Summary
Polymeric Biomaterials with Targeted Applications

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The present thesis is composed of 190 pages, a third part (50 pages) is dedicated for a complex literature review and the other two parts are dedicated for the research directions. In this regard, the thesis starts with the literature review and continues with the two research directions as following:

- The literature review is composed of several parts. A general introduction about polymeric biomaterials with medical applications. Here are presented the main achievements of this domain in the last 30-40 years. Starting from the first biomaterials difinition, the chapter addresses the developed smart materials in the present. The nanotechnology contribution for new materials and nanomaterials synthesis into pharmaceutical domain is presented. The detalied presentation of the main polymers category as natural polymers and synthetic polymers, offers a wide view on the polysaccharides (cellulose, alginate, chitosan, starch, etc.), proteins (collagen, gelatin, wool, sericin, fibtoin, etc.), poliesters (polyhydroxyalkanoates) or polyamides applications. Furthermore, this chapter reveals the other materials importance (metals, alloys, ceramic) besides polymers category for nanotechnology progress.

- The second part of the literature review was dedicated for drug delivery field contribution. This part is divided in three main parts (introduction into drug delivery field; micro-and nanoparticles evolution and nanoparticles for cancer therapy). There are detalied information about drugs conventional adminitration by various routes and information about polymeric nanoparticles evolution. The nanoparticles evolution history from the Macro era begining in the 1970 until the last top nanoparticles preparation technologies were presented. There are detailed the main pharmaceutical domain targets: disease prevention; dose administration frequency reduction; the disease conditions delay; the body condition stablization. The main administration routes were revealed: local administration (available and approachable method); systemic administration (less available method. The systemic route was presented as direct and indirect administration. The chapter focuses on the systemic administration by intravenous and intra-arterial administration. These methods were detailed discussed because polymeric nanoparticles are most of the time administrated by systemic approach. There were revealed the main advantages brought by polymeric nanoparticles usage or nanotechnology

development. The main nanotechnology goals to be achieved by continuously development have been showed. Thus, there are revealed issues such as: drug delivery bioavailability increase; drug toxicity reduction with maintaining of therapeutic effect; higher biocompatibility and biodegradability; new preparation methods, etc. The nanoparticles administration routes were classified into oral and intravascular with main advantages and disadvantages. Among main advantages of the intravascular administration the EPR effect (enhanced permeability and retention), high targeting capacity or high bioavailability appear due to nanoparticles ability for extravasation. Theoretical information regarding nanoparticles internalization, opsonization or biodegradation inside tumor cells were revealed. The nanoparticles action mechanism was presented as an active mechanism and a passive mechanism together with main diference between them.

The nanoparticles general preparation methods were classified into: polymerization methods (nanoparticles prepared during polymerization reactions such as emulsion or suspension polimerization) and methods based on preformed polymers (the initial synthetized polymer is used for nanoparticles preparation). The second category of the general preparation methods exposed two important methods, namely nanoprecipitation and emulsification-diffusion. These two methods were detailed because due to usage for nanoparticles preparation during thesis research. Between the two methods, nanoprecipitation was presented as main procedure providing nanoparticles with sizes below 100 nm.

- The polymers used in the research part were detailed in the third part of the literature review. The chapter is divided in three parts revealing the natural polyesters (PHAs), proteins (silk proteins) and synthetic polymers (PNIPAM). The Polyhydroxyalkanoates (PHAs) category is represented by the Poly-(3-hydroxybutyrate) (PHB and Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV). General information about PHBHV and PHB synthesis procedure, structure, mechanical properties, biocompatibility, biodegradation or main applications were revealed. This part focused to show the PHBHV major application for nanoparticles development. Several literature studies considering PHBHV as nanoparticles preparation polymer have been revealed. The major polyester advantage is related with the bacterial origin. This approach offers highly biocompatibility for PHBHV biomedical applications. The second type of natural polymers, namely silk proteins, are extracted from silk sources. Silk fibroin and silk sericin were presented as main proteins with unique properties. These properties are related

to fibroin and sericin aminoacid composition and intrinsic structure (crystalline and random domains). As in case of PHBHV, the proteins application for nanoparticles development were disscused in detail. The last category of polymers, synthetic polymers, is represented by Poly-(N-isopropilacrylamide). This polymer exhibits thermoresponsive properties with high impact for use in polymeric nanoparticles development.

