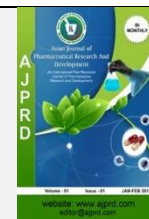


Available online on 15.06.2020 at <http://ajprd.com>

## Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

© 2013-20, publisher and licensee AJPRD, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited

Open  Access

Research Article

## Formulation Development and Evaluation of Gum Damar Based Sustained Release Matrix Tablet of Metoprolol Succinate

Nilima Thombre\*, Archana Aher, Pranali Shimpi

Department of Quality Assurance Techniques, M.E.T.'s Institute of Pharmacy, Bhujbal Knowledge City, Nasik, Maharashtra, India

### ABSTRACT

**Background:** An oral dosage form containing sustained released matrix tablet used the natural polymer in the replacement of synthetic polymer.**Objective:** To prepare sustained release matrix tablet using natural polymer damar & drug Metoprolol Succinate.

**Material and method:** Metoprolol Succinate using Gum Damar as release retardant were prepared by wet granulation technique using  $2^3$  factorial design with the quantity of gum damar, concentration of the microcrystalline cellulose & dicalcium phosphate (DCP) as variables. Matrix tablets were prepared using different strengths of GD (10% to 30% w/w) with respect to total tablet weight and two different excipients DCP & microcrystalline cellulose as a diluents. The physicochemical properties such as hardness, thickness, friability, uniformity of weight and the drug content of the formulated tablets were estimated. The physico-chemical properties gum Damar was evaluated for its oral toxicity. Acute oral toxicity study was carried out according to OECD guideline 425 in the albino mice and study revealed that gum was nontoxic.

**Result & discussion:** Developed sustained released matrix tablet possesses hardness of  $4.93 \pm 0.25$ , Thickness of  $4.74 \pm 0.15$ , Content Uniformity (%) of  $98.78 \pm 0.52$ , Weight variation of  $248.51 \pm 2.12$ , Friability (% w/w) of  $0.41 \pm 0.024$ , In vitro drug release was found to be  $95 \pm 0.84$  % in 12 hrs time, DSC thermogram study sharp endothermic peak was observed. The kinetics of release of Metoprolol Succinate was found diffusion. In vivo oral toxicity study showed no animal was died during study and it was observed that gum is nontoxic.

**Conclusion:** A systematic study revealed that by selecting a suitable composition of Damar gum & dicalcium phosphate as a diluent, the desired dissolution profile could be achieved. The mechanism of drug released was found to be diffusion.

**Keywords:-** Natural polymer, Metoprolol Succinate, Gum Damar, Sustained release matrix, Dicalcium phosphate.

**ARTICLE INFO:** Received 17 April 2020; Review Completed 04 May 2020; Accepted 10 June 2020; Available online 15 June. 2020**Cite this article as:**

Thombre N, Aher A, Shimpi P, Formulation Development and Evaluation of Gum Damar Based Sustained Release Matrix Tablet of Metoprolol Succinate, Asian Journal of Pharmaceutical Research and Development. 2020; 8(3):81-86.

DOI: <http://dx.doi.org/10.22270/ajprd.v8i3.752>**\*Address for Correspondence:**

Nilima Thombre, Department of Quality Assurance Techniques, M.E.T.'s Institute of Pharmacy, Bhujbal Knowledge City, Nasik, Maharashtra, India

### INTRODUCTION

In recent years, polymers those are derived from plant origin have evoked tremendous interest because of their diverse pharmaceutical applications such as diluents, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels, and bases in suppository. Due to advances in drug delivery technology, currently, excipients are included in novel dosage forms to fulfil specific functions and in some cases they directly or indirectly influence the extent and/or rate of drug release and drug absorption. Recent trends towards use of plant based and natural products demand the replacement of synthetic additives with natural ones. Today, the whole world is increasingly interested in natural

drugs and excipients. These natural materials have many advantages over synthetic ones as they are chemically inert, nontoxic, less expensive, biodegradable, and widely available.<sup>1</sup> Gums and mucilages are interesting polymer for the preparation of pharmaceutical formulations, because of their high water-swelling, non-toxicity, low cost and free availability. Gums and mucilages are polysaccharides or complex carbohydrate containing one or more monosaccharides or their derivatives linked in bewildering variety of linkages and structures.<sup>2</sup> Gums and mucilages find applications in tablet formulation as binders because of their adhesive nature. They impart cohesiveness to the powder mass and convert them into granules, which are further compressed into tablets.

