https://doi.org/10.31925/farmacia.2025.2.8 **ORIGINAL ARTICLE**

FORMULATION AND CHARACTERISATION OF PLGA-DOXORUBICIN COMPOSITES CONJUGATED WITH BISPHOSPHONATES IN ONCOLOGICAL THERAPY

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Abstract

The study investigates the characteristics of PLGA-based particles encapsulating doxorubicin (Doxo) and functionalised with alendronate (Aln) for controlled drug delivery. Zeta potential measurements indicated moderate colloidal stability of both PLGA-Doxo and PLGA-Doxo-Aln particles, with values of -7 mV and -10 mV, respectively. This negative charge suggests weak electrostatic repulsion between particles, potentially promoting aggregation in complex biological environments. Numerical and volumetric distributions showed that most particles were in the 550 - 822 nm size range, with a predominant particle size of 817.2 nm for PLGA-Doxo-Aln. The morphology of the particles revealed a porous structure, which could influence the gradual release of Doxo. Antibacterial activity tests showed significant inhibition of *S. aureus* by PLGA-Doxo, while PLGA-Doxo-Aln showed no activity against *E. coli* due to functionalization with Aln. Release studies indicated a controlled release of both Aln and Doxo, with gradual degradation of the PLGA matrix contributing to prolonged drug release. The results suggest that PLGA-Doxo-Aln particles could serve as a promising system for targeted drug delivery, particularly for bone-related cancers.

Rezumat

Studiul investighează caracteristicile particulelor pe bază de PLGA care încorporează doxorubicină (Doxo) și sunt funcționalizate cu alendronat (Aln) pentru cedarea controlată a substanțelor medicamentoase. Măsurătorile potențialului Zeta au indicat o stabilitate coloidală moderată a particulelor PLGA-Doxo și PLGA-Doxo-Aln, cu valori de -7 mV și -10 mV, respectiv. Această sarcină negativă sugerează o repulsie electrostatică slabă între particule, ceea ce ar putea favoriza agregarea acestora în medii biologice complexe. Distribuțiile numerice și volumetrice au arătat că majoritatea particulelor se încadrează în intervalul de dimensiuni 550 - 822 nm, cu o dimensiune predominantă a particulelor de 817,2 nm pentru PLGA-Doxo-Aln. Morfologia particulelor a relevat o structură poroasă, care ar putea influența eliberarea treptată a Doxo. Testele de activitate antibacteriană au arătat o inhibare semnificativă a *S. aureus* de către PLGA-Doxo, în timp ce PLGA-Doxo-Aln nu a prezentat activitate împotriva *E. coli* din cauza funcționalizării cu Aln. Studiile de eliberare au indicat o eliberare controlată atât a Aln, cât și a Doxo, cu degradarea treptată a matricei PLGA contribuind la eliberarea prelungită a medicamentului. Rezultatele sugerează că particulele PLGA-Doxo-Aln ar putea reprezenta un sistem promițător pentru livrarea țintită a medicamentelor, în special pentru cancerele osoase.

Keywords: PLGA, doxorubicin, alendronate, drug delivery, controlled release

Introduction

To enhance the therapeutic performance of cytostatics, researchers have explored various strategies for functionalising PLGA nanoparticles, either with specific ligands [1], through the simultaneous delivery of two or more drugs, or by using stimulus-sensitive release systems [2]. These particles can be designed to release the drug only in the presence of specific factors, such as the low pH of the tumour micro-environment or enzymes specific to cancer cells.

Drug stability can be increased through structural modifications, such as adding hydrophilic polymers or magnetic nanoparticles, and its transport to target tissues can be improved [3]. Several drugs used as cytostatic agents in combination with PLGA have been studied (*e.g.*, cisplatin [4, 5], curcumin [6]) to enhance antitumour activity, improve drug stability against blood components, and extend circulation time in the bloodstream.

In current therapeutic practice, the use of doxorubicin is optimised through various approaches aimed at improving treatment efficacy and reducing associated toxicity. Besides liposomal formulations and the use of dexrazoxane for cardioprotection, recent research focuses on new drug delivery systems and combination

therapies that can enhance doxorubicin's selectivity and minimise adverse effects [7].

On the other hand, bisphosphonates represent an essential therapeutic class in managing conditions characterised by excessive bone resorption, with extensive applications in oncology, rheumatology, and endocrinology. Their use has evolved significantly in recent decades, integrating standardised protocols and innovations in administration and molecular targeting. These findings support the use of the bisphosphonate-functionalised nanoparticles in therapies targeting bone and oncological diseases, providing a promising direction for developing more effective and better-targeted treatments.

Beyond the advantages of using PLGA as a transport vector, its functionalization with polydopamine and bisphosphonates opens new perspectives for more efficient targeted therapy, especially for patients diagnosed with bone metastases [8, 9]. Bisphosphonates are known for their ability to selectively bind to bone tissue and inhibit osteoclast activity, making them suitable for the selective targeting of cytostatics in oncological diseases with bone localisation. Thus, combining doxorubicin with PLGA and bisphosphonates may represent an innovative solution for increasing therapeutic efficacy and reducing side effects.

Two new materials based on PLGA, doxorubicin (Doxo), and alendronate (Aln) were formulated and characterised as advanced controlled drug delivery systems for treating severe conditions such as bone cancer. Incorporating doxorubicin, a potent chemotherapeutic agent, into the PLGA matrix enabled its controlled release, reducing systemic adverse effects and improving therapeutic efficiency [10]. Adding a bisphosphonate used for treating osteoporosis and bone metastases provides an additional benefit by allowing specific targeting of the composite to bone tissue and enhancing the anticancer effect through therapeutic synergy [11].

