RESEARCH
www.commprac.com
ISSN 1462 2815

CHITOSAN-BASED FLOATING MICROSPHERES OF TOPOTECAN HCL FOR IMPROVED BIOAVAILABILITY

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DOI: 10.5281/zenodo.10017471

Abstract

Topotecan hydrochloride (TPT) is an anti-cancer agent used in the treatment of ovarian scanner, cervical cancer and small cell lung cancer. TPT is prone for pH-dependent reversible hydrolysis. It is present as therapeutically active lactone form below pH 4. Whereas, it gets converted into inactive carboxylate form above pH 7. The equilibrium between both these form is favoured towards inactive carboxylate form in the physiological pH. Hence, in this present work it was aimed to develop extended release gastro-retentive floating microspheres with the objective of improved bioavailability of therapeutically active lactone form. Chitosan-based porous microspheres by phase separation-coacervation technique were developed. These microspheres were studied for particle size, surface morphology studies, drug entrapment, floating parameters, in vitro drug release and in vivo bioavailability studies in rats. The in vitro characterization testing results demonstrated that the microspheres were obtained with good entrapment efficiency of 78.2%, particle size of 365.8 µm, and drug release control up to 16 hours. In vivo pharmacokinetic studies of the microspheres in comparison with pure drug were performed. These results demonstrated that the bioavailability of the active lactone form of TPT in terms of AUC was improved by times for the microspheres against the pure drug. These results altogether signified that the slow and extended release of the TPT from the gastric region provided higher concentrations of lactone form in the blood. This could improve its bioavailability which might further improve its therapeutic efficiency.

Keywords: Topotecan hydrochloride, Anti-cancer, Extended release, Floating microspheres, Bioavailability.

INTRODUCTION

Topotecan hydrochloride (TPT) is an anti-cancer agent used in the treatment of ovarian scanner, cervical cancer and small cell lung cancer. It is considered as topoisomerase I inhibitor and its cytotoxicity might be due to damaging the double strand DNA produced during DNA synthesis resulting in apoptosis [1]. It is a water-soluble analogue of natural anti-cancer agent camptothecin [2]. It is taken most commonly at a dose of 1.5 mg/m^2/day without exceeding 4 mg per day [1]. It is available in the dosage forms of oral capsules and reconstituted powder containing vials for IV administration.

TPT is prone for pH dependent reversible hydrolysis. It is stable with containing the pharmacologically active lactone form below pH 4. But above pH 7 i.e., at the physiological pH, it undergoes ring opening and is converted into carboxylate form. This conversion between lactone and carboxylate forms is reversible and pH dependent. Only the lactone form is pharmacologically active, whereas the ring-opened carboxylate form is not having any anti-cancer activity [3]. Hence, the therapeutic efficiency of TPT is lesser in case of conventional formulations. IV

administration of reconstituted solution of TPT makes the drug readily exposed to the slight alkaline conditions of blood that results in conversion of the drug readily into its inactive carboxylate form. The conventional oral capsule dosage forms result in rapid dissolution of TPT in the stomach followed by absorption. Total dose is dissolved readily and absorbed into blood stream makes the entire dose exposed to the slight alkaline conditions of the blood resulting in conversion of the total absorbed drug into its inactive ring-opened carboxylate form. So, both the conventional dosage forms result in inactivation of most of the TPT with in short time after entering into the blood stream.

This limitation of the TPT necessitates its development into an extended release (ER) dosage form [4]. Because, the slow and prolonged release of lactone form of TPT and its continuous absorption/presence for prolonged period makes the drug available in the blood stream in therapeutically active lactone form for long time. ER formulations of TPT reported in this regard were liposomes [5-8], polymeric nanoparticles [9], and solid lipid nanoparticles [4] for both oral and parenteral routes. But the oral ER dosage form resides in the gastric region for a period of about 2-3 hours and then it passes into small intestine. As the small intestine environment is slightly alkaline, the TPT released in this region converts into inactive carboxylate form even before absorption. Hence, an ER dosage form which resides in the stomach only until the total amount of TPT released would be more beneficial and more effective formulation strategy for TPT.

In this present work, porous floating microspheres for ER of TPT were aimed to develop. For this purpose, chitosan-based porous microspheres were developed using sodium tripolyphosphate (NaTPP) as the coacervating material. The obtained microspheres were characterized for several in vitro characterization studies including the surface morphology, entrapment efficiency and in vitro drug release studies. further, the optimized formulation was subjected to in vivo bioavailability studies in rats in comparison with pure TPT. The obtained data were treated by non-compartmental analysis using PK Solver software.

MATERIALS AND METHODS

TPT was obtained from Hetero Labs Pvt. Ltd.; chitosan, NaTPP and sodium alginate were bought from Sigma-Aldrich; xanthan gum was obtained from CP-Kelco and all the other materials of analytical grade were employed.

