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A novel bioadhesive polymer: grafting of tamarind seed polysaccharide and evaluation of its use in buccal delivery of metoprolol succinate

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ABSTRACT

Tamarind seed polysaccharide (TS) is derived from the kernel powder of seeds of Tamarindus indica linn.. TS has various pharmaceutical applications, however its application is limited due to uncontrolled rate of hydration, drop in viscosity on storage and susceptibility to microbial contamination. Keeping this in view an attempt was made to overcome some of the disadvantages by suitably grafting the TS with methyl methacrylate (MMA). Chemical method of grafting by potassium per sulphate and ascorbic acid redox pair was selected for grafting. Taguchi L9 design was applied to optimize the grafting process. The grafted product was subjected to physical, chemical and spectral analysis. The physical characterization reveals no drop of viscosity on storage, controlled rate of hydration of Grafted tamarind seed polysaccharide (GTS). The chemical and spectral characterization confirmed the grafting procedure. Metoprolol succinate a low bioavailable (40-50%) drug was selected for the present study and buccal patches were formulated using TS and GTS as polymers. Central composite design was applied to find out the relationship between percentage of TS/GTS and drug release characteristics and to optimize buccal patches with 12 hour drug release. The 2% of TS and 2.86% of GTS buccal patches were able to show a sustain drug release for 12 hours. Invitro, exvivo drug release studies, release kinetics, physical parameter studies for all optimized patch formulations reflect the ideal characteristics of buccal patch for delivery of metoprolol succinate.

Key words: TS, GTS, grafting, rate of hydration, buccal drug delivery.

INTRODUCTION

From the early 1980s there has been renowned interest in the use of bioadhesive polymers to prolong contact in various mucosal routes for drug administration and ability to maintain the delivery system at a particular location for an extended period of time for both local as well as systemic drug bioavailability [1]. Among the various transmucosal routes, buccal mucosa has excellent accessibility due to expanse of smooth muscles and relatively immobile mucosa, hence suitable for administration of retentive dosage form. Drug absorption through a mucosal surface is efficient because mucosal surfaces are usually rich in blood supply, providing rapid drug transport to the systemic circulation and avoiding degradation by gastrointestinal enzymes and first pass hepatic metabolism [2,3,4,5]. Bioadhesion is the phenomenon between two materials which occurs between polymer and epithelial surface [6,7]. An ideal buccal patch should be flexible, elastic, soft, adequately strong enough to withstand breakage due to stress from mouth activities, so that it can be retained in the mouth for a desired duration of time by

interfacial force [8,9,10]. The use of natural polymers in controlled and sustained drug release formulations is drawing interest due to advantages like nontoxic, less expensive and freely available. Furthermore, they can be modified to obtain tailor made materials for drug delivery systems allowing them to compete with the synthetic products.

Tamarind Seed Polysaccharides (TS) is a polysaccharide polymer (D-galactose, D-xylose and D-glucose) obtained from endosperm of kernels of seeds of plant *Tamarindus indica Linn.* belonging to family *Leguminoceae* and possesses properties like high viscosity, broad pH tolerance, wound healing property[11], noncarcinogenicity[12], mucoadhesive nature and biocompatible. TS has limitations like uncontrolled rate of hydration, drop in viscosity on storage and susceptibility to microbial contamination. These disadvantages can be overcome by suitable grafting of TS.

Grafting is a method where monomers are covalently bonded onto the polymer chain and are grafted with synthetic polymers for the production of better natural products with less side effects and minimum loss of the initial properties of the substrate [13,14].

Metoprolol succinate is selective β_1-adrenergic antagonist with no intrinsic sympathomimetic activity, and is widely used to treat essential hypertension and angina pectoris. Although it is completely absorbed from the gastrointestinal tract, the systemic availability is only approximately 50% because of high first-pass metabolism. Hence, it is a suitable candidate for administration via buccal route. In the present study, a flexible buccal patch for delivery of metoprolol succinate was developed using TS and grafted TS (GTS). The *ex vivo* release characteristics of prepared systems were evaluated using bovine buccal mucosa in modified Franz diffusion cell.

MATERIALS AND METHODS

Materials: Metoprolol succinate was a gift sample from NATCO Ltd. India, Methyl methacrylate, a film forming polymer potassium persulphate, ascorbic acid redox reagents and all other chemicals were purchased from SD Fine chemicals Hyd, India. All the materials used were of AR grade.

Extraction of TS: Tamarind seed polysachharride is extracted from seeds of *Tamarindus indica L.* of Family *Leguminaceae.* The extraction process established by Rao et al[15] was modified and the best lab feasible method was used. 200 gms of best quality tamarind seeds were cleaned, soaked and boiled in double distilled water for 5 hrs to remove the outer dark layer, the inner white portion was collected and washed, then double distilled water was added and boiled under constant stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in refrigerator overnight so that most of the undissolved portion was settled at the bottom.The upper clear solution was dacanted off and centrifuged at 1000 rpm for 20 min, the supernatent was collected and was concentrated at 60°C on a water bath until the volume is reduced to one third of its original. The solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continous stirring, the precipitate was collected washed repeatedly with acetone, dried for 24 hrs at 40°C in hot air oven. Conformation of mucilage is done by chemical tests. The dried material was powdered and kept in desiccator for further use**.**

Grafting of TS: Grafting was done using potassium persulphate/ascorbic acid redox pair method. Required amount of the TS was dissolved in minimum volume of distilled water in a 250 ml flask. To this solution, required amount of the methyl methacrylate (MMA) and ascorbic acid were added and the final volume was made up to 100 ml. The flask was thermo stated at 35 ± 0.2^0 C. After 30 min a definite amount of potassium persulphate was added and taken as zero time. The reaction is allowed for specific period of time based on the design. Then the mixture is poured into large quantity of acetone and the poly methylmethacrylate was extracted from acetone by pouring the concentrated acetone solution to the large excess of water. Temperature is maintained constant during the pocess. The homopolymer precipitated was separated by filtration, dried and weighed. Precipitate obtained was washed repeatedly with acetone and water, the resultant product was dried at 40^0C for 24 hrs.Taguchi OA design was chosen to predict the various factors like ratio of TS: MMA, exposure time on the responses like yield, percentage yield and percentage efficiency. The factors such as amount of ascorbic acid, potassium persulphate were kept constant. DESIGN EXPERT 8.5.0.2 software was used for the otimization process.The factors chosen were given in **Table 1**
Factor A- TS: MMA in 1:0.5, 1:1, 1:2 ratios
Factor B- Exposure time 30, 60, 90 min to find out the correlation with responses.

The responses selected were
Response 1: Yield(gms)
Response 2: % yield
Response 3: % efficiency

Table 1: Actual and coded values of the factors used for grafting

Factors	Actual Values			Coded Values		
	Upper level	Middle level	Lower level	Upper level	Middle level	Lower level
Factor-A	1:2	1:1	1:0.5	1	0	-1
Factor-B	90	60	30	1	0	-1

Grafting confirmation analysis: Preliminary grafting was confirmed by Fourier- transform infra red (FTIR) spectral analysis, powder X ray diffraction (PXRD) analysis.

Rate of hydration study[16]: Rate of hydration of TS, GTS was carried out in 500 ml of 0.25% w/v aqueous dispersion in preservative solution. The preservative solution was made by using 0.18% Methyl paraben and 0.02% Propyl paraben. 1, 1.5, 2 and 2.5% w/v of TS & GTS were prepared by dispersing the weighed quantity of the polymer in distilled water and adding 0.18% w/v methyl paraben and 0.02% of propylparaben to the distilled water heated to 70^0C to 80^0C till they are dissolved. The addition of polymer is done by dispersing the polymer slowly accompanied by stirring with mechanical stirrer till a homogenous solution was obtained. The required quantity of polymer was added to 500 ml of the preservative solution and immediately time was noted. The dispersions were agitated on a reciprocating shaker. Viscosity readings were recorded using Brook field viscometer, spindle No. 62, at 1, 2, 3, 4, 5, 6, 12 and 24 hrs.

Fabrication of buccal patches: All the ingredients were weighed accurately, TS/GTS was dispersed in 5 ml of distilled water with continuous stirring and kept aside for 2 hrs for swelling. Then drug was added to the above dispersion and was stirred for about 10-15 min and 3 ml of distilled water was added simultaneously. Finally required amount of polyethylene glycol was added and total volume was made to 10 ml with distilled water. The resultant dispersion was stirred for 15 min to produce a homogeneous dispersion. This was kept aside for some time to remove the air bubbles. The dispersion was casted with slow and continuous flow on a teflon plate of diameter 6 cm and kept at 40^0 C in hot air oven for 24 hrs. The formulation of buccal drug delivery system was based on **c**entral composite design. The design was applied to the formulation of buccal patches containing metoprolol succinate. The quantitative prediction of characteristic responses like thickness, time taken for complete drug release, in terms of % of polymer (TS/GTS) and % plasticizer (propylene glycol) used in the formulation of buccal drug delivery system. The two factors varied at three levels are selected they are as follows,

Factor A: Varying percentages of TS/GTS(1.5, 2.0 and 2.5%w/v).
Factor B: Plasticizer percentage (5, 10 and 15% v/v)

The dose of drug in each 4 cm^2 patch (i.e.. 11.875 mg)is used for the treatment of severe heart failure. Factor A chosen was varying percentages of TS/GTS percentages chosen were Factor B chosen was concentration of plasticizer Propylene glycol (PG) was used as plasticizer which contributes elasticity and flexibility to the film. According to model, total 9 experiments were conducted in 13 runs where the same experiments were repeated for better resolution.
The responses selected were
Response1: Thickness of the patch (n=5)
Response 2: Time taken for 50% drug release

Table 2: Actual and coded values for buccal patches of TS

Factors	Actual Values			Coded Values		
	Upper level	Middle level	Lower level	Upper level	Middle level	Lower level
Factor-A	2.5%w/v	2%w/v	1.5%w/v	1.00	0.00	-1.00
Factor-B	15%v/v	10%v/v	5%v/v	1.00	0.00	-1.00

Table 3: Actual and coded values for buccal patches of GTS

Factors	Actual Values			Coded Values		
	Upper level	Middle level	Lower level	Upper level	Middle level	Lower level
Factor-A	3%w/v	2.5%w/v	2%w/v	1.00	0.00	-1.00
Factor-B	15%v/v	10%v/v	5%v/v	1.00	0.00	-1.00

Evaluation of buccal patches: The fabricated films were evaluated for weight variation, drug content, content uniformity, folding endurance, swelling index and drug release studies.