Materials and Methods

Chemicals and reagents

Doxorubicin and alendronate (purity > 99%) were purchased from Sigma. Solvents (LiChroSolv water, acetonitrile, formic acid, dichloromethane, and all chromatographic purity) were obtained from Merck, Germany. PLGA (65:35) with a molecular weight of 40,000 - 75,000 was sourced from Sigma, while PVA (molecular weight 30,000 - 700,000) was acquired from Merck, Germany.

Synthesis

The synthesis of the PLGA-Doxo (PLGA-doxorubicin) composite was carried out using the double emulsion $W_1/O/W_2$ method. The aqueous phase W_1 was prepared by dissolving 5 mg of doxorubicin in 5 mL of an aqueous solution containing 0.5% polyvinyl alcohol (PVA). The oil phase was obtained by dissolving 100 mg of PLGA in dichloromethane. These phases were mixed at 4500 rpm using a Heidolph Silent Crusher vortex, forming the primary emulsion W_1/O. The aqueous phase W_2 consisted of a 95 mL aqueous solution containing 0.5% polyvinyl alcohol as an emulsifier (w/w). The primary emulsion was added to the secondary aqueous phase and stirred at 900 rpm for 3 hours to evaporate DCM. The final suspension underwent a lyophilisation process. The primary emulsion W_1/O for the PLGA-doxorubicin composite was mixed using a Heidolph Silent Crusher vortex (Figure 1).

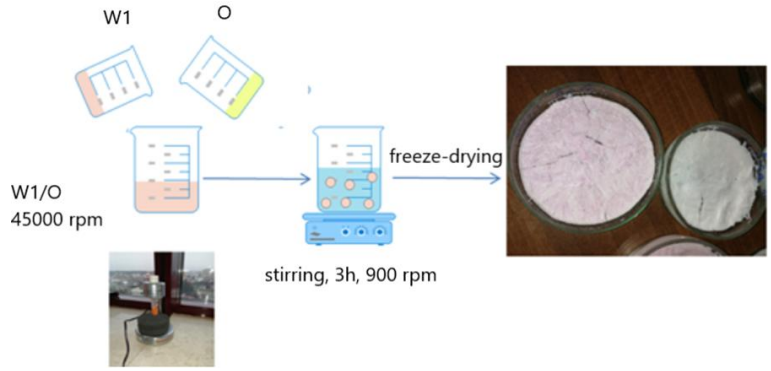


Figure 1.
Synthesis of the PLGA-Doxo composite material

Surface functionalization of PLGA and PLGA-Doxo with polydopamine and conjugation with Aln

The obtained material (PLGA-Doxo) was added to 1 mL of an aqueous solution at pH 8.5 (0.01 M Tris buffer) containing 2 mg of dopamine and stirred for 3 hours at room temperature to coat the PLGA-doxorubicin surface with polydopamine (PDA). The particles turned black due to polydopamine oxidation, after which alendronate was conjugated onto the nanoparticles through incubation as follows: 20 mg of alendronate dissolved in 50 mL of water, and 100 mg of PLGA particles coated with PDA were added under continuous stirring for 5 hours. The final nanoparticle suspension, conjugated with polydopamine

and alendronate, was centrifuged at 1700 rpm and washed (Figure 2).

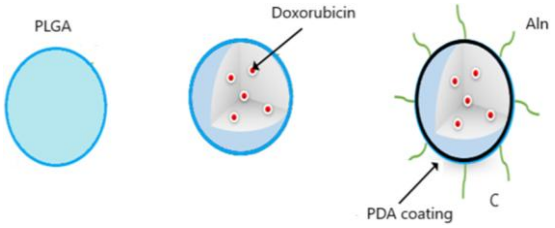


Figure 2.
Surface functionalization of PLGA-Doxo with Aln

Morphological characterization

SEM images were acquired using a high-resolution scanning electron microscope (FEI Inspect F50) at 30 kV and various focus settings.

Numerical and volumetric distribution determination by DLS

The analysis used a Zeta Potential Analyzer, precisely the Brookhaven 90 PLUS/BI-MAS Multi-Angle Particle Sizing option.

Determination of doxorubicin encapsulation efficiency in PLGA

The HPLC instrument used for separation and detection was a Vanquish Core LC system with a manual injector, coupled with a diode array detector (DAD) from Thermo Fisher Scientific (USA). A chromatographic Acclaim™ column (4.6 mm inner diameter × 100 mm length, 5.0 µm particle size) was used for separation.

An isocratic elution was applied, involving two solvents: solvent A (water with 0.1% formic acid v/v) and solvent B (acetonitrile), in a 45:55 (v/v) ratio. The mobile phase flow rate was set at 1 mL *per* min. The column temperature in the dedicated compartment was maintained at 40°C. The injection loop volume was 20 µL, and injections were performed at room temperature.

For quantification, the analyte peak areas were integrated at a wavelength of 233 nm. The UV spectrum was also recorded for both compounds within the 190 - 800 nm range. Instrument parameter control, data acquisition, and result processing were performed using the Chromeleon 7 Chromatography Data System software.