Development of TPT Floating Microspheres (TFMs)

A number of four different formulations of TFMs were prepared as shown in the Table 1. Total polymer was taken 2.67 times to that of the TPT (these quantities were based on the optimization studies performed earlier, whose results were communicated for publication elsewhere). Chitosan (CS) was dissolved in 20 mL of 2% w/v glacial acetic acid. In this CS solution, 40 mg of TPT and 60 mg of sodium bicarbonate (a gas-generating agent) were added and subjected to vortexing on a cyclomixer for 10 minutes. This dispersion was labelled D1. Separately, sodium alginate (SA) or xanthan gum (XG) was added to 20 mL of water and mixed to dissolve the polymer. NaTPP (1-3% w/v) was gradually added to this. This dispersion was labelled D2. Then, D1 was slowly added dropwise into D2, which was kept under stirring at 250 rpm. The microsphere generation was observed as and when the D1 was added to the D2, but it was kept under stirring for 30 minutes to allow rigidization. Later, the microspheres

were collected by filtration and kept for drying. The dried microspheres were stored properly until further use.

Table 1: Formulation compositions of TFMs

Ingredients	Quantities (mg)			
	TFM1	TFM2	TFM3	TFM4
TPT	40	40	40	40
CS	26.7	53.4	26.7	53.4
SA	80.1	53.4	--	--
XG	--	--	80.1	53.4

Characterization of the TCMs

Entrapment Efficiency

EE was quantified using the reverse bag dialysis method. Briefly, 2 mL of microsphere suspension was packed in a dialysis membrane and placed in 50 ml of 0.1N HCl for 8 hours, and the media was analysed using UV-visible spectroscopy [10] for the quantity of free TPT in the microspheres. The amount of drug entrapped and the percentage of drug loading can be calculated using the below-mentioned formula.

$$\%\ Entrapment\ efficiency = \frac{D_T - D_F}{D_T} x100$$

D_T - Total drug content & D_F - Free drug content

Particle Size

PS was determined using microscopy techniques [11]. An optical microscope was used as an instrument to determine the particle size.

Scanning Electronic Microscopy (SEM)

The surface morphology of microspheres was determined using SEM. Briefly, the samples were glued to the stubs and sputtered with gold-palladium alloy at 150–200 °C [12]. The SEM was operated at 20 KV acceleration with a working distance of 12–14 mm.

Floating Time

The floating time of the microspheres was evaluated along with the drug release studies in the dissolution media to know the behaviour of the formulation [13].

Swelling Index

The swelling index was evaluated by soaking the microspheres in water for about 3 hours. The swollen and dried weights of the microspheres were measured, and the swelling index [14] was calculated as the ratio of weight gain by the microspheres to their dry weight and expressed in percentage.

In-Vitro Drug Release

This study was performed for the floating microspheres using the specification mentioned in the USFDA website. 500 mL of Acetate buffer pH 4.5 with 0.15% w/w sodium lauryl sulphate was taken as the dissolution medium in the paddle apparatus. The rotation speed was set as 50 rpm and the temperature was maintained at 37±0.5^{o} C. At regular time intervals, samples were withdrawn and analysed spectrophotometrically.

In vivo Bioavailability Studies

Development of calibration curve for the estimation of TPT in plasma

Linearity or calibration curve was developed after making necessary modifications to the method reported by Yoshikawa N *et al.* [15]. A mixture of ammonium acetate (75 mM, pH 4.5) and acetonitrile at a combination of 56.25% and 43.75% was taken as the mobile phase. Camptothecin was employed as the internal standard (IS). Stock solutions of TPT and IS were prepared at a concentration of 1 µg/mL in dimethyl sulfoxide. Calibration samples were prepared by diluting the TPT stock solution with water, and equal volume of plasma to obtain the concentrations in the range of 2 – 100 ng/mL. Considering the pH-dependent ring opening of the TPT into its carboxylate form, the above plasma spiked samples were analyzed in two different ways. In one case, the above samples were directly extracted for the drug. In this case, 150 µL of a calibration sample was mixed with 300 µL of the mobile phase and 10 µL of IS stock solution. This mixture is vigorously vortexed for 20 s. The mixture was centrifuged (14,000 x g, 4^{o} C, 5 min), to separate the plasma proteins and 20 µL of the supernatant obtained was injected into the chromatograph. The mobile phase was pumped at a flow rate of 0.8 mL/min. the detector was adjusted for an excitation wavelength of 380 nm and emission wavelength of 520 nm. The same method was repeated for all the other calibration samples. In this case, both the lactone and carboxylate forms of the TPT would be present.

In another case, the calibration samples were acidified with equal volume of 0.1M HCl. Due to this acidification of the plasma spiked samples, the carboxylate form is converted back into lactone form. 150 µL of the acidified calibration sample was mixed with 300 µL of the mobile phase and 10 µL of IS stock solution. This mixture is vigorously vortexed for 20 s. The mixture was centrifuged (14,000 x g, 4^{o} C, 5 min), to separate the plasma proteins and 20 µL of the supernatant obtained was injected into the chromatograph. The mobile phase was pumped at a flow rate of 0.8 mL/min. the detector was adjusted for an excitation wavelength of 380 nm and emission wavelength of 520 nm. The same method was repeated for all the other calibration samples. In this case, the total TPT would be present only in the lactone form. The results obtained in both cases were developed into linearity or calibration plots.

