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

Formulation and Characterization of PLGA-quercetin Hybrid Material with Pharmaceutical Applications

BEATRICE ANNE-MARIE POENARIU[1], DENISA-CONSTANTINA AMZOIU[1], MARIA VIORICA CIOCILTEU[1]*, VLADIMIR LUCIAN ENE[2], FELICIA CIULU-COSTINESCU[3], CLAUDIU NICOLICESCU[4], ALEXANDRA COSTACHI[1], LIVIU CHIRIGIU[1], MARIANA POPESCU[1], OANA ELENA NICOLAESCU[1]

[1] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, 2 Petru Rareş Str., 200349, Craiova, Romania
[2]Politehnica University of Bucharest, Faculty of Chemical Engineering and Biotechnologies, 1-7 Polizu Str., 011061, Bucharest, Romania
[3] University Titu Maiorescu of Bucharest, Faculty of Pharmacy, 22 Dâmbovnicului Str., 031593, Bucharest, Romania
[4]University of Craiova, Faculty of Mechanics, 1 Călugăreni Str., 220037, Drobeta Turnu-Severin, Romania

Abstract. *A new bicomponent hybrid material was obtained by encapsulating quercetin in PLGA using solvent evaporation method. The morphological aspects of the samples were established by scanning electron microscopy (SEM). Particle size distribution was determined by dynamic light scattering (DLS), numerical distribution showing that the particles are micrometric, with two granulometric intervals: [385-479] nm and [662-918] nm. Morphology was typical for PLGA, spherical one; the obtained particles are porous with micrometric pores. Zeta potential (ZP, -4.84mV) showed that the particles have electronegative surface charge. The loading efficiency was determined spectrophotometric and it was 18.3% in the mentioned synthesis conditions.*

Keywords: *PLGA-Quercetin, solvent evaporation, hybrid material*

1. Introduction

Quercetin (Que) is an abundant plant flavonol with two aromatic nuclei connected by a chain of three carbon atoms forming a pyranic nucleus (Figure 1).

Quercetin is considered a metabolite present in various parts of many plants in the human diet [1]. The richest sources of Que are red onion, grapes, cherries, apples, broccoli, citrus fruits, tomatoes and tea [2, 3]. Quercetin exhibits strong antioxidant activity due to the presence of phenolic hydroxyl groups and ketone bonds in its structure. Que easily captures reactive oxygen species (ROS) and therefore protects the body against oxidative stress [4]. In addition, Que can exert antioxidant effects by chelating cupric or ferrous ions [5].

Numerous studies have shown that Que interacts with DNA by binding covalently to it [6], but it is not exactly known whether it repairs DNA or protects it from oxidative damage.

Que combined with various small molecule drugs has a synergistic inhibitory effect on cancer cells, and can reduce the dose of anticancer agents, improving their efficacy and safety by regulating signaling molecules and blocking the cell cycle [7].

Despite the many advantages, Que can be prone to degradation, especially when exposed to light, heat, or *p*H changes [8]. Additionally, Que has limited solubility in biological fluids, which can hinder its applications [9].

Hybrid materials represent a scientific approach that combines two or more constituent materials to create a new material with properties superior to those of the original components [10, 11]. A straight forward solution researchers are exploring is incorporating Que into polymer matrices to improve solubility and prevent degradation, making it easier to handle and formulate into different products, such as pharmaceuticals, nutraceuticals, and functional foods [12].

**email: maria.ciocilteu@umfcv.ro*

In order to encapsulate Que, we chosed polylactic-co-glycolic acid (PLGA) because is FDA approved and used for pharmaceuticals and biomedical applications due to biocompatibility [13], biodegradability and controlled delivery capability.

We used for encapsulation of Que via the well-established solvent evaporation technique [14] with slight modification of it. Solvent evaporation is a cost-effective technique for producing microparticles with desired characteristics that can be easily scaled up to industrial-scale without major changes [15].

The method developed by us for the formulation of the hybrid material is slightly modified compared to other methods [16, 17], using a small volum of a single organic solvent (in order to reduce environmental impact and lower operating costs). As an emulsifier an aqueous solution with reduced concentration of polyvinyl alcohol (PVA) 0.5% w:v) was used. The method modification took into account our experience and and other research in the field in formulating composite materials with PLGA by various methods [18, 19], which showed that a higher concentration of PVA as emulsifying agent affects particle size and has a negative effect on the encapsulation efficiency [20].

2. Materials and methods

2.1. Materials

Quercetin (Que, p>99%), $AlCl_3$, poly(lactic-co-glycolic acid) (PLGA , 65:35) M_w-40,000–75,000, were purchased from Sigma; Solvents (LiChroSolv water, acetone) and Poly(vinyl alcohol) (PVA), molecular weight 30000-700000 from Merck, Germany; buffer borate 1M form Sigma. PBS (*p*H=7.4) was achieved from Invitrogen (Fisher Scientific Baltics UAB).

2.2. Methods

Synthesis

Firstly, we dissolved 100 mg PLGA in 10 mL acetone. Then, we added 50 mg Que. This mixture was vortexed at 45000 rpm in a Vortex SilentCrush for 5 min in order to obtain the primary solution (A1) (Figure 1). A1 was then added in drops into a larger aqueous solution (A2) containing PVA 0.5% (50 mL). This results in the formation of small droplets of the core solution dispersed within the aqueous phase. The mixture was stirred (200 rpm) for 5 h at 40°C to evaporate the acetone. This process leads to the precipitation of PLGA around the active substance, forming particles.

