Design and Characterization of Colon Targeted Tegaserod Microspheres by Ionotropic Gelation.

Nemade M. S.*, Chaudhari R. Y. and Patil V. R.

TVES's HLMC College of Pharmacy,
Neharu Vidya Nagar, Savada Road, Faizpur 425 503, India.
E-mail: msnemade@gmail.com
Tel.: +91-2585-245574; Fax: +91-2585-245574

ABSTRACT

The aim of study was to develop and evaluate tegaserod maleate (TM) microspheres for colonic drug delivery system. Microspheres of TM were prepared by with little modification in the ionotropic gelation method by using polymer tamarind seed polysaccharide (TSP). These microspheres were evaluated for angle of repose, bulk density, tapped density, particle size, percentage drug entrapment, swelling behavior and *in-vitro* drug release studies. All the micrometric properties of the microsphere were within the range. The mean particle size of prepared microspheres was found to be in a range of 715.66 - 747.00 µm. Percentage drug entrapment observed in all formulations was between 74.03 - 76.69 %. The % water uptake in the pH 1.2 and pH 7.8 was between 69.66 - 73.33 and 181 - 192 respectively. *In-vitro* drug release studies showed that the release of tegaserod maleate from the microspheres was mainly influenced by the polymer concentration. Among all the formulations, F1 and F3 shows 94.98 and 88.52% better controlled release at the end of 12 hr respectively. The results indicated that a decrease in release of the drug was observed by increasing the polymer concentration. It is concluded from the present investigation that TM loaded microspheres were promising controlled release carriers for colon targeted delivery.

Keywords: Tegaserod Maleate, Tamarind Seed Polysaccharide, Colonic Drug Delivery System, Microspheres.

INTRODUCTION

The colon-specific drug delivery could allow local treatment of inflammatory bowel disease, e.g. ulcerative colitis or Crohn's disease. Such inflammatory conditions are usually treated with glucocorticoids and sulphasalazine. Lower doses will be adequate and if so, systemic side effects will be reduced. A number of serious diseases of the colon, e.g. colon cancer, can also be capable of being treated more effectively if drugs are targeted to the colon. Sustained colonic release of drugs can also be useful in the treatment of nocturnal asthma, angina and arthritis.[1]

The drug release from hydrophilic matrices can be controlled through their physical properties. Polysaccharides are the choice of materials among the hydrophilic polymers used, because they are nontoxic and acceptable by the regulating authorities. The natural polysaccharide, Tamarind seed polysaccharide obtained from the seed kernel of *Tamarindus indica*, possesses properties like high viscosity, broad pH tolerance, noncarcinogenicity, mucoadhesive nature and biocompatibility. It is used as stabilizer, thickener, gelling agent, and binder in food and pharmaceutical industries. [2]

Tegaserod is being developed as a treatment for constipation-predominant irritable bowel syndrome (IBS).[3,4] Tegaserod is a potent partial agonist of serotonin type-4 (5-HT_4) receptors located in the GI tract. The mechanism of action of Tegaserod is reflected in its stimulation of the peristaltic reflex intestinal secretion, as well as inhibition of visceral sensitivity via activation of 5-HT_4 receptors in the gastrointestinal tract. Tegaserod acts as a partial agonist at neuronal 5-HT_4 receptors triggering the release of further neurotransmitters such as calcitonin gene related peptide from sensory neurons. [3-5]

The recommended dosage of Tegaserod is 6 mg (i.e equivalent to 8.3 mg of Tegaserod maleate) taken twice daily orally before meals for 4-6 weeks. Tegaserod is rapidly absorbed following oral administration; peak plasma concentrations are reached after approximately 1 h. Tegaserod is approximately 98% bound to plasma proteins, primarily to α1-acid glycoprotein. The plasma clearance of Tegaserod is 77 ±15 L/h, with an estimated terminal half-life of 11 ± 5 h following intravenous administrations. Approximately two-thirds of the orally administered dose of Tegaserod is excreted unchanged in the feces, with the remaining one third excreted in the urine. [3-6]

The aim of present study is to design and characterization of colon targeted tegaserod maleate microspheres containing tamarind seed polysaccharide with little modification in the ionotropic gelation method.

