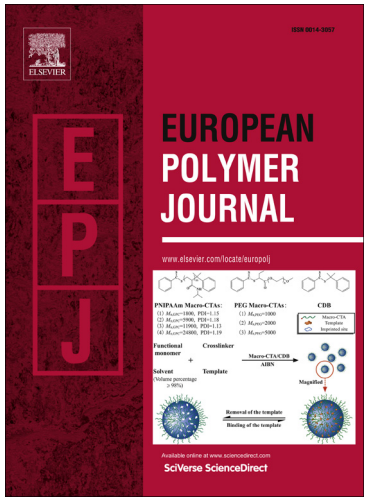

Review article

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Designing polymeric microparticles for biomedical and industrial applications

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Abstract

Nowadays, there are almost limitless applications for microparticles. Microparticles are used as components in many advanced materials and composites, in the healthcare and personal grooming industries, and in many research and development applications. This review presents an overview of recent and current studies carried out at the Particles, Polymers and Biomaterials Technology Group of the University of Coimbra which aims to design and prepare novel polymeric microparticles. Microparticles herein described were prepared from natural polymers, namely, polyhydroxyalkanoates, cellulose, starch, and chitosan and from synthetic polymers, namely polyurethanes, poly(vinyl chloride), silanes, and methacrylates. These studies intended different applications for microparticles, mostly as delivery systems and coatings. The results of our studies confirm the outstanding potential of microparticles in different fields, and emphasize the importance of these systems for the future.

1. Introduction

1.1. Definition and general features

"Microparticle" is the term used for spherical particles with diameters in the micrometer range (typically from 1μm to 1000μm). Polymeric microparticles are usually formed by a polymer matrix in which a smaller amount of an active compound can be immobilized. With respect to the distribution of the active compound, two different categories of microparticles can be distinguished: "microspheres" and "microcapsules" (Fig. 1). "Microspheres" refers to microparticles composed of a homogeneous mixture of active compound and raw material, while "microcapsules" is the name given to microparticles that present a core (where the active compound is placed) which is delimited by a different material (usually the raw material). The core

may be solid, liquid or even gas. Furthermore, one or more discrete domains of active compound may be found in the microcapsule core [1].

Throughout this review, we will use this nomenclature. However, it should be noted that in the literature and other technical sources, the term "microsphere" is used as a synonym for "microparticle". In these cases, the terms "solid microspheres" and "hollow microspheres" are used to distinguish between different types of core structures.

Microparticles can be manufactured from a large variety of starting materials, both natural and synthetic, and by many different preparation techniques [2, 3]. Both starting materials and preparation techniques allows the preparation of an enormous variety of microparticles, in terms of size, size distribution, composition, surface chemistry, topography and morphology [4].

There are a number of interesting features of microparticles that make them particularly suitable for microencapsulation: (i) controlled release of encapsulated materials, (ii) protection of the encapsulated materials against degradative reactions (e.g., oxidation, dehydration, UV, heat, acids and bases) in the external environment, which can also result in an improved shelf life, (iii) masking the organoleptic properties such as color, taste and odor of encapsulated materials, (iv) easy handling of the resulting powder-like materials, and (v) safe handling of toxic encapsulated materials [5]. Many different types of materials, including drugs [6], proteins [7, 8], food materials [9, 10], pesticides [11] and herbicides [12], as well as cells [13, 14] can be encapsulated.

Microparticles do however have some limitations that must be considered. The large particle size, compared to alternative additives, can result, for instance, in surface texture or gloss reduction, in the case of coatings/paintings. It can also result in products

that are not as aesthetically appealing, for example, an unusual appearance or texture in the case of food products. In addition, the use of this technology still results in increased costs to the manufacturer; there is an increased complexity of production process and/or supply; stability of the encapsulated material during processing may present additional challenges; storage of the final product may require changes in packaging or conditions; finally staff would require proper training and safety/handling equipment [15, 16].

1.2. Historical perspective

The concept of microparticles as encapsulation systems date back to 1932 with the work of the Dutch chemist H.G. Bungenberg de Jong on the origin of life [17]. Bungenberg de Jong used the term "coacervate" (from the Latin *acervus*: heap, mass) to describe droplets containing a colloid, rich in organic compounds, surrounded by a tight layer of water, providing a locally segregated environment. These coacervates could have differentiated surfaces and thus be compared to cellular components such as membranes or vacuoles [18].

The first industrial product employing microencapsulation was carbonless copy paper developed by the chemists L. Schleicher and B. Green, in 1953, while working for the National Cash Register Company [19]. They developed an improved copying paper by undercoating sheets of paper with microcapsules containing a colorless dye precursor. The application of pressure, via a pen or a typewriter type hammer, caused microcapsules to rupture, exposing the dye precursor to a reagent that coated the top of the lower sheet [20].

In 1970, W. M. Holliday and collaborators patented the first use of microparticles in the pharmaceutical industry [21], as an orally administered, sustained-release composition consisting of acetylsalicylic acid encapsulated within a continuous thin

coating of ethyl cellulose. This novel pharmaceutical composition allowed for the gradual release of acetylsalicylic acid into the blood by a diffusion mechanism over a period of 4 hr following oral administration and affording 8 hr of analgesic relief. It also served as a strategy to reduce the irritant effect of acetylsalicylic acid on gastric mucosa [22], to reduce the frequency of administration and improve patient compliance.

1.3. Current market

In 2010, BCC Research published an extensive market research report entitled "Microspheres: Technologies and Global Markets" [23]. This report analyzed the global market for microparticles from both the manufacturing and demand points of view, in 2010, and forecast its direction through 2015. The global market for microspheres in 2010 was estimated at $2 billion and it was predicted to reach $3.5 billion by 2015. According to the same report, the medical technology and life sciences industries were mentioned as emerging industries (Fig. 2).

Microparticles have found use in many applications over the years. As the primary and more promising fields, microparticles are utilized in composites, paints and coatings, oil and gas exploration, adhesives, cosmetics and personal grooming products, life sciences and biotechnology, medicine and medical devices [23]. Currently there are several companies producing exclusively microparticle-based products. Nevertheless, microparticles are still an area of ongoing research efforts, and outstanding work continues to be produced by researchers in this field.

In our research group, we have been developing polymeric microparticles with different features for a variety of applications. Here, we outline microparticle production and applications based on work performed in our laboratory.

2. Solvent extraction/evaporation method

The solvent extraction/evaporation (SEE) method is the most commonly used method to prepare drug loaded microparticles from preformed water-insoluble polymers, such as poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA) and polycaprolactone [6, 24-27]. The simplest version of this method, which involves the formation of an oil-in-water (O/W) emulsion, is widely used to encapsulate insoluble or poorly water-soluble drugs. This process, depicted in Fig. 3, can be divided into four steps [27]: 1) The dissolution of the polymer in an appropriated volatile organic solvent, followed by the addition of the active compound (the active compound can be dissolved or simply dispersed in the organic phase); 2) The emulsification of the organic phase in an immiscible aqueous phase, in order to form the O/W emulsion; 3) The removal of the solvent from the dispersed phase by solvent evaporation, with consequent transformation of the dispersed phase into solid particles; 4) The harvesting and drying of the microparticles.

The characteristics of the microparticles loaded with the active compound, such as size distribution, internal structure, and surface morphology, ultimately determine the release profile of the immobilized active compound. In microparticles produced by this method, these characteristics are affected by a large number of formulation and process variables, such as the relative quantities and physical-chemical properties of the materials used and the operational conditions of the production process. The influence of these variables on the final properties of the microparticles has been the subject of several reviews [6, 24-27].

Usually, the encapsulation of hydrophilic active compounds by the O/W emulsion SEE method results in low encapsulation efficiencies and release profiles that are characterized by a burst release. Accordingly, in order to eliminate these undesired

features, several variations of the O/W emulsion SEE method have been developed. Some examples are the water-in-oil-in-water (W/O/W) double emulsion method and the oil-in-oil (O/O) non-aqueous SEE method. In the W/O/W method, an aqueous solution with the hydrophilic active compound is emulsified with the organic phase (W/O emulsion). This W/O emulsion is then dispersed into a second aqueous solution, in order to form a double emulsion (W/O/W). In the O/O method, the aqueous phase is replaced by oil (such as mineral oil) [27].

3. Microparticles based on natural polymers

Natural polymers are formed in nature during the growth cycles of living organisms. Accordingly, they are available in large amounts from renewable sources. Synthetic polymers, on the other hand, are produced from non-renewable petroleum resources [28]. Structurally, natural polymers are highly organized. This feature contributes to their most striking properties such as biocompatibility and biodegradability, which have attracted researchers towards the widespread application of natural polymers [29]. In addition, natural polymers also offer ease of processing [30] and of chemical modification [31]. Although several natural polymers exist, in our research group, we have been mostly focusing on investigating polyhydroxyalkanoates and polysaccharides such as cellulose, starch, and chitosan (Fig. 4).

3.1. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) (Fig. 4a) are polyesters synthesized by many bacteria usually under conditions that are limiting for growth (e.g., lack of nutritional elements such as N, P, S, O, or Mg or lack of oxygen) but in the presence of an excess of carbon sources. These polymers are accumulated as intracellular inclusions to levels

as high as 90% of the dry cell weight and act as a carbon and energy reserve [32]. PHAs have attracted attention as they are biodegradable and biocompatible polymers [33]. There are several PHAs available, with a wide range of molecular weights and chain substitutes. The most commonly used up until now are the homopolymer poly(3-hydroxybutyrate) (PHB) (Fig. 4b) and the copolymer, poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) (Fig. 4c).

In the human body, several metabolic processes produce hydrogen peroxide. The accumulation of hydrogen peroxide in cells affects their normal function, promoting oxidation processes that effect DNA (resulting in DNA damage), proteins and lipids [34, 35]. Catalase is an antioxidant enzyme, capable of protecting the cells from the toxic effect of hydrogen peroxide. Catalase acts to decompose hydrogen peroxide into oxygen and water, without the resulting production of free radicals [36]. Interestingly, this effect can be applied in cancer treatment, namely anti-metastatic therapy. It was demonstrated that high concentrations of hydrogen peroxide are generated in tumor tissues [37]. Furthermore, changes in certain types of genes involved in carcinogenesis sometimes result in a reduced level of antioxidant defence (as low activity of catalase [38]) in cancer cells compared with their normal counterparts [39]. These findings strongly suggest that hydrogen peroxide is not efficiently removed in most tumor tissues, leading to an increase in its level compared with that in surrounding normal tissues [39]. This concentration of hydrogen peroxide activates the transcription of various genes [40], which may accelerate the growth of tumor cells in metastasis [41]. Therefore, the sustained delivery of catalase to sites where tumor cells metastasize seems to be a promising approach for inhibiting or preventing tumor metastasis.

If an enzyme is sensitive to external conditions, it is necessary to develop an appropriate system to deliver them to the specific target. Several enzymes were already

encapsulated and have been applied *in vivo*, in order to treat various diseases [36, 42]. In the case of catalase, its encapsulation within a permeable and biodegradable polymer will protect the enzyme from external conditions, and will allow its delivery to cancer cells [43]. In our research group, we used PHBV to encapsulate catalase [44]. In this study, the PHBV microparticles were prepared by SEE method, using a W/O/W emulsion to encapsulate the enzyme. We concluded that mechanical stirring (at 8,000 rpm) led to larger sized microparticles (mean particle size between 22 and 26 μm) compared to the microparticles produced using a homogenizer (at 11,000-24,000 rpm) (mean particle size between 1.5 and 17.2 μm).

As observed by scanning electron microscopy (SEM), the microparticles obtained were spherical in shape and presented a rough surface (Fig. 5). This feature is typical of PHB and PHBV microparticles [45], and is attributed to the highly crystalline character of these polymers [46]. When encapsulating/immobilizing an active compound, it is also important to determine its encapsulation efficiency (EE). This parameter highlights the percentage of compound lost during encapsulation but it also helps to foresee the duration and dosage of treatment [47]. Usually, EE is determined according to EE = $\Delta D/D_T$, where D_T is the total amount of active compound employed and ΔD is D_T minus the amount of unloaded active compound [48]. For catalase encapsulated in PHBV, higher values for EE (up to 58%) were obtained for the microparticles prepared using the homogenizer compared to the microparticles prepared using mechanical stirring (up to 11%). The enzymatic activity was also higher in the homogenizer prepared microparticles (0.01 μmol H_2O_2/min μg), being tenfold larger than the activity observed with microparticles prepared by mechanical stirring.

The development of an optimized microparticle-based drug delivery system for a specific application is a hard and time-consuming task due to the great number of

variables involved in the particles' formulation and in the production process that directly affect the properties and drug release characteristics of the microparticles. Usually, researchers resort to literature and to their own experience to define a basic set of conditions and a production process and then study the effect of a restricted number of variables by varying them one at a time (the so called one factor-at-a-time approach). To study all of the relevant variables, using the one factor-at-a-time approach, involves performing an unrealistic number of experiments, and does not reveal any information about the presence of interactions, i.e., the influence of one or more factors on others. For these reasons, the use of this methodology hardly ever leads to an optimized formulation and production process [49].

A more rational approach to develop an efficient and adequate microparticulate drug delivery system is the employment of statistical design of experiments (DoE). This methodology permits the planning of experiments in such a manner that appropriate data, that can be analyzed by statistical methods, is collected resulting in valid and objective conclusions. In DoE terminology, the controlled, independent variables under investigation are called factors, while the measured dependent variables are called responses [50]. Using a screening DoE, it is possible to evaluate, with an affordable number of experiments, which process factors significantly affect the response and in what way. Additionally, this type of experimental design also reveals the possible existence of any interactions between factors. After the identification of the critical factors, and through the implementation of an optimization DoE, the optimum levels of the factors, i.e., the values of the independent variables that lead to an optimum response, can be identified [49, 50].