The determination of Doxo incorporation efficiency was calculated using the formula:

$$\text{Encapsulation Efficiency (\%)} = \frac{m_{Doxo}}{m_{Doxo\ total}} \times 100.$$

Determination of the percentage of alendronate bound to PLGA

HPLC analysis was performed using a Thermo Finnigan Surveyor System equipped with a DAD detector and a Thermo Finnigan Xcalibur data acquisition system. Compound separation was achieved using a reversed-phase C18 column (Thermo Scientific Hypersil GOLD, 250 mm × 4.6 mm I.D., 5 µm).

The mobile phase consisted of an isocratic mixture of acetonitrile (65%) and 25 mM sodium citrate solution (35%), with a constant flow rate of 1 mL *per* min. All experiments were conducted at room temperature.

For sample preparation, 5 mg of PLGA-Aln/PLGA-Doxo-Aln were weighed, and 0.5 mL of ultrapure water was added. To facilitate compound hydrolysis, five drops of concentrated HCl were added, and the sample was incubated at 37 - 60°C for 7 days to ensure complete PLGA hydrolysis. After hydrolysis, the solution was neutralised with NaOH, centrifuged at 7000 rpm for 10 minutes, and the supernatant was collected for encapsulated compound analysis.

In the supernatant, 0.5 mL of FMOC (2 mg/mL) and 1 mL of sodium citrate were added, and the sample was left to react for 10 minutes. The final volume was adjusted to 5 mL with ultrapure water, after which 20 µL of the obtained solution was injected into the HPLC system.

To confirm the experimental data, pure alendronate was subjected to the same protocol to identify its specific chromatographic peak and corresponding maximum absorption wavelength.

The percentage of Aln in the composite material was calculated using the formula:

$$\text{Aln (\%)} = \frac{m_{AL}}{m_{Aln\ total}} \times 100.$$

Antibacterial analysis

Using the agar diffusion method, the antimicrobial activity of the composite materials was evaluated against standard bacterial strains (*Staphylococcus aureus* ATCC®25923 and *Escherichia coli*). Inocula with a turbidity of 0.5 on the McFarland scale, obtained from the previously mentioned standardised strains, were applied to a Muller-Hinton agar medium within 15 minutes of preparation. Paper discs for antimicrobial susceptibility testing, with a diameter of 6 mm (Thermo Scientific, UK), were impregnated with the tested samples (abbreviated as 1 - reference doxorubicin, 2 - PLGA-Doxo, 3 - PLGA-Doxo-Aln). These samples were carefully handled using sterilised tweezers and then placed onto the surface of the Muller-Hinton agar plates. The Petri dishes were then incubated at 37°C for 24 hours. The antimicrobial activity of each sample was determined by measuring the total diameter of the bacterial and fungal growth inhibition zones, including the surface of the disc. The diameter of the inhibition zones was expressed in millimetres (mm).

In vitro Release Studies

The dissolution studies of Aln and Doxo from PLGA compositions were performed at a constant temperature of 37 ± 0.5°C using an incubator, with sealed flasks to minimise the evaporation of the dissolution medium. Initially, 10 mL of PBS was added to each flask, and then samples weighing 100 mg of PLGA-Aln and PLGA-Doxo were introduced.

At predetermined time intervals (3, 6, 10, 24, 48, 72, 96, 240, 408, 280 hours), 500 µL of the sample was withdrawn and analysed by HPLC to determine the amount of Aln and Doxo released. The withdrawn volume was replaced with 500 µL of PBS to avoid supersaturation of the solution with the drug. Dilution was considered in the calculations.

Statistical analysis

All experiments were conducted in triplicate for all samples (except for Zeta potential determinations, where n = 5), along with calibration curves and their corresponding concentrations. Statistical analysis was performed using the Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, WA, USA) and expressed as mean ± SD. Graphical figures were created using ConceptDraw Diagram software.

Results and Discussion

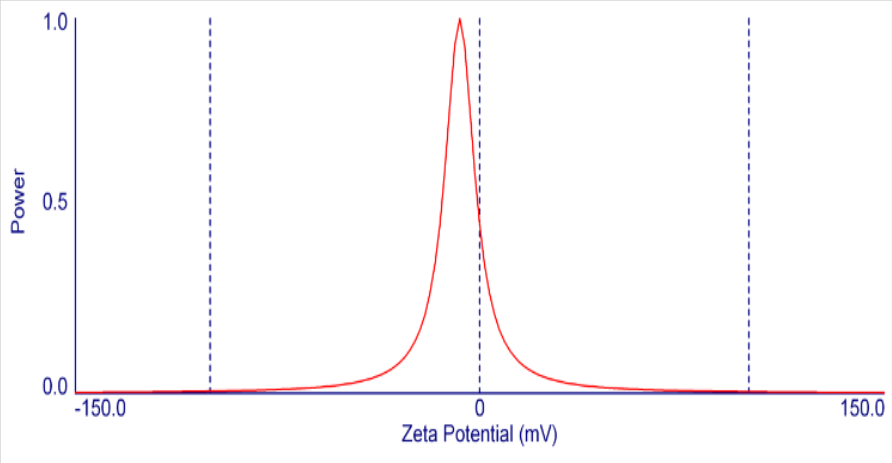
Zeta Potential Determination

The micrometric particles with negative zeta potential, with values of -7 mV (PLGA-Doxo, Figure 3) and -10 mV (PLGA-Doxo-Aln, Figure 4), exhibit moderate suspended colloidal stability. This zeta potential range indicates a weak electrostatic repulsive force between particles, which may promote a tendency for aggregation in complex environments such as biological fluids. As the Figure 1 and Figure 2 showed, encapsulating Doxo and functionalising the surface with Aln lowers the zeta potential to smaller values. A negative zeta potential gives particles a reduced affinity for cell membranes, as most cells also carry a negative charge, which decreases nonspecific interactions. However, these particles can be functionalised to increase specificity toward certain cells or receptors.