- The fourth part of the literature review is dedicated for cancer therapy approach. General information about cancer surgery, chemotherapy and radiotherapy were exposed. Being one of the thesis approach, the chemotherapy domain was detailed. The main antitumor drug administration limitation were presented as top aspect to be addresed by the nanoparticles domain.
- The nanoparticles future perspective together with new challenges and requirements were exposed in the last part of the literature review.

The second thesis part was dedicated for original contributions and detailed research results. This research part is divided into three chapters: **Development of natural polymer nanoparticles based on polyesters (Poly-hydroxybutyrate- co-hydroxyvalerate); Development of natural polymer nanoparticles based on proteins (silk proteins); General conclusions and original contributions.** The first two chapter represent independently research directions.

- The first research direction (chapter II) is also divided into three studies regarding PHBHV nanoparticles development. During first research direction the thesis aims to develop new polyester (PHBHV) nanoparticles with smaller size and size distribution by methods unapproachable for this polymer (emulsification-diffusion and nanoprecipitation methods). Furthermore, the new nanocarriers load a new adjuvant drug (silymarin) in cancer therapy besides classically 5-Fluorouracil.

The first study of this research directions aims to develop PHBHV nanoparticles by emulsification-diffusion method. The PHBHV micro and nanoparticles preparation method by emulsification-diffusion starts from literature data. The preparation method was further optimized to develop a novel protocol for particles preparation with highly controllable size and morphology. During experiment novel nanoparticles with a core-shell structure (nanocapsules) were obtained. The experiment involved two phases preparation by main parameters variation to

control the nanoparticles size, size distribution or shape. In this regard, morphological characterization is one of the leading techniques aiming to reveal nanoparticle characteristics.

- The prepared micro-and nanoparticles as suitable carriers for 5-FU protection were morphologically characterized. The results revealed that one formulation (PHBHV 2% w/v – PVA 2%) was considered suitable to display nanoparticles with smaller size and optimal morphology.
- The biological investigation showed that formulation achieved a therapeutic effect which can be based on nanoparticles accumulation and encapsulated drug release inside tumor cells.
- The first study of this research direction aims to develop PHBHV nanoparticles by nanoprecipitation method. The study reveals significant differences between preparation methods regarding particles characteristic and encapsulation efficiency for drugs with different nature.
- Morphological investigation was performed on the nanoprecipitation prepared nanoparticles. The results showed nanoparticles with narrow size distribution and size around 100 nm.
- The drug release tests revealed higher release efficiency for silymarin compared with 5-FU. The formulation showed excellent compatibility with hydrophobic silymarin and good encapsulation efficiency.
- The loaded nanoparticles were tested in order to reveal the capacity for silymarin deliver into HT-29 human colon cancer cell culture. The in vitro assays, morphological characterization as well as HT-29 multicellular spheroids culture model confirmed the PHBHV nanoparticles capacity to penetrate the tumor cells and drug deliver.
- The last study of this research chapter addressed the nanoparticles biodegradation investigation. More detailed studies regarding drug release behavior of developed PHBHV nanoparticles are required. The studies revealed the activity of two esterase enzymes by interaction with macromolecular chains and generation of novel products with lower molecular weight. The study showed further investigation of the drug release mechanism for the two binary systems, PHBHV/ 5-Fluorouracil and PHBHV/silymarin, due to esterase enzymes biodegradation activity.
- The drug release profiles under enzymatic pathways were evaluated by UV-VIS spectrometer and the novel degradation products were characterized by FTIR-ATR. The drugs

release investigation showed clear higher release efficiency compared with normal *in vitro* conditions (without enzymatic activity) due to chains cleavage for both PHBHV and PVA.

