

Article

Tetronic[®] 1307-Based Polymeric Micelles and Thermoresponsive Gels for the Co-Delivery of Pentamidine and Miltefosine

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Abstract

Background: Pentamidine isethionate (PTM) and miltefosine (MF) are clinically relevant antiparasitic agents whose use is limited by toxicity, emerging resistance, and the lack of effective co-delivery strategies. Tetronic[®] 1307 (T1307), an amphiphilic and thermoresponsive block copolymer, was investigated as a carrier to enable their combination therapy. **Methods:** PTM and MF were formulated in T1307-based micelles and thermoresponsive gels. The systems were characterized by small-angle neutron scattering (SANS), dynamic light scattering (DLS), and nuclear magnetic resonance spectroscopy (NMR). Antiparasitic activity was evaluated against *Leishmania major* promastigotes. **Results:** MF formed stable micelles that efficiently incorporated PTM, generating a “drug-in-drug” architecture. While T1307 alone showed limited PTM loading, MF promoted mixed micelle formation and enhanced PTM incorporation. At physiological temperature and adequate copolymer concentrations, drug-loaded micelles formed thermoreversible gels suitable for topical application. The combined formulations preserved drug activity and exhibited synergistic effects against *L. major*. **Conclusions:** T1307 is a promising platform for the co-delivery of PTM and MF, enabling synergistic combination therapy and thermoresponsive gel formation with potential to reduce systemic toxicity and improve treatment administration.

Keywords: polymeric micelles; gels; miltefosine; pentamidine; Tetronic; leishmaniasis; drug delivery; SANS



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1. Introduction

Miltefosine (MF) is an alkylphospholipid derivative of eldefosine, originally developed as an antineoplastic agent. Due to its amphiphilic nature, it self-assembles into micelles at concentrations below 50 μM [1–3]. The pharmacological activity of MF is primarily attributed to its ability to disrupt cell membrane integrity through intercalation into the lipid bilayer. In tumor cells, MF induces apoptosis mainly by inhibiting phosphatidylcholine (PC) biosynthesis via suppression of phosphocholine cytidyltransferase, which impairs sphingomyelin and diacylglycerol synthesis and promotes ceramide accumulation,

a key mediator of cell death [4,5]. Additional reported mechanisms include the activation of the SAPK/JNK pathway [6,7] and the inhibition of the insulin-PKB/Akt signaling cascade, resulting in cell cycle arrest [5]. Beyond its applications in oncology, MF was repurposed as the first and only oral drug approved for the treatment of leishmaniasis, a vector-borne disease caused by more than 20 species of *Leishmania* parasites [8]. This neglected tropical disease, endemic in many developing countries and increasingly present in industrialized regions, affects 700,000 to 1 million people annually and manifests as cutaneous, mucocutaneous, or visceral forms. Cutaneous leishmaniasis (CL) is the most prevalent form and is characterized by skin lesions, mainly ulcers on exposed body areas, which often result in permanent scarring, severe disability, or social stigma. Approximately 95% of CL cases are reported in the Americas, the Mediterranean basin including several European countries, the Middle East, and Central Asia. Regarding visceral leishmaniasis, it is the most severe and potentially fatal [9–14].

In recent decades, pentamidine (PTM), a diamidine derivative originally synthesized in 1930 as a hypoglycemic agent against trypanosomatids [15], has been explored for potential applications in Alzheimer's disease, Parkinson's disease, cancer, diabetes, and muscular dystrophy [16–25]. PTM has been reported to antagonize NMDA (N-methyl-D-aspartate) receptors, inhibit histone acetyltransferase and calmodulin, and interfere with polyamine synthesis and RNA polymerase activity [21–23,26,27].

Both PTM and MF present significant limitations due to adverse effects and the development of drug resistance [28–31]. Thus, MF is contraindicated in pregnancy due to teratogenicity, it cannot be administered intravenously because of hemolytic activity, and its oral use is often associated with gastrointestinal disturbances, hepato-nephrotoxicity, and frequent emergence of resistance [32,33]. On the other hand, PTM, administered parenterally, can cause nausea, vomiting, hypotension, and injection site pain, and it has been linked to glucose metabolism disorders, acute hepatic injury, and cardiovascular complications [15,32,34].

To overcome these drawbacks, different approaches based on novel drug delivery systems have been attempted [35]. Only a limited number of studies have investigated the use of liposomal formulations for PTM or MF. These include sugar-grafted liposomes and liposomes containing ergosterol or cholesterol for PTM delivery [36,37], as well as thermosensitive liposomes developed for the encapsulation of MF [38,39]. Although an overall improvement in the toxicity profile of these liposomal formulations has been reported, the available evidence is largely derived from preliminary “in vitro” studies. In this context, it is especially important to characterize the drug release profile following administration into the bloodstream, as this parameter will largely determine the extent and severity of adverse effects. In the case of thermoreversible liposomes, a rapid release of approximately 50% of the total encapsulated drug has been reported, suggesting that the development of formulations capable of providing a more sustained and controlled release over time may be necessary to improve their therapeutic profile [40–42].

Research on polymeric nanoparticles has been also intensified, as demonstrated by the ample number of studies developing different polymeric architectures for the encapsulation of both drugs. Commonly used vehicles for the encapsulation of both PTM and MF include polymethacrylate, polycaprolactone (PCL), chitosan, and poly(D,L-lactide) (PLA) [43–47]. These nanoparticles can enhance the poor solubility of hydrophobic molecules and easily, stabilize degradable compounds [48]. Likewise, they can enhance drug retention in tissues, increase the amount of drug reaching the target site, and improve specificity [49–51]. Nevertheless, nanoparticles present certain limitations, including low drug encapsulation efficiency, particularly for hydrophilic compounds such as pentamidine (PTM) [52]. In addition, the identification of materials that enable controlled drug release while avoid-

ing initial burst release phenomena or, conversely, excessively slow release profiles, is challenging [53,54]. Furthermore, many nanoparticle-based formulations require surface functionalization to improve stability, biodistribution, or targeting capability [55].

A different approach relies on polymeric micelles, which form by the self-assembly of amphiphilic polymers above their critical micelle concentration and temperature [56,57]. Amphiphilic diblock copolymers (e.g., polystyrene–poly(ethylene glycol)) and triblock copolymers (e.g., poloxamers) have been widely studied, although graft copolymers (e.g., g-chitosan) and ionic copolymers (e.g., poly(ethylene glycol)-poly(ϵ -caprolactone)-g-polyethyleneimine) are also employed [58,59]. Polymeric micelles are characterized by smaller sizes compared with other nanosystems, such as liposomes or nanoparticles, which makes them attractive for various administration routes [48,60]. Their size also facilitates drug penetration into solid tumors and enhances drug internalization [61,62]. They can improve drug bioavailability and modulate release kinetics, enabling targeted delivery and minimizing side effects. However, their adequate thermodynamic and kinetic stability is essential to prevent premature or uncontrolled drug release, particularly during administration and dilution in the bloodstream [63–65]. Over the last few years, tocopheryl polyethylene glycol succinate (TPGS) and poloxamines [66,67] have been employed as carriers for MF, whereas Pluronic F127 has been recently used for PTM reformulation [68].

In this work, Tetronic® 1307 (T1307) has been proposed as a carrier for both drugs, PTM and MF. T1307 is an X-shaped copolymer composed of polyethylene oxide (PEO) and polypropylene oxide (PPO) with a central diamine group. In solution, it self-assembles into micelles and forms thermoreversible gels depending on the temperature and concentration. These characteristics reinforce Tetronic copolymers as promising candidates for biomedical applications since they can solubilize hydrophobic drugs within both micelles and gels, enabling controlled drug release, enhancing permeability across biological barriers, increasing bioavailability, and reducing potential adverse effects [69–71]. In fact, some recent studies have proven that T1307 forms mixed micelles with MF and reduces its toxicity [2].

To the best of our knowledge, no clinical trials have been conducted recently with both drugs in patients. In 2018, a study explored the combination of oral MF plus intralesional PTM as a treatment for leishmaniasis. Additive adverse effects were reported, and the efficacy of the two drugs was additive [72]. In this context, the present work proposes an integrated strategy to overcome the main limitations associated with PTM and MF therapy, particularly regarding safety and efficacy. A dual-drug approach is introduced, aiming to combine both therapeutic agents to potentially reduce the administered doses without impairing their therapeutic effectiveness or increasing adverse effects. Furthermore, a novel delivery system is proposed to enable the simultaneous encapsulation of both drugs, thereby innovating their administration and improving overall treatment efficacy.

2. Materials and Methods

2.1. Materials

Miltefosine (MF, purity ≥ 98.0 , $M_w = 407.57$ g/mol) and pentamidine isethionate (PTM, purity ≥ 98.0 , $M_w = 592.07$ g/mol) were purchased from Glentham Life Sciences (Wiltshire, UK). Tetronic® 1307 (T1307, 18,000 g/mol average molar mass) was a gift from BASF (Ludwigshafen, Germany), with a reported composition of 72 and 23 ethylene oxide (EO) and propylene oxide (PO) units, respectively. Micellar solutions were prepared by dissolving the drugs and T1307 in water, while gels were prepared by dissolving first the drugs in water and then adding the polymer, followed by several cycles of cooling down to 4 °C and stirring until the complete dissolution of T1307. Unless otherwise stated, the concentrations are expressed as mass percentages.

2.2. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were acquired with a 400 MHz Bruker Avance Neo 400 spectrometer (Bruker Corporation, Billerica, MA, USA), using the default pulse sequences for the 1D and 2D-NOESY spectra. For the diffusion experiments, bipolar pulse longitudinal eddy current delay (BPLED) ledbgpg2s1d and ledbgpg2s pulse sequences were used. Initial 1D-proton spectra were recorded for each applied gradient, and the attenuation of the selected resonances in each spectrum was analyzed by integration. The gradient spacing and duration (Δ and δ , respectively) were optimized to ensure complete exponential signal decay. A 2D diffusion-ordered spectroscopy (DOSY) representation, displaying the NMR spectra on the x-axis and the diffusion coefficient on the y-axis, was generated using MestReNova 14.2 software through the Bayesian transformation of the spectra recorded at different gradients. All samples were prepared by dissolving the required amounts of PTM, MF, and T1307 in D₂O (Aldrich, deuterium content > 99.96%).

2.3. Dynamic Light Scattering (DLS)

Intensity size distributions were obtained using a Protein Solutions DynaPro photon correlation spectrometer (Wyatt Technology LLC, Santa Barbara, CA, USA) operating at 822 nm laser wavelength. The temperature control was provided by the built-in Peltier unit. Experiments were conducted at 20 °C and 37 °C using type II water to prepare all samples. Before measurements, all samples were filtered with 0.45 µm pore-size syringe filters made of PVDF, coupled to 0.02 µm inorganic membrane filters. The intensity size distributions were obtained using regularization analysis with DynaLS version 2.8.2 (Alango Ltd., Tirat Carmel, Israel) software.

2.4. Small-Angle Neutron Scattering (SANS)

SANS experiments were conducted on the LARMOR diffractometer at the ISIS Neutron and Muon Source (Rutherford Appleton Laboratory, UK). Larmor is a fixed-configuration time-of-flight, pinhole SANS instrument with a sample-to-detector distance of 4 m. The usable wavelength (λ) range is $0.9 < \lambda < 13.5$ Å, which gives an accessible q range of $0.004 < q < 0.7$ Å^{−1}, where q is the magnitude of the momentum transfer vector ($q = 4\pi\sin(\theta)/\lambda$, where θ is half the scattering angle). Two different wavelength ranges were used for data processing: a broad one for dilute samples ($0.9 < \lambda < 13.5$ Å) to study the widest range of length scales and a reduced range ($6.0 < \lambda < 13.5$ Å) for gels to improve the resolution of peaks. Samples were loaded into either 1 or 2 mm pathlength quartz (“banjo”) cells, which were placed in the Larmor’s temperature-controlled sample changer, and illuminated by an 11×11 mm² neutron beam. Data were converted from raw data to reduced data of scattering intensity (the scattering cross section per unit volume, $d\Sigma/d\Omega(q)$) as a function of q by correcting for detector efficiency and sample transmission using the instrument-specific software Mantid [73,74]. The raw data were placed on an absolute scale (cm^{−1}) by measuring the scattering of a mixture of hydrogenous and deuterated polystyrene with a known radius of gyration and scattering cross section [75,76]. The scattering from the solvent was measured, processed the same way, and subtracted from the sample data. SANS curves were fitted using SasView 5.0.6 software [76]. The information on the models used and fitting procedures is described in detail in the Results and Discussion section and in the Supporting Information, including statistical information about the goodness of the fits (Figures S9 and S10).

2.5. Steady State Fluorescence

An FLS920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, UK) was used to perform steady state fluorescence measurements of aqueous solutions of PTM in

the presence of T1307 and/or MF at 20 and 37 °C. The emission spectra were recorded by excitation at 270 nm, adjusting the excitation and emission slits to remain within the linear range of the intensity at the concentrations used. All samples were prepared in type II water.

2.6. Rheology

Small-amplitude oscillatory experiments were performed on a dynamic strain-controlled rheometer Discovery HR20 (TA Instruments, Milford, MA, USA) using parallel-plate geometry (25 mm, stainless steel) with a temperature-controlling Peltier unit. All solutions were prepared by weighing the necessary amounts of T1307, MF, PTM, and water followed by magnetic stirring combined with cooling cycles (4 °C) until complete dissolution. The samples were left to rest at least one day at room temperature after preparation before conducting the rheological measurements. After loading, a thin layer of low-viscosity paraffin oil was applied to the edges of the geometry to prevent evaporation. Samples were allowed to rest for a few minutes before the start of the experiments to ensure dissipation of any pre-shearing due to manipulation and loading. Temperature sweeps at a frequency of $6.28 \text{ rad} \cdot \text{s}^{-1}$ and 1% strain amplitude, within the limit of the linear viscoelastic range as measured by strain amplitude experiments, were conducted, at a heating rate of 2 °C per minute, to cover the temperature range from 20 to 80 °C.

2.7. Biological Assessment

2.7.1. Cells and Culture Conditions

Leishmania major promastigotes (Lv39c5, Rho/SU/59/P) were cultured at 26 °C in M199 medium supplemented with 25 mM HEPES (pH 7.2), 0.1 mM adenine, 0.0005% (*w/v*) hemin, 2 mg/mL bioppterin, 0.0001% (*w/v*) biotin, 10% (*v/v*) heat-inactivated fetal bovine serum (FBS), and an antibiotic cocktail (100 U/mL penicillin, 100 µg/mL streptomycin).

