University POLITEHNICA of Bucharest
Faculty of Applied Chemistry and Materials Science
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PhD Thesis summary

Nanocomposites based on polymeric nanoparticles with medical application

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Mulțumiri

Cuvintele nu pot exprima cât de recunoscătoare le sunt tuturor celor care mi-au fost alături în toți acești ani, pentru înțelegerea, răbdarea și prietenia pe care mi-au arătat-o.

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Autoarea

"Excellence is an art won by training and habituation. We do not act rightly because we have virtue or excellence, but we rather have those because we have acted rightly. We are what we repeatedly do. Excellence, then, is not an act but a habit"

Aristotel 384 – 322 î. Hr.

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Key words: poly (lactic-co-glycolic) acid, vegetable oils, hybrid polymeric nanoparticles, lipophilic agents, encapsulation efficiency, release profile, cellular viability, biological activity, polyethylene glycol, modified surface, molecular modelling, Flory-Huggins parameter, polyplexes, complexation reaction.

INTRODUCTION

The PhD thesis entitled „**Nanocomposites based on polymeric nanoparticles with medical application"** was elaborated in the Department of Bioresources and Polymer Science, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest (UPB) and contains original contributions in the field of polymer nanoparticles with potential applications in biomedicine.

Polymeric nanoparticles represent a real revolution in modern medicine, registering remarkable progress in the drug delivery and controlled release field of different therapeutic agents. Exceptional features such as biocompatibility, biodegradability, versatility and functionality as well as the possibility to be associated with other different compounds to form a hybrid system with personalized characteristics, have increased their interest and determine their intensive study as nanoformulations with applications in various fields (imaging, diagnostics or theranostic properties).

Despite all the remarkable progress, there are still some limitations in using polymeric nanoparticles as nanocarriers for challenging drugs (lipophilic or unstable in biological environment) due to low encapsulation efficiency, unpredictable release profile, or low cellular penetration, factors which considerably reduce the bioavailability, consequently the therapeutic effect of the system.

In this context, the **major objective** of this doctoral thesis is to obtain polymeric nanoparticles with potential biomedical applications as nanocarriers for drug delivery and controlled release. The achievement of the main objective addresses **two research directions**: **(1)** Designing and obtaining new hybrid nanoparticles based on biocompatible polymer (PLGA) - vegetable oils with well-established therapeutic activity for potential applications as nanocarriers for lipophilic drug delivery; **(2)** Obtaining a new type of polymeric nanoparticles – polyplexes, non-viral vectors with optimal characteristics for applications in the field of gene therapy.

The thesis is structured in two parts and comprises 12 Chapters. PART I THEORETICAL CONTRIBUTIONS (4 Chapters) and PART II - ORIGINAL CONTRIBUTIONS (7 Chapters) with GENERAL CONCLUSIONS (Chapter 12). The thesis contains 208 pages: 60 pages represent the first Part and 125 pages embody the second Part with Annexes and at the end of the paper are enlisted 323 bibliographic references. The results presented in this PhD thesis have been disseminated within the scientific community, published in important scientific journals and presented at international conferences.

The first part of the thesis (Chapters 1-4) represents the state of the art in the field of polymeric nanoparticles with potential biomedical applications. Chapter 1, entitled "***Polymer Nanoparticles***" is an introduction in the field of polymeric nanoparticles as drug delivery systems for different bioactive compounds. An overview of the preparation methods and the main polymers used in the preparation/synthesis of nanoformulations was offered. Also there is presented a classification of polymeric nanoparticles and the main mechanisms involved in the drug delivery and release processes. It is also highlighted the significance of polymer nanoparticles in biomedical applications and the areas where they have achieved remarkable results, data being accompanied by the *in vitro / in vivo* studies or clinical trials reported in the literature. Chapter 2, entitled "***Polymer-lipid/oil-based hybrid nanoparticles***" presents theoretical aspects related to poly (lactic-co-glycolic)- PLGA- based hybrid nanoparticles as well as their classification. In this chapter is described a new hybridization concept of PLGA-based nanoparticle with vegetable/synthetic oils for delivery of bioactive lipophilic compounds. The

results of the latest research on the performances of PLGA-vegetable/synthetic oils formulations in the biomedical field are presented. In chapter 3, entitled ***"PEG-modified PLGA-nanoparticles – a new strategy for improving performances of the drug delivery systems***" are described the main contributions as nanocarriers of surface modified PLGA-based nanoparticles with biocompatible polyethylene glycol (PEG), also there are presented the PEGylation strategies. Chapter 4, entitled ***"Polyplexes – non-viral vectors in gene therapy"*** is dedicated to a new type of polymeric nanoparticles - polyplexes. The efficiency of polyplexes as non-viral vectors used to transfer nucleic acids (DNA, RNA) in the field of gene therapy is emphasized. Also there are presented and described the most important polymers used in the complexation reaction with nucleic acids.

In the second part of the thesis (Chapters 5-11) are emphasized the original contributions of this research project. Chapter 5 describes the particular aspects of the experiments: In the equipment, the chemical reagents, and the methodology used to perform the experiments. In Chapter 6, entitled ***"Objectives and Originality***" are presented the motivation and the main objective of this PhD thesis as well as the original contributions in the field of hybrid nanoparticles with applications in controlled delivery and release of special drugs (lipophilic, unstable in hydrophilic environments, with low penetrability of the cellular membrane). Chapter 7 of original contributions entitled " ***Theoretical and experimental contributions in the synthesis of PLGA nanoparticles with excellent properties for drug delivery: investigation of key parameters"*** presents the original contribution in optimizing the preparation method of the PLGA-based nanoparticle by studying and modifying the key parameters involved in the synthesis process. In Chapter 8 of the original contributions entitled ***"Hybrid nanocarriers based on PLGA-vegetable oil: a novel approach for high lipophilic antibacterial drug delivery"*** is proposed to extend the idea of hybrid formulation through a new design by combining a biodegradable polymer such as PLGA (poly-lactic-co-glycolic acid) with a vegetable oil (NSO) for nanocarriers for high lipophilic drugs. Two essential properties of the vegetable oil have been explored, firstly as an important component of the nanocarrier matrix, capable of creating the ideal microenvironment for encapsulating a lipophilic drug - Izohidrafural (IHF), and secondly, therapeutic properties, especially the antibacterial activity. In Chapter 9 entitled ***"Novel PEG-modified hybrid PLGA-vegetable oils nanostructured carriers for improving the performances of Indomethacin delivery"*** are obtained and advanced characterized (DLS, SEM, TEM) new PEG-coated hybrid nanoparticles based on PLGA-vegetable oils (NSO, EQ and PMG) for improving stability, bioavailability and, at the same time, reducing side effects of encapsulated lipophilic drug (Indomethacin). The objective of the Chapter 10 entitled ***"Predicting the capacity of hybrid nanocarriers based on PLGA-vegetable oil to encapsulate/release a bioactive compound"*** was to rationalize the encapsulation/release performances of the hybrid formulations based on PLGA-vegetable oil for different drugs using the most important thermodynamic parameter Flory-Huggins theory (χ_{dm}) in combination with computational approach, the mesoscopic (MesoDyn) and Atomistic Modeling. Chapter 11 of the original contributions entitled ***"Polyplexes – a new type of nanoparticles with potential applications in gene delivery therapy"*** is dedicated to study and to optimize the key parameters involved in obtaining a new class of polymeric nanoparticles named polyplexes – non-viral vectors for gene delivery, obtained by the complexation of the DNA with different types of cationic polymers.

Further there are presented the general conclusions of the PhD thesis and its annexes. The thesis ends with a Bibliography chapter containing 323 bibliographic references also the original contributions of the author and the dissemination of the results.

II. ORIGINAL CONTRIBUTIONS
CHAPTER 5. MATERIALS AND METHODS

5. 1. Materials used to obtain polyplexes, standard PLGA or hybrid PLGA- vegetable oils based nanoparticles for lipophilic drug delivery

5. 1. 1. Polymers

Poly (lactic-co-glycolic) acid PLGA – 50:50, acid terminated with Mol. wt. 40000–75000 Da and Mol. wt. 30000-60000 Da, aliphatic polyester, biocompatible and biodegradable.

Poly (ethylene glycol) PEG – hydrophilic, biocompatible polyether with Mol. wt. 3000 Da

Poly (ethylenimine) PEI linear structure - biocompatible polycation with Mol.wt. 10000 Da and 232.55 cationic charges per polymer molecule.

Poly (ethylenimine) star polymer with three arms PEI-3 and four arms PEI-4 with Mol. wt. 25000 Da and 8000 Da, respectyvely 293.77and 94.01 cationic charges per polymer molecules, synthesized by the Institut Parisien de Chimie Moléculaire (IPCM), Chimie des Polymères.

Polyvinyl alcohol PVA – synthetic polymer with excellent emulsifying properties, Mol. wt. 31000-50000 Da and hydrolysis degree 87-90%, PVA with Mol. wt. 30000-70000 Da and hydrolysis degree 87-90%, PVA with Mol. wt. 88000 Da, and hydrolysis degree 87-90% and PVA with Mol. wt. 80000-1240000 Da, and hydrolysis degree 87-89%.

5. 1. 3. Vegetable oils

Nigella sativa oil (NSO) – vegetable oil extracted from *Nigella sativa* seeds, is one of the most extensively used vegetable oil in the medical field, owing to its remarkable anti-inflammatory, antibacterial, and anticancer properties.

Punica Granatum oil (PMG) – vegetable oil extracted from Punica Granatum seeds, exhibits remarkable antibacterial, anticancer, anti-inflammatory and immunosuppressive properties attributed to punicic acid which is the main constituent in the oil composition (237).

Echium oil (EQ) – is a vegetable oil extracted from the Echium plantagineum seeds, with significant amounts of omega-3, omega-6 fatty acids, stearidonic acid, and γ-linolenic acid playing a role in regulating many inflammatory disorders like atherosclerosis, cancer, and rheumatoid arthritis.

5. 1. 4. Bioactive compounds

Indomethacin (IMC), Nitrofurantoin (NFI), Curcumin (CM), Resveratrol (RSV), (±)-α-Tocopherol (TC), Retinol (R), Hydrocortisone (HC), 5-Fluorouracil (5-FU), and Izohidrafural (IHF) and Deoxyribonucleic acid (DNA).