- FTIR analysis revealed the macromolecular chains backbone and side chains breaking with generation of new products with lower molecular weight. The possible generated chemical structures were presented in the second chapter.

- The second research direction (chapter III) is divided into three studies regarding silk proteins nanoparticles development. The chapter addressed challenges in breast cancer therapy besides colorectal cancer therapy. In this regard there were used two antineoplastic drugs, 5-fluorouracil for colorectal cancer and doxorubicin for breast cancer. Both drugs were encapsulated in nanoparticles formulation based on silk proteins. Among natural polymers, proteins represent a wide category of materials used for development of various polymeric nano-carriers. Silk proteins including silk fibroin (SF) and silk sericin (SS) possess unique properties such as biocompatibility, excellent processability or self-assembling ability which promote them for application in biomedical domain.

- The first study assumes fibroin nanoparticles procedure optimizatio by setting a preparation protocol in order to modulate the nanoparticles characteristics. The optimization procedure starts from literature data about nanoprecipitation method. The generated nanoparticles are water stable without revealing further dissolution.

- Nanoparticles morphological characterization revealed the range size around 100 nm with low variations for all formulations as SF 1%, SF 2 %, SF 3% and SF 4% but with differences regarding morphology. Dimensional distribution characterization revealed a poly-disperse distribution for each system with significant differences for average size and polydispersity index (PDI).

- Three biological assessment methods including MTT viability, LDH cytotoxicity and cytoskeleton morphology showed relevant results with certain influences and activity of 5-FU loaded nanoparticles within tumoral line. Therefore, the investigation revealed biological efficiency of developed drug loaded silk fibroin nanoparticles against HT – 29 colorectal tumoral line.

- The second study revealed the silk fibroin modification by grafting reaction. The grafting reaction of NIPAM monomer within fibroin was carried out by radical polymerization

mechanism via redox reaction. The redox formulation was obtained in the presence of ammonium cerium nitrate Ce^{4+} and an oxidizing agent (acid) in an equilibrium (Ce^{4+}/Ce^{3+}).

- The physico-chemical characterization showed a new bonding mechanism on aromatic site compared with literature. Therefore, reaction started from hydroxyl tyrosine instead of typical hydroxyl serine. This part of chapter III offered an interesting overview on SF-g-PNIPAM structure. The modified silk fibroin was further used in smart drug delivery system development. These systems aim to show a better control over 5-FU and doxorubicin release.
- The developed nanoparticles based on modified SF-g-PNIPAM were nanosized with a narrow size distribution according to morphological investigation.
- Fibroin grafting modification with PNIPAM showed a better controlled release over doxorubicin drug compared with nanoparticles prepared from native silk fibroin.
- The last study of this research direction is focused on sericin nanoparticles development by the nanoprecipitation method. The parameters influence over nanoparticles characteristics was presented as a main study approach. The obtained results revealed an unusual behavior of the nanoparticles preparation stages resulting a particular manner of sericin nanoprecipitation.
- The developed formulations revealed sericin nanoparticles with sizes ranging between 15-40 nm depending on solution concentration. These size values are below usual sizes regarding polymeric nanoparticles. These results are revealed by morphological investigation (TEM).
- The release investigation revealed that doxorubicin showed a relative high release efficiency depending of pH environment. The acid media showed that doxorubicin and sericin achieved specific physical interactions based on amino and carboxyl groups.
- The biological investigation performed on MCF – 7 breast cancer cells line revealed high activity of the doxorubicin loaded sericin nanoparticles. The viability, cytotoxicity and morphological cytoskeleton assessment results place the developed systems based on sericin nanoparticles as considerable candidate for usage within cancer domain.
- The last thesis chapter is dedicated for final conclusions and original contributions.