2.7.2. Activity Against Promastigotes

Exponentially growing cells (5×10^6 *L. major* promastigotes/mL) in M199 were seeded in 96-well plates (100 µL per well) and first treated with free PTM (0–10 µM) and MF (0–20 µM), as well as with combinations of the two drugs. In addition, parasites were also exposed to both drugs previously incorporated into T1307 micelles. The plates were incubated at 26 °C. After 48 h, 20 µL of MTT was added per well and kept 4 h under the same conditions. Then, DMSO (80 µL) was added to each well to dissolve the formazan crystals. The optical density was measured at 540 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA), and cell viability was calculated relative to untreated cells. Three independent biological replicates were performed for all biological assays, each with four technical replicates. The half maximal inhibitory concentration (IC₅₀) was obtained with OriginPro version 8.5 using the Boltzmann model. The results were expressed as mean ($\pm$ standard deviation, SD) from three independent experiments (all fits yielded R^2 values greater than 0.97).

2.7.3. Synergy Score

The Bliss independence model was applied to quantify the degree of pharmacological interaction between two drugs [77,78]. This model enables comprehensive evaluation of drug combination dose–response data and facilitates the calculation of synergy scores according to the Bliss framework. The Bliss independence model operates under the assumption that each drug within a combination exerts its effect independently, without mutual influence. In this theoretical framework, each agent is presumed to act as though the other drugs are absent, neither enhancing nor inhibiting their respective activities. The model predicts the expected combined effect based on the individual dose–response

profiles of each drug. When the observed combined effect aligns with the expected value, the interaction is considered independent. Conversely, any significant deviation from this expectation indicates a synergistic (greater than expected) or antagonistic (less than expected) interaction, reflecting interdependent pharmacological behavior between the agents [78].

The Zero Interaction Potency (ZIP) model is a reference framework used to assess synergy, antagonism, or additivity in drug combinations [77,79]. This model assumes the absence of interactions between compounds and posits that the combined effect arises from the independent contribution of their individual potencies, without altering the shape of the dose–response curves. Based on the fitting of the single-agent dose–response curves, the expected combination effect under a zero interaction scenario is estimated. Synergy is quantified as the difference between the experimentally observed effect and the effect predicted by the ZIP model. Positive ZIP scores indicate synergy, values close to zero indicate additivity, and negative values indicate antagonism. This approach is particularly suitable for the analysis of full dose–dose matrices and is widely used in high-throughput drug combination screening studies.

The web-based application Synergy Finder Plus version R-3.10.3 [80] was used to calculate the synergy scores for the tested compound combinations. A positive score indicates that the observed combined effect exceeds the expected additive effect, signifying a synergistic interaction between the tested agents, while a negative score reflects an antagonistic interaction.

2.7.4. Statistics and Data Analysis

OriginPro (version 8.5, OriginLab Corporation, Northampton, MA, USA) was used for the calculation of the half maximal inhibitory concentration (IC_{50}). The statistical analysis for the biological assays was also carried out with the same software. A two-tailed unpaired *t*-test was used for the comparison between two groups. The data were presented as means $\pm$ SD. *p*-Values < 0.05 (*), < 0.01 (**), and < 0.001 (***) were considered significant.

3. Results and Discussion

3.1. MF Micelles as Carriers for PTM

A previous step to understand the ternary systems formed by the drugs and the polymeric surfactant necessarily encompasses the structural characterization and biological activity when MF and PTM are combined. Due to its amphiphilic nature, MF self-aggregates in the form of micelles in aqueous medium [81]. MF micelles consist of a “dry” core formed by the hydrocarbon tails of the molecule and a shell comprising the zwitterionic head. The aggregation number and size remain stable with temperature, both in water and in buffered medium [82], and the critical micelle concentration (CMC) varies with the pH and ion composition, ranging from 60 μ M in water to 50 μ M in phosphate buffer and 35 μ M in phosphate buffer saline (pH 7.4) [2,81].

The interactions between MF and PTM are reflected in the changes observed in the corresponding ^1H -NMR spectra (see signal assignation in Figure S1a,b). In D_2O , the aromatic protons of PTM (H6, H4, H16, H18) appear at 7.65 and 7.03 ppm as two distinct doublets, with protons adjacent to the ether group producing a triplet at 4.11 ppm, while those of the aliphatic chain (H9–H11) appear at 1.79 and 1.56 ppm. In MF, the protons of the tail produce signals at 0.80, 1.22, and 1.54 ppm, with the methyl groups of the amino group resonating at 3.17 ppm. The methylene protons of the head resonate at 3.77 and 3.60 ppm, respectively, while the terminal methyl adjacent to the phosphate group appears at 4.19 ppm. Figure S2a,b show the proton spectra of a 0.1% PTM–1% MF mixture, recorded at 20 and 37 $^{\circ}C$. The most noticeable change is the upfield shift (0.193 and 0.182 ppm at

20 and 37 °C, respectively) of the signals from the aromatic protons (Figure 1a,b). Aliphatic protons also shift in the presence of MF to a lesser extent ($\Delta\delta = 0.039$ and 0.032 ppm, respectively). The changes in the magnetic environment of the PTM are patent in view of the NOESY spectrum (Figure 1c), which reveals strong cross-peaks between the signals of the aromatic rings of the PTM and the aliphatic chain of MF, indicating the closeness between both domains [83], with the PTM lodged in the MF micelle core. This evidence is also supported by the blueshift (12 nm) in the fluorescence band of the PTM in the presence of MF and the pronounced increase in the emission intensity (Figure S3), a phenomenon that usually occurs when the microenvironment of the emitting species becomes more hydrophobic [84]. The reduction in the intensity with the temperature that can be observed in both cases can be attributed to dynamic/collisional quenching.

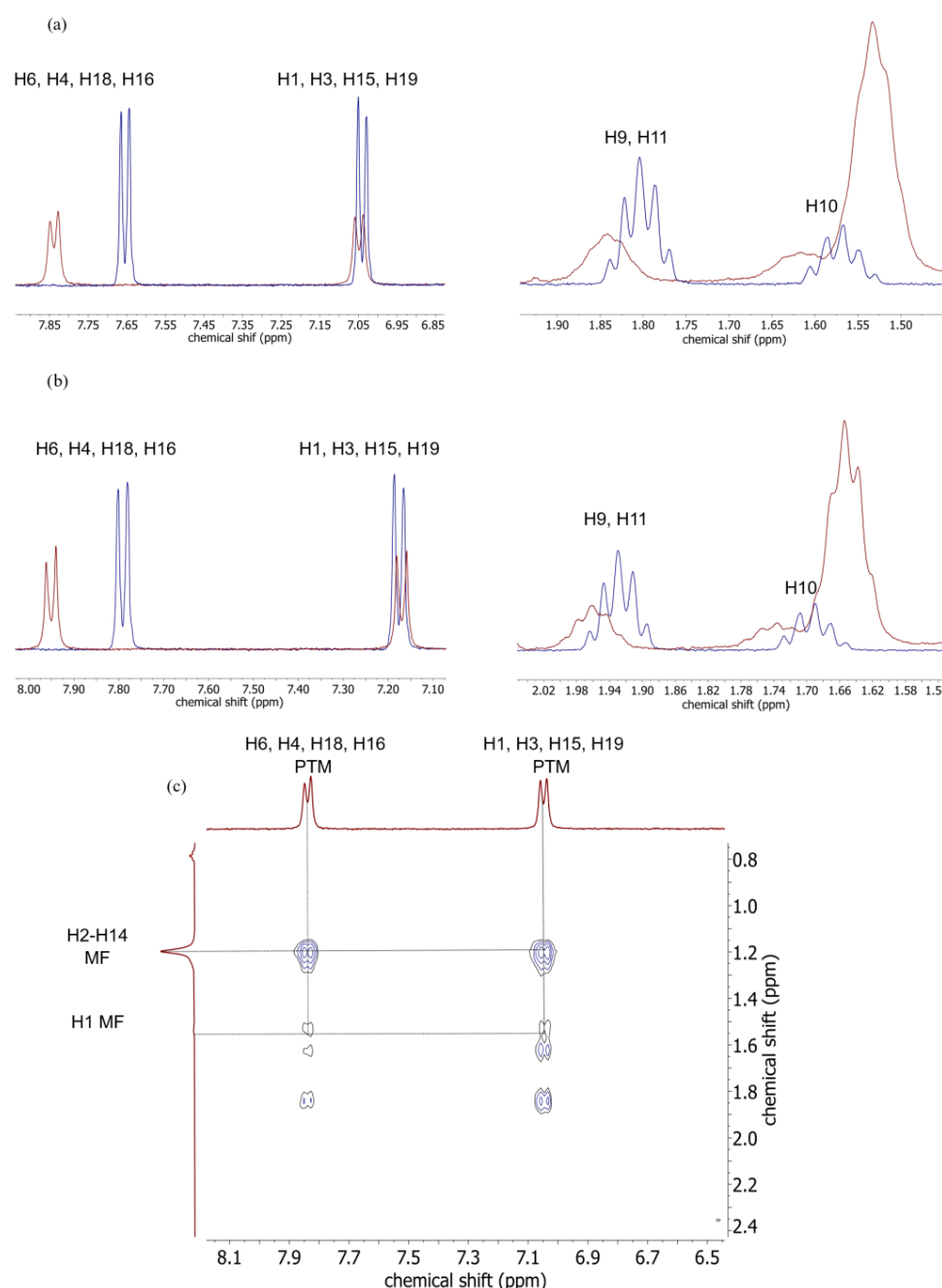


Figure 1. Aromatic and aliphatic region of the ¹H-NMR spectrum of PTM in D₂O in the presence (red) and absence (blue) of MF at 20 °C (a) and 37 °C (b); (c) expanded view of the NOESY spectrum of 0.1% PTM + 1% MF at 20 °C.

Figure S4 shows the size distributions obtained with DLS of MF in water, alone and when combined with PTM. The size of the MF micelles is 3.3 nm at 20 °C and 3.1 nm at 37 °C, in agreement with previous data [2]. The presence of PTM led to a reduction in micellar dimensions, with the average size decreasing to 2.6 nm and 2.8 nm at 20 °C and 37 °C, respectively, while the size distributions broaden (Figure S4).

A deeper insight into the structure of these micelles can be obtained from SANS experiments. Figure 2 shows the reduced scattering data for MF and its combination with PTM at different temperatures. The plots present an upward curvature at low q ($\text{\AA}^{-1}$) due to the presence of large aggregates, particularly in the samples without PTM. The DLS experiments reveal that these unspecific aggregates, albeit large, represent a small contribution in mass. To account for this effect, a power law function was included into the scattering of the MF micelles, modelled by core-shell spheres with a hard-sphere potential, in which the effective radius of interaction has been left free (see “Theoretical models for micelle description” in the Supporting Information [85]). Following the work by Puig-Rigall et al., the scattering length density of the micelle core (corresponding to the hydrocarbon tail) was fixed ($\rho_c = -0.37 \times 10^{-6} / \text{\AA}^2$, “dry” micelle core is assumed) [82], but it was left to float in the analysis of the PTM and MF mixtures to account for the preferential localization of the PTM in the core, as evidenced by NMR.

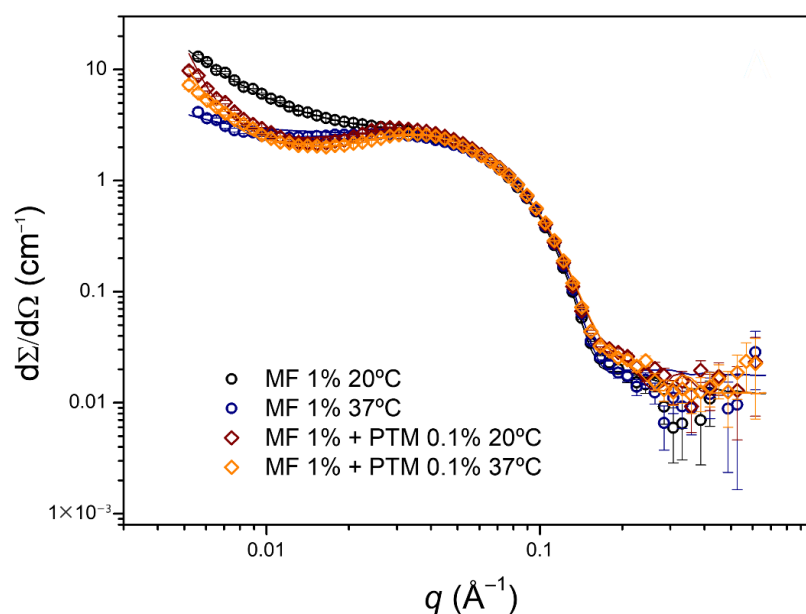


Figure 2. SANS patterns of 1% MF and combinations 1% MF + 0.1% PTM in D_2O , measured at 20 and 37 °C. Solid lines are fits to the model used (core-shell with hard spheres + power law).

The aggregation number (N_{agg}) of MF was obtained following the procedure described by Puig-Rigall et al. [85]. For the MF:PTM micelles, a similar approach was used, considering that the PTM is located in the micellar core (Equations (1)–(3)):

$$\rho_c = \rho_{MF-t}\chi_{MF-t} + \rho_{PTM}(1 - \chi_{MF-t}) \quad (1)$$

$$N_{agg} = \chi_{MF-t} \frac{V_c}{V_{MF-t}} \quad (2)$$

$$N_{PTM} = (1 - \chi_{MF-t}) \frac{V_c}{V_{PTM}} \quad (3)$$

Here, ρ_{PTM} and ρ_{MF-t} were set as $1.7 \times 10^{-6} \text{\AA}^{-2}$ and $-0.37 \times 10^{-6} \text{\AA}^{-2}$, respectively. The values of V_{MF-t} ($275 \text{ cm}^3/\text{mol}$) and V_{PTM} ($301 \text{ cm}^3/\text{mol}$) represent the molar volumes

of the MF hydrophobic tail and the PTM, respectively. Once the number of MF molecules is determined, the hydration of the shell can be obtained. To assess whether the model and fit are reasonable, the volume fraction obtained from experimental data can be compared with the theoretical value, showing good agreement (see Table 1).

