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# *In situ* Formed Implants, Based on PLGA and Eudragit Blends, for Novel Florfenicol Controlled Release Formulations

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ABSTRACT

Drug controlled release technologies (DCRTs) represent an opportunity for designing new therapies. Main objectives are dose number optimization and secondary effects reduction to improve the level of patient/client acceptance. The present work studies DCRTs based in blended polymeric implants for single dose and long-term therapies of florfenicol (FF), a broad spectrum antibiotic. Polymers used were PLGA and Eudragit E100/S100 types. Eudragit/PLGA and FF/PLGA ratios were the main studied factors in terms of encapsulation efficiencies (EEs) and drug release profiles. In addition, morphological and physicochemical characterization were carried out. EEs were of 50−100% depending on formulation composition, and the FF releasing rate was increased or diminished when E100 or S100 were added, respectively. PLGA hydrolytic cleavage products possibly affect Eudragit solubility and matrix stability. Different mathematical models were used for better understanding and simulating release processes. Implants maintained the antimicrobial activity against *Pseudomonas aeruginosa* up to 12 days on agar plates. The developed DCRTs represents a suitable alternative for florfenicol long-term therapies.



## Introduction

The main objective of the studies on drug controlled release technologies (DCRTs) is the development of systems with the capacity to reach and maintain the active principles (PAs) levels in a therapeutic range, during a specified time and at a particular site of the organism. Between the many possibilities, biodegradable-biocompatible polymers are one of the most interesting due to the versatility without toxicity risk and end-therapy dispositive removal. In addition, polymeric DCRTs can be a platform for controlled drug delivery of many PAs characterized by different physicochemical properties. In this sense, several investigations deal with the determination of synthesis conditions in order to obtain devices with controlled and predictable release rates.[1–3] Furthermore, polymeric DCRTs can be designed with multiple sizes and geometries as particles, beads or implants, allowing the use of different administration pathways like parenteral, oral or by inhalation.[3–5] Depending on the components and synthesis technique selection, the systems can be pre-fabricated (*ex situ*) or prepared in the application site (*in situ*).

In the last years, *in situ* formed polymeric implants (ISFIs) have been widely investigated for many biomedical applications such as tissue repair, scaffolds, cellular encapsulation and drug controlled release.[6] These kinds of DCRTs show important characteristics as easy application, long-term releases, improved patient compliance and site-specific release without disadvantages associated with oral or intravenous administration pathways.[7,8] ISFIs allow injecting or depositing a low viscosity material that solidifies to form a solid or semisolid drug deposit. The formation technique for ISFIs can be principally classified into phase separation, crosslinking and organogel solidification.[8]

Between the phase separation systems, the ISFIs prepared by solvent exchange (ISFIs-SE) are being widely studied by the pharmaceutic industry. The precursors of ISFIs-SE are hydrophobic polymers and PAs combined with partially water-soluble organic solvents. When the solution comes in contact with aqueous organism fluids, the solvent-water exchange causes the polymer precipitation and the PAs entrapment, forming a dispositive which

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can release the drug in a controlled manner and avoiding critical formation conditions as temperature, ions presence or specific pH.[6] However, an important drawback is the time required for complete the polymer precipitation, allowing a fast release of drug associated not only to matrix diffusion but also to the solvent exchange. This phenomenon is known as *burst* effect and it is usually undesirable when strict PAs levels are needed.[8,9]

The hydrophobic polymer PLGA (poly(lactic-*co*-glycolic) acid) is an interesting candidate for ISFI-SE due to its biocompatibility, biodegradability and FDA approved condition.[9–11] Even though different characteristics of PLGA can be obtained depending on lactic/glycolic ratio, the glass transition temperature is normally above 37 °C, maintaining a relative solid structure in a wide range of biological conditions. In addition, the PLGA hydrolytic degradation produces the erosion of the polymeric matrix. This process is enhanced by the accumulation of the resultants acidic groups (autocatalytic effect) and depends on implant shape and size.[12–15] Polymer degradation and matrix erosion are important factors that influence the drug release rates. Different applications of ISFI-SEs for controlled release of many PAs such as proteins,[11] antibiotics,[16] analgesics[9] and opioids[17] are reported.

Combination of various polymer with different physicochemical features can improve the control over the release of PAs and properties of the matrixes as solubility, viscosity and glass transition temperature. In this sense, Eudragit polymers are good candidates for blending due to their wide range of properties with interesting applications in pharmaceutics.[18,19] Particularly, the use of ionic Eudragit polymers in blends can contribute to enhance and tune attributes related with the matrix behavior in aqueous media. Eudragit E100 (EuE100, Supplementary material Figure SM1-A) and Eudragit S100 (EuS100, Supplementary material Figure SM1-B) are cationic and anionic, respectively. EuE100 presents an increased solubility under pH = 5 while EuS100 solubilizes over pH = 7.[6] In the bibliography, investigations about PLGA-Eudragit blending are found but no one investigated possible application for ISFI-SE synthesis.[20,21]

Antibiotics are an important group of PAs for controlled release technologies due to problems related with concentrated formulation preparation and high number of doses.[3,22] In particular, florfenicol (FF) is a widely used antibiotic in veterinary applications and presents certain drawbacks associated to poor water solubility and reduced plasmatic half-life.[23–25] Note that these kind of problems are reported in many PAs used in human health fields. Some attempts have been made to design FF controlled release systems but low FF encapsulation efficiencies and fast drug release were reported.[26–30] In this regards, ISFI-SE technologies are an effective alternative for long-term FF therapies. The FF state of art on FF release systems does not show studies for this kind of DCRTs neither the use of PLGA and Eudragit polymer blending.

