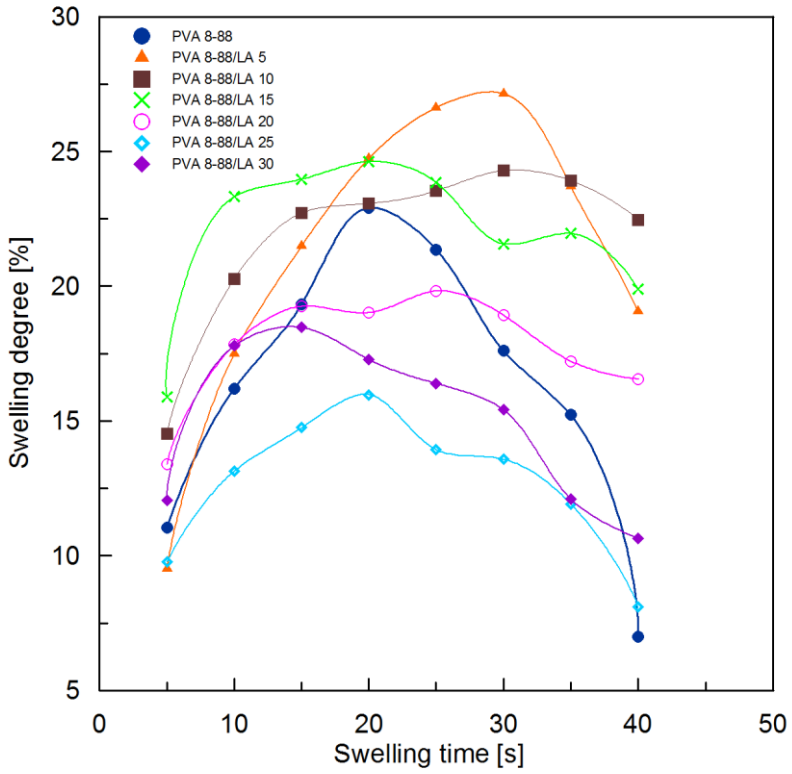


Figure 37: Water-swelling behaviour of the PVA 8-88 based polymer films modified with LA

The matrix with the highest investigated hydrolysis degree, PVA 6-98, (98 mol %) proves totally different behaviour in comparison with PVA 80 and PVA 8-88 based samples. Figure 38 shows time dependence of water-swelling degree of pure PVA 6-98 and PVA 6-98 modified with various content of LA. The swelling ration is riding steeply with the time. There were not any evidences of dissolution even after 600 s of the experiment. It is typical feature of fully hydrolyzed PVA. The influence of the modification with LA shows possible accordance with the assumption of the esterification reaction between PVA and LA. The observed swelling degree values of the PVA6-98/LA films are lower or on the comparable level with unmodified matrix up to 20 wt. % of LA. The higher additions lead to the increased swelling ratio. It may mean that random PVA chain grafting by LA could reduce the mobility of the modified macromolecules and reduce the transport of the solvent molecules during the

material-solvent interaction. On the other hand, the excess of LA can lead to preferable release of the non-grafted LA into the liquid medium.

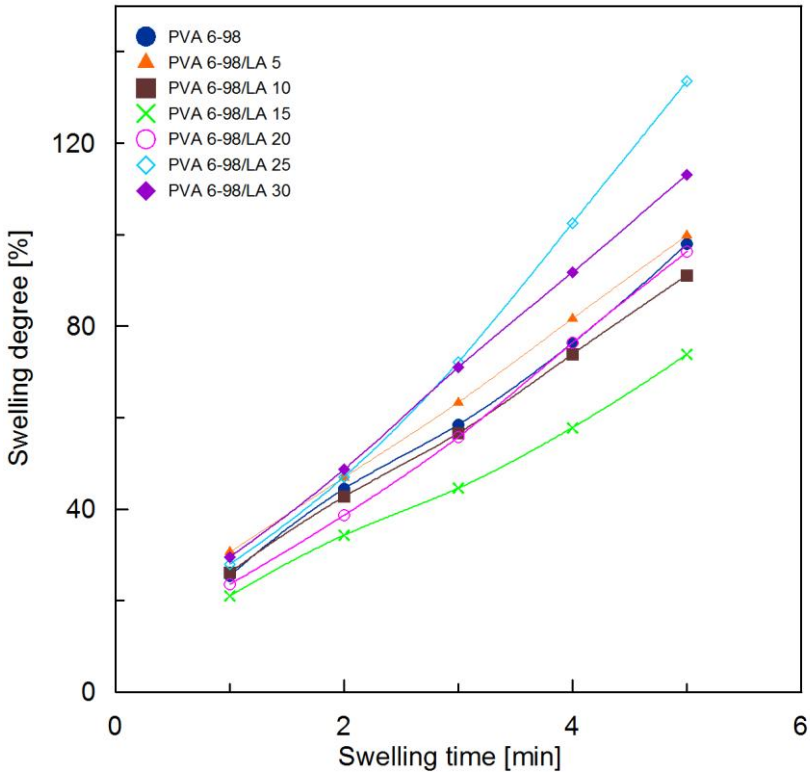


Figure 38: Water-swelling behaviour of the PVA 6-98 based polymer films modified with LA

Antibacterial activity

The antibacterial activity of the prepared samples was studied by using the agar diffusion method as well as dilution and spread plate technique against the representatives of both Gram positive (G+) and Gram negative (G-) bacterial strains. The methodology of the used methods is described in chapter 2.2.6. Since PVA/LA antibacterial activity has been already reported [92], the main goal of this study was to determine the influence of HD on antibacterial properties of the PVA films containing various amount of LA.

The results obtained from the first method, agar diffusion test, are shown in Figures 39 and 40 for G+ *Staphylococcus aureus* and G- *Escherichia coli*, respectively.

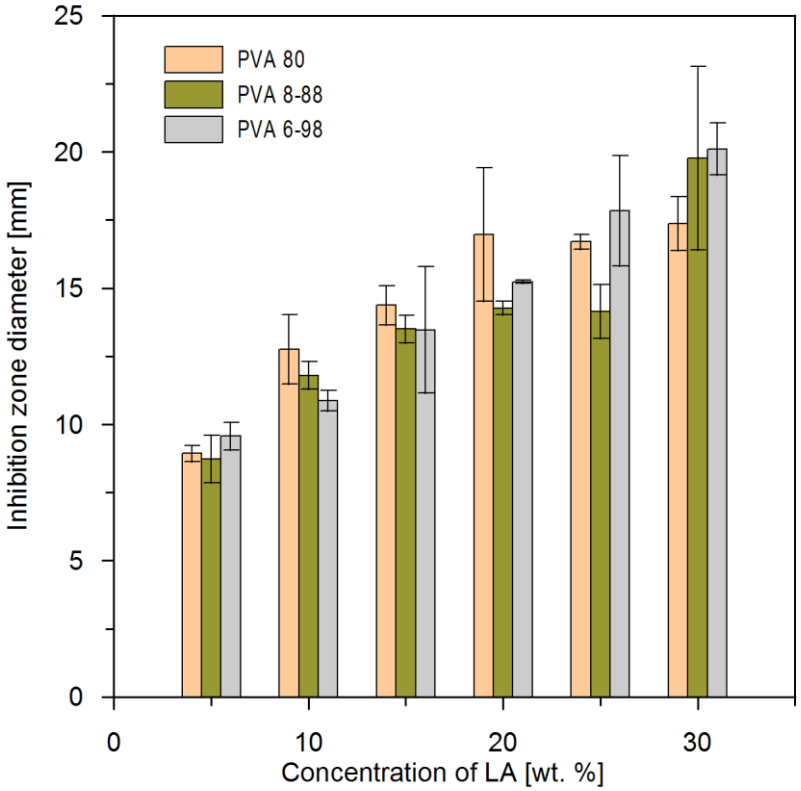


Figure 39: Inhibition zone diameter versus concentration of LA in the PVA based films for *Staphylococcus aureus*

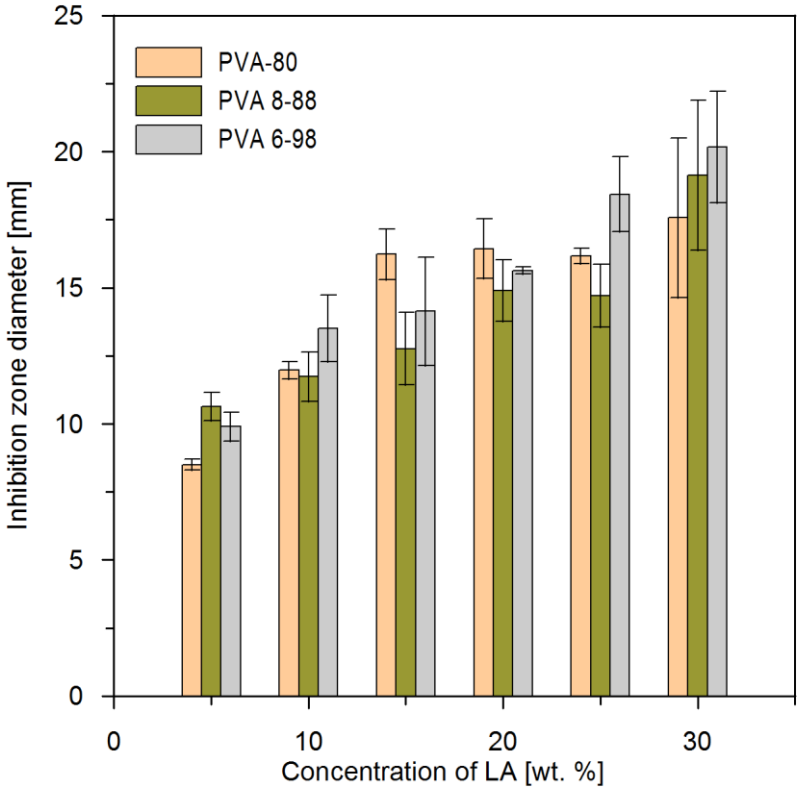


Figure 40: Inhibition zone diameter versus concentration of LA in the PVA based films for *Escherichia coli*

The results reveal that LA has more or less similar inhibition effect against both *Staphylococcus aureus* and *Escherichia coli*. Generally, the higher concentration of LA the larger growth inhibition area is represented by inhibition zone diameter in Figures 39 and 40. The effect of HD on antibacterial activity of the prepared materials is not obvious due to the methodology settings selected for this study.

The second method used for the assessment of antibacterial properties of the prepared samples can describe the activity of a material in liquid medium where the diffusion of an antibacterial agent from the polymer is not reduced by the solidified agar medium i.e. this method offers relatively higher sensitivity and accuracy in comparison to the simple measurement of inhibition zones diameters. On the other hand, this method is more time consuming. The results in form of the CFU number were used for calculations of effectiveness of antibacterial activity (EAA) according to the Equation 9. These data can clearly demonstrate whether the material has certain antibacterial activity [118]. The results are shown in Table 5. It can be noticed that G- *Escherichia coli* shows higher sensitivity against LA in case the films based on partially hydrolyzed PVA 80 and PVA 8-88 at the LA content up to 20 wt. %. The samples with LA presence 25 and 30 wt. % were 100 % effective in all cases.

The role of HD cannot be fully discovered because of the solubility of the samples and high antibacterial efficiency of LA itself. The standard procedure for this kind of experiment includes 24 hours incubation of the inoculated agar plates. However, the swelling-solubility studies (see above) showed that all material can be dissolved during several minutes. Thus, possible differences which could occur due to various HD are overlaid by long period needed for bacterial incubation. Nevertheless, it can be conclude that all samples proved significant antibacterial action against tested bacterial strains.

Table 5: Antibacterial activity of polymeric films based on partially and fully hydrolyzed PVA modified with LA

PVA	Sample index	EAA [%]	
		Staphylococcus aureus	*Escherichia coli*
Partially hydrolyzed	**PVA 80**	0.00 ± 0.0	0.00 ± 0.00
	PVA 80/LA 5	19.95 ± 11.49	16.77 ± 10.75
	PVA 80/LA 10	37.52 ± 6.78	62.77 ± 4.12
	PVA 80/LA 15	46.75 ± 11.24	95.23 ± 0.85
	PVA 80/LA 20	58.79 ± 8.88	97.42 ± 0.81
	PVA 80/LA 25	90.34 ± 0.52	99.00 ± 0.00
	PVA 80/LA 30	95.73 ± 0.91	100.0 ± 0.00
	PVA 8-88	0.00 ± 0.00	0.00 ± 0.0
	PVA 8-88/LA 5	23.73 ± 10.77	12.84 ± 5.31
	PVA 8-88/LA 10	33.01 ± 11.04	63.58 ± 8.84
	PVA 8-88/LA 15	46.51 ± 13.73	96.42 ± 0.74
	PVA 8-88/LA 20	64.11 ± 7.96	99.69 ± 0.11
	PVA 8-88/LA 25	89.38 ± 1.50	100.0 ± 0.00
	PVA 8-88/LA 30	95.89 ± 1.33	100.0 ± 0.00
Fully hydrolyzed	**PVA 6-98**	0.00 ± 0.00	0.00 ± 0.00
	PVA 6-98/LA 5	28.27 ± 11.09	14.50 ± 5.62
	PVA 6-98/LA 10	35.82 ± 6.79	56.81 ± 8.09
	PVA 6-98/LA 15	62.49 ± 7.53	99.67 ± 0.11
	PVA 6-98/LA 20	69.42 ± 12.22	100.0 ± 0.00
	PVA 6-98/LA 25	95.98 ± 3.24	100.0 ± 0.00
	PVA 6-98/LA 30	97.16 ± 0.50	100.0 ± 0.00

3.2 The effect of crosslinking on mechanical properties and solubility of the PVA/LA polymer films

Since PVA is characteristic by its water-solubility, the crosslinking reaction is needed to ensure it protection against surrounding solvent, prolong the time of water resistance or even control this property according the required conditions. PVA is chemically polyolic compound. Its hydroxyl groups offer several possibilities for crosslinking reaction with a multi-functional compound. One of the possibilities is reaction with isocyanates to form polyurethane-like structure. However, the most popular way of PVA chemical crosslinking is reaction with aldehydes. Polyvinyl alcohol reacts with aldehydes in acid media to form water insoluble polyacetals [119]. Chemical reaction is shown in Figure 22.

The aim of this study is to determine the effect of LA modification on crosslinkability of the PVA films by glutaraldehyde (GAD). The results could provide important information, which could help during designing of the microcapsule preparation experiment. It should be reminded that highly reactive GAD is toxic. Thus, the least amount of crosslinker is needed to be used for microcapsule preparation due to the health concern about the toxic effects of the non-reacted GAD. On the other hand, the sufficient amount of GAD must be used to ensure required stability and mechanical properties of the prepared systems.

For further experiment only PVA 8-88 was chosen due to its medium water-solubility and work-friendly properties.

Mechanical properties testing

The results from tensile properties testing are shown in the Figures 41 and 42 as LA concentration dependences of tensile strength and tensile strain, respectively. It can be noticed that mechanical properties of the pure PVA matrix and crosslinked PVA are totally different as could be expected. The comparison presented in Figure 41 can prove the crosslinking ability of GAD (0.25 wt. % - related to PVA mass). Interestingly, half addition of GAD (0.125 wt. %) leads to reduction of tensile strength value. It can be also noticed that increasing concentration of LA causes decrease in tensile strength. This reduction has gradual trend. The crosslinked samples containing above 10 wt. % of LA have significantly enhanced tensile strength in comparison with not-crosslinked films.

