MATERIALE PLASTICE
https://revmaterialeplastice.ro
https://doi.org/10.37358/Mat.Plast.1964

PLGA - gallic Acid Advanced Drug Delivery System as New Functional Material

ANNE-MARIE BEATRICE POENARIU[1], ROBERTO ANGELO POSTELNICU[1*], MARIA VIORICA CIOCILTEU[1], GABRIELA RAU[1], MARIANA POPESCU[1], ANDREEA SIMIONESCU[4], IONELA ANDREEA NEACSU[2], CLAUDIU NICOLICESCU[3], DENISA CONSTANTINA AMZOIU[1*]

[1]University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Str., 200638, Craiova, Romania
[2]Politehnica University of Bucharest, Faculty of Chemical Engineering and Biotechnologies, 1-7 Polizu Str., 011061, Bucharest, Romania
[3]University of Craiova, Faculty of Mechanics, Department of Engineering and Management of the Technological Systems, 1 Călugăreni Str., 220037, Drobeta Turnu-Severin, Romania
[4] University of Craiova, Faculty of Exact Sciences, Department of Chemistry, 107 Calea Bucuresti Str., 200515, Craiova, Romania

Abstract: *Combining polimers with polyphenols such as gallic acid opens up new directions in healthcare system. By encapsulating secondary metabollites within PLGA nanoparticles, we tried to enhance their stability, solubility, and obtain a targeted delivery system. In this study, we synthesized a PLGA-gallic acid sustained release system, using the solvent evaporation method. This approach improved the therapeutic efficacy of gallic acid. The numerical distribution showed that most PLGA-GA nanoparticles have a size of 10 nm. Through the method of solvent evaporation, an incorporation efficiency of 49% was obtained.*

Keywords: *PLGA, gallic acid, nanocomposite material, solvent evaporation method*

1. Introduction

Researchers from all over the world use nanocarriers to deliver bioactive compounds for disease prevention and treatment. By incorporating secondary metabolites into nanomaterials new functional materials with applications in drug delivery or diagnostics were developed [1-3].

Gallic acid (GA) or 3,4,5-trihydroxybenzoic acid (Figure 1) is one of the most common natural polyphenols [4], extremely widespread in plants, especially in fruits such as berries, grapes, and tea leaves [5, 6]. The first time it was obtained by extraction and isolation from the *Punica* species and in the laboratory it was obtained from tannic acid, through a reaction of its hydrolysis under the action of the enzyme tannase (glycoprotein esterase) [7].

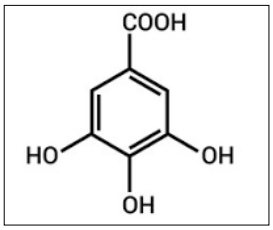


Figure 1. Structure of gallic acid

GA has many applications different industrial branches such as food or pharmaceutical. For example, it is used as an oil additive because it prevents its rancidity through its antioxidant activity [8].

In addition to its use as a flavoring and preservative agent in the food industry, there is scientific evidence of its biological and pharmacological actions as an anti-inflammatory, antimicrobial, antioxidant and anticancer agent, having gastroprotective, neuroprotective and cardioprotective effects as well as being an anti-diabetic and anti-obesity agent [9].

**email: robertopostelnicu4@gmail.com, denisa.amzoiu@umfcv.ro*

GA has shown selective cytotoxicity to cancer cells and very low toxicity to normal cells [10]. This property makes GA a useful nutritional supplement, along with vitamins, to prevent the risk of cancer [11].

GA has a broad antibacterial activity against numerous *gram-positive* (*Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa)* acting both by reducing their motility and by adhesion [12]. It was found that GA also has an action against biofilm formation, the antibacterial mechanism involving the alteration of membrane permeability [13].

GA formulations with chitosan have a synergistic action (antibacterial, antioxidant), noting that they can even increase the activity of antibiotics such as penicillin, ampicillin, oxacillin etc. [14].

The use of these properties in the medical and pharmaceutical world can however be very limited because polyphenols show great instability at the gastric level (*p*H, gastric enzymes, other nutrients) if they are introduced through food intake (extract, plant powder) but this instability is also present in pharmaceutical processes, storage (temperature, light, air) [15]. The encapsulation of these compounds through the nanotechnological approach can solve the problems mentioned above and even improve certain of their properties [16].