The aim of the present work was the synthesis and evaluation of polymeric ISFI-SE based on PLGA and Eudragit polymer blends for FF controlled release. The performances of the systems were studied by measurements of encapsulation efficiencies and release profiles. Morphological and physicochemical characterizations were carried out using scanning electron microscopies, FTIR, DSC and XRD studies. Empirical/semi-empirical equations were implemented to simulate drug release and study involved release processes. Furthermore, *in vitro* biological activity was tested against *Pseudomonas aeruginosa.*

## Materials and Methods

### *Materials*

PLGA 75:25, average molecular weight (Mw) 15 kDa (Shanghai Easier Industrial Development Co., Ltd.), Eudragit$^{R}$ E100 with a Mw of 47 kDa and Eudragit$^{R}$ S100 with a Mw of 125 kDa (Evonik industries, Germany) were utilized. Florfenicol (FF, 99.1%, Romikin S.A.), 2-pirrolidone (pro-analysis grade, Ciccarelli) and methanol (HPLC-grade, Sintorgan) were used. All reagents were utilized as received. Ultrapure water was used for all solutions and dilutions.

### *FF Quantification*

An HPLC instrument (Prominence Series 20A, Shimadzu, Japan) equipped with a UV detection diode array (SPD- M20Avp) was used for FF analysis. The wavelength was set at 224 nm. Mobile phase was a methanol:water (50:50) solution at pH = 2.5 with a flow rate of 0.75 mL/min (LC-20AT pumps). Zorbax Eclipse XDB-C18 column (4.6 × 150 mm; 5 μm) was used and maintained at 35 °C using a CTO-10Asvp column oven. The calibration curve ($R^2$ = 0.998) was performed using FF standard solutions.

### *Preparation of* In Situ *Formed Implants*

Implants were prepared *in vitro* by solvent exchange method. First, 0.110−0.220 g of FF and 0.220−0.400 g of PLGA were co-dissolved in 1.1 mL of 2-pirrolidone, under constant agitation and using a 70 °C water bath. For blended matrixes, a mass of EuE100 or EuS100 was added to the organic solution reaching proportions of 15 or 28% (w/w[1] of PLGA). Then, cylindrical glass molds with 0.5 g of the polymeric solution were immersed in 100 mM phosphate buffer (pH 7.4), at 37 °C and under orbital agitation, allowing to solvent exchange. After 24 h, the finished implants were retired and the supernatants were filtered and stored at 4 °C until non-encapsulated FF determination. The different prepared formulations are listed in Table 1.

**Table 1**
Formulations for the IFIS-IS Syntheses, Using 2-Pirrolidone as Organic Solvent.

| Formulation ID | PLGA (% w/v) | Florfenicol/PLGA (% w/w) | Eudragit Type | Eudragit/PLGA (% w/w) |
|---|---|---|---|---|
| PLGA20 | 20 | 25 | – | – |
| PLGA20 | 20 | 50 | – | – |
| P25 | 40 | 25 | – | – |
| P50 | 40 | 50 | – | – |
| PE15-25 | 40 | 25 | EuE100 | 15 |
| PE15-50 | 40 | 50 | EuE100 | 15 |
| PE28-25 | 40 | 25 | EuE100 | 28 |
| PE28-50 | 40 | 50 | EuE100 | 28 |
| PS15-25 | 40 | 25 | EuS100 | 15 |
| PS15-50 | 40 | 50 | EuS100 | 15 |
| PS28-25 | 40 | 25 | EuS100 | 28 |
| PS28-50 | 40 | 50 | EuS100 | 28 |

*Encapsulation Efficiency*

The synthesis supernatants were analyzed by HPLC to quantify non-encapsulated FF. Encapsulation efficiencies (EEs) were calculated as follows:

$$\boldsymbol{EE}\ (\%) = \frac{\boldsymbol{w}_{FFi}\ (g) - \boldsymbol{w}_{FFne}\ (g)}{\boldsymbol{w}_{FFi}\ (g)} x100$$

where $\boldsymbol{w}_{FFi}$ is the initial mass of drug added and $\boldsymbol{w}_{FFne}$ is the non-encapsulated drug.

In Vitro *Release Assays*

With the aim of evaluate the release kinetics, implants were immersed in 50 mL of fresh 100 mM phosphate buffer (pH 7.4), at 37 °C and under constant orbital agitation at 150 rpm. Every 24–48 h, the release medium was sampled and replaced with fresh medium for maintain sink conditions. Samples were filtered and stored at 4 °C for FF determination by HPLC. Assays were done by triplicate. Statistical analyses were performed using f1-f2 test for the determination of significant differences between release profiles. The f1 parameter (difference factor) measures the percentage error between two profiles along a time period while the f2 parameter (similarity factor) is the logarithmic transformation of the squared sum of the errors of the differences between two profiles in different time points. Release profiles are considered similar when f1 and f2 are between 0-15 and 50–100, respectively.[31]

*Structural and Morphological Analyses*

The morphology of implants was investigated by Scanning Electron Microscopy (SEM; Phenom World PRO X). Lyophilized implants were placed in a graphite tape and observed at 15 kV.

*DSC, FTIR and X-Ray Diffraction Studies*

Fourier Transform Infrared (FTIR) spectra were recorded in the range of 4000–400 $cm^{-1}$ in a FTIR-8001 PC spectrometer (Shimadzu, Japan). Sample (3.0–4.0 mg) and potassium bromide were blended and compressed to obtain suitable discs for FTIR analyses.

Differential Scanning Calorimetry (DSC) spectra were carried out using a DSC Q2000 calorimeter (TA Instruments, Tx, USA). Hermetic aluminum sealed capsules with 4.0–5.0 mg of sample were heated at a rate of 10 °C/min under nitrogen atmosphere (50 mL/min flow rate). The heat flow was recorded in a temperature range of 0–300 °C.