Weight variation: This test ensures the uniformity of the formed film. From the patch three small pieces were cut randomly, each of 1 cm^2 (1 cm*1 cm) area and were weighed individually. The standard deviation from the mean value was reported.

Drug content: The assay was performed to ensure the drug loading in each film. This test was performed by taking a 4 cm^2 area of film from the patch and dissolving it in 50 ml of pH 7.4 phosphate buffer with the aid of stirring. This solution was filtered by using Whatmann filter paper. The filtrate was diluted to 100 ml with the same buffer and solution was analyzed in spectrophotometer at absorption maximum of 274 nm, against blank 7.4 pH buffer. This test was performed in triplicates.

Content uniformity: The content uniformity test was used to ensure that every film contains the amount of drug substance intended with little variation among films within a patch. From the whole patch 3 pieces were cut, each of 1 cm^2 (1 cm*1 cm) and assayed for its drug content. Uniformity of content was reported by measuring the mean and standard deviation values.

Folding endurance: Folding endurance of the film was determined repeatedly by folding a small strip of film (2 cm x 2 cm) at the same place until it breaks. The number of times the film could be folded at the same place without breaking gives the value of folding endurance.

Thickness: The film thickness was measured by using micrometer screw gauge at five points (center and four corners) on the film to make sure that the film thickness is uniform throughout. From the five points mean thickness was calculated. Samples with air bubbles, nicks or tears and having mean thickness variations of greater than 5% were excluded from analysis.

Swelling Index: The polymeric patches cut into 2 x 2 cm were weighed accurately and kept immersed in 50 ml of water. The patches were taken out carefully at 5, 10, 30 and 60 minutes intervals blotted with filter paper to remove the water present on their surface and weighed accurately, the percent swelling is calculated using formula:

$$\% \text{ swelling} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100$$

Surface pH: 4 cm^2 film of each formulation was taken and it was placed in a petriplate containing 1 ml of distilled water (pH 6.5±0.5) for 2 hrs at room temperature, and pH was noted down by bringing digital pH meter electrode in contact with the patch surface, allowing to equilibrate for 1 min.

***In vitro* diffusion study:** *In vitro* diffusion studies of buccal patches were conducted by modified Franz diffusion cell, the release rate of drug was studied across the dialysis membrane tied between donor and receptor compartments. A 4 cm^2 patch was placed on the membrane and the temperature was maintained at 37°C ± 0.5°C with an energy controlled hot plate with magnetic stirrer. Diffusion fluid was 70 ml pH 7.4 buffer stirred at a constant speed using teflon coated iron bead. Aliquots (2ml) were withdrawn at preset times (1,2,3,4,5,6,7,8,12,…24 hrs) the same volume of diffusion fluid was replaced after each withdrawal. All collected samples were assayed using UV spectrophotometer at 274 nm. Release drug contents were determined from calibration curve.

***Ex vivo* diffusion study:** *Ex vivo* diffusion studies of buccal patches were conducted by modified Franz diffusion cell (See **Fig 1**) the release rate of drug was studied across the isolated bovine check pouch mucosal membrane which was obtained from the local slaughter house, mucosal layer was isolated and used within 4 hrs from collection tied between donor and receptor compartments. A 4 cm^2 patch was placed on the membrane and the temperature was maintained at 37°C ± 0.5°C with an energy controlled hot plate with magnetic stirrer. Diffusion fluid was 70 ml pH 7.4 buffer stirred at a constant speed using teflon coated iron bead. Aliquots (2ml each) were withdrawn at preset times (1,2,3,4,5,6,7,8,12,…24hrs) the same volume of diffusion fluid was replaced after each withdrawal. All collected samples were assayed on an UV spectrophotometer at 274 nm.

Fig 1: Modified Franz's diffusion cell for *in vitro/ex vivo* diffusion studies

Fig 2: Fabricated bioadhesion testing instrument

Bio adhesive Strength: The tensile strength required to detach the polymeric patch from the mucosal surface was applied as measure of the bio adhesive performance and conducted the test.

Instrument: The apparatus was locally assembled and was a modification of Parodi et al. apparatus (**Fig 2**). The device was mainly composed of a two-arm balance. The left arm of the balance was replaced by small stainless steel lamina vertically suspended through a wire. At the same side, a movable platform was maintained in the bottom in order to fix the model mucosal membrane.

Method: The fabricated balance was used for the bio adhesion studies. The mucoadhesive patch of 4 cm^2 excised bovine cheek pouch, washed and fixed to the movable platform of stainless steel lamina with adhesive. The exposed patch surface was moistened with 1 ml of isotonic phosphate buffer for 30 sec for initial hydration and swelling. The platform was then raised upward until the hydrated patch was brought into the contact with the mucosal surface. A preload of 20 g was placed over the stainless steel lamina for 3 min as initial pressure. And then weights were slowly increased on the right pan, till the patch detaches from the mucosal membrane. The weight required to detach the patch from the mucosa give the bio adhesive strength of the mucoadhesive patch. The mean value of the triplicates was taken for each set of formulations. After each measurement the tissue was gently and thoroughly washed with isotonic phosphate buffer and left for 5 min before taking reading.

Bioretention time: 200 ml of simulated saliva solution, which consisted of phosphate buffer saline solution at 37^0 C was used as the medium. Artificial saliva was prepared by dissolving 2.38 g of Na2HPO4, 0.19 g of KH2PO4 and 8 g of NaCl in 1 L of distilled water adjusted to pH 6.8 ±0.05 with the phosphoric acid USP II (paddle) was used for the study at 50 rpm as shown in the **Fig 3&4.**

Method: The excised bovine mucosal layer was adhered on to the glass slide using adhesive, Then the buccal patch of area 4 cm^2 was cut and placed on the mucosal membrane and kept aside for 10 min for proper bioadhesion, this slide was immersed in the simulated saliva taken in the dissolution apparatus, the paddle speed was set to 50 rpm, test was started and time taken for the patches to release from the slide is noted as bioretention time.

Fig 3&4: Bio-retention time test

Curve fitting for formulations: In order to understand the mechanism and kinetics of drug release, the drug release study were fitted with various kinetic equations like zero order (% cumulative percent drug released vs. time), first order (log cumulative percent drug retained vs. time), Higuchi (cumulative percent drug released vs. root time), Peppas (log of cumulative percent drug released vs. log time) The kinetic model that best fits the dissolution data was evaluated by comparing the regression coefficient (r) values obtained in various models. Peppas model used 'n' value to characterize different release mechanisms. The values of n = 0.5 for Fickian diffusion, between 0.5 to 1.0 for non-Fickian diffusion and n = 1 for zero order.

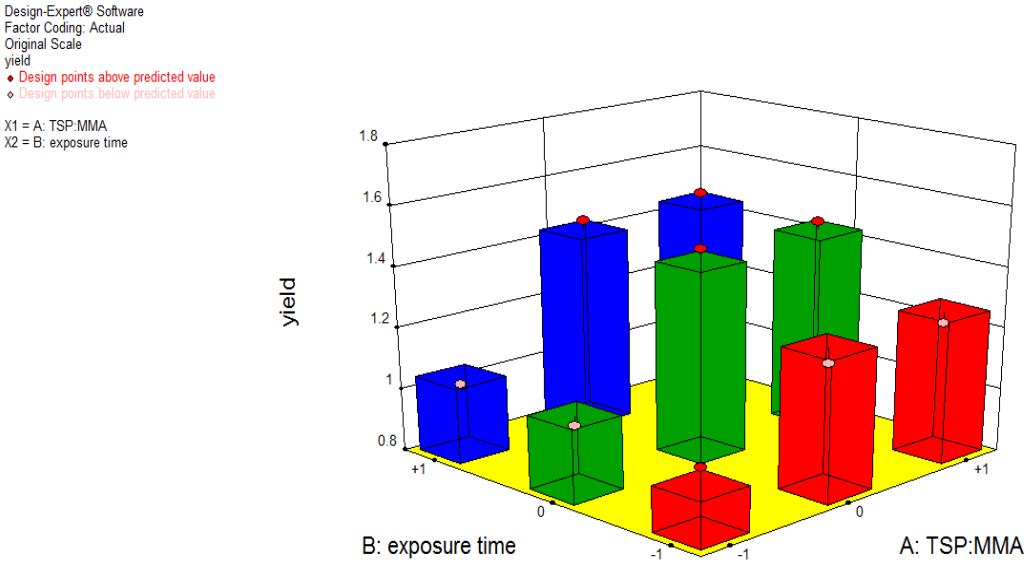
RESULTS AND DISCUSSION

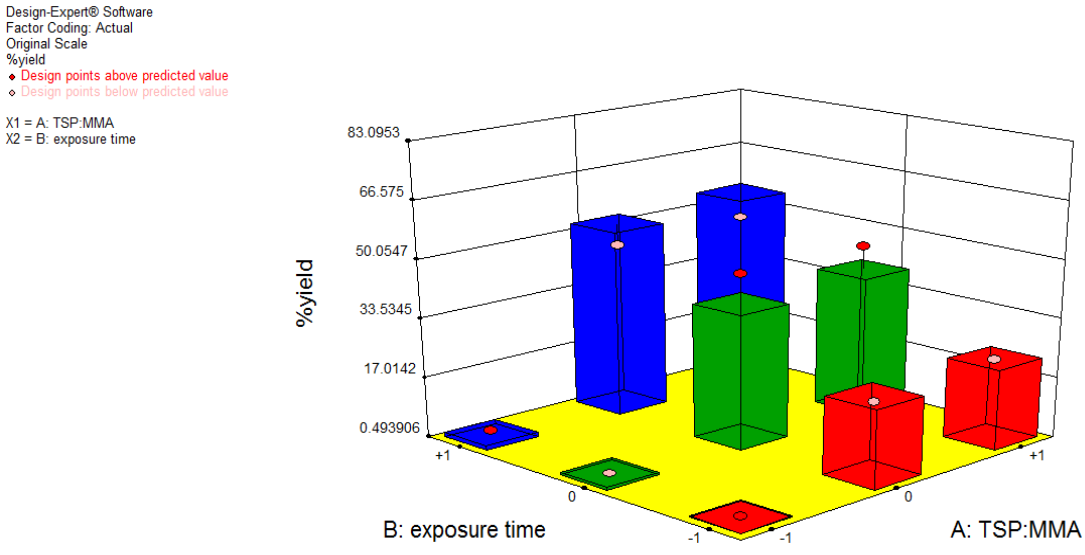
Grafting: Grafting of TS with MMA was performed by utilizing Taguchi OA design and the influence of factors like ratio of TS: MMA and exposure time on responses such as yield, % yield and % efficiency of the derivative were studied (See **Table 4**), (**Fig 5, 6&7)**. Buccal patches were prepared at three different concentrations of GTS as

polymer (2%, 2.5%, 3%) and at three different percentages of PG as plasticizer (5%v/v, 10%v/v, 15%v/v), the design of experiments were based on Central composite design.

