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Optimization of carboxymethyl tamarind seed polysaccharide based glipizide matrix tablets using 3^2 full factorial design and response surface methodology

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ABSTRACT

The present study was undertaken to assess the potential of Carboxymethylated Tamarind seed polysaccharide (CM-TSP) as a matrix former in sustained release matrix tablets of Glipizide. Carboxymethylation of Tamarind kernel powder (TKP) was carried out and evaluated for its micromeritic properties viz. bulk density, tap density, angle of repose, Hausner's ratio, Carr's index and the results indicated good flow properties. CM-TSP was also evaluated for various physicochemical properties such as solubility, swelling index, melting point and viscosity. The drug and CM-TSP were found to be compatible as confirmed by IR spectral studies and Differential Scanning Calorimetry. Sustained release matrix tablets of Glipizide were prepared by direct compression method using CM-TSP as matrix former. A 3^2 full factorial design with two independent variables and three dependent variables was employed to optimize drug release profile and evaluated using Response Surface Methodology. Concentration of CM-TSP (X1) and type of diluent (X_2) were taken as independent variables. The dependent variables selected were percent of drug release at 4 hr (Y_1), 8hr (Y_2) and swelling index (Y_3). Response surface plots were developed, and optimum formulation was selected. The Formulation F8 showed a slow and complete drug release of 98.35±0.57% over a period of 20 hr with 'n' value 0.642 indicating that the release mechanism was Non-Fickian. The polymer CM-TSP had significant effect on drug release from the tablet ($p>0.05$). Polynomial mathematical models generated for various response variables using multiple regression analysis, were found to be statistically significant ($p>0.05$).

Key words: Carboxymethyl tamarind seed polysaccharide, Glipizide, 3^2 full factorial design, response surface methodology, sustained release matrix tablet.

INTRODUCTION

Hydrophilic matrices are an interesting option while developing an oral sustained-release formulation. They can be used for controlled release of both water-soluble and water insoluble drugs. The release behaviour of drugs varies with the nature of the matrix and it is the complex interaction of swelling, diffusion and erosion process [1]. Polysaccharides are the choice of material which has been evaluated as hydrophilic matrix for drug delivery system due to their non-toxicity and acceptance by regulating authorities. Tamarind seed polysaccharide is a natural polysaccharide isolated from seed kernel of *Tamarindus indica* family Leguminoseae. It has been significantly evaluated for use in hydrophilic drug delivery system. It possesses high viscosity, broad pH tolerance, swelling and binding properties [2]. This led to its application as release retardant polymer and binder in pharmaceutical industry. In addition to these other important properties of xyloglucan have been identified recently, which include non-

carcinogenicity [3], mucoadhesivity, biocompatibility [4], high drug holding capacity [5] and high thermal stability [6]. This led to its application as excipient in hydrophilic drug delivery system [3-6].

Carboxymethyl TSP is a derivative of xyloglucan and the microbial resistance of CM-TSP is much better than that of plain powder. The viscosity of CM-TSP in solutions is higher compared to native gum. Derivatization of TSP i.e. CM-TSP disrupts the organization and exposes the polysaccharide network for hydration which results in higher viscosity due to which it's swelling index is also higher as compared to TSP. The presence of carboxymethyl groups makes the molecule resistant toward enzymatic attack [7]. Since CM-TSP is having improved properties which are required for the retardation of release, the present study was undertaken to elucidate release kinetics of Glipizide, a hypoglycemic drug from the matrix tablets containing CM-TSP as matrix former. The model drug chosen to assess the release behaviour was Glipizide that is BCS class-II drug, with low aqueous solubility and high permeability. Glipizide, is an effective oral antidiabetic (100 times more potent than Tolbutamide in evoking pancreatic secretion of insulin (8,9) requires controlled release formulation owing to its short biological half-life (10) of 3.4 ± 0.7 h and is rapidly eliminated. Hence sustained release formulation is needed for Glipizide for better control of blood glucose levels to prevent hypoglycemia and enhance clinical efficiency, to reduce G.I disturbances and to enhance patient compliance.

MATERIALS AND METHODS

Tamarind kernel powder was obtained as gift sample from Meckoni Impex, Surat, Gujarat. Glipizide was obtained as gift sample from Horizon Bioceuticals Pvt. Ltd. Magnesium Stearate, Lactose, Starch and Microcrystalline cellulose were purchased from CDH (P) Ltd, New Delhi, India. Methanol, monochloroacetic acid, sodium hydroxide were purchased from Merck Ltd., India. All the chemicals used were of A.R grade.

Procedure for Carboxymethylation of TKP:
Carboxymethylation of TSP was carried out using the method reported by Goyal et al [7]. TSP (0.05 mol) was dispersed in 80 ml alkaline aqueous methanol (0.158 mol sodium hydroxide). To this dispersion monochloroacetic acid (0.09 mol) was added in solid form with continuous stirring for 15 min. The flask was immersed in a thermostatic water bath and the temperature was maintained at 70°C for 60 min. The contents of the flask were shaken occasionally during the course of the study. The reaction product was filtered, dissolved in water and neutralized with dilute acetic acid. The reaction product was precipitated in ethyl alcohol and washed twice with aqueous methanol (80 %, v/v) followed by pure methanol. The products were initially dried at room temperature and then in vacuum oven at 40°C for 4 hr to obtain carboxymethyl tamarind seed polysaccharide (CM-TSP). The CM-TSP was characterized by FTIR analysis.

Evaluation of CM-TSP:
1. **Micromeritic properties of CM-TSP:** Bulk density, tap density, angle of repose, Hausner's ratio and Carr's index was determined.
2. **pH of 1% solution:** The pH was measured using a digital pH meter.
3. **Physicochemical Properties of CM-TSP**: Various physicochemical properties such as solubility, swelling index, melting point, moisture content and viscosity were determined.
4. **Compatibility studies:** Pure drug (Glipizide) and physical mixtures drug and CM-TSP were examined by Fourier Transform Infrared (FT-IR) spectra. The spectra were recorded in a Thermo-IR 200 FTIR spectrophotometer. Each spectrum was derived from 25 single average scans collected in the range of 4000-400 cm-1 at the spectral resolution of 20 cm-1. DSC curve of pure drug (Glipizide) and physical mixtures drug and polymer were obtained by a Differential Scanning Calorimeter at heating rate of 10°C/min from 30 to 300°C in nitrogen atmosphere (30mL/min).