They can also be used as disintegrates in tablets<sup>3</sup>. Different mucilages have been used as binding agent in pharmaceutical formulations. Mucilage has good binding properties as compared to many synthetic compounds<sup>4</sup>. Among various dosage forms, matrix tablets are widely accepted for oral sustained release as they are simple and easy to formulate. Matrix system is the specific type of release system, which prolongs and controls the release of drug that is dissolved or dispersed. Making drug-embedded matrix tablets through the direct compression of a blend of drug, retardant material and additives is one of the simplest formulation approaches. The inclusion of polymeric materials in a matrix system is a common method of modulating drug release. Various natural gums and mucilages have been examined as polymer for sustained release formulations. The use of natural polymers and their semi-synthetic derivative in drug delivery continues to be an area of active research. Drug-release retarding polymers are the key performers in matrix systems. Various polymers have been investigated as drug retarding agents, each presenting a different approach to the matrix system. Based on the features of the retarding polymer, matrix systems are usually classified into three main groups: hydrophilic, hydrophobic and plastic.<sup>5</sup>

To reduce the frequency of administration and to improve the patient compliance, a sustained release formulation of Metoprolol Succinate is desirable. Metoprolol Succinate has short biological half-life (about 3 to 4hrs) and dosing frequency of tablet more than one per day make Metoprolol Succinate an ideal candidate for development of sustained release dosage form. To improve therapeutic efficacy, patient compliance and better utilization of Metoprolol

Succinate will be beneficial for formulation. The present study was deals with formulation and evaluation of oral drug delivery system for using suitable materials and methods.<sup>6</sup>

## MATERIAL & METHODS

### Collection of materials

Metoprolol succinate was obtained from Universal Medicament Nagpur, India. Microcrystalline cellulose (MCC, Avicel pH 101) and dibasic calcium phosphate were purchased from S. D. Fine Chem. Labs. (Mumbai, India), gum damar were received as a gift sample from Imex Inc. (Chennai, India). All other ingredients used throughout the study were of analytical grade and were used as received.

### Wet granulation method :

Matrix tablets, metoprolol succinate were prepared by a conventional non-aqueous wet granulation technique. In each formulation, the amount of the active ingredient is 100 mg and the total weight of a tablet is 250 mg. The ingredients were passed through a 60-mesh sieve. A blend of all ingredients except glidant and lubricant was mixed, a particular attention had been given to ensure thorough mixing and phase homogenization. Granulation was done manually with a solution of isopropyl alcohol. The wet masses were passed through a 26 mesh sieve and the wet granules produced were first air dried for 10 min and after lubrication with magnesium stearate. Tablets were prepared using 7.5 mm concave punch of the tablet compression machine (Karnavati, rotary compression machine); compression force was kept constant for all formulation.<sup>6,7</sup>

**Table 1:** The formula for the preparation of the Metoprolol succinate tablet

Pharmaceutical Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
Metoprolol Succinate	100	100	100	100	100	100	100	100
Gum Damar	25	50	62.5	75	25	50	62.5	75
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Microcrystline cellulose	-	-	-	-	120	95	82.5	70
Dicalcium Phosphate	120	95	82.5	70	-	-	-	-

### Evaluation of tablets:

The prepared tablet were evaluated for Hardness, Thickness, Friability, Weight variation, Drug content, Fourier transform infrared spectroscopy analysis, Differential scanning calorimetric analysis, In vitro drug released study, In vivo oral toxicity study, Stability study.

