ORIGINAL ARTICLE

Optimization and Pharmacokinetic Evaluation of Gastroretentive Mucoadhesive Microspheres of Propranolol Hydrochloride using Thiolated Tragacanth: A Novel Strategy for Enhanced Drug Delivery

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Abstract

Background: Gastroretentive mucoadhesive drug delivery systems offer a promising approach to enhance the therapeutic efficacy of drugs with low bioavailability and short half-lives. The current study aims to the formulation and pharmacokinetic evaluation of propranolol hydrochloride-loaded gastroretentive mucoadhesive microspheres using thiolated tragacanth (TTG) – a chemically modified natural polymer with improved mucoadhesive capacity. **Materials and Methods:** TTG was prepared by esterification and characterized for thiol content, swelling ability, and mucoadhesive properties. Propranolol hydrochloride-loaded microspheres were prepared employing ionic gelation with cross-linking using calcium chloride and barium chloride. The prepared microspheres were characterized for particle size, drug entrapment efficacy, swelling index, and *in vitro* drug release profiles. A pharmacokinetic profile in Wistar rats was done to identify increases in bioavailability. **Results:** Thiolation of the polysaccharide resulted in 3.5-fold greater thiol levels, greatly improving the mucoadhesive character of tragacant. Optimum microspheres were spherically shaped with excellent entrapment efficacy of 84.2 ± 2.7%. Release studies conducted under *in vitro* conditions indicated controlled drug release over a period of 12 h following Korsmeyer–Peppas model, indicative of non-Fickian or anomalous diffusion. Pharmacokinetic evaluation showed a 2.8-fold relative bioavailability increase over the standard propranolol solution, with delayed Tmax and increased Cmax values, indicating improved systemic absorption. **Conclusion:** The TTG-based gastroretentive microspheres developed in this work showed enhanced drug retention and bioavailability, indicating their potential as a new drug delivery system for propranolol hydrochloride. This work highlights the promise of thiolated natural polymers in sustained drug delivery systems and invites further research in this direction.

Key words: Design of experiments, microspheres, mucoadhesion, optimization, propranolol hydrochloride, thiolation

INTRODUCTION

Over the past few years, mucoadhesive drug delivery systems have been widely studied, as they prolong the presence time of the drug at the site of action and therefore enhance therapeutic efficacy.[1,2] These systems bind to mucosal tissues, providing advantages such as enhanced bioavailability, diminished dosing frequency, and enhanced patient compliance. Localized drug delivery, enabled by interactions with the mucus layer (made up largely of mucins and glycoproteins) and sustained retention in the gastrointestinal (GI) tract, can be particularly useful for drugs with poor GI stability or extensive first-pass metabolism.[3-5]

The key component in the preparation of mucoadhesive drug delivery systems is polymers. Natural polymers such

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as tragacanth, thiolated tragacanth (TTG), and alginate have already been studied extensively as carriers because of their biocompatibility and biodegradability.[6] Of these, tragacanth, a polysaccharide obtained from the dried sap of certain species of *Astragalus*, is a widely used emulsifier, thickener, and stabilizer. However, its isolated mucoadhesive ability is modest, requiring chemical modifications to boost its mucoadhesion.

Thiolation has been proven to be a successful approach to enhancing the mucoadhesive property of natural polymers. This method includes the modification of the polymer backbone by introducing thiol (-SH) groups to form thiomers (thiolated polymers). By forming covalent disulfide bonds with mucin glycoproteins, these thiomers greatly enhance the GI tract retention time of drug delivery systems. All these properties of thiolated polymers provide them with a higher swelling capacity, enzymatic resistance, and controlled drug release abilities, which support their potential applications for advanced mucoadhesive formulations.[7]

Propranolol hydrochloride, a non-selective beta-adrenergic blocker, is commonly prescribed to treat cardiovascular disorders, including hypertension, angina pectoris, arrhythmias, and myocardial infarction. Notably, propranolol hydrochloride is an effective drug, both clinically and as a result of its pharmacokinetic drawbacks, which account for its short half-life (around 3–6 h) and significant first-pass metabolism with limited oral bioavailability (≈25%).[8] These limitations lead to signing doses, which can lead to suboptimal human compliance and plasma drug concentration variability. The above challenges can be addressed with a new drug delivery approach by prolonged release of the drug and long-lasting gastric residence time.

To overcome the above limitations, the development of a gastroretentive mucoadhesive drug delivery system using TTG has promising applications.[9,10] Thiolation enhances physicochemical properties and mucoadhesive strength of tragacanth: Device for sustained release of propranolol hydrochloride-loaded microspheres. Use of thiolated polymers in gastroretentive drug delivery systems will lead to prolonged gastric residence time, improved drug absorption, and minimal first-pass metabolism.[11,12]

Microspheres have been recognized as a promising carrier for sustained drug release in mucoadhesive systems.[13,14] Such spherical particles can entrap drugs and afford sustained release of encapsulated drugs with the possibility of extended contact with the mucosal tissues. The addition of bio-adhesive polymers facilitates enhanced absorption and optimization of the overall therapy.[15] The objective of this study was to formulate and characterize propranolol hydrochloride-loaded mucoadhesive microspheres using TTG as a novel excipient. The mucoadhesive properties of tragacanth when thiolated are greatly enhanced, making it an excellent candidate as a pH-sensitive drug release system and a gastric retentive device. If successful, this system could lead to wider use of thiolated natural polymers in pharmaceutical formulations, overcoming challenges that inhibit conventional oral therapies, ultimately benefiting patients.

MATERIALS AND METHODS

Materials

Propranolol hydrochloride (99% purity) was obtained from Aurobindo Pharma, Hyderabad, and tragacanth gum (TG) was obtained from Yarrow Chemicals, Mumbai. Thioglycolic acid (TGA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were obtained from SD Fine-Chem Ltd., Mumbai, and utilized for thiolation of tragacanth. Calcium chloride and barium chloride were obtained from Loba Chemie Pvt Ltd., Mumbai, for cross-linking. All other chemicals and solvents utilized in this research were of analytical grade, and distilled water was used throughout the experiments.