Preparation of Glipizide matrix tablets:
Matrix tablets were prepared by direct compression method. Glipizide (10mg) was blended with CM-TSP (10%, 20% and 30%)) with diluents (Lactose, Starch and MCC). The mixture was blended with 1% Magnesium stearate and mixed for 5 minutes. This mixture was compressed using 8 station rotary tabletting machine (Cadmach) with flat punch of 8mm diameter. The compositions of tablets were varied by using polymer in different ratios and using different diluents and is represented in Table-2

Evaluation of Tablets

1) **Granular analysis**: Bulk density, tap density, angle of repose, Carr's index and Hausner's ratio of the prepared granules were determined.

2) **Post compression analysis**: The prepared tablets were evaluated for weight variation test, hardness, friability and content uniformity. Hardness was determined by using Monsanto hardness tester. Friability was determined using Roche friability testing apparatus. Uniformity of weight and content uniformity were performed according to the I.P method

Measurement of swelling index:

Six tablets were weighed individually (W1) and placed separately in Petri dishes containing 25 ml of phosphate buffer pH 7.4. At regular intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hr. the tablets were removed carefully from the petridishes and excess water was removed using filter paper without pressing (11). The swollen tablets were re-weighed (W2) and the swelling index of each tablet was calculated using the equation:

$$Swelling\ Index = \frac{W2 - W1}{W1} \times 100$$

***In-vitro* drug release:**

Drug release studies were carried out using USP dissolution test apparatus-II. The study was conducted at 37ºCand 50 rpm. The dissolution medium used was 900ml of phosphate buffer pH 7.4 and study was carried up to 24 hours. 5ml of sample was withdrawn at different time intervals and replaced with fresh medium in order to maintain sink condition. The withdrawn samples were diluted suitably and drug content was estimated spectrophotometrically at 223 nm.

Release Kinetics:

To analyze the mechanism of drug release from the matrix tablets, the release data was fitted into various mathematical models viz., Zero order, first order and Higuchi equation.[12] The dissolution data was also fitted to the well known experimental equation (Korsmeyer Peppas equation), which is often used to describe the drug release behaviour from polymer systems.[13]

$$log\ (M_t - M_f) = log K + n log t$$

Where, Mt is the amount of drug release at time t, Mf is the amount of drug release after infinite time; K is a release rate constant incorporating structural and geometrical characteristics of the tablet and n is the differential exponent indicative of the mechanism of drug release. To clarify the release exponent for the different batches of matrix tablets, the log value of % drug was plotted against log time for each batch according to the above equation. A value of n=0.5 indicates Fickian (case I) release; >0.5 but <1.0 for non Fickian (anomalous) release; > 1.0 indicates super case II type of release. Case II gradually refers to the erosion of the polymeric chain and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled drug release. [14]

Experimental design:

A full 3^2 factorial design was developed where the concentration of the CM-TSP (X1) and the type of diluent (lactose, starch and MCC) (X2) were selected as factors. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the factorial design. The percent of drug release in 4hr (Q4), 8hr (Q8) and swelling index (SI) were taken as response variables. The factors and levels of experimental design were given in Table 7 and 8. The response surface graphs and mathematical models were obtained from DOE software.

Statistical analysis:

The results from statistical analysis of the factorial design batches were performed by multiple regression analysis using Microsoft excel. To evaluate contribution of each factor with different levels on responses, two way analysis of variance (ANOVA) was performed using Microsoft Excel. To graphically demonstrate the influence of each factor on responses, the response surface plots were generated using DOE software.

Similarity Factor (f2) analysis:
In-vitro release profile of Glipizide from selected CM-TSP matrix tablet formulation and the marketed sustained release tablets were performed under similar conditions. The similarity factor between the two formulations was determined using the data obtained from the drug release study. The data was analyzed by the formula:

$$f_2 = 50\log \{[1 + (1/N) \Sigma (Ri - Ti)^2]^{-0.5} \times 100\}$$

where; N = number of time points, Ri & Ti = dissolution of reference and test products at time 'i'. If f_2 is greater than 50 it is considered that the two products share similar drug release behaviors.

RESULTS AND DISCUSSION

Carboxymethylation of TSP was carried out with monochloroacetic acid in the presence of alkali as a catalyst under heterogeneous conditions. The product was brownish white in colour and soluble in water. The micromeritic properties of CM-TSP are tabulated in Table no.1. The viscosity of 1% w/v solution of CM-TSP was found to be 1225-715 cps at various shear rates. The pH of the isolated polysaccharide was found to be 6.9 and melting point was 252-256º.The glipizide matrix tablets using CM-TSP as matrix former were prepared by direct compression method. (Table 2) The granules were characterized with respect to micromeritic properties. (Table 3) The angle of repose of 23-31° indicates satisfactory flow behaviour.