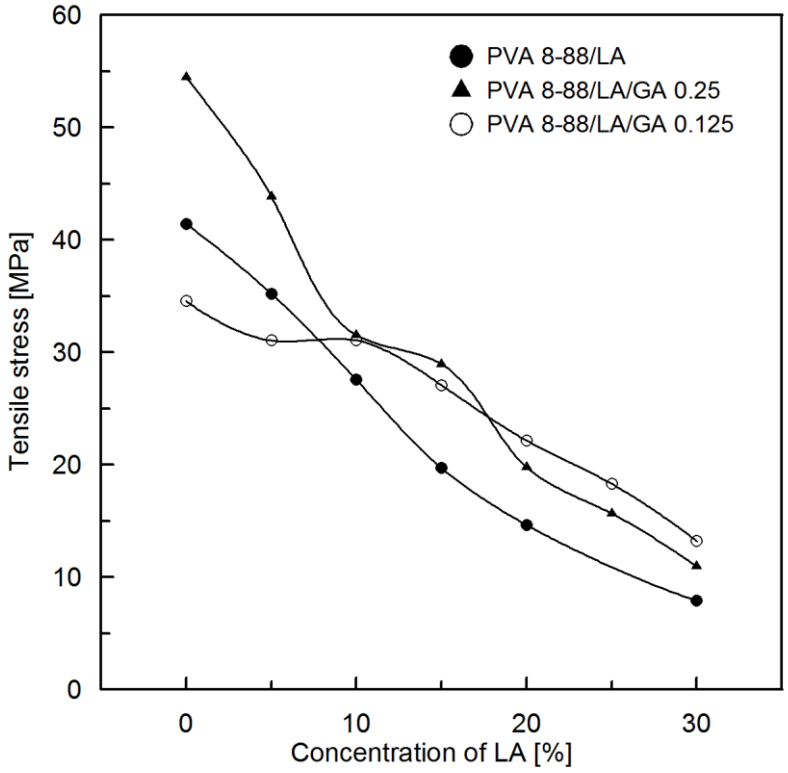


Figure 41: Effect of crosslinking on tensile strength of PVA 8-88/LA films

The maximal elongation observed at the tensile experiment for the same specimens is presented in Figure 42. The crosslinking addition leads to reduced

tensile strain in case of pure PVA 8-88 and PVA8-88/LA up to 15 wt. %. The excess of LA (above 20 wt. %) causes significant rising of maximal elongation value, which was out of range of the used tensile testing machine (above 500 %), which is represented by dashed line in the Figure 42.

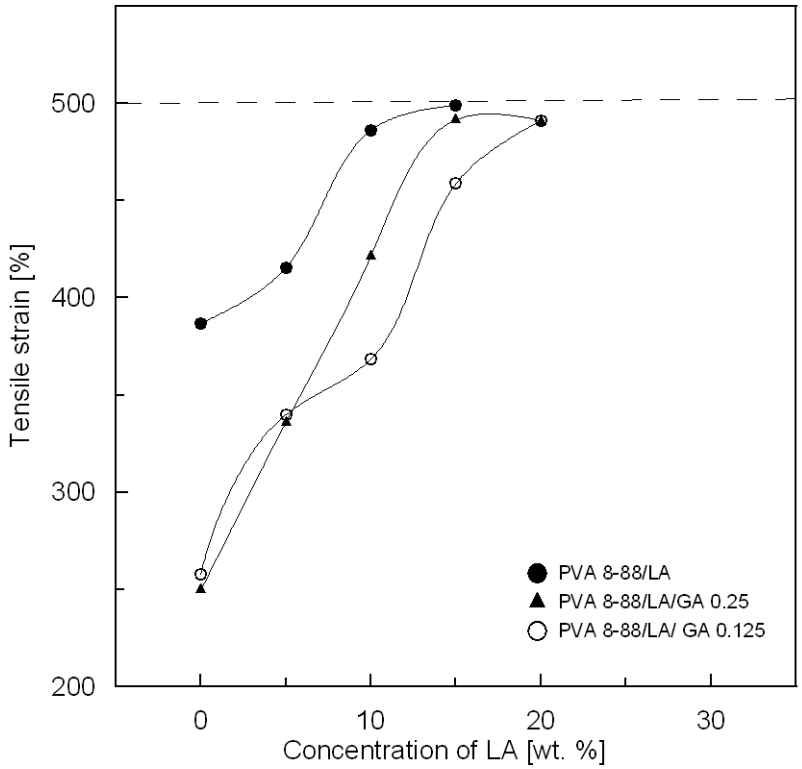


Figure 42: Effect of crosslinking on tensile strain of PVA 8-88/LA films

Water-swelling and solubility testing

The results from the mechanical properties of the PVA/LA films crosslinked with GAD showed that 0.25 wt. % presence of GAD can ensure a sufficient crosslinking action. This series of samples were further investigated on water-solubility. The obtained data are presented in Figure 43 for pure PVA 8-88 and Figures 44 and 45 for PVA 8-88 films modified with various amount of LA. The reason for separation of the results were in substantial differences in solubility as will be shown further.

As it has been already mentioned, Figure 43 presents time dependencies of swelling degree and solubility of the unmodified PVA 8-88 films crosslinked

with 0.25 wt. of GAD. The degree of swelling increases up to 5 min of the experiment period and then drops steeply. The solubility data are in accordance with that. The longer testing times will lead to further reduction of degree of swelling and solubility rising as it has been described in Figure 37. However, the time to reach the point of swelling degree drop is ten times as high as in case of non-crosslinked PVA 8-88 (Figure 37).

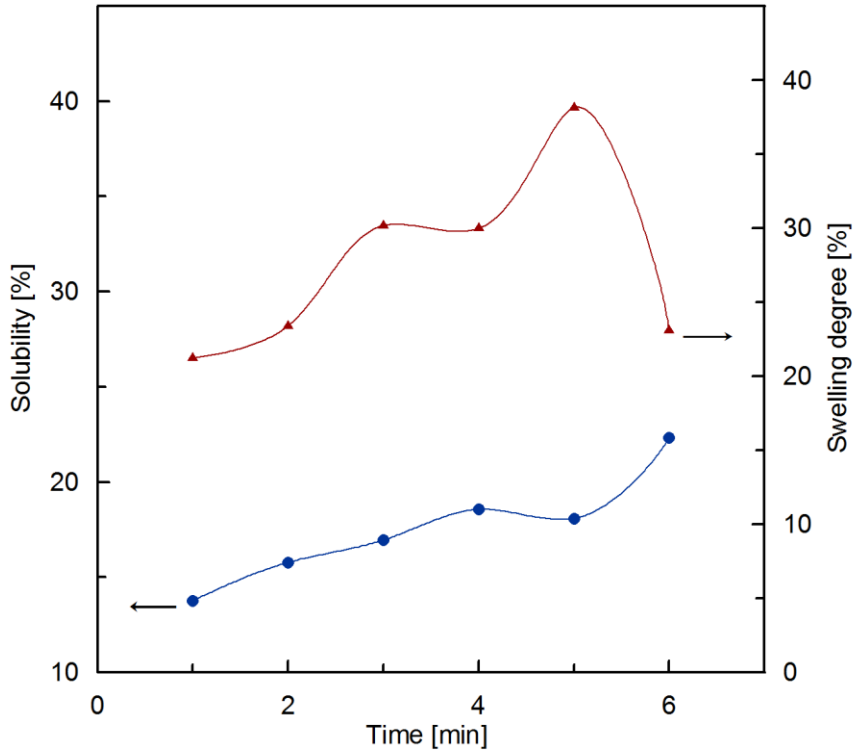


Figure 43: Time dependences of degree of swelling and solubility for unmodified PVA 8-88

To keep the certain level of good arrangement, the results collected for PVA 8-88/LA based films are shown separately in Figures 44 and 45. The clear evidence of a positive effect of LA presence on crosslinking occurrence can be found in Figure 44. The swelling degree of the film was dramatically increased already at 5 wt. % concentration of LA in the system. The further LA additions (10 wt. % and more) lead to enhancement of this characteristic up to approximately 1500 % (i.e. almost twice as high as for the film containing 5 wt. % LA).

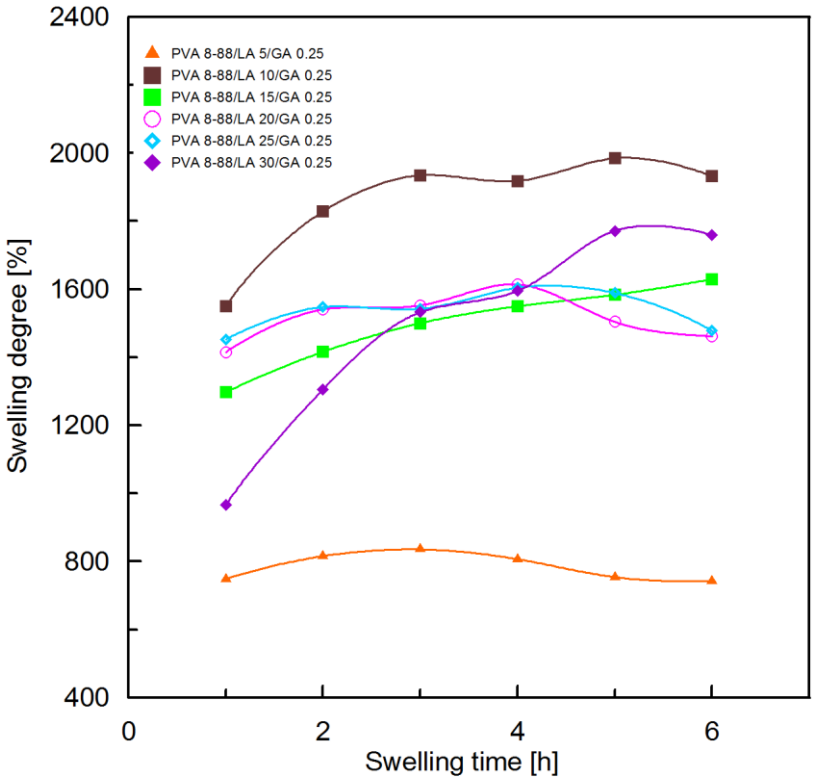


Figure 44: Time dependences of degree of swelling for PVA 8-88/LA films

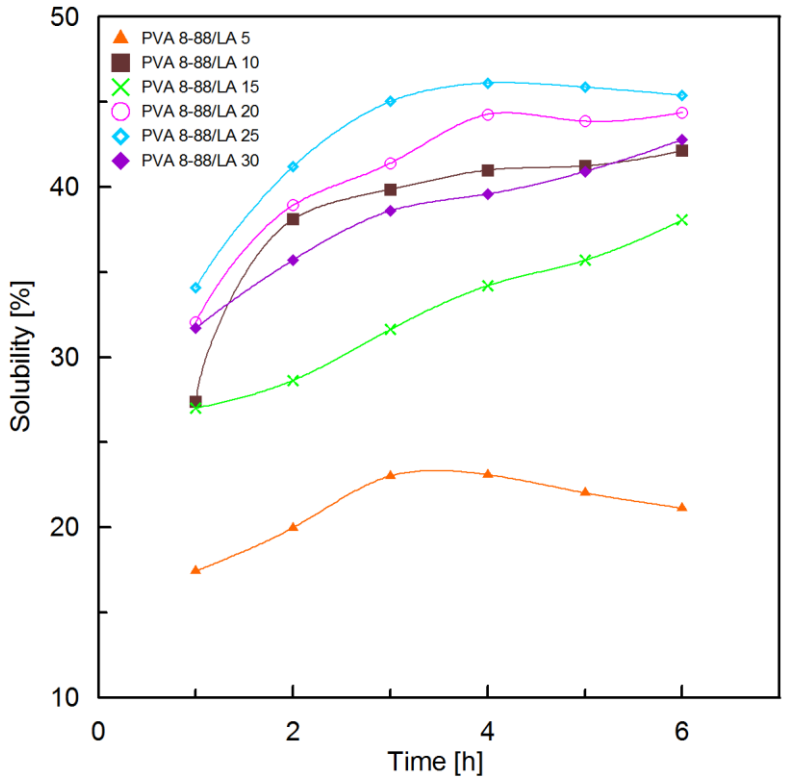


Figure 45: Time dependences of degree of solubility for PVA 8-88/LA films

The solubility versus time dependence (Figure 45) keeps similar trend as could be seen in the previous Figure 44. The sample containing 5 wt. % of LA has the lowest solubility in comparison with the films with higher amount of modifier. These results could indicate the formation of 3D polymer network due to GAD crosslinking action. This phenomenon can be expected since PVA has polyol structure and already bi-functional crosslinking agent is sufficient to form a network structure. In addition, GAD is known to be highly reactive at low pH. This is the reason, why all crosslinking reaction mediated by GAD is proceeded in acidic conditions in presence of a strong acid. Despite LA does not belong into the group of the strong acids, it can decrease pH and make more favourable conditions for the crosslinking reaction in comparison with unmodified PVA where pH of its water solution is higher. The experimental measurements showed that PVA solution containing 30 wt. % of LA proves pH=2.77. On the other hand, excess of LA, which is not chemically incorporated into the polymer structure, can cause its fast release into water (solvent) and thus increase the solubility values apparently.

3.3 Optimization of crosslinking agent concentration in PVA/BCAR microcapsules preparation

The following chapter deals with the process of microencapsulation of BCAR dissolved in silicone oil. PVA with various concentrations of acetyl moieties in polymer backbone chain was used as a shell material. The microcapsules characteristics such as encapsulation efficiency, size distribution of the microcapsules, structural studies, in-vitro release studies and stability testing were the object of the investigation.

As mentioned above, simple coacervation technique was used for PVA/BCAR microcapsules. This method has been described elsewhere. In this work, the microcapsules were prepared according to T. K. Maji *et. al.*[120]. It

must be mentioned that only PVA 8-88 and PVA 6-98 were used for further experiments due to the same reasons as indicated above.

Encapsulation parameters

The parameters of the capsules such as dimensions, size distributions, efficiency of encapsulation etc. depend on many factors. Intensity of mixing (emulsification), type of mixing device (e.g. propeller geometry), concentration of polymer, crosslinker, and encapsulated (core) material belong among them [121]. This experiment is focused on the investigation of PVA matrix HD effect on encapsulation parameters for hydrophobic BCAR containing compound in dependence on amount of crosslinker (GAD). The design of the experiments is shown in the Tables 6 and 7 below.

Table 6: Design of the microcapsules preparation experiment for PVA 8-88

Experiment	PVA (8-88) (0.25 wt. %) [ml]	Na_2SO_4 (20 wt. %) [ml]	Si.oil-BCAR (0.46 mg/ml) [ml]	Crosslinking solution [ml]	GAD concentration [mmol]
A	100	50	15	25	11.4842
B				23	10.5673
C				21	9.6484
D				19	8.7295
E				17	7.8106

Table 6: Design of the microcapsules preparation experiment for PVA 6-98

Experiment	PVA (6-98) (0.25 wt.%) [ml]	Na_2SO_4 (20 wt. %) [ml]	Si.oil-BCAR (0.46 mg/ml) [ml]	Crosslinking solution [ml]	GAD concentration [mmol]
F	100	50	15	25	11.4842
G				23	10.5673
H				21	9.6484
I				19	8.7295
J				17	7.8106

Following optical micrographs (OM) (Figures 46-49) show the visualization of the prepared microcapsules morphology at two magnifications (100x and 40x).