Table 1. Parameters obtained from the analysis of SANS data using a core-shell spheres model for MF 1% and combinations of MF 1% and PTM 0.1% at 20 and 37 °C in D₂O. R_c , radius of the core, Å; R_{eff} , radius effective; t , thickness of shell, Å; ρ_s , shell scattering length density (sld), $\times 10^6$ Å²; ρ_c , core scattering length density, $\times 10^6$ Å²; ϕ , particle volume fraction; ϕ_t , theoretical particle volume fraction; $X_{D2O\ shell}$, water volume fraction in the shell; N_{agg} and N_{PTM} , number of MF and PTM molecules per micelle, respectively.

	T (°C)	R_c (Å)	t (Å)	R_{eff} (Å)	ϕ	ϕ_t	ρ_s	ρ_c	X_{D2O}	N_{agg}	N_{PTM}
1% MF	20	23 ± 1	12.2 ± 0.2	58 ± 1	0.018 ± 0.001	0.026	4.74 ± 0.11	−0.37	0.78	116 ± 17	-
	37	22.4 ± 0.7	13.4 ± 0.2	53.0 ± 0.6	0.023 ± 0.001	0.030	5.01 ± 0.15	−0.37	0.81	104 ± 12	-
1% MF + 0.1% PTM	20	18.2 ± 0.6	19.4 ± 0.3	69.3 ± 0.2	0.067 ± 0.001	0.083	5.38 ± 0.06	0.08 ± 0.32	0.94	44 ± 10	11 ± 7
	37	17.5 ± 0.4	18.7 ± 0.2	62.4 ± 0.3	0.069 ± 0.001	0.091	5.37 ± 0.08	0.21 ± 0.28	0.95	36 ± 7	13 ± 6

The calculated parameters according to this model are summarized in Table 1. Regarding the unloaded MF micelles, the aggregation number, micellar size, and hydration of the shell match those reported by Puig-Rigall et al. obtained with a different experimental set-up [82]. Differences emerge in the combinations with PTM. Specifically, the MF molecules per micelle diminishes slightly with temperature, which, in turn, favors the incorporation of more PTM. Likewise, the presence of PTM leads to a reduction in both the core size and N_{agg} , in line with the DLS results, where a slight decrease in micelle radius is observed upon PTM incorporation. Additionally, the incorporation of PTM leads to an increase in both the shell hydration and the volume fraction. This effect can be explained by the fact that PTM disrupts the packing of MF hydrophobic tails, resulting in a decrease in the aggregation number. Finally, it is worth mentioning the differences between the geometric micelle size and the effective radius. The R_{eff} value refers to the minimum center-to-center distance at which two micelles can approach each other, as defined in the hard-sphere structure factor. According to the SANS data, this distance is larger than the micelle size. This phenomenon is also reported for similar systems with SDS [86,87], an effect relevant in particles with charges on the micelle surface, where electrostatic repulsion between micelles plays a significant role.

DOSY experiments can provide precise information on the amount of PTM loaded in the micelles, using the diffusion coefficients of the components in the mixture. When a molecule binds or is incorporated into a larger structure, such as nanoparticles or micelles, its mobility decreases, and the variation in the self-diffusion coefficient provides an indirect measure of the extent of the association [88]. In this study, several samples containing 0.4% MF and increasing concentrations of PTM (0.05–0.9%), corresponding to MF:PTM molar ratios between 11.5:1 and 0.5:1, were analyzed. The fraction of free PTM (χ_{PTM}) was calculated using Equation (4), where D is the measured PTM diffusion coefficient, D_{PTM} the diffusion coefficient of the free PTM, and D_{PTM-MF} is the diffusion coefficient of PTM in the MF micelles (set to the diffusion coefficient of the micelle).

$$D = D_{PTM}\chi_{PTM} + D_{PTM-MF}(1 - \chi_{PTM}) \quad (4)$$

Equation (5) relates the number of micelles to the number of molecules of PTM and MF in solution. Considering the low CMC of the MF, it is reasonable to assume that all MF is in aggregated form, and for each solution, the fraction of PTM bound to micelles is determined using Equation (4). The water content within the micelles can be taken from

SANS data, as mentioned above, and its value is assumed to remain constant irrespectively of the sample composition.

$$N_m V_m 0.15 = V_{MF} N_{MF-s} + V_{PTM} N_{PTM-s} \chi_{PTM-MF} \quad (5)$$

Here, N_m is the number of micelles; N_{PTM-s} and N_{MF-s} are the total PTM and MF molecules in solution; χ_{PTM-MF} is the fraction of PTM loaded in the micelles; and V_m , V_{PTM} , and V_{MF} are the volumes of the micelle, PTM, and MF, respectively. The results in Figure 3 show the number of MF and PTM molecules per micelle (Figure 3a) and the fraction of PTM in the micelles (Figure 3b) (complete information about data analysis can be found in the Supporting Information). The results are consistent with those obtained from SANS analysis, and differences are attributable to the degree of hydration of the shell, which was assumed to be constant with the PTM concentration. Overall, the results obtained using both methods demonstrate that PTM is loaded in the MF micelles to a large extent, located mainly in the core of the aggregates, and increasing the PTM concentration produces a reduction in the micelle size.

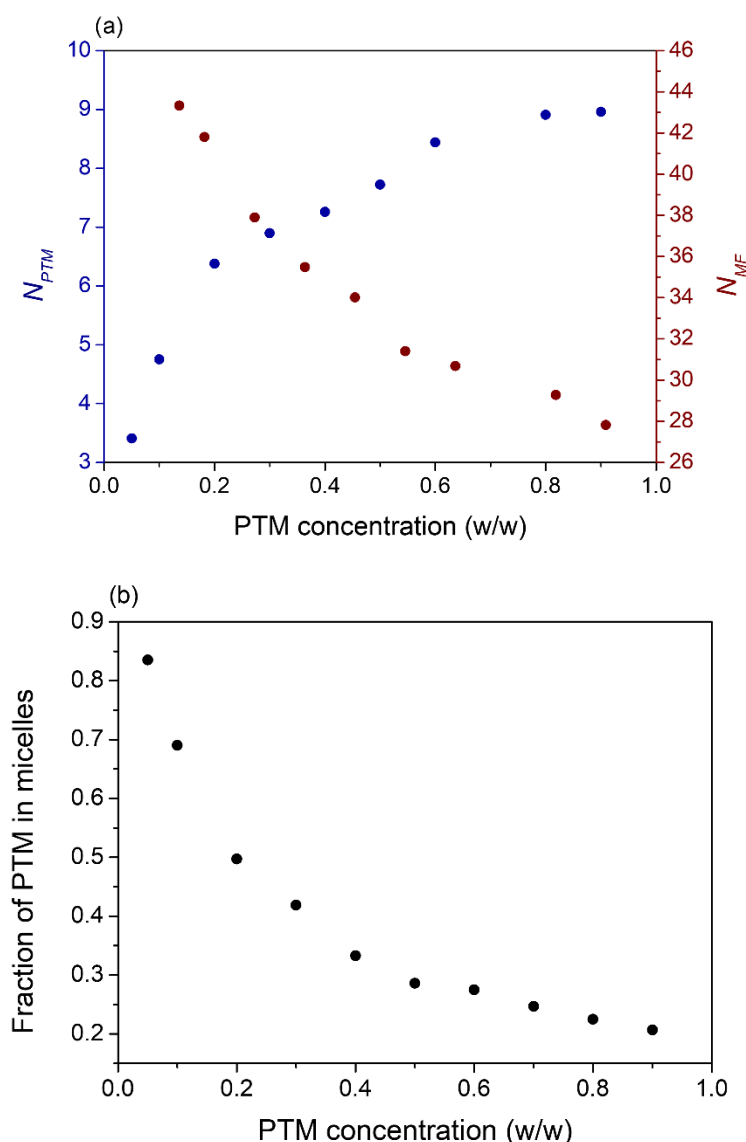


Figure 3. (a) Number of molecules of PTM (blue dots) and MF (brown dots) per micelle as a function of PTM concentration; (b) fraction of PTM in MF micelles (concentration of MF fixed at 0.4% w/w).

3.2. The Combination MF + PTM (Dual Therapy Approach) Exhibits a Significant Synergistic Effect Against Cell Proliferation

This “drug-in-drug” formulation constitutes an interesting system for drug delivery, in which both carrier and cargo are the active agents against cell proliferation. The biological activity was then evaluated, and synergistic interactions between MF and PTM were also assessed accordingly.

On the one hand, the activity of each drug was tested against *L. major*. PTM and MF were used at concentration ranges of 0–10 μ M and 0–20 μ M, respectively. The IC₅₀ values obtained (mean $\pm$ SD), 3.9 $\pm$ 0.3 μ M for PTM and 13.5 $\pm$ 0.7 μ M for MF, are in agreement with those previously reported [89–92]. The MF:PTM molar ratios obtained above (DOSY experiment) were used when studying the biological activity, namely: 11.5:1 to 0.5:1 (MF:PTM). Initially, PTM concentrations were set at 2 and 3 μ M, while those of MF varied from 0 to 20 μ M. The results shown in Figure 4a reveal that combinations of both drugs exhibit higher activities against *L. major* compared to the effect of the single drugs.

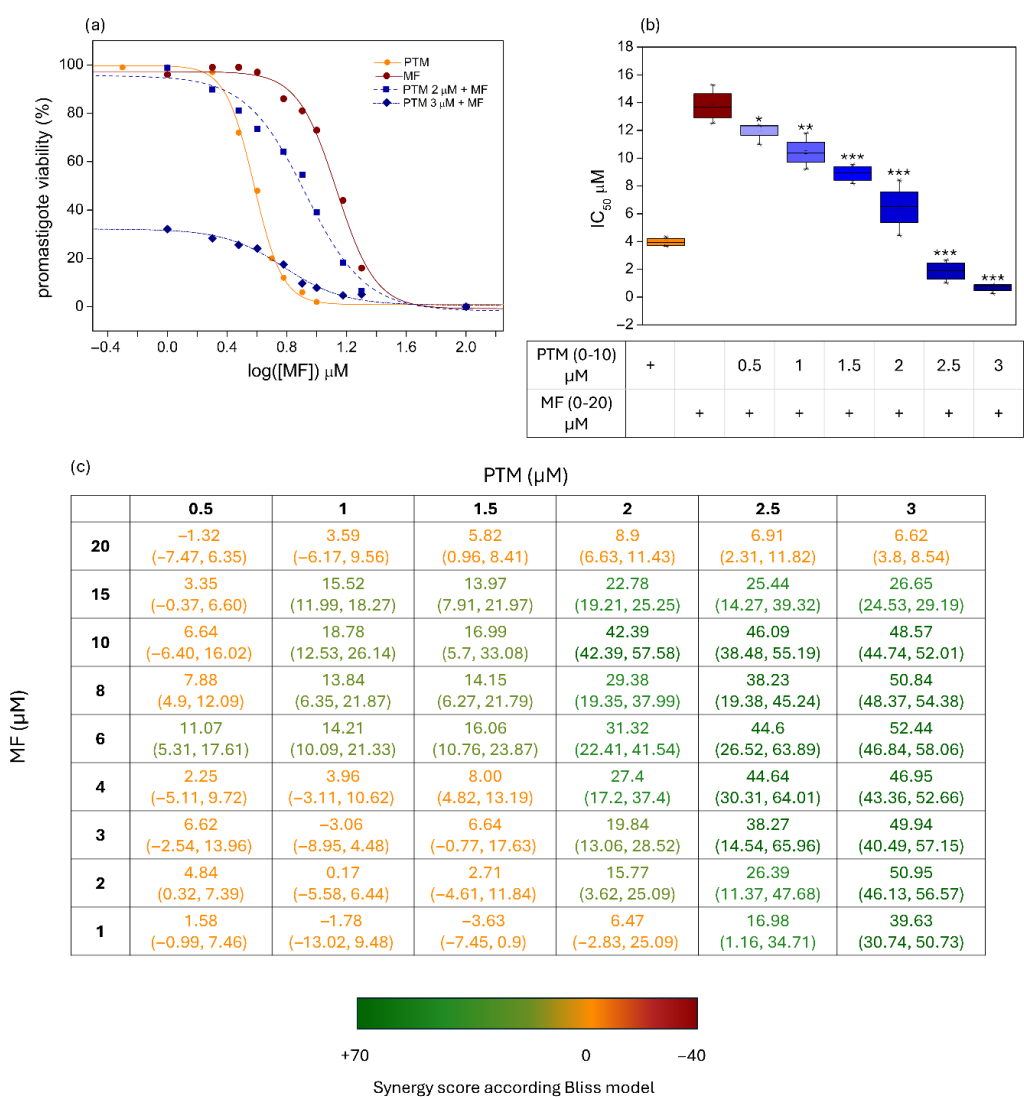


Figure 4. (a) *L. major* promastigote viability after treatment with PTM, MF, and combinations of both drugs. (b) Variation in IC₅₀ values of MF in the presence of different concentrations of PTM (0–3 μ M). Each PTM:MF combination is compared to MF alone. *p*-Values < 0.05 (*), < 0.01 (**), and < 0.001 (***) were considered statistically significant. (c) Synergy score values for each PTM:MF combination according to the Bliss model.

Moreover, the study was extended to a larger number of MF + PTM combinations (Figure 4b), supporting that the presence of PTM leads to a significant reduction in the IC₅₀ values of MF. Interestingly, at specific concentrations, the leishmanicidal activity is dramatically enhanced (Tables 2 and S1). While the percentage of survival of *L. major* after single treatments with PTM (2–3 µM) or MF (6–8 µM) was >70% or >80%, respectively, a substantial synergistic effect was observed when combining both drugs (Table 2). In fact, the percentage of cell viability was $7.16 \pm 1.9\%$ with the combination of 3 µM PTM plus 8 µM MF. However, single treatments showed moderate antiparasitic activity (at 3 µM PTM, leishmania survival was $71.3 \pm 0.3\%$; similarly, at 8 µM MF, parasite viability was $82.7 \pm 5.0\%$) (Table 2).

Table 2. Viability of *L. major* expressed as percentage (mean $\pm$ SD) when treated with PTM (0, 2, and 3 µM) and MF (0, 6, and 8 µM), alone and combined (see Tables S1 and S2 for the full data sets on *L. major* viability).

