UNIVERSITYOF BIRMINGHAM

Development of a thermosensitive hydrogel based on Polaxamer 407 and gellan gum with inclusion complexes (Sulfobutylated-β-cyclodextrin-Farnesol) as a local drug delivery system

de Dios-Pérez, Inmaculada; González-Garcinuño, Álvaro; Tabernero, Antonio; Blanco-López, Marcos; García-Esteban, Juan A.; Moreno-Rodilla, Vidal; Curto, Belén; Perez Esteban, Patricia; Valle, Eva M. Martín del

[Link to publication on Research at Birmingham portal](#)

Development of a thermosensitive hydrogel based on Polaxamer 407 and gellan gum with inclusion complexes (Sulfobutylated-β-cyclodextrin–Farnesol) as a local drug delivery system

Inmaculada de Dios-Pérez [a], Álvaro González-Garcinuño [a,b], Antonio Tabernero [a,b], Marcos Blanco-López [a], Juan A. García-Esteban [c], Vidal Moreno-Rodilla [c], Belén Curto [c], Patricia Pérez-Esteban [d], Eva M. Martín del Valle [a,b,*]

[a] Department of Chemical Engineering, University of Salamanca, Spain
[b] Institute for Biomedical Research of Salamanca (IBSAL), Spain
[c] Department of Computer Science and Automation, University of Salamanca, Spain
[d] Institute of Translational Medicine, Heritage Building, Mindelsohn Way, Birmingham, B15 2TH, England

ARTICLE INFO

ABSTRACT

This work proposes the development of a thermosensitive local drug release system based on Polaxamer 407, also known as Pluronic® F-127 (PF-127), Gellan Gum (GG) and the inclusion complex Sulfobutylated-β-cyclodextrin (CD) with Farnesol (FOH). Rheological properties of the hydrogels and their degradation were studied. According to the rheological results, a solution of 20% w/v of PF-127 forms a strong gel with a gelling temperature of about 25 °C (storage modulus of 15,000 Pa). The addition of the GG increased the storage modulus (optimal concentration of 0.5 % w/v) twofold without modifying the gelling temperature. Moreover, including 0.5% w/v of GG also increased 6 times the degradation time of the hydrogel. Regarding the inclusion complex, the addition of free CD decreased the viscosity and the gel strength since polymer chains were included in CD cavity without affecting the gelling temperature. Contrarily, the inclusion complex CD-FOH did not significantly modify any property of the formulation because the FOH was hosted in the CD. Furthermore, a mathematical model was developed to adjust the degradation time. This model highlights that the addition of the GG decreases the number of released chains from the polymeric network (which coincides with an increase in the storage modulus) and that the free CD reduces the degradation rate, protecting the polymeric chains. Finally, FOH release was quantified with a specific device, that was designed and printed for this type of system, observing a sustainable drug release (similar to FOH aqueous solubility, 8 μM) dependent on polymer degradation.

1. Introduction

Cancer has become the main cause of death worldwide, with a five-year net survival rate depending on the type and stage of cancer. Based on these factors, the appropriate treatment is decided, with surgery being the main strategy for localised tumours. The disadvantages of surgery are that it involves many possible complications, and the conventional complementary treatments (radiotherapy and chemotherapy) are very aggressive, with high toxicity, low efficacy, and numerous side effects. Furthermore, some tumours are unresectable or require adjuvant therapy before or after surgical removal (to reduce tumour size or remove cell debris in order to prevent tumour regeneration) (Rafael et al., 2021). To minimise these problems, new approaches have focused on the use of local drug delivery systems. These systems are available in different physical forms (wafers, gels, foams, enemas, fibres, or microparticles) (Rafael et al., 2021; Mohammadi et al., 2022; Chen et al., 2021), but from a physicochemical point of view, hydrogels might be the most interesting because they are similar to tissue and extracellular matrices (Mohammadi et al., 2022). There are currently examples of these systems in anticancer treatments with good results in clinical trials, but most of them are not completely biodegradable, requiring surgery to remove them after treatment (Rafael et al., 2021; Mohammadi et al., 2022).

Specifically, hydrogels are 3D network structures formed by

---

* Corresponding author.
   E-mail address: emvalle@usal.es (E.M. Martín del Valle).

hydrophilic polymers capable of retaining a high amount of water (Rafael et al., 2021; Kesharwani et al., 2021; Xiao et al., 2021). Depending on the type of crosslinking, they are classified as chemical or physical crosslinking, the second method being more biocompatible, as the chemical process leaves residues that generate compatibility problems (Rafael et al., 2021; Mohammadi et al., 2022). Moreover, based on their physical properties they can be conventional gels, preformed gels, or smart gels, which respond to external triggers changing their structure from solution to gel under physiological conditions; this feature allows the therapeutic compound to be added to a liquid hydrogel, which will be injected and then solidify at the application site and release the drug over time (Mohammadi et al., 2022; Chen et al., 2021; Nieto et al., 2022; Mohanty et al., 2018).

Smart hydrogels are a great option for use as local delivery systems after tumour resection, as they allow a uniform application that conforms to the irregular shape of the post-surgical cavity, with minimal invasiveness and a mild gelation process, allowing for prolonged release and degradation over time (Mohammadi et al., 2022; Xiao et al., 2021; Marabelle et al., 2018). They have numerous advantages for use as controlled release systems to prevent tumour regeneration: (i) easy and minimally invasive application (Mohammadi et al., 2022; Xiao et al., 2021; Mohanty et al., 2018), (ii) good adaptability to irregular surfaces (Mohammadi et al., 2022), (iii) easy incorporation of the drug into the system (Kesharwani et al., 2021; Mohanty et al., 2018), (iv) controlled and sustained drug release in the target tissue (Xiao et al., 2021; Mohanty et al., 2018), (v) greater efficacy of the drug (Mohanty et al., 2018), (vi) reduction in dosing frequency and drug toxicity (Mohanty et al., 2018), (vii) protection of the drug against changes in environmental conditions (Kesharwani et al., 2021; Mohanty et al., 2018), (viii) greater bioavailability and residence time of the drug (Mohammadi et al., 2022), and (ix) do not induce immune response (Mohanty et al., 2018).

Smart hydrogels that respond to temperature are called thermosensitive, and their study is interesting because their gelling process can be performed at conditions homogeneous to those of the human body (Rafael et al., 2021; Mohammadi et al., 2022). There are numerous studies of thermosensitive gels for anticancer treatments: Li et al. and Liu et al. prepared a hydrogel based on Poly(N-isopropylacrylamide (PNIPAM) and another polysaccharide by loading them with 5-fluorouracil (Li et al., 2018) or doxorubicin (Liu et al., 2017); Soo Gil et al. loaded poly(ethylene glycol)–poly(β-aminoester urethane) (PEG-PAEU) nanogels with cisplatin for release (Gil et al., 2017); Hang et al. developed an antitumour therapy based on an intratumoral injection using hydrogels made of dialdehyde-functionalized polyethylene glycol (DF-PEG) and chitosan cross-linked with β-glycerophosphate that release doxorubicin (Wu et al., 2014); and Wu et al. prepared polymeric micelles with cisplatin and which were loaded with paclitaxel (Xiao et al., 2021; Yu et al., 2021). In all these studies interesting results regarding release and gelation temperature were obtained, however, all of them have the same drawbacks in common: firstly, the gel preparation processes were long; secondly, many organic compounds (such as 2-propanol) had to be used; and, finally, it is necessary carrying out purification processes.

Most of the polymers for the preparation of thermosensitive hydrogels are poloxamers, which are amphiphilic triblock copolymers, having a hydrophobic polypropylene oxide (PPO) central block and two hydrophilic polyethylene oxide (PEO) ends. PEO groups provide biocompatibility and water solubility, while PPO groups confer hydrophobic drug loading capacity. The solutions of these polymers gel with increasing temperature, due to the dehydration of the PPO and the production of hydrophobic interactions, forming micelles that eventually aggregate. The higher the polymer concentration, the lower the required temperature (Rafael et al., 2021; Mohammadi et al., 2022).