Co-glycolic-lactic acid polymers are part of the so-called delivery systems (DDS - Drug Delivery Systems) of a multitude of active principles, such as drugs, proteins, etc. Among the advantages of using polymeric nanoparticles we mention: protection of the active principle from degradation, controlled release, pass the gastric barrier unaffected, improvement of certain physical properties of the drug such as solubility, etc. The use of synthetic polymers is preferred over natural ones (collagen) because they have a much higher purity and the results obtained when using them are reproducible [17].

Polylactic-co-glycolic acid (PLGA) is FDA approved, many drugs encapsulated in it have been on the pharmaceutical market for almost two decades, and also a large number of drugs are in the stage of clinical trials (Figure 2).

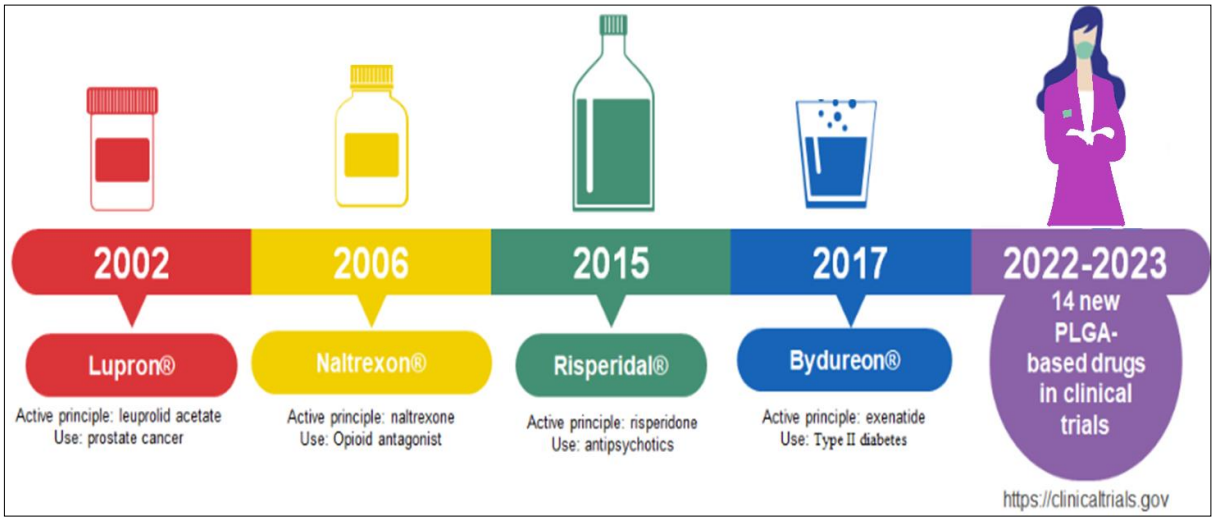


Figure 2. Drugs with PLGA as polymeric matrix approved or in the process of approval

2. Materials and methods

Chemicals and reagents

GA (p>95%) was purchased from Sigma; solvents (LiChroSolv water, acetone, ethanol, methanol, dichloromethane, chromatographic grade) were purchased from Merck, Germany; PLGA (65:35), molecular weight 40,000-75,000 from Sigma; PVA, molecular weight 30000-700000-Merck, Germany; Folin-Ciocâlteu reagent 2 M was made by Sigma.

Synthesis

The GA: PLGA ratio was 1:10 for nanoparticle synthesis initially, and then other ratios were tested to observe differences in incorporation efficiencies. Thus, 20 mg of GA and 200 mg of PLGA were dissolved in 7 mL of acetone and mechanically stirred at 45000 rpm for 3-5 min (Vortex SilenCrush). The obtained solution was added over an aqueous solution with PVA (0.5-5% wt) and stirred (1000 rpm) to evaporate the organic solvent for 5 h at 40°C. The formed nanoparticles were centrifuged at 11000 rpm for 10 min, the supernatant containing unencapsulated GA was removed and washed with ultrapure water three times to remove residual GA and PVA. The final suspension obtained was subjected to a lyophilization process (Alpha 1-2 LSCbasic Martin Christ freeze dryer): PLGA-GA particles were frozen at -55°C overnight and kept at 0.02 mbar 48 h;

In Figure 3 the steps of the PLGA - gallic acid (PLGA-GA) material synthesis by the solvent evaporation method are shown.