Synthesis of TTG

TTG was prepared by esterifying TG with TGA using hydrochloric acid (HCl). First, 6 g of TG was homogenized with 50 mL of hot distilled water. Afterfv addition of 2 mL of TGA and 7N HCl was followed by stirring the mixture to allow the reaction to proceed at 80°C for about 2 h and 30 min.[7] The reaction mixture obtained was treated with 0.5 L of methanol, and the precipitate formed was washed several times with methanol, dried, and kept in a desiccator. The whole synthesis process was carried out under a nitrogen atmosphere (argon environment).

Characterization of TTG

Thiol content determination

The immobilized thiol groups of the polymer backbone were analyzed by Ellman's reagent (5,5-dithiobis (2-nitrobenzoic acid)) according to the procedure given by Werle *et al*. The disulfide content was also found after subsequent reduction with sodium borohydride.[16]

Subacute toxicity study of TTG

Animals for the study

The animal study was carried out according to ethical guidelines for animal experimentation and was approved by the Institutional Ethical Committee. The experimental animals were cared for as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Animals were procured from a recognized breeder and acclimatized under in-house conditions of controlled temperature (25 ± 2°C), relative

humidity of 50 ± 5%, and a light/dark cycle of 12 h. They were kept in polypropylene cages and fed a standard pellet diet (Hindustan Lever Ltd., Mumbai, India) supplemented with ad libitum water.[17]

Microbial load study

Microbial load of the samples was assessed by the standard pour plate method. For bacterial and fungal growth, soybean-casein digest agar and potato dextrose agar media were employed, respectively. New TTG and a 10-month-old TTG sample were inoculated on Petri plates with the corresponding media aseptically. The plates were then placed at 37°C for 24 h after solidification, and colony numbers were enumerated using the Coulter counter method. Fungal enumeration was conducted using the same method.[18]

Formulation of microspheres

Microspheres were formulated through the spray-drying technique. TTG was dissolved in deionized water with 1% v/v acetic acid, and then the drug solution was added gradually with constant stirring at 800 rpm for a maximum of 2 h to form a clear solution.[19] The solution was then spray-dried with a constant drug-to-polymer ratio of 1:1. Optimization of the spray-drying conditions was achieved at an inlet temperature of 140°C, outlet temperature of 90°C, feed rate of 6 mL/min, and atomization air pressure of 2×10^5 Pa. The drying air was regulated by an aspirator filter vessel with a noted pressure of −40 mbar.

Cross-linking of TTG microspheres

Microspheres thus prepared were cross-linked using glutaraldehyde. This was performed through alteration in cross-linking duration (30–120 min) and in concentration of glutaraldehyde (5–15 mL for every 100 mg TTG). The microspheres obtained from cross-linking were then filtered and intensively washed using ethanol, followed by an initial acetone treatment (30 s). Ultimately, the vacuum dried the microspheres for a period of not <24 h.

Optimization of grafting process – Design expert approach

- Design name: Central composite statistical design (Design Expert, Version 13; Stat-Ease Inc., Minneapolis, MN, USA)
- Design: 2 × 5 {2-factor, 5-level}
- Independent variables: Cross-linking duration, glutaraldehyde concentration, and exposure time
- Dependent variables: Encapsulation efficiency (EE) and particle size (PS) [Table 1].

Drug entrapment efficiency

EE was calculated after grinding 100 mg of microspheres in a glass mortar and suspending the powder in 10 mL of 0.1 M HCl (pH 1.2) and then allowing the mixture to stand for 24 h. The mixture was then filtered, and the filtrate was scanned at 228 nm on a Systronic 1700 ultraviolet (UV) spectrophotometer (Japan) to calculate drug content.[20]

PS of microspheres

Microsphere diameter was determined with a Brookhaven Instruments Corp. (USA) laser PS analyzer. A small amount of dry microspheres was suspended in 10 mL of ethanol, and 2–3 mL of this suspension was poured into disposable cuvettes.[21] A 35-mW laser diode was employed to determine PS distribution.

Desirability approach

A desirability function was utilized to maximize cross-linking of TTG microspheres, maximizing EE and PS. EE was maximized for maximum drug loading, whereas PS was optimized for uniformity and controlled release. The overall desirability value (from 0 to 1) was utilized to select the most ideal combination of glutaraldehyde concentration and cross-linking time for an optimal microsphere preparation.[22,23]

Preparation of optimized formula

Design-Expert® software determined the optimum glutaraldehyde concentration and cross-linking time for TTG microspheres from the highest desirability score. The optimized microspheres were wet milled, washed, and vacuum dried to get the final product.

Characterization of microspheres for PS and surface morphology

The size of the optimized microspheres was measured using laser diffraction to verify even distribution and regulated drug release. Scanning electron microscopy (SEM) was used to examine surface morphology, smoothness, and porosity, which determine drug loading and release rate.

Mucoadhesive property of microspheres

The mucoadhesion of microspheres was measured by the wash-off method of Lehr *et al*.[24] A 2 × 2 cm rat stomach tissue sample was fixed on a plastic slide, and ~100 microspheres were dispersed on the wet tissue. The slide was fixed on a United States Pharmacopeia disintegration test apparatus, in which it was subjected to vertical motion in distilled water. The microspheres held back by the tissue were counted hourly for a period of 4 h.