One of the most common polymers is Poloxamer 407, also called Pluronic® F-127 (PF-127), which has a hydrophilic-lipophilic balance of 20–29 and a PPO chain of 62 units (Diniz et al., 2015). Besides forming thermosensitive solutions, PF-127 helps improve cell union and collagen formation, which facilitates the formation of blood vessels, contributing to post-surgical healing. In addition, PF-127 is approved by the U.S. Food and Drug Administration (FDA) for use in humans, since it is non-toxic, biocompatible, and biodegradable (Turabee et al., 2019). Therefore, PF-127 can be used for the formation of biocompatible and biodegradable thermosensitive hydrogels for localised therapy to develop potential systems for cancer treatment, taking advantage of the benefits of traditional depot systems while avoiding systemic drug exposure (Rafael et al., 2021). In this context, PF-127 has been studied for the formation of hydrogels, however, these gels have inadequate mechanical strength and stability, causing rapid degradation of the gels, and therefore cannot be used in certain biomedical applications. Consequently, some modifications are needed to increase the degradation time. One possibility is to add other polymers to the solution, developing copolymeric gels, instead of homopolymer gels (Mohammadi et al., 2022; Chen et al., 2013). With this purpose, some authors added N,N,N-trimethyl chitosan or hexamethylene diisocyanate to solutions of PF-127, forming gels that would be used to release docetaxel (Turabee et al., 2019; Chen et al., 2013). Specifically, Turabee et al. added N,N,N-trimethyl chitosan, but it had no effect on the degradation time of the gels. On the other hand, Chen et al. were able to increase the degradation time by synthesizing a copolymer of PF-127 with hexamethylene diisocyanate. However, they used petroleum ether as a solvent, that might be a problem for a future application in biomedicine due to its toxicity (Turabee et al., 2019; Chen et al., 2013).

In this sense, our hypothesis is that the degradation time of the gel can be modified by adding a polysaccharide, forming a polymeric network. Both strategies aim to achieve a controlled and extended drug release over time. For this reason, a thermosensitive gel using PF-127 combined with gellan gum (GG) is proposed for anticancer therapy. GG is a natural polysaccharide that presents characteristics necessary to hydrogels (biocompatibility, biodegradability, stability, and ionotropic sensitivity). GG is a water-soluble anionic linear tetrasaccharide with a high molecular weight. Its solutions undergo thermally reversible gelation, its spiral structure becomes random at temperatures above 80 °C and changes to double helical when cooled. This process is ionotropic, that is, the presence of cations promotes the aggregation site by binding between pairs of carboxylate groups in neighbouring helices. In addition, the viscoelastic properties of GG facilitate its easy administration by injection, gelling into a fluid gel once the application is complete (Nieto et al., 2022; Sweety et al., 2022).

In order to prevent the proliferation of cancer cells remains, the thermosensitive hydrogel was loaded with a cytostatic agent. Farnesol (FOH) is a natural isoprenoid that can arrest the cell cycle at the G0/G1 phase in various types of carcinoma cells. FOH controls the localisation and activity of phosphocholine cytidylyltransferase (CTP), an enzyme involved in the biosynthesis of phosphatidylcholine (PC). In this way, FOH inhibits the action of CTP disrupting PC synthesis and, as a result, mammalian cells cannot produce their membrane and proliferate (de Dios-Pérez et al., 2022). However, due to its low solubility in aqueous media (approximately $8 \cdot 10^{-6}$ M), the possibility of forming an inclusion complex with cyclodextrins to improve FOH solubility was studied in our previous work. In that work, authors worked with Sulfobutylated-β-cyclodextrin Sodium Salt (CD), due to its high aqueous solubility and because it is a FDA approved compound. Our previous results showed that the solubility of FOH was improved since a concentration of 100 mM of SBE-β-CD allows a solubilization of 50 mM of FOH. Another relevant result of that study was that the half maximal inhibitory concentration (IC50) of cyclodextrin in healthy and cancer cells is higher than 1 mM; while free and complexed FOH is 71 μM and 127 μM, respectively, in the HCT-116 colon cancer line and 113 μM and 204 μM, respectively, in the HS-5 normal cell line (de Dios-Pérez et al., 2022).

Summarising, taking into account the selective action of FOH, the main objective of this work was the preparation of a thermosensitive hydrogel that incorporates a complex CD with a highly hydrophobic

drug (FOH in this case) and GG, studying how the addition of the different compounds can modify the formulation properties.

2. Materials and methods

2.1. Reagents

Pluronic® F-127 (PF-127, #2443), Gelzan™ CM, Gelrite® (GG, #G1910, low acyl, molecular weight 1000 kg/mol), Phosphate Buffer Saline (PBS, pH 7.4) and FOH 95 %, were purchased from Sigma-Aldrich whereas Sulfobutylated-cyclodextrin Sodium Salt (SBE-β-CD) was purchased on Cyclolab (CY-2041.2).

2.2. Formation of gels

To develop formulations, it is necessary to determine the optimal percentage of different polymers and drug concentrations.

2.2.1. PF-127 gel formation

Due to the thermosensitive nature of PF-127, it dissolves better in solutions at low temperatures. Consequently, solutions were prepared in PBS at 4 °C and 15 %, 20 %, 25 %, and 30% w/v PF-127. After stirring overnight, at least 12 h, the homogeneous solutions were stored in tubes at 4 °C until use.

2.2.2. GG gel formation

The optimal GG concentration was studied in the presence and absence of PF-127. The solutions were prepared by adding 0.125 %, 0.25 %, 0.50 %, and 1.00% w/v GG to PBS at 80 °C with stirring for 1 h, after which they were cooled at room temperature for a further 1 h, shifted to 4 °C, and PF-127 was added with stirring overnight, at least 12 h. The homogeneous solutions were stored in tubes at 4 °C until use.

2.2.3. PF-127-GG-CD-FOH gel formation

Finally, after determining the optimal concentrations of PF-127 and GG, the influence of drug addition was studied. A solution was prepared by adding GG to PBS at 80 °C and stirring for 1 h, after which it was cooled at room temperature for a further 1 h, then shifted to 4 °C and PF-127 was added with stirring overnight at least 12 h. Once homogeneous solutions were obtained, the solution was separated into other bottles where the corresponding drug was incorporated in each case. Solutions were stirred overnight (at least 12 h) again and, the next day, stored in tubes at 4 °C until use.

However, since FOH has poor solubility in aqueous solvents, the CD-FOH complex was studied using the same molar concentration as free FOH. A stock solution of CD-FOH was prepared, 200 mM FOH was added at a solution of 400 mM CD on PBS, mix and filter to remove free FOH. Free CD was also studied to verify that it did not interfere with rheology.

The drugs studied were free FOH at a final concentration of 400, 200, 40, 20, and 4 mM; CD-FOH at a concentration of 40–20 and 8–4 mM; and, CD at a concentration of 40 and 8 mM. It was considered that the rheology could change when preparing the solutions with the different drug concentrations, due to the addition of different volumes of the stock solution. To avoid this problem, it was decided to work with a final volume of 10 % v/v, supplementing with PBS if necessary.

2.3. Rheology

Rheological measurements were performed using an AR 1500 Rheometer (TA instrument, Newark, DE), equipped with an acrylic plate geometry (with a diameter of 40 mm and a gap distance of 1 mm).

The lower critical solution temperature (LCST) is the value at which the solution changes from a liquid to a gel by heating. Therefore, for injectable hydrogels, the ideal condition is to use a thermosensitive gel that changes by heating, being the LCST higher than 25 °C and lower than 37 °C.

Briefly, the solution was pipetted into the diameter plate and 3 types of tests were determined: (i) Temperature sweep from 10 °C to 45 °C with an increase of 1 °C/min to determine the LCST, at a constant frequency (1 Hz) with a controlled strain percentage (0.2 %). (ii) Frequency sweep by varying the angular frequency from 0.5 to 500 rad/s and applying a constant strain (0.2 %), to evaluate its storage modulus G' and viscous modulus G'' as a function of the angular frequency [ω]) at body temperature (37 °C). (iii) Steady-state flow test by varying the shear rate from 0.1 to 200 s^{-1} at 10 °C and 37 °C to determine the deviations from ideal Newtonian behaviour at different temperatures.

2.4. Gel degradation testing and modelling

2.4.1. Gel swelling and degradation experiments

The swelling and degradation time of hydrogels was investigated in vitro. 5 mL of the solutions were plated in

35 mm diameter Petri dishes and incubated at 37 °C overnight, at least 12 h. After that, the Petri dishes were placed in beakers containing 70 mL of PBS (pH 7.4) at physiological temperature and subjected to magnetic stirring. The gels were weighed every hour to determine the swelling and degradation time.

2.4.2. Modelling, parameter estimation, and statistical analysis

Degradation results were used to build a model to explain the contribution of both polymers (GG and PF-127) to the stability of the gels as well as the release of the compounds inside them (FOH in this case). According to previous publications [1; 2], the percentage of mass loss can be calculated according to the Eq. (1), as a function of the relative mass of both polymers involved (W) and the fraction of chains released from the internal structure of the hydrogel (F):

$$\% \text{ Mass loss} = W_{plur} \cdot F_{plur} + W_{GG} \cdot F_{GG} \qquad (1)$$

In order to estimate the F value for each polymer, it is necessary to define another parameter (P), which is a variable function of time according to Eq. (2), and that depends on the degradation parameter k'.

$$P = 1 - \exp(-k' \cdot t) \qquad (2)$$

From this parameter, it is possible to define the fraction of chains released for PF-127 (Eq. (3)) and for GG (Eq. (4)).

$$F_{GG} = \left(1 - (1 - P)^2\right)^N \qquad (3)$$

$$F_{plur} = P^2 + P(1 - P) \cdot F_{GG} \qquad (4)$$

Therefore, two parameters have to be estimated (k' and N) from the experimental data. Initially, preliminary estimation is carried out in order to analyse if both parameters are lineally independent and the range of values. After this preliminary estimation, it can be observed that the N value has similar results for all simulations (value around 10). Therefore, it can be considered as a fixed value for the following simulations, with more accuracy (N = 10).