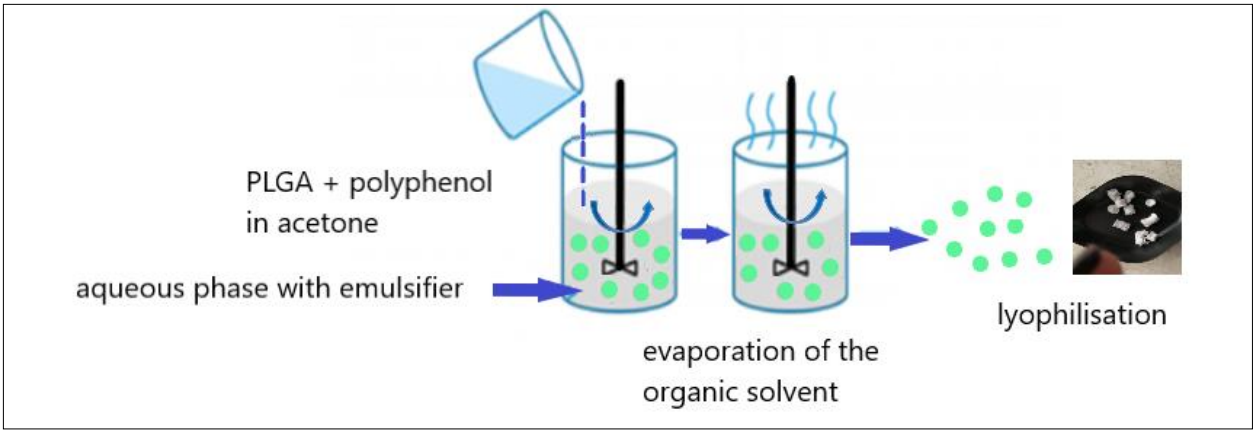


Figure 3. Stages of the synthesis process

Characterization

The morphological characterization of the nanoparticles was carried out by scanning electron microscopy (FEI Inspect F50, at the voltage of 30 KeV and various magnifications), and their sizes were measured by DLS (Brookhaven 90 Plus, Brookhaven Instruments Corporation, USA, equipped with a 35 mW and σ = 660 nm).

Determination of encapsulation percentage

The experiments were carried out using a spectrophotometer DLAB SP-UV1000. From the sample to be analyzed, solubilized in dichloromethane (DCM) and extracted in 70% ethanol, 200 µL were taken, over which 2.5 mL 0.2 M of Folin Ciocalteu reagent was added. After four minutes, 2 mL of Na_2CO_3 75 g/L were added. The mixture was kept at rest for two hours, at room temperature (26-27°C), in the dark, until the spectrophotometric analysis was performed [18].

The calibration curve was made using gallic acid as a standard, with concentrations of 0-100 mg/L. The absorbance was measured at λ = 765 nm. The results were expressed as a percentage, with the formula below. All measurements were performed in triplicate, calculating the standard deviation.

$$GA(\%) = \frac{mass\ of\ recovered\ GA\ (mg)}{\boldsymbol{initial\ mass\ of\ GA}(mg)} x100$$