Table 1: Central composite optimization design for formulation optimization of microspheres

Independent variables	The level used, actual and coded			High (+1)	Alpha (+1.414)
	Alpha (–1.414)	Low (–1)	Medium (0)		
X1=cross-linking duration (min)	11.3604	30	75	120	138.64
X2=glutaraldehyde concentration (5-15 mL)	2.9289	5	10	15	17.0711
Dependent variables			Desirability		
EE (Y1)			Maximize		
PS (Y2)			Minimize		

EE: Encapsulation efficiency, PS: Particle size

Table 2: Effect of TTG at different doses on blood hematological and biochemical parameters

Parameters	Control	Treatment with TTG (mg/kg body weight)		
		200 mg	300 mg	400 mg
Hemoglobin (g%)	14.57±0.45	14.28±0.41	14.02±0.42	19.85±0.57
RBC (10^6/μL)	8.69±0.041	8.26±0.037	8.11±0.032	7.75±0.041
WBC (10^3/μL)	13.63±0.045	13.25±0.044	12.98±0.041	12.44±0.032
Neutrophil (10^3/μL)	20.62±0.697	19.44±0.547	19.22±0.597	19.01±0.458
Monocyte (10^3/μL)	2.17±0.368	1.88±0.351	1.67±0.321	1.23±0.332
Lymphocyte (10^3/μL)	70.89±0.379	69.05±0.359	68.55±0.315	68.15±0.387
Eosinophil (10^3/μL)	2.22±0.129	1.77±0.109	1.60±0.138	1.52±0.145
Platelets (10^3/μL)	1268.15±1.526	1249.25±1.508	1239.45±1.408	1231.69±1.308
SGOT (U/L)	91.26±0.362	90.11±0.302	89.86±0.349	88.05±0.308
SGPT (U/L)	35.29±0.567	34.87±0.425	34.10±0.528	33.78±0.518

TTG: Thiolated tragacanth, RBC: Red blood cells, WBC: White blood cells, SGOT: Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

Swelling study

Swelling characteristics were determined by placing microspheres in deionized water (pH 6.0, 37°C) and monitoring weight gain as a function of time. A predetermined amount of microspheres was added to a constantly stirred medium (50 rpm) for 120 min. Swollen samples were taken at regular intervals (0, 1, 5, 10, 15, 30, 60, 90, and 120 min), blotted to remove excess water, and immediately weighed. Duplicate experiments were performed, and the average swelling ratio was noted.[25]

Drug release study

The *in vitro* release of the drug was carried out employing a USP XXPS (17) basket apparatus under the conditions of 900 mL of 0.1 M HCl (37 ± 0.5°C) as the dissolution medium stirred at 100 rpm. Aliquots were withdrawn at definite intervals, filtered through a ≤1.0 mm membrane, and quantitatively estimated using UV spectrophotometry. The first 1 mL of the filtrate was discarded for accuracy.

In vivo pharmacokinetic studies

Pharmacokinetic trials of Optimized - Thiolated Tragacanth-Microspheres were conducted following CPCSEA norms and approved by the Institutional Ethical Committee 20/IAEC/SLSRPL/2024. Twelve male rabbits of New Zealand (1.5–2.0 kg) were selected and divided equally into two groups. Group I was administered the intravenous bolus of propranolol hydrochloride (1 mg/kg), whereas Group II received the oral administration of O-TTG-MS formulation (being equivalent to 20 mg propranolol hydrochloride-10 mg/kg for the 2-kg rabbits). Blood from the marginal ear vein was sampled at fixed times, and the plasma was processed for analysis.

Pharmacokinetic parameters such as half-life, clearance, volume of distribution, and area under the curve (AUC) were determined with PK solver (Excel add-in). Quantitation of propranolol was done on an Agilent high-performance liquid chromatography system with a UV detector at 289 nm and a reversed-phase C18 column. The mobile phase was 0.01 M dipotassium phosphate and methanol (50:50), with the flow rate of 1.0 mL/min. The column was kept at 25°C, and 20 μL of the injection was injected. Linearity 10–200 ng/mL, $r^2 = 0.9991$; limit of detection 2.1 ng/mL; limit of quantitation 6.4 ng/mL; intra-day precision <2%; recovery 98.4–101.2%; bench-top stability 24 h has been validated.

The current study offered insights into the bioavailability and distribution of O-TTG-MS and compared it to intravenous administration as usual.[26,27]

RESULTS AND DISCUSSION

Synthesis of TTG

The thiolation of extracted TG was successfully performed through a covalent bond between the formed ester bond of hydroxyl groups (-OH) of TG and carboxyl groups (-COO) of TGA, leading to the formation of TTG with an average yield of 75%. This modification enhanced the chemical as well as physical characteristics of TG and made it more suitable for different pharmaceutical applications. The product was yellow-brown in color, indicating that modification had occurred without any significant degradation.

The flow properties of thiolated TG gum were found to be significantly improved compared to unmodified TG. The angle of repose is an important parameter for evaluating powder flowability; it decreased from 32° for TG to 27° for TTG, indicating that particle cohesiveness or particle-particle interaction decreases, improving flowability. The Hausner's ratio, also an apparent indicator of flow properties, decreased from 1.16 (TG) to 1.06 (TTG), further leading to a reduction of inter-particle friction and better compressibility. Such advancements in flow characteristics render TTG a more suited excipient for application in drug delivery systems, where flowability and processing ease play a pivotal role in the success of the formulation. These modifications further allow for the promising enhancements of mucoadhesive and controlled-release properties of drug delivery systems based on TG gum.

Thiol content determination

The immobilized Thiol groups on TTG were quantified; the content of thiol groups per gram of TTG is 134.67 ± 6.34 µmol. By contrast with thiolated polymers such as CH (where the presence of disulfide bonds is characteristic), the formation of a significant number of disulfide linkages was not recorded. Regarding TTG, this result suggests a marked change in its properties. Consequently, enough thiol groups on it are available for further modification into drug delivery systems that would be safe and bio-compatible for human beings. It turns out that the formation of disulfide bonds was insignificant, a contrast to the usual behavior of thiolated polymers, where thiol groups are oxidized, and that in turn leads to disulfide bond formation.

This finding can be attributed to the controlled, oxygen-free environment maintained during the synthesis of TTG, which likely prevented the oxidation of thiol groups into disulfide bonds. The stability of thiol groups is crucial for their functionality in various biomedical and pharmaceutical applications, particularly in drug delivery systems where they can provide mucoadhesive properties or interact with other biomolecules. The absence of significant disulfide bond formation is advantageous in preserving the reactivity of the thiol groups, which can be used for further modification or cross-linking with other molecules. Moreover, the thiolation reaction itself is dependent on the extent of hydroxyl groups available on the TG gum backbone. The efficient modification of the gum with TGA, leading to a high thiol content, enhances the potential of TTG as a versatile excipient in controlled-release and mucoadhesive drug delivery systems, and the prolonged gastric residence is consistent with the known mucoadhesive behavior of thiolated polymers.