5. 1. 6. Chemicals and other materials

Dichloromethane (CH_2Cl_2) DCM, Acetone (CH_3COCH_3), Dimethyl sulfoxide ($(CH_3)_2SO$) DMSO, Petroleum ether (fr. 40-60) PE, Ethanol (C_2H_5OH), Trizma base ($NH_2C(CH_2OH)_3$), Hydrochloricacid (HCl), Sodium chloride (NaOH), cellulare line L929 fibroblaste from mouse adipose tissue, the ECACC collection, Bacterial strains: Enterococcus faecalis – EF ATCC 29212, EF 2328, Streptococus aureus – SA ATCC 25923, SA 14, Escherichia Coli - EC ATCC 25922, EC 2041, Pseudomonas aeruginosa – PA ATCC 27853, PA 1908 ATCC (American Type Culture Collection).

5. 2. Methods of preparation and characterization of polyplexes and standard /hybrid PLGA- vegetable oils based nanoparticles for lipophylic drug delivery

5. 2. 1. 1. Preparation of standard PLGA or hybrid nanoparticles based on PLGA-vegetable oils

The organic phase containing a specific amount of PLGA or PLGA/oils (NSO, EQ and PMG) in different ratios (1/0.25, 1/0.5, 1/1 w/w) disolved in 4 mL DCM was slowly added to 20 mL aqueous solution of PVA under constant stirring. The mixture was subjected to sonication for 15 min (Vibra-Cell CVX 130, 20 kHz, 220 V, CT, USA) in an ice bath; afterwards it was stirred 3 h at 1000 rpm to evaporate the organic solvent. The obtained formulations were separated from the aqueous phase by centrifugation (8000 rpm - 30 min). The unbound PVA and unencapsulated oil were removed from the system by washing three times with ultra-pure water at 37^{o} C.

Drug – loaded formulation were prepared following the same protocol, the drug concentration was 5% from the PLGA or PLGA and oil amount employed in the initial preparation step.

PEGylation strategy – The surface modification of the formulations was achieved by physical PEG adsorption (in the post-production step). All standard or hybrid nanoparticles were incubated in a 4.5% w/v PEG3000 solution for 4 h under constant stirring, the formulations were then washed to remove unbound PEG and were used freshly or lyophilized (Freeze Dryers, D-37520, Osterode am Harz, Germany) for further experiments.

5. 2. 1. 2. Preparation of DNA/PEI complexes

The performances of DNA/PEI complexes are regularly driven by strong electrostatic interactions which appear between anionic phosphate groups (P) from DNA and cationic amino groups (N) presented in the polymeric structure.

The complexation reaction was performed at different N/P molar ratios by dropping the polycationic solution (pH=7.4, the concentration depends on the N/P ratio) into the DNA solution (c (DNA) = 0.05 mg/mL, pH=7.4, 10 mM Tris buffer). The obtained mixture (200µL) was homogenized 10 seconds under vortex and after it was left 15 minutes at the room temperature for the complexation.

The N/P molar ratio is defined as the molar ratio of the nitrogen atoms in PEI to the phosphorus atoms in DNA double helix in the solution mixture (232). It should be emphasized that the N/P ratio is not the nitrogen/phosphorus ratio inside each polyplexes because some of PEI chains are free in the solution regardless the value of N/P ratio.

5. 2. 2. Nanoparticles characterization techniques

Dynamic light scattering (DLS) - hydrodynamic characteristics were determined by Malvern Zeta Sizer ZEN 3600 (Worcestershire, UK, DLS). The principle of DLS consists in calculation of the diffusion coefficient of particles undergoing Brownian motion employing a He-Ne linear polarized laser operating at a wavelength of λ=632.8 nm and an angle of 173°. The samples were diluted in ultra-pure water and 12 successive cycles were run at 25°C, for complexes characterization DNA/PEI samples with 200µL of mixtures were used.

Electrophoretic mobility (Zeta potential measurements) - The electrophoretic mobility of all the formulations was determined employing the principle of laser Doppler velocimetry using Malvern Sizer ZEN 3600 (Worcestershire, UK, DLS) equipped with a He-Ne linear polarized laser operating at a wavelength of λ=632.8 nm and an angle of 13^{0}. The measurements were performed in ultra-pure water at 25°C performing 40 successive cycles.

Stop flow light scattering (LS) – the complexation kinetics of the polyplexes was studied by the means of Bio-Logic SAS (SFM-3000, France). Samples containing different amount of polycations and a constant DNA concentration were injected in two separate channels of the

instrument, then the DNA/PEI complexation was studied in the main reservoir in which volumes of 75μL mixture were introduced.

Scanning Electron Microscopy (SEM) - the morphological features were studied employing the microscope, Quanta Inspect F, SEM. The dried samples were sputter-coated with a thin gold layer and their morphology was observed with a field emission gun operated at 30kV.

Transmission Electron Microscopy (TEM) - TEM investigations were performed using a high-resolution transmission electron instrument (HR-TEM) TECNAI F30 G2 S-TWIN. A small amount of each sample was deposited on a TEM copper grid covered with a thin amorphous carbon film with holes. After that, the TEM grid was placed on a sample holder and the geometrical evaluation (size and shape) of the samples.

Atomic Force Microscopy (AFM) - a drop of dried suspension was placed on a lamella and was analyzed through contact method using AFM microscope (Agilent 5500, AFM).

Ultraviolet Uisible spectroscopy (UV-VIS) - UV-VIS spectra were performed using UV-VIS NIR Spectrometer (UV-3600), then drug loading, encapsulation efficiency, drug release profile were determined.

Fourier-transform infrared spectroscopy (FTIR) - FTIR measurements were performed on a Vertex 70 Bruker FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. For all the formulations, the FTIR spectra were registered in the ATR-FTIR mode, at a resolution of 4cm^{-1} in 600–4000cm^{-1} wavenumber region and 32 scans were averaged for each sample.

Differential scanning calorimetry (DSC) - DSC analyses were carried out on a Netzsch DSC 204 F1 Phoenix differential scanning calorimeter under a constant nitrogen flow rate (20mL/min) at a heating rate of 5°C/min.

In vitro cytotoxicity using MTT assays – the MTT test is a colorimetric assay for assessing cell metabolic activity which uses the ability of succinate dehydrogenase to reduce the tetrazolium dye to its insoluble formazan. The assessments were performed on L929 (Cell culture ECACC, fibroblasts from mouse adipose tissue) cells were seeded in culture flasks to 18th passage, using Tripsin - EDTA 1× solution with Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS - Biochrom) and penicillin - streptomycin 1× antibiotic solution. The cells were seeded in 96 - well plates with a density of 1×105/ml and incubated for 24 h at 37°C in a humidified atmosphere of 95% air and 5% CO2 to allow cell attachment. After 24 h a sequence of binary dilutions of the samples were prepared and added to the culture wells. After 24h the cells were incubated with 5 mg/mL MTT solution for additional 3hat 37°C in the same atmosphere. Then, the medium was replaced with 100μL acidified buffer (10% SDS, 0.01% HCl, 50% DMF) and incubated overnight at 37°C under orbital shaking to solubilize the purple formazan produced by the metabolically active cells. The UV absorbance of the dye produced was measured at 570 nm. The untreated cells were used as positive control.

In vitro antibacterial activity

The antibacteria activity of loaded-standard/hybrid nanoparticles was assessed on Gram-negative and Gram-positive strains (Enterococcus faecalis – EF ATCC 29212, EF 2328, Streptococus aureus – SA ATCC 25923, SA 14, Escherichia Coli - EC ATCC 25922, EC 2041, Pseudomonas aeruginosa – PA ATCC 27853, PA 1908). The microbial suspensions of 1.5×108 CFUmL−1 (0.5 McFarland density) were obtained from 8 bacteria strains developed in physiological sterile medium on agar at 37°C for 24h.

The qualitative assay: was achieved on Mueller–Hinton Agar medium by an adapted disc diffusion method (240). Spots containing microbial inoculum and 10 μL sample suspensions of

each stock solution were incubated for 24 hours at 37 ° C then the antibacterial activity of the formulation was assessed by determining the ability of the sample to diffuse into the medium with the microbial cultures and to inhibit their germination and growth.
For quantitative analysis: the minimal inhibitory concentration (MIC) values for all analyzed formulations were determined in 96 multi-well plates using the broth microdilution method (240). Each well containing sample (were used binary dilutions of compound solutions, starting from750μg/mL) and 100μL volume of broth was seeded with 20μL microbial inoculum. Thereafter the plates were incubated for 24 h at 37°C, and MIC values were considered as the lowest concentration of the tested samples that inhibited the growth of the bacteria culture. The results were compared to the positive control (wells containing culture medium seeded with the microbial inoculum) measuring and comparing the absorbance at 600 nm.
The anti-biofilm activity: was assessed by the plate microtiter assay with violet crystal. Following the reading of the liquid cultures absorbance at 600nm for establishing the MIC value, the content of the plates was removed, the plates were washed three times by phosphate buffered saline, and the biofilms adhered to the plastic walls were fixed with cold methanol and stained by violet crystal solution for 15 minutes and finally resuspended in a 33% acetic acid solution. The density of the microbial biofilm harvested from the plastic wells was measured by reading the optical density at 490 nm for the colored suspensions. The minimal biofilm eradication concentration (MBEC) value was corresponding to the concentration found in the well in which the absorbance values were inferior to those of the positive control.

CHAPTER 7. THEORETICAL AND EXPERIMENTAL CONTRIBUTIONS IN THE SYNTHESIS OF PLGA NANOPARTICLES WITH EXCELLENT PROPERTIES FOR DRUG DELIVERY: INVESTIGATION OF THE KEY PARAMETERS

In this chapter were designed and obtained PLGA nanoparticles (PLGA-Np) employing standard emulsion-solvent evaporation method. The influence of the key parameters which drive the preparation process, respectively the final hydrodynamic characteristics of colloids were investigated and optimized and then the preparation protocol was established.

Flow chart showing the main steps involved in the preparation of PLGA-Np by standard emulsion-solvent evaporation method is presented in Figure 7. 1.