For the estimation of k' (degradation rate parameter), the Solver add-in in Excel has been used to minimise the error value between the predicted (theoretical) value and the experimental value. This average absolute deviation (AAD) is calculated as the summation of the absolute value of the difference between the experimental and theoretical values for each time, according to Eq. (5).

$$AAD \ (\%) = \frac{\sum_i |T_{v_i} - E_{v_i}|}{n} \cdot 100 \qquad (5)$$

Where T_{vi} is the theoretical value for each time, E_{vi} is the experimental value for each time and n is the number of experimental points.

2.5. In vitro FOH release from hydrogels

In order to simulate more realistically the drug delivery process in

the tumour microenvironment, a 3D structure was designed and printed to simulate the geometry and the hydrodynamic conditions. This section will describe the details of the modelling and printing of the device used in the testing and validation processes of the created/used drug.

2.5.1. Drug release device

The main objective in the design of the device is to maximise the contact surface of the fluid channel with the gel, avoiding overflows at the interface, as there would be uncertainty in the contact surface. The cylindrical device (Fig. 1) created has a square base with a side of 50 mm and a height of 13 mm. The body (central cylinder) in which the gel is deposited has an internal diameter of 35 mm and a height of 8 mm. Regarding the feeding, there is an inlet tube of 4 mm inner diameter with a wall thickness of 1 mm and a length of 20 mm. The outlet channel was created in an open, semi-circular shape because in that area (effluent) there were surface overflows problems due to surface tension. The contact channel has a lateral surface of 443.75 mm^2, a total surface of 703.46 mm^2, and a volume of 692.86 mm^3.

The device has been designed and modelled with Sketchup, a computer-aided design (CAD) software from the Trimble company, which is widely used for its numerous utilities for modelling and designing 3D objects and functionalities for exporting to various file formats, such as STL.

Using the 3D CAD model, the device has been built by additive manufacturing with a poly lactic acid material, resistant to possible chemical alterations, with a Witbox-1 3D printer from the BQ company. As a 3D-printing slicer app, Ultimaker Cura version 4.12.1 has been used to generate the G-code file to control the 3D printer. Regarding the printing parameters that have been defined, the device has been built at a printing temperature of 220 °C and a speed of 60 mm/s. Finally, to obtain a good quality print, a 0.1 mm high layer, 1 mm thick walls, and an infill with a density of 30 % have been used to ensure the robustness of the design.

2.5.2. Drug release experiments

Firstly, the device was placed in an incubator at 37 °C and connected to 2 containers by tubing, which will pass through a peristaltic pump (Mehrkanal Pumpenkopf C4 524–80420–00, Heidolph, Germany) that was assembled with a pump drive (Heidolph Pumdrive PD 5201) and a cassette (Heidolph cassette small 524–90022–00). One container was empty and the other had PBS, which caused the PBS to enter the device and exit into the empty container.

In the same way as for the degradation (see Section 2.4.1.), the gels were prepared on 35 mm plates which contained a double bottom to facilitate their extraction. Once the gel was formed, it was removed from the plate and inserted into the device. To prevent the spaces from clogging with the gel, a 35 mm nylon filter (11 μM pore size) was placed into the device beforehand. The pump was activated at a flow rate of 2 mL/min and samples were collected every hour until complete degradation of the gel.
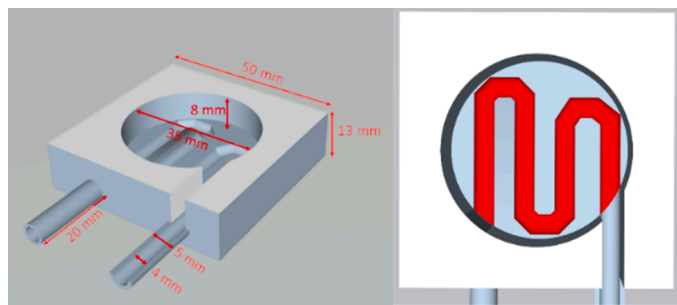

Fig. 1. 3D model of the device: (a) isometric view; (b) top view.

2.5.3. Drug quantification

The concentration of drug that was released in the experiment described in the previous section (2.5.2) was corroborated by high-performance liquid chromatography-tandem mass spectrometry (HPLC MS/MS). To quantify the amount of FOH, the samples were analysed and the corresponding calibrated mass spectrometer in the Agilent 7890 GC chromatograph equipment coupled to a 220 ion TRAP-MS, using a VF5MS column of 30 m length, 0.25 mm internal diameter, and 0.25 microns thick stationary phase layer, applying an initial temperature of 50 °C that was increased by 10 °C/min up to 270 °C, hold time was 5 min and run time 32 min per sample. To determine the amount of cyclo-dextrin, the samples and the corresponding calibration were analysed in a Thermo Q-Exactive equipment Focus Orbitrap using a Poroshell 120 EC—C18 column, working at a 0.2 ml/min workflow using an eluent A of 0.1 % formic water constant at 58 % and an eluent B of acetonitrile constant at 42 %.

3. Results and discussion

3.1. Rheology of gels

3.1.1. PF-127 gel formation

The main characteristic of solutions with PF-127 is that the higher the concentration of the polymer, the lower the temperature at which gelation will occur (Rafael et al., 2021; Sworn et al., 1995). So, the first step was to study the effect of temperature in solutions with different concentrations of PF-127 (15 %, 20 %, 25 %, and 30% w/v).

The oscillatory analysis is illustrated in Fig. 2. Specifically, Fig. 2A shows the storage modulus (G') versus temperature for PF-127 solutions at different concentrations. All the samples showed a G' less than 50 Pa below the LCST temperature, which was lower as the concentration increased (15 %, 20 %, 25 %, and 30 %): 26 °C, 20 °C, 18 °C, and 15 °C, respectively. Above LCST, the G' increased with temperature until it reached a maximum, after which it remained stable, having a constant G' at 37 °C: in the case of the lowest concentration (15 %) this stability was not reached, being found at very low G' values (2468 Pa); while at the 20 % concentration, stability was reached at 27 °C, with G' being 15 kPa; finally, the highest concentrations, 25 % and 30 %, reached stability at 22 °C and 18 °C, respectively, with G' being 25 kPa and 33 kPa, respectively. The second plot (Fig. 2B) represents the frequency sweep of the PF-127 solutions at 37 °C (G' vs. angular frequency), all having a constant G' value, unaffected by the angular frequency, indicating the formation of a strong gel at physiological temperature. This fact can also be observed in the supplementary material (Fig. S.1), which shows the physical difference between PF-127 solutions at 15 % and 20% w/v before and after incubation at 37 °C for 24 h.

Steady state analysis is illustrated in Fig. 2, showing different results at 37 °C and 10 °C. At 37 °C (2C) all samples decreased their viscosity with the shear rate, varying from 100 to 1000 Pa s to 1 Pa s, and showing a strong pseudoplastic character. At 10 °C (2D), in the samples with the lowest concentrations (15 and 20 %), the viscosity was less than 1 Pa s and decreased slightly with shear rate, but remained almost constant, showing Newtonian behaviour; however, the samples with the highest concentrations (25 and 30 %) were predominantly pseudoplastic, decreasing in viscosity from 10 Pa s to 1 Pa s.

According to these results, PF-127 solutions with a concentration of 15 % was discarded because it had a low G' and did not form a stable gel, as well as the samples with the highest concentrations (25 % and 30 %), as they formed stable gels below 25 °C, making them difficult to administer in the body. Therefore, it was decided to work with a concentration of 20 % PF-127, since it was in liquid form below 20 °C and did not reach the maximum G' until 27 °C, being a stable gel at 37 °C with a G' of about 15 kPa.