MATERIALS AND METHODS

Materials:

Tegaserod maleate, sodium alginate (SA) and tamarind seed polysaccharide were obtained as gift sample. All other chemicals/reagents used were of analytical grade.

Compatibility study by FT-IR: [7]

Drug-excipients compatibility was studied by using FT-IR spectral analysis. A preliminary study was carried out with formulation excipients to determine drug-excipients interaction or compatibility. TM was uniformly mixed in 1:1 ratio with the excipients separately and the mixture was placed in glass vials. Vials were sealed by carnauba wax were kept at room temperature and 40°C and 75 % RH. After 30 days sample were withdrawn and observed for change in colour and chemical change by recording FT- IR spectrums. The scanning range was 4000 to 400 cm^{-1}.

Preparation of Microspheres: [8-10]

SA and TSP aqueous dispersion were prepared separately using distilled water. This dispersion was well mixed with stirring for 10 min. at 1000 rpm using electronic stirrer. Afterwards TM was added to this dispersion mixture. The ratio of drug to polymer was maintained 1:1 in all formulations. The final SA-TSP dispersion containing TM was homogenized till it completely mixed together at 1000 rpm. The resulting dispersion were sonicate for 5 min. to de-bubbling. The resulting dispersion was then added via a 26 gauge needle. The added droplets were retained into $CaCl_2$ solution for 20 minute complete the curing reaction and to produced spherical rigid microsphere. The microspheres were collected by decantation and washed thrice with distilled water and dried at 45^0 C for 12 h. The formulation details were given in Table No.1.

The preliminary study performed for microspheres formulation with 1:0.5, 1:1, 1:1.5 ratio for drug to polymer. It was found that 1:0.5 ratio for drug to polymer release the drug faster and 1:1.5 ratio for drug to polymer retard the release of drug, while 1:1 ratio for drug to polymer release the drug appropriately so, 1:1 ratio was decided for the final batches of microsphere formulations by using 5%, 10% calcium chloride as crosslinking agent. (8-10)

Table No. 1: Formulation Batches of TM- SA-TSP Microspheres.

Formulation Code	Drug Polymer Ratio	$CaCl_2$
F1	1:1	5%
F2	1:2	
F3	1:1	10%
F4	1:2	

Evaluation of Microspheres

The microsphere was formulated with above composition were evaluated for following micromeritics properties:

Angle of repose: [3, 11-16]

The angle of repose for the microsphere of each formulation was determined by the funnel method. The microsphere was allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface, this forms a pile of microspheres on the paper. The angle of repose was calculated by substituting the values of the base radius 'R' and pile height 'H' in the following equation.

$$\text{Tan } \theta = H / R$$

Where, H = pile height, R = radius of pile

$$\text{Therefore; } \theta = \tan\text{-}1 (H / R)$$

Bulk density and tapped density: [3, 11-16]

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2g of microsphere from each formula was lightly shaken to break agglomerates if any and then was introduced into a 10 ml-measuring cylinder. It was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2- second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulae.

LBD = weight of the microsphere / volume of the packing

TBD = weight of the microspheres / tapped volume of the packing

Compressibility index: [3, 11-16]

The compressibility indices of the formulation blends were determined using Carr's compressibility index formula.

Hausner's ratio: [3, 11-16]

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation.

Hausner's Ratio = Tapped density / Bulk density

Particle size analysis: [3, 11-16]

The particle size was measured using a stage micrometer, and the mean particle size was calculated by measuring 200 particles with the help of a calibrated stage micrometer. A small amount of dry microspheres was suspended in liquid paraffin (10 ml). A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and diameter of at least 100 particles was measured using a calibrated optical micrometer.