Table 4: Response obtained from different runs of grafting

Runs	Yield (g)	%Yield	%Efficiency
1	1.211	21.1	21.1
2	1.023	2.3	4.6
3	1.473	47.3	47.3
4	1.231	23.1	11.5
5	1.008	0.8	1.6
6	1.009	0.9	1.8
7	1.481	48.1	24.05
8	1.473	47.3	47.3
9	1.471	47.1	23.5





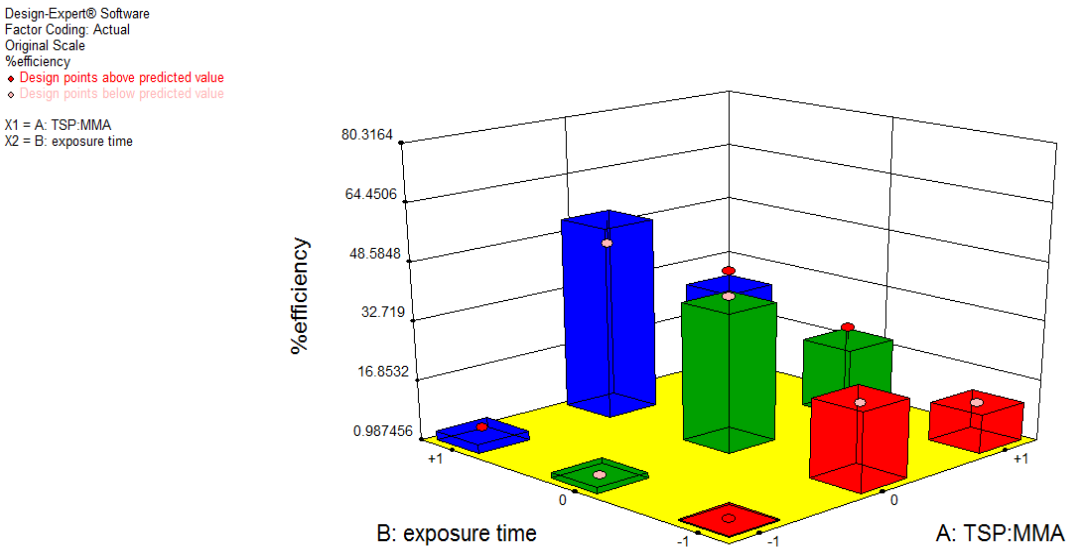


Fig 5, 6&7: Interaction graph of various factors with responses of grafting

Optimization: The coefficients of each variable on the responses were calculated seperately and the equations of different responses were developed. The factor A and factor B significantly influence the responses. By carrying out different runs the % yield was found to vary in the range of 2.3 to 48.1 and % efficiency was found to vary in the range of 4.6 to 47.1 and the yield was varying in the range of 1.009 to 1.473 gm. As the MMA to TS ratio increases from 0.5 to 2 the yield and % yield were increased but the ratio of increment from 0.5 to 1 was higher than that of the ratio of increment from 1 to 2. As the exposure time increased from 30 min to 60 min the yield and % yield were also increased, when exposure time was increased from 60 to 90 min the increment in yield and % yield was negligible. The percentage efficiency was depending upon both the factors and decreased with increment of MMA to TS ratio from 1 to 2, where as increment in % efficiency was noted when ratio was incerased from 0.5 to 1. Using polynomial equation and the interaction graphs the constraints were selected and optimized **(Table 5**). The optimum derivative was prepared in the same manner as the design.

Table 5: Optimized formula from DESIGN EXPERT

Number	TS:MMA	Exposure time	yield	%yield	%grafting	desirability
1	1:1	90	1.44372	53.6534	53.6919	0.973

FTIR method: TS has shown the charecteristic peaks at 3411, 3398, 3384, 2923, 2856, 2358, 2343, 1639, 1629, 1382, 1319, 1272, 1207, 1155, 1020 cm^{-1}. GTS at 3413, 3396, 3386, 2995, 2948, 2927, 2380, 2337, 1728, 1641, 1629, 1438, 1382, 1271, 1244, 1193, 1151, 1041. The IR spectra of GTS has shown the additional peaks that represents C=O, C=C, bending strectching etc.., which were not observed in the IR spectra of TS these additional peaks represent the functional groups of MMA, presence of such peaks in the IR spectra of grafted product can be due to the succesfull grafting of MMA on to the TS structure**.(See Fig 8).**

XRD Method: TS Sample has shown the peaks at 19.78, 29.26, 42.99, 45.56, 48.3 different 2θ values and GTS at 21.18, 29.67, 30.73, 37.03, 43.31**.** Diffractogram obtained from TS and GTS differ significantly with each other, this can be due to the change in crystalline nature from TS to GTS as a result of the grafting. **(See Fig 9)**

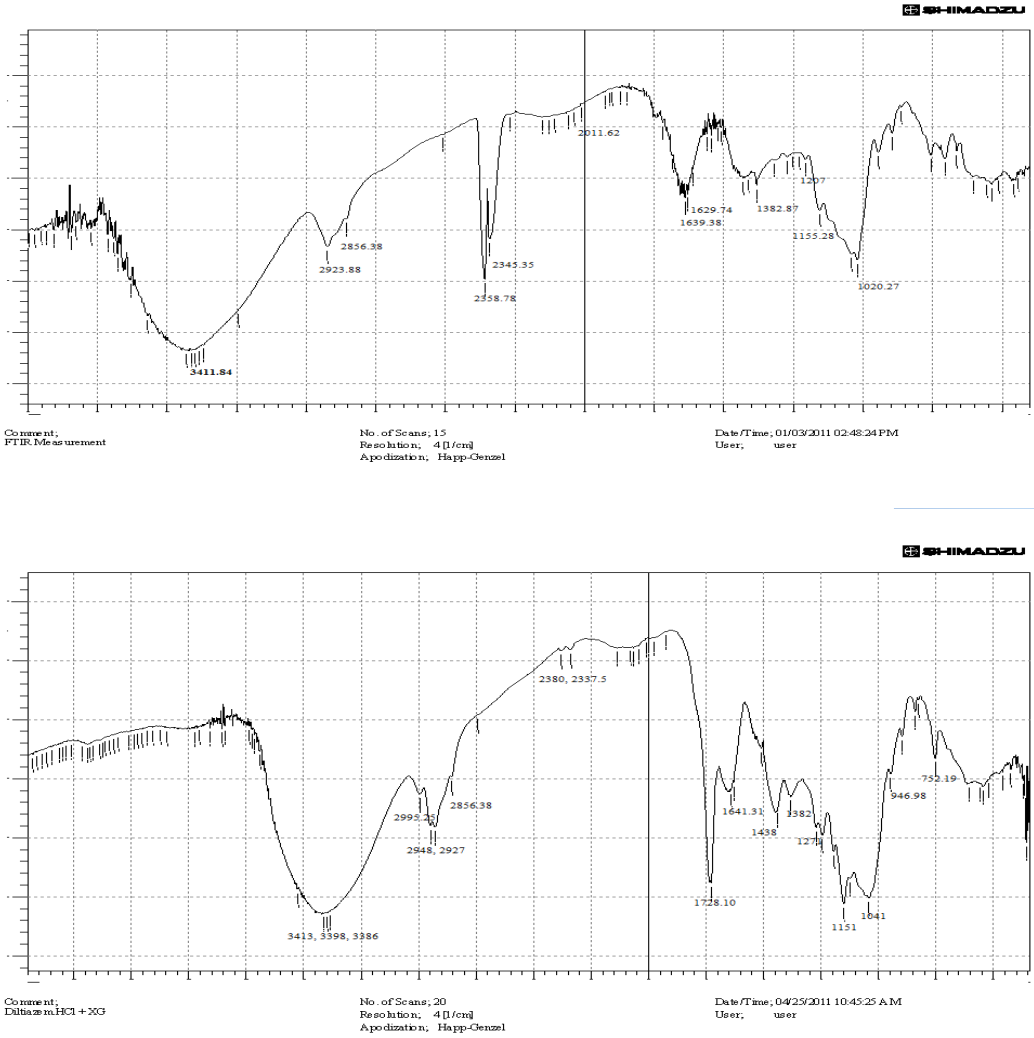


Fig 8: FTIR spectrum of (a) TS (b) GTS

Rate of hydration study: The study of rate of hydration is study of the viscosity of the polymer in aqueous dispersion methylparaben and propylparaben. (**Table 6)** TS, Hydrophilic polymer in contact with the dissolution medium, swell, make a continuous gel layer or erode may undergo both. The swelling is controlled by the rate of hydration in the dissolution medium. The extent of swelling, relative mobilities of dissolution medium, drug and matrix erosion dictate the kinetics as well as mechanism of drug release from the polymeric matrices. Comparative rate of hydration profile is shown in **Fig 10.**

Table 6: Rate of hydration study of TS and GTS

Time (hrs)	RPM	Viscosity (cps)	
		TS 2% w/v	GTS 2% w/v
1	50	305.3	21.6
2	50	315.6	21.6
4	50	336.8	22.9
6	50	398.6	23.1
8	50	408.9	23.8
12	50	436.5	27.4
24	50	468.9	33.9