In our research group, we employed a DoE methodology to investigate the encapsulation of flurbiprofen, a non-steroidal anti-inflammatory drug, in PHBV

microparticles prepared by an O/W emulsion SEE method [45]. A central composite experimental design was employed to evaluate the effect of two process variables: i) the polymer concentration in the organic phase, and ii) the surfactant (poly(vinyl alcohol), PVA) concentration in the aqueous phase, on microparticle properties, specifically the EE of the drug, the mean particle size, the particle size distribution (PSD) and the required time for the *in vitro* release of 50% of the encapsulated drug ($t_{50\%}$). The statistical analysis of the implemented experimental design revealed that the two investigated variables had significant and opposite effects on the EE of the drug (Fig. 6). We found that microparticle mean size increased with PHBV concentration in the organic phase and that the surfactant concentration in the aqueous phase played a critical role in the degree of aggregation of the microparticles. We also concluded that a minimum PVA concentration was required to stabilize the O/W emulsion and thus obtain non-aggregated microparticles. The *in vitro* flurbiprofen release profiles from PHBV microparticles were very similar for all the prepared formulations, showing that more than 90% of the drug was released in the first 8 h of the assay. The $t_{50\%}$value was not significantly influenced by any of the two investigated variables. The comparison between the release profiles of flurbiprofen from the PHBV microparticles and the dissolution profile of the pure drug led to the conclusion that the drug was mostly dispersed at the surface of the microparticles, rather than effectively entrapped in the polymeric matrix.

3.2. Polysaccharides

Polysaccharides occur in nature in large quantity and with a wide variety of chemical structures and physical properties. They are usually hydrophilic and possess numerous functional groups, such as free carboxyl and hydroxyl groups, which make

them susceptible to chemical modification. Also, they are, in general, non-toxic and biodegradable. For these reasons, polysaccharides and their derivatives are widely used in a large number of industries, including the pharmaceutical industry. For these reasons, the encapsulation of active compounds in polysaccharide-based microparticles has been the subject of great interest [51]. In our research group, we have already encapsulated several active compounds in microparticles obtained from different polysaccharides, using several microparticle preparation techniques.

3.2.1. Cellulose derivatives

Cellulose is the major component in the rigid cell wall of plants. It is formed by repeating D-glucose units and is a highly functional, linear yet stiff, polysaccharide chain. This homopolymer is characterized by its chirality, biodegradability and broad capacity for chemical modification. In its chain structure, it presents a high number of hydroxyl groups suitable to be converted, by chemical modification, into cellulose esters [52]. These cellulose derivatives are more suitable to be applied in different areas, such as enteric coatings, hydrophobic matrices, and semi-permeable membranes for applications in pharmaceuticals, agriculture, and cosmetics [52-54]. Furthermore, the cellulose esters can be applied in the field of controlled release systems, due to their well established preparation processes, application safety and good handling properties [55].

The preparation and characterization of cellulose derivative microparticles as delivery systems, usable either in cancer treatment or in coatings has been previously reported by our research group [56, 57]. In this study, microparticles were produced by the SEE method from cellulose acetate butyrate (CAB) (Fig. 4d). Fluorouracil, a tumor treatment drug [58], and two fluorinated compounds used to improve hydro and

oleophobicity in coatings – Zonyl®321 and pentafluorotoluene (PFT), were encapsulated in the CAB microparticles by the double emulsion method (W/O/W). We studied the influence of the production method (mechanical stirrer or homogenizer) and stirring speed in the final properties of the microparticles. From this study [56, 57], we concluded that a uniform stirring method using the homogenizer, promotes the formation of smaller microparticles (mean particle size ranging from 1.1 μm to 4.4 μm). Immobilization of the different substances into the CAB microparticles promoted an increase in the final microparticles size. These microparticles were stable and did not undergo significant hydrolytic degradation in aqueous media at pH 2.0 or pH 7.4, over a period of 30 days, meaning that they are suitable for use in an aqueous release medium. The morphology of the microparticles was assessed by SEM. Fig. 7a) and 7b) present CAB microparticles with and without the drug entrapped, respectively.

The EE and drug release studies were assessed by UV-Visible spectroscopy. The EEs for fluorouracil and for PFT were ~60% and ~90%, respectively. Release studies from the CAB microparticles revealed that fluorouracil was released in two phases. The first phase probably corresponded to the drug being released from the external particle surface. The second phase, after a week, was most likely due to the entrapped drug. The release occurred during a period of 45 days. In the PFT release study, it was verified that only a small amount of the entrapped quantity was released to the water medium, probably the residual PFT in the external surface of the CAB particles. This behavior was attributed to the low affinity of the PFT (hydrophobic) for the water medium.

3.2.2. Starch

Starch is a polysaccharide consisting of glucose units joined by α(1→4) glycosidic bonds in amylose and by α(1→4) and α(1→6) glycosidic bonds in

amylopectin.[59] Fig. 4e) shows the general representation of the starch chain structure. The preparation of starch microparticles for drug delivery applications has been the subject of many publications, especially in the last decade [60-62]. Generally, the methods used to prepare starch microparticles include a cross-linking step, during or after particle formation [60, 61, 63]. Since starch is a hydrophilic polymer, the cross-linking of the polyssacharide chains is required to obtain microparticles that are resistant to dissolution in the physiological environment.

We prepared microcapsules from starch derivatives by chemical cross-linking in water-in-oil (W/O) emulsion (W/O) [64]. In a first approach, the water soluble starch was modified with 2-vinyl-4,4-dimethyl-2-oxazolin-5-one to introduce vinyl groups into the starch chain (Scheme 1). Then, the modified starch was used to obtain microcapsules, through an interfacial cross-linking reaction with dipropyleneglycol diacrylate (DPGDA) (Scheme 2), by a W/O emulsion polymerization. The modified starch was characterized by ^{1}H NMR and differential scanning calorimetry (DSC). The ^{1}H NMR spectra showed high degrees of substitution and the DSC thermograms suggest a low crystallinity in the modified starch. Also from the ^{1}H NMR spectra, substitution in the starch chain was determined to be around 38.6%. The prepared microcapsules were observed by optical microscopy (Fig. 8). The obtained microcapsules presented spherical shape with a mean particle size around 150 μm.

3.2.3. Chitosan

Chitosan is a natural cationic polysaccharide, which is a copolymer formed by units of 2-deoxy-*N*-acetyl-D-glucosamine and 2-deoxy-D-glucosamine linked by β-1,4 glycosidic bonds (Fig. 4f). Since chitosan occurs rarely in nature, it is generally obtained a by partial *N*-deacetylation of chitin, the second most abundant natural

biopolymer. It is usually found in the exoskeleton of shrimps, fungi, insects, annelids and mollusks. Chitosan has great potential for biomedical applications since it is biocompatible, biodegradable, nontoxic, mucoadhesive, haemostatic, hypocholesterolemic, hypolipidemic, antimicrobian, immunoadjuvant, antiviral and antitumoral [65]. Recently, the applications of chitosan have been extended to other scientific and industrial fields such as soil remediation [66], waste water treatment [67] , food packaging [68], and cosmetics [69], among others.

The design of new materials based on blends of two or more polymers constitutes nowadays an interesting and promising challenge in Material Sciences. This is the case of blending synthetic and natural polymers [70]. In fact, synthetic polymers are characterized by their good physicochemical and mechanical properties but they are not sufficiently biocompatible, most likely due to the presence of residues of initiators and other compounds/impurities. On the other hand, natural polymers have good biocompatibility but their mechanical properties are often inadequate [71-73]. For chitosan, among the several blends with synthetic polymers described [74], we have been focused on the blending with poly(vinyl alcohol) (PVA).

Recently, we prepared glutaraldehyde cross-linked chitosan–PVA microparticles by an improved water-in-oil emulsion technique using corn oil as organic phase [75]. Our procedure constitutes a more environmentally friendly alternative to the use of toxic volatile solvents, such as cyclohexane [76], toluene and chloroform [77] as organic phase. Moreover, since the organic phase viscosity determines the size of microparticles produced by the emulsion technique [2], the use of a more viscous organic phase was advantageous. Among the several vegetable oils commercially available, we used sunflower, soya bean, and (mainly) corn oil. The preparation of these microparticles included two main steps: i) production of a dispersion of small droplets of polymeric

(chitosan and PVA) solution in vegetable oil; ii) gradual hardening of the droplets into the corresponding microparticles, by glutaraldehyde cross-linking. In this study, glutaraldehyde was added to the organic phase as a saturated solution in toluene [78], improving its solubility in that phase. Therefore, glutaraldehyde induced the reaction between aldehyde groups and amino groups (from chitosan) or hydroxyl groups (from PVA) on the matrix of the globlets, forming either an imine (Schiff's base) or an acetal bond, respectively, as analyzed by infrared spectroscopy. Moreover, free aldehyde groups are likely to exist on the surface of the microparticles. These aldehyde functionalities may enhance, for example, tissue immobilization and attachment of drugs, immunoglobulins or enzymes [78].

As a consequence of both the use of vegetable oil and efficient glutaraldehyde cross-linking, and also of the addition of methanol to the polymeric phase, well-formed microparticles, with regular spherical shape, without aggregation and, apparently, homogeneously distributed were obtained (Fig. 9). This constitutes a remarkable achievement over other published works [79-81].

Particle size is known as a key property of microparticles, since processes such as matrix degradation, and the release or adsorption of active compounds [82], are related to the surface area:volume ratio. In this study, we observed that the increase of stirring speed resulted in smaller particles since a more efficient dispersion of polymeric phase in the organic one was achieved. At 1,370 rpm, microparticles with a mean particle size of 16 μm were obtained.

The use of glutaraldehyde as a cross-linking agent is still a cause for concern due to its toxicity [83]. Accordingly, in order to assess the cytotoxicity of glutaraldehyde cross-linked chitosan–PVA microparticles an MTT assay was performed. Wistar mice peritoneal macrophages were incubated for 20 hr with microparticle suspensions in

complete RPMI medium at 0.2, 2.0 and 10% (v/v) (final concentration). Since cell viability was > 70% of the negative control in all tested concentrations, according to the guidelines of ISO 10993-5:2009 [84] for biological evaluation of medical materials, we assumed that these microparticles had no cytotoxic potential. Therefore, despite the use of toxic glutaraldehyde, results suggested that its toxicity was minimized most likely due to the cross-linking reaction [85] that occurred during the outlined procedure.

4. Microparticles based on synthetic polymers

Thus far, numerous studies on the synthesis of microparticles based on natural polymers have been described. However, it is important to keep in mind that both the properties and performance of microparticles prepared from natural polymers may be less predictable than those of microparticles prepared from synthetic polymers. This fact is mainly due to the heterogeneity of chemical composition and therefore physical and chemical characteristics of natural polymers. Also, these polymers are often immunogenic. On the other hand, it is possible to prepare synthetic polymers in a controlled manner, resulting in the production of materials with well-established composition and molecular weight and therefore, with predictable properties such as solubility and degradability. Among synthetic materials, polyurethanes (produced from polycaprolactone), poly(vinyl chloride), silanes, and polymethacrylates (Fig. 10), have been successfully used in our group to prepare particles for different applications.

4.1. Polyurethane

Polyurethanes (PUs) are a very versatile family of polymers. They are characterized by the presence of an urethane linkage (Fig. 10a), which is formed via a polycondensation reaction between an isocyanate (with at least two isocyanate end

groups) and a hydroxylated compound (with at least two hydroxyl groups).[86] PUs have been the target of many studies, mostly in the biomedical field. The main reason for interest in PUs is because of their excellent physical properties, such as elasticity, abrasion resistance, durability, chemical stability and easy processability [87]. These properties enable the use of PUs in several biomedical applications [86], including pacemaker lead insulation [88, 89], breast implants [90, 91], heart valves [92, 93], vascular prostheses [94, 95], bioadhesives [96, 97] and vehicles for controlled delivery of active compounds [98, 99].

We synthesized PU-based microparticles by O/W emulsion polymerization with poly(caprolactone) diol (PCL) and poly(propylene glycol), tolylene 2,4-diisocyanate terminated (TDI) or poly(propylene oxide)-based tri-isocyanated pre-polymer (TI) [100]. In this work, we studied mainly the effect of the mass ratio between isocyanate and PCL (20/80, 50/50, and 80/20), the presence of a surfactant (Tween 80®) and the stirring type and speed on the physicochemical properties of these microparticles. The polymeric matrix of these PU-based microparticles was formed via the reaction of the hydroxyl endgroups from the PCL and the isocyanate endgroups from the TDI or TI. The urea linkage was also formed through the reaction of the non-reacted isocyanate end groups with water from the continuous phase (Fig. 11) [101]. Both urethane and urea groups were confirmed by attenuated total internal reflection Fourier transformed infrared spectroscopy (ATR-FTIR). According to dynamical mechanical thermal analysis (DMTA) results, the extent of the reaction between the hydroxyl groups and the isocyanate groups was higher in the 50/50 and 80/20 formulations, since only one peak was observed in the respective thermograms, confirming a homogeneous polymeric matrix. Moreover, we observed that the glass transition temperature (T_g) was higher for TI-based formulations than for TDI-based formulations, suggesting a more

crystalline and/or rigid polymeric matrix in the TI-based formulations. We concluded that the use of Tween 80®, as a surfactant, did not significantly influence the PSD of the microparticles prepared. We also observed that only the microparticles prepared according to the 80/20 and 50/50 formulations presented a spherical shape, (the 80/20 formulation produced microparticles with a more regular shape and with a smoother surface - Fig. 12). Thus, considering the chemical, morphologic and granulometric assays, the microparticles prepared according to the 80/20 formulation without Tween 80® were selected as the most promising for further studies. In this study, we also concluded that the size and the PSD were directly related to the stirring type and speed used during the emulsification step in the microparticle preparation. In fact, for TDI/PCL 80/20 microparticles, the use of mechanical stirring at 1,400 rpm led to a mean particle size of 24.34 μm, whereas the use of the homogenizer at 1,600 rpm led to mean particle size of 5.82 μm (results for the same volume ratio of 1:100); for TI/PCL 80/20 microparticles, mean particle size decreased from 19.4 μm to 4.7 μm, under the same conditions.