For X-Ray diffraction (XRD) studies, samples were scanned at speed of 1°/min, using 1.54098 Å wavelength at 40 kW and 45 mA in a Panalytical Empyrean X-ray diffractometer in the 2θ of 4–40° range.

*Mathematical Modeling*

FF release kinetics were modeled with zero order, first order, Higuchi and Korsmeyer-Peppas equations. Models which achieved $R^2 \geq 0.9$ were considered adequate.

*Microbiological Studies*

With the aim of evaluating the antimicrobial efficiency in a semi-solid medium, selected implants were immersed in 50 mL of inoculated Mueller-Hinton agar. *Pseudomonas aeruginosa* ATCC 15422 was chosen as bacteria strain due to its widely reported association with injury infections, biofilm production and generalized sepsis, among others.[32–34] During 1–12 days, photographic registers of the inhibition zones were obtained for qualitative analyses. Control of bacteria growth without formulations was performed.

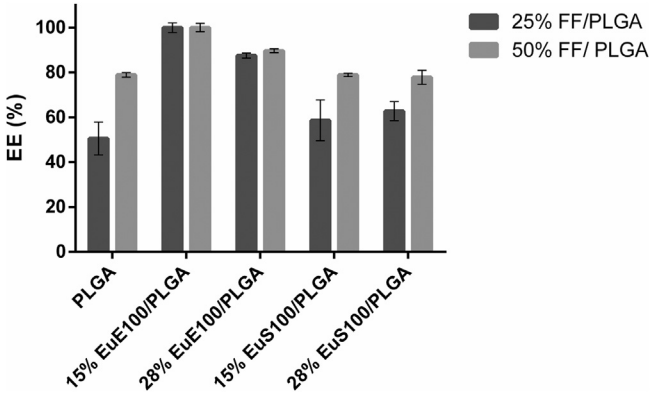


**Fig. 1.** Encapsulation efficiencies (EE%) obtained for ISFI-SE formulations (PLGA 40% w/w) using different florfenicol/PLGA and Eudragit/PLGA proportions.

## Results and Discussions

During ISFI-SE preparation, two stages are well defined: the process of polymer precipitation during the exchange of organic solvent by water, where polymeric matrix and organic solution coexist, and the release of the PA after the complete precipitation of the polymer, where the processes involved are more related to the properties of the matrix.[7]

Fig. 1 shows the encapsulation efficiencies (EEs) obtained for the different studied formulations. The PLGA concentration in the organic phase was defined taking into account that the formation of the implants was impeded with 20% (w/v). When PLGA was used alone at 40% (w/v), the increment of drug/polymer proportion from 25% to 50% (w/w PLGA) caused an improvement in EE from 50.6 to 78.9%, respectively. These results could be associated to the higher FF concentration that promotes an increase of precipitated drug. The bibliography presents different attempts to develop PLGA *in situ* formation systems for PA controlled release. It was reported that increments of the PA concentration allow to obtain higher EEs during implant formation because solid drug presence and increased viscosity.[7–9,17] In this way, the solid drug dissolution process can act as a release control step diminishing the burst effect.[35] The burst effect is an undesirable and uncontrolled release,