Percentage yield: [3, 15-16]

The percentage yield of different formulations was determined by weighing the microspheres after drying. The percentage yield was calculated as follows

% Yield = (Total weight of microspheres / Total weight of drug and polymer) X 100

Drug entrapment: [3, 15-16]

The various batches of the microspheres were subjected to estimation of drug content. The microspheres equivalent to 100 mg of Tegaserod, were accurately weighed and crushed. The powdered of microspheres were placed in 100 ml of methanol for overnight. This solution is then filtered through whatmann filter paper. After filtration, use this clear supernatant solution to measured absorbance at 291 nm by using UV-visible spectrophotometer. The percentage drug entrapment was calculated.

% Drug entrapment = (Calculated drug concentration / Theoretical drug concentration) X 100

Swelling behaviour study: [15-16]

Water uptake of the microspheres loaded with the drug was determined by measuring the extent of swelling of the matrix in pH 1.2 and phosphate buffer 7.4 solutions. The samples were allowed to swell in pH 1.2 buffer solution for 2 hr and then at pH 7.4 phosphate buffer for 10 hr. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to an accuracy of 0.01 mg using an electronic microbalance. The hydrogel microspheres were then dried in an oven at 50 ^{0}C for 5 hr until there was no change in the dried mass of the samples.

$$\% \text{ water uptake} = \frac{\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}}{\text{Mass of dry microspheres}} \times 100$$

In-vitro release studies: [14-17]

The *in-vitro* drug release studies of the microsphere formulation were carried out using USP dissolution test apparatus type-II [Electrolab (TDT-08L)]. Weighed amount of microspheres equivalent to 400 mg to the total weight of drug used in microsphere formulation, they were packed in muslin cloth and placed in the basket. The dissolution medium consisted of 900 ml of 0.1N HCL (pH 1.2) for the first 2 h, followed by pH 7.4 phosphate buffer for the remaining time period up to 12 h. The temperature of the medium was maintained at 37±0.5^{0}C. The speed of rotation of the basket was kept at 50 rpm. Aliquots of 10 ml were withdrawn after every hour for a total of 12 h. The samples so withdrawn were replaced with the fresh dissolution medium to maintain the sink condition throughout the experiment. The collected aliquots were diluted with suitable medium to determine the absorbance at 314 nm for microspheres by using U.V. visible spectrophotometer. The % cumulative release from the microspheres was noted.

RESULTS AND DISCUSSION

Compatibility study by FT-IR:

The below IR spectra shows the peaks of major functional groups, these peaks were nearly unchanged as compared in spectra of TM with SA-TSP. So, from the below spectra it can be concluded that there was no interaction between drugs and excipients used in formulation of microspheres. The characteristic frequencies of TM with SA-TSP were shown in Table No. 2.

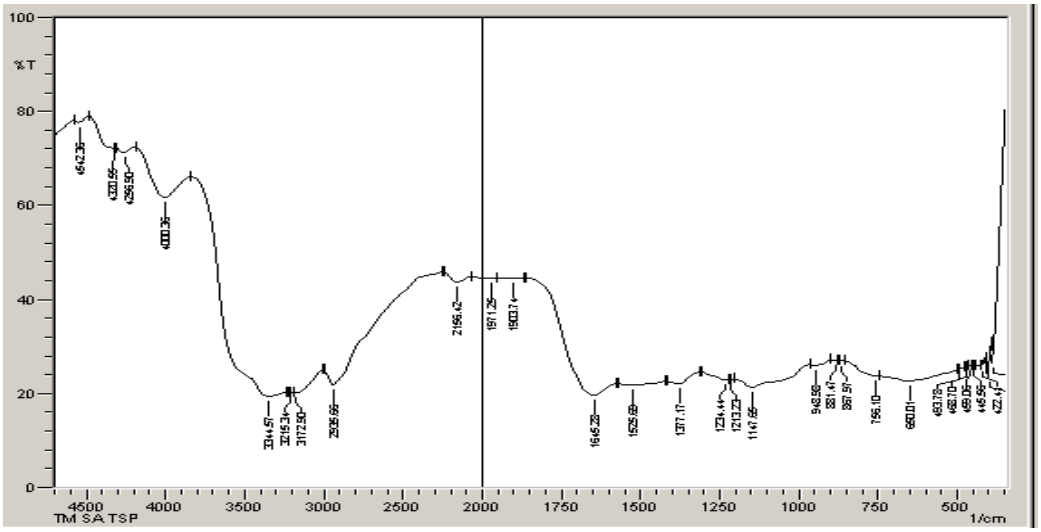

Figure No.1: IR Spectrum of TM-SA-TSP.