3. Results and discussions

Encapsulation efficiency

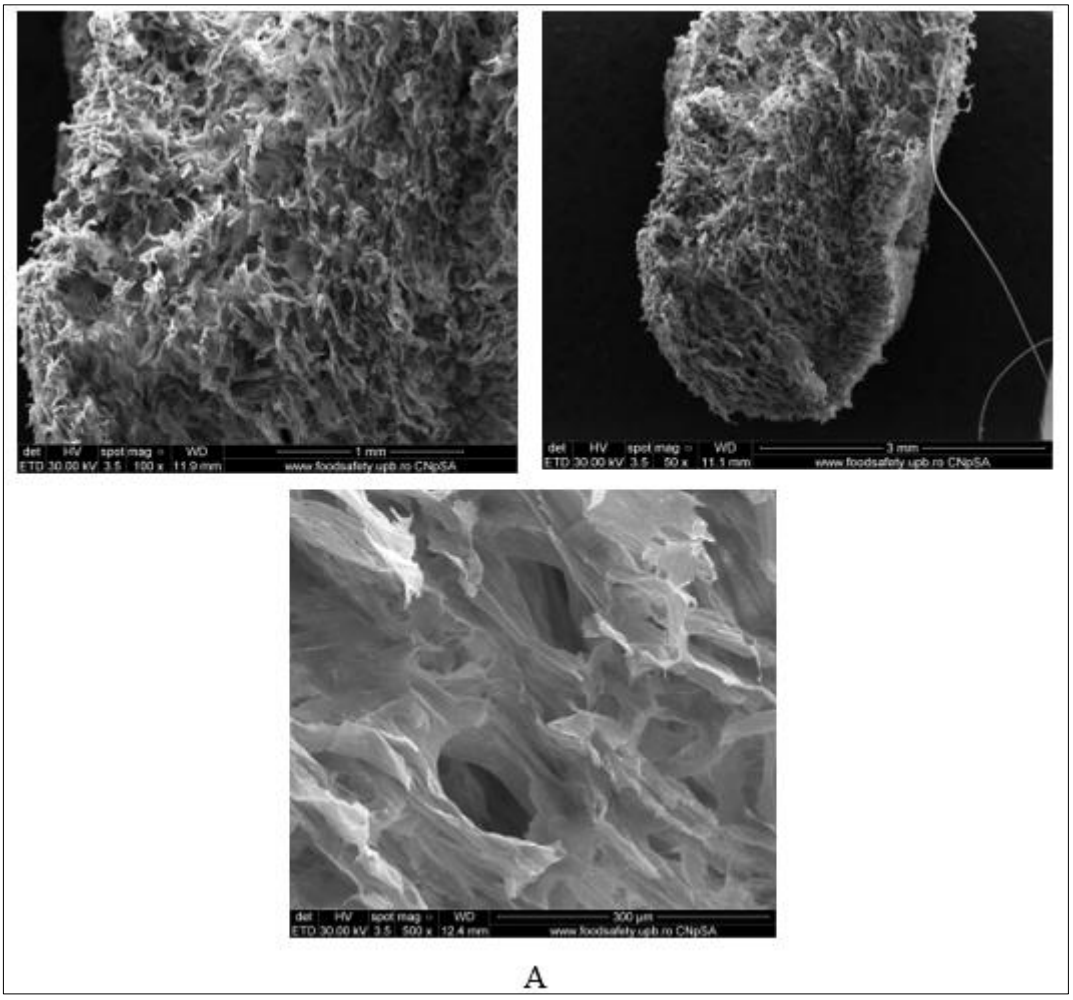
Encapsulation efficiency is a crucial parameter in PLGA-GA drug delivery system. The percentage of the active GA that was successfully encapsulated within the intended carrier material during the encapsulation process was 49±0.3%. We achieved a good encapsulation efficiency in the synthesis

conditions mentioned above. The percent depends on various factors such as the compatibility between PLGA and GA, the emulsification process, the solvent evaporation conditions and subsequent purification of the obtained material. Higher encapsulation efficiency is often associated with better control over the release of the active substance, improved therapeutic efficacy, and reduced wastage of the active substance.

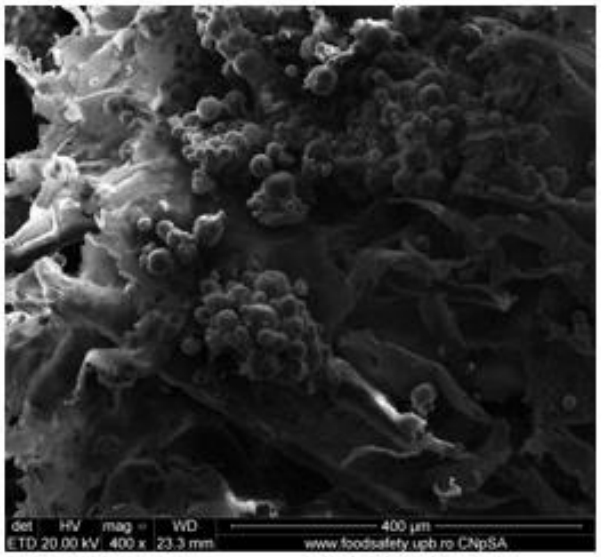
Morphological aspects

PLGA-GA nanoparticles obtained by the solvent evaporation method have certain morphological characteristics and properties, depending on the experimental conditions and the parameters used in the manufacturing process.

After the lyophilization process, the PLGA-GA material presented a spongy, porous appearance with micrometric pores (Figure 4 A). Similar to most nanoparticles obtained by the solvent evaporation method, in the case of PLGA, they tend to be spherical (Figure 4 B). This is a common form due to the process of emulsification and evaporation [19, 20]. The spherical shape was only visible in centrifuged and washed particles, in this case, lyophilization having a profound effect on the morphology of particles. The freeze-drying probable increased the surface area of PLGA-GA. This occurs when the ice crystals create cracks or fissures in the material during freezing and sublimation. Increased surface area explains the rapid rehydration and dissolution we have observed later. In our previous studies we found that the material often maintains its original particle shape and size [22] better than other drying methods, such as spray drying or air drying, but not in this particular case.