In controlled drug delivery applications, this slightly negative potential can contribute to a slower and more controlled release of the active substance, preventing premature binding to cellular structures or plasma proteins. Furthermore, particles with this type of charge are less likely to be rapidly captured by the reticulo-endothelial system, thus increasing their circulation time in the body.

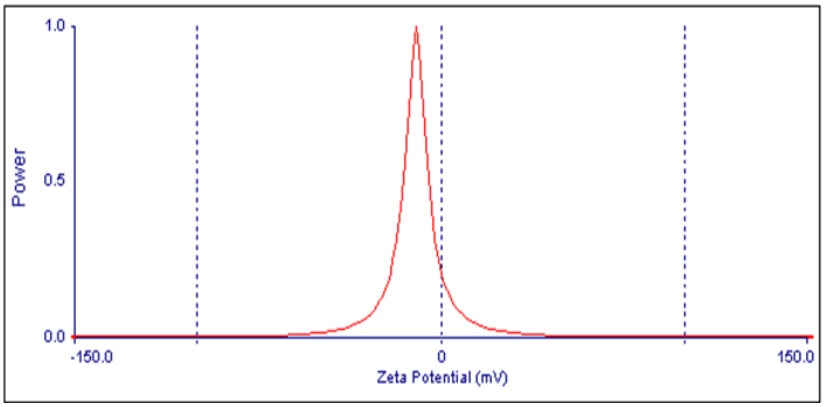
Numerical distribution

In the case of the numerical distribution of PLGA-Doxo particles, particles smaller than 400 nm have a very low or zero frequency (G(d) = 0) and contribute up to 14% of the cumulative distribution (C(d) ≤ 14%). Small particles are few in number and contribute only a minor amount to the total particle volume (Figure 5). The frequency of the particles begins to increase slightly, reaching values of G(d) = 5 - 100 around the size of 817.2 nm. The cumulative distribution reaches up to 98%, indicating that most of the particles are medium sized within this range. Particles larger than 800 nm contribute to the remaining 2% of the cumulative distribution, reaching 100% at a size of 3151.9 nm and above. The frequency of these particles is low (G(d) = 0 - 1), suggesting they are rare but contribute significantly to the total volume (Figure 6).



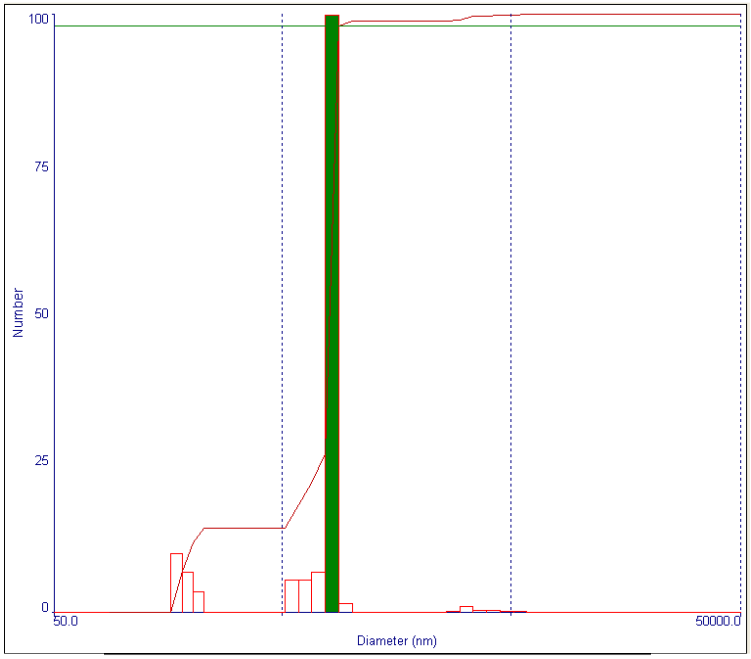
Run	Zeta Potential (mV)	Half Width (mV)
1	-15.30	10.70
2	-3.49	3.90
3	-6.03	3.26
4	-6.44	3.30
5	-7.36	6.66
Mean	-7.73	5.56
Std. Error	2.00	1.43

Figure 3.
Zeta Potential of PLGA-Doxo



Run	Zeta Potential (mV)	Half Width (mV)
1	-17.75	3.34
2	-8.49	2.83
3	-4.60	6.39
4	-9.26	6.16
5	-10.19	5.29
Mean	-10.06	4.80
Std. Error	2.15	0.73

Figure 4.
Zeta Potential of Lyophilized PLGA-Doxo-Aln



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
94.3	0	0	416.1	0	14	1836.9	0	99
107.9	0	0	476.3	0	14	2102.3	0	99
136.5	0	0	545.1	5	18	2406.2	0	99
152.8	0	0	623.9	5	22	2753.9	0	99
171.0	10	7	714.1	7	27	3151.9	1	100
191.3	7	12	817.2	100	98	3607.4	0	100
214.1	3	14	935.3	1	99	4128.7	0	100
239.6	0	14	1070.5	0	99	4725.3	0	100
277.6	0	14	1225.2	0	99	5408.2	0	100
317.7	0	14	1402.3	0	99	6189.8	0	100
363.6	0	14	1604.9	0	99	7084.3	0	100

Figure 5.
Numerical distribution of PLGA-Doxo particles, depending on d (nm) – particle size in nanometers, G(d) – frequency of particles of that size, C(d) – cumulative percentage distribution of particles