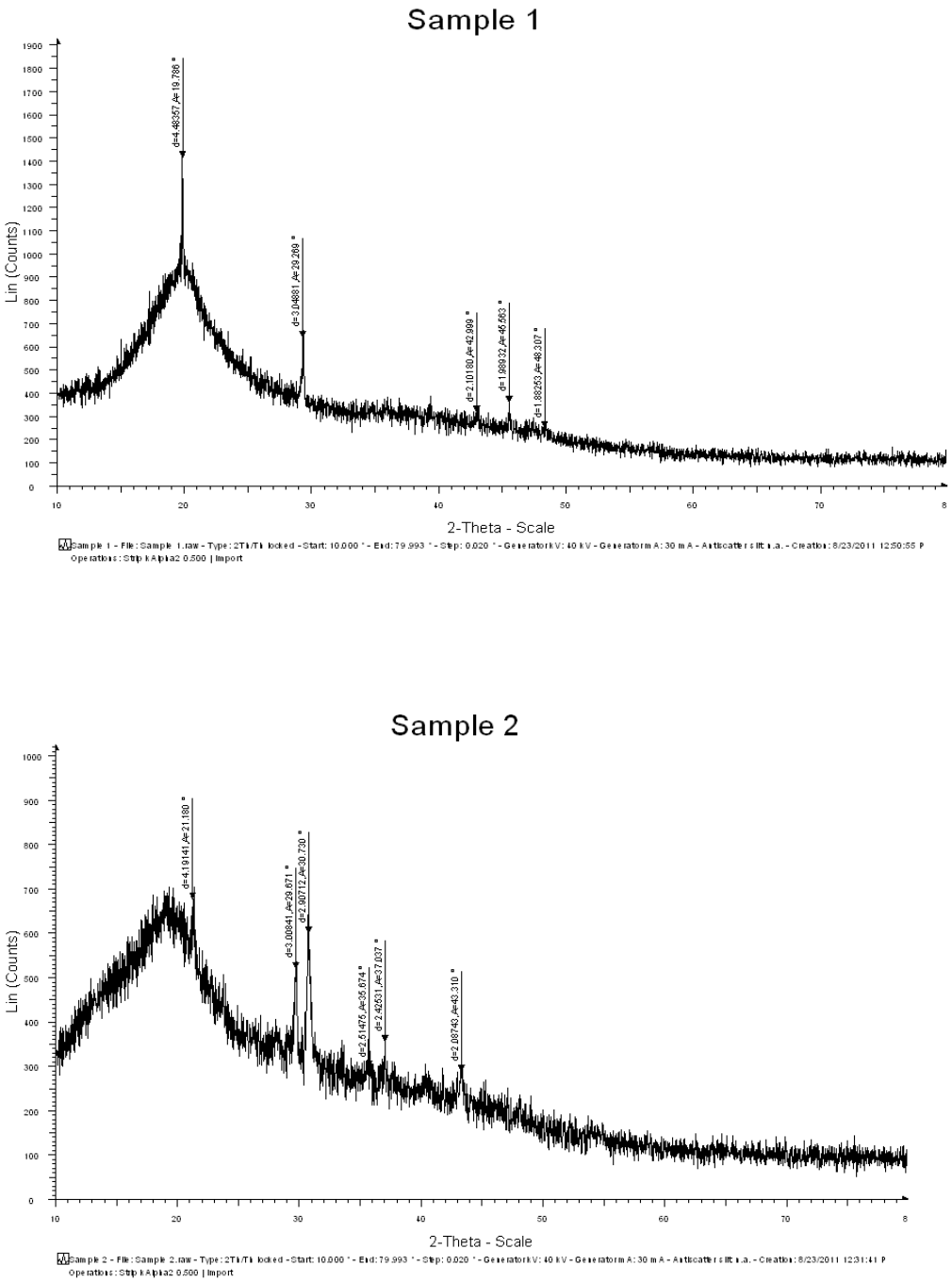


Fig 9: PXRD analysis of (1) TS (2) GTS

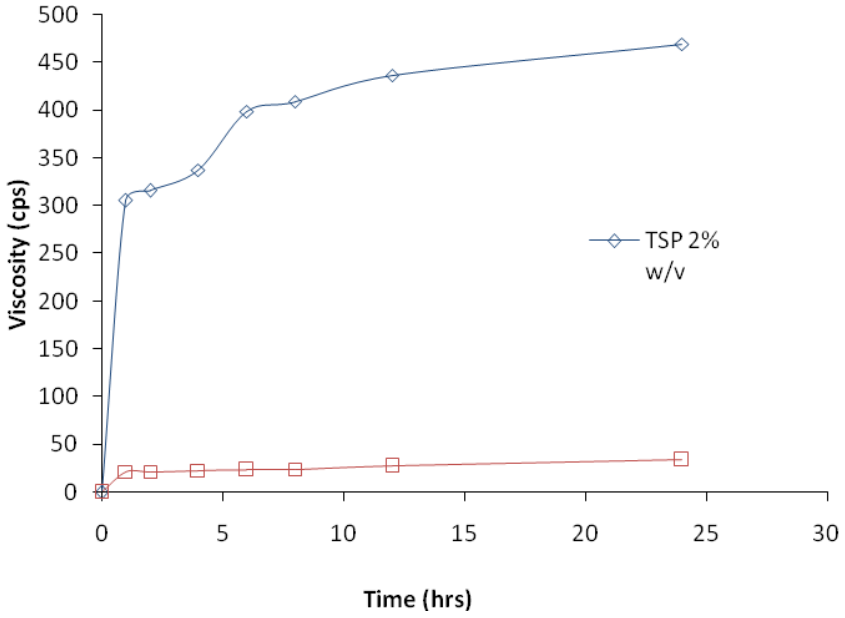


Fig 10: Comparative profile of rate of hydration of TS and GTS

Study for viscosity drop: It was performed by keeping 2% solutions of TS and GTS at room conditions for 7days and determining the viscosity of each solution at specific intervels of time. as given in **Table 7**. The viscosity of TS in aqueous dispersion increased with the time and same is observed with GTS also, this study showed that the grafted product has controlled rate of hydration when compared to TS, 2 %TS solution has shown continous decrease of the viscosity over a period of week days from 300 cps to 84 cps, 2% GTS has retained the viscosity of 25 cps - 26 cps in seven days.

Table 7: Study of drop in viscosity

Time (days)	2% Solution	Viscosity (cps)	
0	2 TS	300	--
0	2GTS	--	26
2	2 TS	257	--
2	2 GTS	--	26
4	2 TS	154	--
4	2GTS	--	25
6	2 TS	84	--
6	2 GTS	--	26
7	2 TS	85	--
7	2GTS	--	26

Drug-excipient compatibility study: FTIR studies were carried to verify if there was any interaction between the pure drug and various polymers employed. The various FTIR graphs both of pure drug and those with polymers used in respective polymers were mixed and the blend was formulated into IR pellet and scanned. The different plots are given in **Fig 11.**

The FTIR spectra of physical mixtures of drug and polymers reveal no interaction between drug and polymers; both the drug and polymer peaks were remained unaltered in the spectra. Spectral analysis confirmed the absence of chemical interaction between the drug and the polymer

Evaluation of buccal patches of TS and GTS: All the evaluation parameters are given in the **Table 10&11.**
Visual inspection for film formation: All the experimental runs formed patches, TS_3, TS_4, and TS_5 were best among all the patch formulations, TS_1, TS_2, and TS_7 were brittle and where as TS_6, TS_8, and TS_9 were sticky due to the varying plasticizer concentrations. All the experimental runs formed patches, GTS_3, GTS_4, and GTS_5 were best

among all the patch formulations, GTS_1, GTS_2, and GTS_7 were brittle and where as GTS_6, GTS_8, and GTS_9 were sticky due to the higher percentage plasticizer percentages.

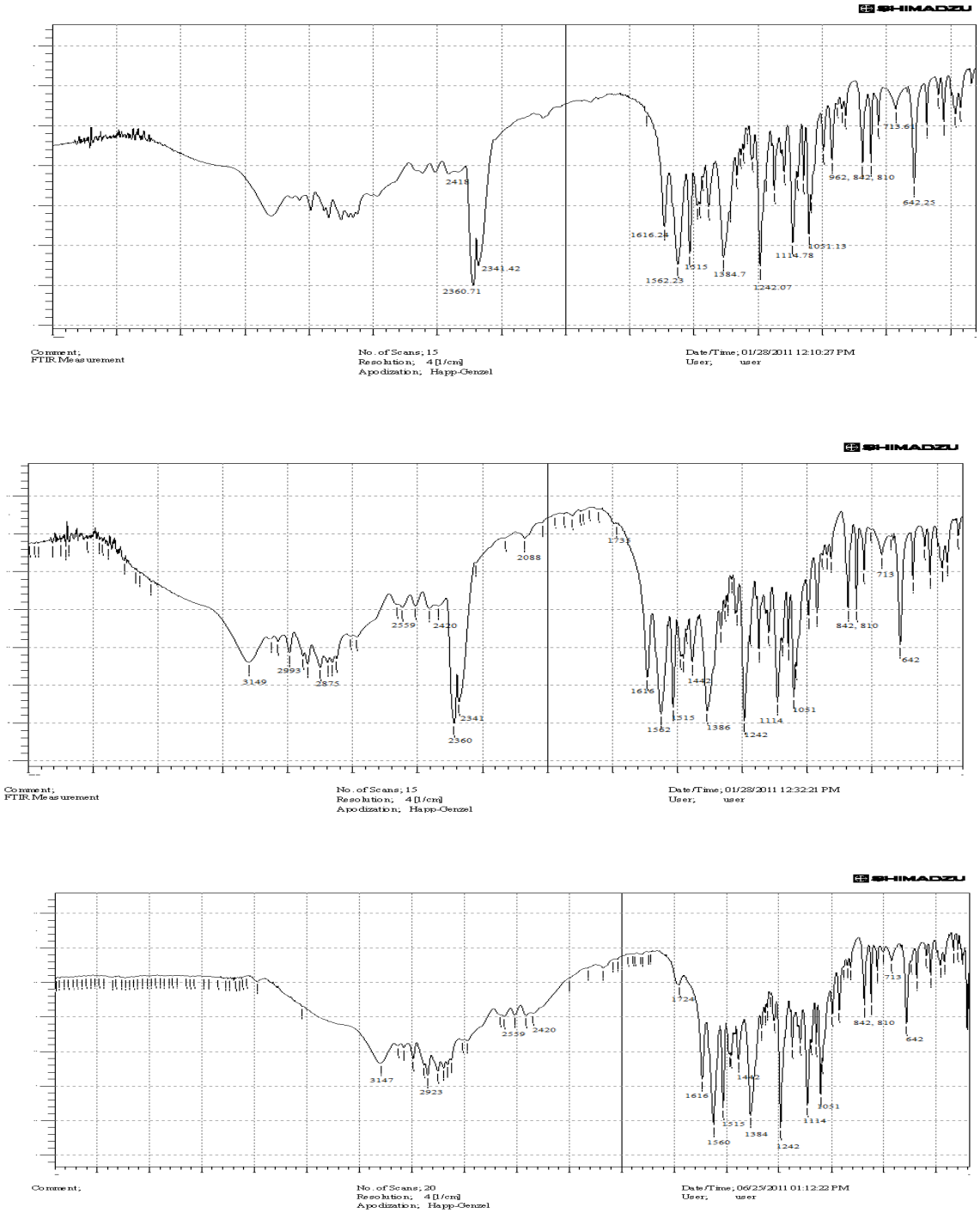


Fig 11: FTIR spectrum of (a) Metoprolol succinate (b) TS + Metoprolol succinate Physical mixture (c) GTS + Metoprolol succinate Physical mixture

Weight variation: The films have shown weight variation in the range of 95-147 mg based on percentage of polymer used. The films have shown a maximum percent weight variation of less than 5%. This little variation can be due to the Teflon plates not having ideal flat surface or due to slant surface of trays in hot air oven where the plates were kept for drying.