Table No. 2: Characteristic Frequencies in IR Spectrum of TM-SA-TSP Mixture.

Sr.No.	Wave No.(cm^{-1})	Infernces
1	3344	N-H stretching
2	2935	C-H stretching
3	1645	C=O stretching
4	1234-1377	C-N bending vibration

Angle of repose:

Angle of repose of TM-SA-TSP microspheres was determined by fixed funnel method. Angle repose of these microspheres was observed in range of 22°.38'- 23°.49' suggesting good flow properties of microspheres. (Table No.3)

Bulk density and tapped density:

Bulk and tapped density was determined by tapping method. The bulk density value of different batches of TM-SA-TSP microspheres was determined which was summarized in Table No.3

Carr's compressibility index:

The Carr's compressibility index values for microspheres were ranged between 12.96-13.36. The value less than 20 for all formulation suggested good flow property of microspheres. (Table No.3)

Hausner's ratio:

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density. It was in ranged from 1.183-1.226; i.e. all the formulation showed that they had excellent flow properties. (Table No.3)

Table No. 3: Properties of TM-SA-TSP microspheres.

Formulations	Parameter	Angle of repose (^{0})	Bulk density (g / ml)	Tapped density (g / ml)	Compressibility Index (%)	Hausner's ratio
		Mean ± SD (n=3)				
F1		22.65 ± 0.83	0.451 ± 0.004	0.580 ± 0.007	13.33 ±0.75	1.217 ±0.012
F2		22.38 ± 0.69	0.449 ± 0.008	0.556 ± 0.009	12.96 ±0.50	1.226 ±0.009
F3		23.49 ± 0.70	0.458 ± 0.01	0.578 ± 0.009	13.16 ±0.90	1.187 ±0.01
F4		23.36 ± 1.09	0.461 ± 0.006	0.555 ±0.0098	13.36 ±0.83	1.183 ±0.01

Particle size:

Particle size of different batches of microspheres was determined by stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was calculated and it was in the range between 715-747 µm. (Table No.4)

Percentage yield:

The percentage yield of different batches was determined by weighing the microspheres after drying. The percentage yields of different formulation of microspheres were in the range of 72.50-74.37 %. (Table No.4)

Drug entrapment efficiency:

The drug entrapment efficiency of different batches of microspheres was determined. The entrapment efficiency was in the range of 74.03-6.69%, as shown in Table No.4. Drug entrapment efficiency was increased when the crosslinking agent increases. 10% of $Cacl_2$ shows the maximum drug entrapment as compared with the 5% of $Cacl_2$.The drug entrapment efficiencies were increased with decreasing polymer blend ratios and increasing cross-linking concentrations. This may be due to the high degree of cross-linking.

Table No.4: Particle Size, Percentage Yield and Entrapment Efficiency of Different Batches of TM-SA-TSP Microsphere.

Formulations	Parameter	Mean particle size (µm)	Percentage yield (%)	Drug Entrapment efficiency (%)
		Mean ± SD (n=3)		
F1		715.66 ±0.51	72.50 ±1.11	76.69±1.30
F2		747.00 ±1.78	74.37 ±1.89	74.66±0.99
F3		725.33 ±1.36	72.76 ±1.89	74.91±0.98
F4		722.00 ±0.89	74.32 ±0.97	74.03±1.03

Swelling Studies:

Swelling behavior of TM loaded microspheres was evaluated in simulated gastric medium 0.1 N HCl (pH 1.2), and intestinal pH 7.4 (phosphate buffer 7.4). The swelling index profile of these microspheres in both the pH was shown in Figure No. 2. The swelling index of TM loaded microspheres was lower in 0.1N HCL in comparison with swelling index in phosphate buffer pH 7.4.