A



B

Figure 4. Images of PLGA-GA material: A) lyophilized and B) centrifuged taken by SEM at different magnitudes

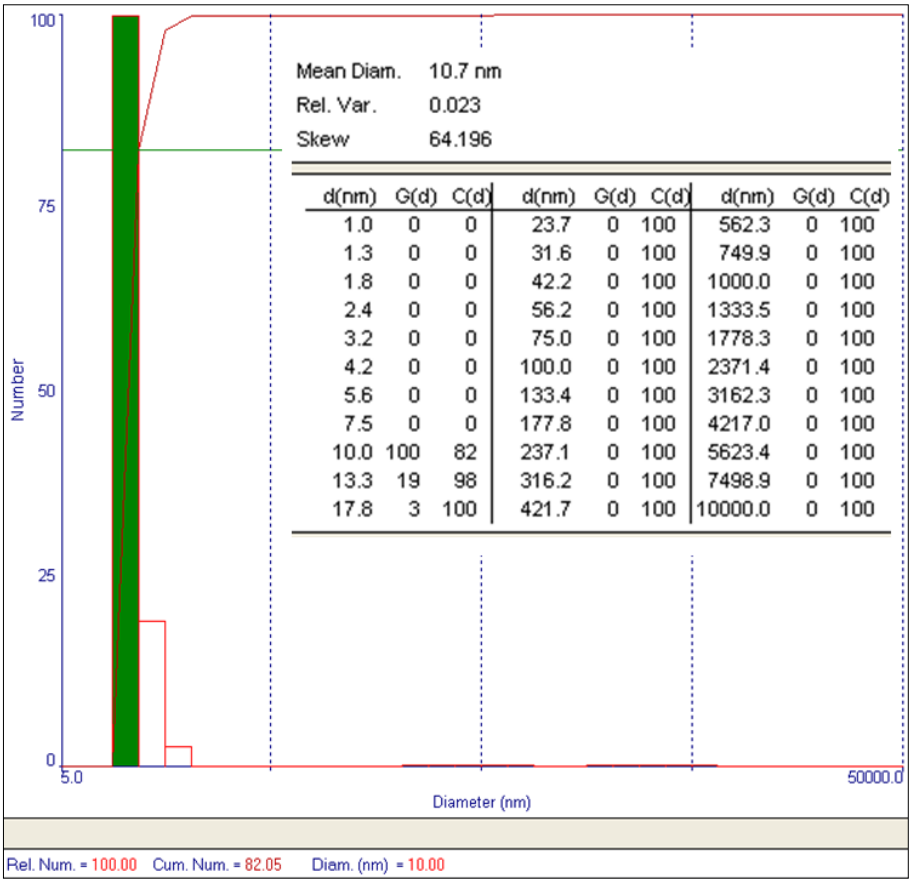
Numerical distribution

DLS was used to measure the hydrodynamic size of particles suspended in water after lyophilisation preocess. This technique measures the fluctuations in intensity of scattered light caused by Brownian motion of particles in the suspension. It provides information about the hydrodynamic diameter of particles. This diameter represents the effective size of particles in a liquid, taking into account the particles' diffusion properties as they move in the liquid medium.

The numerical distribution shows the following structure: 81.97% of the total number of particles is of size 10 nm, 15.57% of the total number of particles is of size 13.3 nm, 2.46% of the total number of particles is of size 17.8 nm (Figure 5).