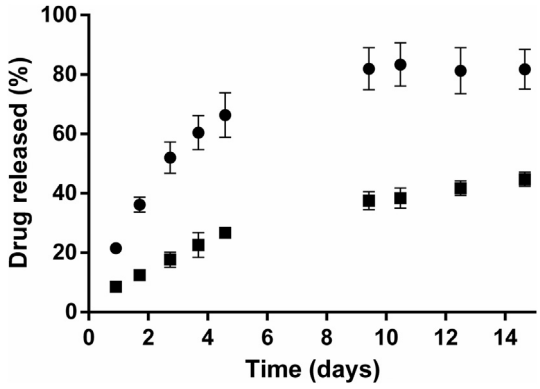


**Fig. 2.** Florfenicol release profiles from (●) P25 and (■) P50 formulations.

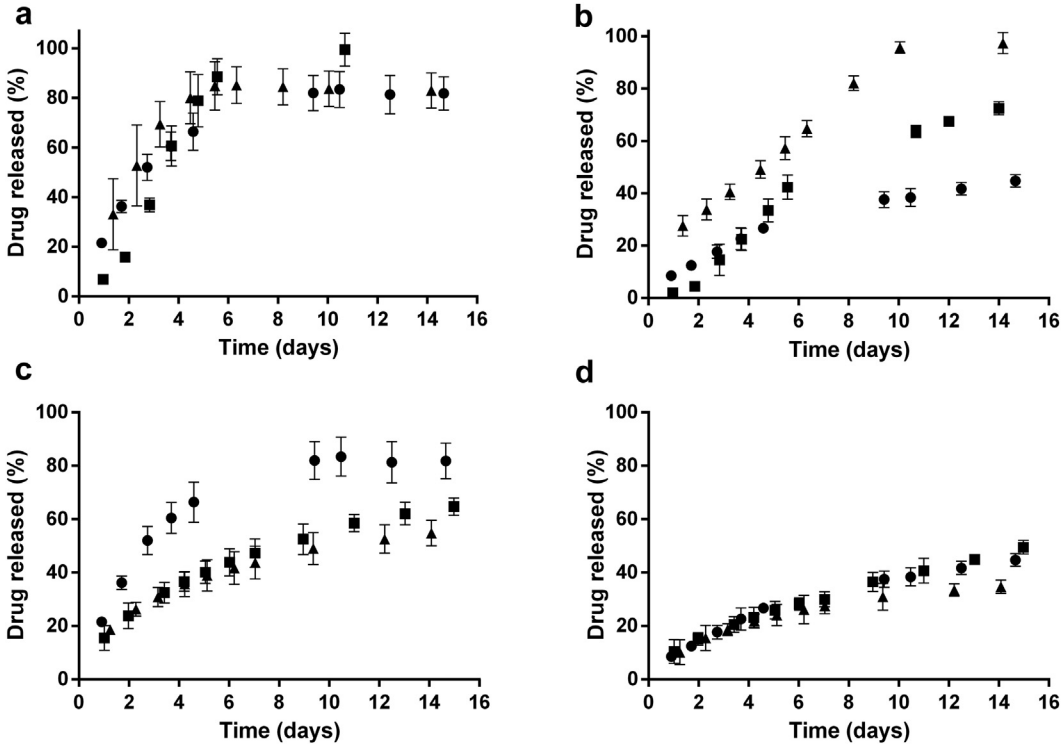


**Fig. 3.** Forfenicol release profiles from PLGA-EuE100 ISFI using (a) 25% and (b) 50% FF/PLGA (w/w) and from PLGA-EuS100 ISFI using (c) 25% and (d) 50% FF/PLGA (w/w). ISFI polymer composition: (●) PLGA; (■) 15% Eu/PLGA; (▲) 28% Eu/PLGA.

which can be associated with many secondary effects as local irritations and toxic drug plasma levels as well as technical drawbacks like low EEs and shorter release times.

With the aim of achieving new technological properties for ISFI-ES, two Eudragit polymer types were added to the PLGA formulations. Encapsulation efficiency results can be seen in Fig. 1. The addition of EuE100 caused a global increment of the EEs, yielding results of 85–100%. However, 28% EuE100 formulations reached lower EEs when are compared to 15% EuE100. On the other hand, the incorporation of EuS100 generated similar EEs to pure PLGA formulations. These striking erratic behaviors related to FF and Eudragit variations suggest multiple factors that affect the encapsulation of PA. Specific information about IFIS-IS synthesized with Eudragit/PLGA blends has not been reported. Solorio et al. (2012) investigated blends of PLGA, with different Mw, for fluorescein encapsulation and concluded that the implant osmolarity, polymers hydrophobicity and solvent affinities play important roles in the EEs obtained.[36] Particularly, Eudragit polymers present different physicochemical characteristics as Mw, ionization, pH-responsivity and aqueous solubility, resulting in different behaviors during the phase inversion when compared to pure PLGA matrixes.

When synthesis stage was completed, FF release from the different formulations was studied. Fig. 2 shows the results obtained for pure PLGA matrixes. At day 3, cumulative FF was 50–55% and 15–20% for 25 and 50% FF (w/w PLGA), respectively. The higher solid drug proportion can be the main factor that explains the release delay when higher concentrations of FF are used because of the dissolution effect on the FF transport from the polymeric matrix.[37]

Fig. 3 shows the release profiles for the ISFI-SE with EuE100 and EuS100 added. With the aim to statistically determine the difference between the different profiles, the f1-f2 test was carried out. In general, values between 0-15 and 50–100 for f1 and f2, respectively, indicate a high similarity degree between two compared profiles.[31] Profiles with equal proportions of FF were compared in order to evaluate the Eudragit effects. Results of the f1-f2 tests are shown in Table 2.

Fig. 3a shows the released FF from the EuE100 implants with the drug proportion of 25% (w/w PLGA). Pure PLGA implants results were included in all figures for comparison purposes. The accumulated FF was of 50% for PE28-25 and P25 while for PE15-25 was of 35%, after 3 days. However, the differences between formulations tend to become less important as release times progress. Release profiles for EuE100 implants with 50% FF (w/w PLGA) are shown in Fig. 3b. As can be seen, the release rate is increased when more EuE100 is used in the formulation. At day 3, PE28-50 showed an increased release rate while P50 and PE15-50 yielded lower and similar results, respectively. At day 10, FF released was of 40, 60 and 90% for P25, PE15-50 and PE28-50, respectively. While the assays were carried out at 37 °C, a considerable polymer relaxation can be expected because the used polymers present a transition glass temperature of 37–48 °C. On the other hand, the solubility of EuE100 is enhanced at pH values below 5.[18] As it is well known, the

**Table 2**
Statistical Comparison Between Florfenicol Release Kinetics from Different IFIS-IS Formulations Using f1-f2 Test.

| Kinetic 1 | Kinetic 2 | f1 | f2 |
|---|---|---|---|
| P25 | P50 | 100.00 | 25.99 |
| P25 | PE15-25 | 26.49 | 50.13 |
| P25 | PE28-25 | 13.92 | 48.70 |
| P50 | PE15-50 | 75.10 | 30.86 |
| P50 | PE28-50 | 44.72 | 29.54 |
| P25 | PS15-25 | 46.24 | 37.82 |
| P25 | PS28-25 | 57.60 | 36.06 |
| P50 | PS15-50 | 6.04 | 85.33 |
| P50 | PS28-50 | 16.14 | 70.19 |

**Table 3**
Matemathical Modeling for the Studied Florfenicol Release Kinetics.

| Formulation ID | Best Adjusted Model | Equation[a] | R[b] |
|---|---|---|---|
| P25 | First order | $DR(\%) = -((-e^{0.20xt(h)})-1)$ | 0.98 |
| P50 | Zero order | $DR(\%) = 4.12 \times t(h)$ | 0.99 |
| PE15-25 | First order | $DR(\%) = -((-e^{0.24xt(h)})-1)$ | 0.98 |
| PE15-50 | First order | $DR(\%) = -((-e^{0.07xt(h)})-1)$ | 0.99 |
| PE28-25 | First order | $DR(\%) = -((-e^{0.28xt(h)})-1)$ | 0.93 |
| PE28-50 | Zero order | $DR(\%) = 10.86 \times t(h)$ | 0.99 |
| PS15-25 | Korsm-Peppas[b] | $DR(\%) = 28,.6 \times (t(h)^{0.23})$ | 0.99 |
| PS15-50 | First order | $DR(\%) = -((-e^{0.05xt(h)})-1)$ | 0.99 |
| PS28-25 | Korsm-Peppas[b] | $DR(\%) = 27.63 \times (t(h)^{0.22})$ | 0.99 |
| PS28-50 | Korsm-Peppas[b] | $DR(\%) = 18.02 \times (t(h)^{0.18})$ | 0.99 |