After the solvent has been evaporated, we centrifuged and triple washed the resulting particles, and then we lyophilized them in an Alpha 1-2 LSC basic freeze dryer at -55°C overnight and then at 0.02 mbar for 48 h.

In Figure 1 the PLGA-Que hybrid material synthesis process by the solvent evaporation method is shown.

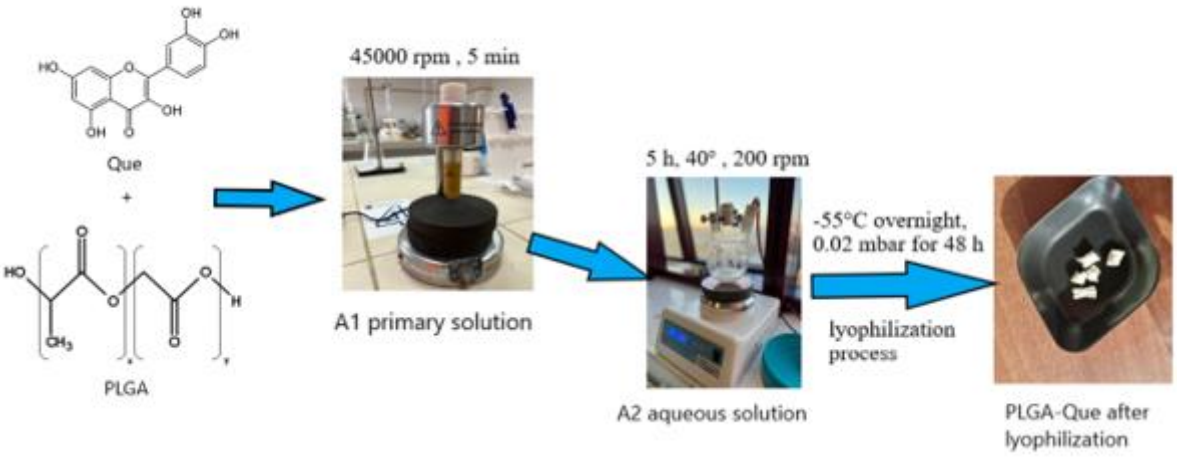


Figure 1. PLGA-Que hybrid material synthesis process by the solvent evaporation method

The morphological analysis was achieved using a scanning electron microscope, FEI Inspect F50 (Hillsboro, Oregon) at an energy of 30 KeV and a resolution of 1.2 nm.

Particle size distribution (DLS) was measured using a Brookhaven 90 Plus apparatus (Brookhaven Instruments Corp., Austin, Texas, USA) with a laser, at 35 mW power and 660 nm.

Quercetin loading efficiency

Quercetin was determined by aluminum chloride colorimetric assay [21, 22].Que stock solution was prepared by dissolving 10 mg quercetin in 1.0 mL methanol. Five solutions with concentrations in the range 12.5 -200 μg/mL were prepared. 0.5 mL from each solution was mixt with 0.5 mL of 2% $AlCl_3$ and left for 1 h at room teperature.

The absorbance was measured at 420 nm with an UV-Vis spectrophotometer (DLAB SP-UV1000, 200 - 1000 nm). The calibration curve y = 0.0028x + 0.0229, R^2 = 0.9981) was used to calculate Que loading efficiency.

150 mg PLGA-Que were dissolved in 5 mL buffer borate (*p*H 8.5 ± 0.2). 0.5 mL solution was mixt with 0.5 mL of 2% $AlCl_3$ and after 1 h, the absorbance was measured. Que loading efficiency was calculated with the formula:

$$\text{Que loading efficiency } (\%) = \frac{\text{Que (mg)}}{\text{PLGA} - \text{Que (mg)}} \text{x} 100$$

where:

Que - experimental mass determined by UV-VIS;

PLGA-Que - mass of synthesized material.

The obtained material showed a quercetin encapsulation percentage of 18.2%.

The obtained material showed a quercetin encapsulation percentage of 18.2%.

Degradation studies

To perform the degradation studies, a disk with the initial weighed mass was placed in screw cap vials with 5 mL mL PBS and then kept at 37°C in an oven. PBS was chosen as a degradation medium because an initial *p*H value close to that of blood (7.35-7.45), slightly alkaline, was desired.

***In vitro* release study**

The temperature was maintained at 37°C ± 0.5°C.

Bottles with screw caps were used to ensure the tightness of the samples and avoid water evaporation.

Initially, 10 mL of ultrapure HPLC water (Li Chrosolv, Merck) were added and then the PLGA-Que discs were weighed and immersed.