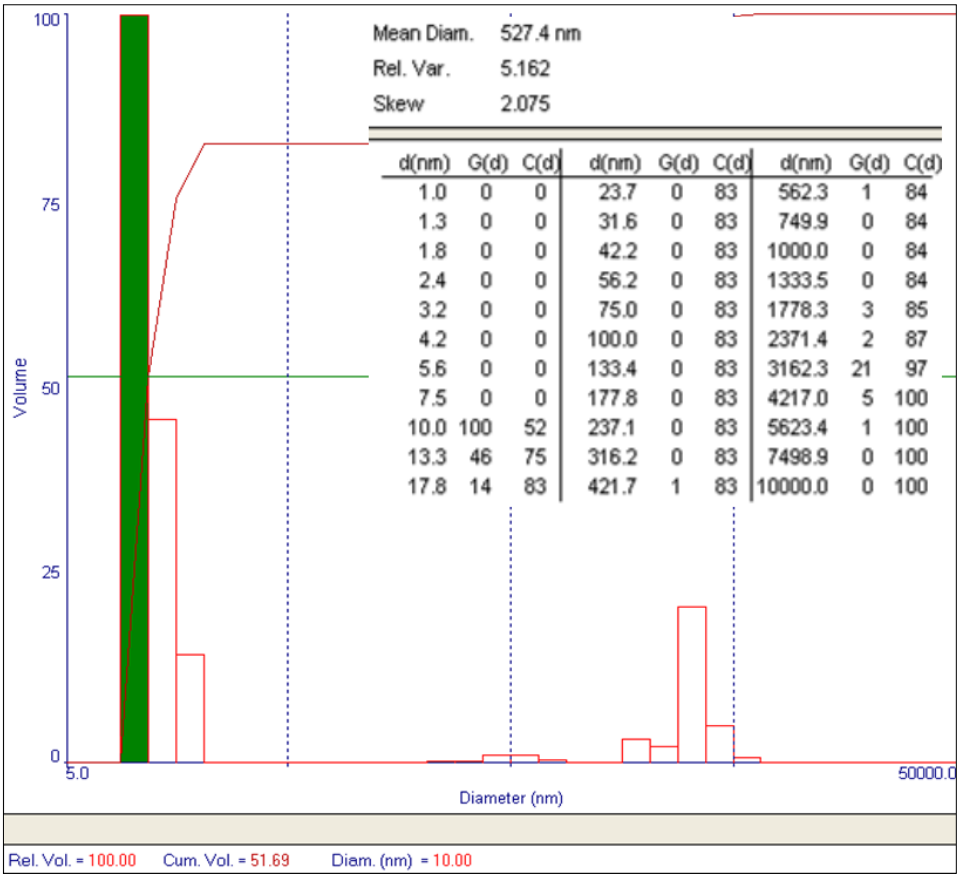
The distribution by volume shows the following structure: 51.55% of the total volume of particles is of size 10 nm, 23.71% of the total volume of particles is of size 13.3 nm, 7.22% of the total volume of particles is of size 17.8 nm, 0.52% of the total volume of particles is of size 421.7 nm, 0.52% of the total volume of particles is of size 562.3 nm, 1.55% of the total volume of particles is of size 1.77 µm, 1.03% of the total volume of particles is of size 2.37 µm, 10.82% of the total volume of particles is of size 3.16 µm, 2.58% of the total volume of particles is of size 4.21 µm, 0.52% of the total volume of particles is of size 5.62 µm (Figure 6).

The higher values that occur in the case of the volume distribution are not present in the case of the numerical distribution. Given the fact that most particles have a size between 10-18 nm, it is possible that the appearance of these volume percentages is due to particle agglomeration.



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
1.0	0	0	23.7	0	100	562.3	0	100
1.3	0	0	31.6	0	100	749.9	0	100
1.8	0	0	42.2	0	100	1000.0	0	100
2.4	0	0	56.2	0	100	1333.5	0	100
3.2	0	0	75.0	0	100	1778.3	0	100
4.2	0	0	100.0	0	100	2371.4	0	100
5.6	0	0	133.4	0	100	3162.3	0	100
7.5	0	0	177.8	0	100	4217.0	0	100
10.0	100	82	237.1	0	100	5623.4	0	100
13.3	19	98	316.2	0	100	7498.9	0	100
17.8	3	100	421.7	0	100	10000.0	0	100

Figure 5. Number distribution of PLGA-GA



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
1.0	0	0	23.7	0	83	562.3	1	84
1.3	0	0	31.6	0	83	749.9	0	84
1.8	0	0	42.2	0	83	1000.0	0	84
2.4	0	0	56.2	0	83	1333.5	0	84
3.2	0	0	75.0	0	83	1778.3	3	85
4.2	0	0	100.0	0	83	2371.4	2	87
5.6	0	0	133.4	0	83	3162.3	21	97
7.5	0	0	177.8	0	83	4217.0	5	100
10.0	100	52	237.1	0	83	5623.4	1	100
13.3	46	75	316.2	0	83	7498.9	0	100
17.8	14	83	421.7	1	83	10000.0	0	100

Figure 6. Volume distribution of PLGA-GA

Electrokinetic potential

The electrokinetic potential of materials influences dispersion properties, colloidal stability, interactions with biomolecules and cells, as well as general material behavior in their application systems [21].

The electrokinetic potential therefore represents the electric potential difference between the nanoparticle surface and the surrounding solution. It is critically influenced by surface properties of the material, functionalization or electrical charge, as well as the characteristics of the environment in which the material is located. In our situation, the material was immersed in phosphate buffer solution (PBS), *p*H=7.4, to simulate the *in vivo* conditions as closely as possible. We obtained a negative Zeta potential, with the average value of -1.74 mV (Figure 7).