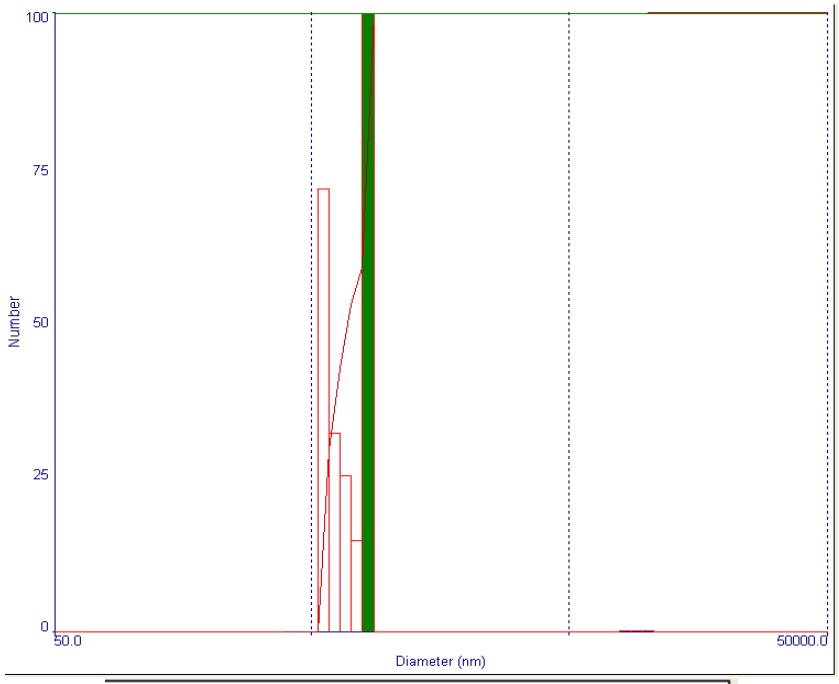
d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
94.3	0	0	416.1	0	0	1836.9	0	52
107.9	0	0	476.3	0	0	2102.3	0	52
136.5	0	0	545.1	2	1	2406.2	0	52
152.8	0	0	623.9	2	2	2753.9	4	54
171.0	0	0	714.1	4	4	3151.9	48	77
191.3	0	0	817.2	100	51	3607.4	15	84
214.1	0	0	935.3	2	52	4128.7	30	97
239.6	0	0	1070.5	0	52	4725.3	5	100
277.6	0	0	1225.2	0	52	5408.2	1	100
317.7	0	0	1402.3	0	52	6189.8	0	100
363.6	0	0	1604.9	0	52	7084.3	0	100

Figure 6.
Volumetric distribution of PLGA-Doxo particles, depending on d (nm) – particle size in nanometers, G(d) – frequency of particles of that size, C(d) – cumulative percentage distribution of particles

In the 94.3 - 363.6 nm range, both the frequency G(d) and the cumulative distribution C(d) are 0%. This indicates that very small particles are absent or present in an insignificant percentage of the total volume (Figure 6). Between 416.1 nm and 935.3 nm, the frequency increases slightly, and the cumulative distribution reaches up to 52% at this size. Notable frequency values (G(d)) appear around the size of 817.2 nm, with a maximum value of 100, suggesting that this is the predominant particle size. Particles in this range represent approximately 52% of the total volume, indicating a significant concentration of medium-sized particles. At sizes over 1000 nm, the cumulative distribution increases rapidly from 52% to 100%. The size of 3151.9 nm has a frequency G(d) = 48, and at 3607.4 nm, the cumulative distribution reaches 84%. At sizes larger than 4725.3 nm, the cumulative distribution reaches 100%, suggesting that the remaining particles contribute significantly to the total volume, although rare.

PLGA-Doxo-Aln

In the case of numerical distribution, it is observed that the particles are in the granulometric range 550 - 822 nm with the following structure: 29.51% of the total number of particles are at a size of 550.5 nm, 13.11% of the total number of particles are at a size of 608.4 nm, 10.25% of the total number of particles are at a size of 672.4 nm, 6.15% of the total number of particles are at a size of 743.1 nm, and 40.98% of the total number of particles are at a size of 821.2 nm (Figure 7).



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
407.9	0	0	1225.1	0	100	3679.5	0	100
450.8	0	0	1353.9	0	100	4066.4	0	100
498.2	0	0	1496.2	0	100	4494.0	0	100
550.5	72	29	1653.6	0	100	4966.5	0	100
608.4	32	43	1827.4	0	100	5488.8	0	100
672.4	25	53	2019.6	0	100	6065.9	0	100
743.1	15	59	2231.9	0	100	6703.7	0	100
821.2	100	100	2466.6	0	100	7408.6	0	100
907.6	0	100	2726.0	0	100	8187.6	0	100
1003.0	0	100	3012.6	0	100	9048.5	0	100
1108.5	0	100	3329.4	0	100	10000.0	0	100