Survival (%) of <i>L. major</i> Promastigotes		MF (µM)		
		0	6	8
PTM (µM)	0	100	91.3 ± 2.7	82.7 ± 5.0
	2	100.0 ± 3.0	59.7 ± 7.9	49.9 ± 9.9
	3	71.3 ± 0.3	10.9 ± 2.1	7.16 ± 1.9

Based on the Bliss independence model, the synergy scores were calculated to assess the existence of synergistic effects. As abovementioned (in Section 2), positive Bliss scores indicate synergistic interactions between both drugs [78,93]. Our results (Figure 4c) confirm a significant synergistic effect of both drugs, particularly when PTM and MF concentrations are >2 µM and >6 µM, respectively. Moreover, the ZIP model was added as a complementary model to assess synergy, antagonism, or additivity in drug combinations. Interestingly, this model yields values that are highly consistent with those obtained with the Bliss independence model (full synergy maps are shown in Table S3).

These findings therefore support the notion that a dual therapy combining PTM and MF could be beneficial for the treatment of leishmaniasis. The synergistic effects may be helpful to reduce the current clinical doses applied to achieve therapeutic efficacy, while potentially minimizing adverse effects associated with single-drug therapies. Nevertheless, further investigation along this research line is required to determine whether the observed synergistic effect translates into therapeutic benefits.

3.3. Polymeric Micelles of T1307 Containing PTM and MF

Previous research has demonstrated that T1307 effectively improves some properties of MF and biological effects by forming mixed micelles, including the reduction of its toxicity [2]. Herein, we have investigated the combinations PTM:T1307, as well as PTM:MF:T1307, aiming to encapsulate both drugs within T1307 polymeric micelles.

Regarding the NMR experiments, no clear interactions were detected between the PTM protons and the PEO or PPO groups of T1307 in the NOESY spectra of T1307:PTM mixtures. However, the ¹H-NMR spectra did present small shifts of the aromatic (0.01 ppm) and aliphatic (0.003 ppm) peaks of PTM, as well as in the EO (−0.005 ppm) and PO (−0.055 ppm) units of the copolymer (Figure S5), suggesting a reduced load of PTM in the micelles, distributed between the micelle shell and core. Interestingly, the presence of MF, which is known to form mixed micelles with T1307, seems to facilitate the load of PTM. This is evidenced in the ¹H-NMR spectra of samples containing different T1307:MF ratios and 0.1% PTM (Figure 5). The shifts in the aromatic protons of PTM increases with the MF

content, strongly suggesting that MF favors the encapsulation of PTM by forming mixed micelles with T1307.

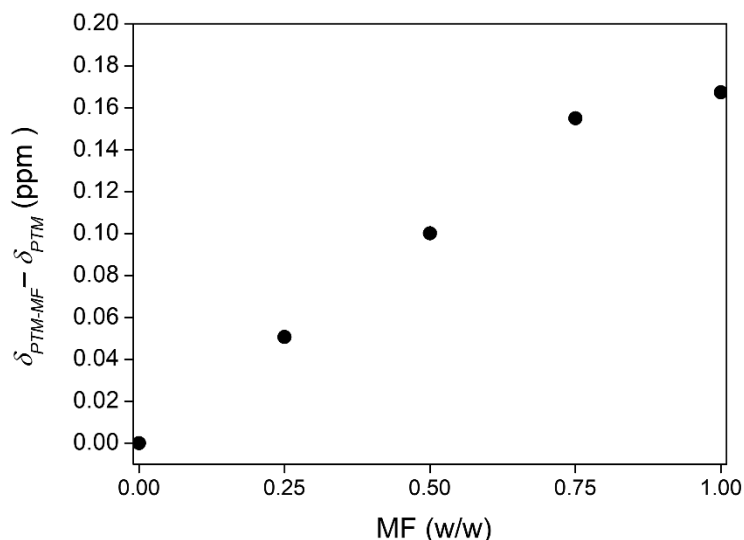


Figure 5. Chemical shifts of the aromatic protons of 0.1% PTM in the presence of MF and T1307, measured in D₂O at 37 °C. The sum of MF and T1307 concentration is 1% w/w.

DLS measurements were carried out to assess the particle size of T1307:PTM and T1307:PTM:MF aggregates. Consistent with a previous report [94], the copolymer is in its unimer form at 20 °C ($R_h = 3.1$ nm, Figure S6a). Upon the addition of 0.1% and 0.3% PTM, the coil expands to 3.2 nm and 3.5 nm, respectively. At 37 °C, T1307 forms micelles with of 10.8 nm, while a fraction of unimers remains, with the same radius as at 20 °C [94–96]. The addition of 0.1% and 0.3% PTM results in a decrease in the micelle size, with radii of 10.4 nm and 10.1 nm, respectively. At 0.1% PTM, the peak corresponding to the unimers remains, but its contribution to the scattered intensity diminishes with a threefold increase in PTM concentration (Figure S6b), suggesting that PTM helps stabilize the T1307 micelles and induces the self-aggregation of the block polymer.

Upon the addition of MF to T1307:PTM solutions, mixed micelles of T1307 and MF are formed [2]. At 20 °C, these systems exhibit notable complexity. The size distributions show a peak around 3.4 nm, which corresponds to T1307 unimers and MF micelles (Figure S7a). Additionally, a peak at 7.5 nm suggests the formation of mixed micelles comprising T1307 and MF. However, at 37 °C, the distributions merge into a single peak at 7.5 nm, due to the mixed micelles T1307–MF (Figure S7b).

SANS was employed to further characterize the binary and ternary systems. The morphology of Tetronics has been usually described by two models: a four-arm star polymer structure to represent unimers, accounting for the branched architecture of the surfactant, and core–shell spheres for the micelles [66,82,97]. Recent studies, however, suggest that core–shell ellipsoids may more accurately represent Tetronic micelles in buffered media [2]. In this work, the scattering patterns of T1307 and T1307:PTM were fitted using a four-arm star polymer structure at 20 °C, and a combined model of core–shell spheres and star polymer (to account for the persistent unimer peak detected by DLS), with a hard-spheres structure factor at 37 °C. Figure 6a,b show the neutron scattering patterns of T1307 unimers and micelles, respectively, with the structural parameters derived from the fits summarized in Table 3 (in the analysis of T1307 and T1307:PTM at 37 °C, the radius of the unimers was fixed at the value obtained at 20 °C).

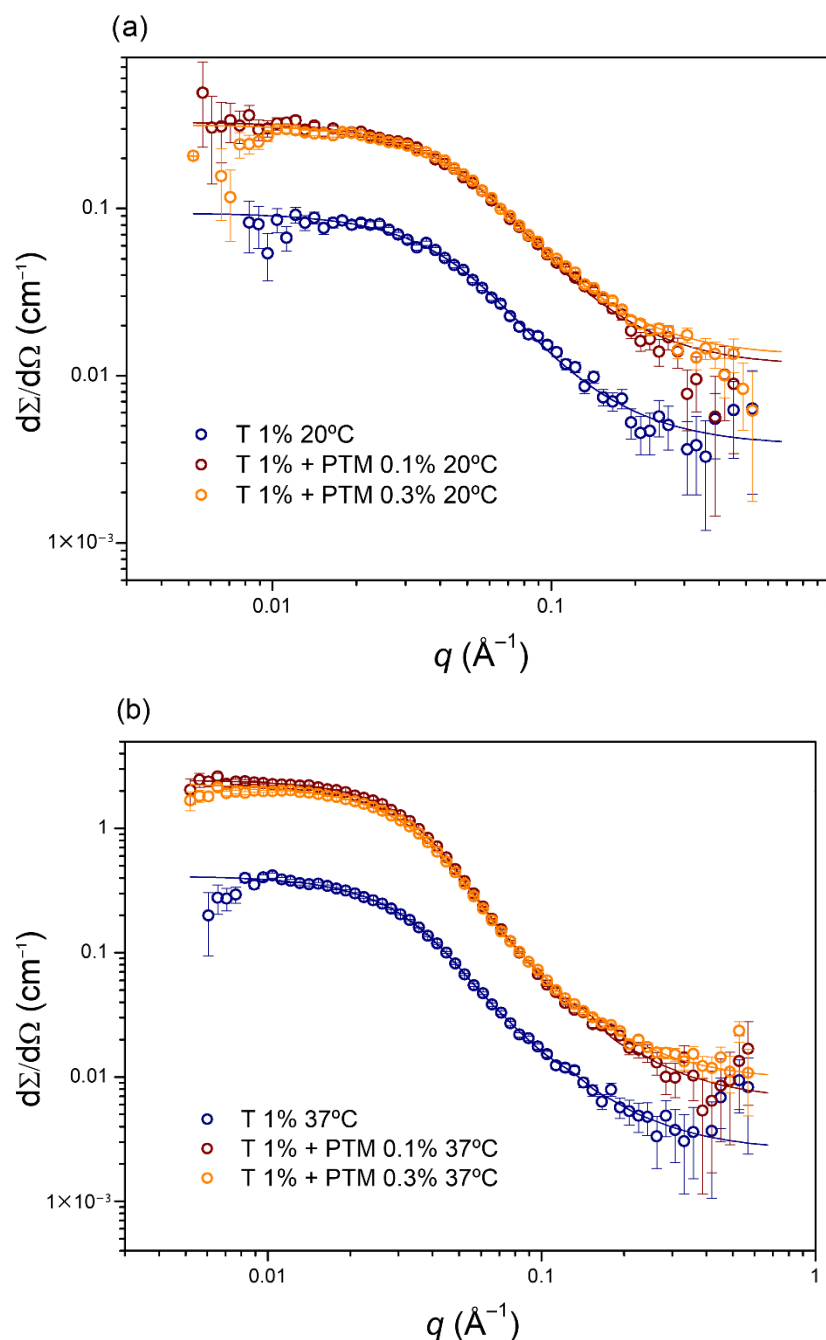


Figure 6. SANS patterns of 1% T1307 and combinations 1% T1307 + 0.1% or 0.3% PTM in D₂O at (a) 20 °C; (b) 37 °C. Solid lines are fits to a four-arm star polymer shape factor (20 °C) and to a combined core-shell spheres and star polymer model (37 °C).

Considering individual T1307 micelles first (at 37 °C), the relatively high ρ_c returned by the fits implies a core with a non-negligible amount of solvent. This finding, combined to the persistence of the unimers signal in DLS measurements (Figure S6), suggests that micelles are not fully structured and justifies the inclusion of the star polymer model in the analysis of SANS data. Upon the addition of PTM, the decrease in ρ_c reflects a less hydrated core, while the overall increase in the scattering indicates that PTM is favoring the self-aggregation of the copolymer.

Next, the ternary systems were studied. The complexity of the mixed systems at 20 °C makes fitting the data to any specific model challenging due to the presence of both unimers and aggregates (Figure S8). At 37 °C, the patterns are more readily interpretable, as only

micelles are present in solution (Figure 7), and the data were modelled using a shape factor of core-shell spheres (micelles) with a power law contribution (aggregates), including a hard-spheres factor (Table 4).

Table 3. Parameters obtained from SANS data analysis using a four-arm star polymer shape factor (20 °C) and core-shell spheres (37 °C) for T1307 and T1307 + PTM. R_u , radius of unimers; R_c , core radius, Å; t , shell thickness, Å; ρ_s , shell scattering length density (sld), $\times 10^6 \text{ Å}^2$; ρ_c , core scattering length density, $\times 10^6 \text{ Å}^2$.

	1% T1307	1% T1307 + 0.1% PTM	1% + T1307 0.3% PTM	1% T1307	1% T1307 + 0.1% PTM	1% + T1307 0.3% PTM
T	20 °C			37 °C		
R_u	36	34	33	36	34	33
R_c	-	-	-	47.5 ± 4.5	40.2 ± 0.6	39 ± 0.7
t	-	-	-	43.0 ± 4.1	45.9 ± 0.6	45 ± 0.6
ϕ	-	-	-	0.009 ± 0.006	0.016 ± 0.002	0.018 ± 0.001
ρ_c	-	-	-	5.02 ± 0.45	2.55 ± 0.24	2.92 ± 0.16
ρ_s	-	-	-	6.17 ± 0.08	5.95 ± 0.03	5.97 ± 0.05

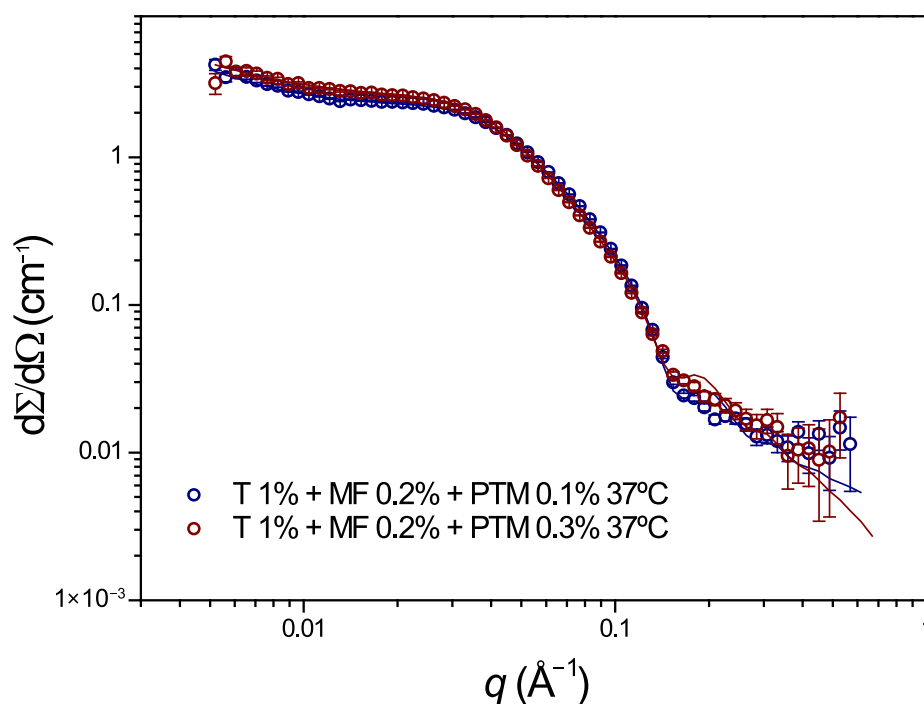


Figure 7. SANS patterns of the ternary systems T1307:PTM:MF at 37 °C in D₂O. Solid lines are fits to the core-shell with hard-spheres structure factor + power law model.