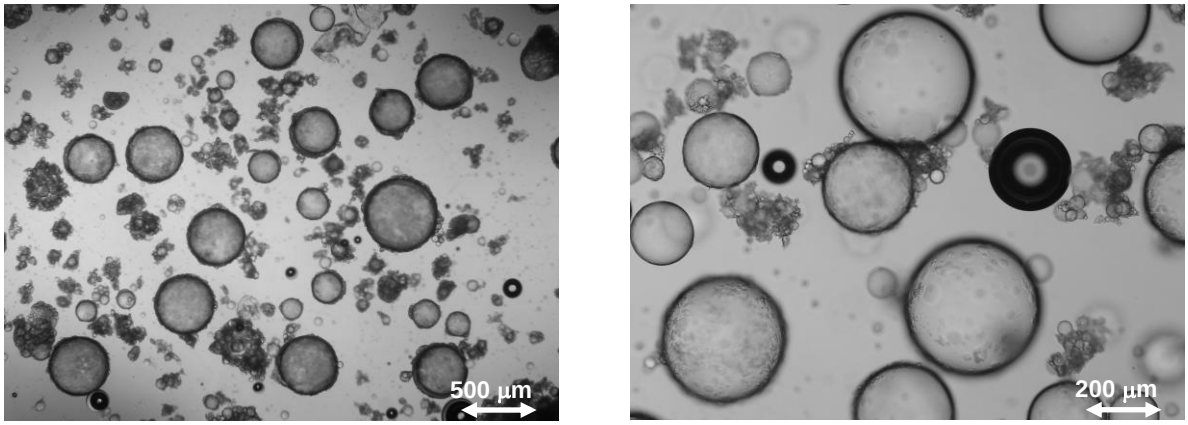


Figure 46: Optical micrographs of PVA 8-88/BCAR microcapsules after preparation
Experiment A

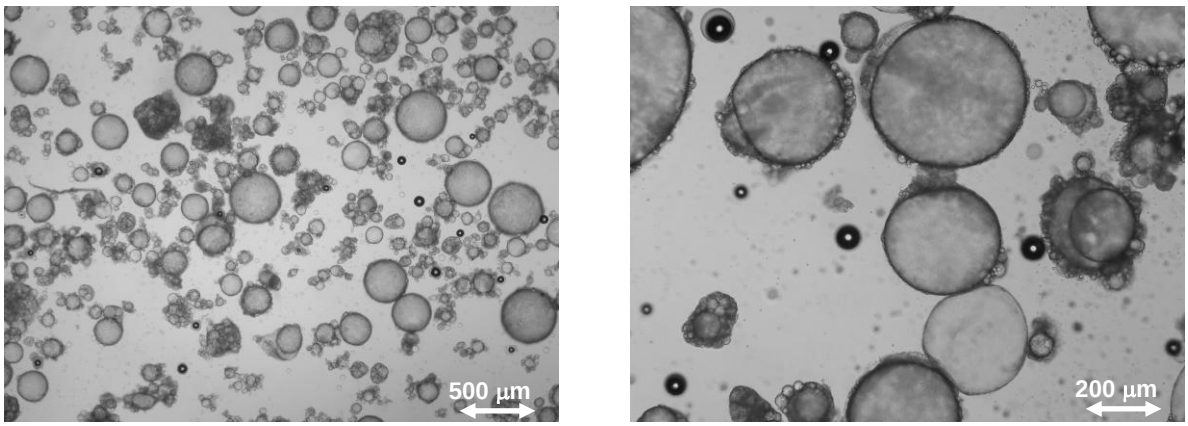


Figure 47: Optical micrographs of PVA 8-88/BCAR microcapsules after preparation
Experiment E

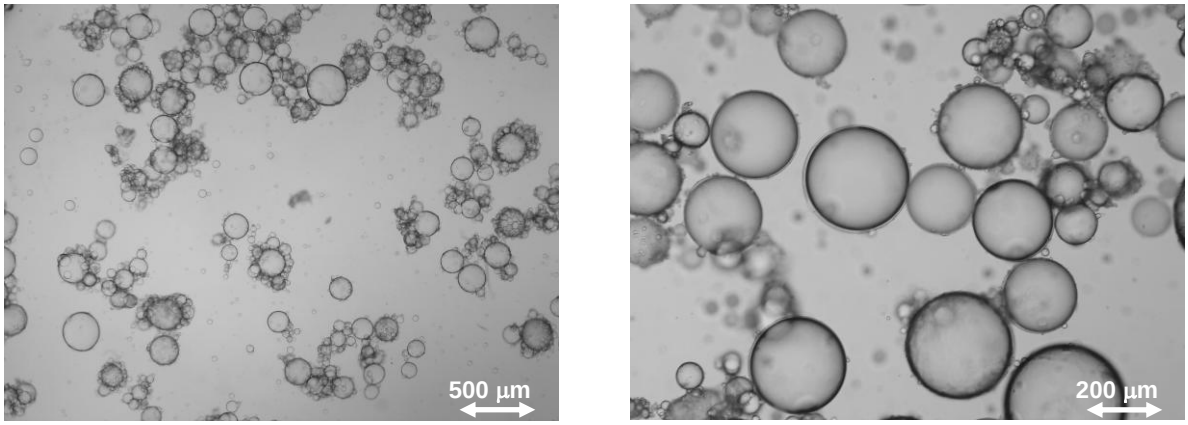


Figure 48: Optical micrographs of PVA 6-98/BCAR microcapsules after preparation
Experiment F

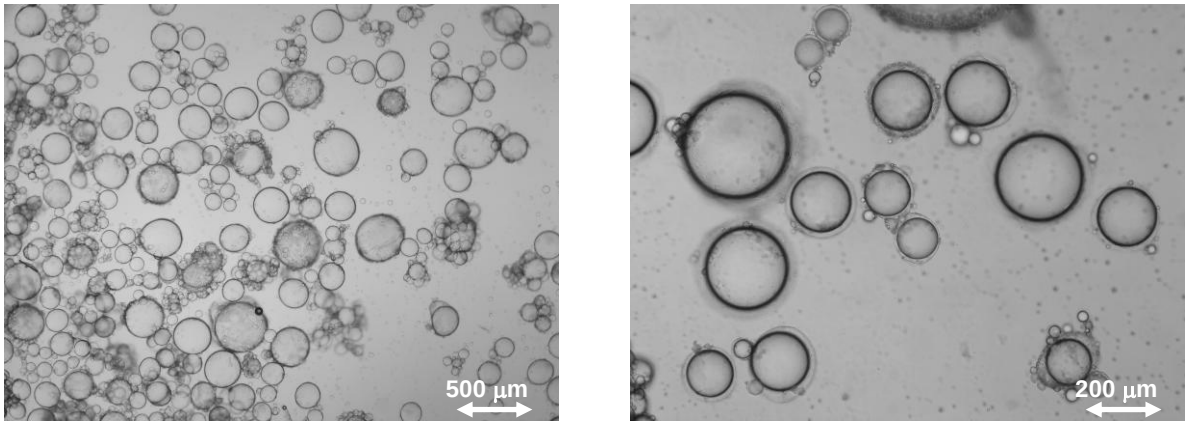


Figure 49: Optical micrographs of PVA 6-98/BCAR microcapsules after preparation

Experiment J

Figures 46 and 47 show the OM of microcapsules consisting of Si. oil with BCAR as a core material and partially hydrolyzed PVA 8-88 as shell. PVA matrix is crosslinked with various amount of GAD (see Table 6). The results of the experiments presenting the limit investigated GAD concentrations are shown here. It can be noticed that higher amount of crosslinker causes formation of more robust microcapsules shell. The microcapsules based on PVA 6-98/BCAR (Figures 48 and 49) prove the same trend. The typical surface morphology of the capsules is shown in Figure 50. It is characteristic by its typical roughness.

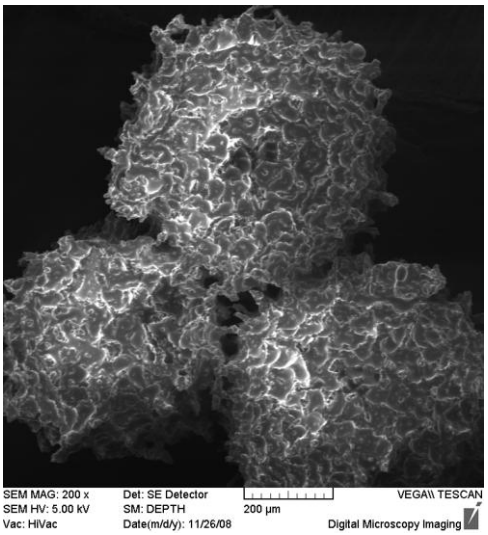


Figure 50: Typical SEM picture of PVA/BCAR microcapsule

The statistical analysis of the microcapsules size dimensions according the method introduced above (subchapter 2.2.7) allows the calculation of average diameters of the microcapsules (Table 8) and depicting of size distribution histograms (Figures 51 and 52). Generally, the data presented in Table 8 reveal that lower HD of PVA matrix may lead to formation of smaller microcapsules at given conditions of their preparation (mixing process, temperature, concentration of polymer etc.). It could be due to various viscosities of polymer solutions. On the other hand, viscosity is dependent on M_w as well. This parameter is higher in case of PVA 8-88 (M_w ~ 67.000 g.mol^{-1} PVA 6-98 with M_w ~ 47.000 g.mol^{-1}). On the other hand, the statistical certainty is too low to consider this assumption as considerable.

Table 8: Average diameters of microcapsules obtained through analysis of OM pictures. Experiment A-J

Experiment	d_n [μm]	d_w [μm]	d_w/d_n	d_z [μm]
A	151.47	188.08	1.24	224.27
B	124.0	154.618	1.25	189.24
C	153.16	179.36	1.17	206.08
D	134.91	165.34	1.22	200.63
E	123.03	148.62	1.21	179.89
F	140.63	155.79	1.11	170.93
G	150.38	169.83	1.13	192.59
H	149.02	164.10	1.10	183.24
I	117.74	142.58	1.21	167.27
J	174.96	198.04	1.13	225.17

In spite of that, it can be concluded that weight average microcapsules diameter (d_w) is in the range 150 – 200 μm and relatively narrow polydispersities (d_w/d_n) were achieved in all cases (1.10 – 1.25).

The histograms showing (Figure 51 and 52) the microcapsules size distributions for all experiments reveal in accordance with the results presented in Table 8. The capsules based on PVA 8-88 are characteristic by dimension distribution with the peak intensity positioned close to 100 μm and noticeable decreasing peak shoulder including particles with diameters within the range 150 – 450 μm (Figure 51). All frequency versus microcapsule diameter dependencies have typical Gaussian distribution shape except for Experiment I (Figure 52), which proved its histogram similar to that ones presented in Figure 51.

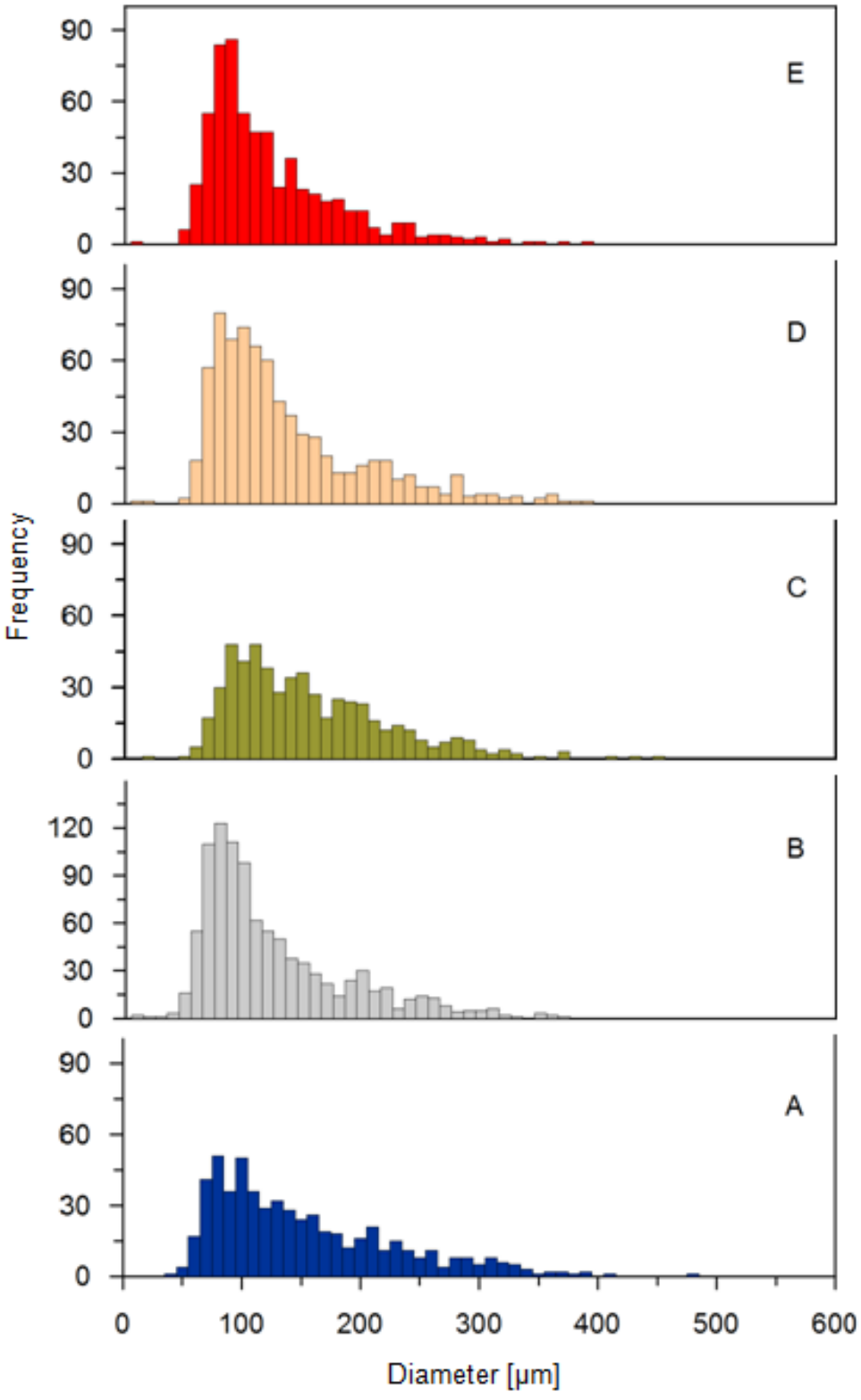


Figure 51: Histograms of PVA 8-88/BCAR microcapsules diameters distribution – Experiments A-E

