1. Introduction

Chitosan is a biodegradable polymer obtained by deacetylation of chitin, which is present in shells of insects and marine crustacean. The unique properties of chitosan, among others biodegradability, bioactivity, non-toxicity as well as good adhesion and sorption, largely contribute to its multiple applications [1]. It must be added that chitosan is a valuable component of polymer blends and composites [2, 3]. Using an appropriate technological process one may obtain films, fibres, gels and foams as well as chitosan beads of different size and morphology.

Spherical chitosan structures (CS) have been started to produce in the 80-ties of XX century [4, 5]. They find applications in medicine as the active substances carriers [6, 7] as well as sorbents in the processes of water purification from heavy metal ions or the decolourisation of textile sewages [9].

There are many methods of CS beads/microbeads producing. Considering medical applications of the structures, the choice of a method depends primarily on a desired shape of the beads and the stability of an encapsulated active substance as well as the whole Controlled Release Systems (CRS). Furthermore, the choice concerns the repeatability of the kinetic release profile and the requirements concerning an acceptable toxicity level of the CRS degradation products.

All the methods of the spherical chitosan structures generation are based on the similar procedure. The first stage encompasses the process of solving chitosan in an organic acid (acetic, formic acid). The next stage is aimed at the formation of solution drops from a solution of CS in the emulsification process [10] or pumping the solution through a nozzle [4] or sieve [11]. The subsequent stage comprises the precipitation of chitosan in the
drops obtained by the addition of a cross-linking agent, the change of pH or, as it occurs in the process of spray-drying, the evaporation of the solvent from a droplet [12].

Other methods of chitosan micro- and nano-particles production base on the coalescence phenomenon or the collision of two dispersed emulsions phase drops of a different pH [13], or the formation of reversed micelles [14]. Another method uses the phenomenon of the opposite charged poly-ions complexation. Chitosan, being a poly-cation in a solution undergoes an ion gelation due to a reaction with an appropriate poly-anion, e.g. sodium tri-polyphosphate (TPP) [15].

The processes of filtration, washing and drying of the spherical structures are aimed at removing from the porous chitosane beads such products as surfactants, cross-linking agents, sodium hydroxide or poly-anions.

It is quite recently that to enhance the mechanical properties of the polymer carriers and taking into account properties of incorporated active substances, the systems composed of two or more different macromolecules, forming complexes have been discussed.

One of the basic motives of the research into the formation of the polymer hybrid systems is a necessity to obtain a desired kinetic model of an active substance release. Elimination of the so-called “burst effect”, being frequently present at the beginning of the release process and leading to the diffusion of a significant amount of an active substance to the environment, has become a basic problem in the formation of CRS polymer systems.

Poly (lactic acid) is a biodegradable polymer originating from such renewable resources as corn, sugar beet or rice. Plant products constitute the source of starch which, to obtain PLA, is first transformed into dextrose on the way of enzymatic hydrolysis. Dextrose proceeds a fermentation process, which results a lactic acid. The condensation of lactic acid using a solvent, under vacuum and at a high temperature leads to obtaining the so-called pre-polymer of low molecular mass (M$_w$ about 5000). PLA of high molecular mass is generated during the polymerisation of dimer - lactide by the process of ring opening [16].

Poly (lactic acid) is a hydrophobic material, easily soluble in organic liquids such as ethyl acetate, dichloromethane or chloroform. To its benefits one may account: high mechanical resistance (resembling popular synthetic polymers such as polystyrene, polypropylene) as well as a possibility of forming porous micro- and nano-structures [17, 18].

An additional factor, which has decided about our choice of poly (lactic acid) as a component of hybrid matrix to control the active substance release, is a negative zeta potential of its microspheres [19], which contrasts with the cationic character of chitosan.

In the paper the preliminary results of the preparation and morphology of two-component spherical chitosan/PLA structures have been demonstrated.
2. Materials and methods

2.1. Materials

The beads were produced using chitosan (CS) powder manufactured by the Sea Fisheries Institute in Gdynia. The degree of deacetylation of the polymer was 72.9%. Poly(lactic acid) (PLA) in the granular form was the product of Cargill Dow Polymers LLC (USA). The solvents of chitosan and PLA were acetic acid and ethyl acetate respectively. In the process of the emulsification of an organic PLA solution in water a surfactant: poly-oxy-ethylene-poly-oxy-propylene glycol of a trade name Pluronic F 68 (PF 68), was applied.

The coacervation of chitosan beads occurred in a 0.5 M solution of sodium hydroxide.

2.2. Analytical methods

The preparation of the hybrid beads in the chitosan/PLA system was a three-stage process (Figure 1). Firstly, PLA microstructures were formed and, next, they were distributed in a 3% solution of CS in acetic acid. The beads were generated by chitosan coacervation in NaOH.

The PLA micro-particles were prepared using the emulsification method combined with the diffusion of a solvent [20]. To achieve the goal, two solutions were used: 0.45 g PLA dissolved in 18 cm$^3$ of an organic solvent (ethyl acetate) and a 2.5% (w/v) aqueous solution of a Pluronic F 68 surfactant.