Results concerning loss modulus (G'') versus temperature and angular frequency are included in the supplementary material (Fig. S.2).
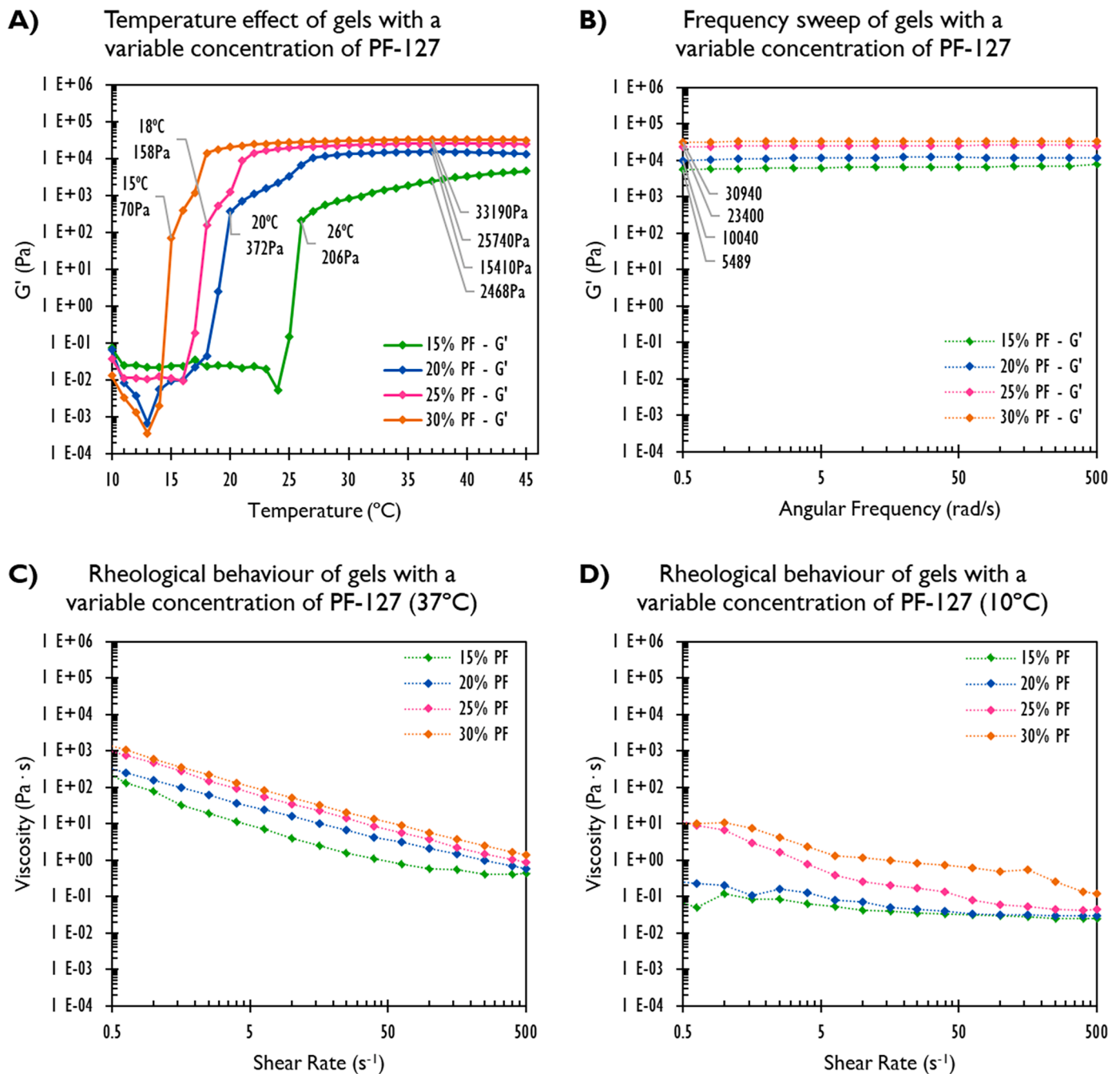
Fig. 2. A) Temperature sweep of the different PF-127 solutions (15 %, 20 %, 25 %, and 30 %) in PBS B) Frequency sweep of PF-127 solutions at 37 °C. Rheological behaviour of PF-127 solutions at different concentrations (15 %, 20 %, 25 %, and 30 %) at 37 °C (C) and 10 °C (D).
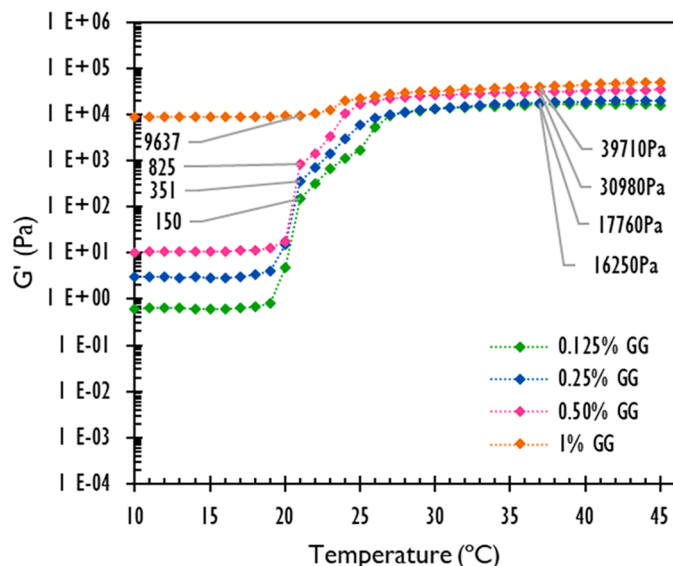
3.1.2. Effect of the addition of GG

In order to increase the gel degradation time by tuning the gel structure without changing the rheological properties, it was proposed to incorporate GG into the PF-127 gels, for which tests with different concentrations of GG (0.125 %, 0.25 %, 0.50 %, and 1% w/v) were carried out. A maximum GG concentration of 1 % was used to prevent potential problems for a potential injectable administration.

On the one hand, oscillatory analysis of GG solutions without PF-127 (see supplementary material, Fig. S.3.A) indicated that G' was not affected by temperature changes between 10 °C and 45 °C, as expected, since, according to the bibliography (Bradbeer et al., 2015; Joseph et al., 2007), GG changes its structure (from double helical to spiral) above 80 °C. When it cools down, it changes its structure as it drops below 42 °C, but in the heating process it needs a higher temperature. The G'
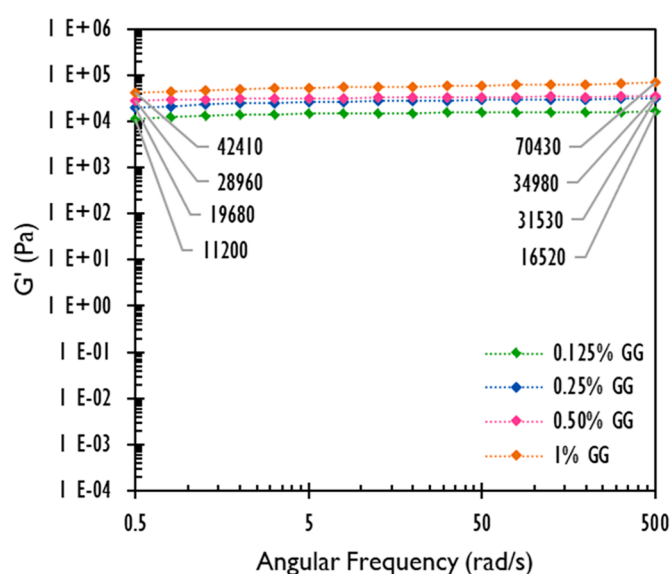
values were 2 Pa, 49 Pa, 415 Pa, and 5280 Pa for each concentration: 0.125 %, 0.25 %, 0.50 %, and 1 %, respectively, at 37 °C. Regarding the rheological oscillatory results of these solutions (Fig. S.3.B), it is important to indicate that GG at a concentration of 0.125 % promoted a crossover point between the G'' and G', noting the weak character of the gel formed and its viscous nature at high frequency; the rest of them were strong gels because G'' was much lower than G'. This was confirmed with the frequency sweep test, since there is a higher dependence of the storage modulus on the frequency for the 0.125 % GG solutions. However, this dependence was not observed for higher concentrations (formation of stronger gels). The G' values for those concentrations at 0.5 rad/s were: 60 Pa, 477 Pa, and 5781 Pa for 0.25 %, 0.50 %, and 1 % GG, respectively.