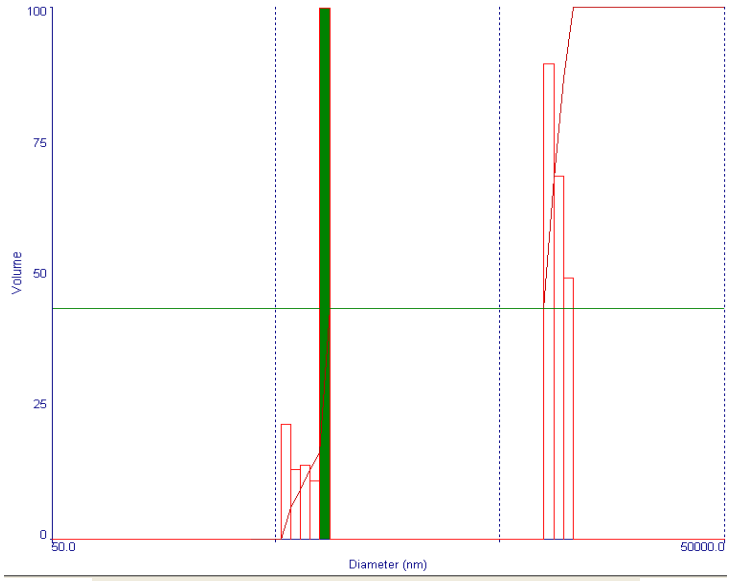
Figure 7.
Numerical distribution of PLGA-Doxo-Aln

The volumetric distribution (Figure 8) presents two granulometric ranges, namely: the first (550 - 822) nm with the following structure: 6.01% of the total particle volume is of size 550.5 nm; 3.55% of the total particle volume is of size 608.4 nm; 3.83% of the total particle volume is of size 672.4 nm; 3.01% of the total particle volume is of size 743.1 nm; 27.32% of the total particle volume is of size 821.2 nm; 24.32% of the total particle volume is of size 8.18 μm; 18.58% of the total particle volume is of size 9.04 μm; 13.39% of the total particle volume is of size 10 μm.

The second granulometric range does not appear in the case of numerical distribution, which means that these particles are present in very small numbers.

Morphological aspects

At a magnification of 100x, the morphology of PLGA-Aln and PLGA-Doxo-Aln particles and their porous structure are visible (Figure 9), highlighting possible characteristics relevant to the drug release profile and interaction with bone tissue. The PLGA particles appear to have a porous and irregular morphology, with numerous thin fibres and network-like structures. This architecture suggests a large specific surface area favourable for interaction with biological fluids. The potential microporous structure influences the release of doxorubicin, and this structural organisation may be advantageous for the gradual release of the drug. Possible modifications of PLGA due to the encapsulation process – the interaction between doxorubicin and the polymer may alter the architecture and porosity of the material, leading to the formation of spherical particle shapes, typical for the synthesis method, with large pores. The presence of alendronate on the material's surface, which has a high affinity for calcium-containing surfaces (such as bone), and how it is distributed, may influence the interaction with the bone matrix. The structures observed in the images suggest a possible diffusion-based release mechanism, where the drug is gradually released through the material's pores.



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
407.9	0	0	1225.1	0	44	3679.5	0	44
450.8	0	0	1353.9	0	44	4066.4	0	44
498.2	0	0	1496.2	0	44	4494.0	0	44
550.5	22	6	1653.6	0	44	4966.5	0	44
608.4	13	9	1827.4	0	44	5488.8	0	44
672.4	14	13	2019.6	0	44	6065.9	0	44
743.1	11	16	2231.9	0	44	6703.7	0	44
821.2	100	44	2466.6	0	44	7408.6	0	44
907.6	0	44	2726.0	0	44	8187.6	89	68
1003.0	0	44	3012.6	0	44	9048.5	68	87
1108.5	0	44	3329.4	0	44	10000.0	49	100

Figure 8.
Volumetric distribution of PLGA-Doxo-Aln

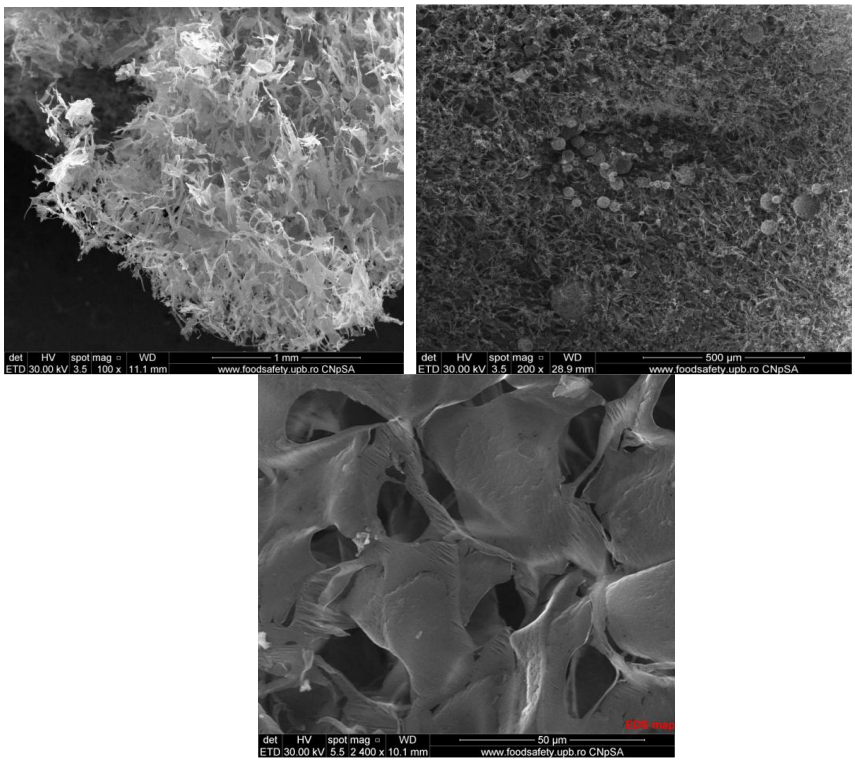


Figure 9.
Morphological aspects of PLGA-Doxo and PLGA-Doxo-Aln at different magnifications

A B

Figure 10.
Antibacterial activity of PLGA-Doxo (2) and PLGA-Doxo-Aln (3) compared to pure Doxo (1)

Antibacterial activity of PLGA-based materials

S. aureus is sensitive to doxorubicin due to its porous cell wall, which allows the antibiotic to penetrate quickly. The mechanism of action involves intercalation into DNA, inhibition of topoisomerase II, and the generation of reactive oxygen species (ROS), leading to cell destruction. Studies show an MIC (Minimum Inhibitory Concentration) for *S. aureus* between 0.5 µg/mL and 4 µg/mL, depending on the strain and experimental conditions [12].