### Hardness:

The hardness of 6 tablets was determined using the Monsanto hardness tester. The tablet was held along its diametrical axis in between the two jaws of the tester. At this point, reading should be zero kg/cm<sup>2</sup>. Then constant force was applied by rotating the knob until the tablet fractured. The value at this point was noted.<sup>8,17</sup>

### Thickness:

The thickness of the tablets was determined using a Dial caliper (Advance). Three tablets from each type of formulation were used and average values were calculated. It is expressed in mm.<sup>9,18</sup>

### Friability:

Roche friabilator (Meta lab) was used to determine friability of the tablet. A sample of preweighed 6 tablets was placed in Roche friabilator which was then operated for 100 revolutions i.e. 4 minutes. The tablets were then dusted and reweighed. A loss of less than 1 % in weight is generally considered acceptable. Percent friability (% F) was calculated as follows.<sup>9,16</sup>

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \quad \text{-----(1)}$$

### Weight variation test:

To find out weight variation, 20 tablets of each type of formulation were weighed individually using an electronic balance, average weight was calculated and individual tablet weight was then compared with average value to find the deviation in weight.<sup>13</sup>

#### UNIFORMITY OF DRUG CONTENT:<sup>13</sup>

Twenty tablets were weighed and crushed in mortar and powder equivalent to 10 mg of Metoprolol Succinate was weighed and dissolved in 100 ml of phosphate buffer (pH 6.8). This was the stock solution from which 1 ml sample was withdrawn and diluted to 10 ml with phosphate buffer (pH 6.8). The absorbance was measured at wavelength 222 nm using double beam UV-Visible spectrophotometer. Content uniformity was calculated using formula

$$\% \text{ Purity} = \frac{\text{Conc. of unknown}}{\text{Conc. of standard}} \times 100 \quad \text{---(2)}$$

#### Fourier transform infrared spectroscopy analysis:<sup>11</sup>

The infrared spectra of metoprolol succinate pure drug, optimized formulation were recorded between 400 to 4000 cm<sup>-1</sup> on FTIR. The IR spectra for the test samples were obtained using KBr disk method using an FTIR Spectrometer (Shimadzu, Japan).

#### Differential Scanning Calorimetry (DSC) Analysis:<sup>12,15</sup>

Thermogram of Metoprolol Succinate was obtained using mettler T100 by Zurich, Switzerland Differential Scanning Calorimeter using aluminum pans. Nitrogen was purged through cooling unit. Indium standard was used to calibrate the DSC temperature. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min, over a temperature range of 0 to 400°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 100 mL/min.

#### In vitro drug release study:<sup>9,14</sup>

The *in-vitro* drug release was determined using USP dissolution testing apparatus type-II (paddle type). The

dissolution test was performed using 900 mL of 0.1 N HCl for 2 hrs and then in phosphate buffer (pH 6.8), at 37 °C ± 0.5 °C and 30 rpm. Sample volume of 10 mL was withdrawn at regular time intervals. The volume withdrawn was replaced by fresh volume of dissolution medium to maintain constant volume of medium. The filtered samples were analyzed spectrophotometrically at 222 nm using 0.1 N HCl and phosphate buffer (pH 6.8) as a blank. Drug content in dissolution sample was determined by calibration curve.

The release data were fitted to various mathematical models as under to know which model is best fitting the obtained release profile. Zero order release kinetics, Higuchi model

#### *In vivo* oral toxicity study

Oral toxicity study was performed on albino mice according to OECD guideline 425. The temperature in the experimental animal room was 22 °C (± 3°C). Although the relative humidity was 30 % and preferably not exceed 70 % The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.<sup>19</sup>

#### Stability study<sup>10</sup>

The optimized formulation was subjected to evaluate the effect of temperature and humidity in environmental stability chamber maintained at 40 °C ± 2 °C/75% ± 5% RH for 30 days. The stability study was performed at 40 °C ± 2 °C and 75 % ± 5 % RH for 30 days.