The zeta potential is an important physicochemical parameter when characterizing particles. It is assumed that particles with zeta potential more positive than +30 mV or more negative than -30 mV normally lead to physically stable suspensions [102]. Therefore, measurement of the zeta potential of the microparticles in suspension helps to foresee the storage stability of the suspension, which is especially important in pharmaceutical formulations. The zeta potential also influences how particles interact with cell membrane [103, 104] and influences how particles behave when undergoing processes such as phagocytosis [105]. We observed that all PU-based microparticle formulations presented negative zeta potential values (between -3 mV and -25 mV) most likely due to the ionization of carbonyl groups that exist in the polymeric matrix.

Moreover, we also observed that the zeta potential of TI-based microparticles was significantly lower (in absolute values) than that of TDI-based microparticles. We assumed that, in the TI-based microparticles, the negatively charged groups represent a lower percentage of the total matrix than in the case of TDI, leading to a decrease in absolute value of the zeta potential. We also studied the influence of the suspending medium composition (distilled water, and PBS solutions at pH 7.4, 6.5 and 2.0, in order to simulate several physiological environments) on the zeta potential of particles. The results suggested that the salt concentration of buffered solutions caused the equalizing of the zeta potential values of the studied formulations, due to the compression of the electrical double layer which surrounds the dispersed microparticles, thereby making their surface charges similar. This significant difference, when the suspending medium changes from water to a saline solution, can be very useful for a pharmaceutical formulation. Higher values in water will lead to better storage stability, while lower values at physiologic pH will make mucoadhesion possible [100].

In order to assess the eventual biomedical application of the PU-based microparticles described above, further studies were carried out [106]. Accordingly, we performed *in vitro* hydrolytic degradation and cytotoxicity assays using TDI/PCL 80/20 and TI/PCL 80/20 microparticles. The obtained results revealed lower hydrolytic degradation values for TI/PCL 80/20 microparticles than for TDI/PCL 80/20 microparticles (5.9% and 6.9%, respectively) after a 28 day incubation in PBS at 37 ºC. We hypothesized that the lower degradation of the TI/PCL 80/20 microparticles was due to the higher content of urethane linkages, a hard segment that degrades more slowly than the soft segments (ester linkages). The TI/PCL 80/20 microparticles also showed lower toxicity for peritoneal macrophages, relative to TDI/PCL 80/20 microparticles, after a 3 day incubation period, with different microparticle

concentrations. We concluded that these microparticles were suitable for biomedical applications. However, since cytotoxicity depends on the microparticle concentration, for any possible biomedical application, the maximum concentration to be used should be 0.08 μg/ml (cell viability less than 80%) [106].

4.2. Poly(vinyl chloride)

Poly(vinyl chloride) (PVC) (Fig. 10b) is produced by addition polymerization of the monomer vinyl chloride (VCM). This polymer is widely applied in industry, namely in construction, since it possesses suitable properties (such as durability), as well as low associated price [107]. Some studies on PVC-based particles were carried out in collaboration with our research group. Tomás and coworkers prepared PVC particles by O/W emulsion polymerization [108]. They studied the effect of the anionic surfactants (concentration and type) and long-chain fatty alcohol (as co-emulsifier) on the PSD of the PVC-based particles prepared. The authors used ammonium laurate (AL), as the main surfactant, and cetyl alcohol (CA), as the fatty alcohol. The experimental work was developed in a pilot plant, in collaboration with the company CIRES, S.A (Estarreja, Portugal), aiming towards industrial application. A tenfold increase of the CA/AL ratio resulted in an increase in particle size (D_{50}) from 267 nm (with PSD monomodal) to 581 nm (with PSD bimodal). According to the authors, this increase in particles size can be attributed to a decrease of the micellar nucleation mechanism. The effect of CA on PVC-based particles size was visualized by transmission electron microscopy (TEM) as shown in Fig. 13. With this study, the authors concluded that the PSD of the particles was influenced by the mixture of anionic surfactant with fatty alcohol (CA) and by the type and concentration of the anionic surfactant.

4.3. Silane

Silanes are chemical compounds that contain silicon in their composition and that are analogues of alkane hydrocarbons. They are widely applied both in industry [109] and in the biomedical field, mainly as adhesion promoters [110].

In our research group, microparticles obtained from a silane-based material, poly(vinyl trimethoxysilane), were prepared by O/W emulsion polymerization of vinyl trimethoxysilane (Fig. 14) [111]. Since the polymerization reaction was carried out in aqueous medium, cross-linking between the hydroxyl groups of water and the free hydroxyl groups of the silane occurred, resulting in a silicone or polysiloxane derivative (Scheme 3). The microparticles obtained were used to immobilize hydrophobic fluorinated compounds, such as PFT, with the purpose of using the particles as a coating. We observed that the immobilization of PFT led to a slight decrease in particle size (1.53 µm *vs* 1.41 µm). This led us to the conclusion that PFT immobilization did not significantly affect particle size. For the microparticles described, the EE value obtained was 91.3%, which means that encapsulation of PFT was achieved with high efficacy by this preparation method.

The silane-based microparticles described above were designed for industrial applications. However, silane-based materials were also used by other researchers in collaboration with our group to prepare microparticles for biomedical applications. During the work developed by Ferreira and co-workers, polymeric particles were prepared to be used as a tool to compare the experimental results of drug release with the simulation results obtained by a mathematical model [112]. The studied system was composed of microparticles loaded with flurbiprofen embedded in a polymeric matrix composed of the copolymer 2-hydroxyethyl methacrylate-*co*-methacrylic acid. The system was designed in order to simulate drug-loaded contact lenses. The microparticles

were prepared based on triethoxy(octyl)silane, as previously described [113]. By comparing the drug release profiles of the two approaches, the authors concluded that for the studied system, the mathematical model used and the software package used to implement it, could be employed in the design of contact lenses for a therapeutic application [112].

4.4. Methacrylate

Methacrylates (Fig. 10c) can be defined as ester derivatives of methacrylic acid. Since they present a carbon-carbon double bound, they are used as monomers in the production of polymers [114]. The most commonly used methacrylate is poly(methyl methacrylate) (PMMA), because of its properties, namely high light transmittance, low weight, transparency, chemical resistance and weathering corrosion [115]. PMMA applications are wide-ranging, from industry (e.g., glass substitute, additives, coatings, binders and sealers) [116] to biomedicine (e.g., bone cements, rigid intraocular and contact lenses and dental fillings) [117]. PMMA has also been used to prepare microparticles. These particles have been applied as fillers and bulking agents in replacing both soft [118] and hard tissues [119] that have been lost because of disease or injury. Since PMMA is a non-biodegradable polymer, it is classified as permanent filler, remaining functional indefinitely in the site of implantation, unless physically removed. Another application for microparticles based on PMMA has been microencapsulation of drugs. Such systems have been used for the controlled delivery of several drugs such as vaccination agents [120], antibiotic filled bone cements [121], chlorpheniramine maleate (an antihistaminic) [122] and chemotherapeutic agent cisplatin [123].

Like PMMA, other acrylates and methacrylates have been used to prepare microparticles. Magnetic non-porous poly(2-hydroxyethyl methacrylate-*co*-ethylene

dimethacrylate) (P(HEMA-*co*-EDMA)), poly(glycidyl methacrylate) (PGMA) and P(HEMA-*co*-GMA) microspheres with hydrophilic properties were prepared by dispersion copolymerization of the respective monomers in the presence of colloidal iron oxides. These particles were used for genomic DNA isolation, in order to improve molecular diagnostics [124]. α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide-graft-polybutylmethacrylate copolymer microparticles were prepared to encapsulate two model hydrophobic drugs, beclomethasone dipropionate (BDP, a glucocorticoid steroid with potent anti-inflammatory and anti-allergic effect), and flutamide (FLU, a non-steroidal antiandrogen drug used in prostate cancer treatment). These microparticles presented *in vitro* mucooadhesiveness, biodegradability and biocompatibility, though the two encapsulated drugs have showed different release profiles [125].

In our research group, we prepared microparticles by O/W emulsion polymerization of two different methacrylate monomers: methyl methacrylate (MMA) and sulfopropyl methacrylate potassium salt (SPM), using ammonium persulfate as the initiator (Scheme 4) [111]. Moreover, we immobilized a hydrophobic fluorinated compound (PFT) in the microparticles. These microparticles were then introduced in commercial paints to assess their influence on the hydrophobicity/hydrophylicity ratio. The purpose of this work was to obtain an improved paint, with hydrophobic properties, since this type of material is highly resistant to staining and easier to clean. The obtained microparticles were observed by SEM (Fig. 15). The size analysis confirmed the presence of micron- and submicron-sized particles, in both MMA/SPM particles without and with PFT. In fact, the average size of the particles without and with PTF was determined to be 170 and 180 nm respectively. The results showed that the immobilization of PFT did not significantly influence PSD. We determined the PFT EE using the same approach as was used for silane-based particles. However, for

MMA/SPM-based particles, the PFT EE (63.5%) was considerably lower than the one obtained with silane-based particles. Finally, we measured the water static contact angles (θ) of films prepared with a commercial paint and with the same commercial paint containing the prepared particles in order to evaluate any changes in paint's hydrophobicity. The water contact value changed from 68.8º to 74.0º, when particles were incorporated into the paint's composition. This means that the hydrophobicity of the initial sample was increased with the embedding of particles, resulting in a coating material with higher staining resistance and easier cleaning once applied.

5. Concluding remarks

Microparticles are now established as an important part of technology, taking new roles and offering a combination of benefits, such as reduced cost and improved product quality and stability. Microparticles are presently used in a wide variety of applications ranging from medical devices to construction materials. The increasing complexity of existing applications, as well as potential applications, of microparticles requires that more sophisticated materials become available to render these systems more successful. Advances in polymer synthesis chemistry are making it possible to prepare more refined microparticles with greater performance. In this review, we presented recent and current studies carried out in our research group in developing new and promising microparticles from either natural or synthetic polymers for biomedical and industrial applications. However, a multitude of challenges in creating polymeric microparticles to fulfill the technological demands still remains, making this an exciting and highly rewarding research field.

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Figures captions

Fig. 1. Different categories of microparticles [1].

Fig. 2. Global market for microparticles, by industry, in 2010 and estimates for 2015 [23].

Fig. 3. Main steps of the O/W emulsion SEE method (see text, for details).

Fig. 4. Generic chemical structure of (a) PHAs, (b) PHB, (c) PHBV, (d) CAB, (e) starch, and (f) chitin and chitosan.

Fig. 5. Representative SEM images of PHBV microparticles prepared using (a) mechanical stirring, and (b) a homogenizer, during the emulsification steps [44].

Fig. 6. Effect of PHBV concentration and PVA concentration on the EE of the flurbiprofen-loaded microparticles prepared by an O/W SEE method. Dots: experimental points; surface: adjusted model [45].

Fig. 7. SEM images of CAB microparticles (a) with entrapped fluorouracil, and (b) without fluorouracil [57].

Fig. 8. Microcapsules prepared from modified starch at 100× magnification [64].

Fig. 9. (a) Optical (magnification, 10×) and (b) SEM (magnification, 5,000×) images of glutaraldehyde cross-linked chitosan-PVA microparticles [75].

Fig. 10. Chemical structure of (a) urethane linkage, (b) PVC, and (c) methacrylate.

Fig. 11. Chemical structure of (a) TDI/PCL and (b) TI/PCL microparticles, with urethane (filled in red) urea linkages (filled in green) shown [100].

Fig. 12. Representative SEM images of (a, b) TDI/PCL 80/20 and (c, d) TI/PCL 80/20 microparticles [100].

Fig. 13. Representative TEM images of PVC-based particles showing the effect of the anionic surfactant CA concentration on particle size (a) without CA, (b) CA:AL = 1, and (c) CA:AL = 10 [108].

Fig. 14. Microparticles of poly(vinyl trimethoxysilane) observed by optical microscopy at 1,000× magnification [111].

Fig. 15. Typical SEM image of MMA/SPM-based microparticles. A representative part of the image was selected to highlight the microparticles [111].

Schemes captions

Scheme 1. Scheme of the chemical modification of starch with 2-vinyl-4,4- dimethyl-2-oxazolin-5-one using 4-dimethylaminopyridine (DMAP) as catalyst [64].

Scheme 2. Scheme of the cross-linking reaction between the starch derivative and the bifunctional cross-linker DPGDA.

Scheme 3. Cross-linking of poly(vinyl trimethoxysilane) in aqueous medium and formation of silicone [111].

Scheme 4. Copolymerisation reaction between MMA and SPM [111].