Study Protocol

The study protocol conducted at Jeeva Life Sciences, Hyderabad and the study protocol was approved with the number CPCSEA/IAEC/JLS/19/02/23/158. Male Sprague–Dawley (SD) rats of 8 – 9 weeks old with a weight of 250 – 280 g were taken for this study. The animals were maintained under 25 ± 0.5^{o}C temperature and 50 ± 5% RH with 12 h light/dark cycles for 2 weeks. During this time, the animals were given free access to food and water. The animals were kept for fasting 12 h before the study.

The animals were divided into three groups with each group containing six animals and categorized as Group G1: Control, Group G2: Pure TPT solution, and Group G3: Optimized TFMs. The animals in the G2 and G3 were administered with the respective formulations at a dose of 0.25 mg/Kg. the dose equivalent formulations were dispersed in 1.5 mL water and administered orally through a feeding tube [16]. After administration, the blood samples (300µl) were withdrawn from the retro orbital of rat at 0.5, 1.0, 1.5, 2.0, 4.0, 8.0, 12.0, 18.0 and 24 h and transferred into heparinized containers. After 24 hours, the animals were injected with sufficient fluids and subjected to rehabilitation and revival.

Preparation Of Biological Samples

The biological plasma samples were also treated in the same manner as like for the *in vitro* plasma spiked samples which was describe in the previous section. Half-portion of the plasma samples at every time were analysed without acidification. This would give the results of the concentrations of only lactone form of the TPT available in the body excluding the carboxylate form. And the remaining half-portion of the plasma samples were analysed after acidification. This acidification treatment would convert the carboxylate form into lactone form and thus would give the results of the concentrations of total TPT available in the body. The obtained plasma drug concentration versus time data in both the cases (with and without acidification) were subjected to non-compartmental analysis for the estimation of pharmacokinetic parameters of the TPT using PK Solver software.

RESULTS AND DISCUSSION

Table 2: Results of characterization studies on the TFMs

Formulation	Observed results (Mean ± Std. dev. for n = 3)			
	% Yield	Swelling index (%)	Particle size (µm)	EE (%)
TFM1	76.6 ± 3.2	122.4 ± 11.5	312.2 ± 19.4	51.8 ± 2.3
TFM2	80.4 ± 1.9	139.8 ± 10.2	341.8 ± 22.1	55.3 ± 3.1
TFM3	81.5 ± 2.6	67.2 ± 4.6	388.5 ± 17.2	75.1 ± 1.8
TFM4	84.2 ± 3.5	61.6 ± 5.1	352.7 ± 20.3	69.4 ± 2.5

The prepared TFMs were characterised for the percentage yield, swelling index, particle size and EE. The results are presented in Table 2. The % yield of the TFMs was found to be in the range of 76.6% to 84.2%, whereas the % entrapment efficiency ranges from 51.8% to 75.1%. The particle size was determined using an optical microscope, and the results were found to be in the range of 312.2 µm to 388.5 µm. The swelling index was determined by the soaking technique, and the results were found to be in the range of 61.6% to 139.8%. The high % yield values demonstrated that the method conditions employed were effective enough to prepare the floating microspheres in good condition. The swelling index was found to be highly variable. This could be attributed to the properties of the colloids used. In case of the CS-SA formulations, the swelling index was found to be increased with an increase in the CS concentration. Whereas in case of CS-XG formulations, the swelling index was found to be decreased with increased CS. This result could be attributed to the order of swelling behaviours of the individual colloids i.e., XG > CS > SA.

Floating characteristics

TFMs of all the formulations were found to float immediately after adding them to water without any lag time. This could be due to their porous nature [10] because of the presence of bicarbonate during preparation. The TFMs of all the formulations were observed for 24 hours and it was found that all were remained floating. This extended floating behaviour could be attributed to the cross-linking of the positively charged CS with the negatively charged SA/XG which resulted in the formation of polyelectrolyte complex that was almost water insoluble. This characteristic was further aided by the inorganic polyelectrolyte, NaTPP. These formulation combinations made the TFMs remain insoluble and floatable for sufficient period until the complete release of the drug.

Particle size

Upon increasing the CS concentration, the particle size of the TFMs was found to be increased in case of CS-SA TFMs, and decreased in case of CS-XG formulations. This could be due to the differences in the molecular weights of the colloids which is in the order of SA > CS > XG [17-19]. In case of the CS-SA TFMs, the overall molecular weight of the mixture is increased upon increase in the CS concentration. But, in case of the CS-XG TFMs, the XG concentration is decreased upon increase in the CS concentration and thus the overall molecular weight of the mixture is decreased. Higher molecular weight of the polymer mixture would yield higher viscosities during preparation which cause lesser degree of size reduction and thus result in bigger size of the microspheres. Even the differences in the sizes in TFMs made with SA or XG were also attributed to the same reason. As the XG has higher molecular weight than that of SA, the TFMs made with XG showed bigger sizes than those prepared with SA.