Acute toxicity studies

The toxicity study of TTG was performed under Organisation for Economic Co-operation and Development guidelines and ethical practices to determine its safety before human use. The research measured the impact of various doses (200, 300, and 400 mg/kg body weight) on hematological and biochemical parameters in experimental animals. The results showed that administration of TTG did not induce severe adverse effects on blood composition, as evidenced by plateaus in hemoglobin values and normal red blood cells, white blood cells, platelets, and differential counts in treated groups compared to control. In addition, biochemical evaluation did not indicate changes in serum indicators such as serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and bilirubin among all treatment groups, supporting the safety profile of the compound. As these enzymes are crucial parameters of liver function, their invariance points toward TTG not inducing hepatic toxicity or influencing essential organ health. Overall maintenance of such physiological factors further confirms that TTG is highly tolerable at the dosing levels examined. Hence, TTG has no substantial effect on hematological or biochemical markers, proving its safety for further study. The findings present a strong background for its future applications in the pharmaceuticals industry, drug delivery systems, wound healing, and other areas of therapy [Table 2].

Microbial load study

Microbial load was assessed through the pour plate method of fresh and old TTG samples to evaluate the microbiological quality of the material. Hence, we pour-plated serial dilutions of fresh and aged TTG samples to determine colony-forming units (CFU/g), we incubated the live and stale wing samples at 37°C for 24 h, and we found 280 CFU/g and 310 CFU/g of microbes in the fresh and stale samples, respectively (as per microbiological count), The fungal (yeast and mold) counts were 69 CFU/g for the fresh sample and 77 CFU/g for the aged sample. The microbial load limit (1000 CFU/g) and the fungal load limit (100 CFU/g), according to the Indian Pharmacopoeia (4th edition, 1996), have both been satisfied in the current study, as it falls within the limit of the control value. Both fresh and old TTG samples exhibited microbial content below the limit and proved their microbiological safety, maintaining the pharmacopeial standards. This supports the

case for TTG use in the pharmaceutical and other industries, where microbiological purity is of utmost concern to ensure product safety and efficacy.

Formulation optimization

On the basis of the proposed experimental design, the impact of any main, interaction, and quadratic effect of selected independent variables regarding Grafting polymer properties (with regard to responses [dependent variables EE (R1) and PS (R2)]) was put into a matrix of 17 runs. The resulted values were fitted to linear, quadratic, cubic, and 2FI (linear two-factor interaction) models to obtain various polynomial equations. Based on the probability that was previously fixed, the software selected the model with the most elevated determination coefficients and significance value. Table 3 depicts 13 unique combinations of formulations. Both PS (2.9–8.2 um) and EE (38.52–86.95%) in all the trial preparations. The responses were additionally analyzed using statistical models such as analysis of variance (ANOVA) and fx to determine how the various factors may have influenced one another. The quadratic model was selected for all of the responses based on the sum of squares (Type I) and fit summary (adjusted and projected R^2) [Table 4]. The high-order polynomial quadratic model was chosen because the addition terms are explicit and it does not alias. As the difference between EE and PS is <0.2, the predicted R^2 values of 0.0.7623 and 0.8540 are analogous to the adjusted R_2 values of 0.9417 and 0.9633. The signal-to-noise (S/N) ratio is estimated accurately enough. Hence, it is mostly better with a larger figure than four. EE and PS with S/N ratios of 22.04 and 23.50, respectively, which tells that the signal is greater than the noise, which clearly indicates that the model is working well in the designed space. Using both responses, model F-values of 39.80 and 6.91 indicate that your theory is correct. Noise could have created the high F-value 0.01% of the time.

The coefficient of variation (CV) ensures the reproducibility of the design. They found that the accuracy of the current model was 10% CV. The study results showed that CV values about the 22-point measure were not too high, indicating the model is accurate and reliable. If the fit is not good enough, the model does not show all the data [Table 4]. Thus, a lack of fit is required to demonstrate that the equations generated by the model approximate the results. The selected model was good for the study because the *P*-values for lack of significance were statistically not significant. The experimental run is compared to the residuals to then find what has an influence on the results [Figure 1]. Within the allowed range, a scattered trend was seen, showing a time-coupled variable in the background.

Study of the effect of independent variables or selected factors on EE and PS

The ANOVA findings offer crucial statistical insights into the significance of both individual and interactive factors on the chosen responses, EE, and PS. The P <0.0500 confirms that the model is statistically significant, indicating that the selected independent factors and their interactions notably influence the responses. For EE, key contributing factors [Table 5] include B, C, AB, A^2, B^2, and C^2, signifying their essential role in determining drug EE. Among these, B, AB, A^2, and C exhibit a synergistic effect, meaning that their increase enhances EE. C^2 had the most substantial impact, suggesting that C (potentially polymer concentration or stirring speed) follows a quadratic pattern, significantly influencing EE. Conversely, other parameters showed an antagonistic effect, implying that their increase negatively affects EE, potentially due to interference in drug entrapment or phase separation issues. The regression equation for EE is as follows:

Table 3: Factors and responses for the CCD for optimization of TTG microspheres

Standard	Run	Factor 1	Factor 2	Response 1	Response 2
		A: Cross-linking duration	B: Glutaraldehyde concentration	EE	PS
		min	mL	Percentage	um
5	3	11.3604	10	38.52	8.2
1	2	30	5	48.5	5.9
3	8	30	15	52.6	7.6
12	10	75	10	65.87	3.65
9	7	75	10	66.42	3.78
10	6	75	10	66.84	3.92
13	1	75	10	67.2	3.8
11	5	75	10	67.49	3.85
4	12	120	15	72.58	3.8
8	13	75	17.0711	75.65	2.9
7	11	75	2.92893	77.85	4.5
2	9	120	5	84.52	6.2
6	4	138.64	10	86.95	6.4

CCD: Central composite design, TTG: Thiolated tragacanth, EE: Encapsulation efficiency, PS: Particle size