At regular time intervals 0.5 mL of solution were taken for the quantitative determination of Que.

$$\text{Cumulative release } (\%) = \frac{\text{Que}_t}{\text{Que}} \text{x} 100$$

where:

Que_t= Amount of Que released at time t;

Que= Total amount of drug initially loaded (or the theoretical maximum drug that can be released, assuming complete release, known from preparation);

3. Results and discussions

3.1. Results

3.1.1. Morphological analysis

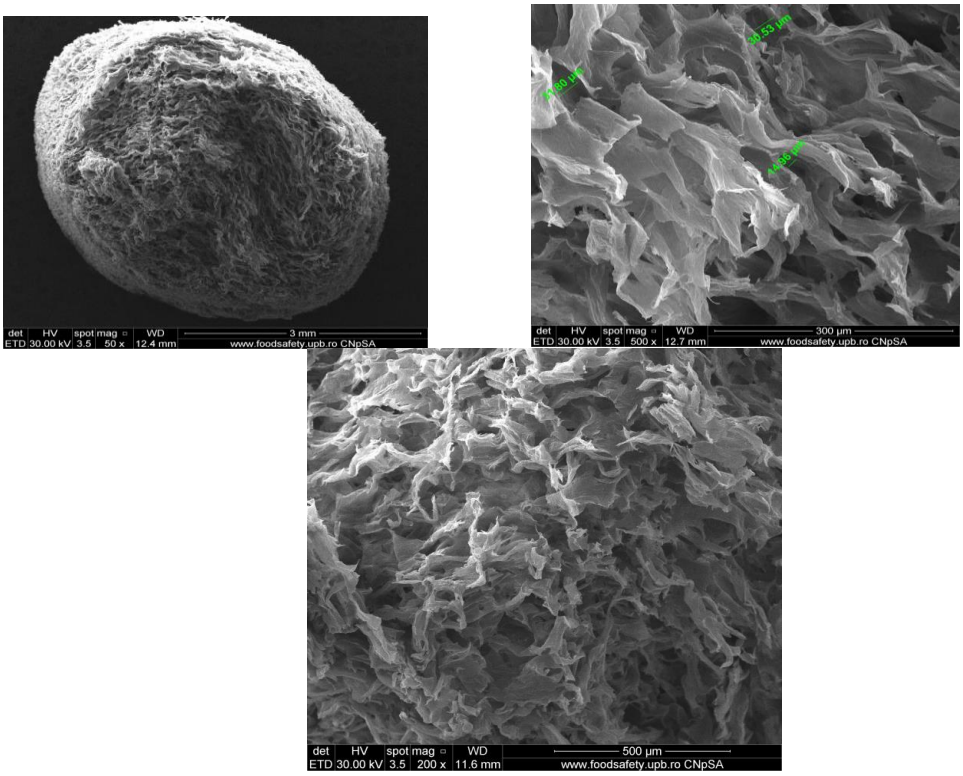


Figure 2. Morphology of PLGA-Que material

As it can be seen in the images taken by SEM, the PLGA-Que material is porous, with large pores of micrometric in size (Figure 2) providing a high surface area and volume for Que loading. The porous material can help stabilize Que, protecting it from degradation caused by different factors such as stomach *p*H [23]. This contributes to the increased shelf life of Que formulations [24].

3.1.2. Particle distribution

In the case of the numerical distribution, there are two granulometric intervals that are very close, namely: the first granulometric interval [385-479] nm structured as follows: 1.11% of the total number of particles have the size of 385 nm, 3.33% of the total number of particles have the size of 429.1 nm, 36.11% of the total number of particles have a size of 478.3 nm; and the second [662-918] nm has the following structure: 0.56% of the total number of particles have the size of 662.5 nm, 2.22% of the total number of particles have the size of 738.5 nm, 55.56% of the total number of particles have size of 832.2 nm, 1.11% of the total number of particles have a size of 917.6 nm (Figure 3, Table 1).

In the case of volume distribution, 3 granulometric intervals can be observed, as follows: the first interval [429-479] nm structured as follows: 0.68% of the total volume of particles have a size of 429.1 nm, 8.78% of the total volume of particles have a size of 478.3 nm; the second interval [738-1023] nm structured as follows: 2.03% of the total volume of particles have the size of 738.5 nm, 67.57% of the total volume of particles have the size of 823.2 nm, 2.03% of the total volume of particles have the size of 917.6 nm, 0.68% of the total volume of particles have a size of 1022.8 nm; and the 3rd interval [3.76-6.47] µm is structured as follows: 1.35% of the total volume of particles have a size of 3.76 µm, 6.76% of the total volume of particles have a size of 4.19 µm, 1.35% of the total volume of particles have the size of 4.67 µm, 4.05% of the total volume of particles have the size of 5.21 µm, 3.38% of the total

volume of particles have the size of 5.81 µm, 1.35% of the total volume of particles have the size of 6.47 µm (Figure 4).

The 3rd interval present in the case of the volume distribution is not present in the case of the numerical distribution, which means that this interval presents an imperceptible number of particles, which most likely agglomerated and thus increased their volume.