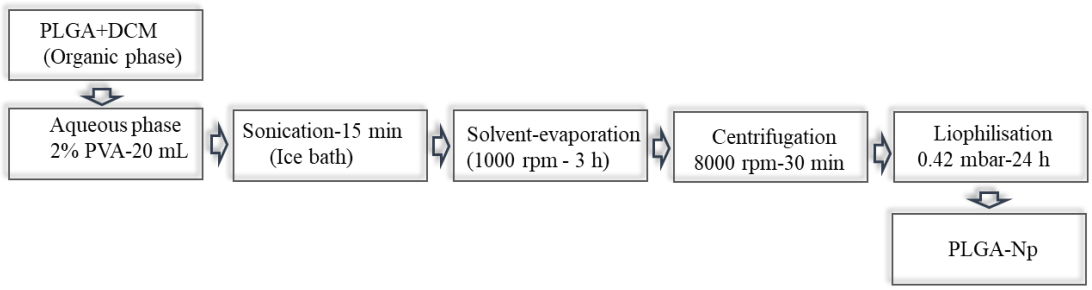


Figure7. 1. The main steps involved in the PLGA-Np preparation

7. 3. ***Results and discussion***

DLS results highlighted colloidal systems with hydrodynamic diameter (d) 108.2 ± 1.4 nm and polydyspersity index (P) 0.21±0.01 suggesting a narrow size distribution of nanoparticles. The negative zeta potential value (-17.6 mV) is owed to carboxylic –COOH end groups from the

chemical structure of PLGA (35, 48) which emphasizes the obtaining of a medium stability population.

7. 3. 2. Study and optimization of the formulation parameters

Particle size and distribution of a colloidal system are ones of the main hydrodynamic parameters which influence the performances of nanoparticles as different drug delivery carriers. Therefore, using emulsion-solvent evaporation method, various parameters having an essential role in final characteristics of obtained carriers were evaluated in order to reach optimal preparation conditions for an ideal device for drug delivery.

7. 3. 2. 1. Surfactant concentration (PVA) - the influence of different PVA concentrations (1.0%, 1.5%, 2.0% and 5%) in the external aqueous phase upon the final hydrodynamic characteristics of PLGA-Np were studied and the results are presented in Figure 7.5

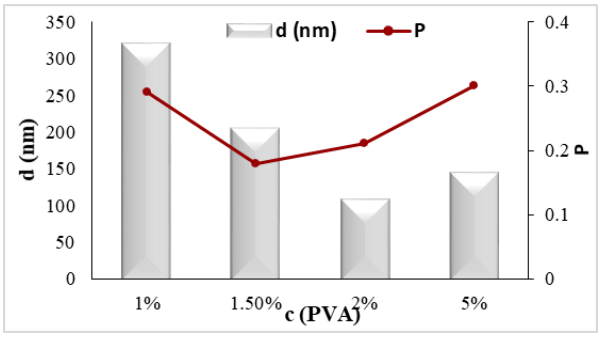


Figure 7.5. The size and P index of PLGA-Np at different concentrations of APV

7. 3. 2. 2. Molecular weight and the hydrolysis degree of PVA – different types of PVA concerning molecular weight (Mw) and hydrolysis degree were employed and their effect on the hydrodynamic characteristics of formulations were studied (Table 7.1)

Table 7. 1. The influence of different type of PVA on the mean diameter and P of PLGA NPs

Mw APV (KDa)	*Hydr. degree of PVA (%)*	*d (nm)*	*P*
80 - 124	87 - 89	416.9 ± 0.4	0.420 ± 0.090
30 - 70	87 - 90	111.4 ± 2.2	0.218 ± 0.012
31 – 50	87 - 90	172.9 ± 15.5	0.219 ± 0.012
88	88	259.4 ± 7.5	0.221 ± 0.031

PVA with Mw 30-70KDa and 87-90% hydrolyzed allowed obtaining nanocarriers with optimum hydrodynamic characteristics, lowest dimension and polydispersity.

7. 3. 2. 5. Polymer concentration – the polymer concentration from the organic phase is another important parameter to consider when forming polymeric PLGA-Np. The experiment was performed at different PLGA concentration into the organic phase and their effect on the mean diameter and polydispersity of nanocarriers was studied (Figure 7.6).

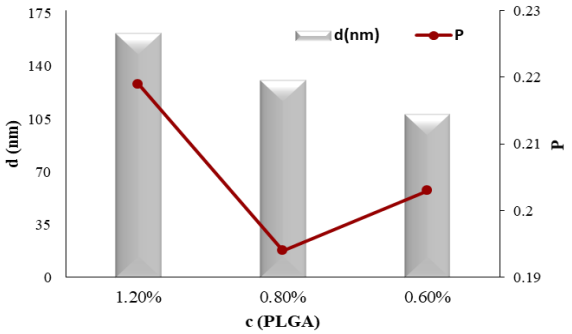


Figura 7. 6. The influence of c(PLGA) on the mean diameter and P of PLGA-Np

7. 3. 2. 6. Method of homogenization - The homogenization process was performed by two methods: sonication and magnetic stirring. (Table 7. 4). The homogenization by sonication determined a better dispersion of droplets into aqueous medium, forming colloids with smaller size and distribution in comparison to magnetic stirring which led to nanoparticles with a mean diameter 10 times higher.

Table 7. 4. The size and polydispersity index of PLGA NPs homogenized by different methods

Homogenization methods	*d (nm)*	*P*
Sonication (70%)	110.9 ± 0.8	0.236 ± 0.019
Magnetic stirring (1000 rpm)	1016.0 ± 543.8	0.758 ± 0.221

CHAPTER 8. HYBRID NANOCARRIERS BASED ON PLGA-VEGETABLE OIL: A NOVEL APPROACH FOR HIGH LIPOPHILIC ANTIBACTERIAL DRUG DELIVERY

The purpose of this research part was to extend the idea of hybrid formulation through a new design of hybrid polymer – vegetable oil nanoparticles as nanocarriers for lipophilic drug delivery and controlled release. The hybrid formulations were obtained by combining biodegradable and biocompatible PLGA (poly-lactic-co-glycolic acid) and vegetable oil (Nigella sativa oil – NSO) with well-established therapeutic activity, using standard emulsion solvent evaporation method.

8. 4. Results and discussion

8. 4. 1. Optimization of the formulation parameters

The hydrodynamic parameters of carriers obtained at different PLGA/NSO ratios were determined and also the possibility of oil aggregation in the presence of the surfactant was investigated (Table 8. 4).

Table 8. 4. The hydrodynamic characteristics of NPHN, PLGA-Np, and NSO-emulsion

No.	**Formulation**	**Optimization**		**d (nm)**	**P**	**ζ (mV)**
		PLGA (mg)	**NSO (mg)**			
1	PLGA-Np	24	-	106.2±1.4	0.21±0.010	-12.2±1.2
2	NPHN -1	24	6	154.4±2.4	0.20±0.009	-14.3±0.8
3	NPHN -2	24	12	152.4±0.9	0.20±0.001	-16.2±1.4
4	NPHN -3	24	24	147.9±2.6	0.14±0.003	-19.7±1.8
5	NSO-emulsion	-	24	132.9±1.5	0.24±0.008	-8.3±1.4

All systems exhibited a mean diameter larger than 100 nm, with a moderate negative zeta potential, ranging from -12.2 to -19.7 mV, specifying a low occurrence of the aggregation

phenomena in the system (60). The emulsion obtained only from NSO presented low size of droplets (132.9 nm) with insufficient zeta potential (ζ = - 8.3 mV). Therefore, the presence of amphiphilic PLGA into primary emulsification process was decisive for preventing the agglomeration of oil nanodroplets and formation of stable hybrid nanoparticles. Based on the results, HPONP with 1/1 PLGA/NSO ratio were considered the optimal combination of polymer-vegetable oil and were selected for preliminary encapsulation/ release experiments of the lipophilic antimicrobial drug – IHF.

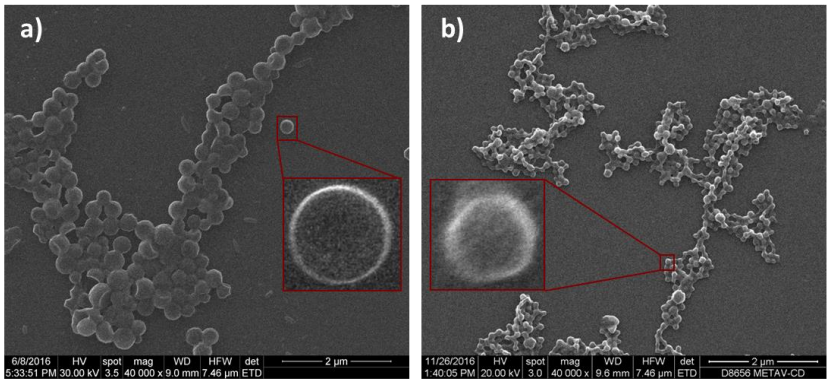
Table 8. 5. The hydrodynamic characteristics registered for PLGA-IHF and NPHN-IHF

IHF	*d (nm)*		*P*		*ζ (mV)*	
(wt.%)	*PLGA-Np*	*NPHN*	*PLGA-Np*	*NPHN*	*PLGA-Np*	*NPHN*
1	125.4 ± 4.4	136.1 ± 0.9	0.24 ± 0.02	0.15 ± 0.02	-7.99 ± 1.5	-12.8 ± 0.3
3	152.0 ± 8.1	133.5 ± 0.6	0.33 ± 0.01	0.17 ± 0.06	-12.1 ± 0.8	-13.5 ± 2.1
5	164.0 ± 2.1	138.8 ± 1.3	0.24 ± 0.03	0.18 ± 0.02	-13.4 ± 1.7	-17.6 ± 4.7

The drug loading determined an increase in the size of nanocarriers, insignificantly for hybrid formulations (~ 2%) but important for PLGA-Np, which show an increase of ~ 30% in the mean diameter. The IHF payload led to a noticeable deterioration in polydispersity of PLGA-IHF, registering with ~15% higher P value as compared to empty PLGA-Np and did not affect the homogeneity of hybrid colloids. The low influence of IHF upon the size and polydispersity of hybrid nanocarriers may be explained by the drug accommodation into already amorphous hybrid nanostructure oily nanopockets similar to NLC. Likewise NPHN registered ~ 1.5 times higher drug loading (DL) and encapsulation efficiency (EE) than standard PLGA-NP at all IHF loadings. The increased values of DL and EE achieved for hybrid formulations may be explained by the higher affinity of IHF to nanostructured hybrid lipophilic matrix (60) and also to the oil's capability to stabilized and incorporate high lipophilic drug (89).

8. 4. *2. Morphological characterization (SEM)*

SEM micrographs highlighted the formation of homogenous population of nanoparticles with smooth, well-defined spherical shape and a mean diameter around 110–140nm (Figure 8. 6).