On the other hand, the results of combining PF-127 (20 %) and GG

A) Temperature effect of gels with 20% PF-127 and a variable concentration of GG

Values labeled: 9637, 825, 351, 150; and 39710Pa, 30980Pa, 17760Pa, 16250Pa

Legend: 0.125% GG, 0.25% GG, 0.50% GG, 1% GG

B) Frequency sweep of gels with 20% PF-127 and a variable concentration of GG

Values labeled: 42410, 28960, 19680, 11200; and 70430, 34980, 31530, 16520

Legend: 0.125% GG, 0.25% GG, 0.50% GG, 1% GG

C) Rheological behaviour of gels with 20% PF-127 and a variable concentration of GG (37°C)

Legend: 0.125% GG, 0.25% GG, 0.50% GG, 1% GG

D) Rheological behaviour of gels with 20% PF-127 and a variable concentration of GG (10°C)
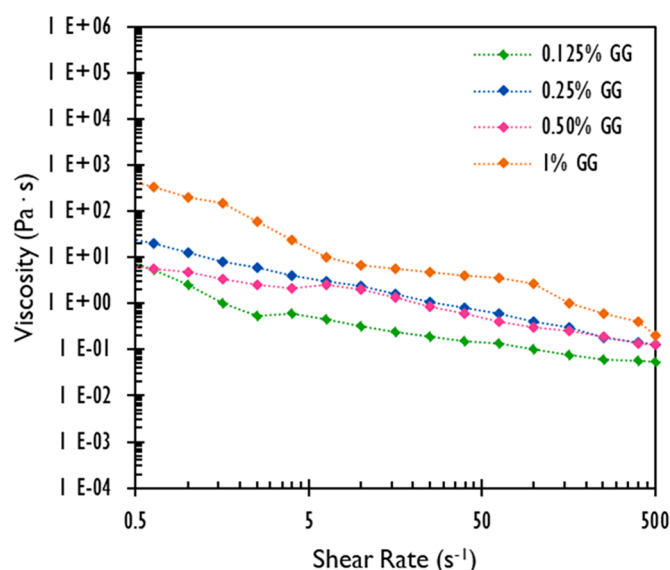
Legend: 0.125% GG, 0.25% GG, 0.50% GG, 1% GG
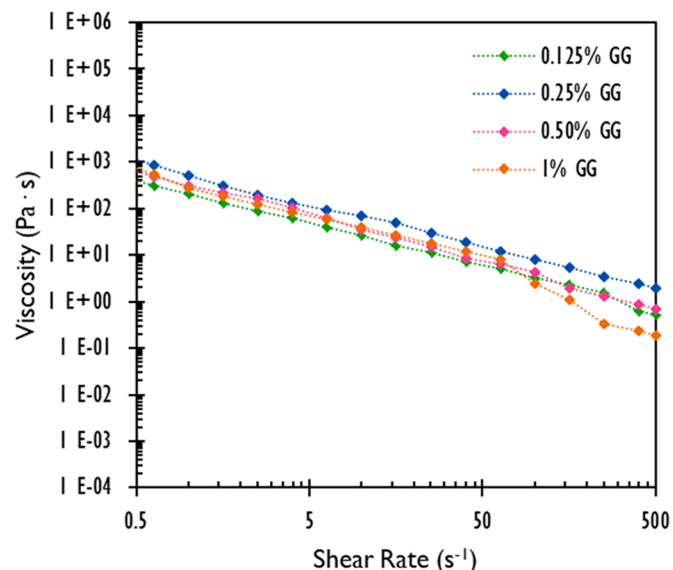
Fig. 3. (A) Temperature sweeps from 10 to 45 °C of samples with PF-127 at 20 % and GG at different concentrations in PBS. (B) Frequency sweep of PF-127 and GG solutions at 37 °C. And rheological behaviour of solutions with PF-127 (20 %) and GG at different concentrations at 37 °C (C) and 10 °C (D).

(Fig. 3A) indicate that at low concentrations of GG, the system is clearly temperature sensitive (from 10 to 45 °C). The LCST was 20 °C for all samples and they were stables gels at 37 °C, the G' values were: 16,250 Pa at 0.125 %, 17,760 Pa at 0.25 %, and 30,980 Pa at 0.50 %. Nevertheless, in the case of 1 % GG, the polysaccharide gel structure predominated over PF-127, keeping the sample in gel form without temperature sensitivity, which could be a problem for future systemic administration. Furthermore, frequency sweep analysis (Fig. 3B), indicated that there is a slight increase of the G' with frequency; showing that the gels still have remaining interactions between the different chains.

Finally, Fig. 3 also shows the rheological behaviour at 37 °C and 10 °C with steady state analysis. In the case of samples without PF-127, similar plots were obtained at different temperatures, since in this range

of temperature these solutions were not affected to a great extent (see supplementary material, Fig. S.4). In all cases, the higher the shear rate, the lower the viscosity, so they behaved as pseudoplastic fluids. When GG was combined with PF-127 at 20 % differences in the graphs of the different temperatures were observed (Fig. 3C and D). Although they continued to be pseudoplastic solutions, the viscosity values were higher at 37 °C than at 10 °C for all solutions due to the formation of a stronger network as a result of the addition of GG; except for the 1 % solution, where the values were similar because, as mentioned before, GG predominates over PF-127.

Results of combining PF-127 (20 %) and GG concerning loss modulus (G'') versus temperature and angular frequency are included in the supplementary material (Fig. S.5).

Taking all these results into account, it was decided that the optimal

concentration of GG was 0.50 %, which allows working with systems in the liquid state at low temperatures (G' is 18 Pa at 20 °C) and makes the gel stronger and more stable at 37 °C (G' is more than 30 kPa).

3.1.3. Study of the effect of the addition of FOH, CD, and CD-FOH

To determine the effect of the addition of FOH on the gel, the rheology was studied by adding FOH at different concentrations (4 mM, 20 mM, 40 mM, 200 mM, and 400 mM) to solutions with 20 % PF-127 and 0.50% w/v GG. Nevertheless, since FOH has poor solubility in aqueous solvents (approximately $8 \cdot 10^{-6}$ M), the CD-FOH complex was also studied, using CD-FOH at 40–20 or 8–4 mM and, as a control of cyclodextrin, CD at 40 or 8 mM without FOH. These concentrations were chosen based on the respective IC50 and the low solubility of FOH, in order to achieve a sustained release (with a proper antitumoral effect) that could be quantified, overcoming instrument's detection limit (Xu et al., 2014). As can be seen in the Fig. 4, above 200 mM of FOH we have an emulsion, not a dispersion.

Fig. 5A shows that the LSCT did not change with respect to the control when FOH was added at the minimum concentration (4 mM), but as the concentration of free FOH increased, the LSCT decreased, reaching a LCST of around 10 °C (G' of 600 Pa) if 400 mM FOH was added. However, once gelled at 37 °C, the FOH concentration increased the G' (from 16 kPa to 20 kPa), and these values remained constant with the angular frequency (Fig. 5B). This also indicated the formation of a strong gel for all FOH concentrations. Based on these results, a concentration of 20 mM FOH was chosen since this is the maximum amount that can be added to overcome the problems of using FOH as a drug, following our explanation in our previous paragraph, without modifying the rheological behaviour of the formulations.

Moreover, as the use of CD may be necessary to increase the solubility of FOH, a rheological study was performed with the respective systems with free CD and CD-FOH with 20 mM FOH. Fig. 5C shows that the addition of FOH or CD at those concentrations did not significantly affect the LSCT; however, at 37 °C the presence of CD drastically reduced the G', which was approximately 20 kPa for the PBS control and the sample with free FOH, compared to approximately 3 kPa for the CD and CD-FOH samples. It seems, therefore, that the addition of the inclusion complex hinders the formation of the polymeric network, as some polymeric chains try to be hosted in the CD cavity. This explanation is consistent with previous works that used cyclodextrins to minimise the assembly in polymeric micelles [26]. Finally, Fig. 5D shows that, for all the studied formulations, the angular frequency did not affect G' (strong gel formation). This figure also indicates that the addition of the free CD reduced the G' because the CD hosts polymeric chains, thus reducing the solid nature of the formulation. However, this reduction was not observed for the other formulations because, as explained above, the CD hosts FOH instead of polymeric chains in the formulation network. In this context, the results concerning the loss modulus (G") of the respective formulations are included in the supplementary material (Fig. S.6).

Finally, Fig. 6 (steady state analysis) illustrates that the system

maintained its pseudoplastic behaviour with the addition of the complex and the single molecules. In the case of free FOH samples at different concentrations, it is observed that at 37 °C (Fig. 6A) all samples had approximately the same behaviour (zero viscosity around 1000 Pa s), while at 10 °C (Fig. 6B) the viscosity values increased with increasing FOH concentration (from 10 to 100 Pa s). This phenomenon at 10 °C was attributed to the poor solubility of FOH in water, forming an emulsion, which contributed to an increase in the initial viscosity with the amount of FOH added. However, at 37 °C, the gel was formed due to the gelation of PF-127, and as a consequence the FOH was entrapped in the gel and its contribution to the viscosity was less significant.

On the other hand, comparing the CD, CD-FOH and FOH values, it is observed that at 37 °C (Fig. 6C) the control sample (PBS) had similar values to those of free FOH, while the samples with CD had a lower viscosity. In this case, these results can be explained by considering the competition between the different compounds to be hosted by the CD (as was explained before) and how polymeric chains can be included in the CD, decreasing their contribution to the viscosity. This fact was not observed at 10 °C (Fig. 6D) because the PF-127 gel was not formed (with no structure) and only free FOH increased the viscosity because of the emulsification phenomenon.

These results indicated the possibility of controlling the LCST of the hydrogel with the addition of different compounds, such as CD, GG or FOH. This phenomenon highlights how the injectable character of a formulation with PF-127 can be modified. This fact can be relevant to define a potential pharmaceutical application of the developed system.

3.2. Gels degradation and drug delivery

According to the method described in Section 2.4.1., the degradation of gels was quantified by measuring the amount of mass loss at different times. The results were used for parameter estimation (Section 2.4.2.) and the adjustment for different sets of samples analysed is represented in Fig. 7.