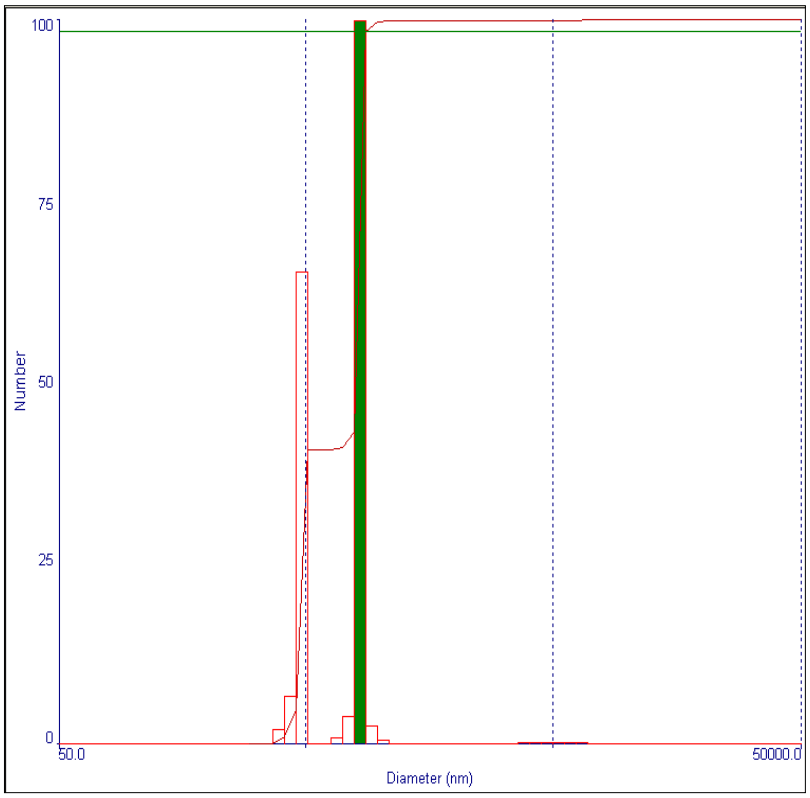


Figure 3. Numerical distribution of PLGA-Que

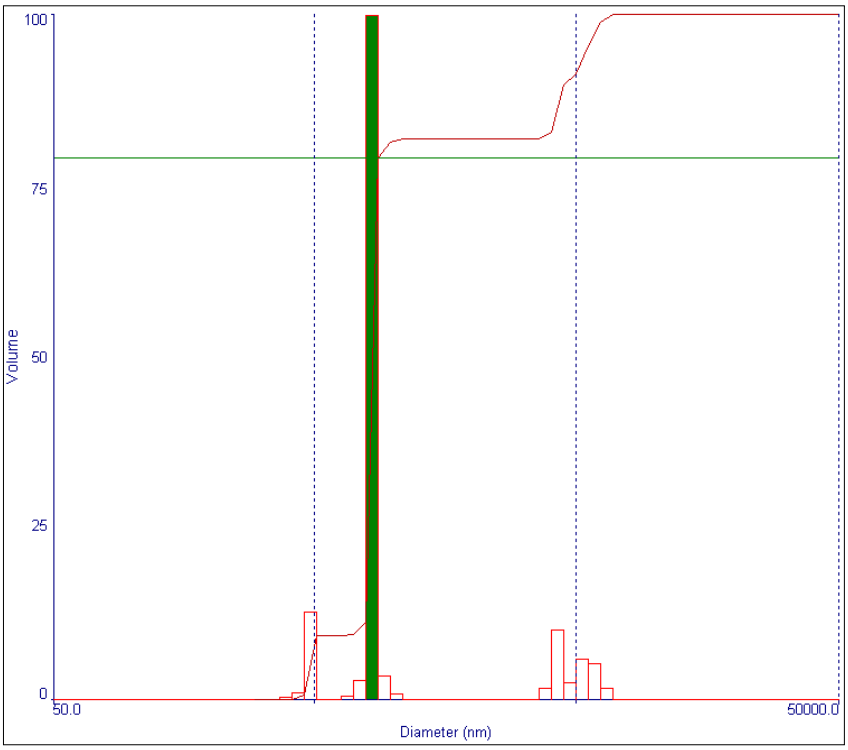


Figure 4. Volume distribution of PLGA-Que

Table 1. Granulometric distribution of PLGA-Que particle

	Numerical distribution		Volume distribution	
d(nm)	G(d)	C(d)	G(d)	C(d)
309.8	0	0	0	0
345.4	0	0	0	0
385	2	1	0	0
429.1	6	5	1	1
478.3	65	41	13	9
533.2	0	41	0	9
594.3	0	41	0	9
662.5	1	41	0	10
738.5	4	43	3	11
823.2	100	98	100	79
917.6	2	100	3	81
1022.8	0	100	1	82
1140.1	0	100	0	82
1270.9	0	100	0	82
1416.6	0	100	0	82
1579.1	0	100	0	82
1760.2	0	100	0	82
1962.1	0	100	0	82
2187.1	0	100	0	82
2437.9	0	100	0	82
2717.5	0	100	0	82
3029.2	0	100	0	82
3376.6	0	100	0	83
3763.8	0	100	2	90
4195.5	0	100	10	91
4676.6	0	100	2	95
5213	0	100	6	99
5810.8	0	100	5	100
6477.3	0	100	2	100
7220.1	0	100	0	100
8048.1	0	100	0	100
8971.1	0	100	0	100
10000	0	100	0	100

3.1.3. Zeta electrokinetic potential

Zeta potential represents the difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particles in a colloidal system. PLGA-Que hybrid system has a negative charge due to the carboxyl groups, that can ionize in the aqueous medium, resulting in the generation of negative charges on the particle surface.