Entrapment efficiency

Upon increasing the CS concentration, the EE of the TFMs was found to be increased in case of CS-SA TFMs, and decreased in case of CS-XG formulations. This pattern, similar to the particle size, also could be due to the differences in the molecular weights of the colloids which is in the order of SA > CS > XG [17-19]. Entrapment of the drug in the microspheres is attributed to the binding nature of the polymers which depends on the molecular weight of the polymer. In a similar class of materials like the hydrocolloids used in this work, higher molecular weight of the colloid has more binding nature and can entrap more amount of the drug. In case of the CS-SA TFMs, the overall molecular weight of the mixture is increased upon increase in the CS concentration and thus the EE was increased. But, in case of the CS-XG TFMs, the XG concentration is decreased upon increase in the CS concentration and thus the overall molecular weight of the mixture is decreased which resulted in decreased EE. On the other hand, the at same concentration of the CS, the TFMs made with XG exhibited higher EE than those made with SA. Again, this could be due to the higher molecular weight and thus the binding nature of the XG than those of the SA [20].

SEM analysis

The SEM images of the TFMs are illustrated in Fig. 1. The surface was found to be slightly protruding, which may be due to the entrapment of CO_2 from sodium bicarbonate during the manufacturing process. SEM images were taken for the cross-sectioned TFMs and are shown in Fig. 1(b). This SEM image illustrated many pores present inside the core of the TFMs, which might be due to gas entrapment, and thus these microspheres can be readily floatable upon adding into water and hence can be called floating microspheres [21].

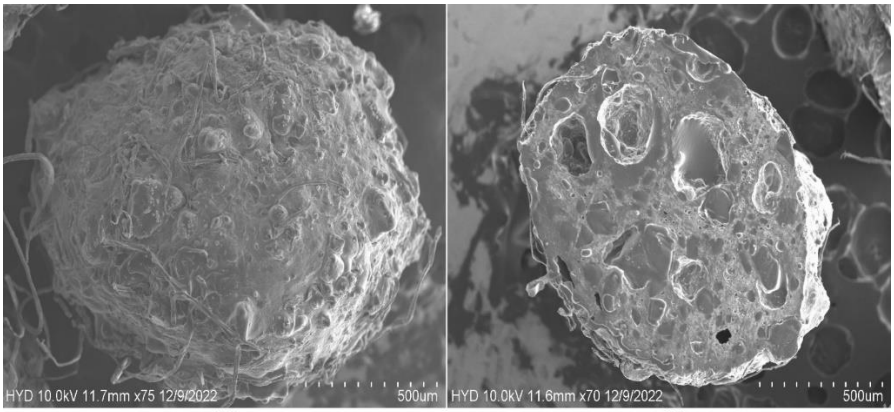

Fig 1: SEM images of the TFM3. (a) Outer surface indicating the blisters, (b) Cros-sectional view illustrating the inner pores

In Vitro Drug Release Studies

All the four formulations of the TFMs were studied for the drug release behaviour. The obtained data was fit into various kinetic models which are illustrated in the Fig 2. From the zero-order and first-order kinetics analysis, it was observed that the release of TPT from the TFMs followed first-order kinetics. This could be attributed to the increased pathlength of the gel matrix and also the decreased drug content in the TFMs upon time which inversely affected the drug release rate. On the other hand, from the Higuchi's and Korsemeyer-Peppas modelling, it was observed that the mechanism of release of TPT from the TFMs followed non-Fickian diffusion model. This observation demonstrated that the polymer gel barriers had influence on the release of TPT from the TFMs.