Fig. 7A shows how the concentration of GG in hydrogel preparation modified the degradation profile. First of all, in all degradation profiles, the swelling phenomenon was not significant and can therefore be considered negligible for modelling and parameter estimation.

This figure also shows that a higher amount of GG in the hydrogel was related to a more stable gel (slower degradation rate), which shows that there are more interactions between the chains and, therefore, more time is needed for a complete degradation of the structure. The degradation is slower, increasing from 3 h, when there is no GG, to 5 h, when the GG concentration is higher than 0.125 %, or even to 6 and 7 h when it is 0.50 % and 1 %, respectively.

Fig. 7B and C indicate that the addition of FOH in free form or complexed with CD modified the degradation of the formulation when 20 % PF-127 and 0.50 % GG were added to the gel. Specifically, Fig. 7B shows how the addition of free FOH to the hydrogel modified the degradation profile, where the slowest rate was achieved with the highest amount of FOH in the sample. One possible explanation for this phenomenon is that the hydrophobicity of FOH, by forming an emulsion, modifies the relaxation process between the chains within the gel, and therefore, more time is needed to obtain the same degree of degradation.

Finally, Fig. 7C shows how the addition of CD, both free and complexed with FOH, modified the degradation rate. The higher the concentration of CD in the sample, the slower the degradation. This phenomenon could be explained by the fact that some polymeric chains may be complexed with the CD inside the gel, making them more difficult to be available for degradation. In the past, CD has proven to be effective in hosting polymeric micelle chains [26]. The same phenomenon can be considered when the inclusion complex is trapped inside the PF-127 gel.

These results can also be corroborated by analysing the values of K' estimated by Excel Solver. They are summarised in Fig. 7 as well as the
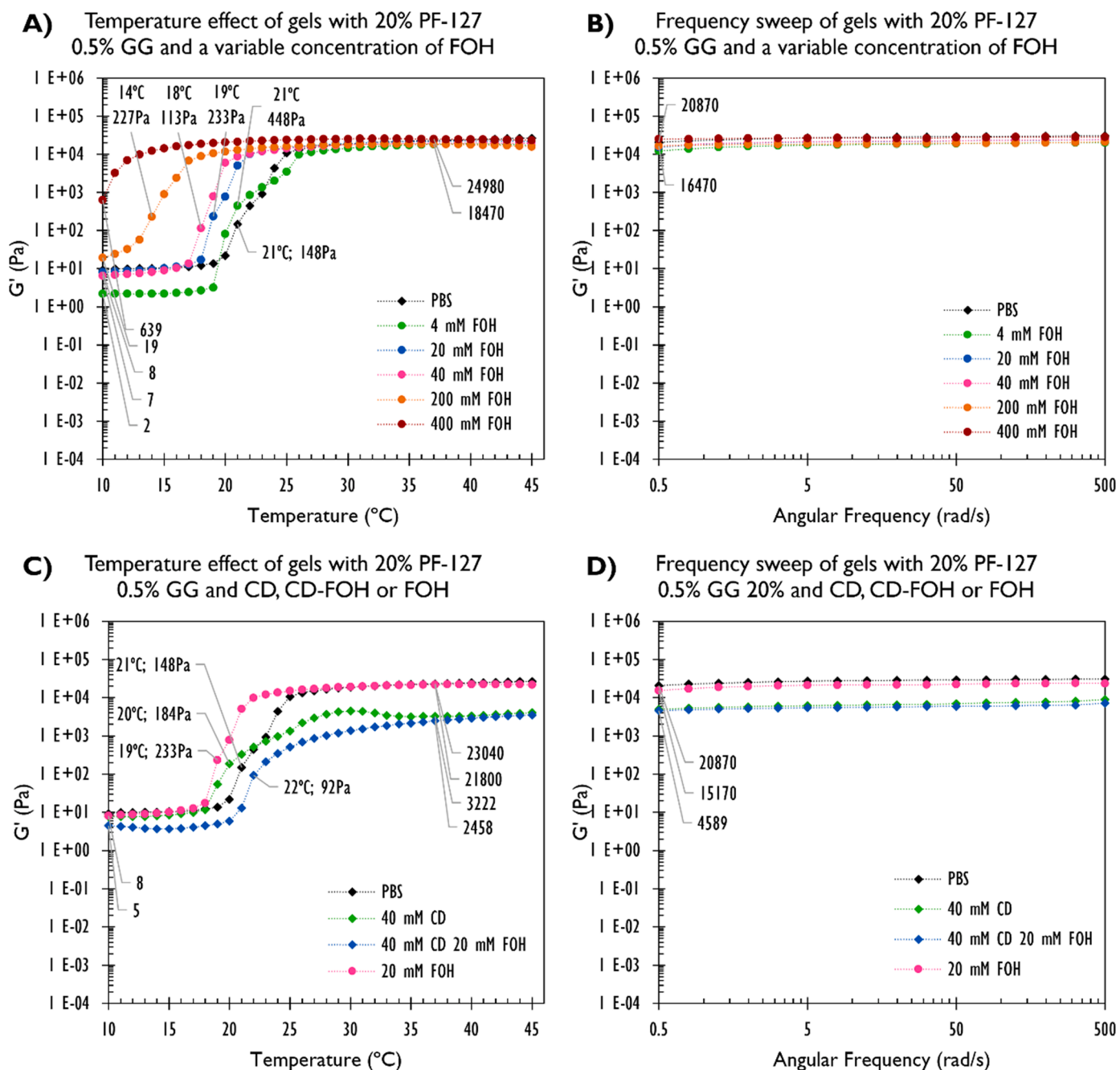

Fig. 4. Hydrogels of PF-127 at 20 % and GG at 0.50 %, with 10 % v/v of different solutions added: CD, CF-FOH (CDF) or FOH at variable concentrations, and PBS as control.

Fig. 5. (A) Temperature sweeps from 10 to 45 °C of samples with PF-127 at 20 %, GG at 0.50 %, and free FOH at different concentrations in PBS. (B) Frequency sweeps of solutions with different concentrations of FOH at 37 °C. (C) Temperature sweeps from 10 to 45 °C of samples with PF-127 at 20 %, GG at 0.50 %, and FOH on different systems (free FOH, CD-FOH, and CD control). D) Frequency sweeps of solutions with PF-127 at 20 %, GG at 0.50 %, and FOH on different systems (free FOH, CD-FOH, and CD control) at 37 °C.

errors calculated for each adjustment. Results in Fig. 7 show that the fitting process has been carried out correctly, as the AADs are less than 10% in all cases and, therefore, there is a statistical significance in the proposed adjustment. The behaviour observed in Fig. 7 was corroborated by analysing the K' values. There was a significant decrease in the K' value as the FOH concentration increased. A similar effect was observed when the proportion of GG in the hydrogel composition was considered. Based on this data, it could be possible to control the degradation profile of hydrogels by modifying their composition according to the intended application.

Although the degradation time for every gel was around 7 h, it is possible to observe different degradation profiles. This phenomenon is

confirmed by the differences in the value of the degradation parameter K'. For instance, this parameter increases from 0.633 (CD-FOH 8/4 mM) to 0.524 (CD-FOH 40/20 mM) that is around a 15 %. Also, around 55% of the gel was degraded in 3 h for CD-FOH 40/20 mM whereas only 30 % of gel mass was found for the formulation CD-FOH 8/4 mM in the same time.