PLGA contains ester bonds, and during its degradation or surface hydrolysis, some of these ester bonds break, exposing carboxyl groups (-COOH) on the surface of the polymer. In an aqueous environment, especially at neutral or slightly basic *p*H, these carboxyl groups can dissociate into negatively charged carboxylate ions ($-COO^-$), contributing to the overall negative surface charge of the PLGA particles. The *p*H of the surrounding medium affect the ionization of surface groups. At a *p*H above the isoelectric point of PLGA (usually around 3), the carboxyl groups become deprotonated ($-COOH \rightarrow -COO^-$), leading to a negative zeta potential.

Negative zeta potential (below -30 mV) is often associated with stability because it promotes electrostatic repulsion between particles of the same charge, thereby inhibiting aggregation or flocculation [25]. Zeta potential also influences interactions with biological systems, such as cellular uptake and circulation behavior, since negatively charged nanoparticles may have reduced clearance by the reticuloendothelial system.

The zeta potential of PLGA-Que was -4.82±0.45 mV (n = 5, Figure 5). Future studies to formulate the optimal synthesis conditions will follow the lowering of the zeta potential as much as possible, towards negative values that will give the material greater stability.

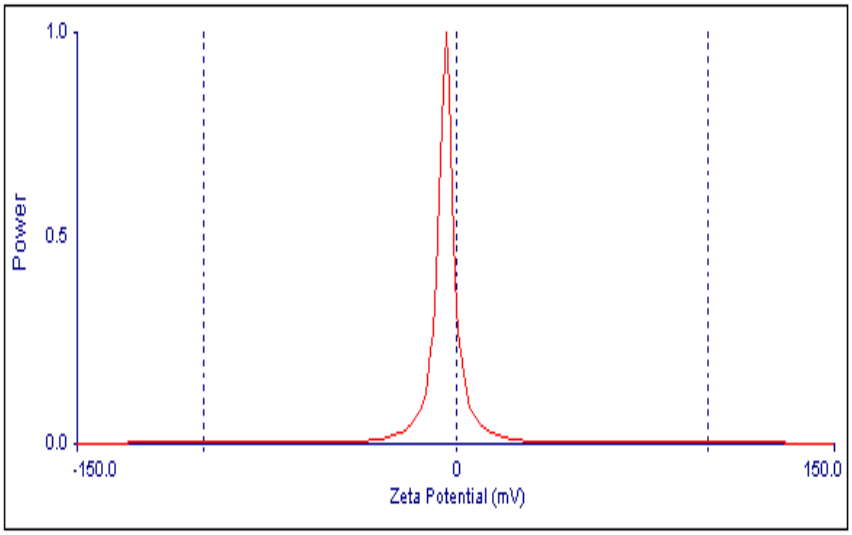


Figure 5. Electrokinetic potential of PLGA-Que

In vitro release studies

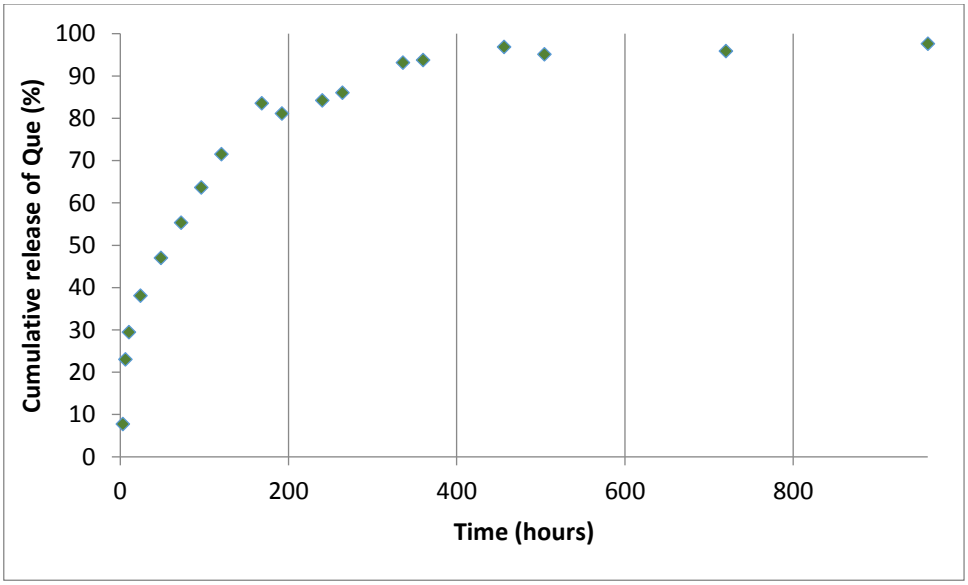


Figure 6. Cumulative release of Que from PLGA-Que in time

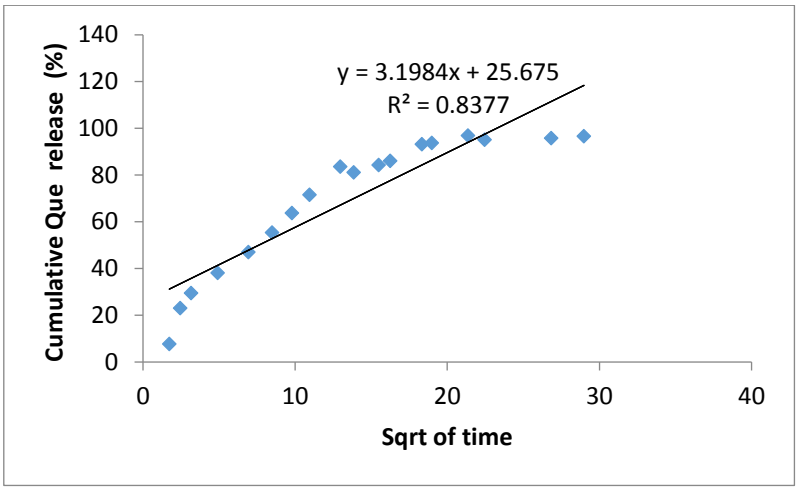
Both our studies and those of other authors have shown that the processes contributing to the degradation of PLGA are diffusion and erosion [26].