The main difference between the ternary and binary (T1307:PTM) systems is the remarkable decrease in ρ_c in the presence of MF, suggesting a “dry” core that consists mainly of PPO, MF, and PTM. The number of molecules per micelle and shell hydration in Table 4 have been determined by following a similar approach to that described in the previous section (see Supporting Information for the description of the model and parameters). In these ternary systems, the water content of the shell represents a 95% and N_{agg} are 2 and 3 in the presence of 0.1 and 0.3 w/w PTM, respectively [94]. The shell was assumed to have negligible contributions of MF and PTM.

The fractions of PTM (χ_{PTM}) and MF (χ_{MF}) within the micellar core can be quantified using the following equations:

$$V_c = \chi_{PPO}V_c + \chi_{PTM}V_c + \chi_{MF}V_c \quad (6)$$

$$\rho_c = \chi_{PPO}\rho_{PPO} + \chi_{MF}\rho_{MF} + \chi_{PTM}\rho_{PTM} \quad (7)$$

The molar volume and scattering length density of MF for this analysis were fixed at 706 cm³/mol and $-0.08 \times 10^{-8} \text{ \AA}^{-2}$, respectively [2], considering that the whole MF molecule was incorporated in the core (not just the hydrophobic tail).

These data strongly support, in agreement with DLS and NMR results, that the presence of MF is required to induce the load of PTM in the micelles, with the PTM occupying mainly the core of the micelle.

Table 4. Parameters obtained from SANS data analysis of core-shell spheres for T1307:PTM:MF at 37 °C in D₂O. R_c , radius of the core, Å; R_{eff} , radius effective; t , thickness of shell, Å; ρ_s , shell scattering length density (sld), $\times 10^6 \text{ \AA}^{-2}$; ρ_c , core scattering length density, $\times 10^6 \text{ \AA}^{-2}$; ϕ , particle volume fraction; $X_{D2O \text{ shell}}$, water volume fraction in the shell; N_{agg} and N_{PTM} , number of MF and PTM molecules per micelle, respectively.

	1% T1307 + 0.2% MF + 0.1% PTM	1% T1307 + 0.2% MF + 0.3% PTM
R_c	28.2 ± 0.1	29.5 ± 0.1
t	39.5 ± 0.2	41.4 ± 0.3
R_{eff}	79.8 ± 0.5	78.4 ± 0.8
ϕ	0.050 ± 0.001	0.049 ± 0.002
ρ_c	0.09 ± 0.1	0.51 ± 0.1
ρ_s	6.13 ± 0.0	6.09 ± 0.0
$X_{D2O \text{ shell}}$	0.96	0.95
N_{agg}	2 ± 1	3 ± 1
N_{PTM}	22 ± 9	49 ± 15
N_{MF}	122 ± 7	104 ± 11

3.4. Biological Assessment of the Combinations T1307:PTM:MF

The biological activity of each drug when combined with T1307 was then evaluated, with IC₅₀ values (mean $\pm$ SD) of PTM ($4.1 \pm 0.1 \text{ \mu M}$) and MF ($14.1 \pm 1.2 \text{ \mu M}$) in the presence of 1% T1307 assessed (Figure 8a). Interestingly, both drugs maintained their biological activity. No significant differences were detected when comparing IC₅₀ values of drugs in micelles and those of the free drugs (PTM: $3.9 \pm 0.3 \text{ \mu M}$ and MF: $13.5 \pm 0.7 \text{ \mu M}$).

Both drugs were then combined with T1307 at the same concentrations (Figure 8a,b and Table 5). Taken together, these data confirm that the combination of PTM and MF with T1307, T1307:PTM:MF, preserved notable antiparasitic effects. Additionally, PTM significantly reduced the IC₅₀ of MF, even in the presence of T1307. For instance, in the presence of T1307 (1%), 2 $\text{\mu M}$ PTM showed very limited antiparasitic activity ($97.7 \pm 2.3\%$ of leishmania survival), and 8 $\text{\mu M}$ MF also showed a modest leishmanicidal effect ($84 \pm 7.9\%$ cell viability). However, when both drugs were combined at the aforementioned concentrations in the presence of T1307 (1%), *L. major* viability was reduced to $57.4 \pm 3.2\%$ (Table 5). The Bliss model also confirms that the synergistic effect observed with the drug-in-drug formulation (MF + PTM) remains relevant when both drugs are combined in T1307 micelles (Figure 8c). Likewise, the ZIP model demonstrates a high level of concordance with the Bliss model (Table S4).

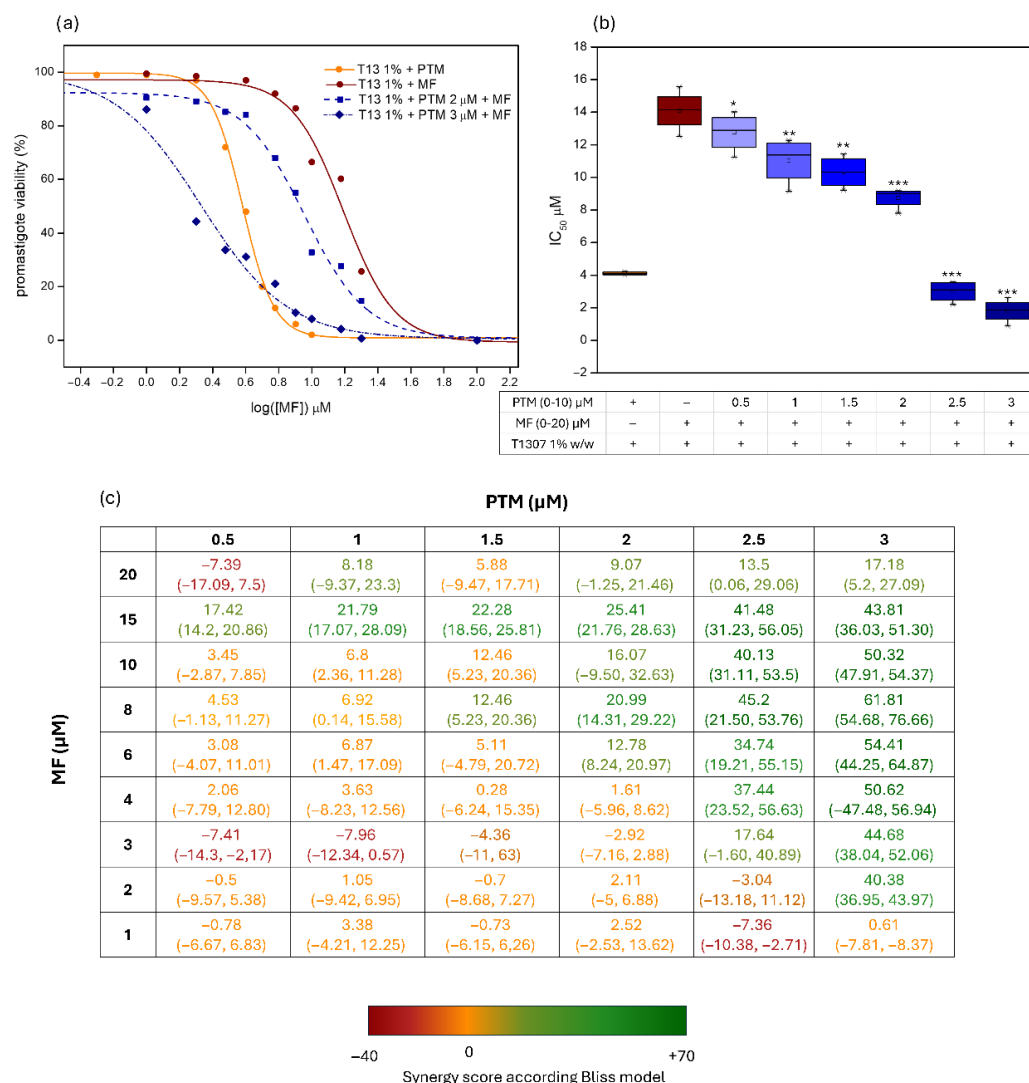


Figure 8. (a) *L. major* promastigote viability after treatment with PTM, MF, and combinations of both drugs with T1307 (1% w/w). (b) Variations of MF IC₅₀ values in the presence of different concentrations of PTM (0–3 μM). Each T1307:PTM:MF combination is compared to T1307:MF alone. *p*-Values < 0.05 (*), < 0.01 (**), and < 0.001 (***) were considered statistically significant. (c) Synergy score values for each T1307:PTM:MF combination, according to the Bliss model.

Table 5. Percentage (mean ± SD) of *L. major* survival. Parasites were treated with combinations of PTM (0, 2, and 3 μM) and MF (0, 6, and 8 μM) in the presence of T1307 (1% w/w). Additional data are available in Supporting Information, Table S2.

% <i>L. major</i> Viability		MF (μM)		
		0	6	8
PTM (μM)	0	100	90.0 ± 4.9	84 ± 7.9
	2	97.7 ± 2.3	73.0 ± 4.0	57.4 ± 3.2
	3	88.1 ± 2.1	21.1 ± 2.9	10.3 ± 8.5

These results are in agreement with a previous clinical study, which also showed the benefits of administering separated doses of both drugs in treatment [72]. Nevertheless, patients experienced pain after PTM intralesional administration, and the adverse effects of MF and PTM were additive. These findings could serve as a starting point for subsequent investigations focused on the development of formulations for the co-administration of MF

and PTM. Altogether, the abovementioned results suggest that formulating PTM and MF in a dual therapy using T1307 may be a promising approach.

Our investigations have shown that PTM and MF could be successfully co-formulated in T1307 micelles, inducing a significant synergistic effect when both drugs are combined. It is well known that both MF and PTM have antiproliferative and antitumoral activity. In fact, PTM has recently been repurposed as a potential treatment for specific types of tumors, inhibiting cell growth through multiple mechanisms. PTM suppresses survival and tumor growth signaling pathways by phosphorylating pAkt and pSTAT3 in glioblastoma [98] and endometrial cancer cell lines [17]. Additionally, it inhibits the PI3K/Akt pathway by activating PTEN in ovarian cancer models [25]. In breast and colon cancer models, PTM counteracts the inhibitory effect of S100 on p53 [99–101], a key tumor suppressor gene involved in cell cycle regulation. Moreover, it has been reported to enhance T-cell reactivation by acting as a PD-L1 antagonist [18], potentially improving immune responses against tumors. In prostate cancer, PTM has been found to reduce the levels of mitochondrial RNA, leading to mitochondrial dysfunction [24]. The biochemical mechanism of action of PTM seems to be a consequence of its dual binding to DNA and proteins, and it specifically modifies the beta-cluster, i.e., the “basic face” of ubiquitin [102]. On the other hand, MF has also been developed as an antineoplastic agent. Taking all this in consideration, this work may serve as an initial starting point for further exploration of the combined therapeutic effects of both drugs, not only in leishmaniasis but also in other pathological contexts.

3.5. Gel Formulations: Structure and Rheology

Concentrated T1307 solutions turn into gels when increasing the temperature. This can be exploited to develop liquid formulations of MF and PTM to be applied on wounds lesions and undergo gelation at body temperature, thereby enabling controlled and localized release of the active compounds. In this section, the structure of T1307 gels and their rheological properties were investigated as an initial step, prior to more applied studies (e.g., release assays, osmolality and pH sensitivity, physical stability, and in vitro assays) that may support their practical application as a topical treatment.

The gel formation is primarily driven by hydrophobic interactions, in which the star-shaped architecture of T1307 likely facilitates inter-micellar bridging. This results in the formation of a three-dimensional network stabilized by hydrophobic interactions, in which the micellar network entraps water within the interstices, giving rise to a macroscopic sol–gel transition [103]. Polymeric micelles usually arrange into macrolattice structures, usually cubic, as evidenced by characteristic diffraction peaks in the SAXS and SANS patterns ($0.05\text{--}0.09\text{ \AA}^{-1}$) [85]. This is the case of T1307 [104], in which micelles arrange in a BCC (body-centered cube) cell [2]. SANS patterns and the corresponding structural parameters are shown in Figure 9a and Table 6. Here, ρ_c was fixed at the value obtained from micelles in the dilute regime.

Table 6. Parameters obtained from SANS data analysis to a BCC lattice of concentrated T1307 solutions, alone and in combination with MF, and MF and PTM 37 °C in D₂O. ϕ , particle volume fraction, dnn , nearest neighbor distance, Å; D , paracrystal distortion factor; R_c , particle radius, Å; R_m , micelle radius, calculated from dnn , Å; ρ_c , core scattering length density, $\times 10^6\text{ \AA}^2$.