[a] DR: Drug released.
[b] Korsmeyer-Peppas.

hydrolytic degradation of PLGA chains can produce acidic groups. PLGA matrixes with reduced sizes cannot accumulate the acidic groups due to their rapid diffusion. However, in systems with sizes above 25 μm, these products can be accumulated producing an acidic microenvironment, catalyzing the hydrolytic degradation and generating new acidic groups and lower pH values (autocatalytic effect).[13–15] Because of the acidic micro environment inside the implants, the number of EuE100 positive charges can be increased, generating a matrix destabilization due to the enhanced solubilization and incremented repulsive forces. These structural phenomena can induce a faster FF release.

The release profiles for the EuS100 formulations with 25% FF (w/w PLGA) are shown in Fig. 3c. When EuS100 is added, the release rate is diminished. At day 5, the implant P25 released 65% of the FF while both blends released 35–40%. The same behavior is observed when the FF proportion is of 50% (w/w PLGA) but the effect is less noticeable (Fig. 3d). This phenomenon could be due to the rigidity contributed by EuS100 ($Tg > 180$ °C), delaying the aqueous media influx and drug diffusion.[6] Furthermore, EuS100 is an anionic polymer with a solubility above neutral pH. In contrast with EuE100, the solubility of EuS100 can be diminished by the low pH values produced by the hydrolytic PLGA degradation products. The

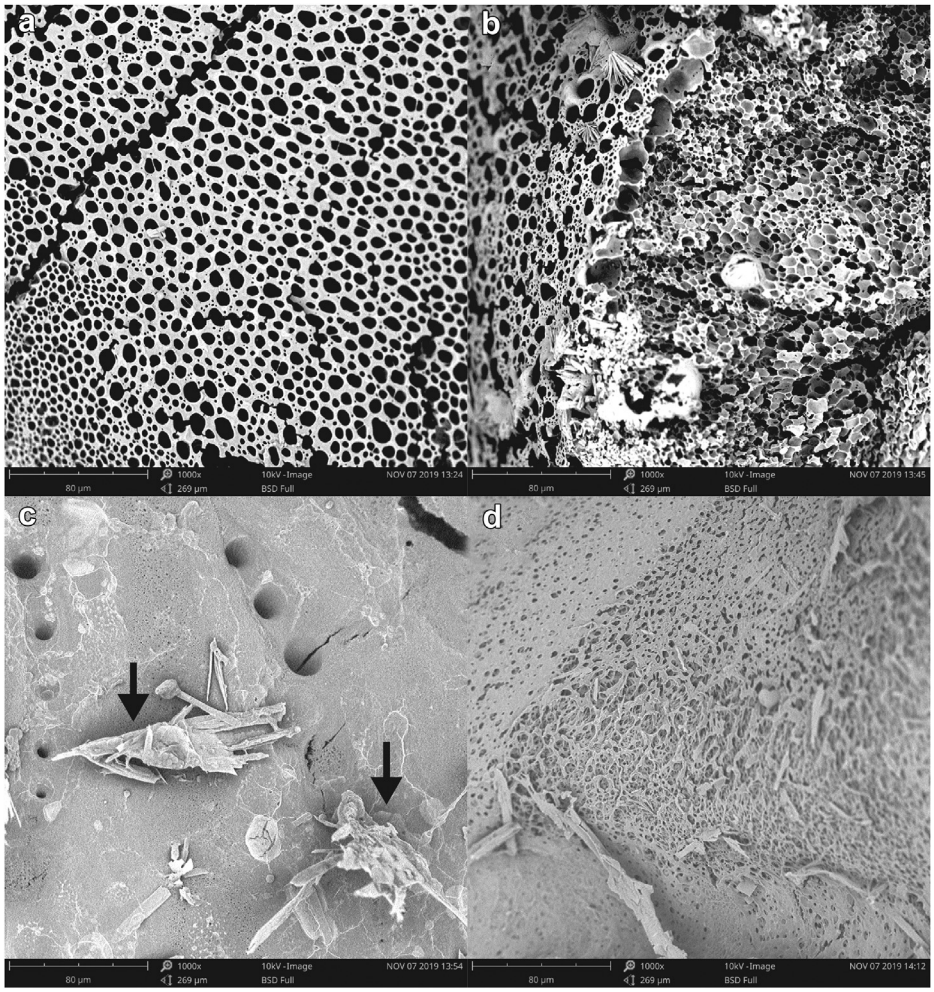


**Fig. 4.** SEM images of P25 ((a) surface and (b) cross-section) and P50 ((c) surface and (d) cross-section) formulations. Black arrows indicate florfenicol solid structures.