Figure1

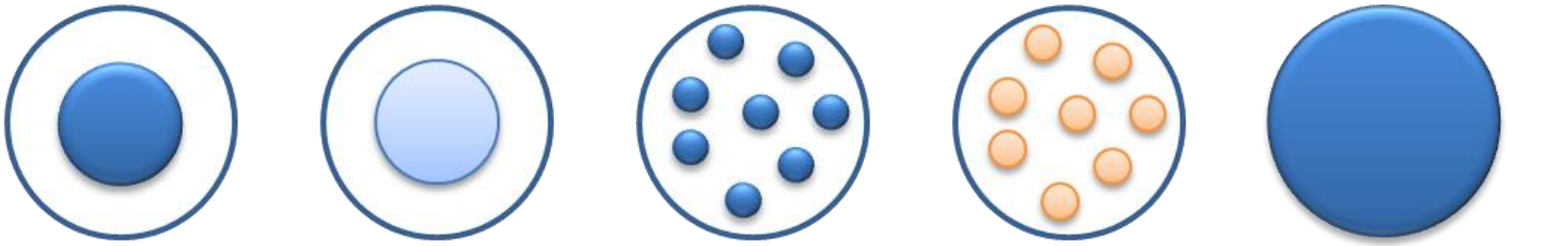

Microcapsule (solid core)
Microcapsule (non-solid core)
Microcapsule (solid micro/ nano domains)
Microcapsule (non-solid micro/ nano domains)
Microspheres (mixture of matrix/ encapsulated agent)

Figure 2

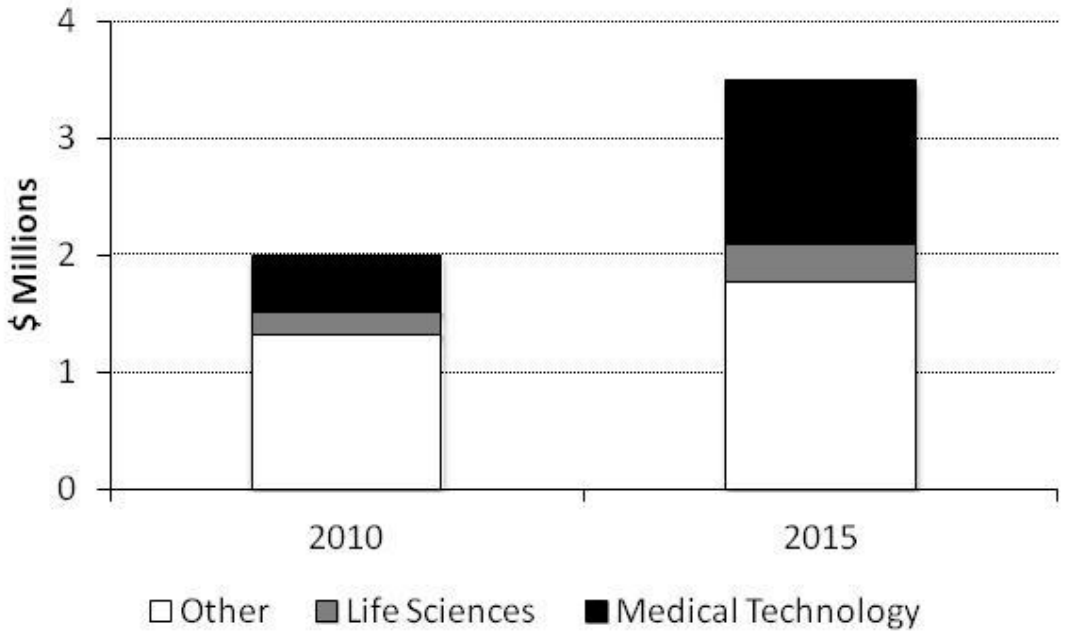

4
3
2
1
0
$ Millions
2010
2015
Other
Life Sciences
Medical Technology

Figure 3

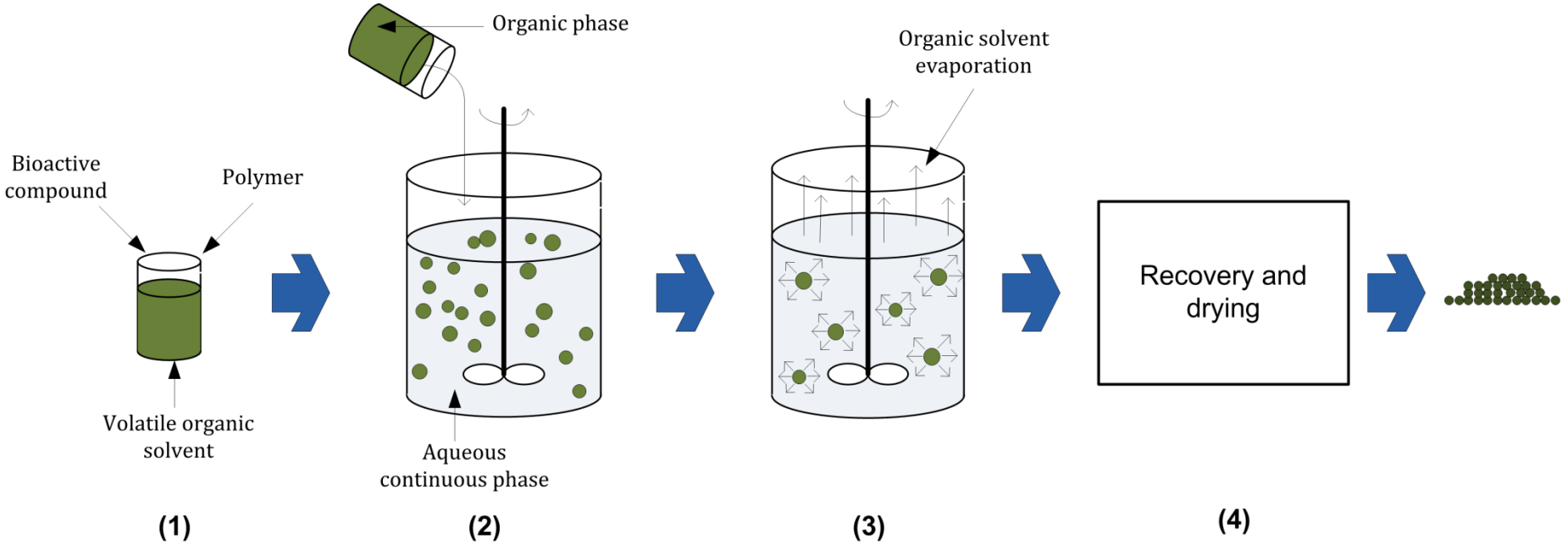
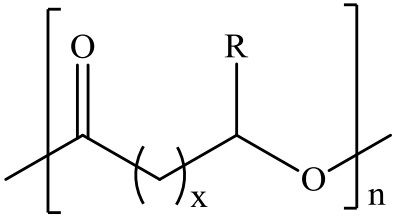

Bioactive compound
Polymer
Volatile organic solvent
Organic phase
Aqueous continuous phase
Organic solvent evaporation
Recovery and drying
(1)
(2)
(3)
(4)

Figure 4

a)

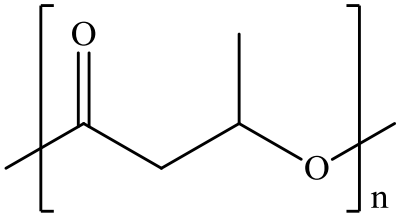


x = 1 to 4

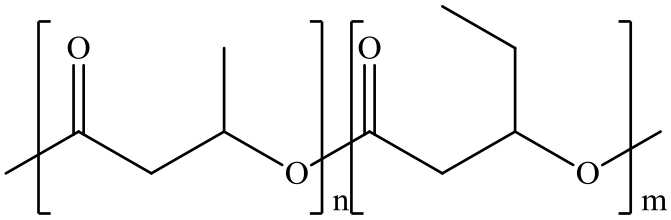
n = 1000 to 10000

R = alkyl group or functionalized alkyl group

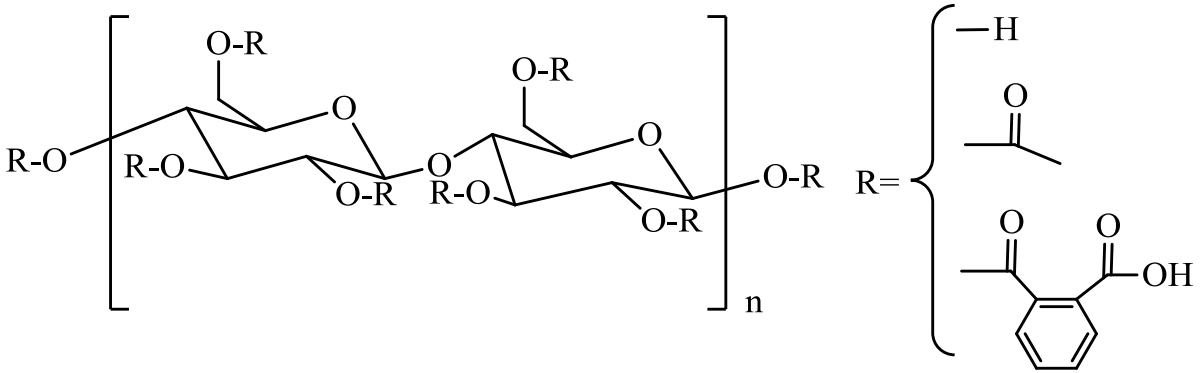
b)



c)

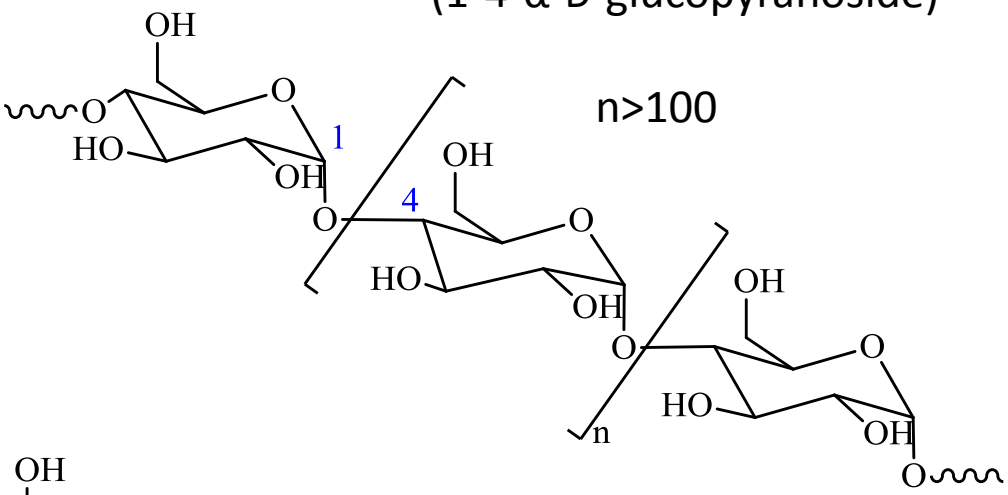


d)

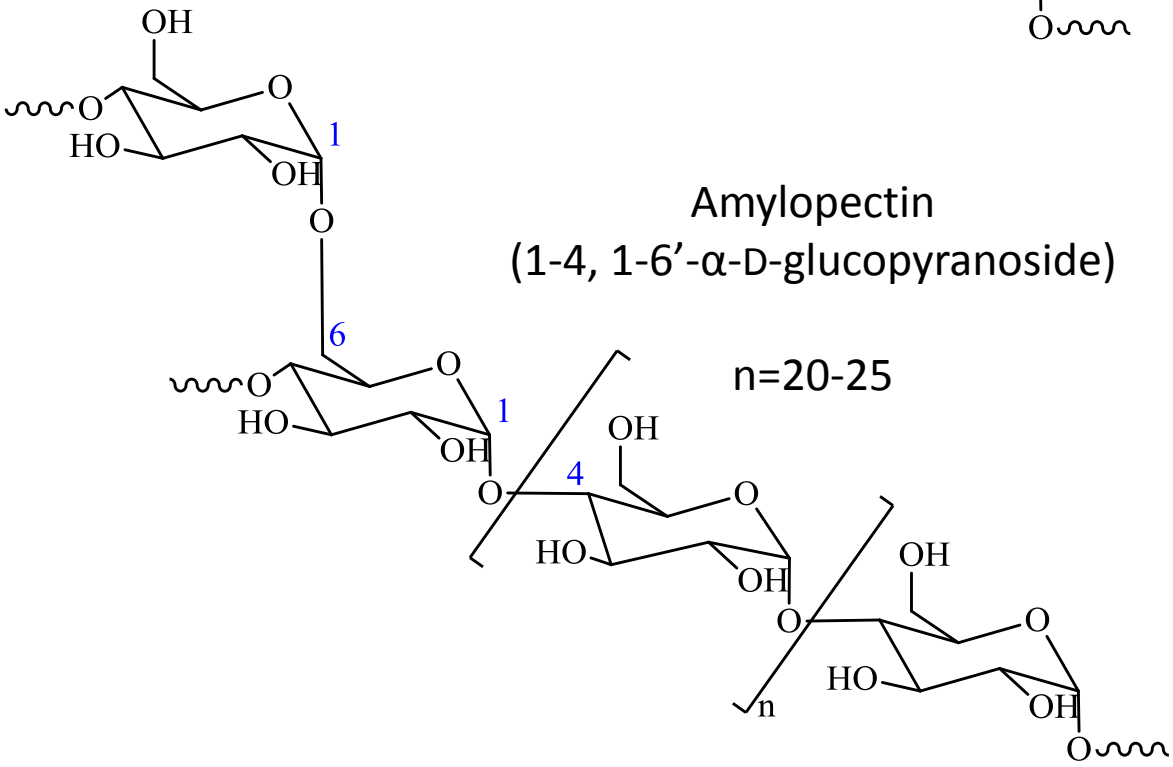


e)