	20% T1307	20% T1307 + 0.2% MF	20% T1307 + 0.2% MF + 0.3% PTM
ϕ	0.513	0.419	0.476
dnn	158.3	156.6	154.9
D	0.080	0.076	0.073
R_c	40.9	39.9	38.4
R_m	79	78	77
ρ_c	1.4	0.6	0.51

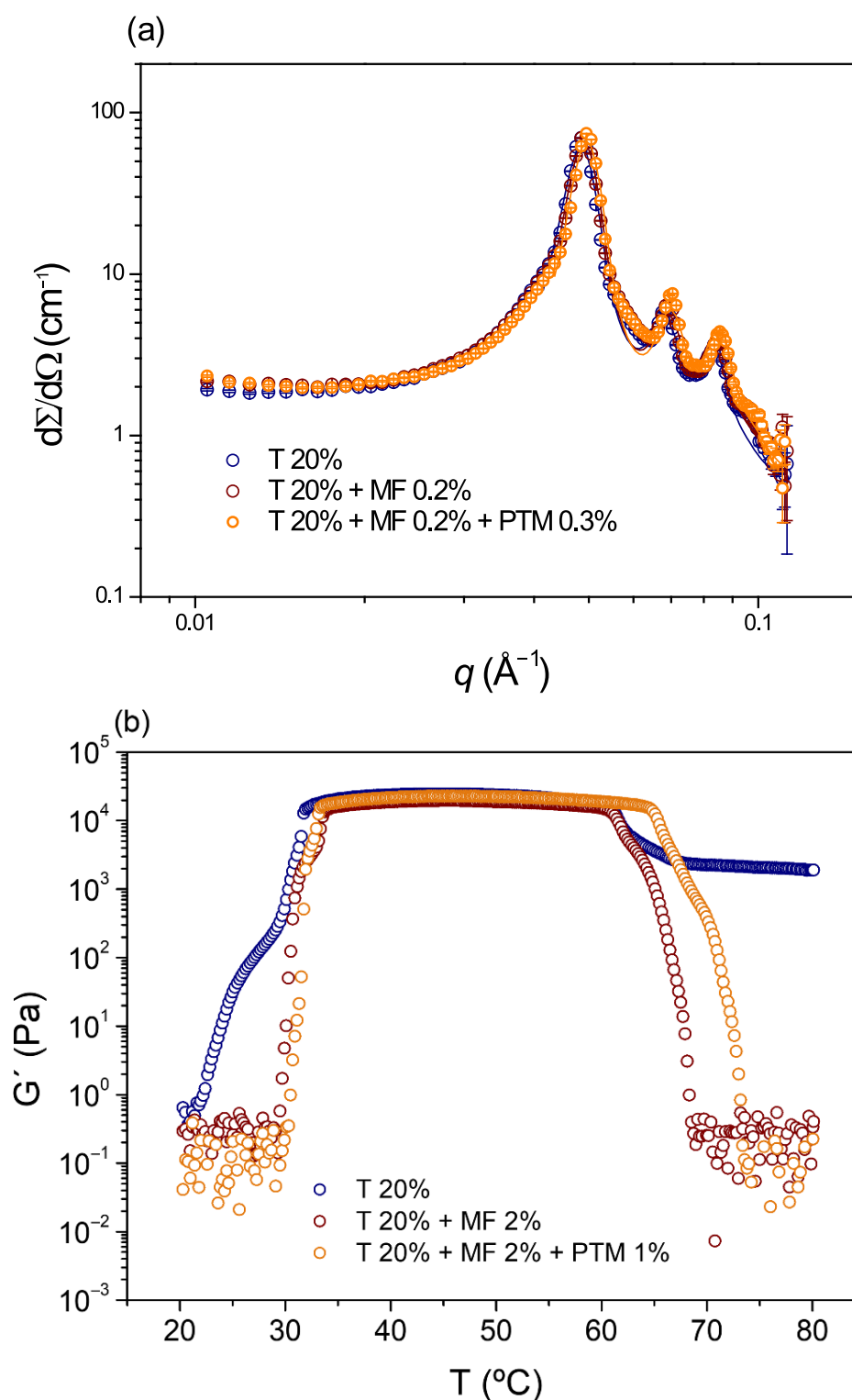


Figure 9. (a) SANS patterns of the T1307 drug-loaded gels at 37 °C. Solid lines are fits to a BCC-type paracrystal lattice. (b) Shear oscillatory temperature sweeps showing the elastic modulus (G') as a function of the temperature.

The presence of small proportions of MF and/or PTM slightly reduces the size of the unit cell, in line with the size diminution of T1307 micelles observed at dilute conditions. The micelle radius is slightly higher than half of the distance between two micelles centers, suggesting a certain overlap of the hydrophilic coronas in the unit cell (Table 6).

Finally, the rheology of the systems was analyzed with shear oscillatory measurements, raising the concentration of PTM and MF to 1 and 2% *w/w*, respectively, in order to detect changes linked to the incorporation of the drugs. Figure 9b shows the thermogelation profile of T1307. The addition of PTM or MF produces a shift of the onset of gelation to higher temperatures, and the range in which the gel is formed increases until nearly 70 °C. At this temperature, excessive dehydration of both PPO and partially the PEO corona reduces micellar stability and weakens the physical inter-micellar interactions. This leads to collapse of the micellar corona, disrupting inter-micellar bridges and the network. As a result, the physical crosslinks are lost, causing disassembly of the three-dimensional structure [103].

Nevertheless, the presence of PTM and MF does not affect the overall profile or value of the elastic modulus. From these results, it can be concluded that even in the presence of relatively high concentrations of PTM and MF, 20% T1307 undergoes gelation at body temperature, while maintaining the gel's mechanical properties.

4. Conclusions

Miltefosine (MF), an amphiphilic compound, incorporates pentamidine (PTM) to a large extent, yielding a “drug-in-drug” assembly in which the host component is itself pharmacologically active. NMR and SANS measurements indicate that PTM is predominantly located within the micellar core. SANS further shows that micelles dimensions decrease as PTM concentration increases, while the aggregate composition is only weakly affected by temperature. The MF + PTM mixtures display high activity against *L. major* promastigotes and a marked synergistic interaction. Co-formulation of MF and PTM was additionally examined using the poloxamine Tetronic® 1307 (T1307), a polymeric excipient that forms micelles and gels. PTM alone exhibits limited association with the copolymer; however, in the presence of MF, mixed T1307–MF assemblies incorporate higher amounts of PTM. Model-based analysis of SANS data, supported by NMR spectroscopy, confirms PTM localization in the core of the mixed MF–T1307 micelles. These findings are consistent with a core–shell architecture in which the hydrated corona is mainly composed of the PEO segments of the poloxamine and water, whereas the interior contains MF, PTM, and the PPO blocks. The encapsulation of MF and PTM within T1307 micelles preserves their antiparasitic and antiproliferative activity against *L. major*. At elevated copolymer concentrations, the dual-loaded micelles organize into a stable body-centered cubic (BCC)-type paracrystalline network. Linear rheology measurements show a clear mechanical response at physiological temperature. The results presented in this study are intended as a preliminary assessment of the proposed systems rather than as definitive conclusions. The experimental approach establishes a basis for further investigation of T1307-based micellar and gel formulations as platforms for the combined delivery of pentamidine and miltefosine. In this context, continued research could address their relevance in specific biomedical settings, such as leishmaniasis, using additional biological studies including activity assays in amastigotes, cytotoxicity evaluations, and more comprehensive safety assessments. Moreover, further work would be required to advance the characterization of T1307 gels, particularly with respect to their suitability for topical or dermal applications, including extended rheological, stability, and formulation-related studies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pharmaceutics18020233/s1>, Figure S1. ¹H-NMR spectra and signal assignment of: (a) 1% PTM; (b) 1% MF (D₂O, 20 °C), Figure S2. ¹H-NMR spectra in D₂O of 1% PTM (blue) and 1% MF + 0.1% PTM (red) measured at 20 °C (a) and 37 °C (b), Figure S3. Effect of the presence of MF in the emission fluorescence spectrum of PTM, measured at 20 °C and 37 °C, Figure S4. Micelle size distributions of 1% MF and 1% MF + 0.1% PTM in water, determined by DLS at 37 °C (a),

and 20 °C (b), Figure S5. ¹H-NMR spectra and expanded views of 1% PTM (blue), 1% T1307 (red) and 1% T1307 + 0.1% PTM (green), measured in D₂O at 37 °C, Figure S6. Micelle size distributions of T1307 and T1307:PTM in water determined by DLS at 20 °C; (a) and 37 °C (b), Figure S7. Size distributions of T1307:PTM:MF mixtures measured in water by DLS at 20 °C (a), and 37 °C (b); SANS patterns of the ternary systems T1307:PTM:MF at 20 °C, in D₂O, Figure S8. SANS patterns of the ternary systems T1307:PTM:MF at 20 °C, in D₂O, Figure S9. Normalized residuals of SANS fits. (a) 1% MF 20 °C, (b) 1% MF 37 °C, (c) 1% MF + 0.1% PTM 20 °C and (d) 1% MF + 0.1% PTM 37 °C, Figure S10. Normalized residuals of SANS fits. (a) 1% T1307 + 0.2% MF + 0.1% PTM 37 °C and (b) 1% T1307 + 0.2% MF + 0.3% PTM 37 °C, Table S1. Percentage of *L. major* viability after treatment with PTM (0–3 µM) and MF (0–20 µM) combinations, Table S2. Percentage of *L. major* viability after treatment with PTM (0–3 µM) and MF (0–20 µM) in the presence of T1307 (1%), Table S3. Full synergy scores for MF + PTM according to ZIP model, Table S4. Full synergy scores for T1307: PTM:MF according to ZIP model, Theoretical Framework for DOSY Data Processing, and Mathematical models used for SANS data analysis.

Author Contributions: Conceptualization, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Data curation, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Formal analysis, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Funding acquisition, P.N. and G.G.-G.; Investigation, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Methodology, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Project administration, P.N. and G.G.-G.; Resources, P.N., G.G.-G., C.A. and C.A.D.; Software, G.N.S.; Supervision, P.N. and G.G.-G.; Validation, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Visualization, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Writing—original draft, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G.; Writing—review and editing, J.C., C.A., G.N.S., C.A.D., P.N. and G.G.-G. All authors have read and agreed to the published version of the manuscript.

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References

1. Eibl, H.; Unger, C. Hexadecylphosphocholine: A New and Selective Antitumor Drug. *Cancer Treat. Rev.* **1990**, *17*, 233–242. [CrossRef]

2. Dirany, Z.; El-Dirany, R.; Smith, G.N.; Nguewa, P.; González-Gaitano, G. Mixed Micelles and Gels of a Hydrophilic Poloxamine (Tetronic 1307) and Miltefosine: Structural Characterization by Small-Angle Neutron Scattering and In Vitro Evaluation for the Treatment of Leishmaniasis. *J. Mol. Liq.* **2023**, *379*, 121654. [\[CrossRef\]](#)
3. Hilgard, P.; Stekar, J.; Voegeli, R.; Harleman, J.H. Experimental Therapeutic Studies with Miltefosine in Rats and Mice. In *Alkylphosphocholines: New Drugs in Cancer Therapy*; Karger Publishers: Basel, Switzerland, 1992; Volume 34, pp. 116–130.
4. van der Luit, A.H.; Vink, S.R.; Klarenbeek, J.B.; Perrissoud, D.; Solary, E.; Verheij, M.; van Blitterswijk, W.J. A New Class of Anticancer Alkylphospholipids Uses Lipid Rafts as Membrane Gateways to Induce Apoptosis in Lymphoma Cells. *Mol. Cancer Ther.* **2007**, *6*, 2337–2345. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Wieder, T.; Orfanos, C.E.; Geilen, C.C. Induction of Ceramide-Mediated Apoptosis by the Anticancer Phospholipid Analog, Hexadecylphosphocholine. *J. Biol. Chem.* **1998**, *273*, 11025–11031. [\[CrossRef\]](#)
6. van Blitterswijk, W.; Verheij, M. Anticancer Alkylphospholipids: Mechanisms of Action, Cellular Sensitivity and Resistance, and Clinical Prospects. *Curr. Pharm. Des.* **2008**, *14*, 2061–2074. [\[CrossRef\]](#)
7. Ruiter, G.A.; Zerp, S.F.; Bartelink, H.; van Blitterswijk, W.J.; Verheij, M. Alkyl-Lysophospholipids Activate the SAPK/JNK Pathway and Enhance Radiation-Induced Apoptosis. *Cancer Res.* **1999**, *59*, 2457–2463. [\[CrossRef\]](#)
8. Croft, S.L.; Engel, J. Miltefosine—Discovery of the Antileishmanial Activity of Phospholipid Derivatives. *Trans. R. Soc. Trop. Med. Hyg.* **2006**, *100*, S4–S8. [\[CrossRef\]](#)
9. Capela, R.; Moreira, R.; Lopes, F. An Overview of Drug Resistance in Protozoal Diseases. *Int. J. Mol. Sci.* **2019**, *20*, 5748. [\[CrossRef\]](#)
10. Singh, V.K.; Tiwari, R.; Rajneesh, N.; Kumar, A.; Chauhan, S.B.; Sudarshan, M.; Mehrotra, S.; Gautam, V.; Sundar, S.; Kumar, R. Advancing Treatment for Leishmaniasis: From Overcoming Challenges to Embracing Therapeutic Innovations. *ACS Infect. Dis.* **2025**, *11*, 47–68. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Mann, S.; Frasca, K.; Scherrer, S.; Henao-Martínez, A.F.; Newman, S.; Ramanan, P.; Suarez, J.A. A Review of Leishmaniasis: Current Knowledge and Future Directions. *Curr. Trop. Med. Rep.* **2021**, *8*, 121–132. [\[CrossRef\]](#)
12. Verma, N.K.; Dey, C.S. Possible Mechanism of Miltefosine-Mediated Death of *Leishmania donovani*. *Antimicrob. Agents Chemother.* **2004**, *48*, 3010–3015. [\[CrossRef\]](#)
13. Rakotomanga, M.; Blanc, S.; Gaudin, K.; Chaminade, P.; Loiseau, P.M. Miltefosine Affects Lipid Metabolism in *Leishmania donovani* Promastigotes. *Antimicrob. Agents Chemother.* **2007**, *51*, 1425–1430. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Tiwari, R.; Kumar, A.; Singh, V.K.; Rajneesh; Chauhan, S.B.; Sundar, S.; Nylén, S.; Engwerda, C.; Kumar, R. The Development and Maintenance of Immunity against Visceral Leishmaniasis. *Front. Immunol.* **2024**, *15*, 1486407. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Sands, M.; Kron, M.A.; Brown, R.B. Pentamidine: A Review. *Clin. Infect. Dis.* **1985**, *7*, 625–6344. [\[CrossRef\]](#)
16. Cirillo, C.; Capoccia, E.; Iuvone, T.; Cuomo, R.; Sarnelli, G.; Steardo, L.; Esposito, G. S100B Inhibitor Pentamidine Attenuates Reactive Gliosis and Reduces Neuronal Loss in a Mouse Model of Alzheimer’s Disease. *BioMed Res. Int.* **2015**, *2015*, 508342. [\[CrossRef\]](#)
17. Lin, L.; Gao, Y.; Hu, X.; Ouyang, J.; Liu, C. Pentamidine Inhibits Proliferation, Migration and Invasion in Endometrial Cancer via the PI3K/AKT Signaling Pathway. *BMC Womens Health* **2022**, *22*, 470. [\[CrossRef\]](#)
18. Gu, T.; Tian, X.; Wang, Y.; Yang, W.; Li, W.; Song, M.; Zhao, R.; Wang, M.; Gao, Q.; Li, T.; et al. Repurposing Pentamidine for Cancer Immunotherapy by Targeting the PD1/PD-L1 Immune Checkpoint. *Front. Immunol.* **2023**, *14*, 1145028. [\[CrossRef\]](#)
19. Huang, T.L.; Mayence, A.; Vanden Eynde, J.J. Some Non-Conventional Biomolecular Targets for Diamidines. A Short Survey. *Bioorg. Med. Chem.* **2014**, *22*, 1983–1992. [\[CrossRef\]](#)
20. Lee, M.S.; Johansen, L.; Zhang, Y.; Wilson, A.; Keegan, M.; Avery, W.; Elliott, P.; Borisy, A.A.; Keith, C.T. The Novel Combination of Chlorpromazine and Pentamidine Exerts Synergistic Antiproliferative Effects through Dual Mitotic Action. *Cancer Res.* **2007**, *67*, 11359–11367. [\[CrossRef\]](#)
21. Chow, T.Y.-K.; Alaoui-Jamali, M.A.; Yeh, C.; Yuen, L.; Griller, D. The DNA Double-Stranded Break Repair Protein Endo-Exonuclease as a Therapeutic Target for Cancer. *Mol. Cancer Ther.* **2004**, *3*, 911–919. [\[PubMed\]](#)
22. Kelly, M.L.; Chu, C.-C.; Shi, H.; Ganser, L.R.; Bogerd, H.P.; Huynh, K.; Hou, Y.; Cullen, B.R.; Al-Hashimi, H.M. Understanding the Characteristics of Nonspecific Binding of Drug-like Compounds to Canonical Stem-Loop RNAs and Their Implications for Functional Cellular Assays. *RNA* **2021**, *27*, 12–26. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Ho, J.M.; Bakkalbasi, E.; Söll, D.; Miller, C.A. Drugging tRNA Aminoacylation. *RNA Biol.* **2018**, *15*, 667–677. [\[CrossRef\]](#)
24. Liu, L.; Wang, F.; Tong, Y.; Li, L.; Liu, Y.; Gao, W. Pentamidine Inhibits Prostate Cancer Progression via Selectively Inducing Mitochondrial DNA Depletion and Dysfunction. *Cell Prolif.* **2020**, *53*, e12718. [\[CrossRef\]](#)
25. Wu, Y.; Zhang, Z.; Kou, Z. Pentamidine Inhibits Ovarian Cancer Cell Proliferation and Migration by Maintaining Stability of PTEN in Vitro. *Drug Des. Dev. Ther.* **2021**, *15*, 2857–2868. [\[CrossRef\]](#)
26. Reynolds, I.; Aizenman, E. Pentamidine Is an N-Methyl-D-Aspartate Receptor Antagonist and Is Neuroprotective in Vitro. *J. Neurosci.* **1992**, *12*, 970–975. [\[CrossRef\]](#)
27. Andreana, I.; Bincoletto, V.; Milla, P.; Dosio, F.; Stella, B.; Arpicco, S. Nanotechnological Approaches for Pentamidine Delivery. *Drug Deliv. Transl. Res.* **2022**, *12*, 1911–1927. [\[CrossRef\]](#) [\[PubMed\]](#)