Table 1: Micromeritic properties of CM-TSP

Sr.no.	Parameters	Values observed
1	Bulk density	0.60 g/cc
2	Tapped density	0.80 g/cc
3	Angle of repose	18.6º
4	Carr's index	32.0º
5	Hausner's ratio	1.15
6	Loss on drying	17%
7	pH	6.8

Table 2: Composition of Glipizide matrix tablets

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Glipizide	10	10	10	10	10	10	10	10	10
CM-TSP	20	40	60	20	40	60	20	40	60
Lactose	168	148	128	-	-	-	-	-	-
Starch	-	-	-	168	148	128			
MCC							168	148	128
Magnesium stearate	2	2	2	2	2	2	2	2	2
Total	200	200	200	200	200	200	200	200	200

Table 3: Pre compression physical parameters of Glipizide granules

Formulation Code	Angle of repose (º)	Bulk density (g/cc)	Tapped density (g/cc)	Carr's index	Hausner's Ratio
F1	29.41±0.031	0.634±0.024	0.721±0.032	15.12±0.192	1.20±0.015
F2	26.35±0.021	0.616±0.025	0.685±0.016	12.55±0.115	1.15±0.014
F3	25.34±0.315	0.634±0.032	0.690±0.024	16.42±0.134	1.24±0.026
F4	29.50±0.200	0.616±0.026	0.694±0.031	18.32±0.118	1.20±0.025
F5	30.45±0.022	0.590±0.014	0.715±0.023	14.12±0.065	1.04±0.040
F6	31.30±0.024	0.595±0.018	0.693±0.027	15.572±0.112	1.06±0.026
F7	29.15±0.026	0.606±0.024	0.715±0.015	13.15±0.115	1.15±0.034
F8	24.65±0.023	0.629±0.024	0.745±0.014	17.25±0.105	1.17±0.056
F9	23.25±0.052	0.652±0.025	0.775±0.022	14.12±0.145	1.14±0.040

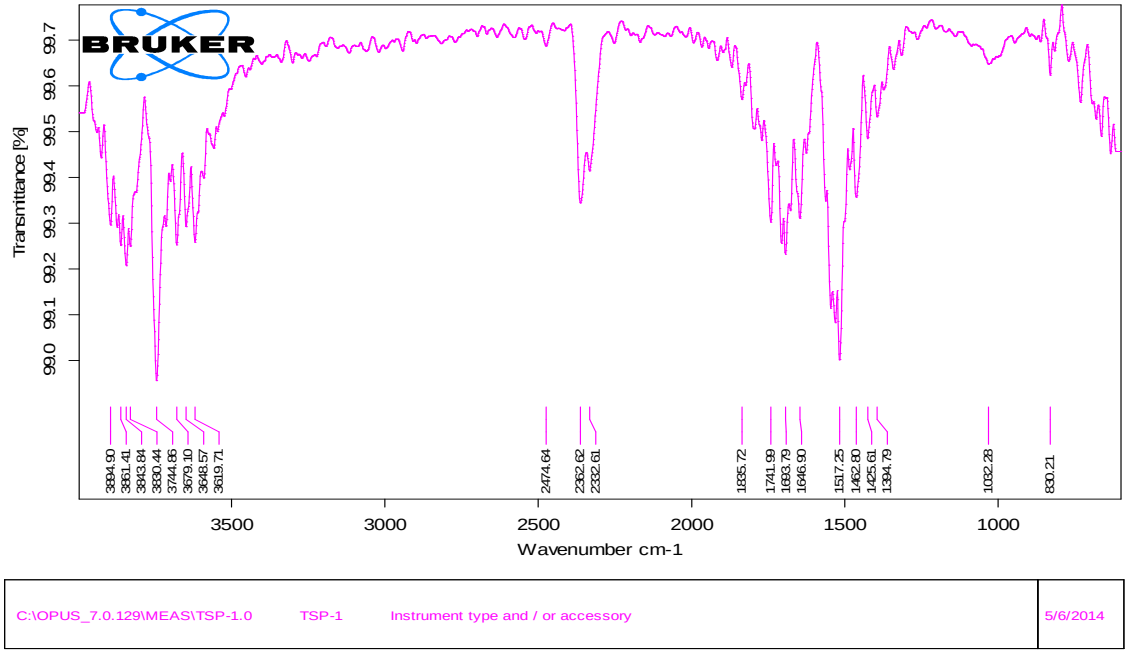
The matrix tablets were evaluated for hardness, friability, content uniformity, uniformity of weight and *in vitro* drug release studies. The hardness of the tablets in all the batches was found to be in the range of 6.5 – 7.1 Kg/cm^2. The friability of the tablets was in the range of 0.24 – 0.65 %. The drug content was found to be uniform for all the batches and was found to be within 95±2% of labeled claim. Evaluation data of the matrix tablets were given in Table 4. The hardness and friability values indicated good handling properties of the prepared matrix tablets.

Table 4: Post compression parameters of Glipizide tablets

Formulation	Weight Variation(mg)	Hardness kg/cm²	% Friability	%Drug content	Thickness (mm)
F1	199.7	6.5	0.45	97.28	2.20
F2	200.2	6.9	0.38	95.65	2.35
F3	199.9	6.6	0.46	97.44	2.20
F4	200.1	7.0	0.50	96.25	2.25
F5	200.5	6.5	0.34	98.80	2.15
F6	200.5	6.9	0.51	97.45	2.12
F7	200.6	7.1	0.24	97.35	2.05
F8	199.5	6.7	0.65	95.15	2.10
F9	202.4	6.9	0.40	96.68	2.14

The compatibility between the drug and CM-TSP was found to be good by the FTIR and DSC studies. The FTIR spectrum of TSP, CM-TSP, Glipizide and physical mixture of TSP and Glipizide is given in Fig.1. It can be used as standard spectrum for quality control and determination of the purity of CM-TSP. The spectra of TSP display a characteristic broad peak at 3260.34 cm^{-1}representing hydroxyl (OH) groups of glucan backbone. Peak at 2918.69 cm^{-1} can be attributed to C-H stretching of alkanes. Peak appearing at 1018.19 cm^{-1} is due to C-O-C stretching of cyclic ether. Cyclic C-H bending was confirmed by the peak at 755.77 cm^{-1} . The IR spectra of CM-TSP shows a reduced intensity of absorption band at 3260.34 cm^{-1} due to OH stretching indicating that some OH groups were carboxymethylated. Peak appearing at 1646 and 1462cm^{-1} may be attributed to the incorporation of carboxymethyl groups into the TSP molecule.

Fig.1: FTIR spectrum of CM-TSP



The DSC thermogram (Fig.2) of TSP shows broad endotherm at 88.13^0 C with heat of fusion of 249.36 J/g followed by an exotherm at 331.83^0 C with heat flow of 63.62 J/g while the thermal curve of CM-TSP shows a sharp endotherm at 128.35º with heat of fusion of 253.65J/g. Thus the shift in the endothermic peak in the thermal curve of CM-TSP indicates the modification of TSP.