These modelling results can also be explained by the oscillatory and steady state rheological results. The K' value decreased with the increase in the storage modulus of the PF-127–GG solutions and with an increase in the initial viscosity of the solutions. The increase in storage modulus favoured the formation of a stronger gel, making the release of the polymeric chains more difficult due to the facilitated interactions.
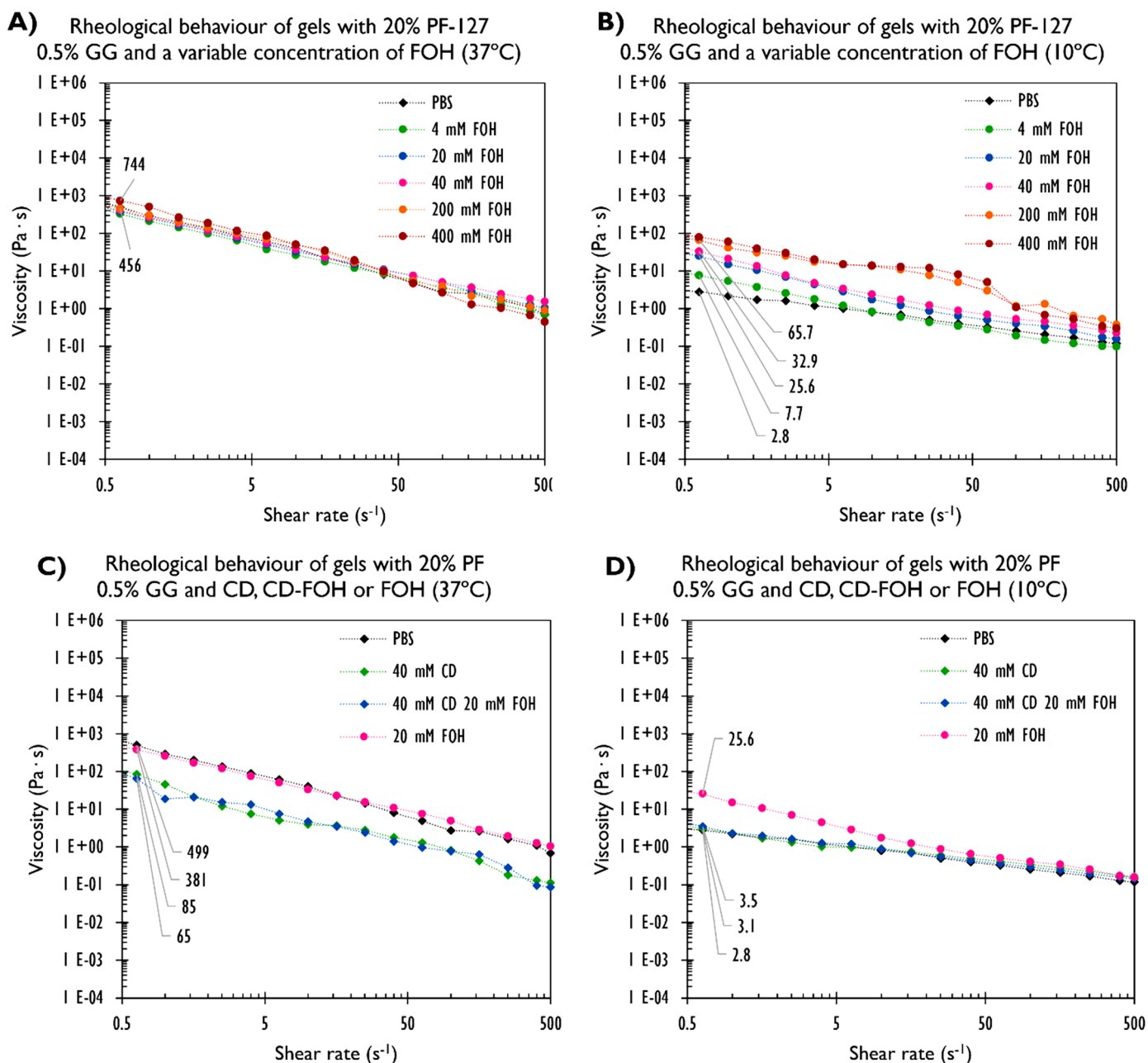
Fig. 6. Rheological behaviour of solutions with PF-127 at 20%, GG at 0.50 %, and FOH at different concentrations at 37 °C (A) and 10 °C (B). Rheological behaviour of solutions with PF-127 at 20 %, GG at 0.50 %, and FOH on different systems at 37 °C (C) and 10 °C (D).

This phenomenon, however, was not observed in the gels with the inclusion complex. The addition of CD-FOH did not modify the storage modulus due to the competition that can occur between CD and the different compounds. However, the entrapping of CD within the PF-127 gel decreased the degradation rate due to the formation of inclusion complexes, as mentioned above, but also reduces the storage modulus of the solutions because the polymeric chains were hosted in the cavity, preventing the possibility of contributing to the solid nature of the system.

After the degradation study, the release assay was performed with the sample with the highest concentration of non-emulsified FOH (lower than 40 mM FOH) without CD, analysing the released of FOH (given in concentration) every hour. A constant amount of FOH was continuously released every hour, up to a saturated concentration around 8 μM FOH. This result indicates that the released quantity of FOH is approximately the maximum solubility of FOH in aqueous media. This causes the FOH

to remain preferentially in the gel and be released only by degradation of the gel. Similar results were found when the FOH release was determined for the CD-FOH complex 40/20 mM (the FOH was sustainedly released according to the FOH aqueous solubility). Therefore, although FOH can be administered in any form, it is best used as a complex with cyclodextrin, ensuring the solubility of FOH in the blood and its permeabilization in cells.

From a pharmaceutical point of view, Fig. 7 indicates that, besides controlling the LCST, the degradation of an injectable hydrogel can be also tuned with the addition of GG and CD. This fact is important, since CD can act as a carrier for a hydrophobic and FDA approved drug, FOH in this case, providing a sustained drug release. The proposed injectable formulation can overcome different drawbacks that have been found in other type of drug release systems.
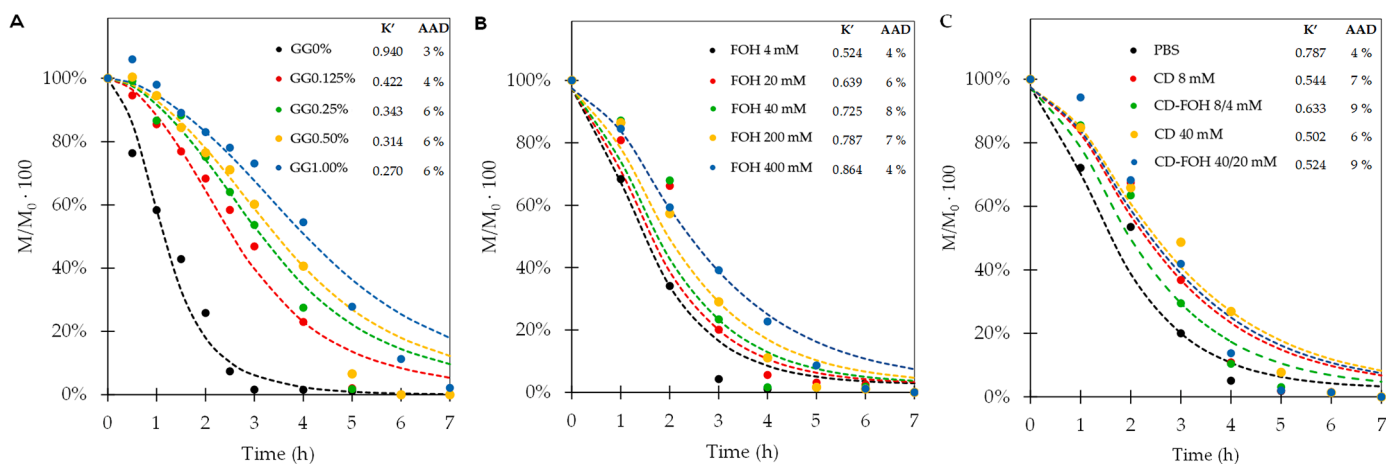
Fig. 7. Results of experimental degradation of different types of hydrogels and their adjustment after parameter estimation. (A) Degradation of hydrogels with different proportions of gellan gum. (B) Degradation of hydrogels containing free FOH at different concentrations. (C) Degradation of hydrogels containing CD at different concentrations and with inclusion complexes CD-FOH. Filled dots represent experimental data and their corresponding adjustment is represented by lines in the same colours. With values of parameters determined by adjusting the experimental results and their average absolute deviation.

4. Conclusions

Thermosensitive gels based on PF-127 and GG were prepared for potential local cancer therapy. The addition of the GG favoured the formation of polymeric interactions, increasing the storage modulus and viscosity, as well as the degradation rate. Furthermore, GG could be useful to control temperature sensitivity. In fact, a concentration of 0.50 % GG doubled the degradation rate (from 3 to 6 h) of a based gel (20 % PF-127) without further modifying the gelation temperature (which started at about 20 °C). Moreover, FOH was incorporated into the gel as a free drug and as an inclusion complex (CD-FOH). These incorporations modified the rheology and the polymeric network, depending on the temperature and also on the addition of the CD. In particular, the inclusion of free CD promoted the formation of polymer-CD complexes, decreasing the storage modulus and viscosity, but also increasing the degradation time. However, free FOH always increases the solid nature of the formulation as well as the degradation rate due to the existence of an emulsion. The complex CD-FOH did not modify the rheological spectrum to a great extent, due to the competition between the previous phenomena.

The degradation results were also modelled, explaining the results based on the release chains of the polymeric system. The release chains matched with the rheological results for the PF-127–GG structures, as the interactions increased the degradation rate and storage modulus. However, the addition of the inclusion complex increased the degradation rate but decreased the storage modulus. This can be explained by the difficulty of the system to release the chains when they are hosted in the CD and as a consequence of the increased degradation rate.