## RESULT & DISCUSSION:

#### Pre-compression parameter:

Bulk density of all batches were ranged from 0.39 to 0.4194 (g/mL), tapped density ranged from 0.46 to 0.488 (g/mL). Carr's index ranged from 14.12% to 15.89%, angle of repose ranged from 28.53° to 29.47° and the Hausner's ratio was found in the range of 1.16 – 1.19. All these results indicated that, the granules showed good flow properties (Table no - 02)

Table: 2 Pre-compression parameters of the formulation F1-F8

Batch code	Bulk density* (g/mL)	Tapped density* (g/mL)	Angle of repose* (°)	Carr's Index* (%)	Hausner's ratio*
F1	0.4089±0.0030	0.4788±0.017	29.63±0.49	14.14±0.39	1.18±0.01
F2	0.3952±0.0043	0.4615±0.014	29.10±0.47	14.12±0.22	1.19±0.015
F3	0.3984±0.0028	0.4654±0.019	28.87±0.36	14.37±0.01	1.16±0.04
F4	0.4194±0.0042	0.4972±0.009	28.73±0.44	15.27±0.34	1.19±0.020
F5	0.4102±0.0037	0.4807±0.006	28.09±0.83	14.85±0.29	1.16±0.011
F6	0.4104±0.005	0.4881±0.008	28.36±0.45	15.89±0.25	1.19±0.010
F7	0.4106±0.0051	0.4857±0.004	28.52±0.52	15.42±0.03	1.17±0.005
F8	0.3971±0.006	0.4716±0.013	29.47±0.56	15.60±0.16	1.19±0.019

(\* mean of three values ± SD)

#### Post compression parameter

The thickness of all batches was found to be 4.02 to 4.23 mm and the hardness of all batches was found to be 4.53 to 5.16 kg/cm<sup>2</sup>. All the tablets showed % friability in the range

of 0.299 to 0.843 % which was within the limit. drug content uniformity were ranged from 97.18 ± 1.31 % to 99.64 ± 1.78 % .No variations in weight of the tablets as all tablets were found to be within the range limit for weight variation. (Table no - 03)



**Table: 3** Post formulation parameter of the formulation F1- F8

Batch No.	Weight variation	Thickness (mm)*	Hardness (kg/cm <sup>2</sup> )	Friability (%)	Content Uniformity (%)
F1	247.36±2.03	4.18 ± 0.13	4.9 ± 0.45	0.68 ± 0.041	97.6±0.99
F2	249.6 ± 1.72	4.02± 0.23	4.93 ± 0.41	0.299 ± 0.051	97.26±0.46
F3	249.72±0.82	4.12±0.15	5.13±0.17	0.652±0.034	98.64±0.240
F4	250.06± 1.10	4.16± 0.27	5.16 ± 0.35	0.443 ± 0.020	98.78±0.55
F5	250.16 ± 1.98	4.21 ± 0.064	4.66 ± 0.11	0.753 ± 0.035	97.09±0.88
F6	250.52± 1.58	4.23 ± 0.27	4.6± 0.2	0.578 ± 0.032	97.47±0.35
F7	249.88±1.87	4.16±0.12	4.83±0.072	0.612±0.043	98.4±0.13
F8	250.77 ± 1.09	4.31 ± 0.062	4.53 ± 0.057	0.843 ± 0.056	99.01±0.36