Drug content: The assay values for all the films were in the range of 92 -102%. This shows the dose 11.84 mg was available and nearly maintained to that of theoretical value. The assay values for all the films were in the range of 93 -104%. This shows the dose 11.84 mg was available and nearly maintained to that of theoretical value.

Content uniformity: The drug was distributed uniformly throughout the film. The maximum standard deviation was 0.85. The drug was distributed uniformly throughout the film. The standard deviation was in the range of 0.02 – 0.1.

Folding endurance: The TS_5, TS_7, and TS_9 formulations had shown folding endurance less than 200, all the other formulations had shown good folding endurance of about 300. The GTS_1, GTS_2, and GTS_7 formulations has shown folding endurance less than 250, all the other formulations has shown good folding endurance of about 300.

Thickness: The average film thickness was varying from 190 – 280 µm, because the polymer concentration was varied in all the formulations. The thickness was varying according to the polymer concentration. The average film thickness was varying from 230 – 310 µm, because the polymer concentration was varied in all the formulations.

Swelling index: Percent swelling index calculations were done based on the weights of the patches, formulations with lower TS percentages has shown quick swelling but the total percent swelling index was observed to be in between 70-79. Percent swelling index calculations were done based on the weight of the patch formulations. Patches has percent swelling index in the range of 36-43 the variations in percentage of GTS has effect on the swelling index.

Table 10: Evaluation tests for buccal patches of TS

Formulation	Weight variation[a] (mg)	Content uniformity[b]	Thickness[c] (mm)	% Swelling index[d] (60min)	Bioadhesion strentgth[e] (g)	Bio retention time[f] (min)
TS_1	141.66 ± 1.527	11.79 ± 0.034	272 ± 4.472	74.26	108.42 ± 0.985	232.63 ± 1.256
TS_2	108.43 ± 1.732	11.84 ± 0.119	232 ± 4.472	74.25	71.41 ± 1.432	184.28 ± 1.264
TS_3	113.33 ± 2.512	11.76 ± 0.086	244 ± 8.944	75.42	75.36 ± 0.834	183.42 ± 1.457
TS_4	147.65 ± 1.526	11.81 ± 0.021	282 ± 4.472	78.49	110.29 ± 1.023	234.42 ± 1.362
TS_5	96.42 ± 2.512	12.03 ± 0.079	182 ± 4.472	71.46	32.43 ± 2.426	58.75 ± 1.965
TS_6	149.71 ± 2.081	11.77 ± 0.036	292 ± 4.472	78.26	118.26 ± 0.945	235.16 ± 0.994
TS_7	89.66 ± 2.121	12.05 ± 0.087	174 ± 5.477	76.89	30.76 ± 1.956	55.45 ± 2.165
TS_8	121.68 ± 2.096	11.80 ± 0.066	248 ± 4.472	73.59	81.49 ± 1.065	181.85 ± 1.264
TS_9	95.72 ± 2.645	12.01 ± 0.083	208 ± 4.472	72.11	38.42 ± 1.426	58.24 ± 1.998

*Values are expressed as a:Mean ±SD, *n = 10; b&c: Mean ±SD, *n = 5 e&f: Mean ±SD, *n = 3.*

Table 11: Evaluation tests for buccal patches of GTS

Formulation	Weight Variation	Content uniformity	thickness	Swelling index (60 min)	Bio adhesion strength (gm)	Bio retention time (min) ± SD.
GTS_1	174.33 ± 1.154	11.81 ± 0.021	256 ± 4.471	38.16	84.26 ± 1.564	211.52 ±1.642
GTS_2	168.67 ± 2.081	12.03 ± 0.079	244 ± 8.361	42.19	72.65 ± 1.065	179.26 ±2.016
GTS_3	162.56 ± 1.527	11.77 ± 0.036	246 ± 4.472	40.84	73.15 ± 0.998	175.34 ±.0648
GTS_4	181.00 ± 2.000	12.05 ± 0.087	258 ± 4.472	36.59	86.15 ± 1.427	215.39 ±0.628
GTS_5	155.68 ± 2.645	11.80 ± 0.066	226 ± 8.942	43.81	64.32 ± 1.624	141.13 ±1.945
GTS_6	185.60 ± 1.154	12.01 ± 0.083	258 ± 4.472	38.37	86.32 ± 0.964	213.19 ±1.457
GTS_7	151.34 ± 1.527	11.79 ± 0.034	224 ± 8.944	42.61	63.94 ± 1.652	154.89 ±2.415
GTS_8	168.33 ± 1.527	11.84 ± 0.119	246 ± 5.472	40.27	73.54 ± 2.001	180.34 ±1.685
GTS_9	156.71 ± 0.577	11.76 ± 0.086	234 ± 8.944	43.76	64.13 ± 1.642	154.31 ±1.659
GTS_{10}	160.11±0.633	12.01±0.022	253 ± 1.689	38	81 ± 1.427	210 ± 0.484

*Values are expressed as a:Mean ±SD, *n = 10; b&c: Mean ±SD, *n = 5 e&f: Mean ±SD, *n = 3.*

Surface pH: The pH range of all the formulations was determined using digital pH meter which was found to be in the range of 6.5-7.0. The pH range of all the formulations was determined using digital pH meter which was found to be in the range of 6-7 as given in the **Table 10&11.**

***In vitro* diffusion study:** *In vitro* diffusion studies were performed using dialysis membrane, all the formulations were studied for drug release up to 24 hrs, Formulations TS_5, TS_7, and TS9 has faster release of drug where 100 percent drug was released within 6-7 hrs. Formulations TS_2, TS_3, TS_8 with 2% TS has shown 100%drug release in the range of 11-13 hrs, Formulations TS_1, TS_4, TS_6 with 2.5 % of TS has shown slow drug release till 18-20 hrs, results revealed that the drug release depends on %of TS used in the formulations. (See **Fig 12&13.**) Formulations GTS_2, GTS_3, GTS_8 with 2% TS has shown 100% drug release in the range of 8-10 hrs, Formulations GTS_1, GTS_4, GTS_6 with 2.5 % of GTS has shown slow drug release till 16-17 hrs. Results reveal that the drug release is depending on the percentage of GTS as shown in the **Fig 14.**

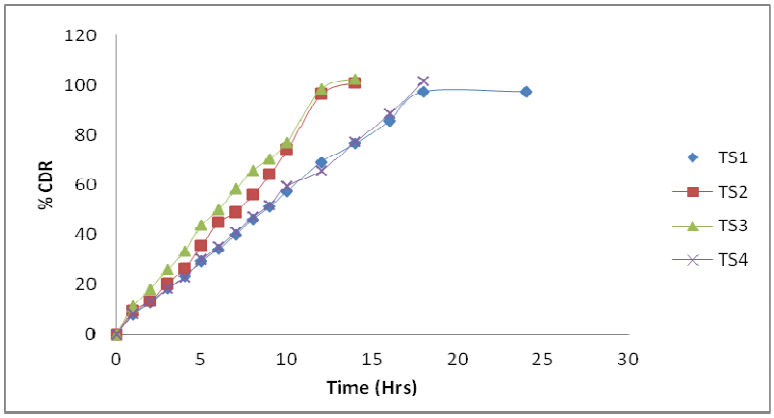


Fig 12: *In vitro* drug release profile for formulations TS_1, TS_2, TS_3, and TS_4

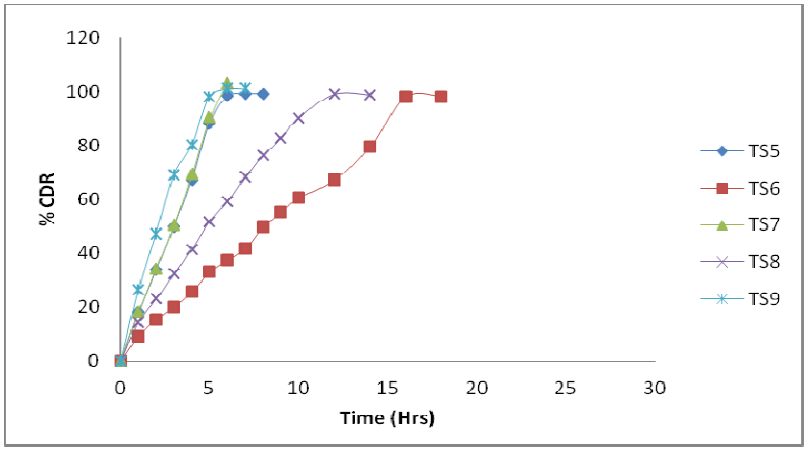


Fig 13: *In vitro* drug release profile for formulations TS_5, TS_6, TS_7, TS_8 & TS_9

***Ex vivo* diffusion study:** Formulations TS_5, TS_7, TS_9 were excluded from the *ex vivo* studies as the patches were brittle, and shown 100 % drug release in the range of 5-7 hrs, hence TS1, TS_2, TS_3, TS_4, TS_6, and TS_8 formulations were subjected for *ex vivo* studies using bovine check pouch. Formulations TS_2, TS_3, TS_8 has shown 100% drug release in the range of 11-13 hrs, Formulations TS_1, TS_4, TS_6 has shown 100 % drug release in the range of 16-20 hrs. The drug release was depending up on % of TS and PG. Formulations having same % of TS with higher % of PG has shown faster drug release and this could be due to the permeation enhancing effect of the used plasticizer PG. Formulations GTS_5, GTS_7, GTS_9 were excluded from the *ex vivo* studies as the patches were brittle, they has 100% drug release in the range of 4-5 hrs, hence only GTS_1, GTS_2, GTS_3, GTS_4, GTS_6, and GTS_8 formulations were subjected *for ex vivo* studies using bovine check pouch, Formulations GTS_2, GTS_3, GTS_8 has shown 100 % drug

release in the range of 8-10 hrs, Formulations GTS_1, GTS_4,GTS_6 has shown 100 % drug release in the range of 15-16 hrs. The drug release was depending up on percentage of GTS and percentage of PG, with in the formulations having same percentage of GTS, patches with higher percentage of PG has shown faster drug release and this could be due to the permeation enhancing effect of the used plasticizer PG. **(**See **Fig 15 & 16)**