Finally, the drug delivery studies indicated that sustained release was achieved, and was proportional to gel degradation, being 2 mM per hour when 400 mM of free FOH was loaded into the gel and 0.2 mM per hour when the gels were loaded with the inclusion complex (40 mM CD and 20 mM FOH). The problem with free FOH is that when it is released, it is at a concentration higher than its solubility in aqueous solvents ($8 \cdot 10^{-3}$ mM), therefore, the use of the CD-FOH complex, which allows total solubility of the released concentration, is recommended.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Supplementary materials

The following supporting information can be downloaded at: _____ S.1. Incubation of PF-127 at a concentration of 15 % and 20 % at 37 °C; S.2. Loss modulus (G'') of gels at a variable concentration of PF-127 versus temperature (A) and angular frequency (B); S.3. Loss modulus (G'') of gels at a variable concentration of GG without PF-127 versus temperature (A) and angular frequency (B) and loss modulus (G'') of gels at a variable concentration of GG with 20 % PF-127 versus temperature (C) and angular frequency (D); and S.4. Loss modulus (G'') of gels (20 % PF-127 and 0.5 % GG) at a variable concentration of FOH versus temperature (A) and angular frequency (B). And loss modulus (G'') of gels (20 % PF-127 and 0.5 % GG) with FOH, CD or CD-FOH versus temperature (C) and angular frequency (D).

CRediT authorship contribution statement

Inmaculada de Dios-Pérez: Methodology, Validation, Formal analysis, Writing – original draft. **Álvaro González-Garcinuño:** Conceptualization, Investigation, Software, Writing – review & editing, Supervision. **Antonio Tabernero:** Conceptualization, Investigation, Software, Writing – review & editing, Supervision. **Marcos Blanco-López:** Methodology, Validation, Formal analysis. **Juan A. García-Esteban:** Methodology, Validation, Software, Writing – original draft. **Vidal Moreno-Rodilla:** Investigation, Supervision, Resources, Writing – review & editing. **Belén Curto:** Investigation, Supervision, Resources, Writing – review & editing. **Patricia Pérez-Esteban:** Supervision, Formal analysis, Writing – review & editing. **Eva M. Martín del Valle:** Funding acquisition, Project administration, Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2023.106618.

References

Bradbeer, J.F., Hancocks, R., Spyropoulos, F., Norton, I.T., 2015. Low acyl gellan gum fluid gel formation and their subsequent response with acid to impact on satiety. Food Hydrocoll. 43, 501–509. https://doi.org/10.1016/j.foodhyd.2014.07.006.

Chen, Y.C., Gad, S.F., Chobisa, D., Li, Y., Yeo, Y., 2021. Local drug delivery systems for inflammatory diseases: status quo, challenges, and opportunities. JCR 330, 438–460. https://doi.org/10.1016/j.jconrel.2020.12.025.

Chen, Y.Y., Wu, H.C., Sun, J.S., Dong, G.C., Wang, T.W., 2013. Injectable and thermoresponsive self-assembled nanocomposite hydrogel for long-term anticancer drug delivery. Langmuir 29 (11), 3721–3729. https://doi.org/10.1021/la400268p.

de Dios-Pérez, I., González-Garcinuño, Á., Del Valle, E.M.M., 2022. An approach to minimize tumour proliferation by reducing the formation of components for cell membrane. Molecules 27 (9). https://doi.org/10.3390/molecules27092735.

Diniz, I.M.A., Chen, C., Xu, X., Ansari, S., Zadeh, H.H., Marques, M.M., Shi, S., Moshaverinia, A., 2015. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. J. Mater. Sci. Mater. Med. 26 (3), 1–10. https://doi.org/10.1007/s10856-015-5493-4.

Gil, M.S., Thambi, T., Phan, V.H.G., Kim, S.H., Lee, D.S., 2017. Injectable hydrogel-incorporated cancer cell-specific cisplatin releasing nanogels for targeted drug delivery. J. Mater. Chem. B 5 (34), 7140–7152. https://doi.org/10.1039/c7tb00873b.

Joseph, J., Dreiss, C.A., Cosgrove, T., Pedersen, J.S., 2007. Rupturing polymeric micelles with cyclodextrins. Langmuir 23 (2), 460–466. https://doi.org/10.1021/la061850g.

Kesharwani, P., Bisht, A., Alexander, A., Dave, V., Sharma, S., 2021. Biomedical applications of hydrogels in drug delivery system: an update. J. Drug Deliv. Sci. Technol. 66 https://doi.org/10.1016/j.jddst.2021.102914.

Li, P., Hou, X., Qu, L., Dai, X., Zhang, C., 2018. PNIPAM-MAPOSS hybrid hydrogels with excellent swelling behavior and enhanced mechanical performance: preparation and drug release of 5-fluorouracil. Polymers (Basel) 10 (2). https://doi.org/10.3390/polym10020137.

Liu, M., Song, X., Wen, Y., Zhu, J.L., Li, J., 2017. Injectable thermoresponsive hydrogel formed by alginate-g-poly(N-isopropylacrylamide) that releases doxorubicin-encapsulated micelles as a smart drug delivery system. ACS Appl. Mater. Interfaces 9 (41), 35673–35682. https://doi.org/10.1021/acsami.7b12849.

Marabelle, A., Andtbacka, R., Harrington, K., Melero, I., Leidner, R., de Baere, T., Robert, C., Ascierto, P.A., Baurain, J.F., Imperiale, M., Rahimian, S., Tersago, D., Klumper, E., Hendriks, M., Kumar, R., Stern, M., Öhrling, K., Massacesi, C., Tchakov, I., Tse, A., Douillard, J.-Y, Tabernero, J., Haanen, J., Brody, J., 2018. Starting the fight in the tumor: expert recommendations for the development of human intratumoral immunotherapy (HIT-IT). Ann. Oncol. 29 (11), 2163–2174. https://doi.org/10.1093/annonc/mdy423.

Mohammadi, M., Karimi, M., Malaekeh-Nikouei, B., Torkashvand, M., Alibolandi, M., 2022. Hybrid in situ- forming injectable hydrogels for local cancer therapy. Int. J. Pharm. 616 https://doi.org/10.1016/j.ijpharm.2022.121534.

Mohanty, D., Bakshi, V., Simharaju, N., Haque, M.A., Sahoo, C.K., 2018. A review on in situ gel. A novel drug delivery system. Int. J. Pharm. Sci. Rev. Res. 50 (1), 175–181.

Nieto, C., Vega, M.A., Rodríguez, V., Pérez-Esteban, P., Martín del Valle, E.M., 2022. Biodegradable gellan gum hydrogels loaded with paclitaxel for HER2+ breast cancer local therapy. Carbohydr. Polym. 294 https://doi.org/10.1016/j.carbpol.2022.119732.

Rafael, D., Melendres, M.M.R., Andrade, F., Montero, S., Martinez-Trucharte, F., Vilar-Hernandez, M., Durán-Lara, E.F., Schwartz, S., Abasolo, I., 2021. Thermo-responsive hydrogels for cancer local therapy: challenges and state-of-art. Int. J. Pharm. 606 https://doi.org/10.1016/j.ijpharm.2021.120954.

Sweety, J.P., Selvasudha, N., Dhanalekshmi, U.M., Sridurgadevi, N., 2022. Gellan gum and its composites. Suitable candidate for efficient nanodrug delivery. In: Nanoengineering of Biomaterials, 2. Wiley, pp. 33–61.

Sworn, G., Sanderson, G.R., Gibson, W., 1995. Gellan gum fluid gels. Food Hydrocoll. 9 (4), 265–271. https://doi.org/10.1016/S0268-005X(09)80257-9.

Turabee, M.H., Jeong, T.H., Ramalingam, P., Kang, J.H., Ko, Y.T., 2019. N, N, N-trimethyl chitosan embedded in situ Pluronic F127 hydrogel for the treatment of brain tumor. Carbohydr. Polym. 203, 302–309. https://doi.org/10.1016/j.carbpol.2018.09.065.

Wu, Z.X., Zou, X.Y., Yang, L.L., Lin, S., Fan, J., Yang, B., Sun, X.Y., Wan, Q., Chen, Y., Fu, S.Z., 2014. Thermosensitive hydrogel used in dual drug delivery system with paclitaxel-loaded micelles for in situ treatment of lung cancer. Colloids Surf. B Biointerfaces 122, 90–98. https://doi.org/10.1016/j.colsurfb.2014.06.052.

Xiao, Y., Gu, Y., Qin, L., Chen, L., Chen, X., Cui, W., Li, F., Xiang, N., He, X., 2021. Injectable thermosensitive hydrogel-based drug delivery system for local cancer therapy. Colloids Surf. B Biointerfaces 200. https://doi.org/10.1016/j.colsurfb.2021.111581.

Xu, J., Feng, E., Song, J., 2014. Bioorthogonally cross-linked hydrogel network with precisely controlled disintegration time over a broad range. J. Am. Chem. Soc. 136 (11), 4105–4108. https://doi.org/10.1021/ja4130862.

Yu, J., Qiu, H., Yin, S., Wang, H., Li, Y., 2021. Polymeric drug delivery system based on pluronics for cancer treatment. Molecules 26 (12). https://doi.org/10.3390/molecules26123610.