Figurr 8. 6. The morphology observed by SEM: a) – NPHN, b) – PLGA-Np

In the inset images the morphological differences between standard or hybrid formulations are highlighted, the first inset (a) shows a formulation with perfect spherical shape and compact structure like liposome (88, 174) compared to PLGA-Np inset, in which some structural defects are emphasized.

8. 4. 5. FTIR analyses

FT-IR analysis was performed to further confirm the NSO dispersion within polymeric matrix and the encapsulation of the IHF into standard or hybrid devices. In Figure 8.9 the registered ATR-FTIR spectra of IHF, NSO, PLGA-IHF and NPHN-IHF with the main characteristics signals, are presented.

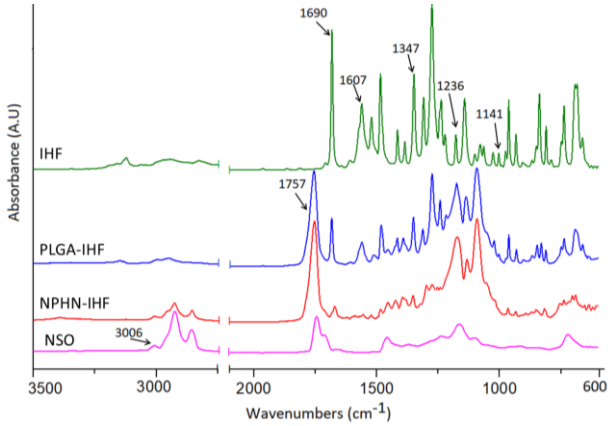


Figure 8. 9. ATR-FTIR specrta coresponding to IHF, PLGA-IHF, NPHN-IHF and NSO

8. 4. 6. In vitro drug release studies of IHF from standard or hybrid polymeric nanoparticles based on PLGA-vegetable oil

The *in vitro* release profiles of IHF from standard or hybrid nanoparticles performed in PBS at 37 ° C under sink conditions are presented in Figure 8.10. The PLGA-IHF showed a standard biphasic release profile characterized by an initial burst release in the first phase and followed by a slower release phase.

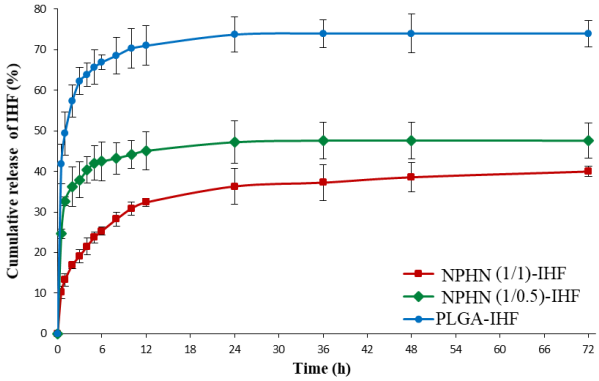


Figure 8. 10. The in vitro release profile of IHF from PLGA-IHF, NPHN (1/0.5)-IHF and NPHN(1/1)-IHF in 72 hours

The incorporation of 50% oil (HPONP (1/0.5)-IHF) determined a visible preservation of the initial burst effect while increasing the oil concentration to 1:1 with respect to the polymer matrix (HPONP(1/1)-IHF) led to a considerable reduction of the initial burst release effect and to a more sustained release drug release kinetics of the drug. This behavior was attributed to the nanostructured amorphous hybrid matrix which facilitated a more uniform distribution of entrapped lipophilic IHF molecules that are also stabilized in the oil nanodroplets.

8. 4. 7. In vitro cytotoxicity assessment

The cytotoxic *in vitro* studies using fibroblast cells line L929 were employed to predict the biological response of the cells on the new obtained hybrid formulations. PLGA is one of the most used polymers in drug delivery systems, approved by FDA, due to its biocompatibility and biodegradability (24, 48). Drug free nanocarriers did not exhibit obvious cytotoxicity on L929 at tested concentrations, demonstrating that the hybrid systems could be vehicles for drugs (Figure 8. 11).

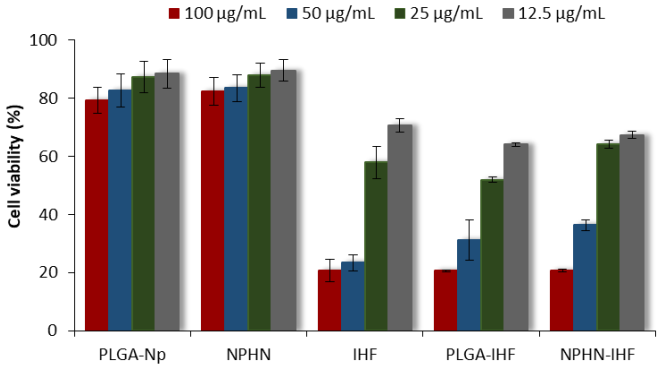


Figure 8. 11. Cellular viability of L929 treated with various concentrations of PLGA-Np, NPHN, PLGA-IHF, NPHN-IHF and IHF

After 24 hours of incubation, PLGA-IHF (IC50 was 39.40 µg/mL) displayed with approximately 13% higher cell toxicity as compared to NPHN-IHF (IC50 was 45.58 µg/mL). The oil dispersion within hybrid matrix, possibly improved the distribution and stability of lipophilic drug, leading to a gradually controlled IHF release (as it was presented in the release studies) and a low cytotoxicity.

8. 4. 8. In vitro antibacterial activity

The antimicrobial activity of the new formulations was assessed by the mean of qualitative and quantitative methods. The quantitative assays were performed using the broth microdilution test, a standardized method recommended by the Clinical Laboratory Standards Institute (CLSI, 2017' Ed.), allowing to establish the minimal inhibitory concentration (MIC) against different Gram-negative and Gram-positive bacterial strains (Figure 8. 13).

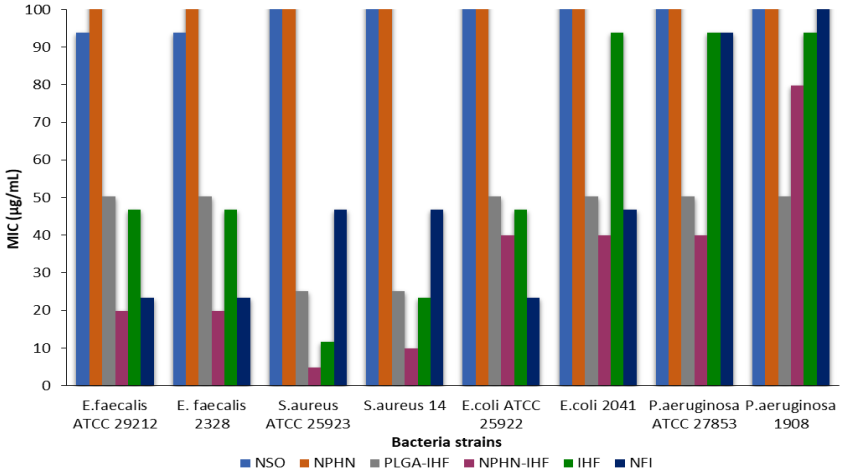


Figure 8. 13. Biological activity of synthesized formulations against different bacterial strains

The NPHN-IHF proved to be highly active against *S. aureus* and *E. faecalis* strains, exhibiting the lowest MIC values ranging from 4.9 to 9.9 μg/mL and 19.9 μg/mL, which are two times lower than that of free IHF (MIC 11.7 μg/mL), five times lower than the standard PLGA-IHF (MIC 25.1 μg/mL) and ten times lower than the reference drug NFI (MIC 46.87 μg/mL).The results also showed that in some experiments concerning Gram-negative bacterial strains all the samples revealed a low antibacterial activity. Effect which might be attributed to the differences in the structure of the tested bacteria, taking into account that the Gram-negative bacteria are protected by a supplemental permeability barrier, represented by the lipid outer membrane (271).

CHAPTER 9. NOVEL PEG-MODIFIED HYBRID PLGA-VEGETABLE OILS NANOSTRUCTURED CARRIERS FOR IMPROVING PERFORMANCES OF INDOMETHACIN DELIVERY

The main objective of the current research study was to prepare new PEG-coated hybrid formulations from biodegradable PLGA and vegetable oils as novel IMC delivery systems. The formulations were prepared by solvent evaporation method and surface modification was achieved by non-covalent PEG adsorption (in the post-production step). The oil dispersion within the polymeric matrix forms a less ordered hybrid nanostructure allowing a higher drug loading capacity and more controlled release as compared to standard nanoparticles, while the high biocompatibility of PEG layer absorbed on the surface of the formulations reduces their side effects.

9. 3. Results and discussion

9. 3. 2. 1. Hydrodynamic characterization

In Table 9. 3 are enlisted the mean diameter, polydispersity and electrophoretic mobility of all the obtained formulations.

Table 9. 3. Hydrodynamic characteristics of the obtained formulations

Nr.	*Formulation*	*d (nm)*	*P*	*ζ (mV)*
1	PLGA-Np	106.2 ± 1.4	0.21 ± 0.01	-12.2 ± 1.2
2	NPHN (1/1)	144.5 ± 0.7	0.16 ± 0.02	-22.0 ± 0.9
3	NPHE(1/0.5)	153.0 ± 2.8	0.16 ± 0.01	-22.1 ± 1.3
4	NPHP(1/1)	141.7 ± 1.4	0.13 ± 0.01	-16.9 ± 0.4
5	PLGA-IMC	165.2 ± 0.7	0.13 ± 0.01	-13.4 ± 0.1
6	NPHN (1/1)-IMC	160.9 ± 1.9	0.11 ± 0.02	-14.2 ± 0.4
7	NPHE(1/0.5)-IMC	167.1 ± 1.8	0.12 ± 0.03	-14.4 ± 0.5
8	NPHP(1/1)-IMC	166.2 ± 0.8	0.14 ± 0.01	2.1 ± 0.2
9	PLGA-IMC-PEG	275.8 ± 2.7	0.23 ± 0.01	-10.1 ± 0.2
10	NPHN (1/1)-IMC-PEG	179.0 ±2.5	0.18 ± 0.01	-14.0 ± 0.8
11	NPHE(1/0.5)-IMC-PEG	200.9 ± 2.2	0.11 ± 0.02	-13.6 ± 1.2
12	NPHP(1/1)-IMC-PEG	193.3± 2.3	0.15 ± 0.04	2.2 ± 0.4

The DL and EE calculated for plain uncoated or hybrid nanoparticles highlighted the remarkable capacity of NPHN-IMC to encapsulate the drug with the highest values of DL = 16.4 ± 0.4 % and EE = 61.4 ± 2.4 %, followed by the hybrid formulations based on PLGA and echium oil NPHE-IMC (DL = 8.5 ± 0.1 % și EE = 37.7 ± 1.9 %), the hybrid matrix containing pomegranate oil NPHP-IMC (DL = 7.6 ± 0.6 % , EE = 32.9 ± 2.8%) and at the end standard formulations PLGA-IMC (DL = 4.6 ± 0.2 % respectiv EE = 28.7 ± 2.4 %).