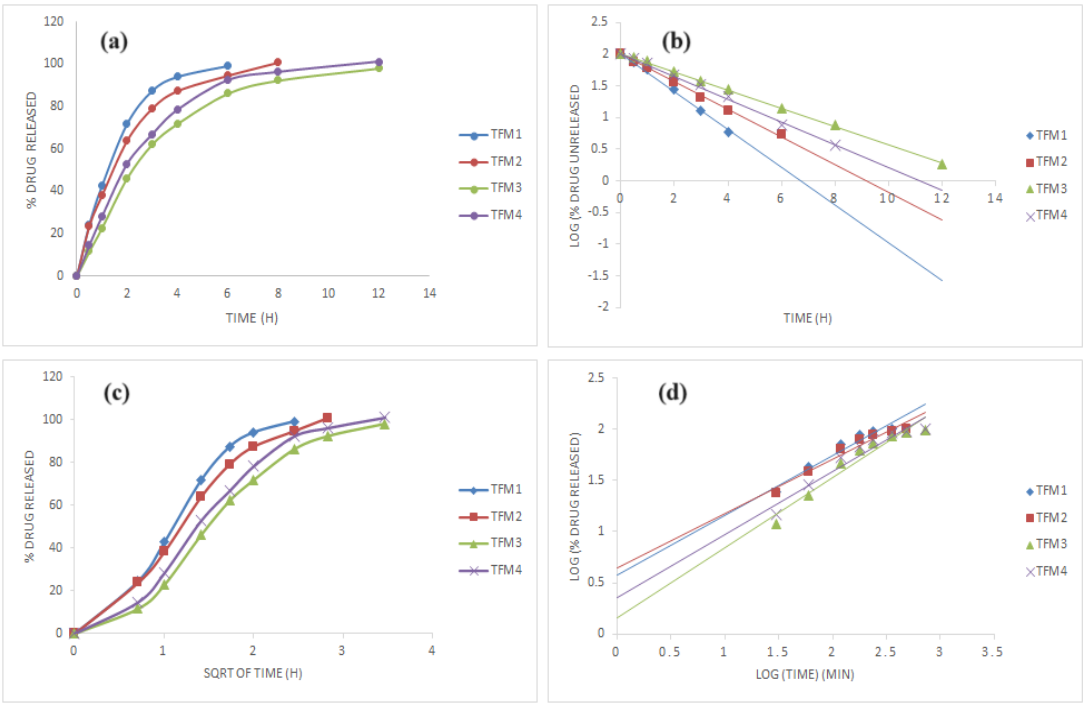

Fig 2: Kinetic modeling of the drug release data of the TFMs. (a) Zero-order profiles, (b) First-order profiles, (c) Higuchi's profiles, and (d) Korsemeyer-Peppas profiles

The drug release rate constant of the four TFM formulations in the order was found to be 0.68, 0.50, 0.33 and 0.41 h^{-1} respectively. These results signified that the concentration of CS and the type of negative charged colloid (SA/XG) had significant influence on the drug release. In case of the TFMs prepared with CS-SA, the drug release was decreased upon increase in the CS concentration. Conversely, in case of the TFMs prepared with CS-XG, the drug release was increased upon increase in the CS concentration. This observation could be attributed to the molecular weights of these colloids as described previously in case of particle size. Higher the molecular weight of the colloid, greater its binding efficiency and thus lesser the drug release [22]. Based on these in vitro observations, the TFM3 was considered as the optimized formulation. This TFM3 along with pure TPT were further subjected to in vivo bioavailability testing in rats to study the efficiency of the TFMs in improving bioavailability of the active lactone form of TPT.

In Vivo Bioavailability Studies

Linearity or Calibration Curve

Linearity plot or calibration curve for the TPT in the rat plasma was developed for the purpose of quantification of the TPT from the *in vivo* bioavailability testing samples. In the acidic pH, TPT is present in the therapeutically active lactone form. But, in the pH above 7, it undergoes reversible ring opening into carboxylate form which is inactive therapeutically. Considering this pH-dependent reversible hydrolysis, majority of the TPT which is absorbed into blood is converted into carboxylate form. The blood samples due to their pH above 7, contain both forms of the TPT. These blood samples can be acidified to convert back the carboxylate form so that total TPT is present in the lactone form only. Hence, calibration curve for the TPT in plasma is required to be developed before and after acidification of the samples. *In vitro* plasma spiked samples containing TPT in the concentration range of 2 – 100 ng/mL were prepared with containing camptothecin at 1 µg/mL as the internal standard. These samples were subjected to quantification directly. Besides, a same set of samples were replicated and were acidified with 0.1N HCl and then quantified. The obtained results were discussed in the following sections.

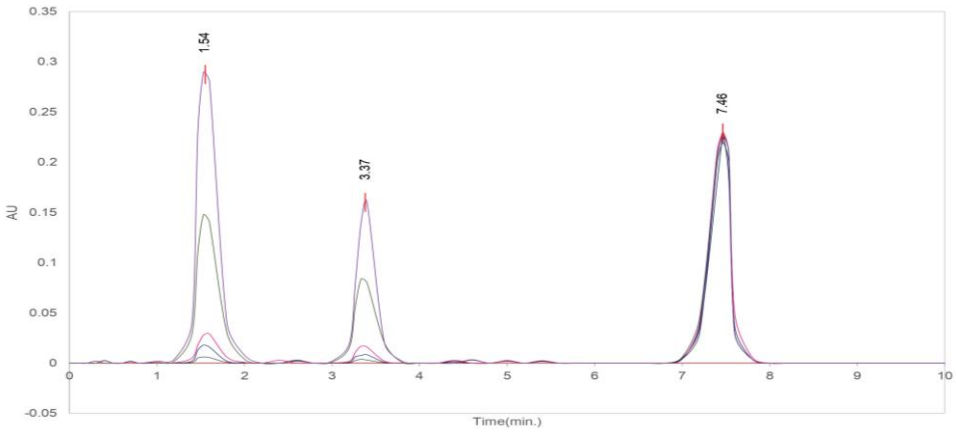

Fig 3: HPLC spectra of various concentrations of TPT without acidification treatment. The carboxylate form was eluted at 1.54 min., the lactone form was eluted at 3.37 min. and the IS was eluted at 7.46 min