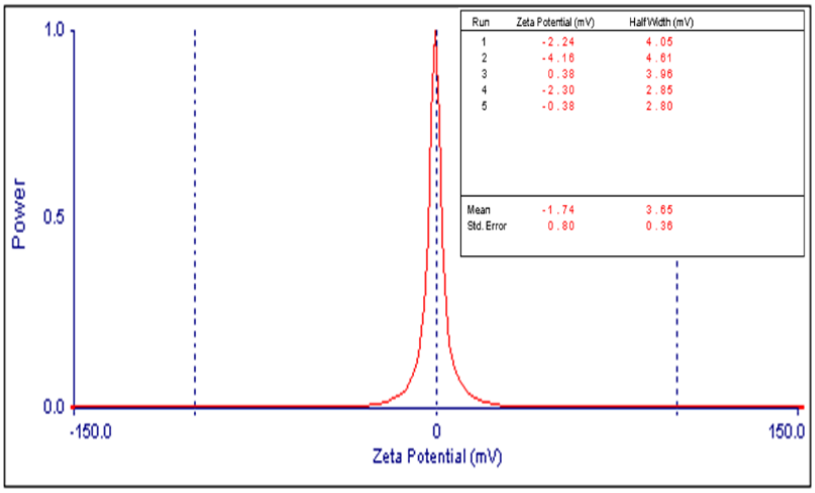


Figure 7. Electrokinetic potential of PLGA-GA

4. Conclusions

The encapsulation of GA in PLGA protects it from degradation and contributes to the growth of system stability. PLGA-GA can be used further as sustained release system, with synergistic effects in regenerative medicine (wound healing). However, a better understanding of the interactions between GA and the PLGA matrix is necessary, in order to establish the kinetic release profile.

References

1. ISOPENCU, G.O., COVALIU-MIERLĂ, C.I., DELEANU, I.M., From Plants to Wound Dressing and Transdermal Delivery of Bioactive Compounds, *Plants*, 12(14), 2023, 2661. https://doi.org/10.3390/plants12142661
2. ANGHELINA, F., MITROI, A.A., CIUCA, E., TUMBAR, L.T., BOICEA, A.R., ANGHELINA, L., VOIOSU, C., CAMEN, A., HAINAROSIE, R., Biomaterials Used in the Prosthetics of Facial Fractures, *Mater. Plast.*, **60**(1), 2023, 62-66. https://doi.org/10.37358/MP.23.1.5645
3. BENCZE, M., ALBU, C., TEODORESCU, E., VASILACHE, A., STANCIU, I., DRAGOMIRESCU, A., IONESCU, E., Clinical Use of Nanoparticles in Orthodontics as Possible Aid to Reduce the Incidence of White Spot Lesions, *Mater. Plast.*, **59**(1), 2022, 156-176. https://doi.org/10.37358/MP.22.1.5569
4. ***National Center for Biotechnology Information. PubChem Database. Gallic Acid, CID=370, 2020
5. ZHANG, J., LI, B., YUE, H., WANG, J., ZHENG. Y., Highly selective and efficient imprinted polymers based on carboxyl-functionalized magnetic nanoparticles for the extraction of gallic acid from pomegranate rind, *J. Sep. Sci.*, 41(2), 2018, 540-547, doi: 10.1002/jssc.201700822
6.SCOCCIA, J., PERRETTI, M.D., MONZÓN, D.M., CRISÓSTOMO, F.P., MARTÍN, V.S., CARRILLO, R., Sustainable oxidations with air mediated by gallic acid: potential applicability in the reutilization of grape pomace, *Green Chem.*, 18(9), 2016, 2647-2650, https://doi.org/10.1039/C5GC02966J