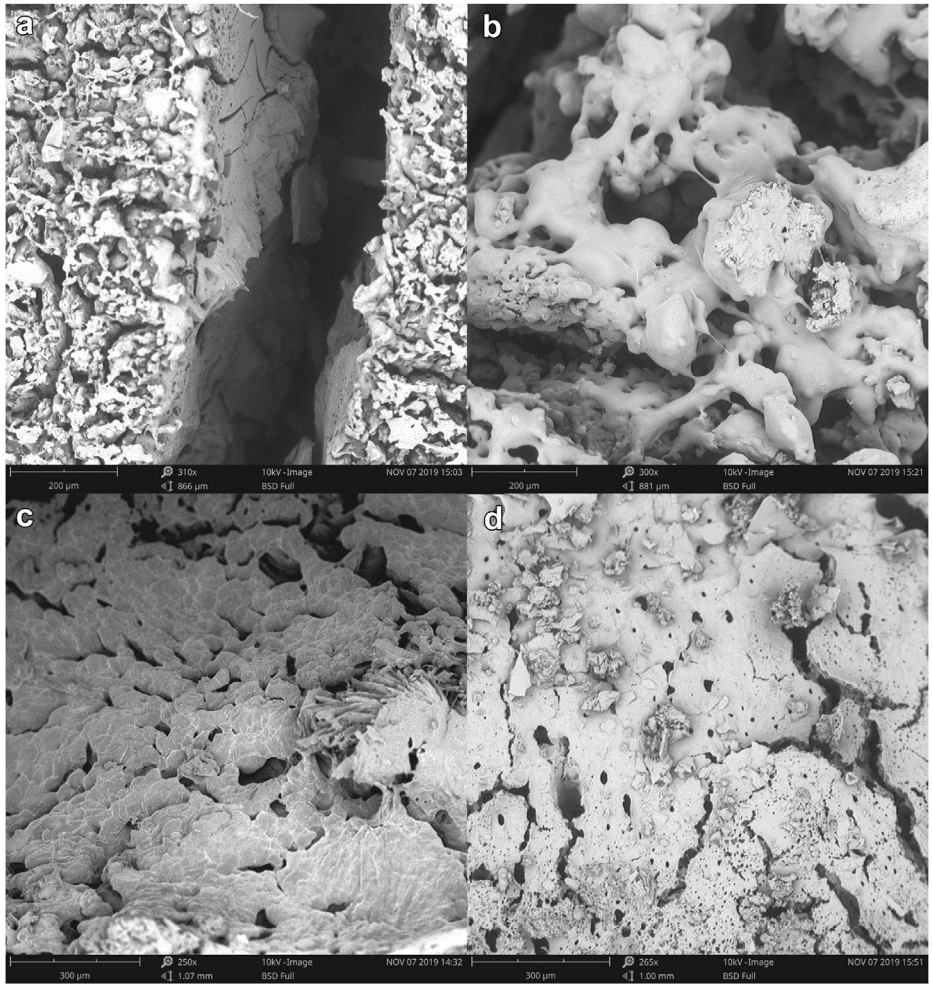


**Fig. 5.** SEM images of (a) PE15-50 and (b) PE28-50 formulations, and (c) PS15-25 and (d) PS28-25 formulations.

bibliography shows various attempts to develop different matrixes based in PLGA-Eudragit blending. For example, nanoparticles based in EuS100-PLGA for capecitabine vehiculization.[20] The principal results showed that, at pH 7.8, the increase of EuS100 proportion produces an enhancement of the drug release. Other example is the encapsulation and release of sodic diclofenac from polymeric nanoparticles using the combination of PLGA with EuL100, a pH responsive polymer similar to EuS100. The usage of this Eudragit also incremented the release rate of the drug.[21] However, both reports utilized nanoparticles systems where the products of the hydrolytic PLGA cleavage are not allowed to accumulate due to their rapid diffusion, preventing low pH microenvironments. In contrast, implant systems allow to accumulate the acidic products which can maintain the EuS100 in the non-ionized form with a reduced aqueous solubility, improving the matrix stability.[13–15]

Table 3 shows the predictions of mathematical models. Most of the profiles followed a first order kinetic, indicating the drug concentration evolution is related to the dissolution of PA particles due to superficial interactions with the aqueous media.[38] However, the results of P50 and PE28-50 formulations presented a good agreement with a zero order model, related to a constant drug saturation with a determined rate of release. These kind of models are interesting due to the possibility of equalize the dosage with the depletion of the drug using the amount of administered PA. In addition, for PS15-25, PS28-25 and PS28-50 the best fitting was achieved with the Korsmmeyer-Peppas model, with the parameter $n > 0.5$, indicating that the FF release possibly depends on a combination of swelling and diffusion processes.[39]

With the objective of performing a structural characterization, the different IFIS-IS were studied by Scanning Electron Microscopy (SEM). Fig. 6 shows the images obtained from P25 and P50 formulations. There is a noticeable difference in surface porosity. P25 implant (Fig. 4a, b) showed intense superficial and internal porosity while P50 (Fig. 4c, d) presented a compact structure and an increased solid FF presence. The composition of the PA precipitate was confirmed by elemental analysis (Supplementary material, Figure SM12). The increased viscosity of the P50 formulation, associated with the higher quantity of PA, could possibly reduce the rate of organic solvent diffusion during synthesis stage, producing a less porous matrix.[6,8] These results are in good agreement with the release profiles where P25 presented a faster FF release possibly due to an increased porosity and a diminished solid drug presence.[37]

SEM images for EuE100 and EuS100 implants are shown in Fig. 5. As was mentioned, an increment in EuE100 proportion induced a faster drug release. This result can also be associated to the differences between PE15-50 and PE28-50 structures (Fig. 5a, b, respectively). When more EuE100 is added, the polymeric matrix presents a less compact structure with an incremented porosity. These characteristics can enhance the drug diffusion to the aqueous media.

Fig. 5c, d shows the SEM images of EuS100 implants. As could be seen in the profiles studies, the EuS100 addition reduced the release rate of FF. When EuS100 proportion is incremented, the implants present less porosity and higher density in the polymeric matrix. The mentioned structural characteristics can reduce the surface exposed to the aqueous media, diminishing the diffusion rate of the PA.