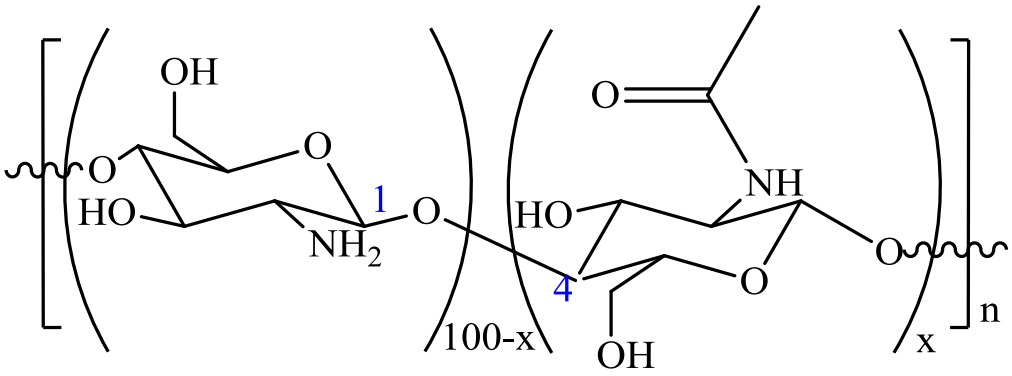
Amylose
(1-4-α-D-glucopyranoside)



Amylopectin
(1-4, 1-6'-α-D-glucopyranoside)



f)



x > 50%: chitin

x < 50%: chitosan

Figure 5

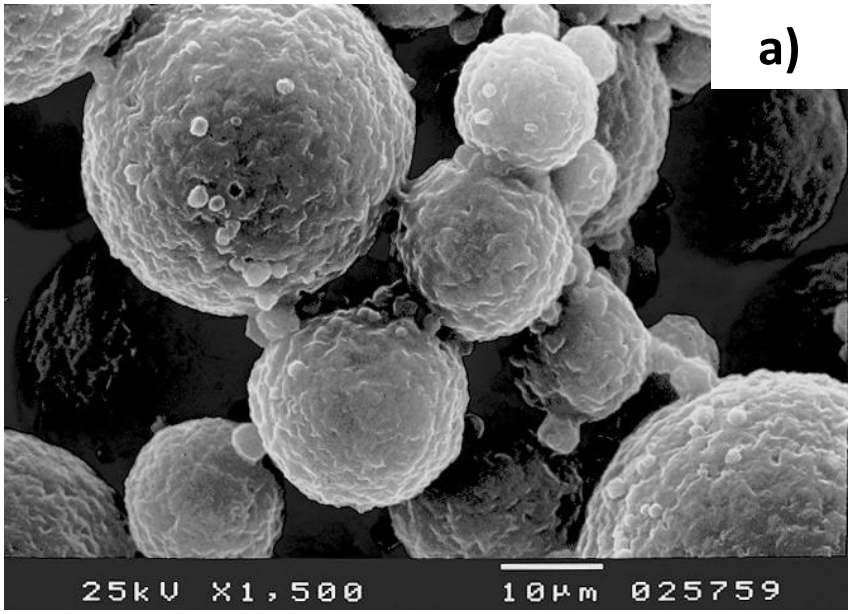


Figure 6

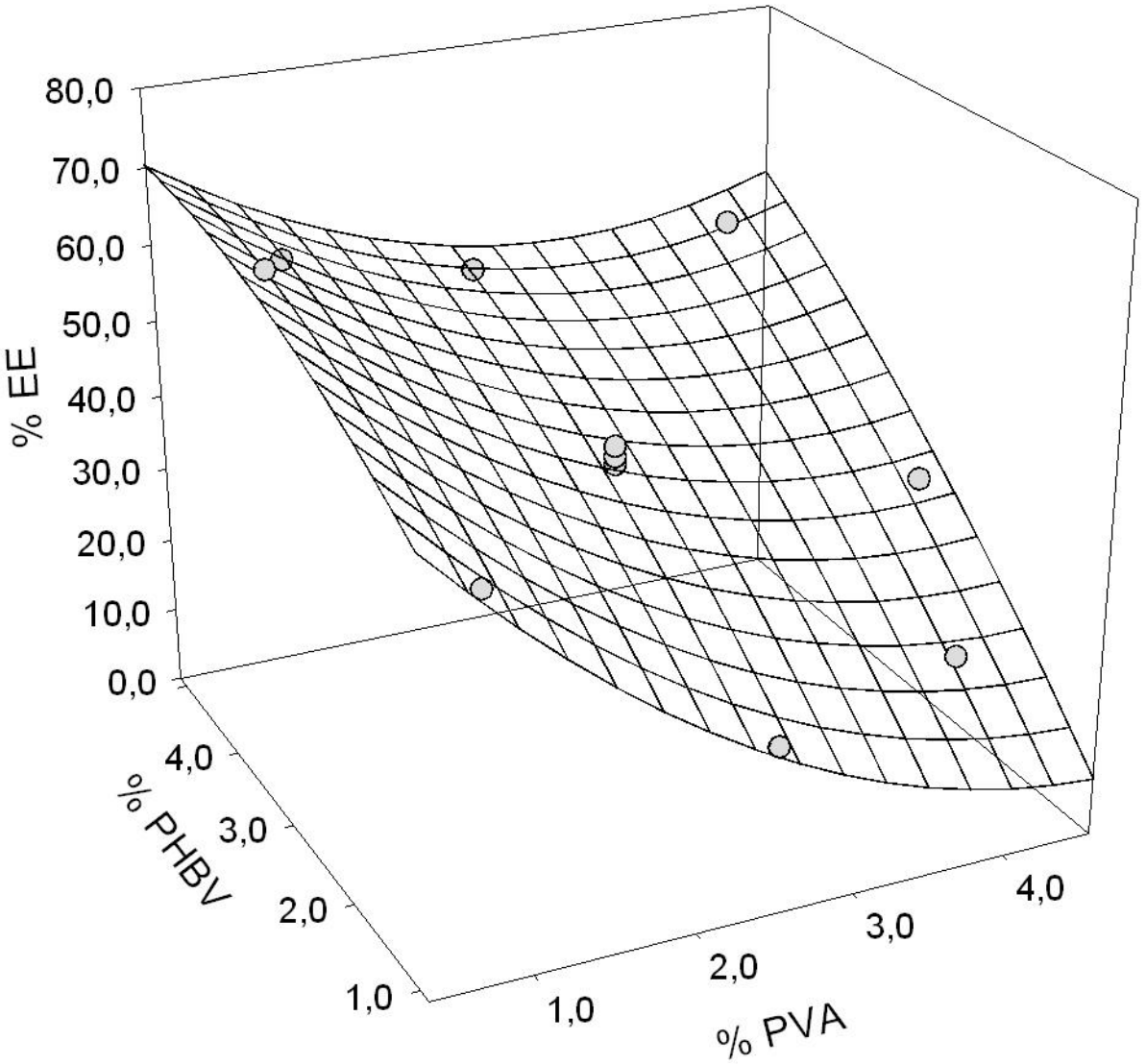
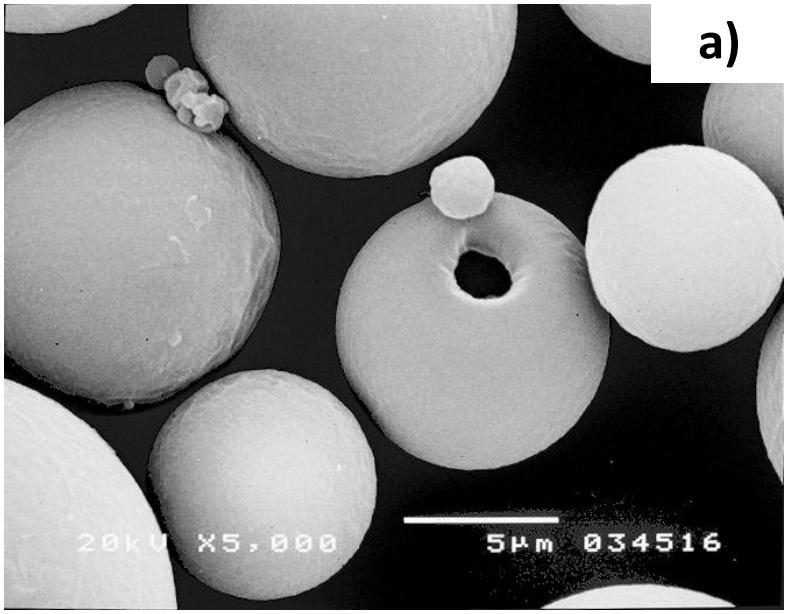

80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
% EE
4,0
3,0
2,0
1,0
% PHBV
1,0
2,0
3,0
4,0
% PVA

Figure 7



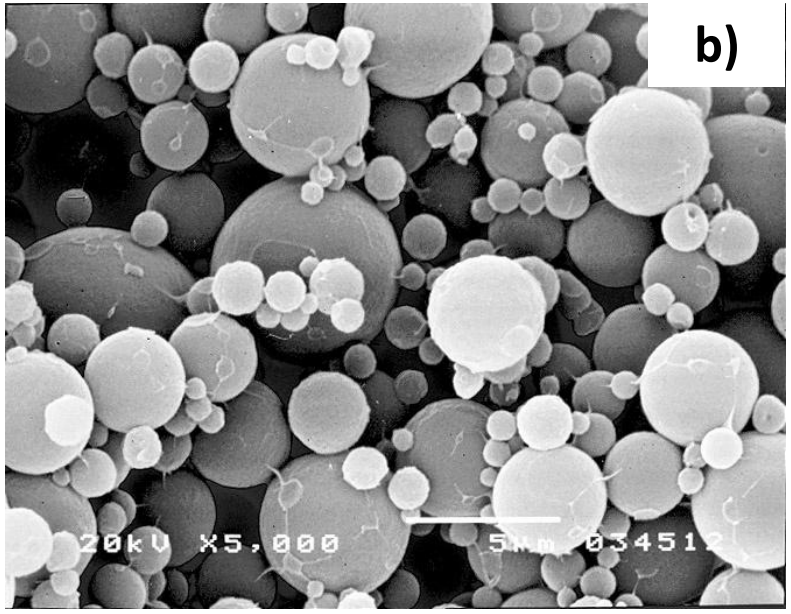


Figure 8

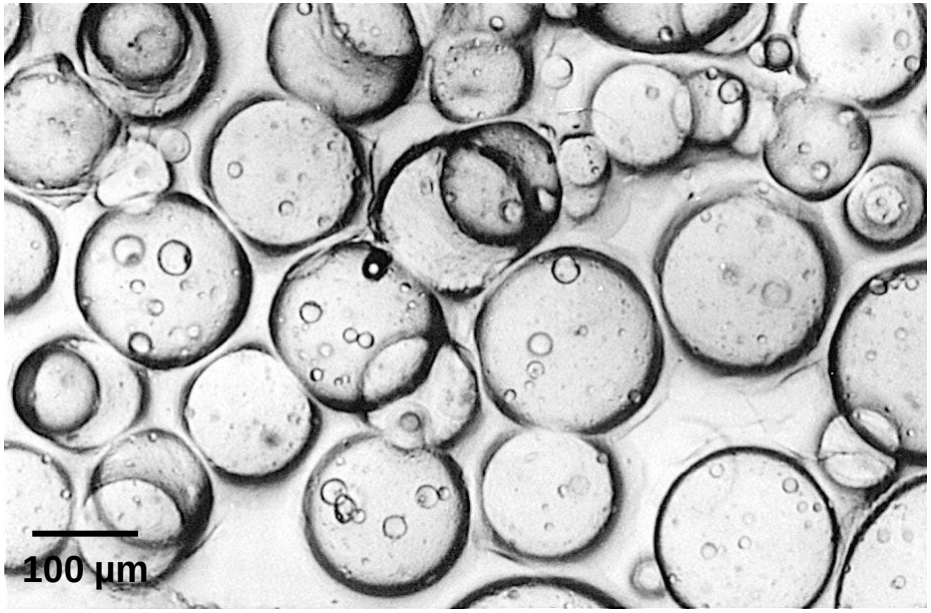
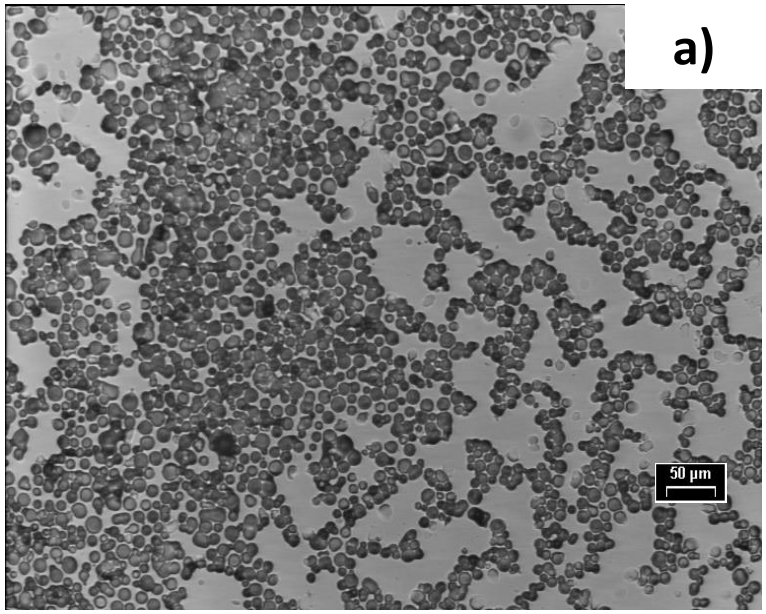