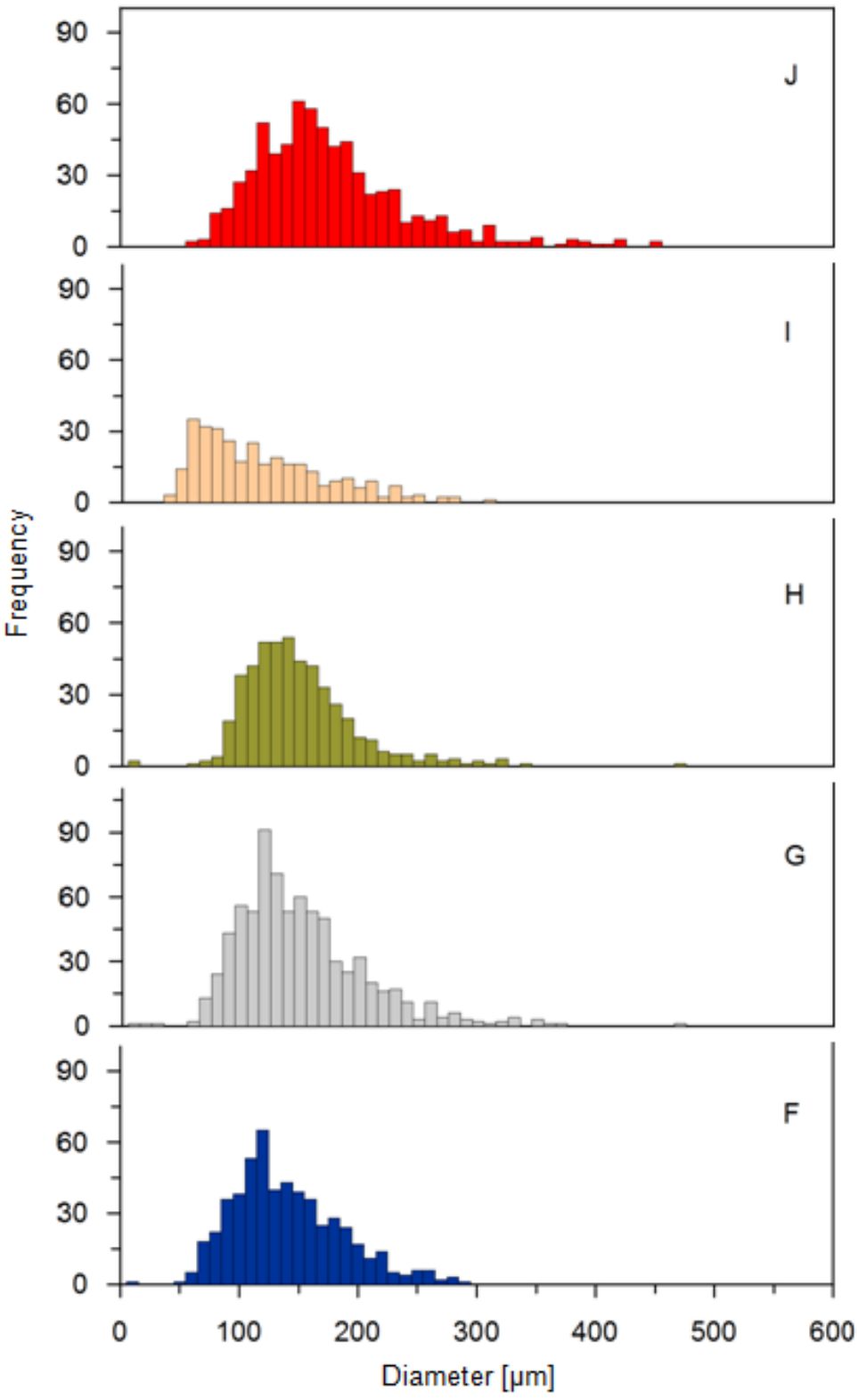


Figure 52: Histograms of PVA 6-98/BCAR microcapsules diameters distribution – Experiments F-J

Each encapsulation based system can be defined by the characteristics describing the efficiency of the encapsulation process itself. This indicator is extremely important from the practical point of view. The parameters showing the oil content, oil loading and encapsulation efficiency (calculated according Equations 10-12) are presented in the Table 9.

Table 9: Characteristics of the prepared PVA/BCAR microcapsules

Experiment	Oil content [%]	Oil loading [%]	Encapsulation efficiency [%]
A	28.43 ± 3.27	340.91 ± 12.52	14.46 ± 1.68
B	34.95 ± 0.80	368.73 ± 12.03	22.82 ± 2.47
C	39.70 ± 2.14	401.50 ± 9.25	21.91 ± 0.71
D	36.45 ± 0.87	440.66 ± 16.52	25.05 ± 1.25
E	37.45 ± 4.78	488.28 ± 10.56	23.23 ± 1.86
F	30.66 ± 4.31	340.91 ± 19.85	10.06 ± 0.40
G	39.93 ± 1.78	368.73 ± 14.20	20.96 ± 1.31
H	40.30 ± 1.10	401.50 ±10.25	22.73 ± 2.98
I	42.10 ± 4.19	440.66 ± 9.55	21.22 ± 0.86
J	35.51 ± 3.17	488.28 ± 8.99	17.84 ± 2.62

It can be noticed that the gravimetrically determined oil content varies from 20 to 40 wt. %. The lowest values were achieved at the highest GAD concentrations in case of both PVAs (Experiments A and E). The same observation can be found for oil loading ration as well as encapsulation efficiency. It reveals certain of these parameters on crosslinker concentration. From this point of view, an optimum can be achieved in the middle of the GAD concentration range used in this study.

Stability studies

As presented above, one of the potential applications of the microcapsules are the systems which are able to release an incorporated bioactive agent according to the given requirements. The release activity itself can be divided into two parts:

a) controlled time dependent release when the bioactive agent is released continuously into the surrounding environment
b) burst release when the bioactive agent is quickly released from the capsules; it is usually connected with controlled collapse of the shell-core system, which can be induced by change of pH, temperature, ionic strength etc. On the other hand, another approach can be in found in stability testing of the microcapsules.

PVA/BCAR microcapsules described in the text above were investigated on both released activities.

BCAR detection

The detection of a BCAR, which is part of the core material, was done by spectrometric measurements in visible range of the light (VIS). However, the methods already published in the scientific papers [122, 123] failed in our case, when various non-organic releasing media were used as will be discusses further. The immiscibility of core material (Si oil+BCAR) with the media (even after surfactant addition) makes the VIS analysis impossible. That is extraction based method was used in release experiments. Its procedure is described in chapter 2.2.7. Four organic solvents were used for construction of calibration dependence of absorbance on BCAR calibration. Table 10 shows absorbance spectra of core material (Si oil+BCAR) in various organic solvents.

Table 10: Absorption peak position maxima of BCAR in various organic solvents

Solvent	1st maximum (nm)	2nd maximum (nm)
Chloroform	462	485
Tetrahydrofuran	458	484
Toluene	462	489
Acetone	454	485

The slight change in absorbance maxima of BCAR in given solvent was observed. The first peak was relative strong in comparison to the second one. The highest absorbance was found for acetone due to poor oil-Ac miscibility. The calibrations for chloroform, tetrahydrofuran, and toluene are shown in Figure 53. It can be seen that linear dependence was obtained in all three cases.

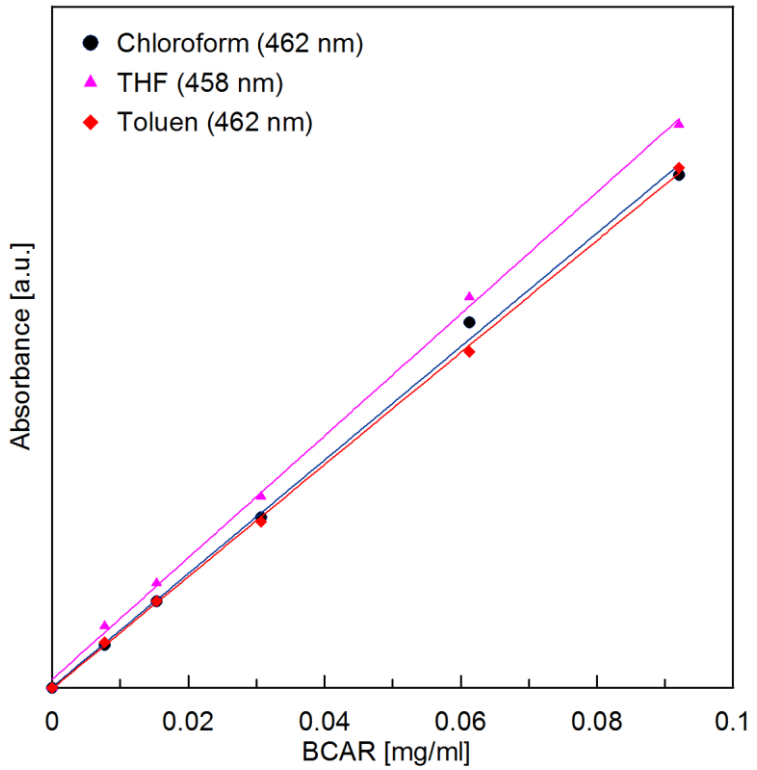


Figure 53: Calibration dependencies of BCAR in chloroform, tetrahydrofuran and toluene at given wavelengths

The linear regressions shown in Figure 53 have the parameters *k* and *q* equal 0 (*k*) and 0.999 (chloroform) and 0.998 (*q*) for tetrahydrofuran. Toluene proves

parameters a=0.002 and q=0.0. Absorbance versus BCAR concentration is not linear in the investigated range (not shown here). In addition, the mixture was not uniform due to poor acetone-Si oil miscibility. Chloroform was chosen for further experiments due to economical reasons.

Ad a) Controlled time dependent release studies

In vitro release studies were carried out in three kinds of release media; phosphate buffer (pH=7.4), hydrochloric acid solution (HCl, pH=2) and distilled water, at 37°C and under continuous shaking (100 rpm) for 48 hours. Each experiment was repeated three times. The results, however, did not show statistically reliable evidence of release activity. Certain release indications were observed in case of release into distilled water for Experiments A-E. Nevertheless, the clear evidence of that was not found and all tested samples (Experiments A-J) seem to be stable under given conditions.

OM (Figures 54-59) analysis of the samples based on PVA 8-88 is in accordance with that except already mentioned release into distilled water, where collapsed microcapsule counterparts were observed for microcapsules prepared by using minimal and maximal GAD amount (see Figures 55 and 58).

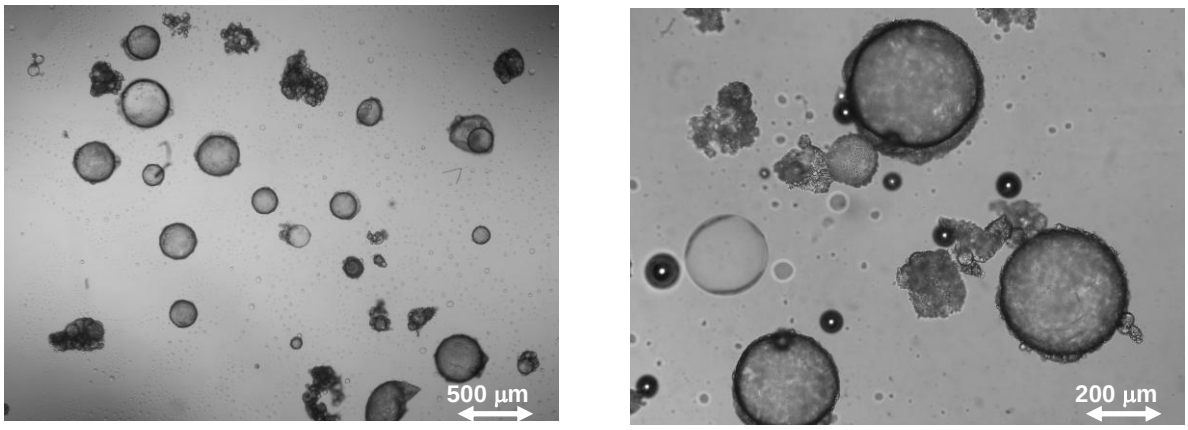
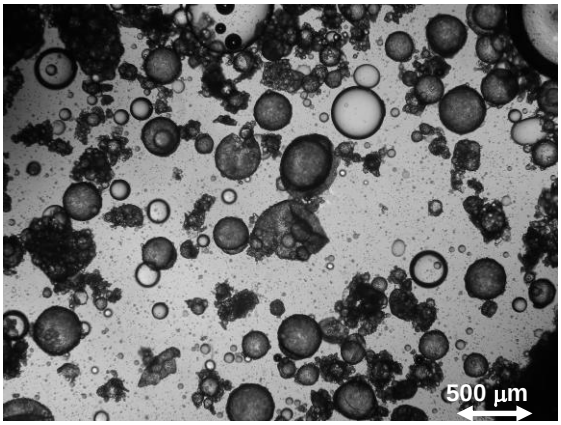


Figure 54: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in phosphate buffer (pH=7.4). **Experiment A**



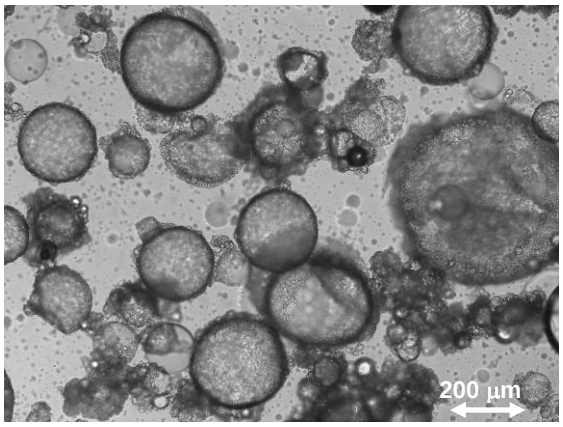
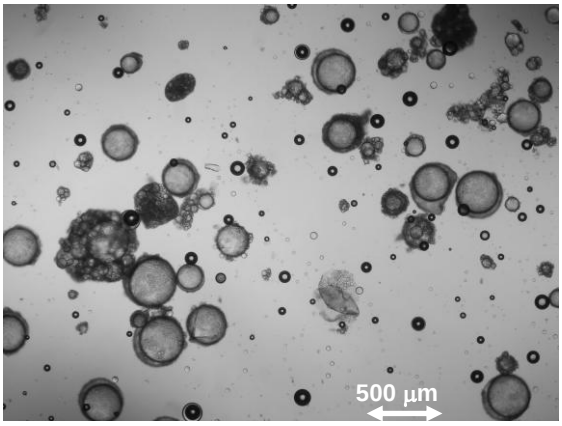


Figure 55: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in distilled water. **Experiment A**



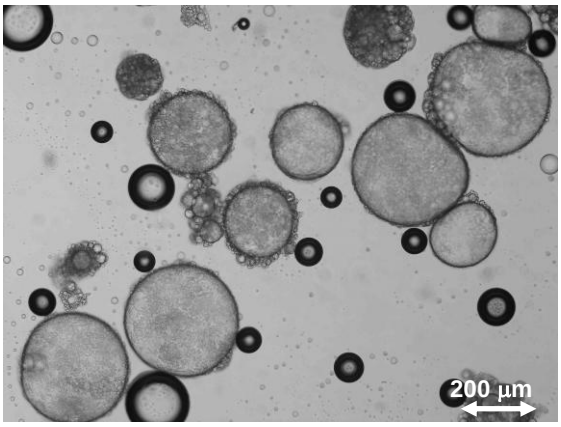
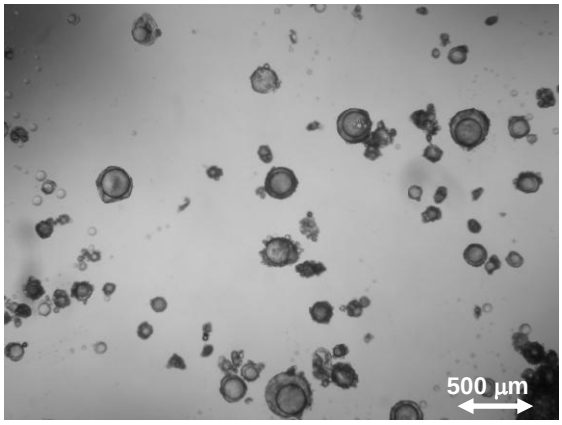