The emulsification of two solutions was carried out by means of ultrasounds of frequency of 21.5 kHz. The obtained system (oil in water) was dispersed in a volume of 215 cm$^3$ of distilled water in the mixing conditions (500 r.p.m.). Consequently, the diffusion of ethyl acetate from a dispersed to continuous phase occurred due to its miscibility with water (1 ml in 9 ml H$_2$O). The result of the process was the suspension of PLA microstructures.

![Figure 1. Scheme of spherical hybrid CS/PLA structures generation.](image-url)
After the sedimentation of the PLA microspheres, a considerable quantity of ethyl acetate, water and the excess of the surfactant were taken from the system with a pipette. Afterwards, the re-dispersion of microstructures in distilled water was carried out using a magnetic stirrer. The whole cycle was repeated several times to remove ethyl acetate and Pluronic F 68 from the system.

After the evaporation of water from the suspension of microspheres at room temperature and additional drying of obtained PLA powder at 85 °C, the microstructures were dispersed in a 3% chitosan solution in 1% acetic acid. The whole mixture was pumped using compressed air through a nozzle of a diameter 0.8 mm into a gently mixed 0.5 M solution of sodium hydroxide. Immediately, the coacervation of CS occurred. As a consequence, the hybrid beads of diameters ranging from 1 to 2 millimeters were obtained. Figure 2a demonstrates a vessel to which a CS solution with the suspension of PLA microstructures was added. Figure 2b presents a photograph of the hybrid beads.

The kinetics of swelling of the structures in distilled water was examined using the gravimetric method. The portions of the hybrid beads and beads made of pure chitosan (as a reference) were dried with a paper towel and put into the laboratory drier at the temperature 50 °C for 15 or 30 minutes. The process of beads drying at higher temperatures resulted in their irreversible deformation and degradation. After the evaporation of the excess water, the beads were weighted ($m_s$) and put into a vessel with distilled water. After 24 h the measurements of the beads mass with water ($m_w$) were carried out and, then, they were placed in the vessel again. The measurements of $m_w$ were continued to the moment of weight statement. The swelling degree $\alpha$ was calculated from the equation (1) which means the percentage quantity of water in the spheres related to their mass before the swelling process.

$$\alpha = \frac{(m_w - m_s)}{m_s} \cdot 100\%$$  \hspace{1cm} (1)

Figure 2. a) Apparatus for the coacervation of CS/PLA hybrid beads; b) Photograph of the hybrid beads.
3. Results and discussion

The microphotographs of the PLA spherical structures (Figure 3a, b) demonstrate a wide range of their sizes. Figure 3b shows a porous structure of the PLA micro-spheres which may contribute to their role as the active substance carriers.

The measurements of the microstructures diameters were performed using the Microscan programme (Figure 4).

![Microphotographs of PLA microspheres](image1)

**Figure 3.** Microphotographs of PLA microspheres; b) Porous structure of PLA microsphere.

![Screen of the Microscan programme](image2)

**Figure 4.** Screen of the Microscan programme – measurements of the diameters of PLA microspheres.
Their size ranged from 1 μm to more than 100 μm. The diameters distribution of the PLA microstructures was demonstrated in the form of a histogram and defined using the Gauss distribution curve (Figure 5).

Analysing the distribution one may notice that more than 70% of all microspheres are the structures of diameters to 20 μm, and the structures greater than 50 μm constitute less than 5% of all.

Figure 6 demonstrates a microphotograph of the hybrid bead cross section with visible PLA microstructures (Figure 6). Lack of visible empty space between chitosan and poly(lactic acid) indicates interactions of two polymers in the system.

The kinetics of swelling (Figure 7) indicates a decrease of water absorption by the hybrid beads if compared with the spheres generated from pure chitosan. Due to the presence of hydrophobic PLA in the system, the hybrid beads demonstrate a higher resistance to swelling. There has not been noticed any visible effect of drying (15 min and 30 min) on a degree of swelling.

![Figure 5. Distribution of PLA microstructures size (the fraction of microstructures of diameter \( \phi \) from a given class division in a random test). The curve – the Gauss function.](image-url)
4. Conclusions

The novelty of preparation is combining two biopolymers into one hybrid system to obtain desired release kinetics of active substances. The substance can be enclosed in the porous PLA microspheres or in the chitosan matrix. The hybrid system demonstrates a high stability in the aqueous environment which indicates some interaction between hydrophobic (PLA) and hydrophilic (CS) composite components.

An increase in the beads stability on swelling, by adding PLA allows to reduce or eliminate the chitosan cross-linking agents, for example, toxic glutaraldehyde.

The future work will be concentrated on optimization of the emulsification method combined with the organic solvent diffusion in order to obtain a narrow size distribution of PLA microstructures. Additionally, it is crucial to carry out the analysis of the loading effectiveness and the kinetics of a model substance release from a system.

The mathematical model of the erosion process with a simultaneous release of an active substance to the aqueous environment of various pH values will be derived.

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6. References