The HPLC spectra obtained for the samples without acidification, were shown as an overlay plot in the Fig 3. These plasma samples contained both lactone and carboxylate form. Hence, in the spectra, the carboxylate form was observed at a retention time (RT) of 1.54 min., the lactone form was observed at an RT of 3.37 min. and the IS was observed at 7.48 min. the obtained results were correlated with those reported by Yoshikawa N *et al.* [15]. The peaks areas illustrated that the carboxylate form was present in more amount than the lactone form in the untreated plasma. The ratio of peak area of the lactone form and the IS at every concentration (presented in the Table 3) were plotted as a calibration curve and is shown in Fig 4. the linear correlation coefficient was found to be 0.999. this result demonstrated that this calibration curve could be employed to quantify the lactone form of TPT in plasma from the in vivo samples without acidification.

Table 3: Ratios of peak areas of TPT to IS at various concentrations of TPT without acidification treatment

S. No.	Concentration of TPT (ng/mL)	Ratio of Peak Areas of TPT to IS*
1	0	0
2	2	0.017273 ± 0.011
3	5	0.037333 ± 0.014
4	10	0.073478 ± 0.019
5	50	0.368421 ± 0.022
6	100	0.707965 ± 0.026

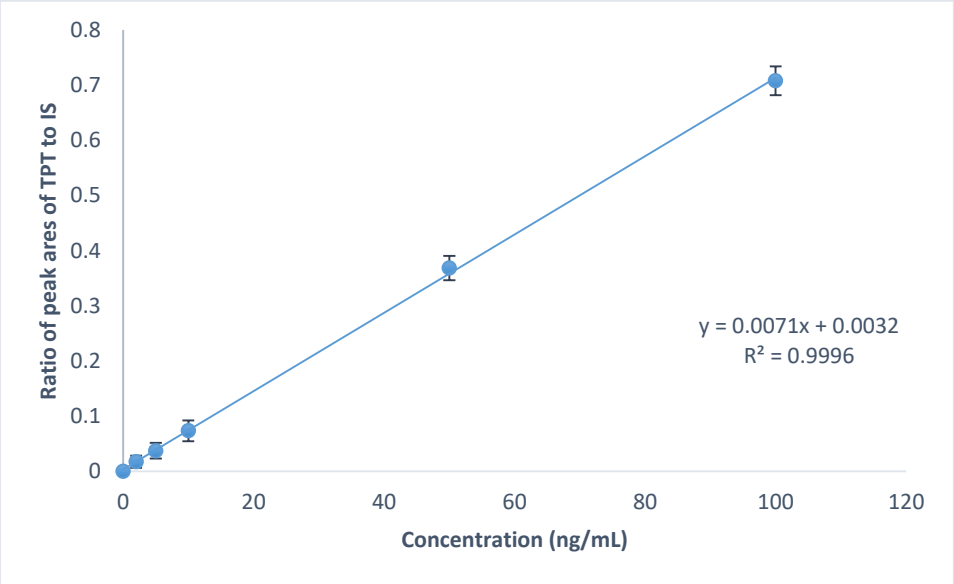

Fig 4: Calibration curve of the lactone form of TPT in rat plasma without acidification

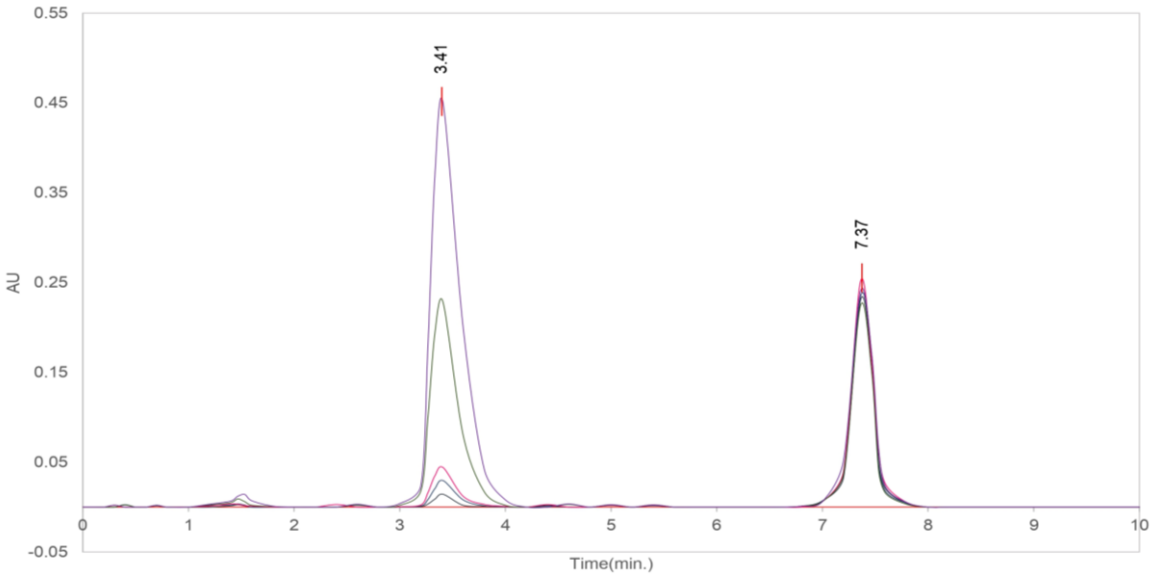

Fig 5: HPLC spectra of various concentrations of TPT after acidification treatment. The lactone form was eluted at 3.41 min., and the IS was eluted at 7.37 min