Table 4: Model statistical summary

Response	Source	R2	Adjusted R2	Predicted R2	Adequate precision	Sequential *P*-value	Remarks
EE	Linear	0.8520	0.8224	0.6742	22.0476	<0.0001	
	2FI	0.8801	0.8401	0.6844		0.1806	
	Quadratic	0.9660	0.9417	0.7623		0.0121	Suggested
	Cubic	0.9757	0.9418	−0.5073		0.4302	Aliased
PS	Linear	0.1641	−0.0031	−0.6420	23.5044	0.4081	
	2FI	0.2858	0.0477	−0.7205		0.2469	
	Quadratic	0.9786	0.9633	0.8540		<0.0001	Suggested
	Cubic	0.9907	0.9777	0.4769		0.1239	Aliased

EE: Encapsulation efficiency, PS: Particle size

Table 5: ANOVA coefficients table

Response	Intercept	A	B	AB	A2	B2
EE	66.764	15.5613	−1.36891	−4.01	−3.31262	3.69488
P-values		<0.0001	0.2837	0.0471	0.0344	0.0223
PS	3.8	−0.755698	−0.370343	−1.025	1.84375	0.04375
P-values		0.0003	0.0146	0.0004	< 0.0001	0.7331

ANOVA: Analysis of variance, EE: Encapsulation efficiency, PS: Particle size

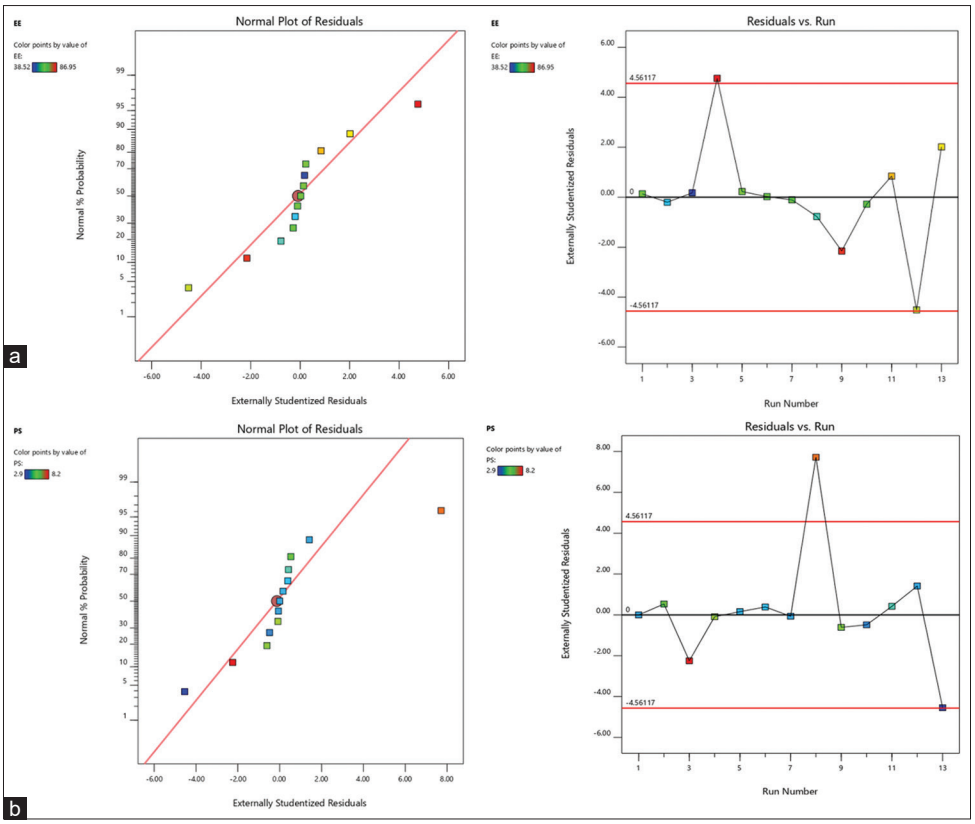


Figure 1: Normal plot of residuals and residuals versus run for (a) response 1 and (b) response 2

$EE = 66.76+15.56A-1.37B-4.01AB-3.31A^2+3.69B^2$

Here, factor A (possibly polymer ratio or surfactant concentration) has the most substantial positive impact on EE (coefficient = 15.56), whereas AB (−4.01) negatively affects EE, likely due to instabilities in formulation. The negative quadratic term A^2 (−3.31) suggests that at higher levels, A leads to encapsulation saturation, thereby reducing EE. For PS, significant influencing factors include A, B, AB, BC, A^2, B^2, and C^2, indicating that both individual factors and their

interactions have a notable impact on PS. Interestingly, A, BC, and C^2 exhibited an antagonistic effect, meaning that increasing these factors led to smaller PS, possibly due to enhanced emulsification, increased shear force, or optimized cross-linking that stabilizes smaller particles. In contrast, the remaining variables had a synergistic effect, where increasing these factors increased PS, likely due to higher viscosity or enhanced particle aggregation. The regression equation for PS is:

$$PS = 3.8 - 0.76A - 0.37B - 1.025AB + 1.84A^2 + 0.043B^2$$

This equation suggests that factor A has the most notable antagonistic effect (−0.76), indicating that an increase in A results in a smaller PS, possibly due to reduced viscosity or improved droplet dispersion. The interaction term AB (−1.025) further supports that these variables work together to reduce PS. On the other hand, the positive quadratic term A^2 (1.84) suggests that higher A concentrations contribute to increased PS, likely due to enhanced coalescence or viscosity-induced limitations.

The 3D surface plots and contour plots provide visual insights into how cross-linking duration (A) and glutaraldehyde concentration (B) impact EE and PS. These plots highlight the synergistic and antagonistic effects of formulation variables, assisting in optimizing the nanoparticle system for improved drug delivery. For EE, the 3D surface plot shows an upward trend, indicating that longer cross-linking duration and higher glutaraldehyde concentration lead to improved EE. This suggests that prolonged cross-linking creates a denser and more stable polymeric network, effectively trapping the drug and minimizing leakage. The contour plot for EE further supports this, transitioning from green (low EE) to red (high EE), marking the regions where encapsulation is maximized [Figure 2a]. However, excessive cross-linking beyond the optimal range may lead to rigidity, potentially affecting drug release behavior. The non-linear nature of the contour lines suggests that these parameters do not have a simple additive effect, and their interactions must be carefully optimized.