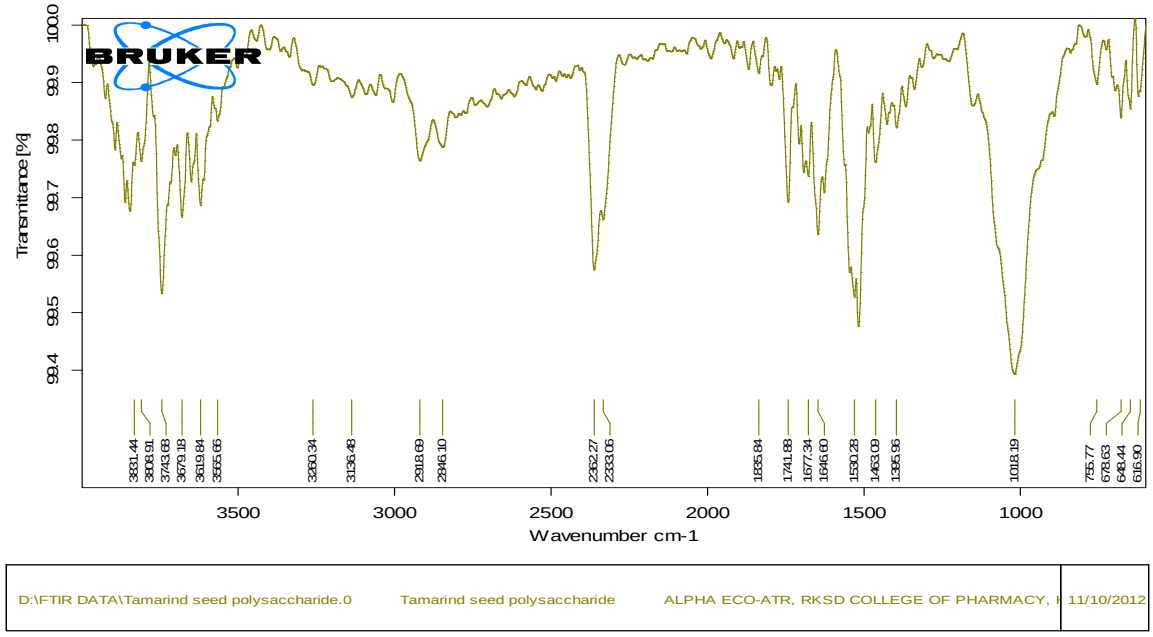
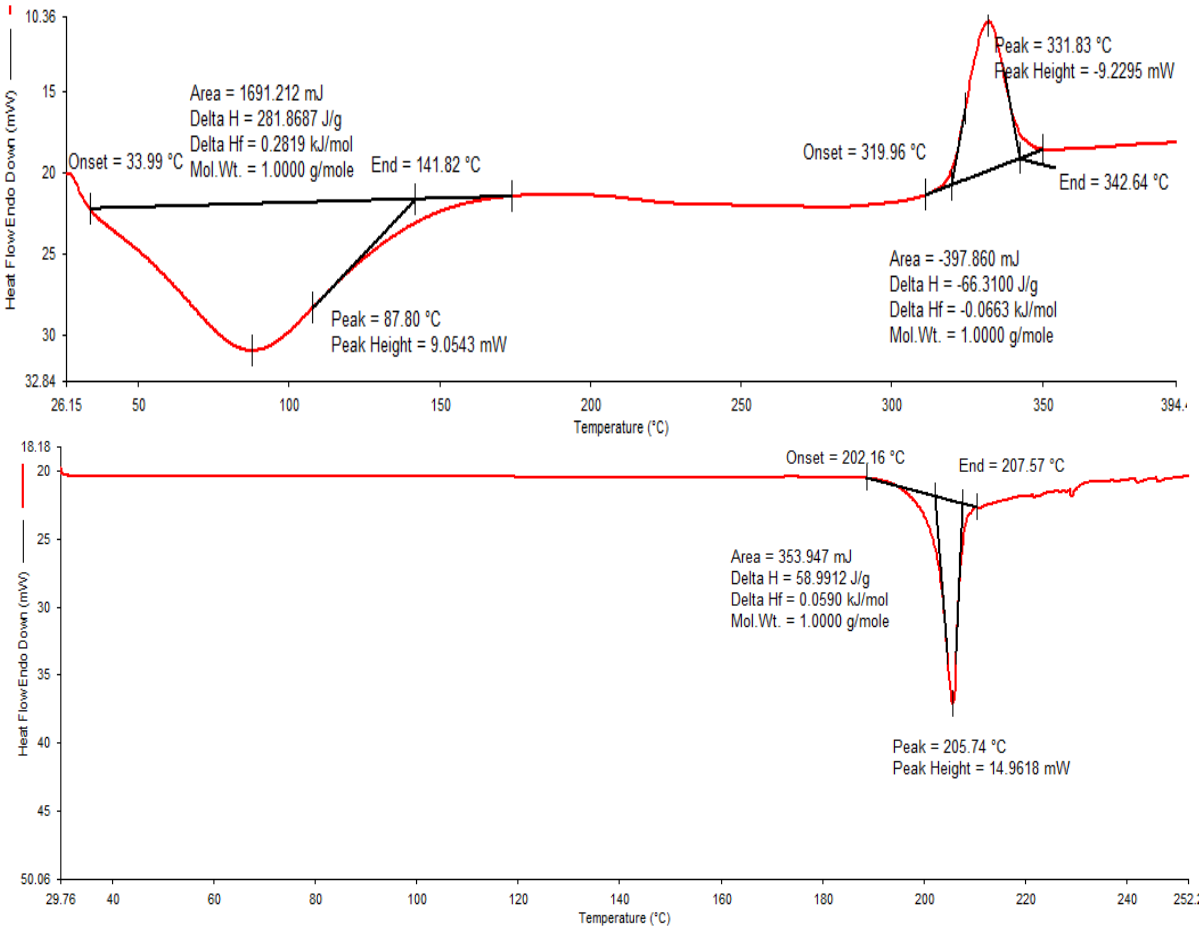