9. 3. 2. 2. Morphological characterization

Standard PLGA-IMC presented uniform, smooth and spherical structure (Figure 9. 4. a) while hybrid systems (Figure 9. 4. b, c) showed a well-defined core-shell structure which was depicted in TEM images as two different compartments: the oil core (dark color) surrounded by an uniform polymeric shell (bright color).

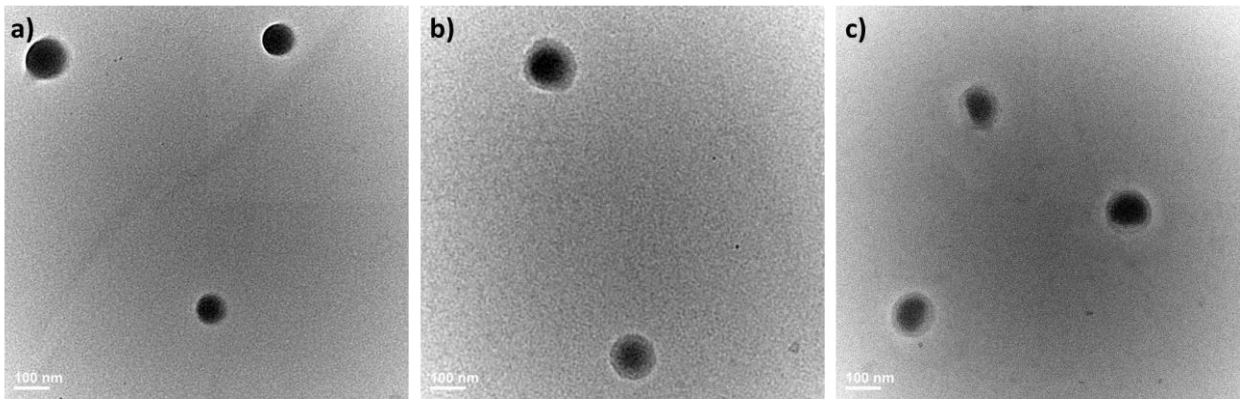


Figure 9. 4. TEM micrographs of a) – PLGA-IMC, b) – NPHN-IMC, c) – NPHE-IMC

9. 3. 3. DSC studies

The assessment of the oil's effect upon the polymeric matrix and IMC encapsulation into the nanocarriers was done by DSC analyses. In Figure 9. 6 are represented the DSC curves registered for IMC, PLGA-IMC, NPHN-IMC and NPHE-IMC in the range of 30 – 180 ^{0}C.

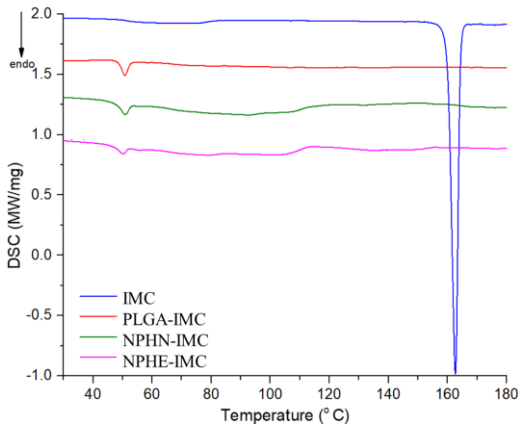


Figure 9. 6. DSC thermograms of analyzed samples

The areas of endothermic peaks which are proportional to enthalpy (ΔH) changes emphasized that the oil dispersion within polymeric matrix increased its amorphosity (87). Highly disordered hybrid matrices were formed requiring a lower energy input to disrupt the more amorphous structures. The value of calculated (ΔH) for PLGA-IMC was 3.48 J/g, the incorporation of 1/1wt. NSO with respect to the polymer matrix reduced it to 2.97 J/g while the incorporation of only 1/0.5 wt. EQ decreased the enthalpy to 1.70 J/g. The DSC thermogram of IMC exhibited a sharp endothermic peak at 162.7°C corresponding to drug melting point (283). After nanoformulation, the characteristic peak of IMC disappeared suggesting that the drug molecules were in a highly dispersed state into the nanocarrier matrix (284).

9. 3. 5. In vitro drug release studies

The in vitro release profile of IMC from all analyzed nanosystems is shown in Figure 9. 8. a, b. PLGA-IMC nanoparticles showed a standard diphasic release profile characterized by initial burst release followed by a slower release phase. By introducing nanostructured oils into the polymeric matrix the initial burst release (HPON-IMC and HPOE-IMC) was visible suppressed (Figure 9. 8. a).

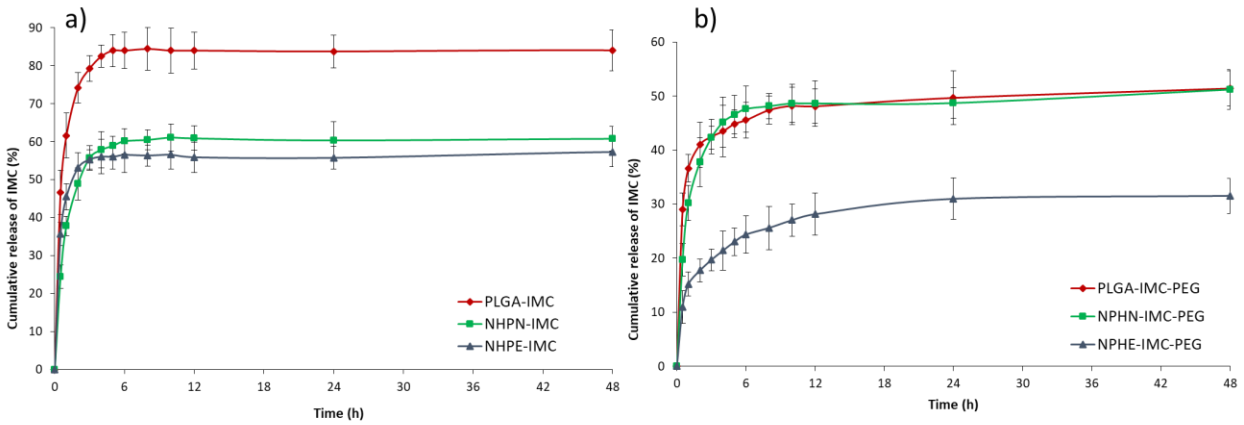


Figure 9.8. In vitro release profile of IMC from a) - plain or hybrid uncoated formulations and b) - PEG – coated nanocarriers

It can be observed that the adsorption of PEG on the surface of nanoparticles determined a decrease of IMC release rate as compared to the corresponding uncoated systems (Figure 9. 8. B) This behavior could be attributed to the presence of PEG layer on the surface acting as an additional barrier to drug diffusion.

9. 3. 6. In vitro drug release kinetics and mechanism

The drug release mechanism from nanocarrier matrix could be classified either in diffusional or erosion controlled, or can be governed by both mechanisms (288). Therefore, to obtain a clear view of the IMC release, the data obtained from the dissolution profile were fitted to different mathematical models such as first order, Higuchi, Hixton-Crowell, Korsmeyer – Peppas and Weibull. The accurateness and prediction abilities of the employed models were compared by calculating the squared correlation coefficient (R^2) and the Korsmeyer- Peppas model was employed to identify the balance between competing release mechanisms.

Table 9. 5. Kinetic modeling of IMC release from all analyzed formulations.

Formulation	*First order*		*Higuchi*		*Hixton-Crowell*		*Korsmeyer-Peppas*			*Weibull*		
	R^2	k	R^2	k	R^2	k	R^2	n	k	R^2	a	b
PLGA-IMC	0.846	0.128	0.916	47.490	0.783	0.153	0.957	0.299	62.452	0.998	0.983	0.534
NPHN-IMC	0.795	0.092	0.971	30.212	0.777	0.196	0.943	0.379	34.925	0.992	1.613	0.275
NPHE-IMC	0.854	0.182	0.868	33.697	0.693	0.138	0.909	0.195	43.489	0.948	1.809	0.170
PLGA-IMC-PEG	0.834	0.085	0.842	20.838	0.777	0.196	0.930	0.178	31.722	0.968	1.548	0.104
NPHN-IMC-PEG	0.855	0.170	0.961	22.547	0.780	0.169	0.946	0.354	27.677	0.994	1.419	0.170
NPHE-IMC-PEG	0.931	0.150	0.944	10.436	0.880	0.122	0.980	0.305	14.216	0.990	1.172	0.060

Among the kinetic models employed, the Weibull and Korsmeyer – Peppas models showed the better fit with $n < 0.43$ specifying a release process predominantly controlled by diffusion

(Fickian type release pattern). The *k* value (Peppas model) decreased with oil dispersion within polymeric matrix and PEG addition, confirming the reduction of the initial burst release as was highlighted by the release studies.

9. 3. 7. Cytotoxicity Assay

The aim of MTT assessments were to determine the intrinsic cytotoxicity of IMC-loaded into plain or hybrid unmodified or PEG - modified formulations, consecutively to obtain a preliminary estimation of the safety of new nanoformulations (Figure 9. 11).