The release profile revealed a sudden release in the first 3 days (55.37% of Que), followed by a sustained release for a long time (at 35 days 97.61% Que was released from PLGA-Que (Figure 6).

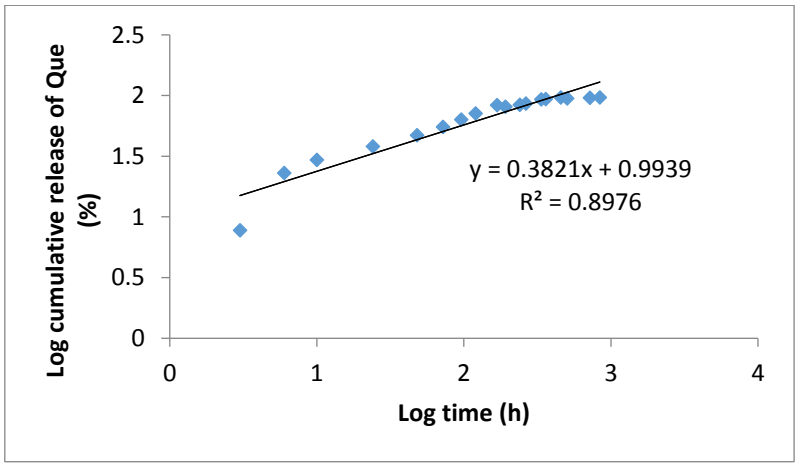
The high percentage obtained in the first 10 days (84.22%)can be explaind by the release of QUE from the polymer surface and its diffusion from the external layer more easily tooutside. The slow controlled release in the range of 10-35 days can be explained by the diffusion process, the drug incorporated inside the polymer diffuses over a greater distance compared to the distance at which diffusion occurs in the first 10 days.

We applied the Higuchi and Korsmeyer-Peppas models to our drug release studies to analyze and describe different mechanisms of Que release from PLGA-Que.

The Higuchi model suggests that the Que release from our formulation is diffusion-controlled (Figure 7A). This is typical for PLGA-based drug delivery systems, where the drug diffuses through the matrix as it degrades. We obtained a good fit also for the Korsmeyer-Peppas model (Figure 7B) suggesting that the Que release mechanism is not purely diffusion-controlled and may involve more complex processes (polymer degradation, swelling). The calculated release exponent (n) from the Korsmeyer-Peppas model is approximately 0.382, less than 0.5. This suggests that the release mechanism follows Fickian diffusion, meaning that the drug release is primarily controlled by diffusion through the polymer matrix.



A



B

Figure 7. A) Higuchi model and B) Korsmeyer-Peppas model for the mechanism of Que release

3.1.4. Degradation study

The degradation rate of PLGA is controlled by choosing an appropriate lactic acid:glycolic acid ratio. The PLGA purchased for our studies has an LA: GA ratio of 65:35, with the higher lactic acid content resulting in slower degradation [27]. PLGA adaptability is important for designing drug delivery systems with specific release profiles [28]. Figure 7 shows the decrease in *p*H during the degradation process. The *p*H of the PLGA-Que had a constant decrease during the experiment, similar to the simple sample, therefore the presence of Que does not affect the degradation process. The decrease in *p*H occurs gradually, as PLGA breaks down into its two constituent acids, lactic and glycolic, through initial

hydrolysis. The hydrolysis reaction is autocatalyzed as the monomers are formed by them. After 35 days, the *p*H remained almost constant, considering that the degradation process of PLGA-Que was completed.