Figure 9



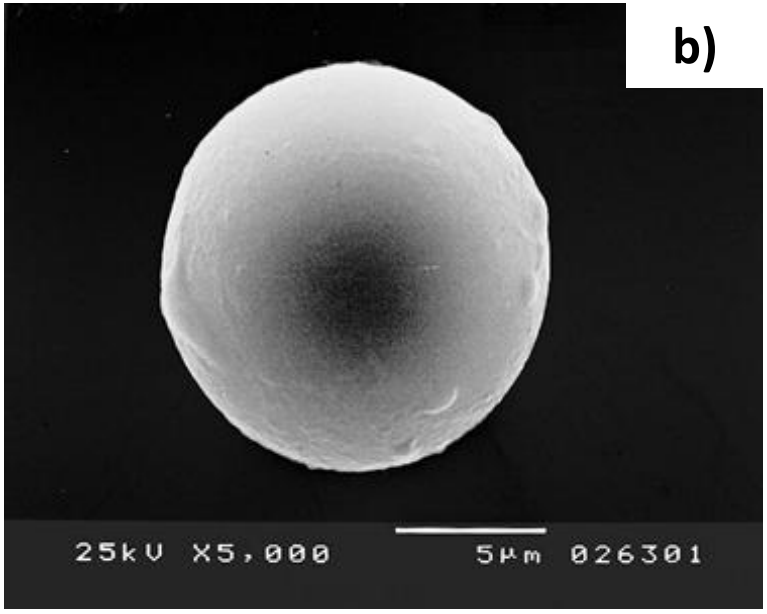
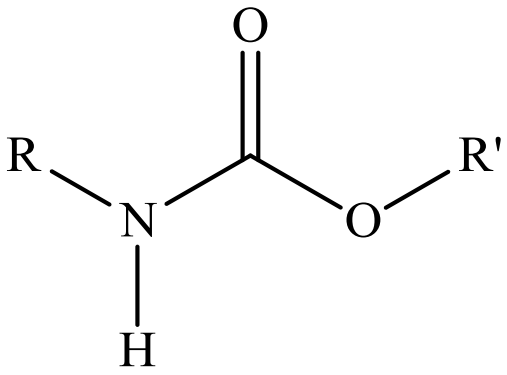
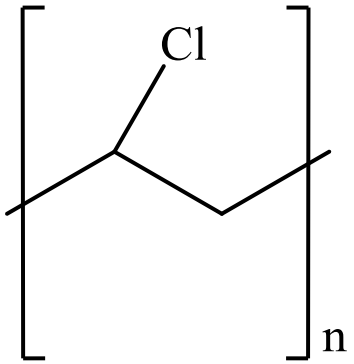


Figure 10

a)



b)



c)

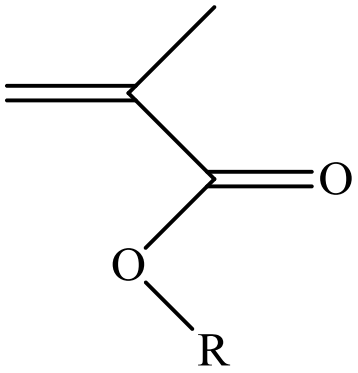
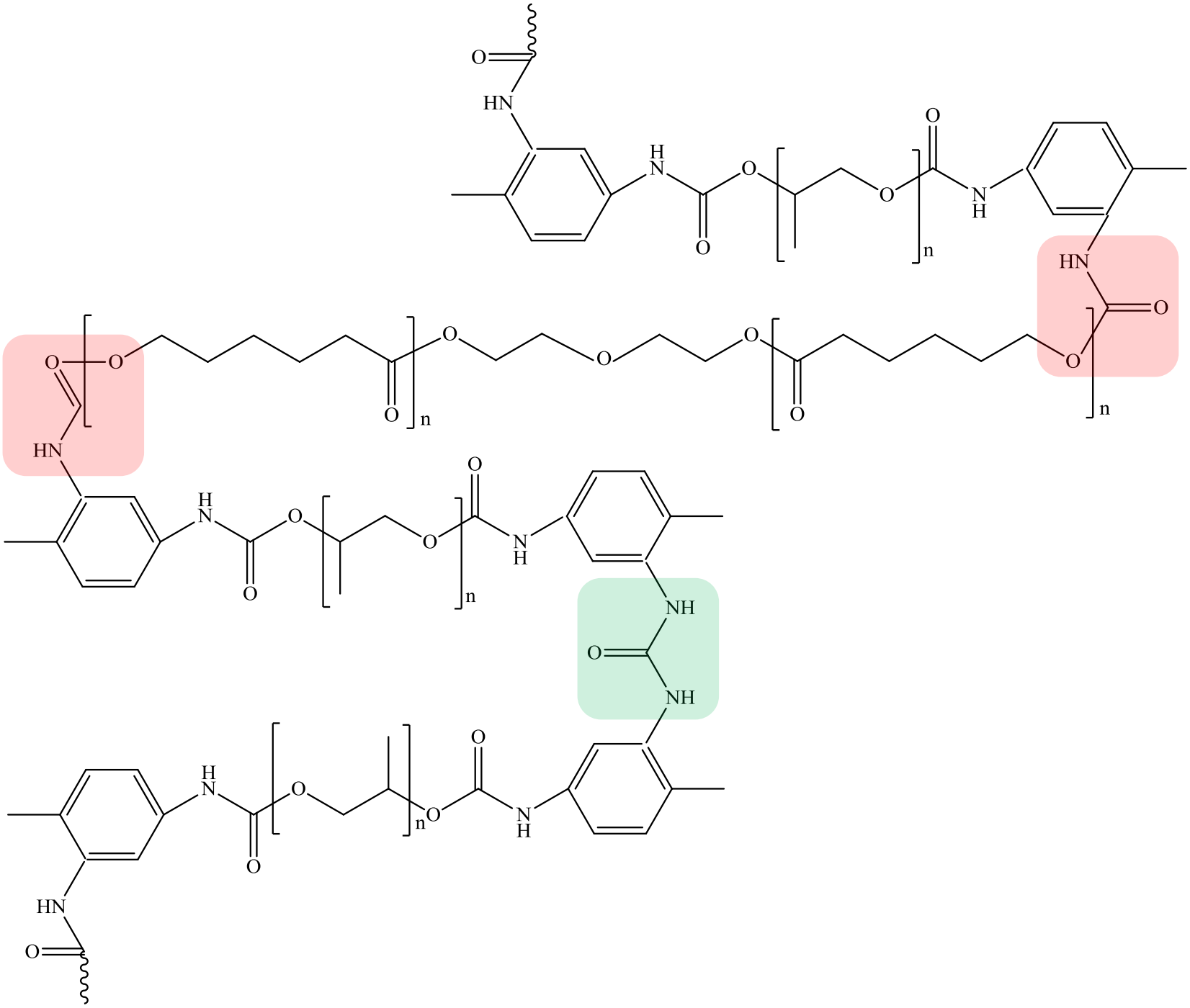


Figure 11

a)



b)

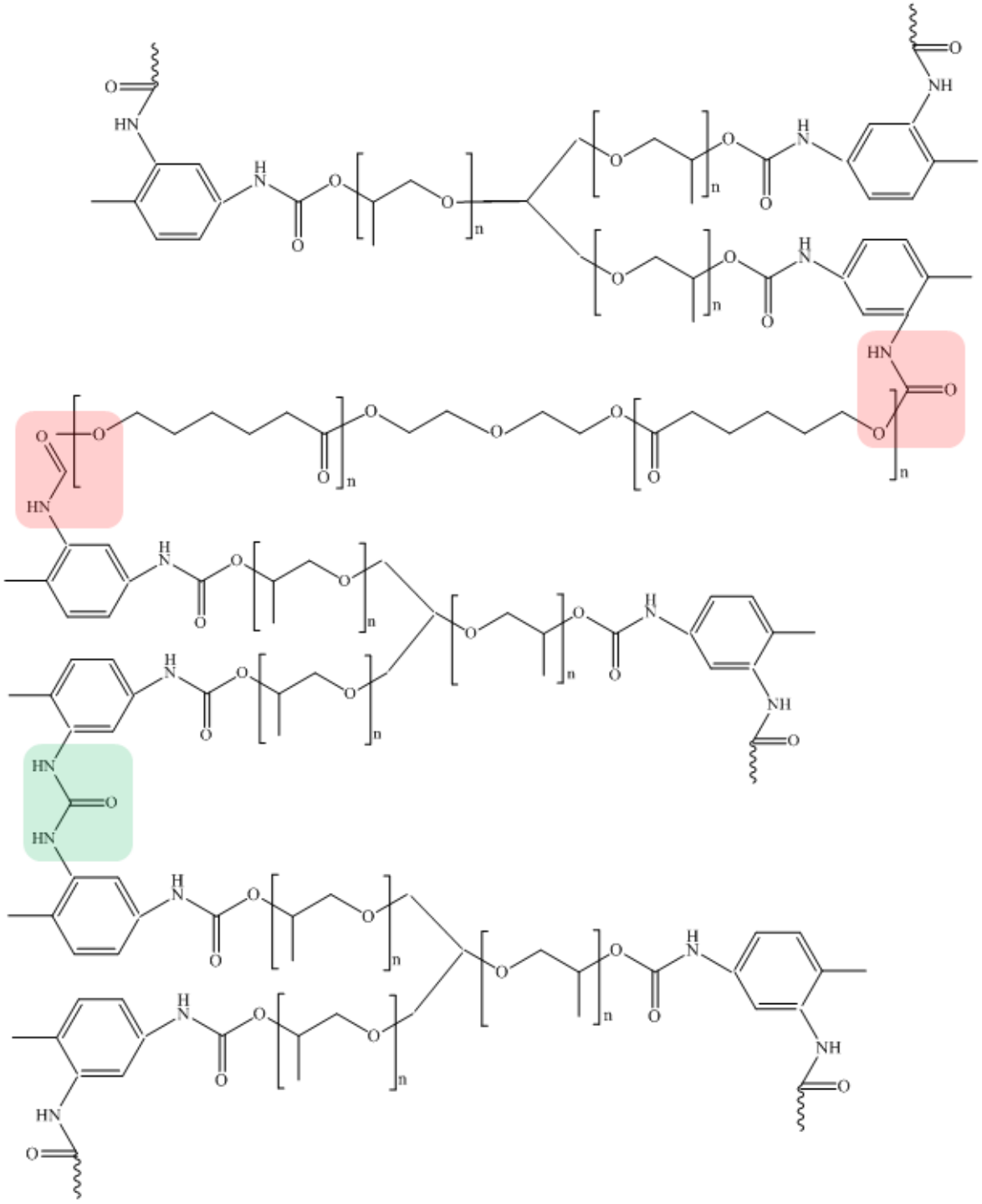
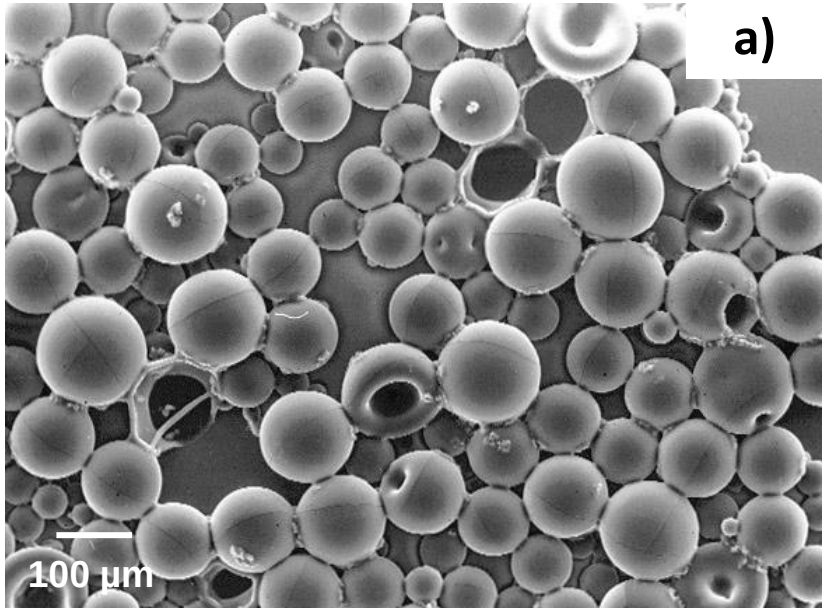
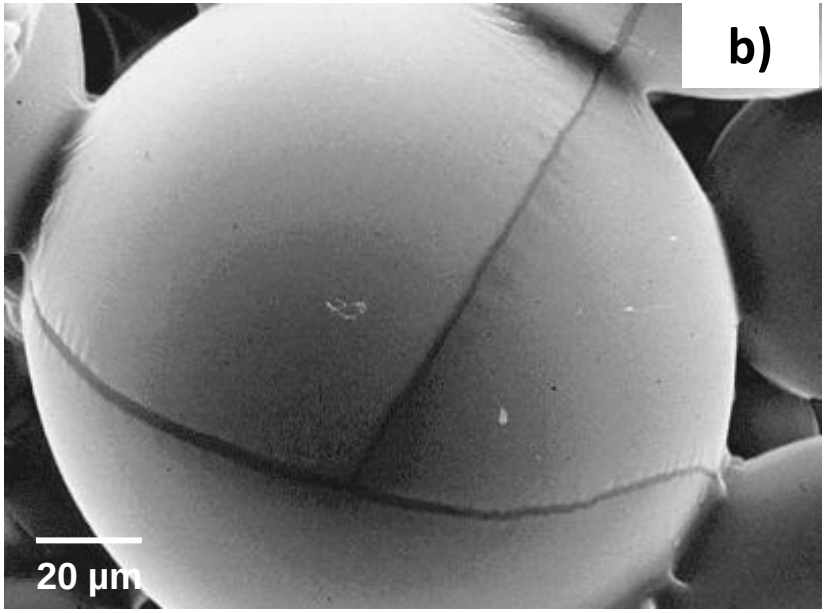
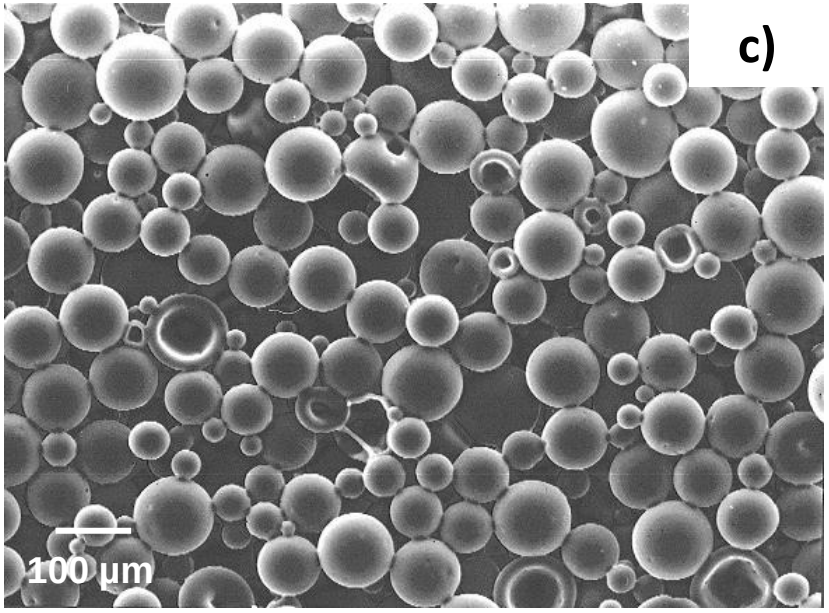