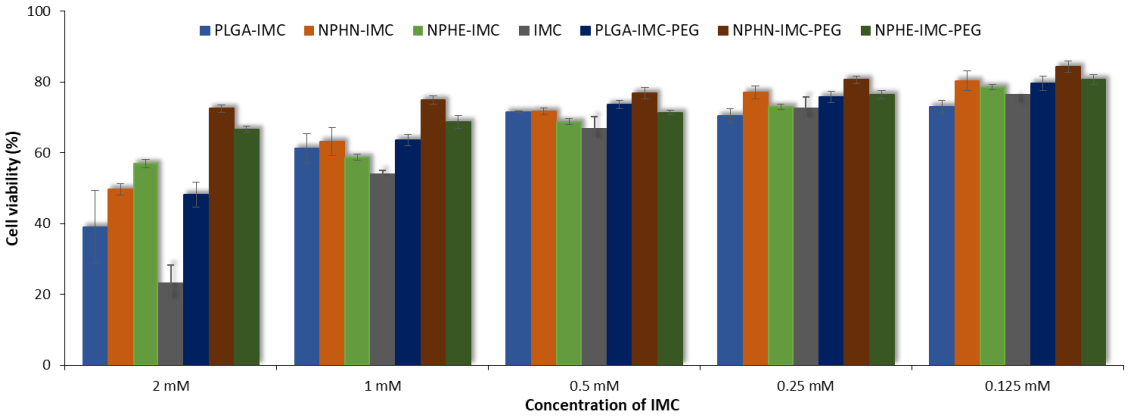


Figure 9. 11. In vitro viability of L929 fibroblast cells line treated with various concentrations of IMC- loaded into formulations

It can be seen that the oils dispersion within polymeric matrix decreased the cells growth inhibition with ~ 2.4 times as compared to standard nanocarriers, while the PEG-adsorption on the surface of the formulations increased the cellular viability with ~1.5 times as compared to uncoated formulations.

CHAPTER 10. PREDICTING THE CAPACITY OF HYBRID NANOCARRIERS BASED ON PLGA-VEGETABLE OIL TO ENCAPSULATE/RELEASE A BIOACTIVE COMPOUND

The main goal of this research study was to predict/explain using the most important thermodynamic parameter Flory-Huggins theory in combination with mesoscopic modelling approach, the limits to encapsulate and the capacity of the hybrid nanoparticles based on poly (lactic-co-glycolic acid) PLGA-vegetable oil to release a bioactive compound. Thus, a series of models of drugs with different chemical structure, solubility, partition coefficient (log P) and therapeutic activity were selected and encapsulated into standard PLGA and hybrid polymer-oil nanostructured formulations, using standard emulsion evaporation method. A wide range of drugs were selected with the aim of establishing the performance limits of the newly developed hybrid nanocarriers.

10. 3. Flory-Huggins Theory.
Determination of the compatibility parameter for binary systems (drug and PLGA matrix) or ternary analyzed systems (hybrid PLGA-vegetable oil matrix and drug)

The compatibility between each encapsulated drug and standard or hybrid polymeric matrices was calculated by Flory-Huggins parameter (χ), which describes the quality of

interactions between encapsulated drug and the nanocarrier matrix from thermodynamic point of view (296, 297).

$$\chi = \frac{(\delta_d - \delta_p)^2 \, v_d}{RT}$$

where δ_d and δ_p are the Scatchard–Hildebrand solubility parameters of the drugs and polymeric matrix, v_d is the molar volume of the solubilized drug calculated by the group contributions method according to Fedors (298), R is the gas constant and T the temperature in Kelvin.
The total solubility parameter of hybrid polymeric-oil matrix was determined according to Burke method (296, 302) the obtained values were centralized in Table 10. 2.

Tabel 10. 2. The solubility parameters of PLGA, NSO, NPHN and PVA

Entity	*δ (MPa)$^{1/2}$*
NSO	17.89
PLGA	19.90
NPHN	18.89
PVA	30.50

10. 4. Results and discussion

10. 4. 1. Study of the effect of compatibility parameter in the systems drug-standard PLGA/hybrid matrix upon the final hydrodynamic characteristics of formulations

The hydrodynamic characteristics of obtained empty/drug - loaded standard or hybrid formulations measured by Photon Correlation Spectroscopy using the principle of dynamic light scattering are presented in Table 10. 4.

Table 10. 4. Hydrodynamic characteristics of all obtained formulations

Bioactive compound	*R_H (nm)*		*P*		*ζ (mV)*	
	PLGA	*NPHN*	*PLGA*	*NPHN*	*PLGA*	*NPHN*
-	53.1±0.8	66.4±0.7	0.21±0.01	0.16±0.03	-12.2±1.2	-22.0±0.9
TC	72.5±0.7	83.2±0.6	0.14±0.04	0.11±0.01	-14.0±0.5	-19.4±0.9
R	72.8±0.5	82.2±0.8	0.10±0.01	0.08±0.06	-11.5±0.1	-18.4±0.4
CM	85.7±1.7	79.9±0.7	0.20±0.03	0.09±0.01	-11.4±0.1	-16.0±0.3
IMC	77.0±0.8	74.1±0.2	0.19±0.01	0.14±0.02	-14.2±0.2	-15.5±1.5
IHF	76.0±4.0	72.4±0.8	0.24±0.01	0.18±0.01	-13.4±1.7	-17.6±2.7
RSV	67.3±0.7	76.2±0.9	0.11±0.01	0.09±0.01	-13.5±0.7	-15.3±0.3
HC	63.3±0.4	69.3±0.7	0.14±0.06	0.13±0.01	-11.6±0.8	-20.5±0.7
NFI	69.2±1.8	74.6±0.1	0.17±0.09	0.13±0.02	-13.5±0.1	-13.8±0.7
5-FU	68.7±1.1	73.0±0.3	0.16±0.02	0.14±0.02	-11.1±0.6	-9.5±0.9

The drug entrapment into standard PLGA or hybrid nanoparticles inclined to increase their mean diameter, the similar effect was also reported in other research studies. However, a trend in reduction of the mean R_H of NPHN under CM, IMC and IHF loading was clearly observed, comparing to corresponding R_H of PLGA nanoparticle. The compaction tendency of hybrid formulations in the presence of these hydrophobic drugs could be a result of preferential interactions between CM, IMC, IHF and hybrid polymer-oil matrix. The dramatically reduction of the zeta potential (HPON-5FU ζ= -9.5) as compared to the empty hybrid matrix could be a

result of low compatibility between drug and polymer matrix (281), as was also reflected by the calculated compatibility parameter (χ_{dp}= 7.65).

10. 4. 2. The influence of the interaction parameter and partition coefficient on the performances of standard PLGA/hybrid matrix to encapsulate and release a selected drug

The capacity of hybrid nanocarrier matrix to encapsulate the lipophilic drugs was found to be the maximum for R 96.14%, followed by IHF 81.69%, IMC 64.01%, TC 61.41% and CM with 54.83% respectively.

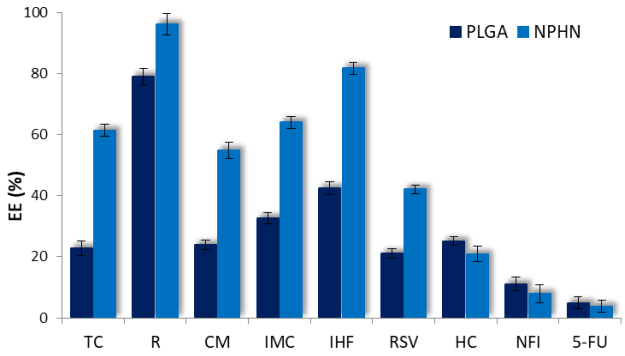


Figure 10. 6 .The drug encapsulation efficiency of PLGA and NPHN nanoparticles

In contrast, the lowest entrapment efficiency (less than 10%) was registered for hydrophilic drugs NFI and 5-FU. Standard PLGA nanoparticles presented an encapsulation capacity higher that 20% for almost all lipophilic drugs excepting R for which the EE has increased to 79.03%. Concerning the hydrophilic drugs (HC, NFI and 5-FU) the encapsulation obviously decreased but was higher comparing with hybrid matrix.

The amount of solubilized drug into polymer matrix is directly correlated with the Flory-Huggins parameter (χ_{dm}) (295, 299). For the above reason it was expected that as stronger are the interactions between nanocarrier matrix and selected drug, the higher will be the drug entrapment efficiency into the formulation (307).

In Figure 10. 7. a, b, are presented the correlations between experimental drug loading and compatibility parameter (χ_{dm}) of the standard PLGA/hybrid matrix – drug systems. It can be seen an increase of the encapsulation efficiency with reduction decreasing the value of compatibility parameter, observations which corroborate with the theory.

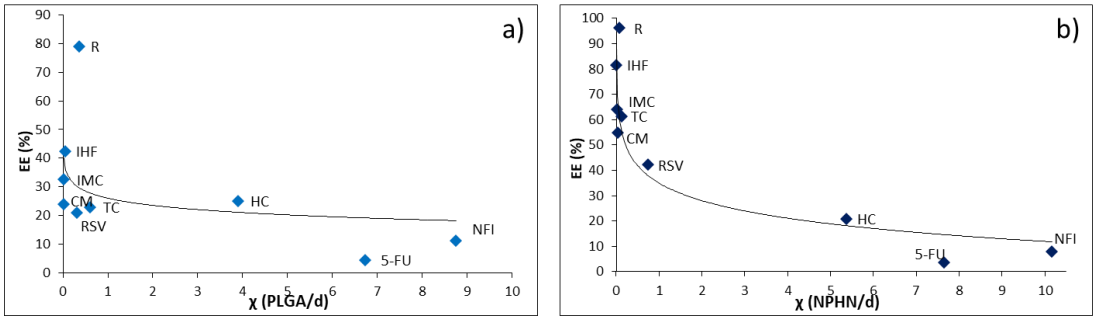


Figure 10. 7. Encapsulation efficiency (EE) vs a) - χ (PLGA/d) as well b) - χ (HPON/d)

Simultaneously, the release profile seems to be more reliant on the partition coefficient of the drug than on their compatibility parameter, as was ascertained regarding the encapsulation

efficiency. In Figure 10. 9. a, b, are illustrated the influence of the partition coefficient on the amount of the released drugs from standard or hybrid formulations.