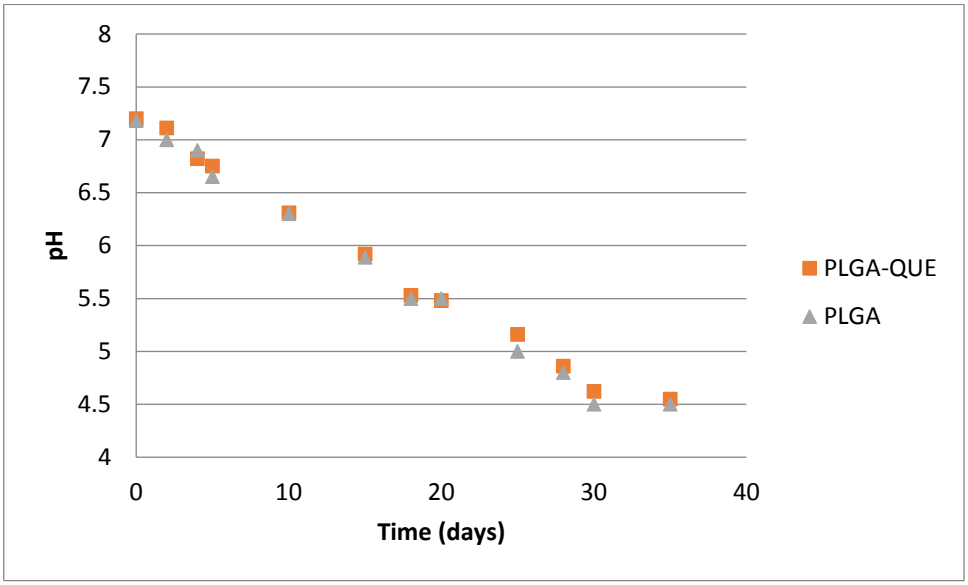


Figure 8. Degradation process of simple PLGA and PLGA-Que

3.2. Discussions

By encapsulating quercetin within PLGA microparticles by the solvent evaporation method, a new hybrid material with improved properties compared to the constituent materials was obtained. Que often exhibit low bioavailability due to factors such as poor solubility, limited absorption, and rapid metabolism [29]. When incorporated into PLGA or other polymers [30], Que is protected from degradation in the gastrointestinal tract, leading to improved absorption and bioavailability in the body.

Also, both quercetin and PLGA are biocompatible and biodegradable materials [31], reducing the risk of adverse reactions in the body. The hybrid material formed maintains these desirable properties, making it suitable for various biomedical applications with minimal risk of adverse reactions or long-term accumulation in the body [32, 33].

There are studies that state polyphenols anti-inflammatory [34] and antioxidant properties can be harnessed in combination with PLGA. This is particularly beneficial in applications targeting inflammation-related diseases or conditions where oxidative stress plays a significant role [35].

The fact that the material obtained by us is porous offers a good control over the release kinetics [36]. The pore size, negative surface charge, and PLGA degradation properties (35 days) allows sustained release over extended periods, leading to improved therapeutic outcomes [37].

PLGA-Que hybrid materials overcome the limitations of quercetin's poor stability and bioavailability and have potential applications in various fields, including drug delivery, wound healing, and regenerative medicine. They can be used to develop novel formulations for the treatment of cancer, cardiovascular diseases, neurodegenerative disorders, and inflammatory conditions.

4. Conclusions

Encapsulating Que in PLGA as hybrid material offers a promising approach to harnessing the beneficial properties of polyphenols for various applications, while addressing challenges such as stability, solubility, and bioavailability. The solvent evaporation method we used is relatively simple, low-cost and can be easily scaled from labs formulations to industrial-scale production. We obtained a porous material with submicron particle size and negative zeta potential that plays a key role in the

particles stability. The PLGA's biodegradable nature allowed sustained release of Que, for approximately 35 days.