Figure 56: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in hydrochloric acid solution, (pH=2.0). **Experiment A**



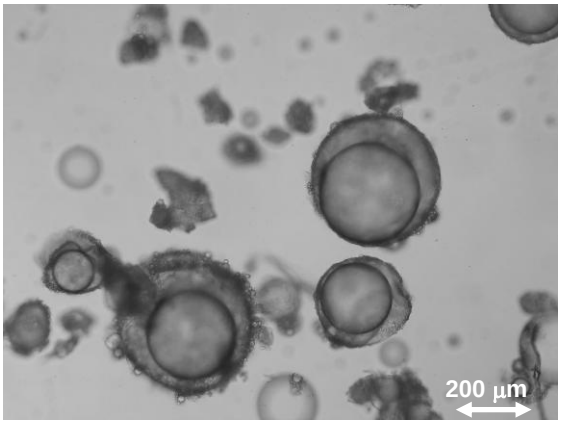
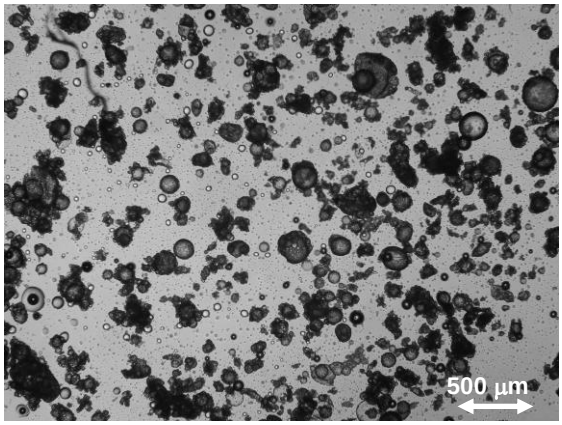


Figure 57: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in phosphate buffer (pH=7.4). **Experiment E**



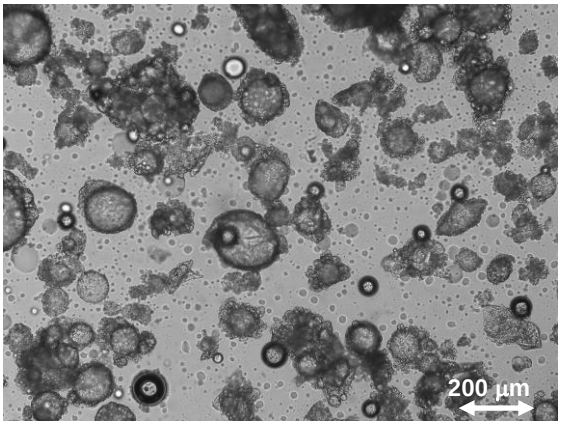
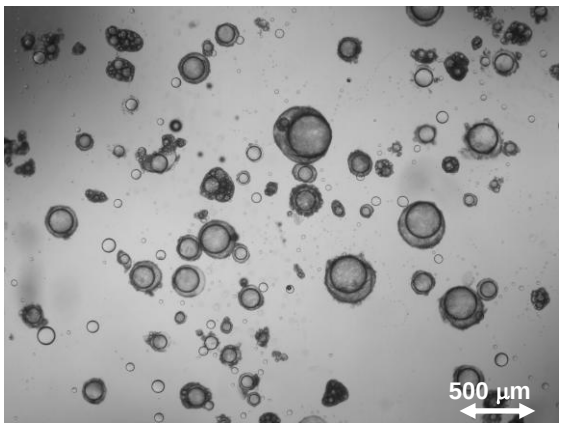


Figure 58: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in distilled water. **Experiment E**



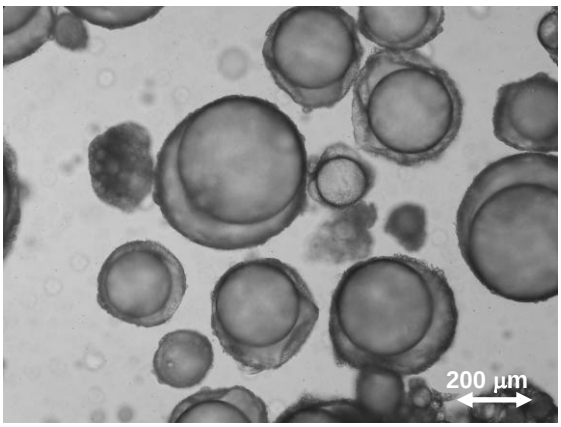
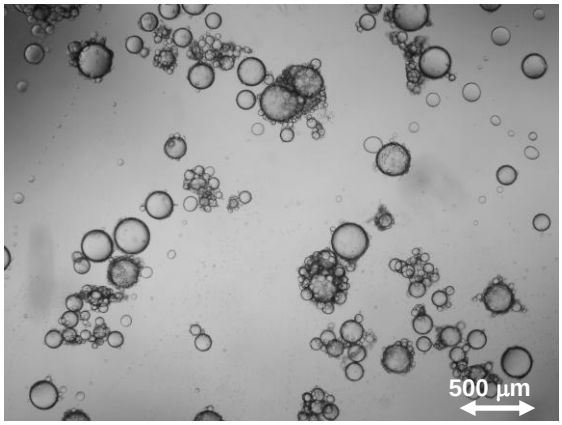


Figure 59: Optical micrographs of PVA 8-88/BCAR microcapsules after 48 hours release test in hydrochloric acid solution, (pH=2.0). **Experiment E**

The microcapsules based on PVA 6-98 (Experiments F-J) showed stability during the whole time of the release experiments and no release evidence was noticed. OM pictures confirm this fact (Figures 60-65). The reason of the apparently increased stability can be explained by chemical structure of the shell material. Higher HD of PVA matrix provides higher possibility for crosslinking reaction with GAD. This assumption has been already proved in case of cast PVA films (chapter 3.1).



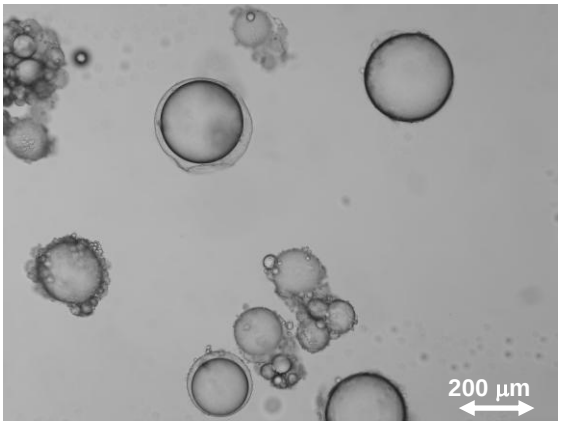
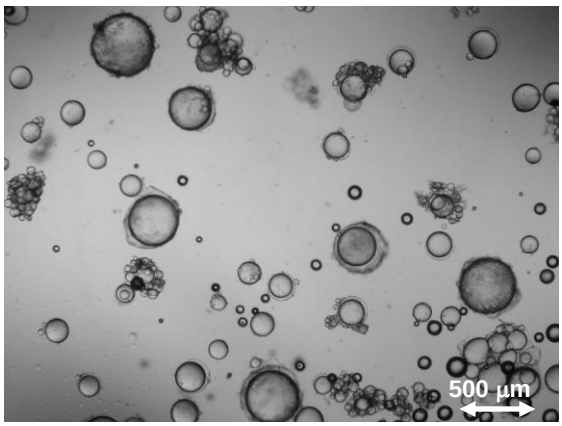


Figure 60: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in phosphate buffer (pH=7.4). **Experiment F**



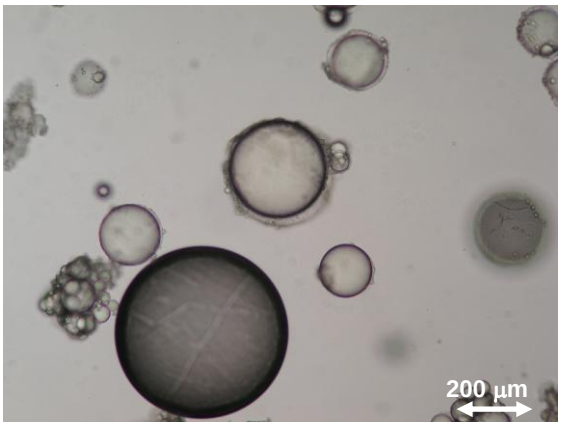
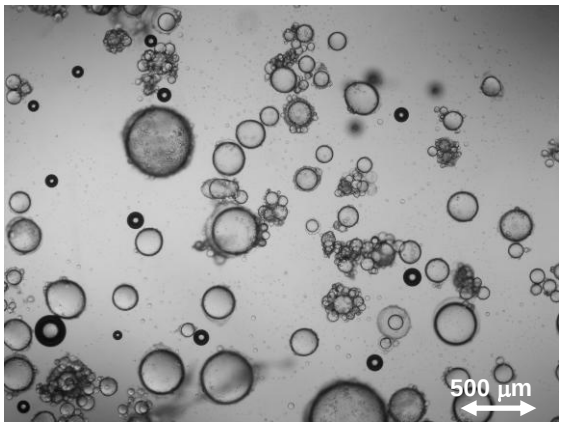


Figure 61: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in distilled water. **Experiment F**



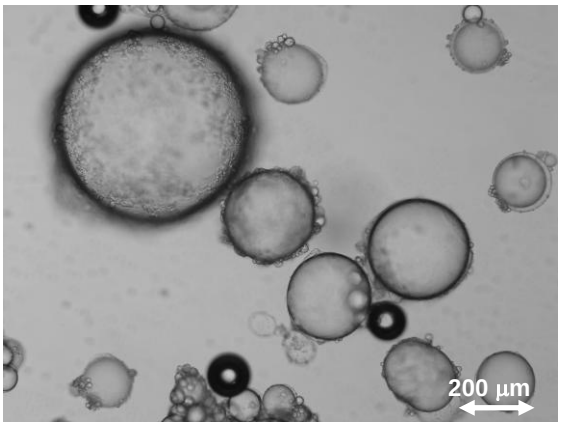


Figure 62: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in hydrochloric acid solution, (pH=2.0). **Experiment F**

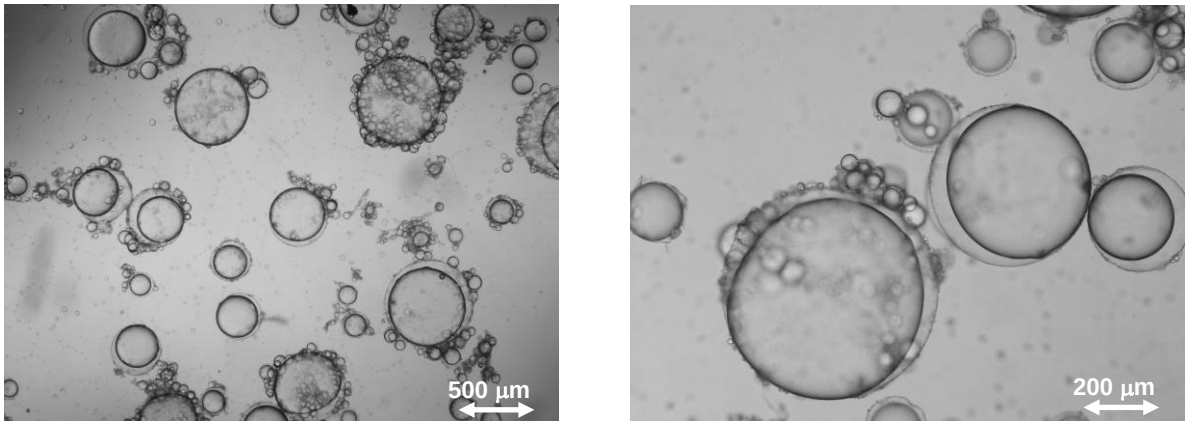


Figure 63: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in phosphate buffer (pH=7.4). **Experiment J**

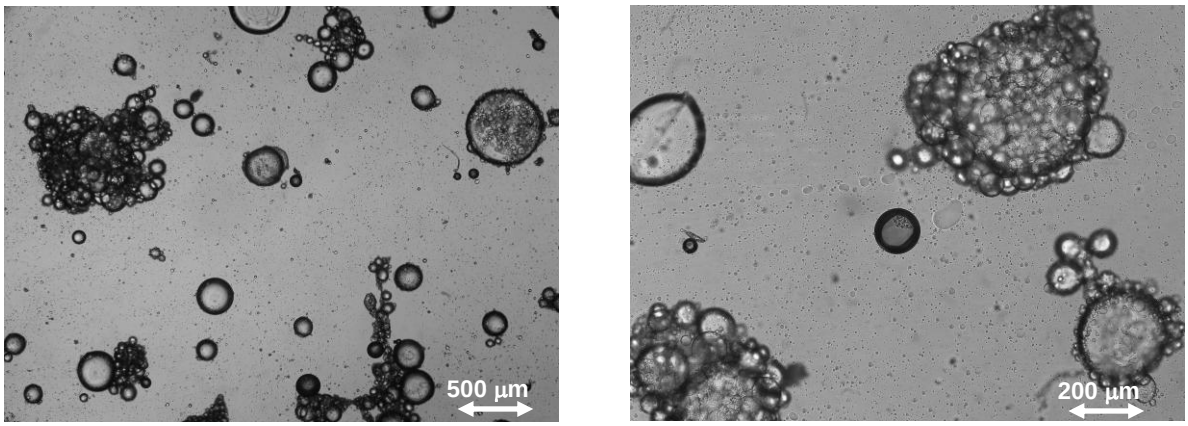


Figure 64: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in distilled water. **Experiment J**

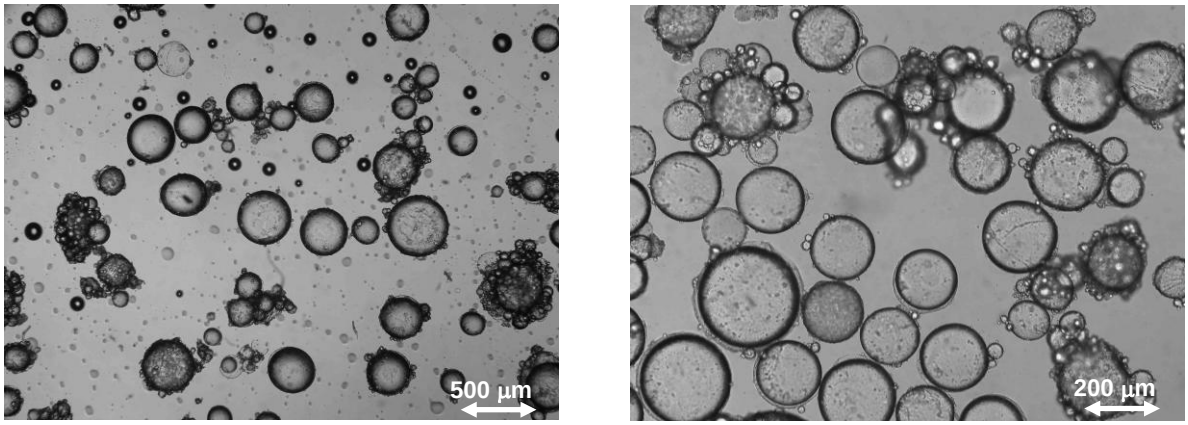


Figure 65: Optical micrographs of PVA 6-98/BCAR microcapsules after 48 hours release test in hydrochloric acid solution, (pH=2.0). **Experiment J**

Interestingly, the swelling of some of the microcapsules tested in distilled water and at pH=2 was noticed. This is evident in Figures 60 and 61 for the microcapsules originated in the Experiment F (the highest GAD amount) and more evidently in Figures 60-64 for the Experiment J, which represents the lowest crosslinker concentration used during microcapsules preparation. It is clear that low crosslinker concentration leads to formation of sparser polymer network, which is more accessible for molecules of a solvent and swelling subsequently. However, microspheres remain stable even in swollen state and core material was not released in the concentration above a detectable limit.