In contrast, PS exhibits an inverse correlation with cross-linking duration and glutaraldehyde concentration. The 3D surface plot for PS reveals a downward slope, suggesting that as cross-linking duration increases, PS decreases due to the stabilization of the polymer structure, which prevents excessive particle aggregation. The contour plot for PS further confirms this trend, with blue regions indicating the lowest PS values, representing ideal formulation conditions for smaller and more uniform nanoparticles [Figure 2b]. The presence of curved contour lines implies that PS does not linearly depend on these factors, emphasizing the need for fine-tuned cross-linking conditions to prevent excessive polymerization and unwanted aggregation.

The response surface analysis demonstrates that increasing cross-linking duration and glutaraldehyde concentration

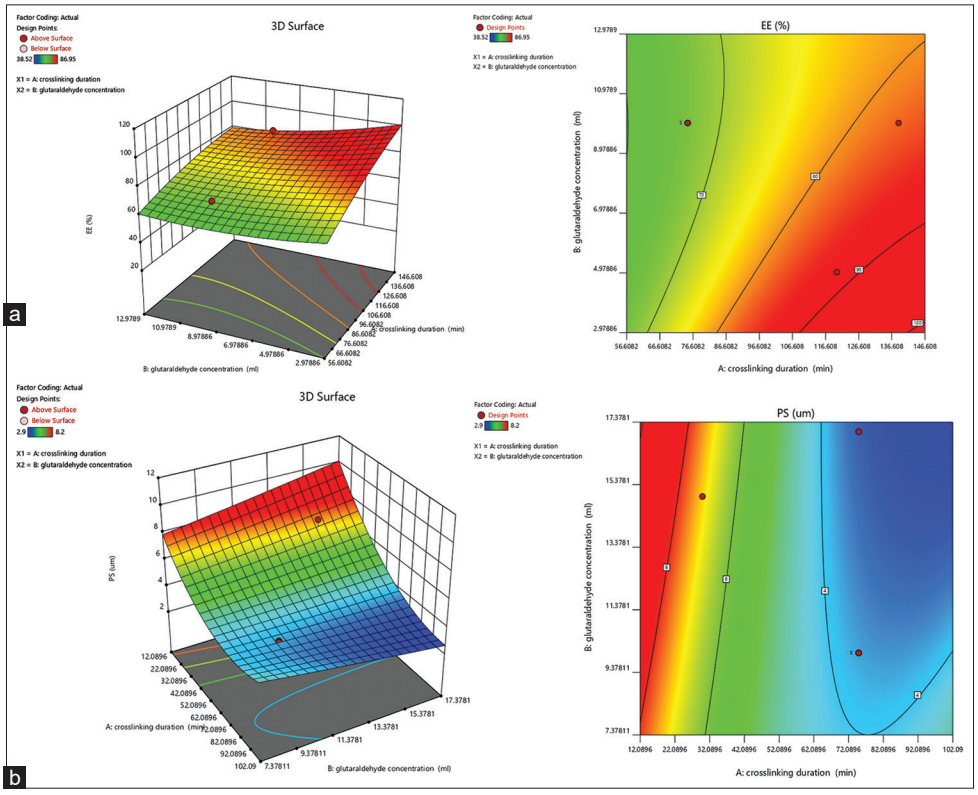


Figure 2: Contour and 3D response surface plots for (a) encapsulation efficiency and (b) particle size

enhances EE while simultaneously reducing PS. However, due to the non-linear interactions between these factors, precise optimization is required to avoid over-crosslinking, which may negatively affect desired physicochemical characteristics. The optimal formulation lies within the transition regions (yellow to red for EE and green to blue for PS) in the contour plots, where maximum drug entrapment is achieved without significantly increasing PS. These findings underscore the importance of response surface methodology in formulating effective drug delivery systems, ensuring a balance between high EE and nanoscale PS for enhanced therapeutic outcomes.

Using the desirability function [D], optimizing various models that came out of an experiment is possible. To make the overlay graph, several limits were set for each response, such as a minimum PS and a maximum zeta potential. The design space had all of the things that were picked. All of the responses combined desirability plots reached a D value of 0.855 at the best concentrations of the independent variables [Figure 3a], and the responses were stacked in the contour plot [Figure 3b]. Using this method, the optimized formulation can witness an EE of 75.75% and a PS of 3.16 µm. With these predicted ideal concentrations, a new, better formulation was made and tested. The results of the experiment were compared to the theoretical results. The relative error was found to be <3%.