Fig. 2 DSC thermograph of TSP, Glipizide and CM-TSP and CM-TSP + Glipizide



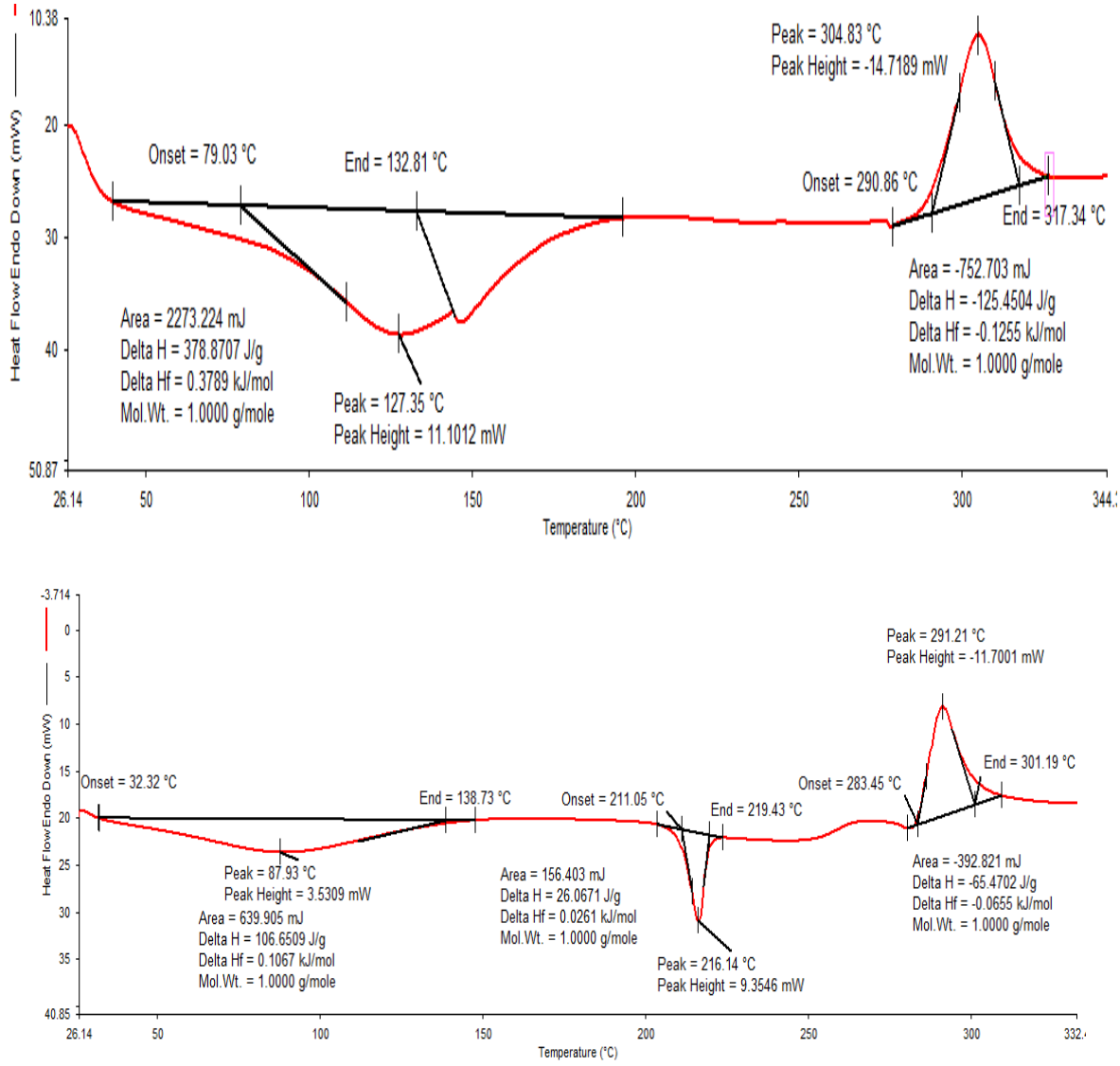


Fig.3 shows the shape and surface of TSP and CM-TSP as examined under Scanning Electron Microscope and the examination reveals that CM-TSP was rougher in comparison to TSP.

Fig.3: Scanning Electron Microscopy of CM-TSP and TSP

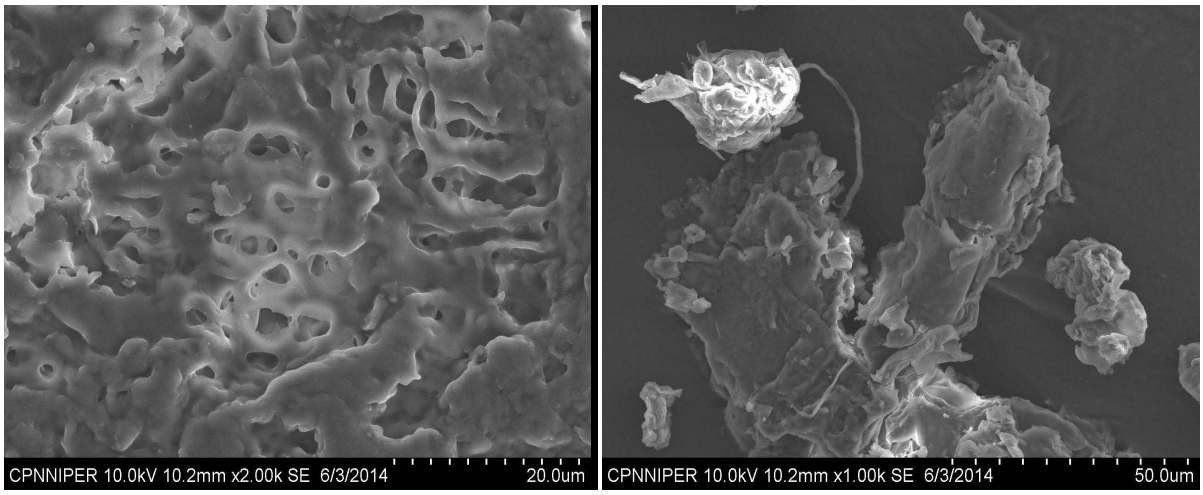


Fig.4: Percentage Swelling Indices of F1–F3 (TSP-Lactose), F4- F6 (TSP-starch), F7- F9 (TSP-MCC)