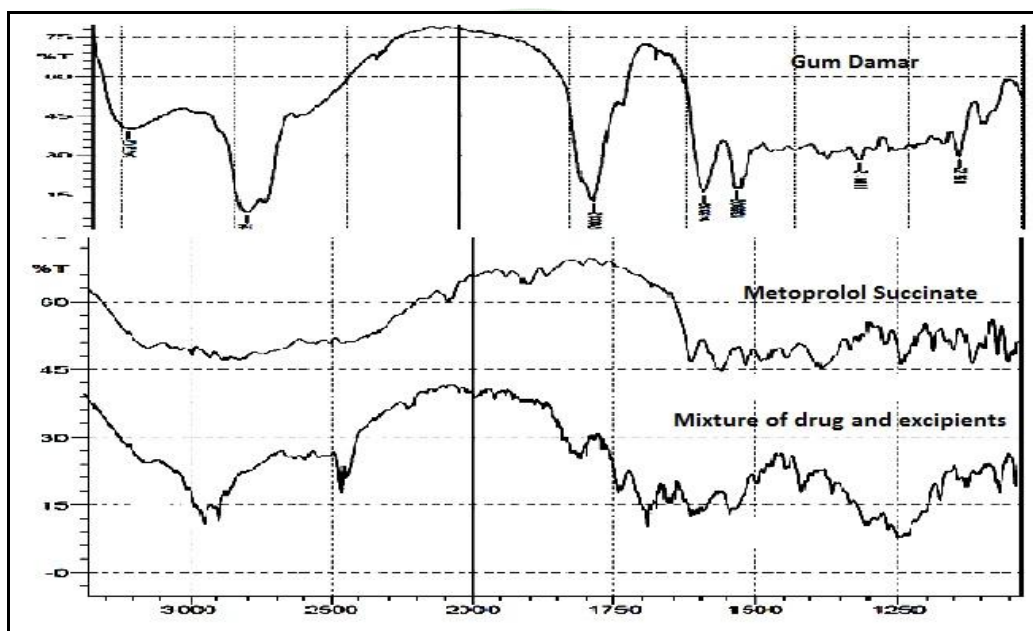
(\* mean of three values ± SD)

### Drug and excipients compatibility study

#### Fourier Transform Infrared Spectroscopy (FTIR)

To assess compatibility study Metoprolol Succinate and excipients mixtures were placed at temperature 40 °C and 75 % RH for one month in 1:1 proportion. On the basis of FTIR spectra it seems there were no possible interaction of Metoprolol Succinate and excipients. The below FTIR spectra showed the peaks of major functional groups of drug. These peaks are nearly unchanged as compared to

spectrum of mixture of drug along with excipients and pure drug. Hence, it can be concluded that there was no interaction between drug, diluents and the polymer used in the formulation. The IR spectra of drug and mixture showed the following characteristic features; broad band at N-H stretch (3500-3100), C-H Stretch (3000-2800), C=O Aldehyde (1740-1720), N-H bend (1640-1550), Aromatic C=C stretching (1600-1475), C-O stretching of alkyl ether (1300-1000). (Figure 1).

**Figure: 1** FTIR spectrum of a) Metoprolol succinate b) damar gum c) Metoprolol succinate & Mixture

#### Differential Scanning Calorimetry (DSC)

A sharp endothermic peak was observed at 128.93 °C. The endothermic obtained at 10 °C /min heating rate. 128.93°C was in close agreement with literature reports for the melting point of Metoprolol Succinate. Hence it can be concluded that there was no interaction between drug and excipients. (Figure 2)

#### In-vitro drug dissolution study:

The release profiles of Metoprolol Succinate from the different formulations were shown in Figures 3 and Table 4. The data clearly indicate that Metoprolol Succinate release can effectively be extended by varying the concentration of polymer. From the above results it was

found to be that the conc. of Damar gum increased in tablets from 10 to 30 % there were decreased in the release of Metoprolol Succinate. Formulation F1, F2, F3, F5, F6, F7, F8 batches shown drug released within 12 hrs and formulation F4 shown drug release upto 12 hrs. Formulation F4 with Damar gum 30% was found to be most promising formulation as it shows sustained release (98.27%) as well as maintained excellent matrix integrity during the period of 12 hrs study. Formulation F4 was selected as the best optimized formulation.

The dissolution data obtained was treated as per the various kinetic models to get release kinetic and results of the analysis have been presented in table 4. The best fit model representing the mechanism of drug release from the matrices was slow zero order and Higuchi.