Figure 12







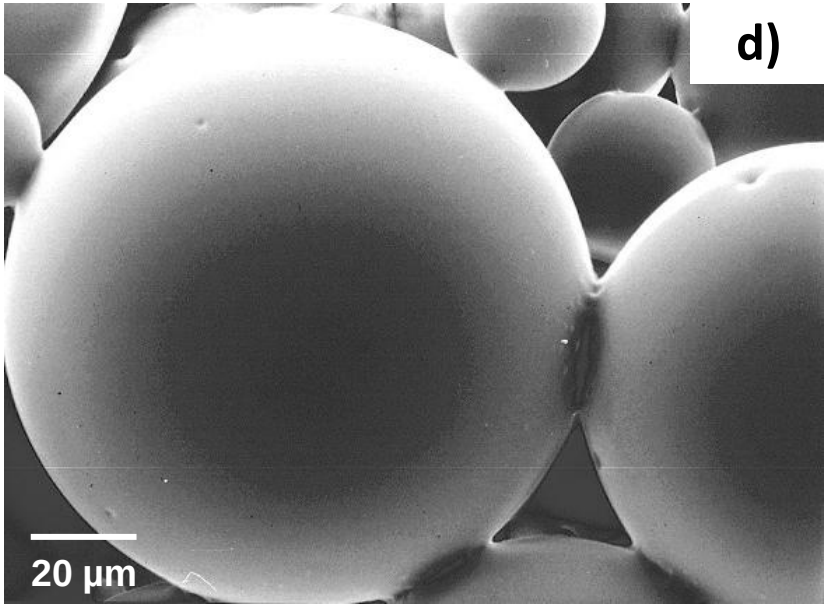


Figure 13

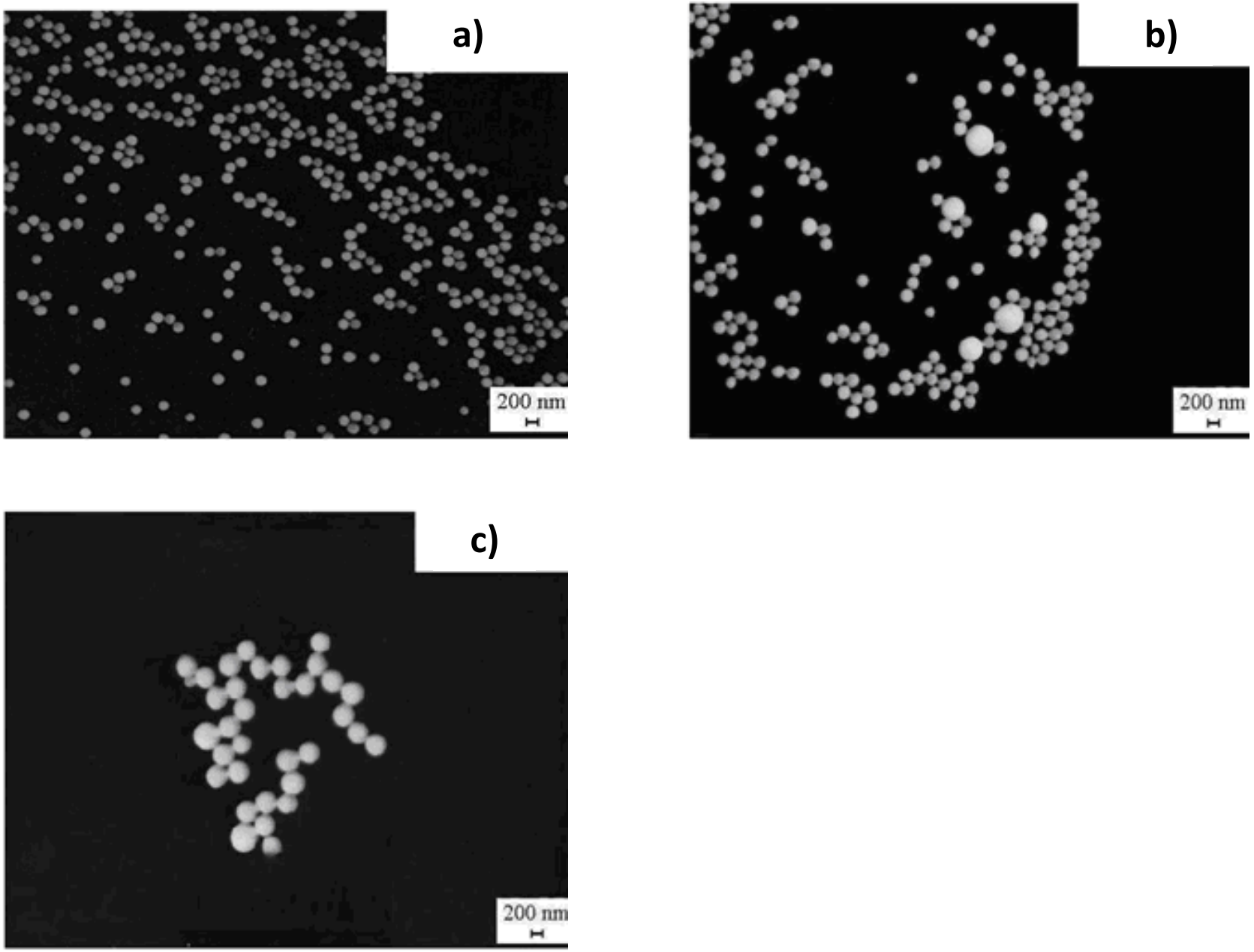


Figure 14

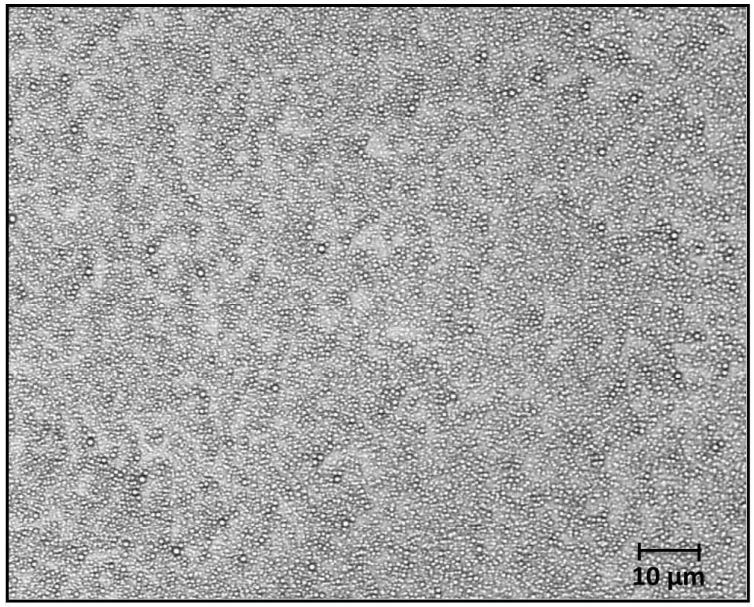
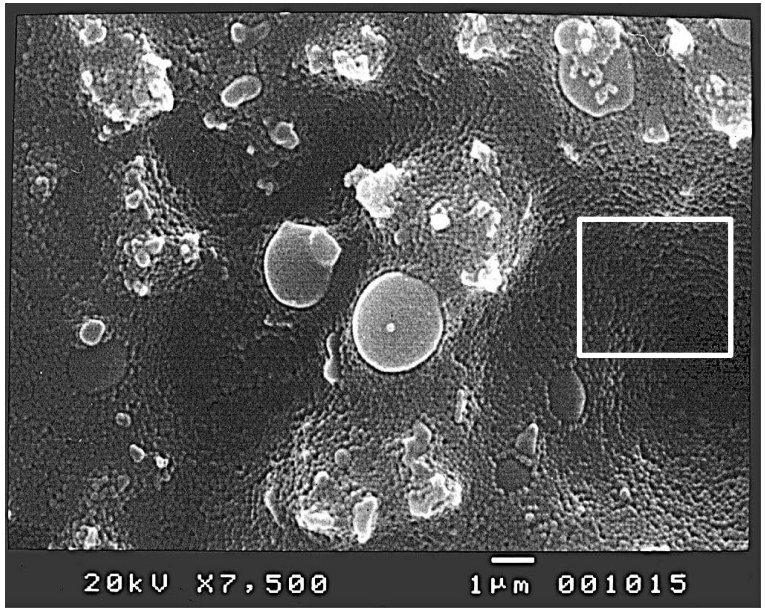
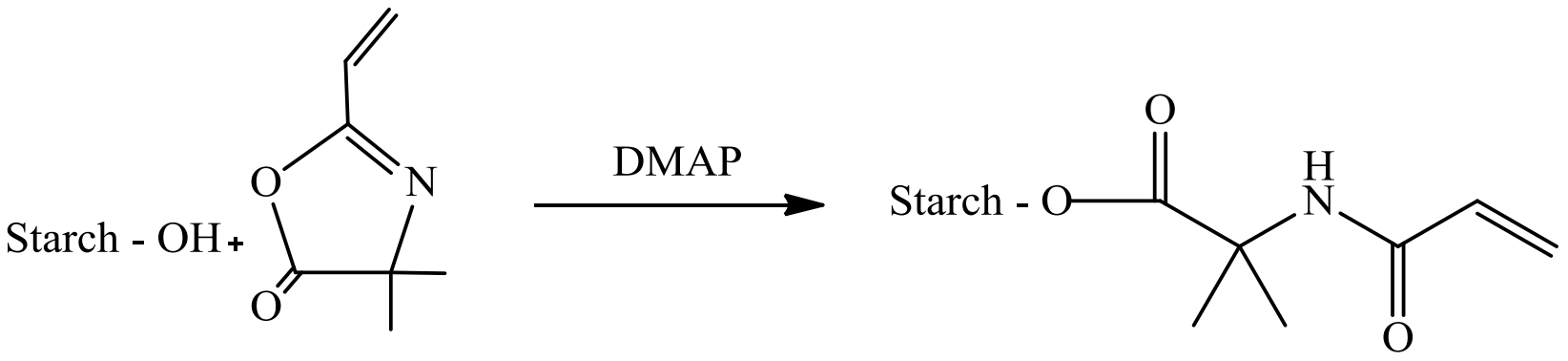