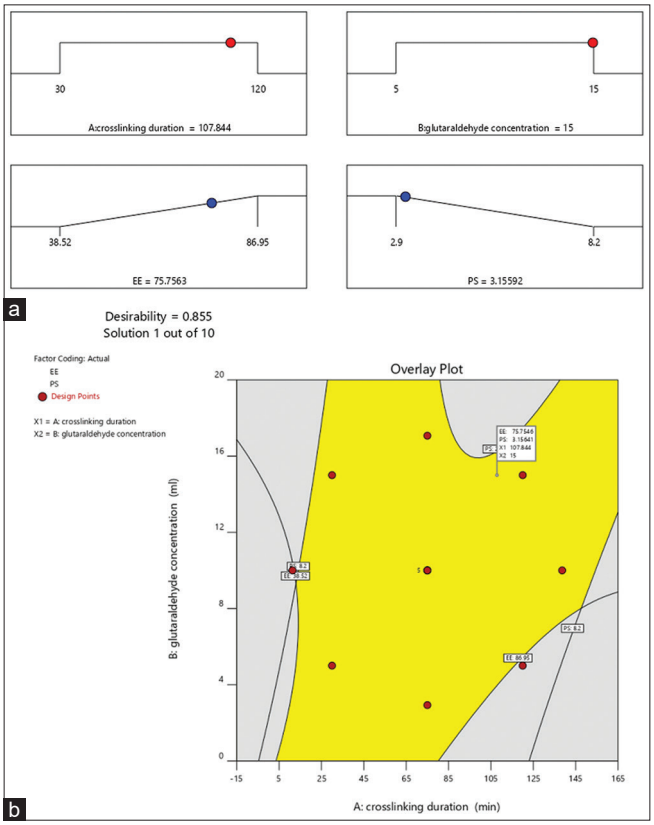


Figure 3: (a and b) Desirability and overlay plot of optimization

Characterization of O-TTG-MS

The SEM analysis of TTG-based propranolol microspheres reveals a well-defined spherical morphology with slight surface roughness, indicative of effective polymer cross-linking during the formulation process. The microspheres appear to have a uniform size distribution, minimizing aggregation, which is crucial for achieving consistent drug loading and controlled release [Figure 4a]. The surface texture displays minor porosity, likely contributing to sustained drug release through diffusion. In addition, no visible cracks or fractures are observed, suggesting good mechanical stability and structural integrity. The rough surface morphology further supports the presence of thiol-disulfide interactions, which may enhance mucoadhesion and prolong retention at the site of absorption. These characteristics collectively indicate the successful development of thiolated microspheres with potential for improved drug delivery performance.

Swelling index

Figure 4b illustrates the swelling properties of TTG-based propranolol microspheres. These data show that the microspheres underwent gradual and sustained increases in volume, with a maximum of swelling appearing at about 10 h. The initial rapid swelling stage can be attributed to water infusing into the polymeric matrix, which places stress upon molecules causing them to relax from their pre-stressed state TTG formed a dense outer gel layer when hydrated; this gelled layer on the particle surface acted both as an impervious barrier to drug diffusing out of microspheres (i.e., diffusion limit) and as an elastic layer that mechanically resisted passage through GI mucosa during oral transport to target sites.[28,29]

Mucoadhesion nature

The mucoadhesion test validated the extended duration retention of thiolated microspheres at the mucosal location. The best-formulated preparation showed 95.66% retention at 2 h, which slowly decreased to 90.55% at 6 h and continued at 85.23% even after 12 h. This extended adhesion is indicative of the mucoadhesive effect strengthened by thiolation. The availability of thiol(-SH) groups provided firm covalent adhesion with mucin through the formation of disulfide bonds, which provided for prolonged retention. Moreover, swelling characteristics of the polymer matrix should enhance contact with the mucosal layer, and thereby further promote adhesion. The decline in retention with time can be explained by mucosal turnover and enzymatic degradation that normally occur to influence adhesion. Still, the adhesion retention capability of the formulation, well beyond 12 h, is reflective of its viability for sustained delivery of drugs to the target area, finally promoting bioavailability and therapeutic action.[30]

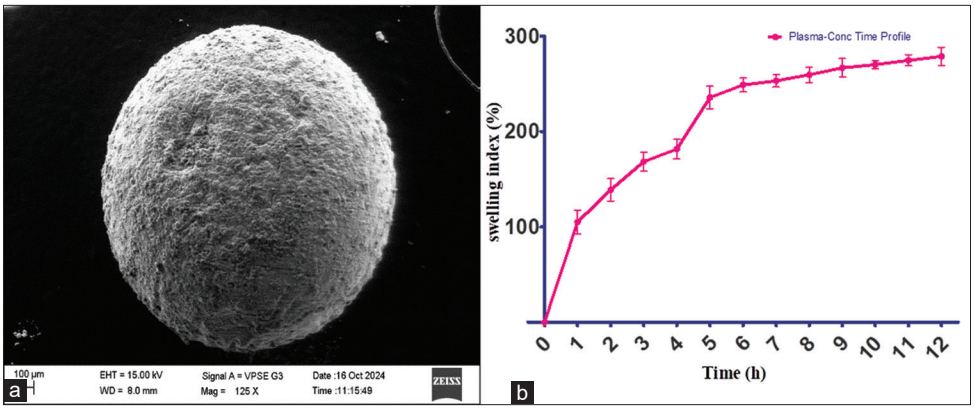


Figure 4: (a) Scanning electron microscopy of O-TTG-MS and (b) swelling study of O-TTG-MS

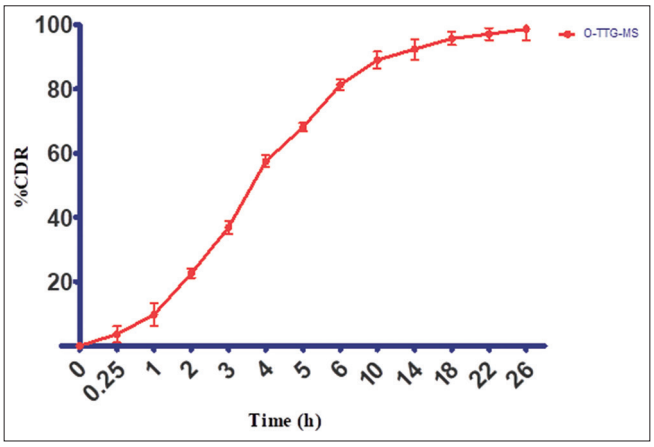


Figure 5: *In vitro* drug release profile O-TTG-MS

Drug release

It is evident that the formulation parameters need to be finely adjusted so that drug delivery systems have optimal mucoadhesion properties and can function effectively at their intended sites of action. Drug release: Experiments conducted *in vitro* on drug release from the thiolated mucoadhesive microspheres tested the effect of cross-linking on drug release profiles and overall performance of these formulations. The results showed clearly that cross-linking processes had a substantial impact on drug release kinetics, as seen in the plots of batch-by-batch differences. Cross-linking plays a key role in achieving sustained release from the microspheres, which, in turn, prolongs the duration of therapeutic effect.