28. Sundar, S.; Singh, J.; Dinkar, A.; Agrawal, N. Safety and Effectiveness of Miltefosine in Post-Kala-Azar Dermal Leishmaniasis: An Observational Study. *Open Forum Infect. Dis.* **2023**, *10*, ofad231. [\[CrossRef\]](#)
29. Planting, A.S.T.; Stoter, G.; Verweij, J. Phase II Study of Daily Oral Miltefosine (Hexadecylphosphocholine) in Advanced Colorectal Cancer. *Eur. J. Cancer* **1993**, *29*, 518–519. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Carnielli, J.B.T.; Monti-Rocha, R.; Costa, D.L.; Molina Sesana, A.; Pansini, L.N.N.; Segatto, M.; Mottram, J.C.; Costa, C.H.N.; Carvalho, S.F.G.; Dietze, R. Natural Resistance of *Leishmania infantum* to Miltefosine Contributes to the Low Efficacy in the Treatment of Visceral Leishmaniasis in Brazil. *Am. J. Trop. Med. Hyg.* **2019**, *101*, 789–794. [\[CrossRef\]](#)
31. Coelho, A.C.; Beverley, S.M.; Cotrim, P.C. Functional Genetic Identification of PRP1, an ABC Transporter Superfamily Member Conferring Pentamidine Resistance in *Leishmania major*. *Mol. Biochem. Parasitol.* **2003**, *130*, 83–90. [\[CrossRef\]](#)
32. Wiwanitkit, V. Interest in Paromomycin for the Treatment of Visceral Leishmaniasis (Kala-Azar). *Ther. Clin. Risk Manag.* **2012**, *8*, 323–328. [\[CrossRef\]](#)
33. Sundar, S.; Jha, T.K.; Thakur, C.P.; Engel, J.; Sindermann, H.; Fischer, C.; Junge, K.; Bryceson, A.; Berman, J. Oral Miltefosine for Indian Visceral Leishmaniasis. *N. Engl. J. Med.* **2002**, *347*, 1739–1746. [\[CrossRef\]](#)
34. Blum, J.; Buffet, P.; Visser, L.; Harms, G.; Bailey, M.S.; Caumes, E.; Clerinx, J.; van Thiel, P.P.A.M.; Morizot, G.; Hatz, C.; et al. LeishMan Recommendations for Treatment of Cutaneous and Mucosal Leishmaniasis in Travelers, 2014. *J. Travel Med.* **2014**, *21*, 116–129. [\[CrossRef\]](#)
35. Jain, K.; Jain, N.K. Novel Therapeutic Strategies for Treatment of Visceral Leishmaniasis. *Drug Discov. Today* **2013**, *18*, 1272–1281. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Banerjee, G.; Nandi, G.; Mahato, S.B.; Pakrashi, A.; Basu, M.K. Drug Delivery System: Targeting of Pentamidines to Specific Sites Using Sugar Grafted Liposomes. *J. Antimicrob. Chemother.* **1996**, *38*, 145–150. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Das, N.; Mahato, S.B.; Naskar, K.; Ghosh, D.K.; Basu, M.K. Targeting of Urea Stibamine Encapsulated in Liposomes to Reticuloendothelial System for the Treatment of Experimental Leishmaniasis. *Biochem. Med. Metab. Biol.* **1990**, *43*, 133–139. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Kavian, Z.; Alavizadeh, S.H.; Golmohamadzadeh, S.; Badiie, A.; Khamesipour, A.; Jaafari, M.R. Development of Topical Liposomes Containing Miltefosine for the Treatment of *Leishmania major* Infection in Susceptible BALB/c Mice. *Acta Trop.* **2019**, *196*, 142–149. [\[CrossRef\]](#)
39. Rysin, A.; Paal, M.; Lokerse, W.J.M.; Wedmann, B.; Hossann, M.; Vogeser, M.; Winter, G.; Lindner, L.H. Evaluation of Release and Pharmacokinetics of Hexadecylphosphocholine (Miltefosine) in Phosphatidylglycerol-Based Thermosensitive Liposomes. *Biochim. Biophys. Acta (BBA) Biomembr.* **2021**, *1863*, 183698. [\[CrossRef\]](#)
40. Seyed, F.; Sharifi, I.; Khosravi, A.; Molaakbari, E.; Tavakkoli, H.; Salarkia, E.; Bahraminejad, S.; Bamorovat, M.; Dabiri, S.; Salari, Z.; et al. Comparison of Cytotoxicity of Miltefosine and Its Niosomal Form on Chick Embryo Model. *Sci. Rep.* **2024**, *14*, 2482. [\[CrossRef\]](#)
41. Román-Álamo, L.; Allaw, M.; Avalos-Padilla, Y.; Manca, M.L.; Manconi, M.; Fulgheri, F.; Fernández-Lajo, J.; Rivas, L.; Vázquez, J.A.; Peris, J.E.; et al. In Vitro Evaluation of Aerosol Therapy with Pentamidine-Loaded Liposomes Coated with Chondroitin Sulfate or Heparin for the Treatment of Leishmaniasis. *Pharmaceutics* **2023**, *15*, 1163. [\[CrossRef\]](#)
42. Siddiqui, R.; Syed, A.; Tomas, S.; Prieto-Garcia, J.; Khan, N.A. Effect of Free versus Liposomal-Complexed Pentamidine Isethionate on Biological Characteristics of *Acanthamoeba Castellani* In Vitro. *J. Med. Microbiol.* **2009**, *58*, 327–330. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Latifi, A.; Esmaili, F.; Mohebbi, M.; Yasami-Khiabani, S.; Rezaeian, M.; Soleimani, M.; Kazemirad, E.; Amani, A. Chitosan Nanoparticles Improve the Effectivity of Miltefosine against *Acanthamoeba*. *PLoS Negl. Trop. Dis.* **2024**, *18*, e0011976. [\[CrossRef\]](#)
44. Khokhar, M.; Shereen, M.A.; Khan, M.; Khan, R.U.; Sohail, A.; Khan, I.U.; Khan, I.U.; Khattak, S. In Vitro Efficacy of Polymer Coated Miltefosine Drug against *Leishmania Tropica*. *J. Parasit. Dis.* **2022**, *46*, 366–376. [\[CrossRef\]](#)
45. Makadia, H.K.; Siegel, S.J. Poly Lactic-Co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers* **2011**, *3*, 1377–1397. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Omarch, G.; Kippie, Y.; Mentor, S.; Ebrahim, N.; Fisher, D.; Murilla, G.; Swai, H.; Dube, A. Comparative in Vitro Transportation of Pentamidine across the Blood-Brain Barrier Using Polycaprolactone Nanoparticles and Phosphatidylcholine Liposomes. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 1428–1436. [\[CrossRef\]](#)
47. Paul, M.; Durand, R.; Boulard, Y.; Fusaï, T.; Fernandez, C.; Rivollet, D.; Deniau, M.; Astier, A. Physicochemical Characteristics of Pentamidine-Loaded Polymethacrylate Nanoparticles: Implication in the Intracellular Drug Release in *Leishmania major* Infected Mice. *J. Drug Target.* **1998**, *5*, 481–490. [\[CrossRef\]](#)
48. Ghezzi, M.; Pescina, S.; Padula, C.; Santi, P.; Del Favero, E.; Cantù, L.; Nicoli, S. Polymeric Micelles in Drug Delivery: An Insight of the Techniques for Their Characterization and Assessment in Biorelevant Conditions. *J. Control. Release* **2021**, *332*, 312–336. [\[CrossRef\]](#)
49. Chen, Z. Small-Molecule Delivery by Nanoparticles for Anticancer Therapy. *Trends Mol. Med.* **2010**, *16*, 594–602. [\[CrossRef\]](#)
50. Caddeo, C.; Manconi, M.; Fadda, A.M.; Lai, F.; Lampis, S.; Diez-Sales, O.; Sinico, C. Nanocarriers for Antioxidant Resveratrol: Formulation Approach, Vesicle Self-Assembly and Stability Evaluation. *Colloids Surf. B Biointerfaces* **2013**, *111*, 327–332. [\[CrossRef\]](#)

51. Irvine, D.J.; Dane, E.L. Enhancing Cancer Immunotherapy with Nanomedicine. *Nat. Rev. Immunol.* **2020**, *20*, 321–334. [[CrossRef](#)] [[PubMed](#)]
52. Paul, M.; Fessi, H.; Laataris, A.; Boulard, Y.; Durand, R.; Deniau, M.; Astier, A. Pentamidine-Loaded Poly(d,l-Lactide) Nanoparticles: Physicochemical Properties and Stability Work. *Int. J. Pharm.* **1997**, *159*, 223–232. [[CrossRef](#)]
53. Valle, I.V.; Machado, M.E.; Araújo, C.d.C.B.; da Cunha-Junior, E.F.; da Silva Pacheco, J.; Torres-Santos, E.C.; da Silva, L.C.R.P.; Cabral, L.M.; do Carmo, F.A.; Sathler, P.C. Oral Pentamidine-Loaded Poly(d,l-Lactic-Co-Glycolic) Acid Nanoparticles: An Alternative Approach for Leishmaniasis Treatment. *Nanotechnology* **2019**, *30*, 455102. [[CrossRef](#)]
54. de Carvalho Moreira, L.M.C.; de Sousa Silva, A.B.A.; de Araújo Medeiros, K.; Oshiro Júnior, J.A.; da Silva, D.T.C.; de Lima Damasceno, B.P.G. Effectiveness In Vivo and In Vitro of Polymeric Nanoparticles as a Drug Release System in the Treatment of Leishmaniasis. *Curr. Med. Chem.* **2024**, *31*, 286–307. [[CrossRef](#)] [[PubMed](#)]
55. Arias, J.L.; Unciti-Broceta, J.D.; Maceira, J.; del Castillo, T.; Hernández-Quero, J.; Magez, S.; Soriano, M.; García-Salcedo, J.A. Nanobody Conjugated PLGA Nanoparticles for Active Targeting of African Trypanosomiasis. *J. Control. Release* **2015**, *197*, 190–198. [[CrossRef](#)] [[PubMed](#)]
56. Owen, S.C.; Chan, D.P.Y.; Shoichet, M.S. Polymeric Micelle Stability. *Nano Today* **2012**, *7*, 53–65. [[CrossRef](#)]
57. Cagel, M.; Tesan, F.C.; Bernabeu, E.; Salgueiro, M.J.; Zubillaga, M.B.; Moretton, M.A.; Chiappetta, D.A. Polymeric Mixed Micelles as Nanomedicines: Achievements and Perspectives. *Eur. J. Pharm. Biopharm.* **2017**, *113*, 211–228. [[CrossRef](#)]
58. Yadav, H.K.S.; Almokdad, A.A.; Shaluf, S.I.M.; Debe, M.S. Polymer-Based Nanomaterials for Drug-Delivery Carriers. In *Nanocarriers for Drug Delivery*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 531–556.
59. Kuperkar, K.; Patel, D.; Atanase, L.I.; Bahadur, P. Amphiphilic Block Copolymers: Their Structures, and Self-Assembly to Polymeric Micelles and Polymersomes as Drug Delivery Vehicles. *Polymers* **2022**, *14*, 4702. [[CrossRef](#)]
60. Duan, X.; Li, Y. Physicochemical Characteristics of Nanoparticles Affect Circulation, Biodistribution, Cellular Internalization, and Trafficking. *Small* **2013**, *9*, 1521–1532. [[CrossRef](#)]
61. Zhang, Y.; Huang, Y.; Li, S. Polymeric Micelles: Nanocarriers for Cancer-Targeted Drug Delivery. *AAPS PharmSciTech* **2014**, *15*, 862–871. [[CrossRef](#)]
62. Lu, Y.; Zhang, E.; Yang, J.; Cao, Z. Strategies to Improve Micelle Stability for Drug Delivery. *Nano Res.* **2018**, *11*, 4985–4998. [[CrossRef](#)] [[PubMed](#)]
63. Trivedi, R.; Kompella, U.B. Nanomicellar Formulations for Sustained Drug Delivery: Strategies and Underlying Principles. *Nanomedicine* **2010**, *5*, 485–505. [[CrossRef](#)]
64. Imran, M.; Shah, M.R.; Shafiullah. Amphiphilic Block Copolymers—Based Micelles for Drug Delivery. In *Design and Development of New Nanocarriers*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 365–400.
65. Ahmad, Z.; Shah, A.; Siddiq, M.; Kraatz, H.-B. Polymeric Micelles as Drug Delivery Vehicles. *RSC Adv.* **2014**, *4*, 17028–17038. [[CrossRef](#)]
66. Puig-Rigall, J.; Blanco-Prieto, M.J.; Aydillo, C.; Radulescu, A.; Molero-Vilchez, D.; Dreiss, C.A.; González-Gaitano, G. Poloxamine/D- α -Tocopheryl Polyethylene Glycol Succinate (TPGS) Mixed Micelles and Gels: Morphology, Loading Capacity and Skin Drug Permeability. *J. Mol. Liq.* **2021**, *324*, 114930. [[CrossRef](#)]
67. Valenzuela-Oses, J.K.; García, M.C.; Feitosa, V.A.; Pachioni-Vasconcelos, J.A.; Gomes-Filho, S.M.; Lourenço, F.R.; Cerize, N.N.P.; Bassères, D.S.; Rangel-Yagui, C.O. Development and Characterization of Miltefosine-Loaded Polymeric Micelles for Cancer Treatment. *Mater. Sci. Eng. C* **2017**, *81*, 327–333. [[CrossRef](#)] [[PubMed](#)]
68. Sanderson, L.; da Silva, M.; Sekhar, G.N.; Brown, R.C.; Burrell-Saward, H.; Fidanboyly, M.; Liu, B.; Dailey, L.A.; Dreiss, C.A.; Lorenz, C.; et al. Drug Reformulation for a Neglected Disease. The NANO-HAT Project to Develop a Safer More Effective Sleeping Sickness Drug. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009276. [[CrossRef](#)]
69. Rodríguez-Évora, M.; Reyes, R.; Alvarez-Lorenzo, C.; Concheiro, A.; Delgado, A.; Évora, C. Bone Regeneration Induced by an in Situ Gel-Forming Poloxamine, Bone Morphogenetic Protein-2 System. *J. Biomed. Nanotechnol.* **2014**, *10*, 959–969. [[CrossRef](#)]
70. Park, J.S.; Lee, S.; Oh, D.H.; Thi, P.L.; Park, K.D. In Situ Forming Hydrogel Crosslinked with Tetronic Micelle for Controlled Delivery of Hydrophobic Anticancer Drug. *Macromol. Res.* **2022**, *30*, 811–819. [[CrossRef](#)]
71. Almeida, M.; Magalhães, M.; Veiga, F.; Figueiras, A. Poloxamers, Poloxamines and Polymeric Micelles: Definition, Structure and Therapeutic Applications in Cancer. *J. Polym. Res.* **2018**, *25*, 31. [[CrossRef](#)]
72. Soto, J.; Soto, P.; Ajata, A.; Rivero, D.; Luque, C.; Tintaya, C.; Berman, J. Miltefosine Combined with Intralesional Pentamidine for *Leishmania braziliensis* Cutaneous Leishmaniasis in Bolivia. *Am. J. Trop. Med. Hyg.* **2018**, *99*, 1153–1155. [[CrossRef](#)]
73. Arnold, O.; Bilheux, J.C.; Borreguero, J.M.; Buts, A.; Campbell, S.I.; Chapon, L.; Doucet, M.; Draper, N.; Ferraz Leal, R.; Gigg, M.A.; et al. Mantid—Data Analysis and Visualization Package for Neutron Scattering and μ SR Experiments. *Nucl. Instrum. Methods Phys. Res. A* **2014**, *764*, 156–166. [[CrossRef](#)]
74. Ahmed, S.; Alvarez, R.; Arnold, O.; Banos, R.; Bilheux, J.; Borreguero, J. *Mantid 3.5: Manipulation and Analysis Toolkit for Instrument Data*, Version 3.5.1; STFC: Daresbury, UK, 2015. [[CrossRef](#)]