Besides, the HPLC spectra obtained for the plasma spiked samples after acidification, were shown as an overlay plot in the Fig 5. As the acidification treatment was done, these samples contained the total TPT only in the lactone form. this lactone form was observed at an RT of 3.41 min. The ratio of peak area of the TPT and the IS at every concentration (presented in the Table 4) were plotted as a calibration curve and is shown in Fig 6. The linear correlation coefficient was found to be 0.998. This result demonstrated that this calibration curve could be employed to quantify the total TPT in its lactone form in plasma the in vivo samples after acidification.

Table 4: Ratios of peak areas of TPT to IS at various concentrations of TPT after acidification

S. No.	Concentration of TPT (ng/mL)	Ratio of Peak Areas of TPT to IS*
1	0	0
2	2	0.062 ± 0.031
3	5	0.126 ± 0.048
4	10	0.175 ± 0.054
5	50	1.018 ± 0.072
6	100	1.875 ± 0.066

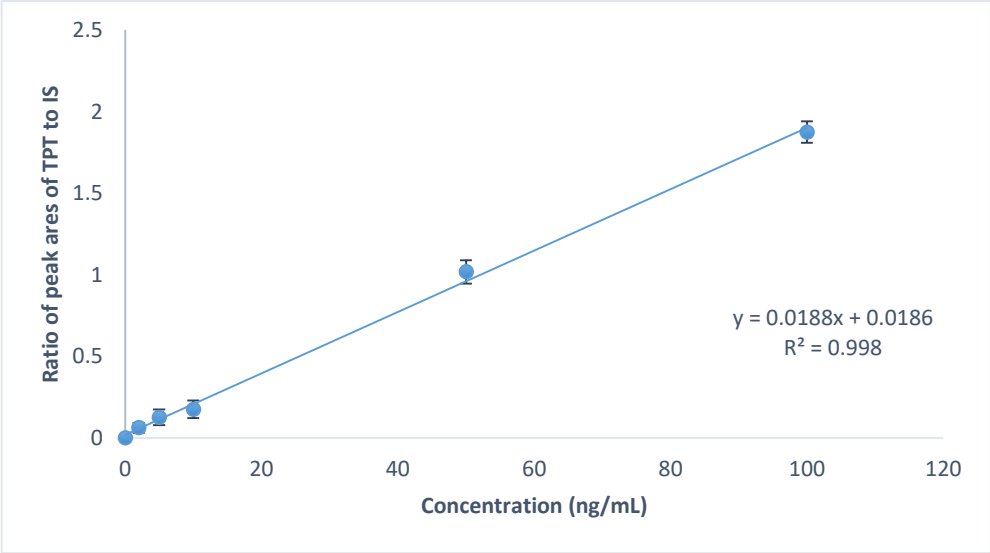

Fig 6: Calibration curve of the lactone form of TPT in rat plasma after acidification

Pharmacokinetic Analysis

The optimized TFM3 formulation along with pure TPT were subjected to in vivo bioavailability studies in rats. The collected plasma samples were separated into two equal portions. One portion of samples were directly quantified for the only lactone form. Whereas, the other portion of the samples subjected to acidification treatment and then quantified to get the total TPT concentration. The obtained data was plotted as time vs plasma concentration and are presented in the Fig 7 (a) and 7 (b).