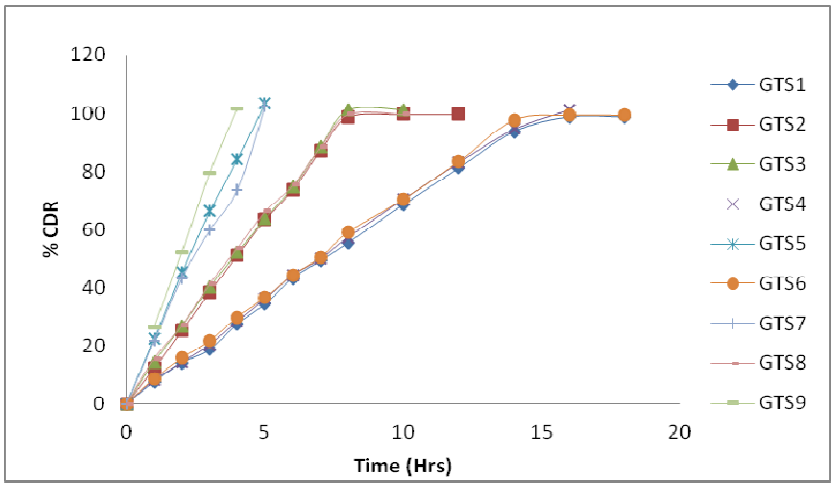


Fig 14: *In vitro* drug release profile of all GTS formulations

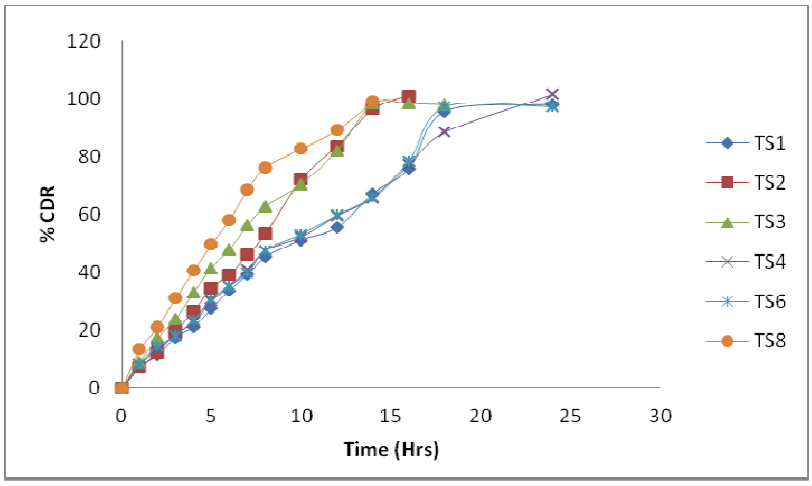


Fig 15: *Ex vivo* drug release profile of all TS formulations

Bioadhesive strength & Bioretention time: Different runs reveal that the bioadhesive strength increased with increased percentage of TS in the formulations, TS_6 has shown highest bio adhesive strength of 118.26 gm. and TS_7 has 30.76 gm. Bio retention time tests were performed in triplicates, mean time was calculated and reported TS_4 and TS_6 formulations has shown highest bio retention time of 235 minutes, the bio retention time was depending on the percentage of TS, with increased TS concentration bio retention time was increased as shown in the **Fig 17** bio adhesive strength increase with increased percentage of GTS employed in the formulations. all the patches has shown adequate bio retention time in the range was 141-215 min, GTS_4 and GTS_6 formulations has shown highest bio retention time of 215 min, the bio retention time was depending on the percentage of GTS, with increased GTS percentage bio retention time was increased as shown in the **Fig 18.**

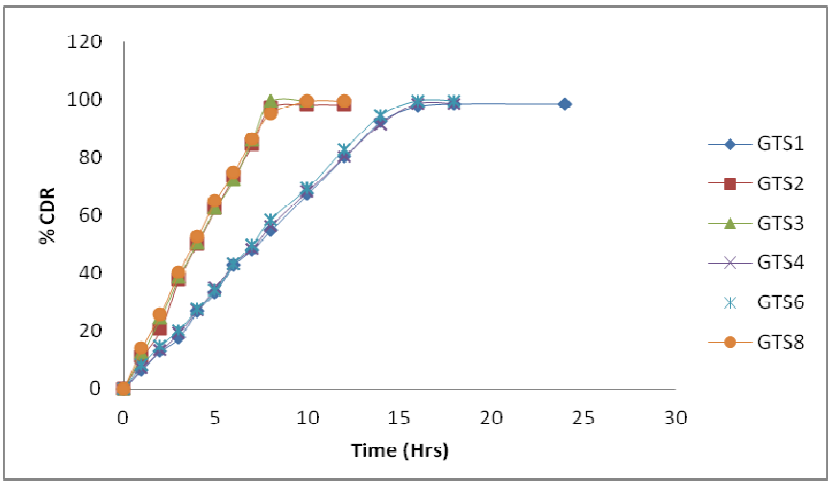


Fig 16: *Ex vivo* drug release profile of all GTS formulations

Optimization: The coefficients of each variable on the responses were calculated seperately and the equations of different responses were devoloped. The factor A and factor B significantly influence the responses. By carrying out different runs, the thickness was found to vary in the range of 174 to 292 µm whereas 224 to 258 µ m for GTS and 50% TDR was found to vary in the range of 3 to 9 hrs for TS and 2 to 8 hrs for GTS. The drug release was found to be depending on both the concentration of TS, GTS and PG this latter may be permeation enhancer. The formulations with lower TS, GTS and PG concentrations were releasing drug slowly than the other formulations. Using polynomial equation, the interaction graphs the constraints are selected and optimized. The optimum formulation was prepared in the same manner as the design.

The optimized formulation obtained from the software was not in the designed trails hence the optimized formulation was prepared again according to the obtained formula and evaluated for all the parameters. **(See Fig 19-24)**

Table 12: Release kinetics for all the formulations of TS and GTS

Formulation	Zero order		First order		Higuchi		Peppas		
	R^2	K	R^2	K	R^2	K	R^2	K	n
TS_1	0.9474	4.478	0.891	-0.0732	0.9746	26.918	0.9894	0.875	0.865
TS_2	0.9901	7.8351	0.772	-0.1053	0.9399	34.869	0.982	0.8906	0.9642
TS_3	0.9906	6.6727	0.7655	-0.105	0.9702	5.0753	0.0262	0.2475	1.2897
TS_4	0.9978	5.3771	0.9322	-0.0543	0.9747	27.39	0.9952	0.872	0.879
TS_5	0.9132	12.702	0.916	0.3258	0.9555	50.435	0.9775	1.272	0.8867
TS_6	0.9992	5.6439	0.7172	-0.0826	0.9639	28.641	0.995	0.9263	0.850
TS_7	0.9961	17.892	0.8806	-0.2178	0.9704	57.377	0.9969	1.2508	0.9825
TS_8	0.9612	7.0003	0.8967	-0.1546	0.9851	34.535	0.993	1.1506	0.7806
TS_9	0.9866	17.629	0.8237	-0.3611	0.9946	57.45	0.996	1.428	0.8131
GTS_1	0.9761	5.8778	0.892	-0.1129	0.9822	31.952	0.9934	0.8712	0.9431
GTS_2	0.9599	10.585	0.8466	-0.2577	0.9808	45.039	0.99	1.1144	0.9581
GTS_3	0.999	12.914	0.9179	-0.1341	0.9852	44.74	0.999	1.156	0.928
GTS_4	0.9981	6.7903	0.889	-0.0837	0.78	32.655	0.9977	0.8833	0.9588
GTS_5	0.9973	20.621	0.9692	-0.2284	0.9974	61.85	0.9987	1.357	0.9586
GTS_6	0.9902	6.4105	0.8396	-0.1231	0.981	32.872	0.9978	0.9247	0.916
GTS_7	0.9898	17.229	0.9959	-0.1564	0.9997	51.938	0.9947	1.3462	0.8891
GTS_8	0.9984	12.035	0.6575	-0.2721	0.986	46.621	0.9985	1.5822	0.9023
GTS_9	0.9997	26.42	0.9664	-0.2754	0.9915	71.676	0.9998	1.4214	0.9963
GTS_{10}	0.9862	7.393	0.7721	-0.2185	0.9732	4.0873	0.9973	0.8954	1.0035