References

1. ANAND DAVID, A.V., ARULMOLI, R., PARASURAMAN, S., *Pharmacognosy reviews*, **10**, 84, 2016.
2. GUPTA, V. K., SHARMA, S. K., *Natural Product Radiance*, **5**, 326, 2006.
3. BATIHA, G. E., BESHBISHY, A. M., IKRAM, M., MULLA, Z. S., EL-HACK, M. E. A., TAHA, A. E., ALGAMMAL, A. M., ELEWA, Y. H. A., *Foods*, **9**, 374, 2020.
4. AGHABABAEI, F., HADIDI, M., *Pharmaceuticals*, **16**, 1020, 2023.
5. QI, W., QI, W., XIONG, D., LONG, M., *Molecules*, **27**, 6545, 2022.
6. WANG, Y., *Chinese journal of biotechnology*, **36**, 2877, 2020.
7. AZEEM, M., HANIF, M., MAHMOOD, K., AMEER, N., CHUGHTAI, F. R. S., ABID, U., *Polymer bulletin* (Berlin, Germany), **80**, 241, 2023.
8.HASSANI, S., MAGHSOUDI, H., FATTAHI, F., MALEKINEJAD, F., HAJMALEK, N., SHEIKHNIA, F., KHERADMAND, F., FAHIMIRAD, S., GHORBANPOUR, M., *International journal of biological macromolecules*, **241**,124508, 2023.
9. COSTEA, T., NAGY, P., GANEA, C., SZÖLLŐSI, J., MOCANU, M. M., *International journal of molecular sciences*, **20**, 1062, 2019.
10.MÎNDRILĂ, I., BUTEICĂ, S.A., MIHAIESCU, D.E., BURADA, F., MÎNDRILĂ, B., PREDOI, M.C., PIRICI, I., FUDULU, A., CROITORU, O., *Romanian Journal of Morphology and Embryology*, **58**, 457, 2017.
11.MÎNDRILĂ, B., BUTEICĂ, S.A., MÎNDRILĂ, I., MIHAIESCU, D.E., MĂNESCU, M.D., ROGOVEANU, I., *Biomedicines*, **10**, 1213, 2022.
12. LI, S.J., LIAO, Y.F., DU, Q., *Zhongguo Zhong Yao Za Zhi*, **43**, 1978, 2018.
13.CIOCÎLTEU, M. V., SCOREI, I. R., RĂU, G., NICOLICESCU, C., BIȚĂ, A., ENE, V. L., SIMIONESCU, A., TURCU-ȘTIOLICĂ, A., DINESCU, V. C., NEAMȚU, J., MOGOANTĂ, L., MOGOȘANU, G. D., *Romanian Journal of Morphology and Embryology*, **64**, 567, 2023.
14. YADAV, N., TRIPATHI A.K, PARVEEN A, PARVEEN S, BANERJEE M., *Pharmaceutics*, **14**, 1326, 2022.
15. ERSOZ, M., ERDEMIR, A., DERMAN, S., ARASOGLU T., MANSUROGLU, B., *Pharmaceutical Development and Technology*, **25**, 757, 2020.
16. ESSA, D., KONDIAH, P.D., KUMAR, P., CHOONARA, Y.E., *Biomedicines*, **11**, 1201, 2023.
17. ANWER, M.K, AL-MANSOOR, M.A, JAMIL, S., AL-SHDEFAT, R., ANSARI, M.N, SHAKEEL, F., *International Journal of Biological and Macromolecules*, **92,** 213, 2016.
18. IONESCU F.O., CIOCÎLTEU M.V., MANDA C.V., NEACSU I.A., FICAI A., AMZOIU E., TURCU STIOLICA A., CROITORU O., NEAMTU J., *Mater. Plast.*, **56**(3), 2019, 534-537
19. POSTELNICU, R.A., CIOCÎLTEU, M.V., NEACȘU, I.A., NICOLICESCU, C., COSTACHI, A., AMZOIU, M., NEAMȚU, J., PISOSCHI, C.G., MOCANU, A.G., RĂU, G., AMZOIU, E., *Farmacia*, **71**, 83, 2023.
20.POENARIU A.B., POSTELNICU R.A., CIOCÎLTEU M.V., RĂU G., POPESCU M., SIMIONESCU A., NEACȘU I., NICOLICESCU C., AMZOIU D.C., *Mater. Plast.*, **60**(4), 2023, 79-86
21. TEFAS, L.R., TOMUȚĂ, I., ACHIM, M., VLASE, L., *Clujul Medical*, **88**, 214, 2015.
22. TRISTANTINI, D., AMALIA R., *AIP Conference Proceedings*, **2193,** 030012, 2019.
23. ARYAL, S., BANIYA, M.K., DANEKHU, K., KUNWAR, P., GURUNG, R., KOIRALA, N., *Nepal Plants*, **8**, 96, 2019.
24. MUNIN, A., EDWARDS-LÉVY, F., *Pharmaceutics*, **3**, 793, 2011
25. HUA, Y., SU, Y., ZHANG, H., LIU, N., WANG, Z., GAO, X., GAO, J., ZHENG, A., *Drug Delivery*, **28**,1342, 2021.

26. VOGEL, R., PAL, A. K., JAMBHRUNKAR, S., PATEL, P., THAKUR, S.S., REÁTEGUI, E., PAREKH, H.S., SAÁ, P., TASSINOPOULOS, A., BROOM, M.F., *Scientific Reports*, **7**, 17479, 2017.
27. RAHMAN, H.S., OTHMAN, H.H., HAMMADI, N. I., YEAP, S.K., AMIN, K.M., SAMAD, N.A., ALITHEEN, N.B., *International journal of nanomedicine*, **15**, 2439, 2020.
28. KIM, J.M., SEO, K.S., JEONG, Y.K., HAI, B.L., KIM, Y.S., KHANG, G., *Journal of Biomaterials Science, Polymer Edition*, **16,** 991, 2005.
29. TOMOU, E.M., PAPAKYRIAKOPOULOU, P., SAITANI, E.M., VALSAMI, G., PIPPA, N., SKALTSA, H., *Pharmaceutics*, **15**, 1656, 2023.
30. MIAO, Y., CUI, H., DONG, Z., OUYANG, Y., LI, Y., HUANG, Q., WANG, Z., *ACS Omega*, **6**, 29254, 2021.
31.BUNLUNG, S., NUALNOI, T., ISSARACHOT, O., WIWATTANAPATAPEE, R., *Saudi pharmaceutical journal*, **29**, 1143, 2021.
32.SU, Y., ZHANG, B., SUN, R., LIU, W., ZHU, Q., ZHANG, X., WANG, R., CHEN, C., *Drug Delivery*, **28**, 1397, 2021.
33. ALOTAIBI, B., EL-MASRY, T.A., ELEKHNAWY, E., EL-KADEM, A.H., SALEH, A., NEGM, W.A., ABDELKADER, D.H., *Drug Delivery*, **29**, 1848, 2022.
34. HUSSAIN, T., TAN, B., YIN, Y., BLACHIER, F., TOSSOU, M. C., RAHU, N., *Oxidative medicine and cellular longevity*, **2016**, 7432797, 2016.
35. DANG, T.T., THAI, A. V., COHEN, J., SLOSBERG, J. E., SINIAKOWICZ, K., DOLOFF, J.C., MA, M., HOLLISTER-LOCK, J., TANG, K.M., GU, Z., CHENG, H., WEIR, G.C., LANGER, R., ANDERSON, D.G., *Biomaterials*, **34**, 5792, 2013.
36. YANG, B., DONG, Y., WANG, F., ZHANG, Y., *Molecules*, **25**, 4613, 2020.
37. PARK T. G., *Biomaterials*, **16**, 1123, 1995.

Manuscript received: 02.04.2024