Ad b) Burst release and stability testing

The long term in-vitro stability of the microcapsules described in the previous text under relatively mild environment is encouraging for their stability testing under more aggressive conditions. The objective of this study was to find the limit of the microcapsules stability. The effect of the temperature and various releasing media (pH, ionic strength, chemical structure) was chosen due the practical reasons from the simulation of the real conditions point of view. Microcapsules prepared according to the procedure designated as Experiment E and J were taken for these experiments as the least stable representative sample (the lowest amount of GAD) of the systems based on both partially and fully HD. The following experiments have been done: testing of thermal stability in various media, autoclave test and organic solvents resistance test.

The first type of experiment was carried out in the five release media

- hydrochloride acid solution, pH=2
- phosphate buffer, pH=7.4
- carbonate buffer, pH=9.0
- physiological solution, NaCl water solution 9 wt. %
- distilled water

The temperature range used for stability testing was 37, 45, 70 and 100 °C. Time of temperature exposure was 6 hours. The following Table 11 shows the results of the experiments. Their evaluation was done by OM analysis.

Table 11: Temperature stability study of the selected microcapsules in various media (+ stable, - collapsed)

Medium	Experiment	Temperature [°C]		
		45	70	100
hydrochloride acid solution	E	+	+	-
	J	+	+	-
phosphate buffer	E	+	+	+
	J	+	+	+
physiological solution	E	+	+	+
	J	+	+	+
carbonate buffer	E	+	+	+
	J	+	+	+
distilled water	E	+	+	+
	J	+	+	+

As can be seen in Table 11, the capsules prove high stability under boiling conditions except for hydrochloride acid solution, which is in synergy with boiling temperature too aggressive for the shells of the capsules. The shells dissolved as a consequence of that.

Providing the testing of the stability is proceeded in water based media, it is rather possible to reach the temperatures above 100 °C under atmospheric pressure. One of the alternatives how to achieve higher temperature is autoclaving. The experiment was carried out in laboratory autoclave Tuttnauer 3870 EN. The microcapsules prepared according the procedure E and J were treated in distilled water containing closed glass bottles according the standard sterilization procedure (121 °C, 15 min, pressure 103.4 kPa).

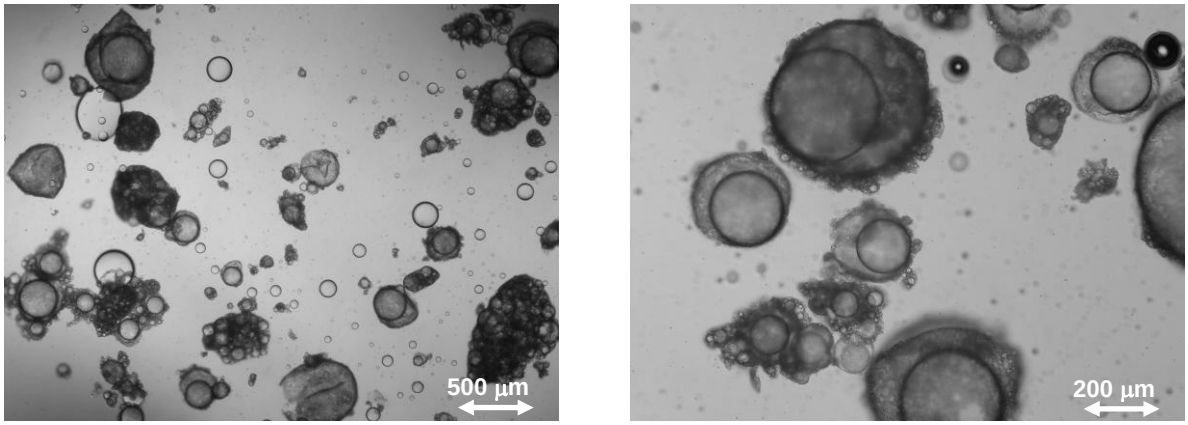


Figure 66: Optical micrographs of PVA 8-88/BCAR after autoclave sterilization
Experiment E

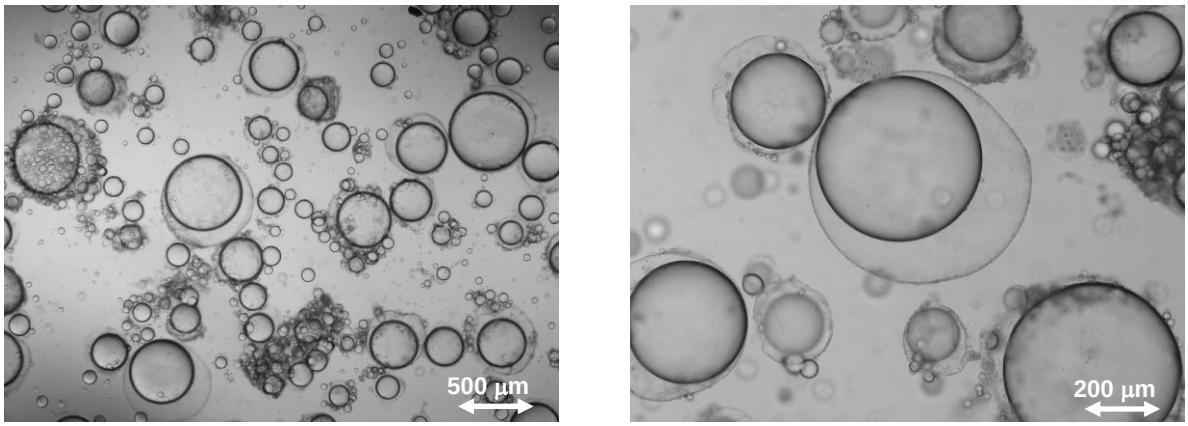


Figure 67: Optical micrographs of PVA 6-98/BCAR after autoclave sterilization
Experiment J

The results in form of OM (Figures 66 and 67) pictures show that some of the microcapsules collapsed due to the combination of heat and pressure. The samples based on PVA 8-88 seem to be more sensitive. On the other hand, relatively many capsules remained stable.

The last experiment is based on exposure of the microcapsules (Experiment E and J) to organic solvent (chloroform, tetrahydrofuran, toluene, dimethylformamide and acetone). The results of such experiment are presented in following OM pictures (Figures 68-77).

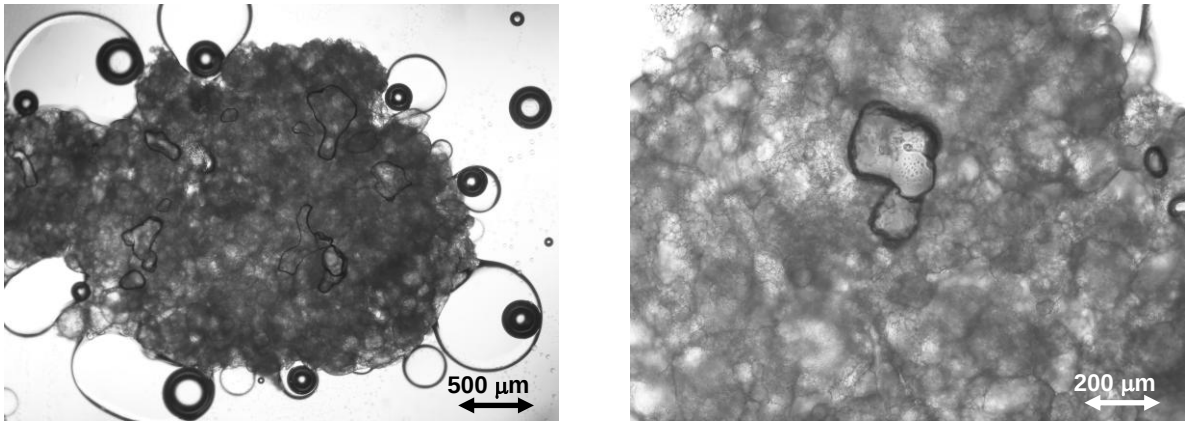


Figure 68: Optical micrographs of PVA 8-88/BCAR after chloroform exposure.
Experiment E

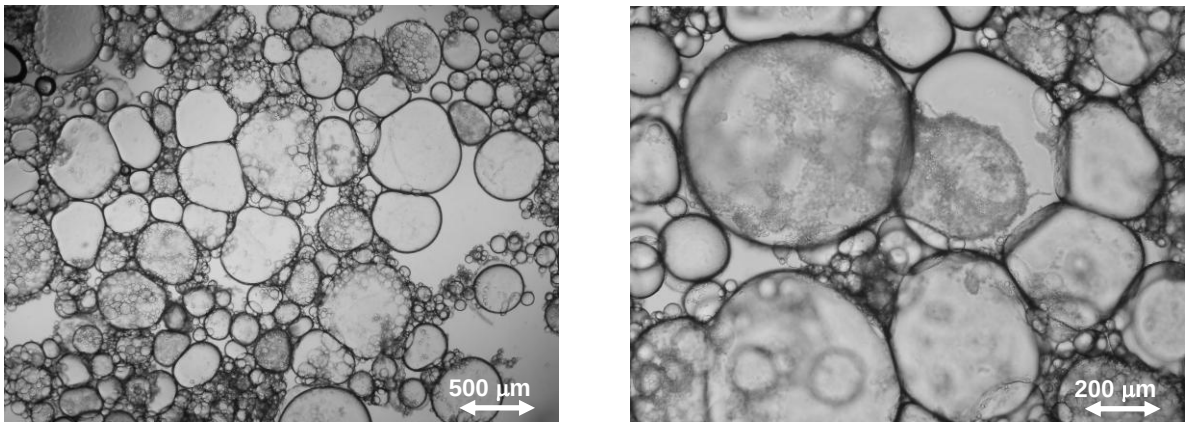


Figure 69: Optical micrographs of PVA 6-98/BCAR after chloroform exposure.
Experiment J

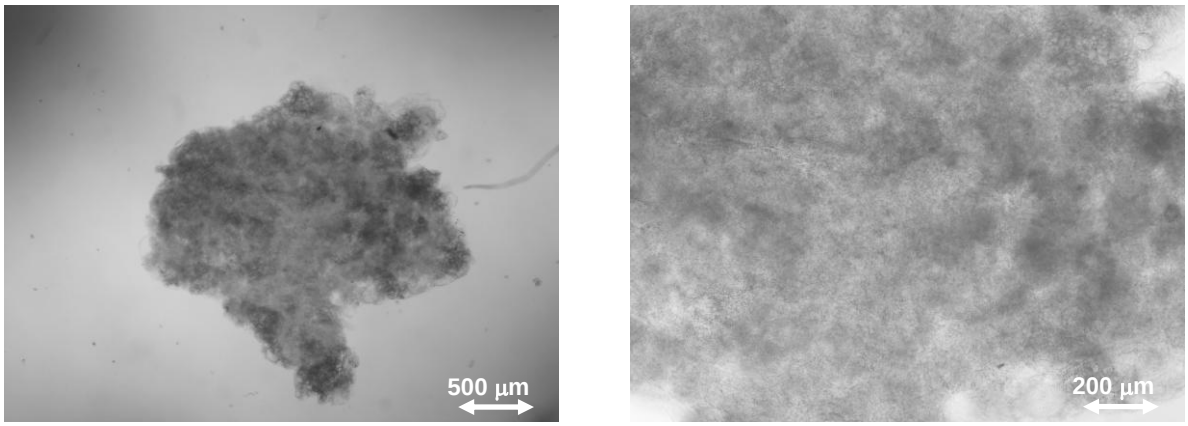


Figure 70: Optical micrographs of PVA 8-88/BCAR after tetrahydrofuran exposure.
Experiment E

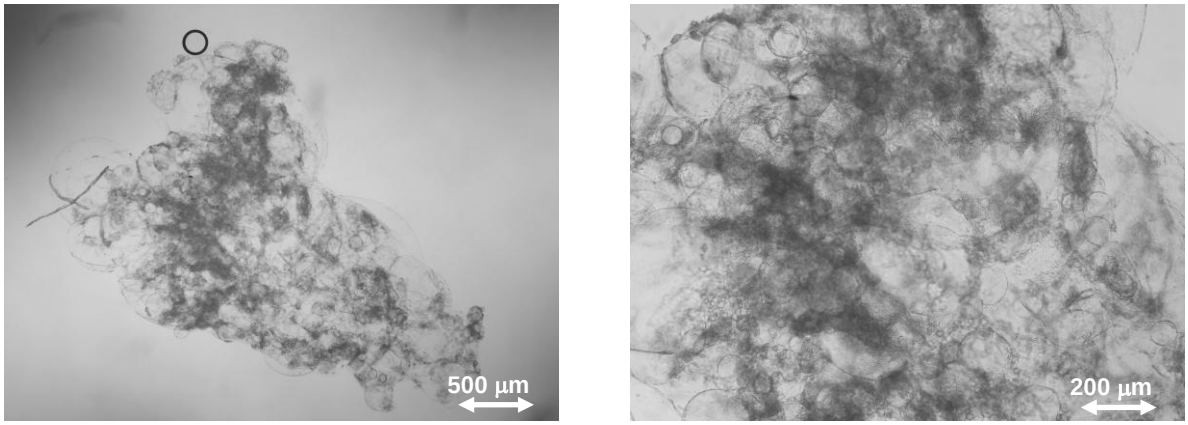


Figure 71: Optical micrographs of PVA 6-98/BCAR after tetrahydrofuran exposure.
Experiment J

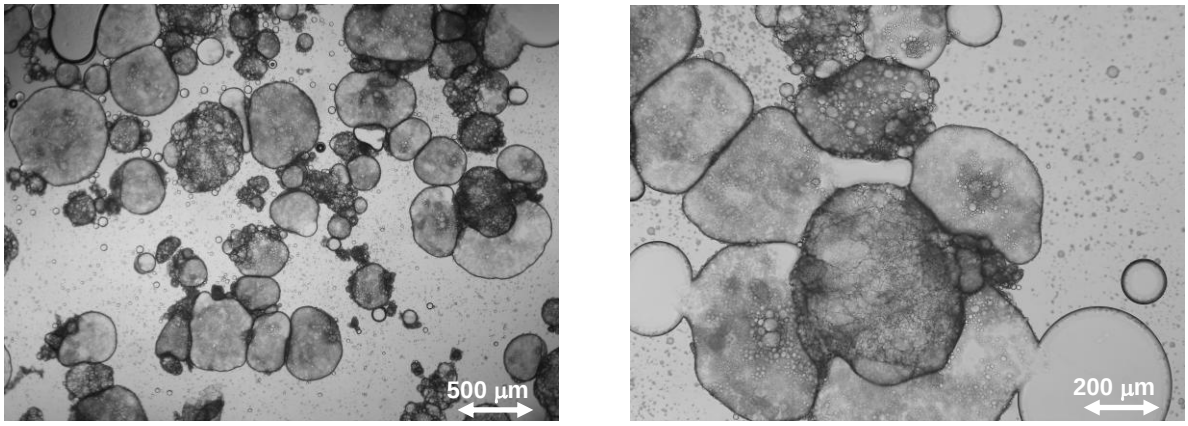


Figure 72: Optical micrographs of PVA 8-88/BCAR after toluene exposure.
Experiment E