Fourier Transformed Infrared Spectra (FTIR) studies for the different implant formulations are shown in Fig. 6. The results for pure PLGA formulations and their components can be observed in Fig. 6a. FF presents the 3450, 3320 and 1680 $cm^{-1}$ characteristic band associated to N-H, O-H and amide vibrations, respectively. PLGA spectra shows the principal bands at 3000-2000 and 1780 $cm^{-1}$ due to C-H and C═O vibrations, respectively. When implant results are analyzed, positional band changes cannot be identified but a notorious increment in the respective FF bands (black arrows) is observed.

FTIR studies for EuE100 and EuS100 are shown in Fig. 6b and c, respectively. EuE100 spectrum presents the characteristic bands at 1730 and 2770-2820 $cm^{-1}$ associated to ester and diethylamine groups.[40] On the other hand, EuS100 peaks are observed: 2950, 1730 and 1450 $cm^{-1}$ due to carboxylic oxydryl, ester and methyl groups, respectively.[41] Black arrows indicate shifts from 1780-1730 to 1760 $cm^{-1}$ which can be associated to polymers interactions related to the blend formation.

X-ray diffraction (XRD) results are presented in Fig. 7. The selected formulations were those with interesting differences in the release profiles. Principal FF signals are observed at 8.10, 16.21 y 26.85°.[42] When EuE100 is added to the formulations (Fig. 7a), the FF signals are less perceptible. This phenomenon indicates that EuE100 can be inducing the amorphous precipitation of FF.[43,44] On the other hand, EuS100 (Fig. 7b) allows the formation of FF crystals during the synthesis of the implants.[45] As the presence of drug crystals can be associated with reduced release times, the release profiles are in good agreement with the XRD results.

Differential Scanning Calorimetry (DSC) results for selected formulations are shown in Supplementary material (Figure SM3) . FF fusion peak can be observed at 152 °C.[42,46] The glass transition temperature of PLGA is 37−38 °C.[47] The formulation P50 presents a fusion peak at 155 °C and can be associated with crystalline FF. The difference with pure FF is possibly related to interactions with the polymeric matrix. The absence of fusion peak, in formulation PE28-50, is in concordance with the presence of amorphous FF. In contrast, the formulation PS28-50 presents a fusion peak at 160 °C associable to crystalline FF. Interactions with the blended matrix can cause the temperature shift. The results of DSC are in good agreement with FTIR and XRD studies. The absence of polymeric events is possibly due to polymer proportions and temperature intervals used.

Microbiological assays were carried out using selected EuE100 formulations (PE15-50 and PE28-50) due to markedly differences

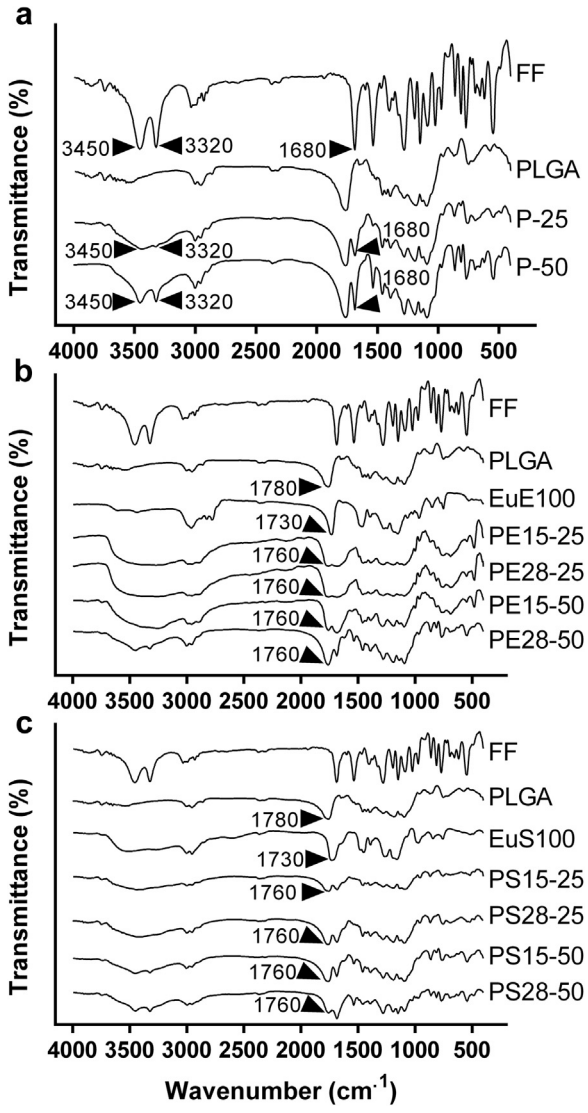


**Fig. 6.** FTIR spectrum results for (a) PLGA, (b) EuE100/PLGA and (c) EuS100/PLGA based formulations. Pure components diffractograms are included.

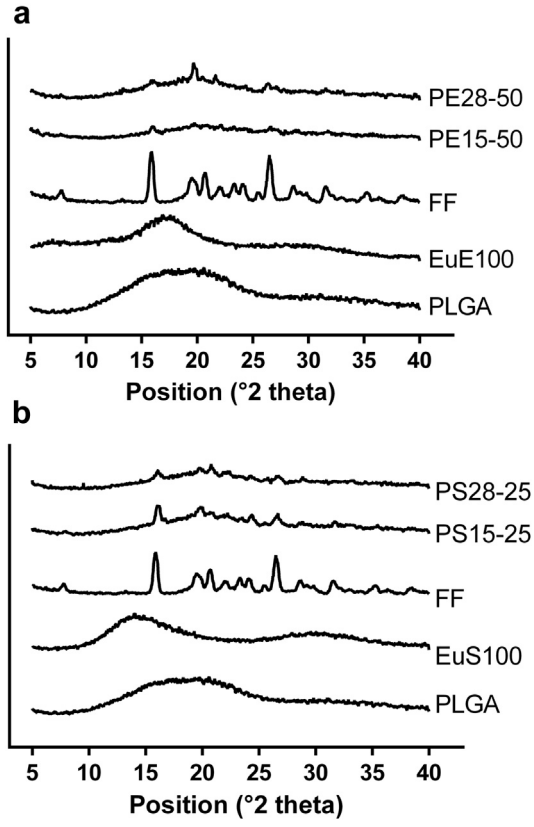


**Fig. 7.** X-ray diffraction studies for (a) EuE100 y (b) EuS100 formulations. Pure components diffractograms are included.