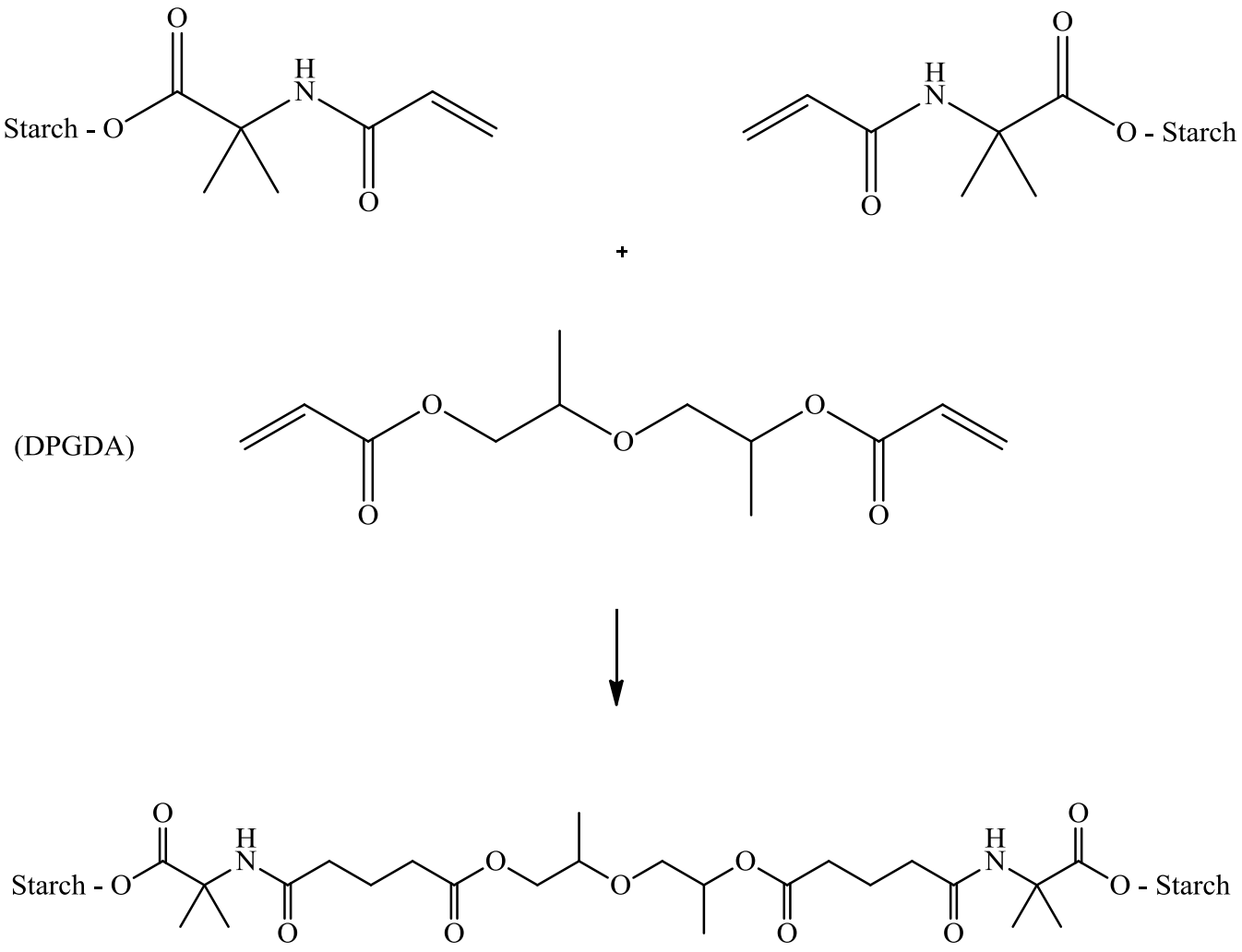
Figure 15



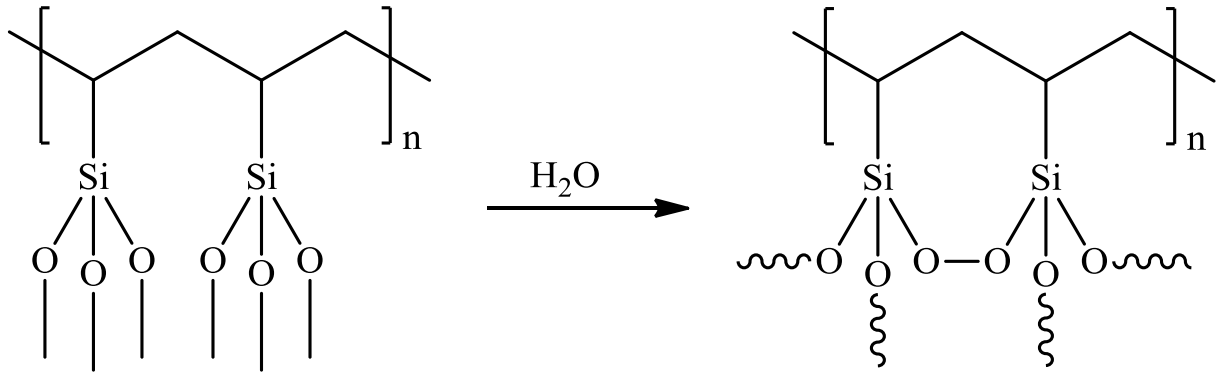
Scheme 1



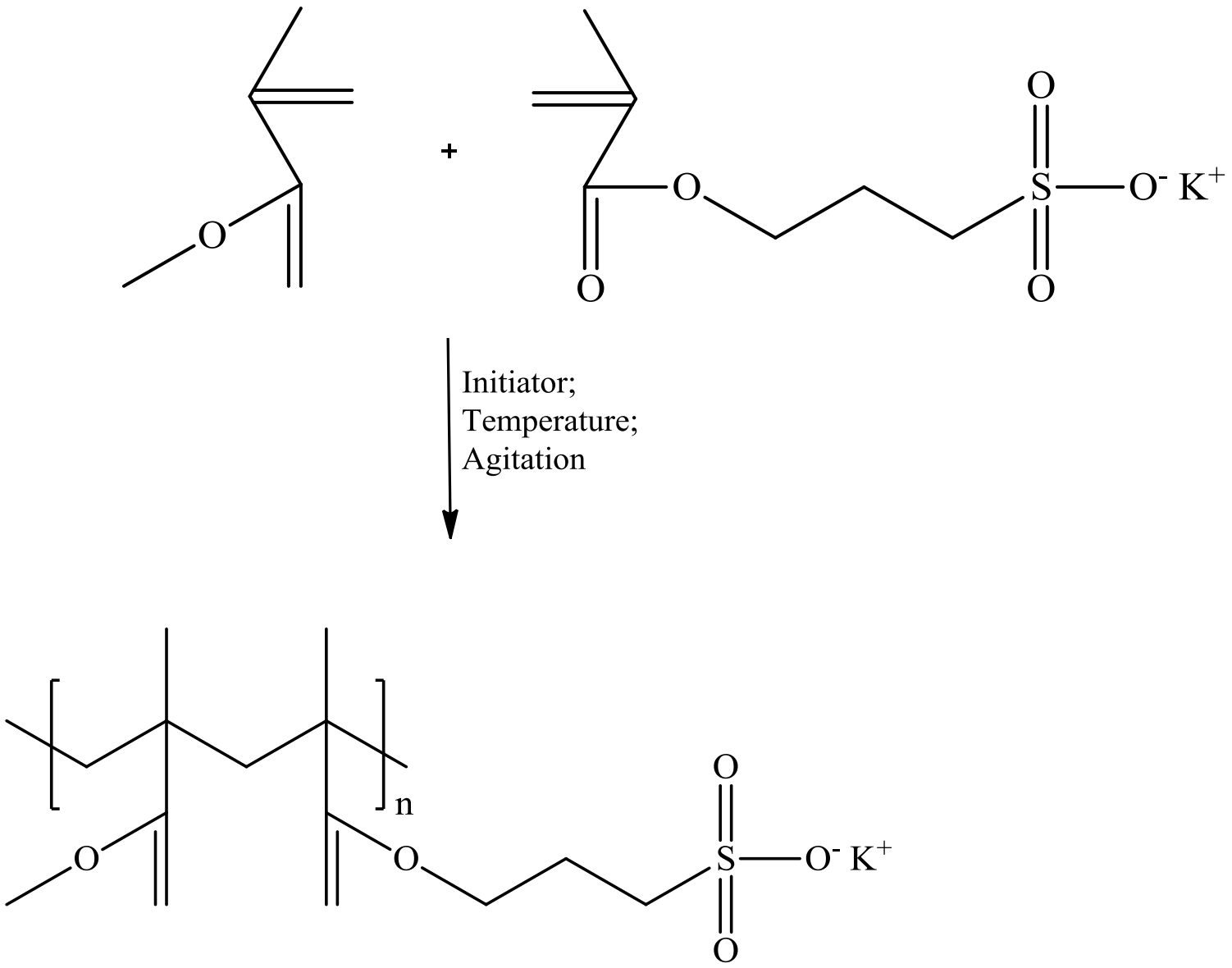
Scheme 2


Starch - O
O
H
N
O
O
H
N
O
O - Starch
+
(DPGDA)
O
O
O
O
O
O
Starch - O
O
H
N
O
O
O
O
O
O
H
N
O
O - Starch

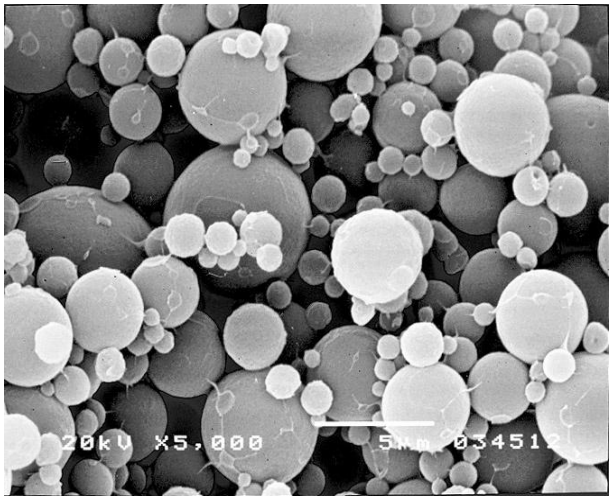
Scheme 3

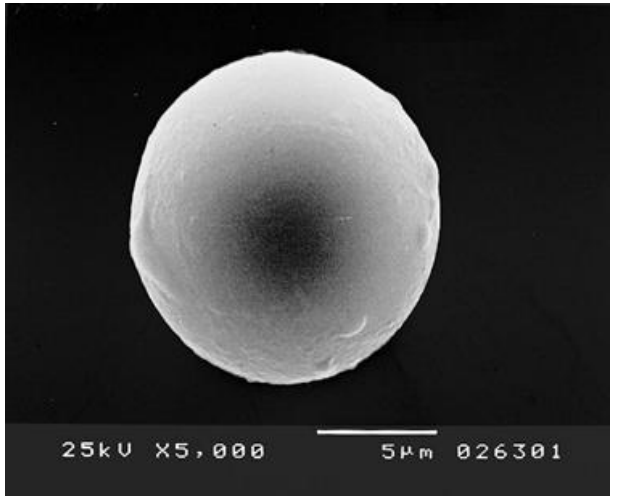


Scheme 4




25kV X1,500 10µm 025759


20kV X5,000 5µm 034512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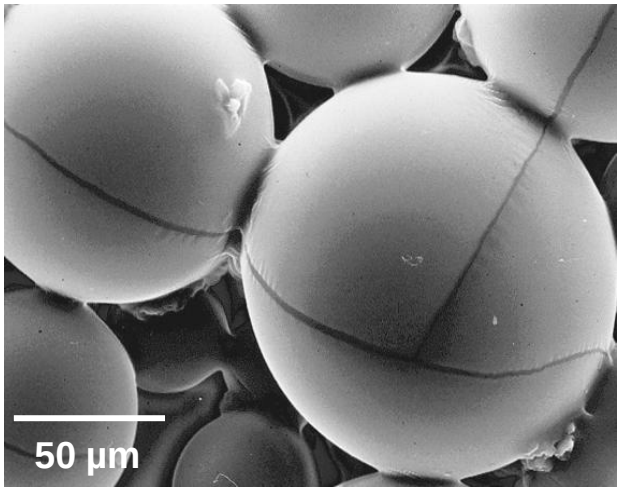
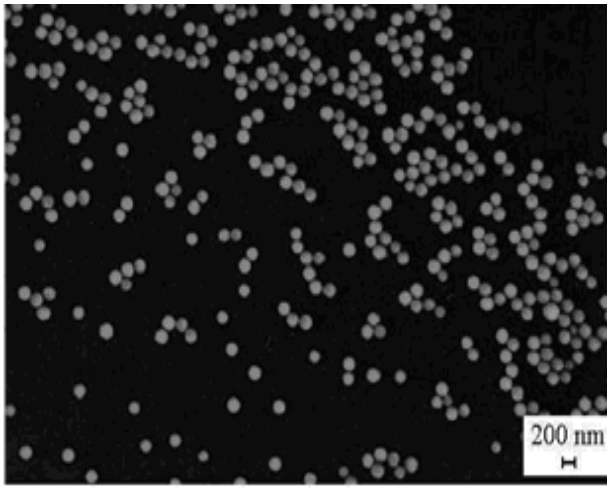
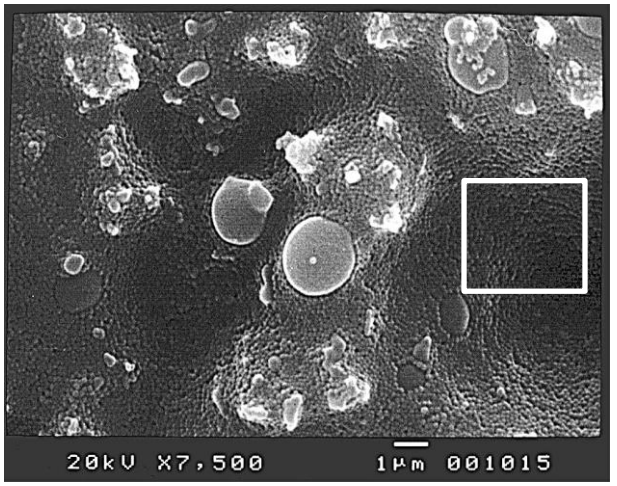

50 µm


200 nm


20kV X7,500 1µm 001015

Highlights

- Microparticles applications range from biotechnology to construction materials.
- There are several companies producing exclusively microparticle-based products.
- Microparticles based on natural polymers aim biomedical applications.
- Microparticles based on synthetic polymers aim biomedical and industrial applications.