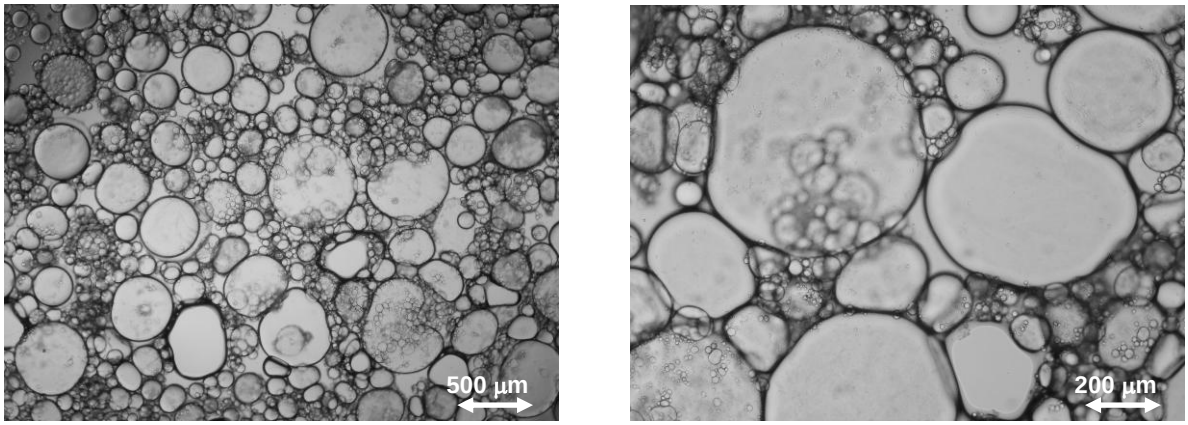


Figure 73: Optical micrographs of PVA 6-98/BCAR after toluene exposure.
Experiment J

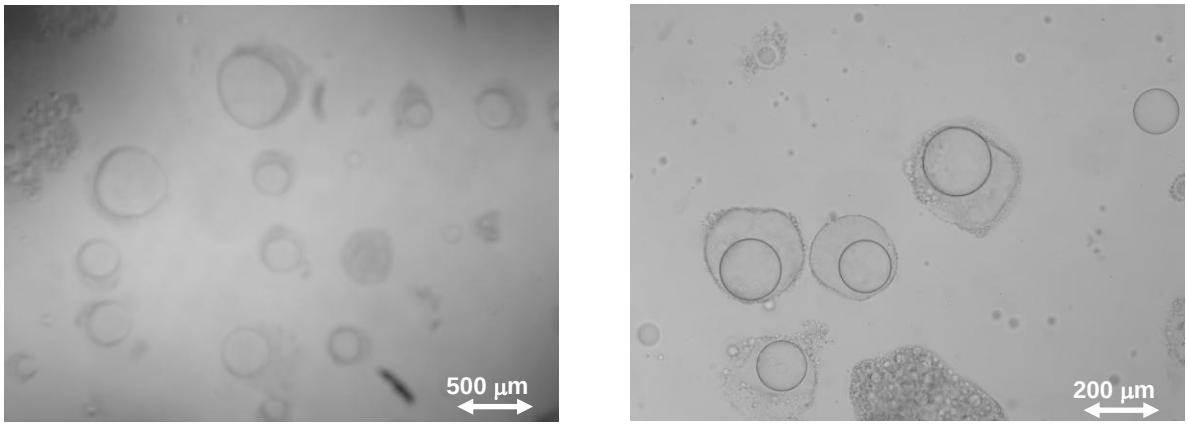


Figure 74: Optical micrographs of PVA 8-88/BCAR after dimethylformamide exposure.
Experiment E

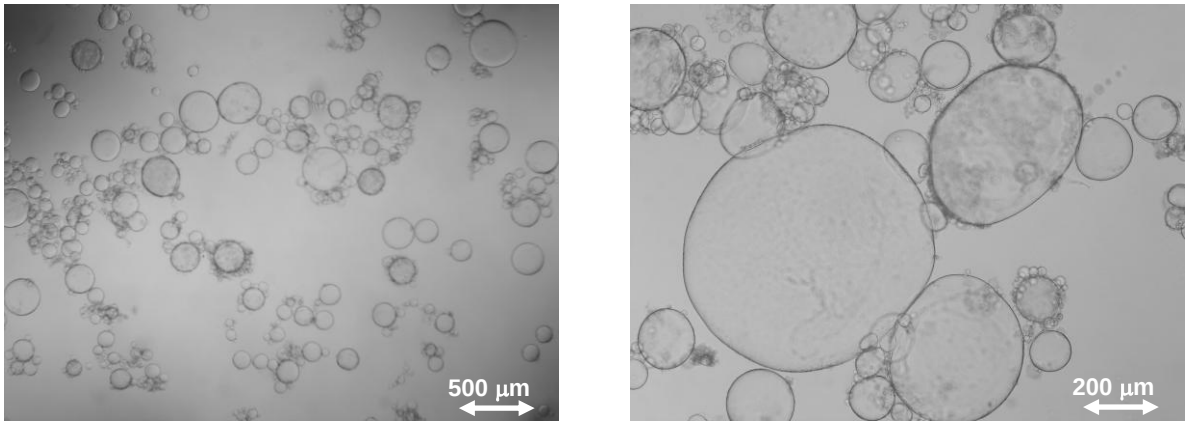


Figure 75: Optical micrographs of PVA 6-98/BCAR after dimethylformamide exposure.
Experiment J

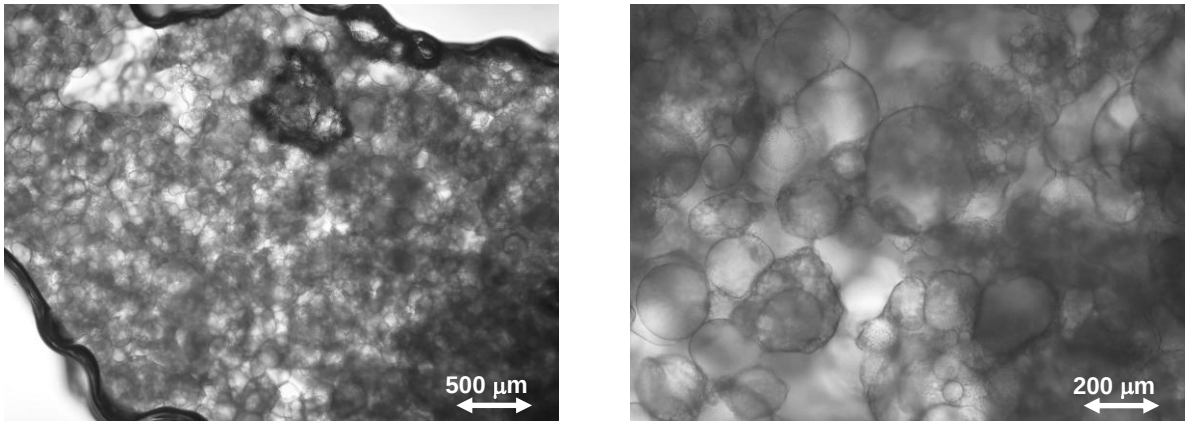


Figure 76: Optical micrographs of PVA 8-88/BCAR after acetone exposure.
Experiment E

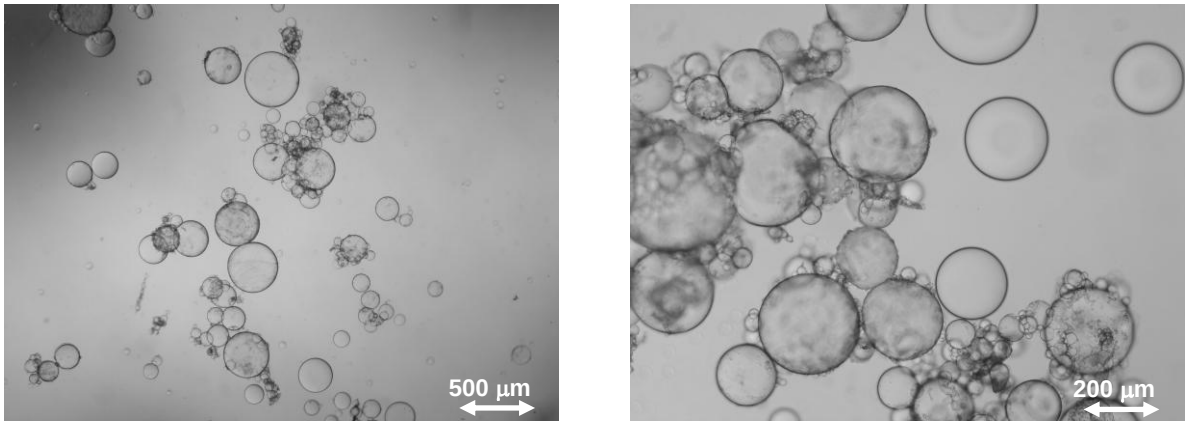


Figure 77: Optical micrographs of PVA 6-98/BCAR after acetone exposure.

Experiment J

The interaction between microcapsules (crosslinked PVA shell) and organic solvent is governed by several factors. The most important are molecular structure of the solvent which is connected with solubility parameter and density of cohesion energy. The order of the solvents used according to the tabulated solubility parameters is following: dimethylformamide (24.80 $J^{1/2}cm^{-3/2}$) > acetone (20.50 $J^{1/2}cm^{-3/2}$) > tetrahydrofuran (19.1 $J^{1/2}cm^{-3/2}$) > chloroform (19.00 $J^{1/2}cm^{-3/2}$) > toluene (18.25 $J^{1/2}cm^{-3/2}$) [124].

PVA HD and density of polymer network also plays considerable work. It can be expected that higher crosslinking level the lower surface energy (hydrophilicity) and better resistance against organic solvents. This can be seen in case of capsules based on fully hydrolyzed PVA 6-98 (Experiment J) in Figures 69, 73, 75 and 77, where the capsules are relatively more stable in comparison with the microcapsules with the shell based on partially hydrolyzed PVA 8-88. Tetrahydrofuran is too aggressive for both kinds of studied systems. In addition, VIS spectrometry of the organic solvents after stability test was carried out. All solvent except chloroform proved absorbance at the wavelengths characteristic for BCAR. This fact together with OM analysis reveals that chloroform can swell the shell of microcapsules. However, the core material remains unreleased.

3.4 Effect of LA on PVA/BCAR microcapsules preparation and properties

This final subchapter is intended to connect the information and knowledge obtained and described in the previous two subchapters. The main goal of this attempt is the preparation and characterization of the PVA/BCAR microcapsules in presence of LA, which is supposed to play the role of shell modifying compound here.

Encapsulation parameters

Two experiments were carried out to prove the effect of LA on properties of PVA based microcapsule shell. The design of the experiments is given below in Table 12. The results of chapter 3.1 reveal that 15 wt. % of LA is the most convenient choice from the both mechanical properties and economical reasons point of view. Figures 78 and 79 provide OM view of the microcapsules designated as Experiments K and L.

Table 12: Design of the microcapsules preparation experiment for PVA/BCAR/LA microcapsules

Experiment	PVA (0.25 wt. %) [ml]	LA* [wt. %]	Na_2SO_4, (20 wt. %) [ml]	Si.oil/BCAR (0.46 mg/ml) [ml]	Crosslinking solution [ml]	GAD concentration [mmol]
K	100[a]	15	50	15	17	11.4842
L	100[b]	15	50	15	17	11.4842
*[a]PVA 8-88, [b]PVA 6-98, * related to mass of PVA,*						

Table 13: Characteristics of the prepared PVA/BCAR/LA microcapsules

Experiment	Oil content [%]	Oil loading [%]	Microcapsule efficiency [%]
K	49.81± 6.84	488.28 ±0	24.69 ± 3.43
L	54.73 ± 1.76	488.28 ± 0	29.05 ± 2.37

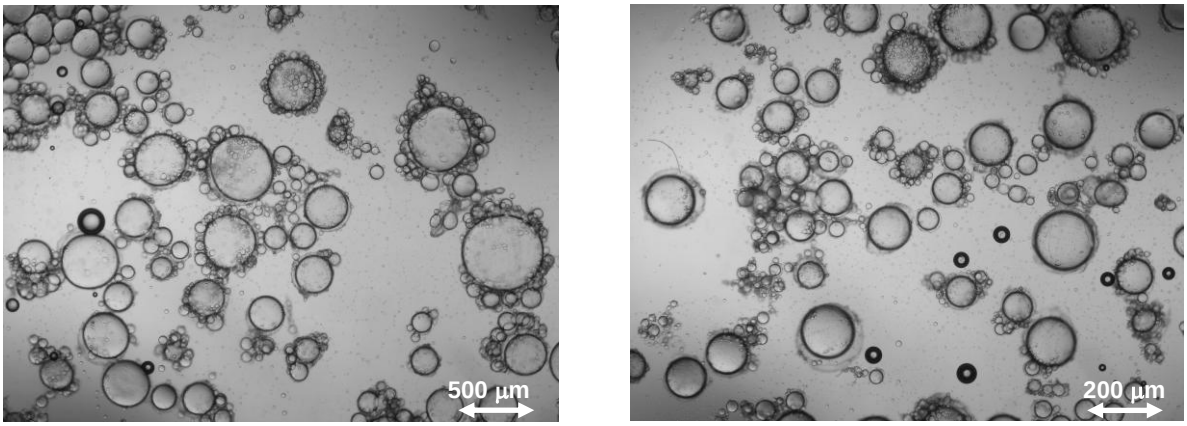


Figure 78: Optical micrographs of PVA 8-88/BCAR/LA 15 wt. % microcapsules after preparation. **Experiment K**

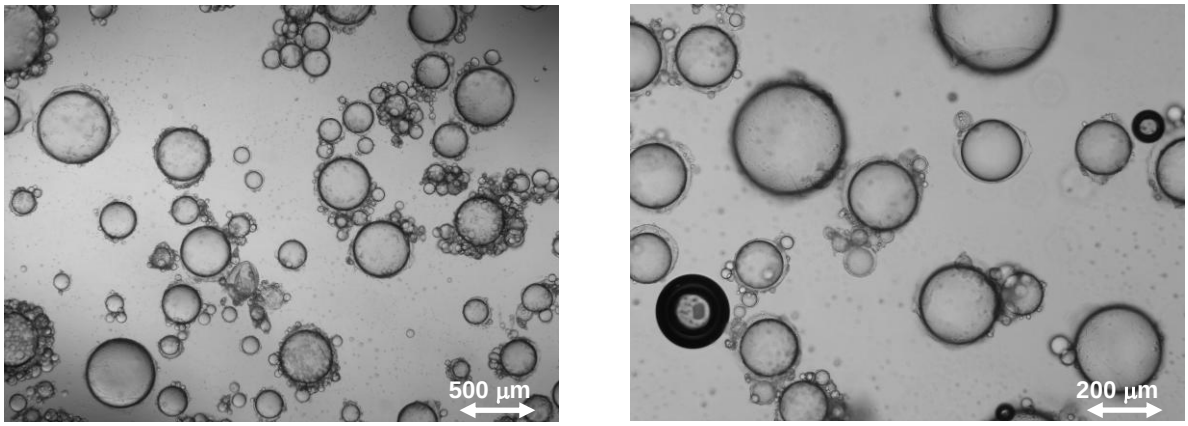


Figure 79: Optical micrographs of PVA 6-98/BCAR/LA 15 wt. % microcapsules after preparation. **Experiment L**

The characteristics of the microcapsules prepared in Experiment K and L are shown in Table 13. It is obvious that all characteristics became enhanced due to introduction of LA into microencapsulating process (compare with Table 9). This fact is important from the practical use of the developed systems point of view.