Due to the outer membrane, *E. coli* is less sensitive to doxorubicin than *Gram-positive* bacteria. Transport through the outer membrane is limited, and active efflux through efflux pumps (*e.g.*, AcrAB-TolC) reduces the intracellular concentration of doxorubicin. The MIC for *E. coli* is higher than for *S. aureus*, around 8 - 16 µg/mL, indicating lower sensitivity [13]. Encapsulation of doxorubicin in PLGA results in a gradual release of the drug, reducing its immediate active concentration in the bacterial culture medium (21.9 mm pure doxorubicin compared to 56.5 mm PLGA-doxo, Figure 10A). Unlike the pure form, which acts rapidly by directly intercalating into bacterial DNA, encapsulated doxorubicin needs longer to diffuse through the bacterial cell wall. PLGA protects doxorubicin and partially isolates it from the surrounding environment. Thus, the drug is less likely to interact with bacterial cells. In the case of PLGA-doxo-Aln, no anti-*E. coli* activity was observed, most likely due to functionalisation with polydopamine and subsequent conjugation with Aln.

Gram-negative bacteria, such as *Escherichia coli*, have an impermeable outer membrane that hinders the penetration of PLGA nanoparticles, thereby reducing antibacterial effectiveness (pure doxorubicin 10.18 mm, Figure 10B). PLGA can interact with various biological components in the culture medium, which may decrease the available concentration of active doxorubicin. Neither PLGA-doxo nor PLGA-Doxo-Aln exhibited any anti-*E. coli* activity.

In vitro release studies

The data in Figure 11 shows the release profile of alendronate (blue) from the polydopamine-functionalized PLGA surface and the release profile of doxorubicin encapsulated in PLGA (red) over time.

In the first 3 hours, the release of alendronate is minimal (0.10089 mg/mL), suggesting strong adsorption to the functionalised surface of PLGA. Between 6 and 10 hours, there is a significant increase in the released concentration, which may indicate an initial phase of rapid release of superficially adsorbed alendronate. After 48 hours, the release of alendronate stabilises around 1.65 - 1.67 mg/mL, suggesting a controlled release phase, likely through diffusion from the PLGA matrix. At 10 days, the value slightly increases to 1.70 mg/mL, and at 21 days it reaches 1.97 mg/mL, indicating that PLGA gradually degrades, allowing the progressive release of alendronate. At 30 days, no value is reported because alendronate has limited solubility in aqueous media. As doxorubicin is not released in the first 3 hours, suggesting effective encapsulation in the PLGA matrix. After 6 hours, a minimal amount begins to be released, only 0.72 mg/mL, and after 10 hours, it reaches 1.06 mg/mL, indicating a more controlled release profile compared to alendronate. Between 48 and 96 hours, the release gradually increases (1.17 - 1.48 mg/mL), confirming that the release mechanism is controlled, likely by diffusion and the gradual degradation of PLGA. After 10 days, the concentration of doxorubicin reaches 1.89 mg/mL, then rises to 2.24 mg/mL at 17 days and 2.26 mg/mL at 21 days. After 30 days, the concentration is 2.11 mg/mL, suggesting that the release slows down, possibly due to the gradual depletion of the encapsulated drug.

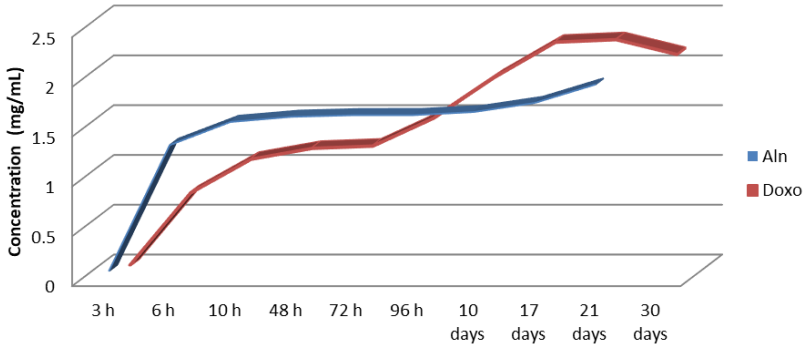


Figure 11.
Release of Aln and Doxo from PLGA

The synthesis was carried out using a copolymer chosen for its remarkable properties, such as biodegradability, non-toxicity and biocompatibility, and it is FDA-approved. This copolymer is PLGA (poly-(lactic-co-glycolic) acid), known for its ability to incorporate various drugs into the structure of nanoparticles [14, 15]. In this study, we chose doxorubicin due to its water solubility, which is essential for the synthesis method used – double emulsion [16, 17].