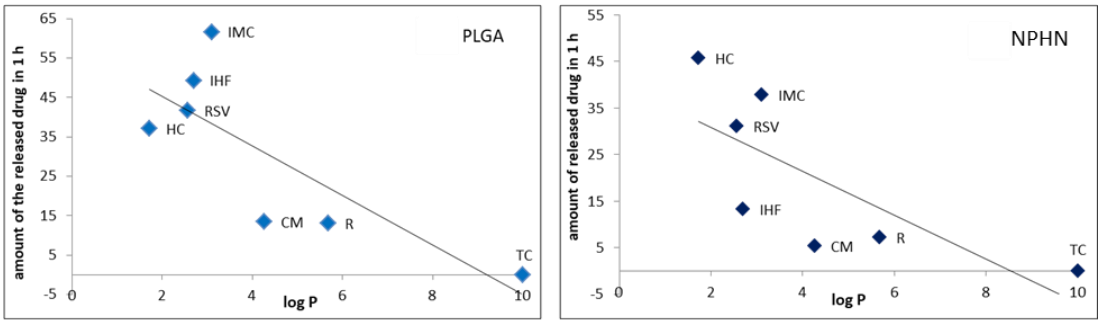


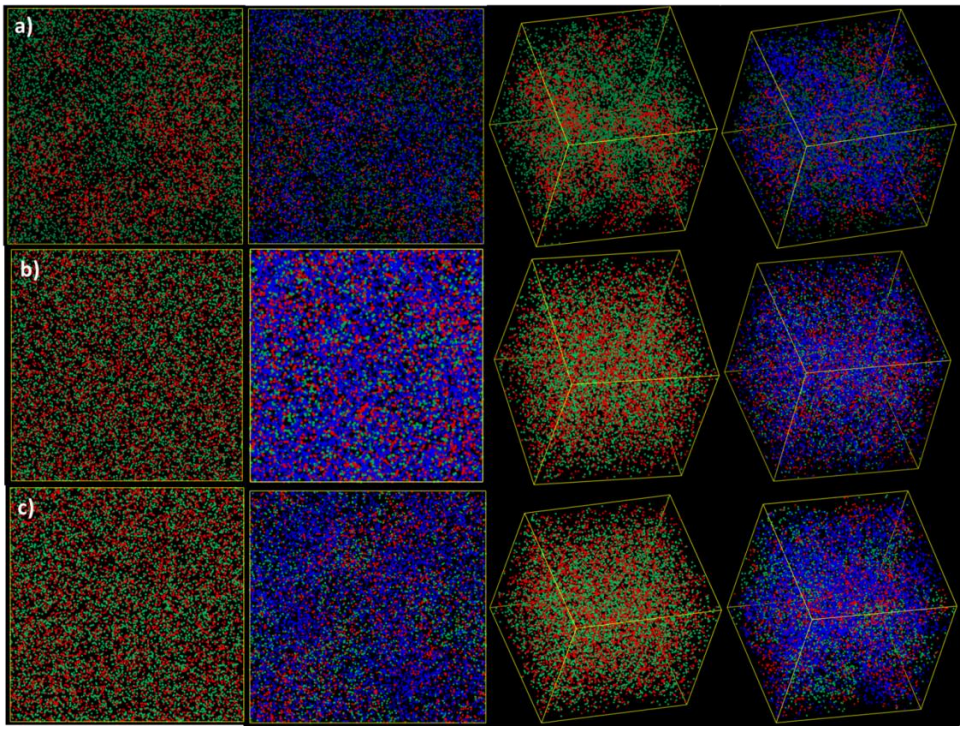
Figure 10. 9. log P vs the amount of released drug in the first hour

It can be seen an indirect dependence between these two parameters, as lipophilic is the encapsulated drug as lower is the initial burst release. This behavior was registered regardless of the type of delivery systems and could be an explanation in the reduction of the initial burst release of high lipophilic drugs from hybrid polymeric matrix as compared to standard ones.

Complete different release manner was noted in the case of NPHN-HC (log P=1.72) where the weak drug-hybrid matrix interactions (χ (PLGA/HC) = 1.77, χ (HOPN/HC) = 2.75) determined a faster release with a prominent initial burst effect. For these systems, it was expected a higher drug affinity to standard PLGA matrix, which is less lipophilic allowing a homogenously drug distribution in the particles and a more controlled release.

10. 4. 3. Molecular modelling of the drug distribution into standard PLGA/hybrid polymeric matrix employing the mesoscopic simulation and atomistic approach

Mesoscale modeling approach (MesoDyn or dissipative particles density-DPD methods) coerced the molecules into beads and it is useful for predicting the interactions which can appear between different chemical species. Mesoscopic modelling can also describe the evolution of a system by predicting the heterogeneity phenomena which can appear into the system (311) when the compatibility between the mixes is low/very low or homogeneity phenomena when the affinity between the compounds is optimal. The simulation describes the dynamics of phase separation by the means of dynamic mean-field density functional theory and Langevin theory and the most important molecular characteristic of the MesoDyn method is associated to the "incompatibility" between the compound of a system determined by Flory-Huggins theory (χ).In Figure 10.10 are presented the most suggestive simulation results, obtained for different types of drug, concerning the compatibility parameter and partition coefficient, dispersed into standard or hybrid matrices (a – HC; b – IMC; c – TC).



PLGA – green color; NSO – blue color; drug – red color

Figure 10. 10. Simulated distribution of a) - HC, b) - IMC and c) – TC into standard or hybrid PLGA-oil based nanocarriers

The molecular simulation of hydrophilic HC molecules (log P=1.72) highlighted systems with some phase separations, characterized by a low distribution of the components, regardless of the presence or absence of the vegetable oil (Figure 10. 10. a). Unlike HC molecules, the mesoscopic modelling of IMC (log P=3.10) – PLGA standard/hybrid matrix interactions, emphasized homogeneous systems with a good distribution of the drug molecules in both matrices/models (Figure 10. 10. b). This homogenous IMC distribution is the result of a good compatibility between the components of the system. Furthermore, when high lipophilic drug TC was used, the mesoscale simulation highlighted a good drug distribution into standard PLGA matrix (Figure 10. 10. c), while a nonhomogeneous phases distribution concerning hybrid system was observed, effect which is probably, a result of preferential partition of lipophilic drug molecules into the lipophilic phase (oil), as indicated by the value of lipophilicity parameter (log P = 10).

CHAPTER 11. POLYPLEXES – A NEW TYPE OF NANOPARTICLES WITH POTENTIAL APPLICATIONS IN GENE DELIVERY THERAPY

In this chapter, aiming to obtain the polyplexes with optimum hydrodynamic characteristics for gene delivery application, the DNA/PEI complexation reaction at different N/P molar ratios was studied. The effect of topology of cationic polymer upon the morphology of complexes was also studied.

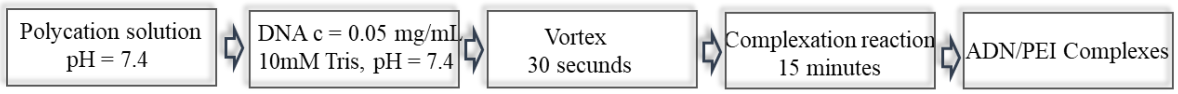


Figure 11.1 The main steps involved in the complexation reaction of DNA/PEI

11. 3. 1. Study of the effect of the N/P molar ratio on the hydrodynamic characteristics of the complexes (mean diameter, size distribution, zeta potential)

The variation of the hydrodynamic parameters and electrophoretic mobility of DNA/PEI complexes in function of N / P molar ratio at constant anionic polyelectrolyte concentration (0.05 mg/ mL) was investigated/ monitored by dynamic light scattering and the results are shown in Figure 11. 2.

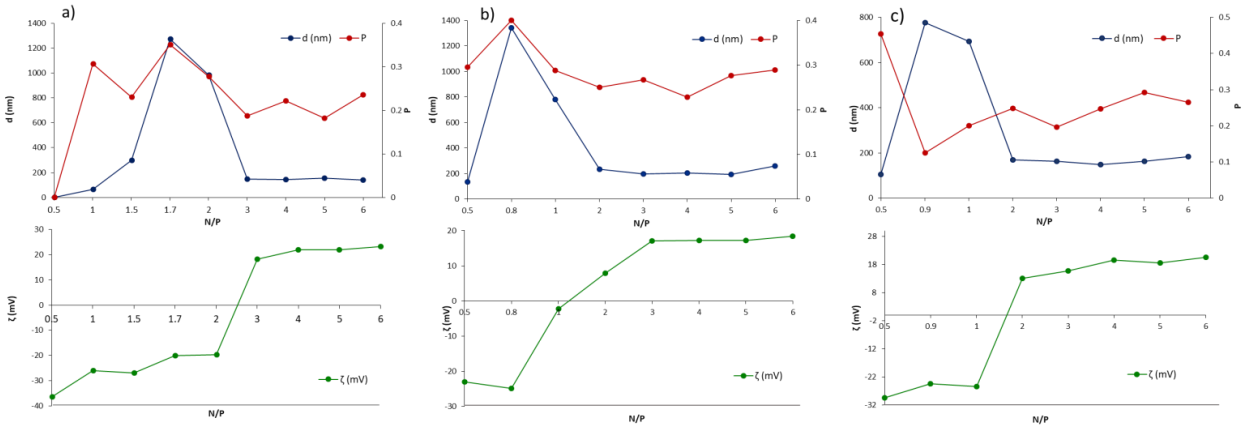


Figure 11. 2. Hydrodynamic characteristics of obtained complexes at different N/P molar ratios a) - PE, b) - PEI-4 and c) - for PEI-3

It can be observed that the mass aggregation process of colloids occurred near to the electroneutrality region, there is no sufficient amount of charge to stabilize the formed colloids (232, 319). Further increasing the amount of cationic polymer (up to N/P =3 for all the samples) determined the formation of complexes with the average diameter around 150-200 nm and reasonable size distribution (PdI < 0.3) and positive zeta potential which has the cross point in the electroneutrality region. Further addition of cationic polymer did not influence the surface charge of the particles. This fact suggests that a part of the PEI chains added above N/P> 3 were free in the solution mixture and might play an important role in promoting gene transfection.

11. 3. 3. Morphological characterization

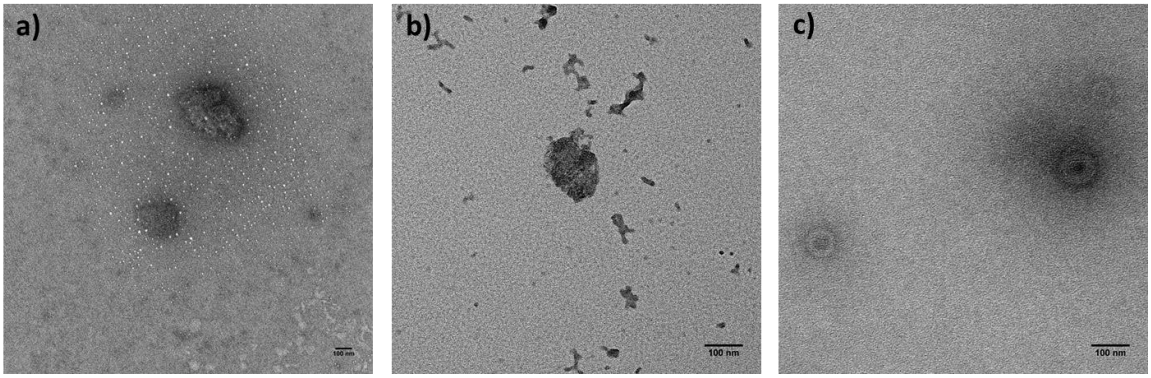


Figure 11. 4. TEM micrographs registered for complexes obtained with a) – PEI, b) – PEI-4, c) – PEI – 3

The morphological structures of polyplexes obtained with different types of polycation at the molar ratio N / P = 5, visualized by TEM microscopy are shown in Figure 11. 4. a -c.