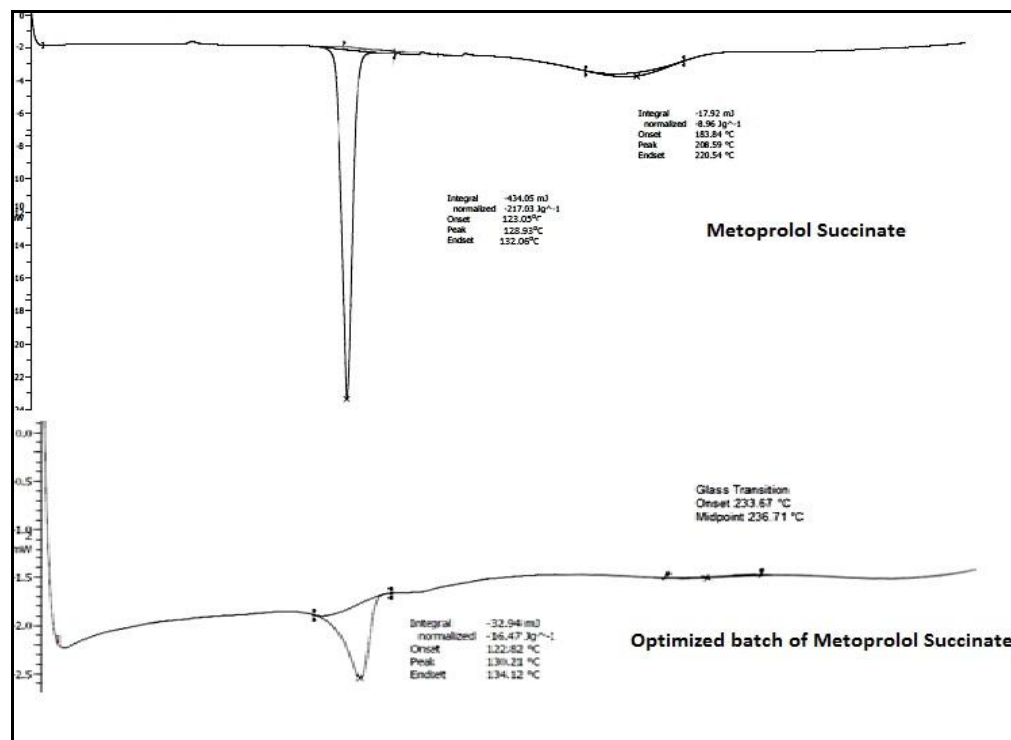


Figure: 2 DSC Thermogram of Metoprolol Succinate and Optimized batch

#### Dissolution Data Treatment:

The dissolution data obtained was treated as per the various kinetic models to get release kinetic and results of the analysis have been presented in Table 4 .

Table: 4 The Dissolution Models for Matrix Tablets (F1-F8) of Metoprolol Succinate

Formulation code	R <sup>2</sup>				
	Zero order	First order	Hixson Crowell	Higuchi	Korsmeyer peppas
F1	<b>0.988</b>	0.881	0.945	<b>0.954</b>	0.677
F2	<b>0.993</b>	0.914	0.966	<b>0.962</b>	0.858
F3	<b>0.992</b>	0.888	0.953	<b>0.967</b>	0.892
F4	<b>0.996</b>	0.874	0.947	<b>0.984</b>	0.853
F5	<b>0.99</b>	0.877	0.936	<b>0.956</b>	0.871
F6	<b>0.992</b>	0.934	0.977	<b>0.966</b>	0.970
F7	<b>0.989</b>	0.926	0.949	<b>0.961</b>	0.866
F8	<b>0.995</b>	0.889	0.964	<b>0.969</b>	0.890

#### In vivo oral toxicity study

Several doses was given in the range of 2000-5000 mg/kg. Animals were observed for mortality for 48 hrs. No animal was died during study and it was concluded that gum is nontoxic.<sup>19</sup>

#### Stability Study

The stability study was performed at  $40^{\circ} \pm 2^{\circ} \text{C}$  and  $75 \% \pm 5 \% \text{ RH}$  for 30 days.