The results microsphere for swelling ability in phosphate buffer pH 7.4 shows better than at acidic medium. The formulation shows higher swelling ability at basic pH because of pH dependant properties of SA and hydrophilic properties of TSP. The calcium chloride cross linking agent was also affect the swelling ability i.e. higher degree of cross linking shows lower swelling ability while at lower degree of cross linking shows higher swelling ability of microsphere.

The swelling index of microspheres in 0.1 N HCL was found to be low because shrinkage of sodium alginate at acidic medium. This might help to avoid drug release at upper part of gastro intestine hence appropriate amount of drug can be deliver to colonic region.

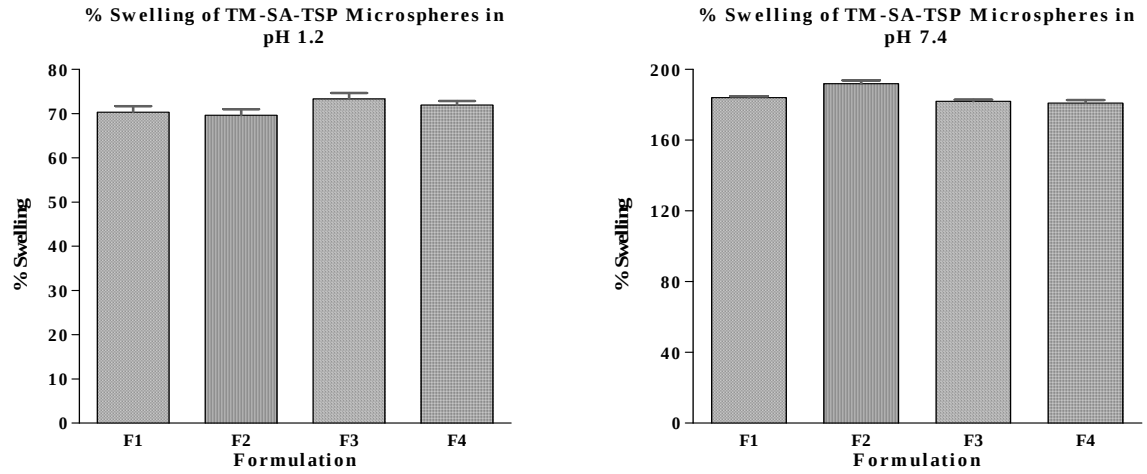

Figure No.2: Percent Water Uptake of TM-SA-TSP Microspheres.

In-vitro drug release study:

The in-vitro drug release study carried out of TM-alginate microspheres for first 2 h in 0.1N HCL and subsequently 10 h for pH 7.4 phosphate buffers to all F1-F4 formulated batches at 370 ± 0.50 C, 50 rpm.

The release of TM from SA-TSP microspheres was found 10.23 ± 0.98 (F1), 13.45 ± 0.37 (F2), 11.62 ± 0.38 (F3) and 14.01 ± 0.33 (F4) separately in 0.1N HCL after 2 h, while 94.98 ± 0.13 (F1), 82.45 ± 0.33 (F2), 88.52 ± 1.33 (F3) and 85.11 ± 0.76 (F4) separately in pH 7.4 phosphate buffer after 12 h. F1 and F3 shows the higher release of drug hence it was better formulation. These results of *in-vitro* drug release shown in Table No.5 and graphical representation shown in Figure No.3.

The release of Tegaserod from SA-TSP microspheres at gastric pH was comparatively slows than intestinal pH. This was due to the shrinkage of alginate at acidic pH (as alginate is pH sensitive), which might slower the drug release from sodium alginate microspheres. The reason of the higher drug release was due to lower the concentration of cross linking agent.

Table No. 5: *In-vitro* Dissolution Study of TM-SA-TSP Microspheres.