75. Wignall, G.D.; Bates, F.S. Absolute Calibration of Small-Angle Neutron Scattering Data. *J. Appl. Crystallogr.* **1987**, *20*, 28–40. [[CrossRef](#)]
76. Doucet, M.; Cho, J.H.; Alina, G.; Attala, Z.; Bakker, J.; Bouwman, W.; Butler, P.; Campbell, K.; Cooper-Benun, T.; Durniak, C.; et al. *SasView*, Version 5.0.4; CERN: Geneva, Switzerland, 2021. [[CrossRef](#)]
77. Yadav, B.; Wennerberg, K.; Aittokallio, T.; Tang, J. Searching for Drug Synergy in Complex Dose–Response Landscapes Using an Interaction Potency Model. *Comput. Struct. Biotechnol. J.* **2015**, *13*, 504–513. [[CrossRef](#)] [[PubMed](#)]
78. Liu, Q.; Yin, X.; Languino, L.R.; Altieri, D.C. Evaluation of Drug Combination Effect Using a Bliss Independence Dose–Response Surface Model. *Stat. Biopharm. Res.* **2018**, *10*, 112–122. [[CrossRef](#)]
79. Vlot, A.H.C.; Aniceto, N.; Menden, M.P.; Ulrich-Merzenich, G.; Bender, A. Applying Synergy Metrics to Combination Screening Data: Agreements, Disagreements and Pitfalls. *Drug Discov. Today* **2019**, *24*, 2286–2298. [[CrossRef](#)]
80. Zheng, S.; Wang, W.; Aldahdooh, J.; Malyutina, A.; Shadbahr, T.; Tanoli, Z.; Pessia, A.; Tang, J. SynergyFinder Plus: Toward Better Interpretation and Annotation of Drug Combination Screening Datasets. *Genom. Proteom. Bioinform.* **2022**, *20*, 587–596. [[CrossRef](#)] [[PubMed](#)]
81. Barioni, M.B.; Ramos, A.P.; Zaniquelli, M.E.D.; Acuña, A.U.; Ito, A.S. Miltefosine and BODIPY-Labeled Alkylphosphocholine with Leishmanicidal Activity: Aggregation Properties and Interaction with Model Membranes. *Biophys. Chem.* **2015**, *196*, 92–99. [[CrossRef](#)]
82. Puig-Rigall, J.; Fernández-Rubio, C.; González-Benito, J.; Houston, J.E.; Radulescu, A.; Nguewa, P.; González-Gaitano, G. Structural Characterization by Scattering and Spectroscopic Methods and Biological Evaluation of Polymeric Micelles of Poloxamines and TPGS as Nanocarriers for Miltefosine Delivery. *Int. J. Pharm.* **2020**, *578*, 119057. [[CrossRef](#)]
83. Butts, C.P.; Jones, C.R.; Towers, E.C.; Flynn, J.L.; Appleby, L.; Barron, N.J. Interproton Distance Determinations by NOE—Surprising Accuracy and Precision in a Rigid Organic Molecule. *Org. Biomol. Chem.* **2011**, *9*, 177–184. [[CrossRef](#)]
84. Orellano, M.S.; Chiappetta, D.A.; Silber, J.J.; Falcone, R.D.; Correa, N.M. Monitoring the Microenvironment inside Polymeric Micelles Using the Fluorescence Probe 6-Propionyl-2-Dimethylaminonaphthalene (PRODAN). *J. Mol. Liq.* **2021**, *343*, 117552. [[CrossRef](#)]
85. Puig-Rigall, J.; Obregon-Gomez, I.; Monreal-Pérez, P.; Radulescu, A.; Blanco-Prieto, M.J.; Dreiss, C.A.; González-Gaitano, G. Phase Behaviour, Micellar Structure and Linear Rheology of Tetrablock Copolymer Tetronic 908. *J. Colloid Interface Sci.* **2018**, *524*, 42–51. [[CrossRef](#)]
86. Tea, L.; Willner, L.; Waldorf, C.; Matsarskaia, O.; Schweins, R.; Förster, S.; Willner, L.; Stellbrink, J. Surface Charged Polymeric Micelles—A Tunable Model System Studied by SANS. *Macromolecules* **2024**, *57*, 5818–5830. [[CrossRef](#)]
87. Hamley, I.W.; Castelletto, V. Sodium Dodecyl Sulfate Micelles: Accurate Analysis of Small-Angle X-Ray Scattering Data through Form Factor and Atomistic Molecular Dynamics Modelling. *Colloids Surf. A Physicochem. Eng. Asp.* **2024**, *696*, 134394. [[CrossRef](#)]
88. Cohen, Y.; Avram, L.; Frish, L. Diffusion NMR Spectroscopy in Supramolecular and Combinatorial Chemistry: An Old Parameter—New Insights. *Angew. Chem. Int. Ed.* **2005**, *44*, 520–554. [[CrossRef](#)]
89. Khademvatan, S.; Gharavi, M.J.; Rahim, F.; Saki, J. Miltefosine-Induced Apoptotic Cell Death on *Leishmania major* and *L. tropica* Strains. *Korean J. Parasitol.* **2011**, *49*, 17–23. [[CrossRef](#)] [[PubMed](#)]
90. Bouabid, C.; Yamaryo-Botté, Y.; Rabhi, S.; Bichiou, H.; Hkimi, C.; Bouglita, W.; Chaouach, M.; Eddaikra, N.; Ghedira, K.; Guizani-Tabbane, L.; et al. Fatty Acid Profiles of *Leishmania major* Derived from Human and Rodent Hosts in Endemic Cutaneous Leishmaniasis Areas of Tunisia and Algeria. *Pathogens* **2022**, *11*, 92. [[CrossRef](#)]
91. Almandil, N.B.; Taha, M.; Rahim, F.; Wadood, A.; Imran, S.; Alqahtani, M.A.; Bamarouf, Y.A.; Ibrahim, M.; Mosaddik, A.; Gollapalli, M. Synthesis of Novel Quinoline-Based Thiadiazole, Evaluation of Their Antileishmanial Potential and Molecular Docking Studies. *Bioorg. Chem.* **2019**, *85*, 109–116. [[CrossRef](#)]
92. Serban, G. Future Prospects in the Treatment of Parasitic Diseases: 2-Amino-1,3,4-Thiadiazoles in Leishmaniasis. *Molecules* **2019**, *24*, 1557. [[CrossRef](#)] [[PubMed](#)]
93. Rao, G.G.; Li, J.; Garonzik, S.M.; Nation, R.L.; Forrest, A. Assessment and Modelling of Antibacterial Combination Regimens. *Clin. Microbiol. Infect.* **2018**, *24*, 689–696. [[CrossRef](#)]
94. González-Gaitano, G.; Da Silva, M.A.; Radulescu, A.; Dreiss, C.A. Selective Tuning of the Self-Assembly and Gelation of a Hydrophilic Poloxamine by Cyclodextrins. *Langmuir* **2015**, *31*, 5645–5655. [[CrossRef](#)]
95. Pillai, S.A.; Lage, E.V.; Casas, M.; Sánchez-Macho, I.; Wang, M.R.; Chen, L.J.; Bahadur, P. A Comparative Study on the Effect of Structural Variations of Co-Solvent on the Tetronic® Micelles with Widely Different Hydrophobicity. *J. Mol. Liq.* **2019**, *282*, 97–104. [[CrossRef](#)]
96. Ribeiro, A.; Sosnik, A.; Chiappetta, D.A.; Veiga, F.; Concheiro, A.; Alvarez-Lorenzo, C. Single and Mixed Poloxamine Micelles as Nanocarriers for Solubilization and Sustained Release of Ethoxzolamide for Topical Glaucoma Therapy. *J. R. Soc. Interface* **2012**, *9*, 2059–2069. [[CrossRef](#)]
97. Ziolk, R.M.; Omar, J.; Hu, W.; Porcar, L.; González-Gaitano, G.; Dreiss, C.A.; Lorenz, C.D. Understanding the PH-Directed Self-Assembly of a Four-Arm Block Copolymer. *Macromolecules* **2020**, *53*, 11065–11076. [[CrossRef](#)]

98. Tamai, S.; Ichinose, T.; Jiapaer, S.; Hirai, N.; Sabit, H.; Tanaka, S.; Kinoshita, M.; Kobayashi, M.; Hirao, A.; Nakada, M. Therapeutic Potential of Pentamidine for Glioma-initiating Cells and Glioma Cells through Multimodal Antitumor Effects. *Cancer Sci.* **2023**, *114*, 2920–2930. [[CrossRef](#)]
99. Parveen, N.; Chiu, W.-J.; Shen, L.-C.; Chou, R.-H.; Sun, C.-M.; Yu, C. The Anti-Cancer Activity of Pentamidine and Its Derivatives (WLC-4059) Is through Blocking the Interaction between S100A1 and RAGE V Domain. *Biomolecules* **2022**, *13*, 81. [[CrossRef](#)] [[PubMed](#)]
100. Katte, R.H.; Dowarha, D.; Chou, R.-H.; Yu, C. S100P Interacts with P53 While Pentamidine Inhibits This Interaction. *Biomolecules* **2021**, *11*, 634. [[CrossRef](#)]
101. Seguela, L.; Rinaldi, F.; Marianecchi, C.; Capuano, R.; Pesce, M.; Annunziata, G.; Casano, F.; Bassotti, G.; Sidoni, A.; Milone, M.; et al. Pentamidine Niosomes Thwart S100B Effects in Human Colon Biopsies Favouring Wtp53 Rescue. *J. Cell. Mol. Med.* **2020**, *24*, 3053–3063. [[CrossRef](#)]
102. Nguewa, P.A.; Fuertes, M.A.; Cepeda, V.; Iborra, S.; Carrión, J.; Valladares, B.; Alonso, C.; Pérez, J.M. Pentamidine Is an Antiparasitic and Apoptotic Drug That Selectively Modifies Ubiquitin. *Chem. Biodivers.* **2005**, *2*, 1387–1400. [[CrossRef](#)] [[PubMed](#)]
103. Zhang, K.; Xue, K.; Loh, X.J. Thermo-Responsive Hydrogels: From Recent Progress to Biomedical Applications. *Gels* **2021**, *7*, 77. [[CrossRef](#)]
104. Alvarez-Lorenzo, C.; Gonzalez-Lopez, J.; Fernandez-Tarrio, M.; Sandez-Macho, I.; Concheiro, A. Tetronic Micellization, Gelation and Drug Solubilization: Influence of PH and Ionic Strength. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 244–252. [[CrossRef](#)] [[PubMed](#)]

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