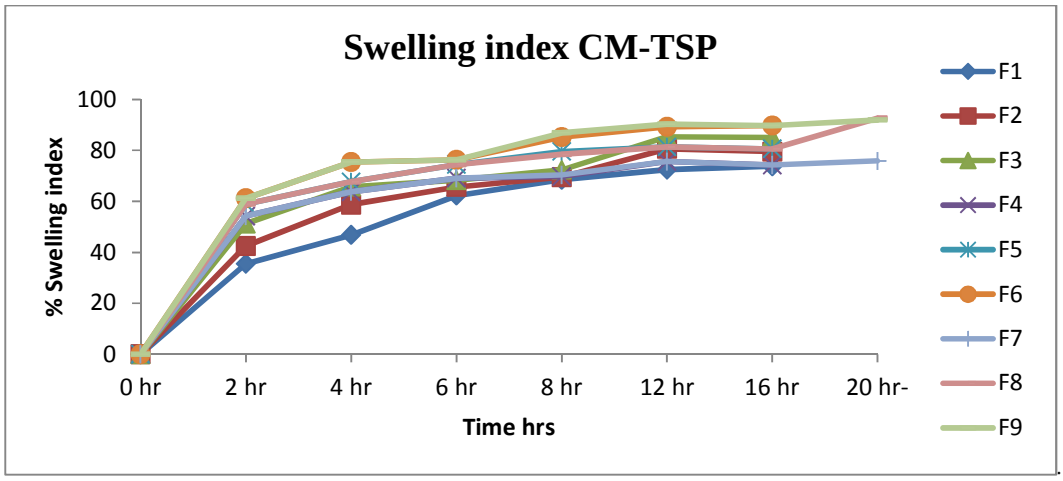
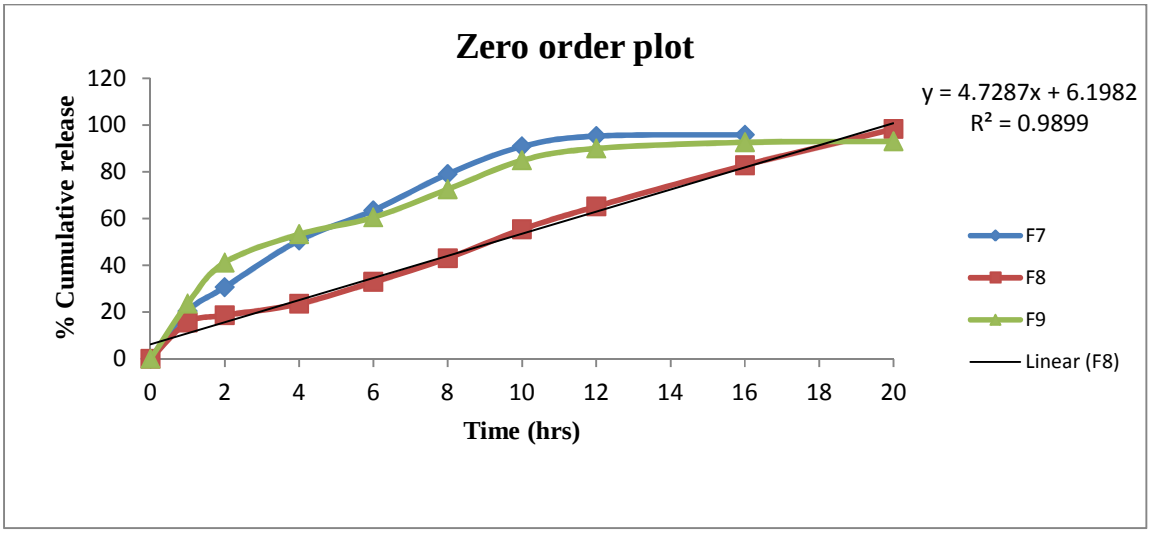


Fig 5: Cumulative % release of Formulations F1–F3 (Lactose), F4- F6 (starch), F7- F9 (MCC)

Fig.4 shows the swelling index of all the formulations which indicates that swelling increases with concentration of CM-TSP using MCC as diluent. The prepared matrix tablets were also studied for *in vitro* drug release studies. Table 5 indicates the data analysis of release profiles according to different kinetic models. Drug release from the matrix tablets was found inversely proportional to the concentration of CM-TSP and depends on type of diluent. The Formulation F8 showed a slow and complete drug release of 98.35±0.57% over a period of 20 hr. The 'n' value of formulation F8 from Korsmeyer-Peppas equation was found to be 0.642 indicating that the release mechanism was non-Fickian or anomalous release ($0.5 < n < 1$). It showed that the release was dependent on both drug diffusion and polymer erosion. R2 value (i.e., 0.989) was maximum for zero order plot, therefore release kinetics fits zero order plot**.**


Zero order plot
y = 6.2744x - 3.1456
R² = 0.9525
% Cumulative release
Time (hrs)
F1
F2
F3
Linear (F3)


Zero order plot
y = 12.347x - 17.682
R² = 0.9686
% Cumulative release
Time hrs
F4
F5
F6
Linear (F4)


Zero order plot
y = 4.7287x + 6.1982
R² = 0.9899
% Cumulative release
Time (hrs)
F7
F8
F9
Linear (F8)

A 3^2 factorial design was adopted to optimize the formulation variables. In the present design, concentration of TSP (X_1) and type of diluent (X_2) were selected as independent variables. Percentage drug release at 4hr, 8hr and swelling index were taken as dependent variables. The application of an empirical polynomial equation to the experimental results facilitates the optimization procedure. The general polynomial equation is as follows:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