Time (hrs)	**Cumulative Drug Release (%) Mean ±SD (n=3)**			
	F1	**F2**	**F3**	**F4**
1	5.72 ± 0.23	7.45 ± 0.37	6.59 ± 0.31	7.39 ± 0.32
2	10.23 ± 0.98	13.45 ± 0.37	11.62 ± 0.38	14.01 ± 0.33
3	17.15 ± 0.96	20.35 ± 0.80	18.82 ± 0.51	23.39 ± 0.40
4	22.51 ± 0.49	27.86 ± 0.25	27.08 ± 0.51	31.42 ± 0.44
5	27.80 ± 0.23	36.39 ± 0.21	35.40 ± 1.13	37.26 ± 0.28
6	35.45 ± 0.58	42.46 ± 0.42	42.64 ± 0.33	43.77 ± 0.22
7	49.36 ± 0.47	49.59 ± 0.64	50.16 ± 0.53	50.71 ± 0.44
8	60.79 ± 0.18	58.00 ± 0.64	56.72 ± 0.39	57.9 ± 0.30
9	72.98 ± 0.81	63.05 ± 0.50	63.14 ± 0.67	64.77 ± 0.43
10	81.24 ± 0.85	68.36 ± 1.01	67.88 ± 0.31	71.10 ± 0.65
11	88.19 ± 0.59	77.29 ± 1.06	77.45 ± 0.59	77.72 ± 0.31
12	94.98 ± 0.13	82.45 ± 0.33	88.52 ± 1.33	85.11 ± 0.76

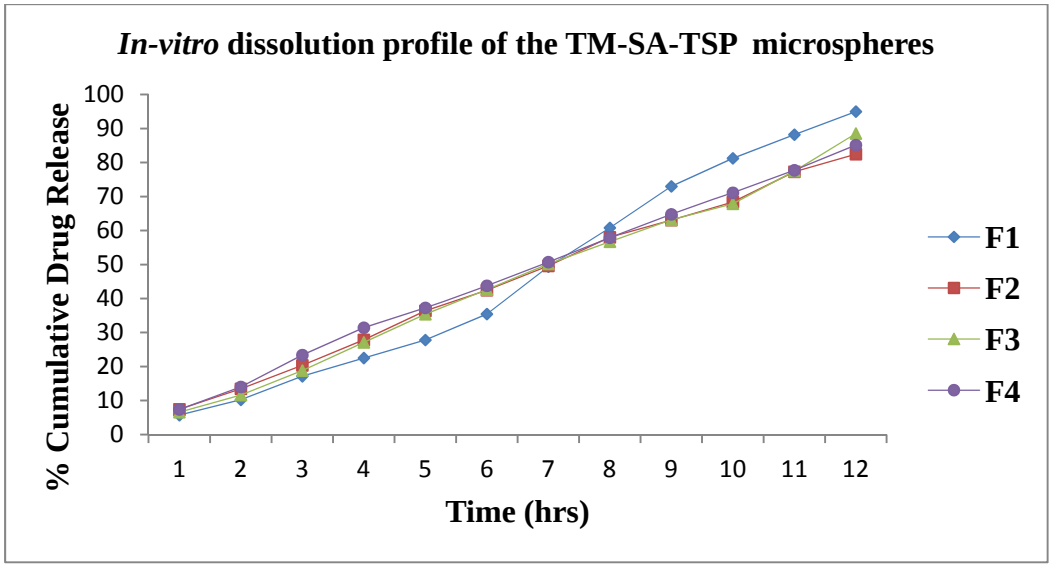

Figure No. 3: *In-vitro* Dissolution Rate Study of TM-SA-TSP Microspheres**.**

CONCLUSION

It can be concluded from the study that, among the prepared formulations with respect to entrapment efficiency, swelling studies and *in-vitro* drug release, the blend microspheres of sodium alginate and tamarind seed polysaccharide prepared by ionotropic gelation method found to be better. Therefore, tegaserod loaded SA-TSP microspheres are promising carrier for oral control release.

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