Surface functionalization of PLGA with polydopamine enabled the attachment of bisphosphonates (alendronate, which represents approximately 9% of the composite mass according to HPLC determination), which play a crucial role in the targeted release of the anticancer drug at bone metastases [18, 19]. This strategy helps to reduce adverse reactions and the systemic toxicity of doxorubicin.

In addition, bisphosphonates are recognised for their benefits in treating conditions such as osteosarcoma [20], demonstrating efficacy even in the case of bone metastases. Based on these premises, we proposed a new method of administration – either local or oral – that could significantly reduce the toxic effects of doxorubicin, providing a safer and more efficient alternative for patients.

Incorporating doxorubicin into a PLGA-based delivery system is an area of great interest, as it could facilitate drug administration while simultaneously reducing the adverse effects associated with intravenous administration [21, 22]. Currently, intravenous administration of doxorubicin can cause tissue destruction when the drug comes into contact with tissues due to improper administration, such as when the drug leaks out of the veins [23]. Additionally, there is the risk of vesicle formation, which can complicate treatment.

A significant issue common to cytostatic agents is that, although they target tumour cells, in many situations, they cannot distinguish between tumour cells and healthy cells with a physiologically high rate of division, such as bone marrow cells [24]. This lack of specificity leads to numerous adverse effects, such as leukopenia and other conditions caused by the reduced ability of the body to defend itself. Moreover, doxorubicin is associated with common side effects like hair loss, nausea, and other unpleasant symptoms [25].

The local administration could enhance the drug's effectiveness at the tumour site while reducing systemic circulation, thereby limiting systemic toxicity. Therefore, combining doxorubicin with PLGA opens the possibility of oral administration, which is currently unavailable, and local administration by directly applying the complex to the tumour site. This could reduce toxicity induced by other routes of administration.

Previous studies have shown that passive targeting of doxorubicin encapsulated in PLGA results in higher antitumoral activity, increases the drug's circulation time in the body, and may improve treatment efficacy [26, 27].

The double emulsion method has proven particularly effective for incorporating Doxo, which is highly water-soluble, into hydrophobic polymers like PLGA. In conventional simple emulsion techniques, water-soluble drugs migrate toward the external aqueous phase, reducing encapsulation efficiency [28]. The double emulsion approach overcomes this limitation by creating an initial water-in-oil (W/O) emulsion [29], in which Doxo dissolves in an aqueous phase and is then emulsified in a PLGA solution dissolved in an organic solvent (dichloromethane in our case, ethyl acetate [30], acetone [31], etc.). This primary emulsion is then emulsified in a second aqueous phase containing a stabiliser (polyvinyl alcohol), forming a W/O/W system. The solvent evaporates, leaving solid PLGA particles encapsulating the water-soluble drug behind.

One of the main advantages of the double emulsion technique is the high encapsulation efficiency for water-soluble molecules like doxorubicin, for which we obtained a percentage of 73%. Because the inner aqueous phase prevents the drug from escaping during emulsification, a significant amount of the active substance remains trapped in the PLGA-Doxo particles. This is especially important not only for drug molecules, but also for other biologically active molecules, such as proteins or peptides that require protection from degradation until they reach the target site [32].

Another benefit is the control over particle size. Factors such as homogenisation speed, the ratio between the organic and aqueous phases, and the concentration of surfactants can be adjusted to produce nanoparticles with sizes ranging from a few tens to several hundred nanometres. Particle size control is crucial for drug release because it affects distribution, cellular uptake, and the elimination of nanoparticles from the body [33].

Our synthesis conditions were chosen so that the particles would be micrometric, with sizes over 100 nm or even 400 - 600 nm after surface functionalization, as the goal was to improve retention and tumour accumulation. Tumours have abnormal vascularisation, with permeable blood vessels and a defective lymphatic system, allowing larger particles (over 200 nm) to accumulate at the tumour site [34]. Particles under 100 nm have a higher risk of rapid elimination through processes such as renal filtration (particles smaller than 50 nm can be quickly excreted by the kidneys), removal by the reticuloendothelial system (very small particles can be recognised and removed by macrophages before reaching the target site), and so on [35].

The method also allows for extended drug release, a desirable feature in therapeutic applications. The degradation rate of PLGA can be adjusted by m0,odifying the ratio of lactic acid to glycolic acid, thus providing a prolonged release of the active substance over several days or even weeks. For our syntheses, we used PLGA 75:25, which is known to have an approximate release time of 20 - 30 days [36] and a degradation time of about 40 - 45 days [37]. This controlled release profile reduces the need for frequent administration, thereby improving patient compliance [38]. The formulation and characterisation of composite materials based on the PLGA, doxorubicin and bisphosphonates are essential for developing advanced controlled drug delivery systems intended to treat severe conditions, such as bone cancer [8, 9].

Incorporating doxorubicin, a potent chemotherapy agent, into the PLGA matrix allows for controlled release, reducing systemic side effects and enhancing therapeutic efficacy [10]. Adding a bisphosphonate, used for the treatment of osteoporosis and bone metastases, provides an additional benefit, enabling the targeted delivery of the composite to bone tissue and potentiating the anticancer effect through the therapeutic synergy [11].

Conclusions

Characterising these composite materials is essential for optimising their physicochemical properties, stability and drug release profiles. Morphology, degradation, controlled release, and cytotoxicity studies have been conducted, which are necessary to validate the use of these new composite materials in clinical applications. Therefore, the formulation and analysis of these innovative systems can significantly contribute to improving therapeutic strategies in oncology and orthopaedics.

Conflict of interest

The authors declare no conflict of interest.

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