Where Y is the dependent variable, b_0 is the arithmetic mean response on nine runs and b_1 is the estimated coefficient for factor X_1. The main effects (X_1 X_2) represent the average values of change in factors from low to high value. The interaction terms ($X_1^2$and X_2^2) are included to investigate nonlinearity. The drug release at Q_4 and Q_8 and swelling index (S.I.) for nine batches showed wide variations and the results are given in Table 8. The data clearly indicates that the values of dependent variables strongly depend on the independent variables. ANOVA data of the dependent variables is given in Table 9.The polynomial equations are given below and the regression coefficients are given in Table 8.

$$Q_4 = 5.08 – 5.63X_1 + 10.09X_2 + 6.90X_1X_2 + 12.28X_1^2 + 19.14X_2^2$$

$$Q_8 = 28.91 – 14.77X_1+ 1.46X_2 + 11.37X_1X_2 + 11.60X_1^2 + 26.79X_2^2$$

$$SI= 78.37 + 5.98X_1 + 4.20X_2 + 3.16X_1X_2 – 0.31 X_1^2 – 3.82X_2^2$$

The high levels of correlation coefficients for the dependent variables indicate a good agreement between the dependent and independent variables. The polynomial equation can be used to draw a conclusion by considering the magnitude of the coefficients and the mathematical sign it carries (+ or –). Positive sign before a factor in the equation represents that the response increases with the factor, while a negative sign indicates that the response and the factor have inverse relationship.

From the above equations it can be concluded that the release of drug from matrix tablets is inversely related to the amount of CM-TSP (X_1) and positively related to the type of diluent (X_2). The magnitude of coefficients indicate that the release of drug after 4hr is dependent on the type of diluent while the release after 8hr and swelling index are dependent on the concentration of polysaccharide. It also indicates that the release of drug initially depends on the diluent but eventually the release is controlled by the concentration of CM-TSP. Similarity factor analysis between the formulation F8 and marketed product (Glynase-XL containing TSP as matrix former) for the drug release showed an f_2 factor of 73.23, which is greater than 50, which confirmed that the release of the drug from the prepared matrix tablets is similar to that of the marketed tablet.

Table 5: Mathematical modeling of matrix tablets

Formulation	Correlation Coefficients (R^2)				Release exponent 'n'
	Zero order	First order	Higuchi	Korsmeyer Peppas	
F1	0.977	0.988	0.878	0.928	0.896
F2	0.946	0.838	0.859	0.874	0.912
F3	0.952	0.899	0.778	0.859	0.904
F4	0.968	0.954	0.891	0.885	0.763
F5	0.910	0.983	0.766	0.864	1.149
F6	0.850	0.960	0.661	0.921	0.999
F7	0.881	0.875	0.924	0.982	0.601
F8	0.989	0.993	0.949	0.965	0.642
F9	0.789	0.843	0.959	0.962	0.458

Table 6: Factors and levels of the factorial design

Factor/level	-1	0	+1
X1(concentration of (CM-TSP)	10%	20%	30%
X2(Type of diluent)	Lactose	Starch	MCC

Table 7: Independent and Dependent variables of formulations in a 3^2 full factorial design

Sr.no.	Formulation code	Coded factor levels		Percent drug released		Swelling index
		X1	X2	Q4	Q8	SI
1	F1	-1	-1	37.15	88.15	68.5
2	F2	0	-1	17.67	61.70	69.53
3	F3	1	-1	12.15	36.10	72.4
4	F4	-1	0	19.47	52.25	69.75
5	F5	0	0	12.30	35.53	79.56
6	F6	1	0	8.05	22.18	85.20
7	F7	-1	1	50.67	79.09	70.35
8	F8	0	1	23.56	43.12	78.40
9	F9	1	1	53.29	72.55	86.9

Table 8: Summary of regression output of significant factors for the measured responses

Parameters	Coefficients of regression parameters						
	b_o	b_1	b_2	b_{12}	b_{11}	b_{22}	R^2
Q_4	5.0822	-5.633	10.091	6.905	12.286	19.141	0.8822
Q_8	28.9177	-14.776	1.46	11.37	11.60	26.79	0.8883
SI	78.3788	5.9833	4.2033	3.1625	-0.3133	-3.8233	0.9692

Table9: Analysis of variance (ANOVA) for dependent variables in factorial design

For Q_4	SS	DF	MS	F value
Regression	2026.904	5	405.380	4.496
Residual	270.458	3	90.15	
Total	2297.363	8		
For Q_8				
Regression	3546.40	5	709.28	4.773
Residual	445.798	3	149.599	
Total	3992.20	8		
For SI				
Regression	390.2425	5	78.049	18.8829
Residual	12.4000	3	4.1333	
Total	402.6475	8		