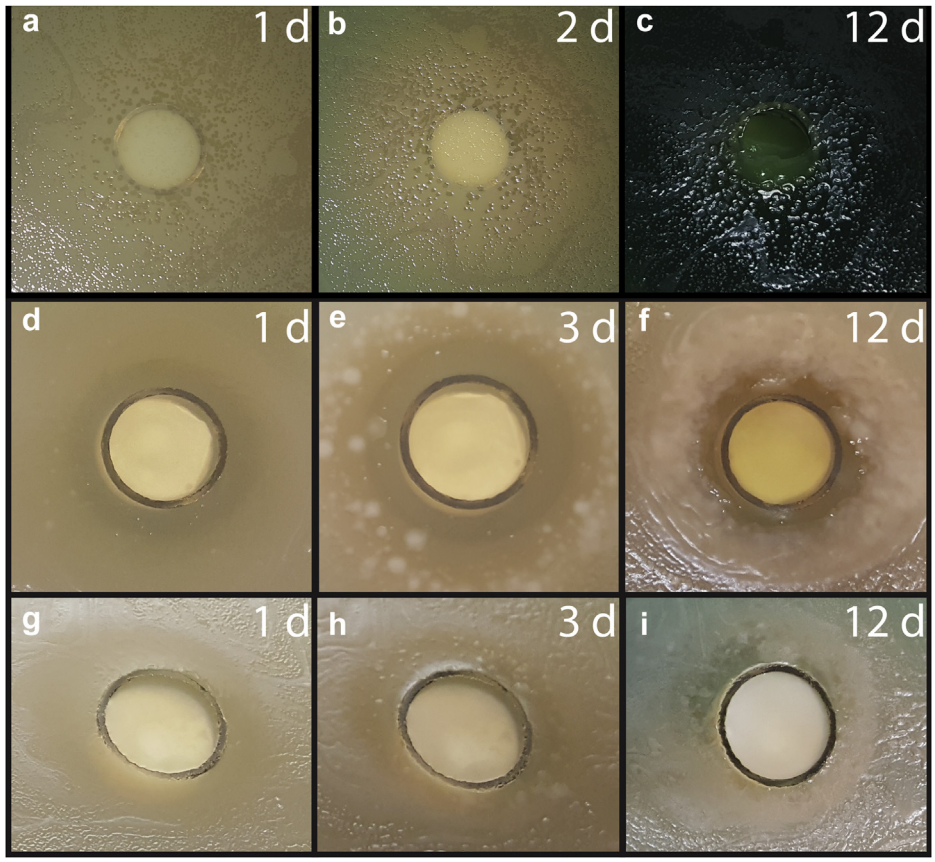


**Fig. 8.** Evolution of antimicrobial activity over Pseudomonas aeruginosa ATCC 15422 for (a–c) implants without FF, (d–f) PE15-50 and (g–i) PE28-50 formulations, at 1, 3 and 12 days. (Color Figure).

observed in the *in vitro* release profiles. Fig. 8a–c shows the evolution of a *Pseudomonas aeruginosa* population in contact with an implant without FF. At 24 h, all agar plate is colonized by the bacteria strain. After 48 h, the production of green pigment (dark zones) can be observed and it is associated to quorum sensing activity, related to intense biofilm formation and bacterial adaptation.[48] Finally, after 12 days, bacteria population colonized the implant. These results are due to the absence of PA and inefficiency of polymers to inhibit bacteria growth in the experimental conditions. Microbiological assays for PE15-50 and PE28-50 are shown in Fig. 8d–f and g–i, respectively. Implants are capable of maintain an inhibition zone during 24 h. In addition, PE28-50 reached a higher diameter in the initial inhibition halo. After 12 days, the microorganisms colonize the surrounding area of the implants but avoid the zone in direct contact with the formulation. These results indicate the effective release of the PA and the conserved activity of FF after ISFI-SE synthesis process.

## Conclusions

Drug controlled release systems, based in polymeric *in situ* formed implants (ISFI), represent important options for optimizing human and animal health therapies. In addition, the combination of different materials allows to obtain versatile technologies. In the present work, ISFI were synthesized using the solvent exchange technique (ISFI-SE). PLGA and ionic Eudragits E100/S100 were used as polymers while florfenicol was the encapsulated drug. The different polymers combinations and drug proportions exhibited various behaviors in terms of encapsulation efficiencies and drug release rates. While PLGA/EuE100 avoided burst effect, PLGA/EuS100 reached encapsulation efficiencies similar to those of PLGA implants. However, FF release rate was increased when cationic EuE100 was used and diminished when anionic EuS100 was applied. This phenomenon can be due to the pH microenvironment generated by acidic PLGA degradation products. On one hand, EuE100 solubility is enhanced by low pH values causing a destabilization of the polymeric implants. On the other hand, EuS100 loses aqueous solubility in acidic environments maintaining the polymeric matrix composition. In addition, qualitative antimicrobial studies show the ISFI-SE capacity for inhibit *Pseudomonas aeruginosa* growth. The antibiotic activity was maintained during 12 days in Mueller-Hinton agar plates. The designed polymeric systems represent an interesting approach to FF single dose and controlled release applications.

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## Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.xphs.2020.11.006.

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