7.CEDÓ, L., CASTELL-AUVÍ, A., PALLARÈS, V., MACIÀ, A., BLAY, M., ARDEVOL, A., et al. Gallic acid is an active component for the anticarcinogenic action of grape seed procyanidins in pancreatic cancer cells, *Nutr. Cancer*, 66(1), 2014, 88-96, doi: 10.1080/01635581.2014.851714
8.CHOUBEY, S., VARUGHESE, L., KUMAR, V., BENIWAL, V., Medicinal importance of gallic acid and its ester derivatives: a patent review, *Pharm. Pat. Anal*., 4(4), 2015, 305-315, doi: 10.4155/ppa.15.14.
9. SCHIMITES, P.I., SEGAT, H.J., TEIXEIRA, L.G., MARTINS, L.R., MANGINI, L.T., BACCIN, P.S., ROSA, H. Z., MILANESI, L.H., BURGER, M.E., SOARES, A.V., Gallic acid prevents ketamineinduced oxidative damages in brain regions and liver of rats, *Neurosci. Lett*., 714, 2020, 134560, doi: 10.1016/j.neulet.2019.134560.
10. CHEN, H.M., WU, Y.C., CHIA, Y.C., CHANG, F.R., HSU, H.K., HSIEH, Y.C., CHEN, C.C., YUAN, S.S., Gallic acid, a major component of Toona sinensis leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells, *Cancer Lett*., 286(2), 2009, 161-171, https://doi.org/10.1016/j.canlet.2009.05.040
11. HO, H., CHANG, C., HO, W., LIAO, S., LIN, W., WANG, C., Gallic acid inhibits gastric cancer cells metastasis and invasive growth via increased expression of RhoB, downregulation of AKT/small GTPase signals and inhibition of NF-B activity, *Toxicol. Appl. Pharmacol*., 266(1), 2013, 76–85, doi: 10.1016/j.taap.2012.10.019.
12. SHAO, D., LI, J., LI, J., TANG, R., LIU, L., SHI, J., et al., Inhibition of gallic acid on the growth and biofilm formation of *Escherichia coli* and *Streptococcus mutans*., *J. Food. Sci*., 80(6), 2015, 1299-1305, doi: 10.1111/1750-3841.12902
13.TEODORO, G., ELLEPOLA, K., SENEVIRATNE, C., Potential use of phenolic acids as anti-candida agents-a review. *Front Microbiol.*, 6, 2015, 1420, doi: 10.3389/fmicb.2015.01420.
14.PERINELLI, D.R., FAGIOLI, L., CAMPANA, R., JENNY, K.W., LAM, BAFFONE, W., PALMIERI, G.F., CASETTARI, L., BONACUCINA, G., Chitosan-based nanosystems and their exploited antimicrobial activity, *Eur. J. Pharm. Sci*., 117, 2018, 8-20, https://doi.org/10.1016/j.ejps.2018.01.046.
15. FANG, Z., BHANDARI, B., Encapsulation of polyphenols: A review, *Trends Food Sci. Technol*., 21(10), 2010, 510-523, https://doi.org/10.1016/j.tifs.2010.08.003
16. ENGINEER, C., PARIKH, J., RAVAL, A., Review on hydrolytic degradation behavior of bio-degradable polymers from controlled drug delivery system, *Trends Biomater. Artif. Organ*., 25(2), 2011, 79-85.
17.MEHROTRA, A., PANDIT, J.K., Critical Process Parameters Evaluation of Modified Nano-precipitation Method on Lomustine Nanoparticles and Cytostatic Activity Study on L132 Human Cancer Cell Line, *J. Nanomed. Nanotechnol*., 3, 2012,1-8.
18. BORNEO, R., LEÓN, A.E., AGUIRRE, A., RIBOTTA, P., CANTERO, J.J., Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their in vitro testing in a model food system, Food Chem., 112(3), 2009, 664–670, https://doi.org/10.1016/j.foodchem.2008.06.027
19.ALDAWSARI, M.F., ALKHOLIFI, F.K., FOUDAH, A.I., ALQARNI, M.H., ALAM, A., SALKINI, M.A., SWEILAM, S.H., Gallic-Acid-Loaded PLGA Nanoparticles: A Promising Transdermal Drug Delivery System with Antioxidant and Antimicrobial Agents, *Pharmaceuticals*, 16(8), 2023, 1090. https://doi.org/10.3390/ph16081090
20. IONESCU (FILIP), O., CIOCÎLTEU, M.V., MANDA, C.V., NEACSU, I.A., FICAI, A., AMZOIU, E., TURCU STIOLICA, A., CROITORU, O., NEAMTU, J., Bone - Graft Delivery Systems of Type PLGA-gentamicin and Collagen - hydroxyapatite - gentamicine, *Mater. Plast*., **56**(3), 2019, 534-537, https://doi.org/10.37358/MP.19.3.5224
21.XU, R., Progress in nanoparticles characterization: Sizing and zeta potential measurement, *Particuology*, 6(2), 2008, 112-115, https://doi.org/10.1016/j.partic.2007.12.002

Manuscript received: 24.08.2023