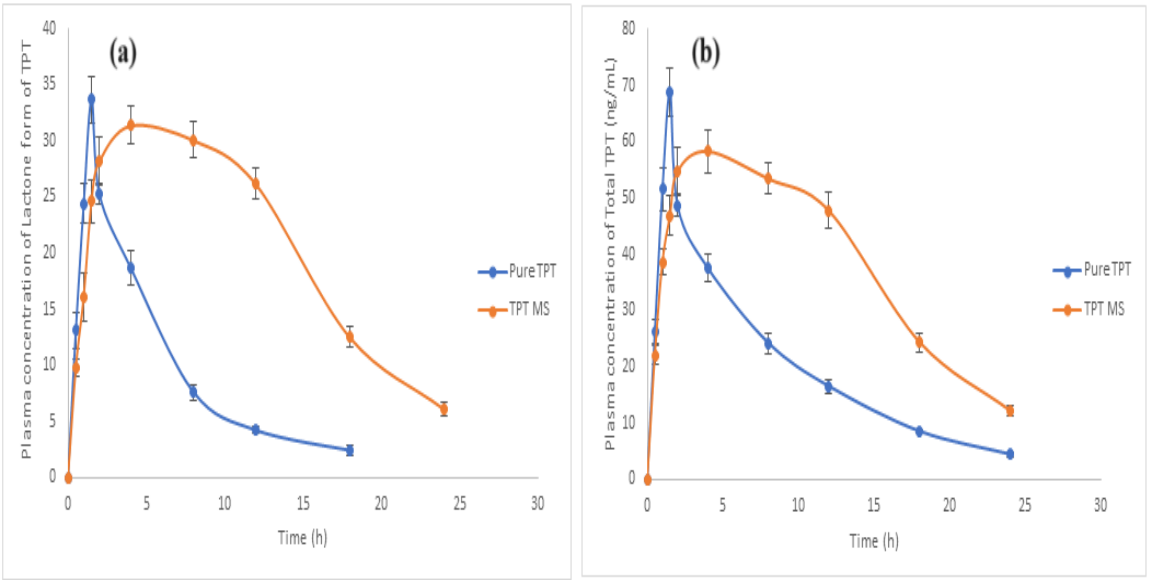

Fig 7: Time – plasma concentration profiles of (a) Only lactone from of TPT, and (b) Total TPT from the *in vivo* studies

This data was further studied by non-compartmental analysis for the bioavailability parameters including area under the curve (AUC). The obtained parameters in both the cases are presented in the Table 5.

Table 5: Results of the pharmacokinetic parameters of TPT from the non-compartmental analysis of the data obtained before acidification (only lactone form) and after acidification (total TPT)

S. No.	Pharmacokinetic parameter	From the data obtained without acidification		From the data obtained after acidification	
		Pure TPT	Optimized TFMs	Pure TPT	Optimized TFMs
1	T_{max} (h)	1.5	4.0	1.5	4.0
2	C_{max} (ng/mL)	33.7	31.4	68.7	58.3
3	AUC_{0-t} (h. ng/ml)	181.92	499.4	489.95	931.4
4	$AUC_{0-\infty}$ (h. ng/ml)	203.06	549.62	529.89	1037.25
5	k_e (h^{-1})	0.114	0.121	0.11	0.114
6	$t_{1/2}$ (h)	6.11	5.71	6.29	6.06

In case of the untreated plasma samples which demonstrate the results of only lactone form, the maximum plasma drug concentration (C_{max}) for the pure TPT was found to be higher than that of the TFMs. The time to reach C_{max} (T_{max}) for the pure TPT was found to be lesser than that for the TFMs. TPT is readily water soluble and thus can be absorbed immediately after administration [23], whereas the TFMs release the drug in a slow manner. This could be the reason for the shorter T_{max} and higher C_{max} from the pure TPT. But, the overall bioavailability of a drug is expressed in terms of AUC. The AUC from the TFMs was found to be 499.64 h. ng/ml which was 2.74 times higher than AUC from the pure TPT. This could be due to the rapid conversion of the absorbed TPT into carboxylate in case of pure TPT [24]. On the other hand, the TPT from the TFMs was absorbed slowly and available in lesser concentrations for inactivation into carboxylate form. This result confirmed that the floating microspheres formulation of TPT would greatly enhance the bioavailability of therapeutically active lactone form of TPT.

Besides, the plasma samples after acidification treatment also demonstrated that the bioavailability was higher for the optimized TFMs when compared to pure TPT. After acidification of the plasma samples, the carboxylate form also converted into lactone form and the total TPT concentration was measured. From the pure drug, The AUC of the total TPT was 489.95 h. ng/ml and the AUC of the only lactone form was 181.92 h. ng/ml. This indicated that the AUC of the therapeutically active form was decreased by 2.69 times in case of pure TPT. On the other hand, from the optimized TFMs, the AUC of the total TPT was 1037.25 h. ng/ml and the AUC of the only lactone form was 499.4 h. ng/ml. This indicated that the AUC of the therapeutically active form was decreased by 2.07 times which was lesser than that in case of pure TPT. This observation further confirmed that the inactivation of TPT into carboxylate form was decreased by formulating it into ER floating microspheres. So, the TFMs were successfully developed with achieving the desired objective.

CONCLUSION

TPT is prone for reversible hydrolysis into carboxylate form which is therapeutically inactive in the physiological pH. Hence, to control this inactivation in the blood, ER floating microspheres were developed in this work. The TFMs were prepared with CS-SA and CS-XG by coacervation with NaTPP. The in vitro characterization results demonstrated good entrapment efficiency and extended drug release over 12 hours. SEM studies revealed that the TFMs were hollow from inside and thus could readily floatable upon addition into water. The in vivo bioavailability studies signified that the bioavailability of the therapeutically active lactone from of the TPT was increased

significantly and also the hydrolysis into inactive carboxylate form was also decreased to a considerable extent. These results concluded that the floating microspheres of TPT were developed effectively and the objectives were achieved successfully.

Acknowledgement

The authors are thankful to the authority of Acharya Nagarjuna University, Guntur for providing the opportunity and facilities to carry out this work.

Conflicts of interest

The authors declare that there are no conflicts of interest with regard to this work.

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