Fig.6: Response surface plot of tablet formulations after 4 hours dissolution

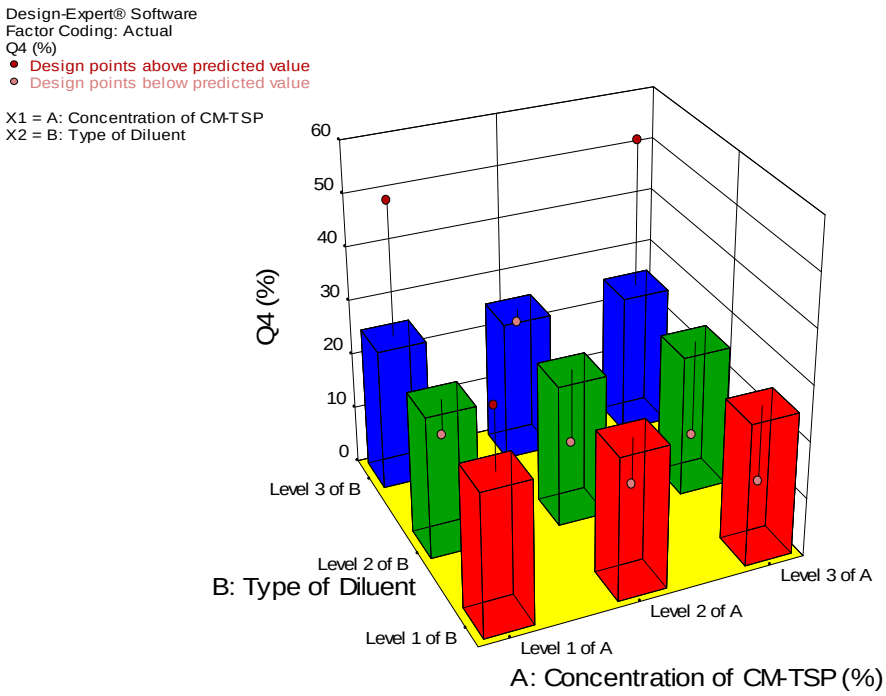


Fig.7: Response surface plot of tablet formulations after 8 hours dissolution

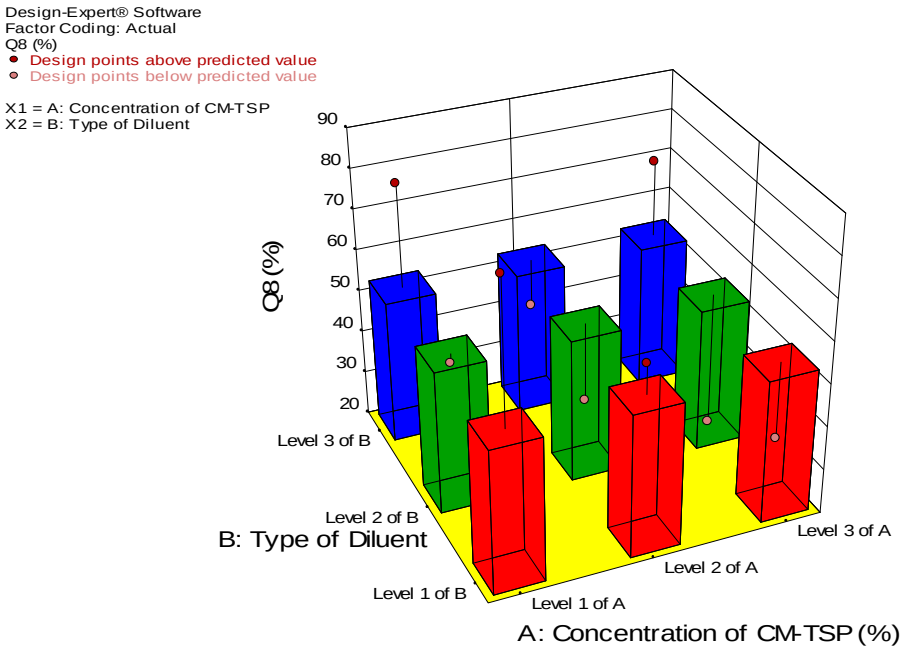
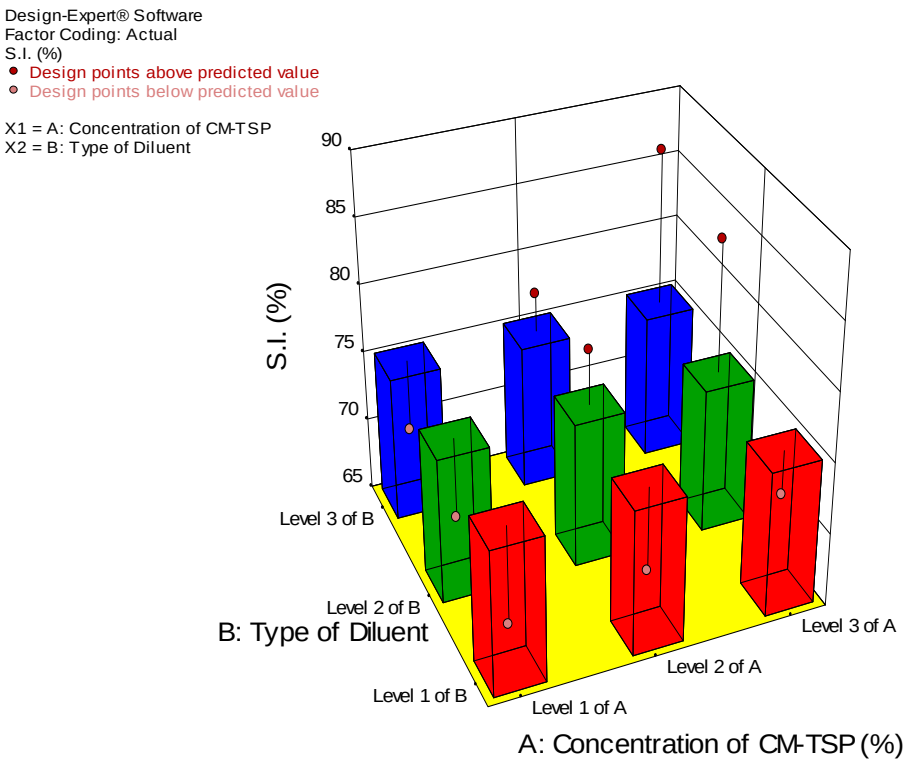


Fig.8: Response surface plot of tablet formulations showing the effect of polymer on Swelling index (S.I.)



CONCLUSION

Sustained release matrix tablets of glipizide with satisfactory release characteristics were successfully prepared by direct compression method using Carboxymethylated Tamarind seed polysaccharide (CM-TSP) as matrix former and different diluents (lactose, starch and MCC). Response surface methodology was adopted for understanding the change of responses and effect of formulation variables. Study indicated that increase in amount of CM-TSP in the tablets resulted in a reduction in the release rate. The calculated release exponent (n=0.642) of F8 was non-Fickian or anomalous release mechanism with zero order kinetics (R^2=0.989). It was concluded that CM-TSP with MCC at high concentrations was able to produce desired effects.

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