Table 14 shows the average diameters of the microcapsules in Experiments K and L. Interestingly, significantly higher microcapsule diameters were obtained in spite of using the method used in Experiments A-J. For instance, d_w is above 220 μm in case of both K and L. It is more than 40 % than in Experiments E and J (see Table 8). Polydispersities, d_w/d_n, are higher as well. It is clearly noticeable

in the histograms presented in Figure 80, where wide distributions of the microcapsules diameters can be observed especially for Experiment L.

Table 14: Average diameters of microcapsules obtained through analysis of OM pictures. Experiment K and L

Experiment	d_n [μm]	d_w [μm]	d_w/d_n	d_z [μm]
L	159.93	224.53	1.40	302.98
K	167.03	221.89	1.33	277.69

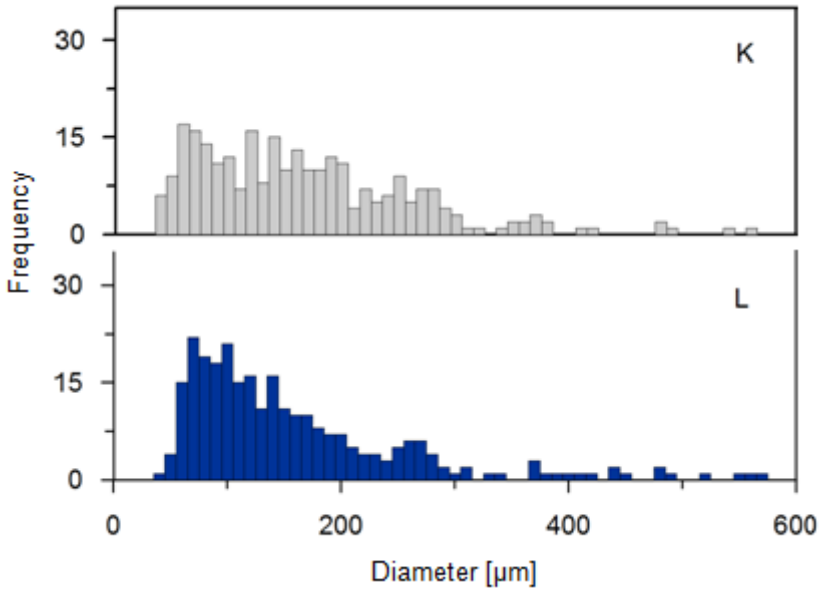


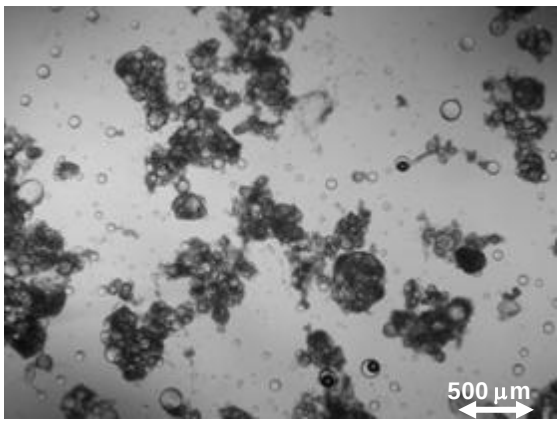
Figure 80: Histograms of PVA 8-88/BCAR/LA and PVA 6-98/BCAR/LA microcapsules diameters distribution – Experiments L and K

The possible reason of such behaviour has been indicated above. LA decreases pH of the system. It is favourable for GAD crosslinking action. It can be supposed that viscosity of the polymer solution (PVA/GA/LA) rises with the number of inter/intra chain connection (Figure 22). Increased viscosity affects the emulsification process of the oily core material in water-based PVA solution. The stability testing of the capsules prepared in Experiments K and L were carried out directly in hydrochloride acid solution at 70, 100 °C for 6 hours and at 100 °C for 72 hours. The results are summarized in Table 15. It can be seen that both LA modified microcapsules are stable up to 100 °C after 6 hours of

testing (Figures 81 and 82). However, a collapse of the microcapsules designated as Experiment K occurred after 72 hours of testing. These results are in agreement with the assumptions (influence of LA on GAD crosslinking activity) presented above.

Table 15: Temperature stability study of the selected microcapsules in the hydrochloride acid solution after various temperature and time periods of the exposure

Experiment	Temperature [°C]		
	70 (6 hours)	100 (6 hours)	100 (72 hours)
K	+	+	-
L	+	+	+



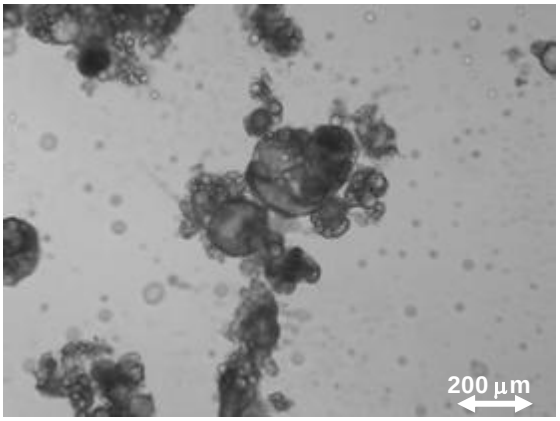
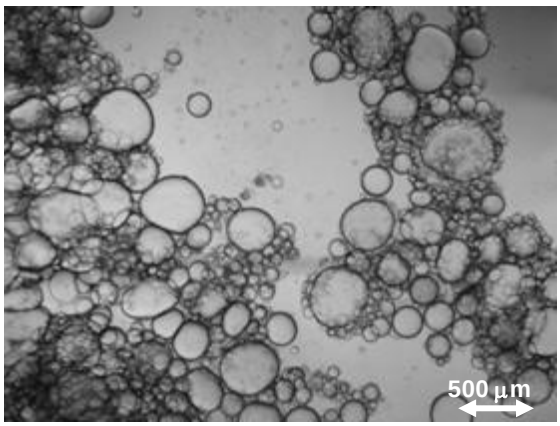


Figure 81: Optical micrographs of PVA 8-88/BCAR/LA 15 wt. % microcapsules after 6 hours in hydrochloride acid solution at 100 °C. **Experiment K**



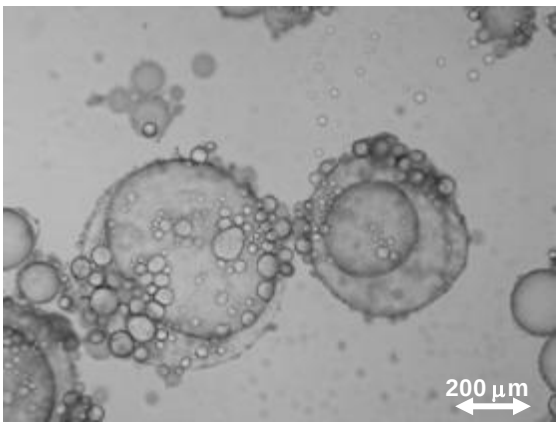


Figure 82: Optical micrographs of PVA 6-98/BCAR/LA 15 wt. % microcapsules after 6 hours in hydrochloride acid solution at 100 °C. **Experiment L**

Conclusions

Polymeric packaging has been the object of both scientific and practical interest already for several decades. Polymers have the potential to impact many aspects of food and pharmaceutical systems. Both natural and synthetic polymers can be used in food (as packaging materials and in construction of product processing plant and equipment) and pharmaceutical industries (as excipients, drug delivery systems, bandage, suture or packaging).

Packaging as a delivery system is a new generation of active packaging that can release active compounds (e.g. antimicrobials, antioxidants, enzymes, flavours, nutraceuticals and drugs) in the controlled way. One of the possibilities to achieve this property is encapsulation of solid, liquid, or gaseous active ingredients with the purpose of protection, controlled release and compatibility of the core materials. The convenient properties of microencapsulation make it technologically important and very attractive for many applications. This technique offers very promising applications in a wide array of biotechnology, biomedical field, micro/ nanotechnology and food industries such as controlling of the release of active agents (e.g. drugs, vitamins, and food supplements), converting liquids to solids separating reactive compounds, providing environmental protection (e.g. heat, humidity and pressure), improved material handling properties (e.g. toxic materials).

Presented work is focused on poly(vinyl alcohol) (PVA) based packaging materials. PVA is suitable for preparation of special packaging systems such as water-soluble films of microcapsules with specific release activities. Unfortunately it is moisture sensitive and the properties of the PVA based materials can vary with time significantly. One of the solutions is using of a plasticizer. The previous research confirmed lactic acid (LA) to be excellent PVA plasticizer. In addition, new valuable properties such as antimicrobial activity can be reached due to the modification. PVA can be available in two chemical versions according to the concentration of the residual acetyl moieties

on the side chains. This parameter is known as hydrolysis degree (HD). It has been reported that HD has crucial effect on the properties of the final product. The main aim of this work is to describe the effect of PVA HD on its interaction with LA. This information is needed for optimisation of the PVA based product preparation. In this case, the influence of LA modification on PVA crosslinkability of the polymer is important for subsequent microencapsulation processes, where PVA/LA material is used as a shell component.

Generally, experimental section of this thesis is divided into two parts. First is dealing with studies of PVA based films. PVA with various HD was modified with LA. The effect of such modification was correlated with the known chemical structure and experimentally observed as the effect of LA presence in the system on mechanical and thermal properties, water interaction and moisture sensitivity and antibacterial activity. Physico-chemical properties were studied as well. The results reveal that HD of PVA matrix plays considerable role due to interaction of hydroxyls present in polymer chain and carboxylic groups coming form LA. The specimens proved excellent antibacterial activity against both Gram positive and Gram negative bacterial strains. The crosslinkability of PVA/LA systems was observed subsequently. Dialdehyde based crosslinking compound, glutaraldehyde (GAD), was used. The results show significant effect of LA on the ability of the polymer network formation. The considerable effect of HD was found as well.

Second section of the experimental part is dedicated to microcapsules preparation by using simple coacervation technique. The knowledge obtained from the first part was utilized to find an optimal reaction conditions for encapsulation of hydrophobic substance (core material) consisting of silicon oil and β-carotene (BCAR). The primary goal was to find the most convenient concentration of the crosslinking agent, glutaraldehyde (GAD) for PVA based shell material with various HD. The results show relatively small effect of PVA matrix and GAD concentration on encapsulation process. On the other hand,

these parameters play considerable role in stability of the prepared microparticles. This parameter was studied as time dependent process as well as simple stability testing in various media and conditions. For this purpose, analytical method of BCAR determination was developed on the basis of the fact already published in scientific periodicals. The results show relatively high stability of the prepared microcapsules even at the lowest GAD concentrations in case of using of both partially and fully hydrolyzed PVA (as a shell material). Thus, no BCAR release was observed even after 48 hours of testing. Stability testing shows interesting data. The prepared microcapsules are stable under wide range of conditions (pH 2-9, temperature 25-100 °C, autoclaving). Generally, it was found that the higher HD the higher stability can be achieved (especially at high temperature and low pH). The resistance of the shell layer against selected organic solvents (chloroform, tetrahydrofuran, acetone, toluene, dimethylformamide) was investigated as well. It was found that PVA HD and density of polymer network also plays considerable work. It can be expected that higher crosslinking level the lower surface energy (hydrophilicity) and better resistance against organic solvents.

The effect of LA on encapsulation process is the last area of the research covered by this thesis. One concentration of LA (15 wt. % related to PVA mass) was selected on the basis of results obtained from the PVA/LA films investigations. The results reveal that LA has significant effect on all studied parameters of the encapsulation process. Firstly, it influences the viscosity of the reaction (water based) mixture due to possible catalysis of GAD-PVA reaction (generally between aldehyde (RC=O) and hydroxyl groups (-OH)). The higher dimensions and wider size distribution of the microcapsules was observed in comparison with the same system without LA. On the other hand, logical consequence of the increased dimensions is improvement of encapsulation characteristics (oil content, oil loading and encapsulation efficiency). Stability of the PVA/BCAR/LA capsules was enhanced noticeable. They are able to sustain

several hours of boiling in hydrochloride acid solution (pH=2). Resistance against organic solvent was concluded to be better for fully hydrolyzed PVA.

It can be conclude that LA is highly potential modifier for PVA matrix from several points of view. Firstly, it works as an excellent plasticizer reducing the brittleness of the PVA films and moisture sensitivity. It brings very good antimicrobial activity. Moreover, it enhances possible crosslinkability of PVA by aldehydic compounds. This fact can be utilized for preparation of microcapsules with multifunctional applications (medicine, pharmaceutical industry, biotechnology, chemistry etc.). Finally, it should be mentioned that LA can be obtained from waste or renewable resources. It makes it potentially attractive form both economical and environmental point of view.

CONTRIBUTIONS TO SCIENCE AND PRACTICE

The presented work brings the overall overview in the field of polymeric materials used for preparation of advanced packaging. All methods used during assigning of the tasks of the given Ph.D. topic were adopted or more often developed on the basis of current scientific works published in reputed impacted journals. In addition, most of the results are under preparation for publication in impacted scientific journals. Contributions of this study to science are the following:

- PVA hydrolysis degree on interaction with LA was described
- new information about PVA chemical crosslinking in dependence on LA concentration and PVA hydrolysis degree was reached
- encapsulation process of liquid material with PVA/LA matrix was described
- methodology for release kinetics determination of the hydrophobic agent into hydrophilic medium was developed and introduced into laboratory practice
- high temperature and low pH resistant microcapsules were obtained

The contribution to practice can be considered finding of an excellent plasticizer for PVA, which makes PVA much user friendly in comparison to virgin polymer. In addition, such modification brings antibacterial activity to the resulting material. The positive effect of LA and high PVA hydrolysis degree on crosslinkability can decrease toxic crosslinker utilization. It can open new areas of PVA application especially in medicine and pharmaceutics packaging production. In addition, this work brings another way of LA utilization as the compound, which can be obtained from renewable resources or waste.

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AP	Active Packaging
BCAR	β-carotene
BP	Bioactive packaging
CFU	Colony forming unit
DMF	Dimethylformamide
DS	Degree of Swelling
DSC	Differential scanning calorimeter
EAA	Effectiveness of antibacterial activity
EMA	Ethylene methyl acrylate
EVON	Ethylene vinyl alcohol
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
GAD	Glutaraldehyde
HD	Hydrolyzation degree
IP	Intelligent Packaging
LA	Lactic acid
MAP	Modified Atmosphere Packaging
MBP	Migratory bioactive packaging
NMBP	Non-migratory bioactive packaging
PBS	Poly (butylene succinate)
PCL	Polycaprolactone
PE	Polyethylene
PEN	Polyethylene naphthalate
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PVA	Poly(vinyl) alcohol
PVAc	Polyvinyl acetate
PVC	Polyvinyl chloride
PVdC	Polyvinylidene chloride
SEM	Scanning electron microscopy
T_g	Glass transition temperature
THF	Tetrahydrofuran
T_m	Melting temperature
WHO	World Health Organization
WVP	Water vapour permeability

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