TEM micrographs highlight colloids with well-defined spherical structures when DNA was aggregated in the presence of PEI (a) or PEI-4 (b) and perfect spherical structures when DNA was assembled with the three-arm star polycation (c). The differences observed in the morphological features of the obtained complexes can be attributed to technical reasons associated to the sample preparation method for analysis, or may be related to the PEI topology used in the complexation reaction. Thus, the anionic polyelectrolyte is assembled with the PEI-3 polycation (Figure 11. 4. c) in perfectly spherical "core-shell" disk structures. This type of polyanion DNA assembly in the presence of a polycation is reported in the literature (322, 323).

CHAPTER 12. GENERAL CONCLUSIONS

Two foremost research directions are explored in the current PhD thesis, the first one is related to a novel type of hybrid PLGA-vegetable oils based nanoparticles as an alternative of nanocarriers with optimum characteristics for lipophilic drugs delivery and the second direction was consisted in studying and optimizing the key-parameters involved in the obtaining process of a new class of nanoparticles based on polymer – non-viral vectors with tailored features and potential applications in gene therapy.

Standard or hybrid nanoparticles based on PLGA-vegetable oils and loaded with different types of bioactive compounds were obtained by standard emulsion evaporation method, using PVA as non-ionic surfactant.

The importance of vegetable oil in improving the hydrodynamic and morphological characteristics of hybrid formulations is revealed in the primary emulsification process by acting as a HLB (hydrophilic-lipophilic balance) moderator.

The hydrodynamic and morphological features of hybrid formulation were studied by various ultra-modern methods. DLS results presented monodispersed populations of colloids while SEM and TEM micrographs highlighted their core-shell, perfectly spherical, liposome-like structures.

Oil incorporation within the polymeric matrix led to a more amorphous nanostructure which enhanced the drug loading capacity of the devices, as was revealed by DSC analysis. Probably this high amorphous structure plays an important role in increasing the capacity of the nanocarriers to encapsulate a lipophilic drug, which might be stabilized in their lipid core. Simultaneously “the amount of disorder” of hybrid structures is firstly related to the nature of dispersed vegetable oil (the content in polyunsaturated acids) and secondly on their assembly method into the polymeric matrix which directly influences the hydrodynamic characteristics and the performance of hybrid formulation to encapsulate/release a bioactive compound.

The drug release studies highlighted the ability of hybrid matrices to discharge in a more sustained and controlled manner the encapsulated drug. Moreover, the cytotoxic assessment emphasized the formation of a more biocompatible hybrid system with the oil dispersion (the cellular growth inhibition was visible reduced).

The PEG layer adsorbed on the surface of the nanocarriers increased their biocompatibility and bioavailability and, at the same time determined a slower release kinetics acting as a supplementary barrier to drug diffusion.

Finally, it is proposed to rationalize the encapsulation/release performances of the hybrid formulations based on PLGA-vegetable oil for different drugs, using the most important thermodynamic parameter Flory-Huggins theory in combination with computational approach, the mesoscopic and Atomistic Modeling.

The second research direction is dedicated to study and to optimize the key parameters (topology of polycation, the N/P molar ratio) involved in obtaining a new class of polymeric nanoparticles named polyplexes – non-viral vectors for gene delivery.

SELECTIVE BIBLIOGRAPHY

12. Kamaly, N., Yameen, B., Wu, J., Farokhzad, O. C. (2016). Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chemical reviews, 116(4), 2602-2663.
24. Prabhu, R. H., Patravale, V. B., Joshi, M. D. (2015). Polymeric nanoparticles for targeted treatment in oncology: current insights. International journal of nanomedicine, 10, 1001
35. Anderson, J. M., Shive, M. S. (1997). Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced drug delivery reviews, 28(1), 5-24.
48. Astete, C. E., Sabliov, C. M. (2006). Synthesis and characterization of PLGA nanoparticles. Journal of Biomaterials Science, Polymer Edition, 17(3), 247-289.
60. Mora-Huertas, C. E., Fessi, H., Elaissari, A. (2010). Polymer-based nanocapsules for drug delivery. International journal of pharmaceutics, 385(1-2), 113-142.
88. Lembo, D., Cavalli, R. (2010). Nanoparticulate delivery systems for antiviral drugs. Antiviral Chemistry and Chemotherapy, 21(2), 53-70
89. Paleos, C. M., Tsiourvas, D., Sideratou, Z., Tziveleka, L. A. (2010). Drug delivery using multifunctional dendrimers and hyperbranched polymers. Expert opinion on drug delivery, 7(12), 1387-1398.
174. Devrim, B., Kara, A., Vural, İ., Bozkır, A. (2016). Lysozyme-loaded lipid-polymer hybrid nanoparticles: preparation, characterization and colloidal stability evaluation. Drug development and industrial pharmacy, 42(11), 1865-1876.
207. Durán-Lobato, M., Martín-Banderas, L., Gonçalves, L., Fernández-Arévalo, M., Almeida, A (2015). Comparative study of chitosan-and PEG-coated lipid and PLGA nanoparticles as oral delivery systems for cannabinoids. Journal of Nanoparticle Research, 17(2), 61.
235. Ramadan, M. F. (2007). Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): an overview. International journal of food science & technology, 42(10), 1208-1218.
237. Spilmont, M., Léotoing, L., Davicco, M. J., Lebecque, P., Mercier, S., Miot-Noirault, E., Pilet, P., Rios, L., Coxam, V. (2013). Pomegranate seed oil prevents bone loss in a mice model of osteoporosis, through osteoblastic stimulation, osteoclastic inhibition and decreased inflammatory status. The Journal of nutritional biochemistry, 24(11), 1840-1848.
283. Nartowski, K. P., Tedder, J., Braun, D. E., Fábián, L., Khimyak, Y. Z. (2015). Building solids inside nano-space: from confined amorphous through confined solvate to confined ‘metastable’polymorph. Physical Chemistry Chemical Physics, 17(38), 24761-24773.
287. Durán-Lobato, M., Muñoz-Rubio, I., Holgado, M., Álvarez-Fuentes, J., Fernández-Arévalo, M., & Martín-Banderas, L. (2014). Enhanced cellular uptake and biodistribution of a synthetic cannabinoid loaded in surface-modified poly (lactic-co-glycolic acid) nanoparticles. Journal of biomedical nanotechnology, 10(6), 1068-1079.
288. Vueba, M. L., De Carvalho, L. B., Veiga, F., Sousa, J. J., Pina, M. E. (2004). Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets. European Journal of Pharmaceutics and Biopharmaceutics, 58(1), 51-59.

296. Van Krevelen, D. W., Te Nijenhuis, K. (2009). Properties of polymers: their correlation with chemical structure; their numerical estimation and prediction from additive group contributions. Elsevier.
297. Eslami, M., Nikkhah, S. J., Hashemianzadeh, S. M., & Sajadi, S. A. S. (2016). The compatibility of tacrine molecule with poly (n-butylcyanoacrylate) and chitosan as efficient carriers for drug delivery: a molecular dynamics study. European Journal of Pharmaceutical Sciences, 82, 79-85.
311. Maiti, A., McGrother, S. (2004). Bead–bead interaction parameters in dissipative particle dynamics: relation to bead-size, solubility parameter, and surface tension. The Journal of chemical physics, 120(3), 1594-1601.
319. Hou, S., Ziebacz, N., Wieczorek, S. A., Kalwarczyk, E., Sashuk, V., Kalwarczyk, T., Holyst, R. (2011). Formation and structure of PEI/DNA complexes: quantitative analysis. Soft Matter, 7(15), 6967-6972.
322. Maury, B., Gonçalves, C., Tresset, G., Zeghal, M., Cheradame, H., Guégan, P., Midoux, P. (2014). Influence of pDNA availability on transfection efficiency of polyplexes in non-proliferative cells. Biomaterials, 35(22), 5977-5985.

PUBLISHED PAPERS

1. Ghitman, J., Stan, R., Iovu, H. (2017). Experimental contributions in the synthesis of PLGA nanoparticles with excellent properties for drug delivery: Investigation of key parameters. *UPB Sci. Bull. Ser. B,* 79, 101-112.
2. Ghitman, J., Stan, R., Cecoltan, S., Chifiriuc, M. C., Iovu, H. (2018). Hybrid nanocarriers based on PLGA-vegetable oil: A novel approach for high lipophilic drug delivery. *Journal of Drug Delivery Science and Technology,* 46, 162-172 (IF = 2.297)
3. Ghitman, J., Stan, R., Ghebaur, A., Cecoltan, S., Vasile, E., Iovu, H. (2018). Novel PEG-Modified Hybrid PLGA-Vegetable Oils Nanostructured Carriers for Improving Performances of Indomethacin Delivery. *Polymers*, 10(6), 579 (IF = 2.935)
4. Ghitman, J., Stan, R., Vlasceanu G., Vasile, E., Iovu, H. " Predicting the drug loading efficiency into hybrid nanocarriers based on PLGA-vegetable oil using molecular dynamic simulation approach and Flory-Huggins theory", ACS. *Soft Matter – in press*

CONFERENCES

1. Ghitman J., Stan R., Iovu H. "New polymer - vegetable oil nanoparticles with potential biomedical applications". 20th Romanian International Conference on Chemistry and Chemical Engineering, University Politehnica of Bucharest, Poiana Brasov, Romania 06.09.2017 - 09.09.2017
2. Ghitman J., Stan R., Iovu H. "Innovative vegetable oil-PLGA hybrid nanoparticles with potential biomedical applications". 7th International Conference „Biomaterials, Tissue Engineering and Medical Devices", University Politehnica of Bucharest, Constanta, Romania, 15.09.2016 – 17.09.2016
3. Ghitman J., Stan R., Cecoltan S., Chifiriuc M., Iovu H. "New polymer - vegetable oil nanoparticles as nanocarriers for antibacterial drugs". 13th International Conference on Materials Chemistry (MC13) Liverpool, United Kingdom, 10.07.2017 – 13.07.2017