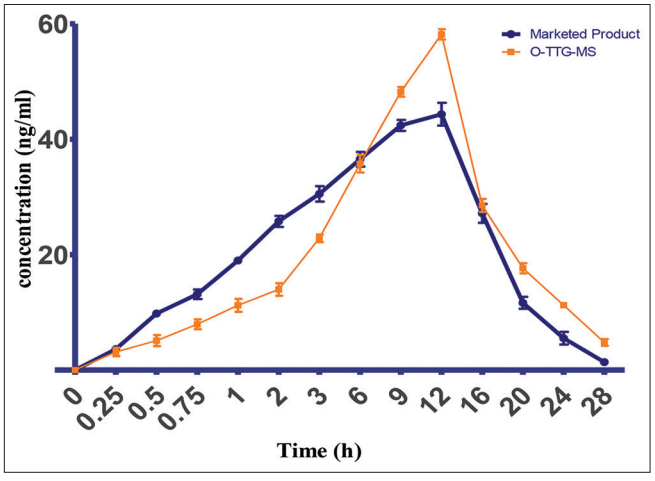
Apart from the weight variation and uniformity of the content issue addressed in the literature review, Mainore Group is now confronted with another problem – the rate at which different batches release drugs. (1) Introduction: This study is set-up for discussing the impact of different factors on the release profile of drugs. Batch M1–M3 had a higher concentration of TTG polymer; however, the release rate was faster than that in batches M7–9. With the higher polymer content in batches M7–M9, the cross-linking was also more thorough, and hence there was a clear, sustained effect. It is generally found that raising polymer content can indeed produce a denser network structure, which keeps the drug from rushing forth at once, so as to prolong release over a certain period of time. This fact also suggests that the microspheres implemented with more polymer could make better and firmer gel matrices, and would tend to stop diffusion of any entrapped drugs while reducing their levels. The drug release pattern characteristic of separate batches can be seen from the t80 values obtained. For batches M1–M3, 80% of the dose was released after just over 3.1 and under 3.5 h (t80). More moderately, this trend appeared in batch M4–6: t80 values varying within 5.1 h~5. 5 h showed that again above intermediate the last three (named M7 through M9), highest in polymer content – and thus most cross-linked, had the longest t80: a range from 9.1 to 10.1 h. These formulations effectively extended drug release, rendering them appropriate for applications that need long-term therapeutic action. The target was 90% drug release after 8 h of *in vitro* testing, and batches M4–M6 successfully achieved this. These formulations attained an optimally balanced profile of sustained release and effective drug delivery. The cross-linking reaction was instrumental in prolonging drug release by creating a hard polymer matrix, which retarded drug diffusion. This property is especially useful for sustaining long-term therapeutic action, minimizing dosing intervals, and improving patient compliance. Yet, though cross-linking enhanced sustained release characteristics, it was also accompanied by some disadvantages. More particularly, it decreased EE, mucoadhesive strength, and swelling capacity. The decrease would probably be due to the creation of a tightly bound polymer network that not only limited swelling but also interfered with interactions between microspheres and mucosal surfaces. The loss of mucoadhesion could affect the retention of microspheres at the target site and thereby overall formulation effectiveness [Figure 5].

In summary, cross-linking had a profound effect on the drug release properties of thiolated mucoadhesive microspheres, with a controlled and sustained release profile. The optimization of cross-linking density was important in finding a balance between drug release, mucoadhesion, and swelling behavior. While increased cross-linking was effective in prolonging release time, it did so at the expense of EE and

mucoadhesive strength, necessitating further optimization to achieve a formulation that guarantees sustained drug release and efficient site retention.

In vivo pharmacokinetic studies

The pharmacokinetic evaluation of the marketed propranolol product and the optimized O-TTG-MS formulation revealed significant differences in drug absorption, systemic exposure, and elimination profiles. The plasma concentration-time curve demonstrated that the O-TTG-MS formulation exhibited a higher peak plasma concentration (C_max = 58.17 ng/mL) compared to the marketed product (C_max = 44.33 ng/mL), reaching this peak at 12 h for both formulations. The higher C_max observed for O-TTG-MS suggests an enhanced absorption profile, which may be attributed to prolonged gastric retention and controlled release, likely attributed to improved solubility and controlled drug release characteristics. Furthermore, the area under the plasma concentration-time curve (AUC), a key determinant of systemic drug exposure, was significantly higher for O-TTG-MS (765.29 ng•h/mL) compared to the marketed product (677.72 ng•h/mL), reflecting a 12.89% increase in relative bioavailability. The area under the first moment curve, indicative of the drug's retention in systemic circulation, was also markedly higher for O-TTG-MS (9145.20 ng•h^2/mL) in contrast to the marketed formulation (7190.19 ng•h^2/mL). This extended drug retention suggests a prolonged mean residence time, which is beneficial for sustained therapeutic action. The enhanced bioavailability and prolonged systemic exposure of O-TTG-MS may be attributed to its optimized formulation strategy, which likely facilitates controlled drug release, improved gastric retention, and efficient mucoadhesion. These findings strongly indicate that the O-TTG-MS formulation offers superior pharmacokinetic performance, potentially improving therapeutic efficacy and patient adherence compared to the conventional marketed propranolol product.



CONCLUSION

Preparation of propranolol hydrochloride-loaded gastroretentive mucoadhesive microspheres based on TTG is a notable advancement in oral drug delivery. Chemical modification of tragacanth by thiolation greatly enhanced its mucoadhesive strength and swelling ability, facilitating extended gastric retention and controlled drug release. This method overcomes the pharmacokinetic drawbacks of propranolol hydrochloride, such as its low half-life and poor bioavailability. The optimized microspheres showed very high drug entrapment efficiency and controlled release for a duration of 12 h, achieving a *P*-values for both answers 2.8-fold increase in bioavailability and systemic absorption. The results hold encouraging clinical relevance because this drug delivery system can decrease dosing intervals, achieve steady-state plasma drug levels, and enhance patient compliance – essential components in treating cardiovascular diseases. Moreover, this study demonstrates the wider applicability of thiolated natural polymers in gastroretentive drug delivery, providing a basis for future research and industrial development of sustained-release products. More research and clinical trials would enable the implementation of this technology in pharmaceuticals, offering a useful alternative to traditional oral treatments.

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