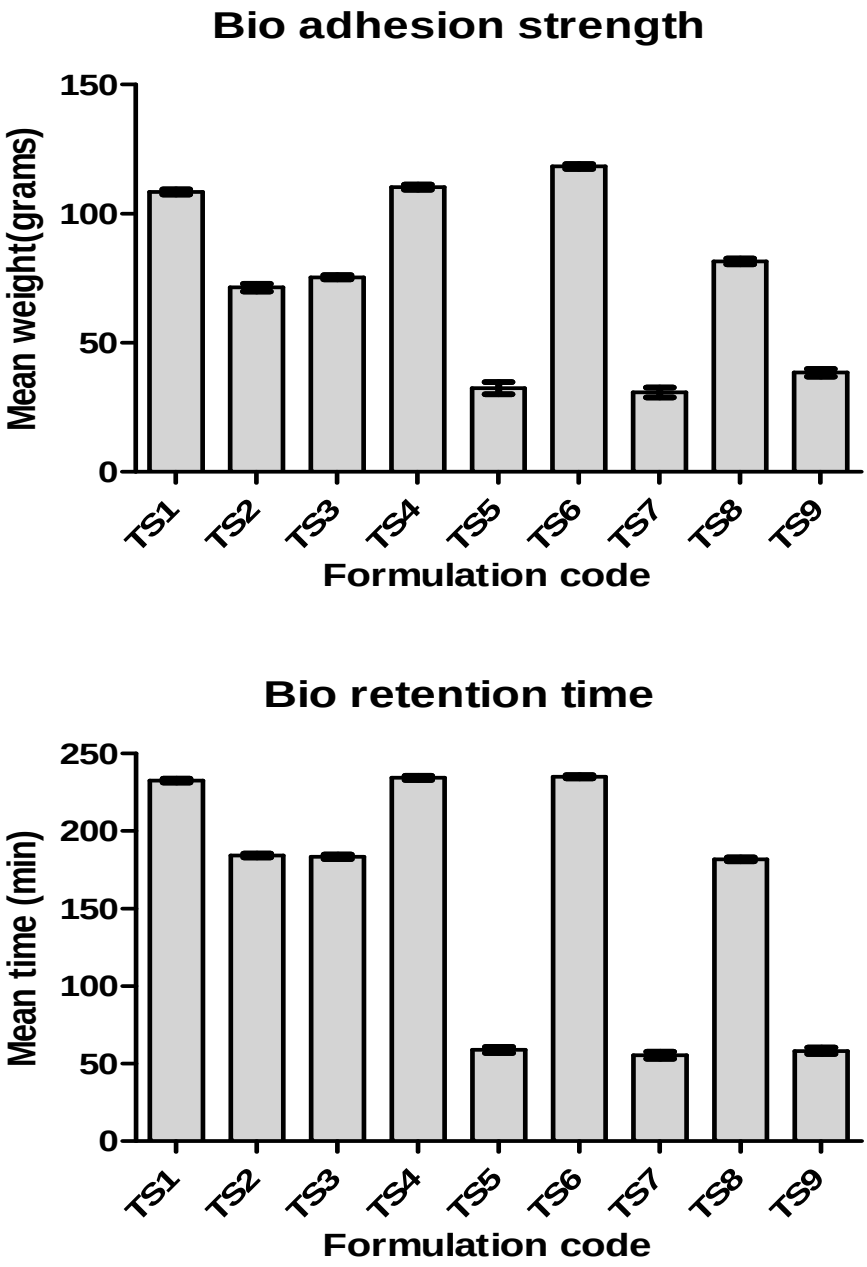


Fig 17: Bar graphs showing Bio adhesion time and Bio retention time of the buccal patches prepared using TS

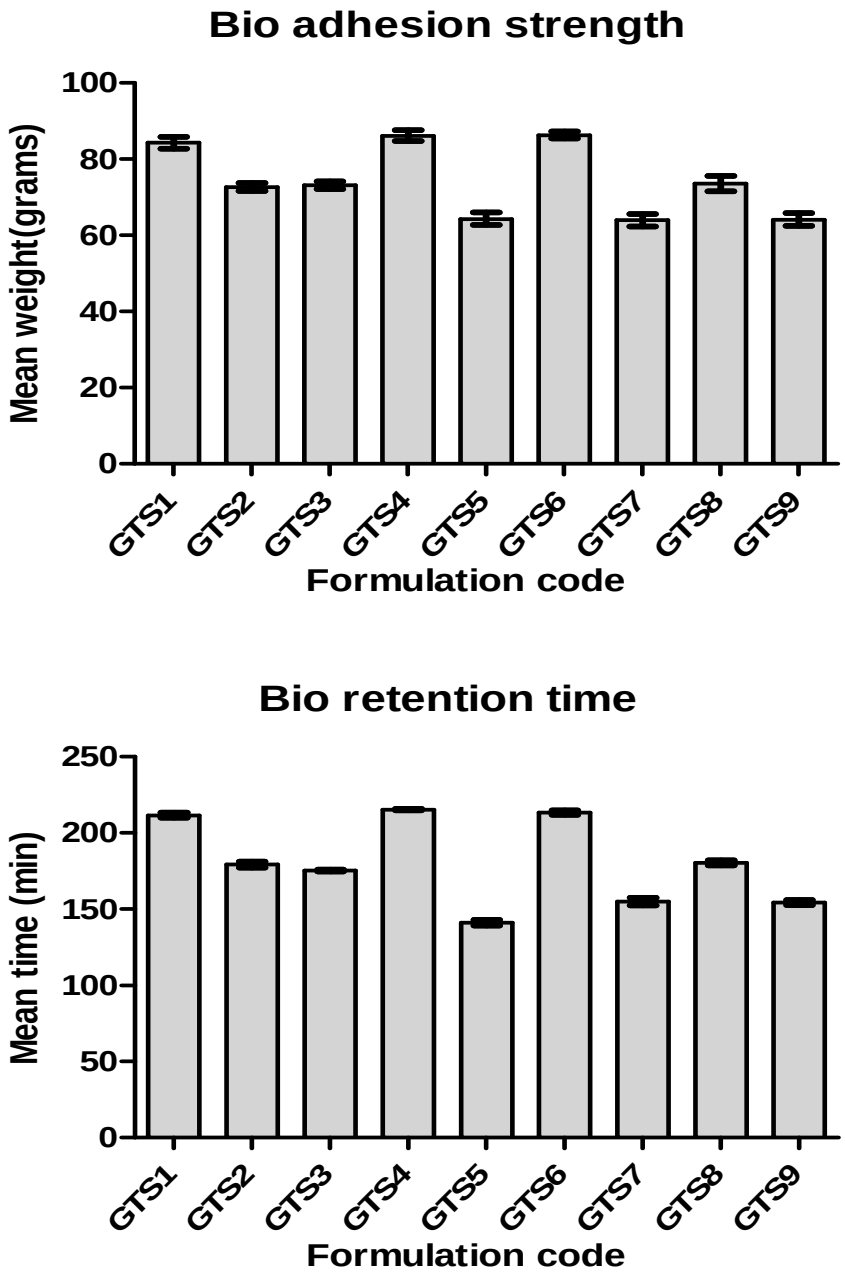
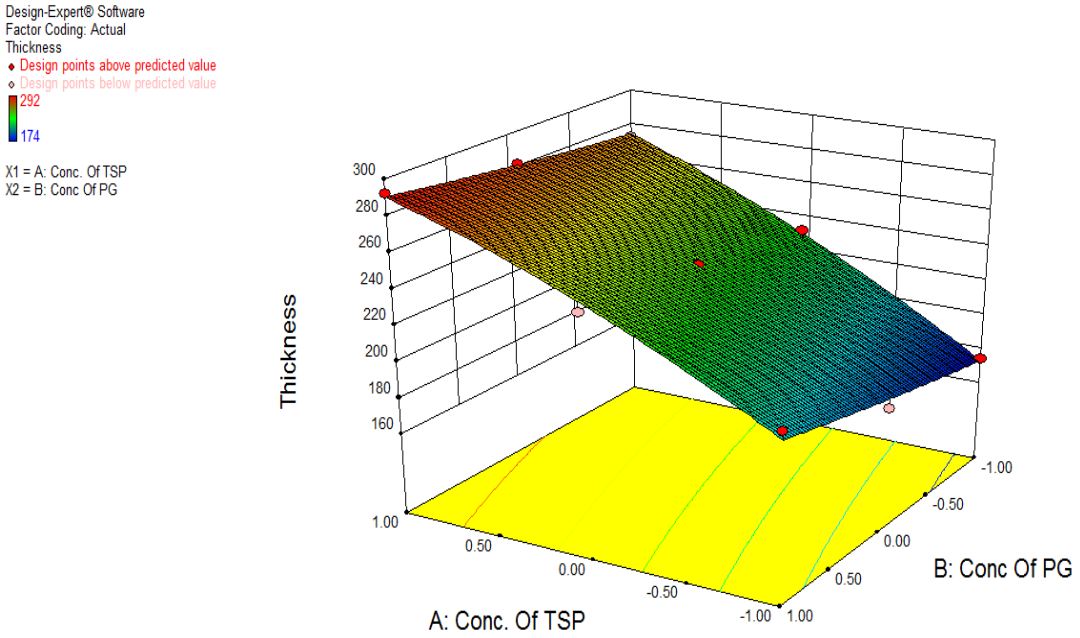


Fig 18: Bar graphs showing Bioadhesion time and Bio retention time of the buccal patches prepared using GTS



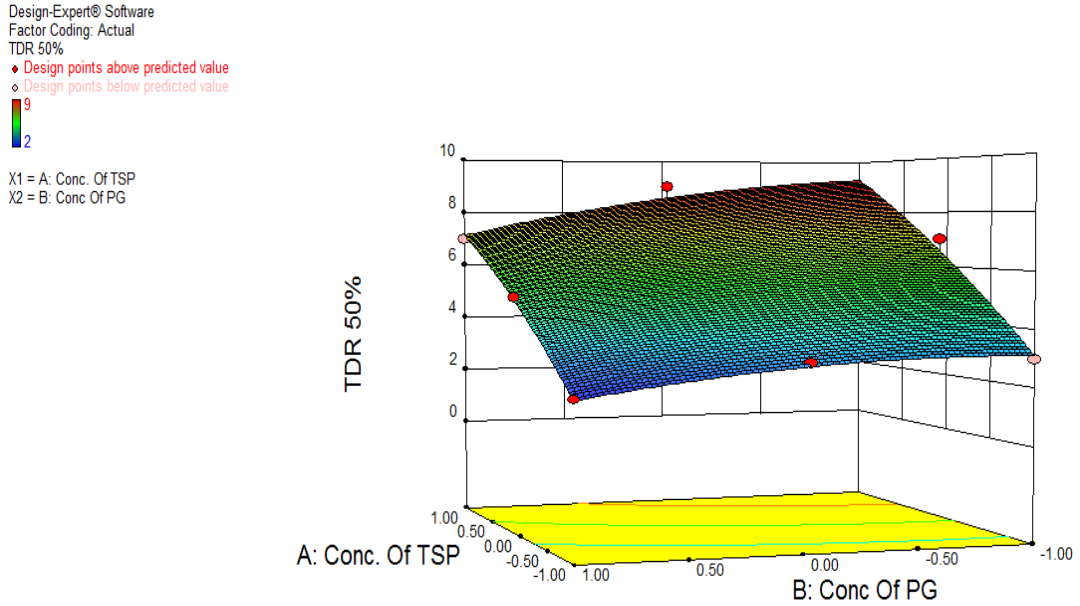


Fig 19&20: Response surface graphs of various factors with responses for buccal patches with TS

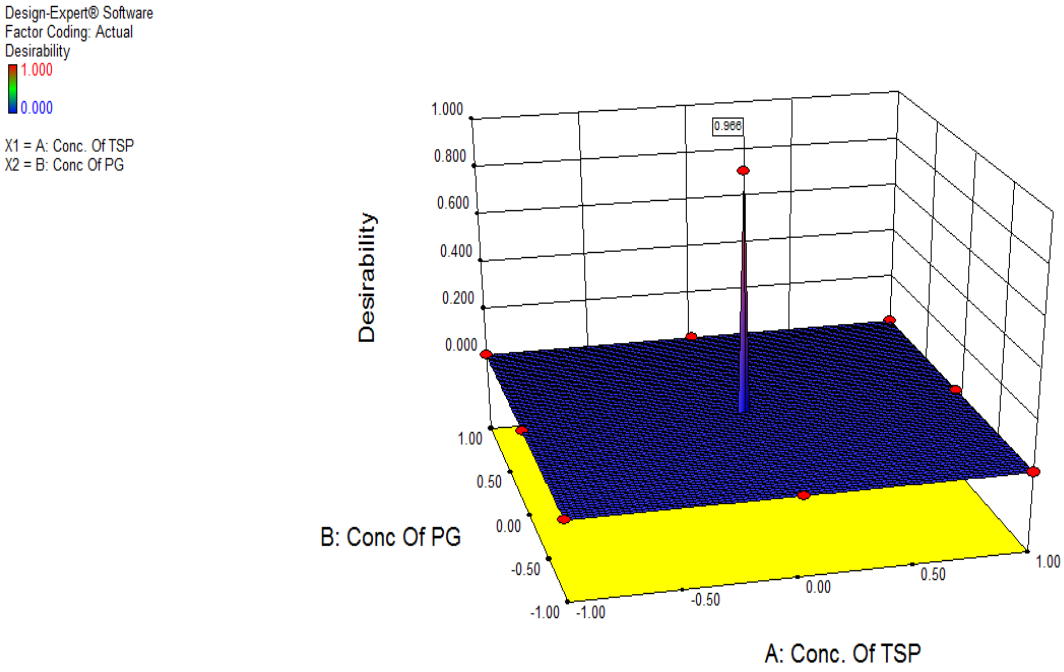


Fig 21: Desirability graph of optimized formula for buccal patches prepared using TS

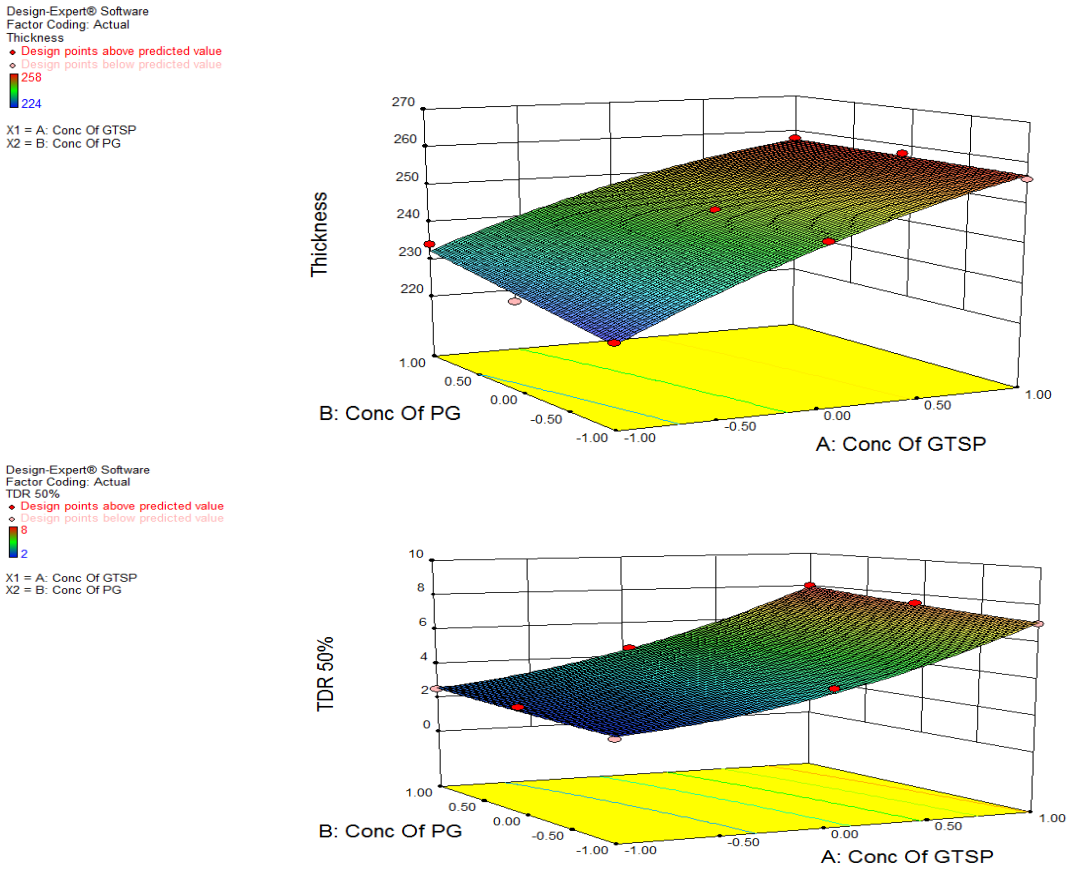


Fig 22&23: Response surface graphs of various factors with responses for buccal patches with GTS

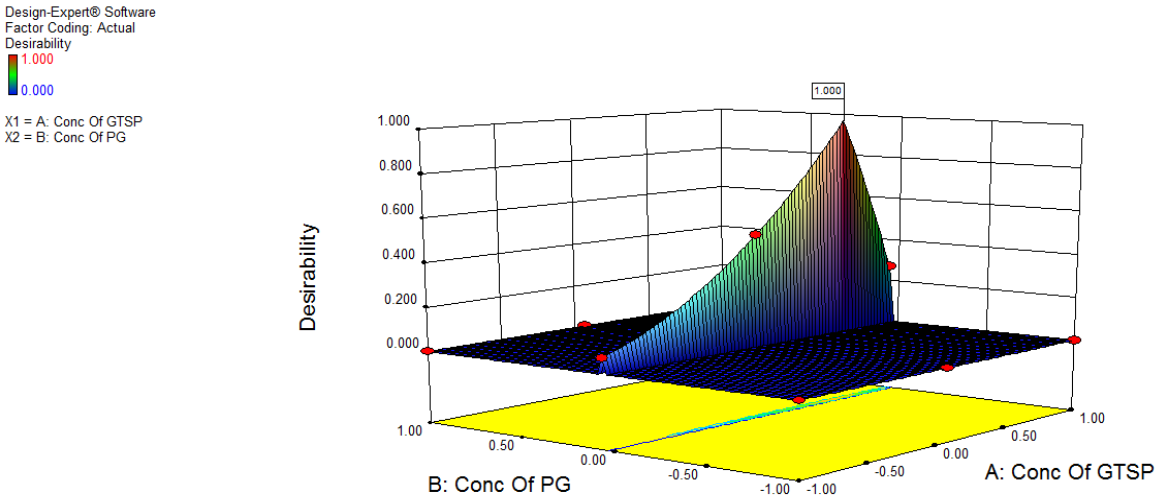


Fig 24: Desirability graph of optimized formula for buccal patches prepared using GTS

Optimized buccal patch GTS_{10}: Optimized formulation was evaluated for all the properties, the drug was uniformly distributed in patches and thickness was in the range of 253-258 µm, folding endurance more than 300 times, bioadhesion strength of 81 gm., bio retention time of 210 min, the *ex vivo* and *In vitro* drug release was observed under the same experimental conditions as other formulations and 100% drug release was in the range of 11to 12 hrs. **(See Fig 25)**

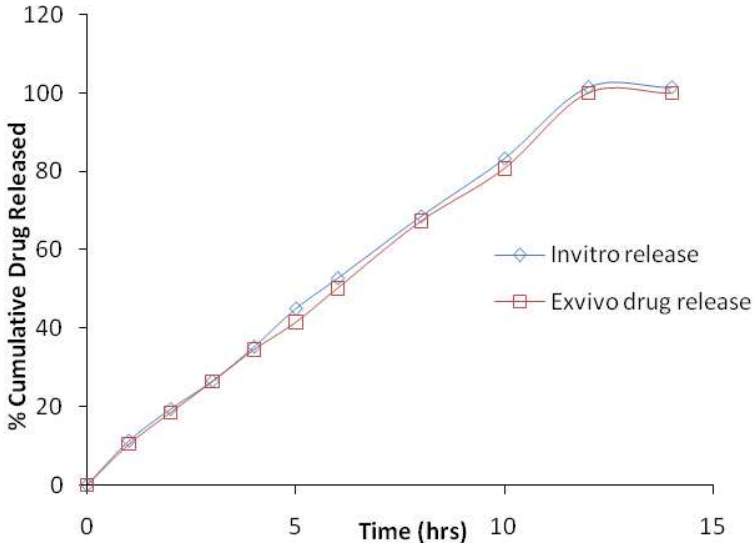


Fig 25: *In vitro* and *Ex vivo* drug release profiles for the optimized formulation GTS_{10}

Curve fitting for formulations: All the formulations followed zero order non fickian type of release. Results revealed that the drug release from optimized formulations followed zero order kinetics with super case II transport mechanism. **(See Table 12)**

CONCLUSION

TS can be grafted to form GTS by a simpler, eco-friendly chemical red ox pair method to overcome disadvantages such as uncontrolled rate of hydration and drop in viscosity. Taguchi OA design was applied for optimizing the grafting process where 1:1 ratio of TS to methyl methacrylate for 60 min of exposure time has shown high yield with good percentage efficiency. Rheological studies of GTS have shown controlled rate of hydration and no drop in viscosity during storage in comparison to TS. The applicability of TS and GTS for film formation and sustained release has been investigated by formulation and evaluation of buccal patches at different percentages of plasticizer and polymers according to central composite design. Patches have shown adequate folding endurance, content uniformity, surface pH, bio adhesive strength and bio retention time. *In vitro* and *ex vivo* studies have shown that TS_3 formulations (2% of TS) and GTS_{10} formulations (2.86% of GTS) were for sustained drug release for 12 hrs. The results indicate that the TS and GTS can be successfully used to develop buccal patches with sustained delivery of metoprolol succinate for 12 hrs.

Future scope: The research can be continued by grafting of TS with other methods to impart thermo sensitive and in situ gelling properties. The research work can be further continued by exploring the pharmaceutical application of GTS in the formulation of suspensions and gels TS and GTS can be further derivatized to impart anti-microbial properties.

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