The results are listed in Table 5.

Table: 5 Effect of Temperature and Humidity

Parameters	0 days	30 days
Appearance	White coloured, Smooth	White coloured, Smooth
Hardness (kg/cm <sup>2</sup> )	4.93 $\pm$ 0.25	5.12 $\pm$ 0.24
Drug Content (%)	98.78 $\pm$ 0.52	98.31 $\pm$ 0.12
Drug Release(at 12 hrs)	98.27 $\pm$ 0.84	97.04 $\pm$ 0. 41

From the above tabulated results it can be concluded that there was no significant physical and chemical changes in the optimized formulation after one month and it suggest

tablets were stable at harsh conditions such as  $40^{\circ} \pm 2^{\circ} \text{C}$  and  $75 \% \pm 5 \% \text{ RH}$ .

## CONCLUSION:

Metoprolol Succinate is  $\beta$ -adrenergic blocking agent drug used mainly to treat high blood pressure (Hypertention). FT-IR and DSC studies revealed that there was no chemical interaction between drug and polymer. An attempt was made successfully to prepare sustained released matrix tablets of Metoprolol Succinate using natural polymer i.e. Damar gum, the release retarding material and dicalcium phosphate and microcrystalline cellulose as diluents. The presence of Damar gum and dicalcium phosphate in the matrix will modulate the drug release to an acceptable pharmacokinetic profile. The main role of Damar gum was to sustain the drug release for longer time due to its strong binding property as well as hydrophobicity. A systematic study revealed that by selecting a suitable composition of Damar gum and Dicalcium phosphate as a diluent, the desired dissolution profile could be achieved. The optimized formulation gives best result in terms of the %

drug release and control of initial release along with sustained the drug release up to 12 hours. Formulation follows zero order kinetic model and Higuchi and the mechanism of drug release was found to be diffusion. No significant change was observed in percent drug release before and after stability studies for three month. Thus, the natural polymer can be used for formulation of sustained drug delivery systems can significantly be facilitated to avoid cost-intensive.

## ACKNOLEGEMENT:

Authors are thankful to MET's Bhujbal Knowledge City, Institute of Pharmacy, Adgaon, Nasik, Maharashtra, India for providing necessary facilities and funds to carry out this work.

**CONFLICT OF INTEREST:** The authors declare no conflict of interest.

## REFERENCE

1. Jani G. K. and Shah D. P. Gums and mucilages: versatile excipients for pharmaceutical formulations, *Asian Journal of Pharmaceutical Sciences*.2009; 4(5):309–323.
2. Avchat AM *et.al*. Recent Investigations of plant based gums, mucilages and resins in novel drug systems. *Indian Journal of Pharmaceutical Education and Research*.2011; 45(1):86-97.
3. Bharat W *et.al* Gums And Mucilages: excipients for modified drug delivery system. *Journal of Advanced Pharmacy Education & Research* 2013;3(4):359-363
4. Asija R *et.al*. Sustained Released Drug Technology: A Review, *International Journal of Research in Pharmacy and Science*,2012; 2(4):1-13
5. Bhargava A. *et.al* Oral Sustained Release Dosage Form: an opportunity to prolong the release of drug. *International journal advanced research in pharmaceutical and bio science*.2013; 3(1):7-14.
6. Sharma VJ. *et.al*. Design and Optimization of Extended Release Metoprolol Succinate Formulation using Melt Granulation Technique *International Journal of Pharmacy and Pharmaceutical Sciences*, 2013;5(3):230-238.
7. Wadher K.J. Formulation and Evaluation of a Sustained-Release Tablets of Metformin Hydrochloride Using Hydrophilic Synthetic and Hydrophobic Natural Polymers, *Indian Journal of Pharmaceutical Sciences*, 2011; 208-214
8. Lachman L, Lieberman HA, Kanig JL. The theory and practice of industrial pharmacy. 3rd ed. Mumbai: Varghese Publishing House; 1987. p. 430-456.
9. Aulton M.E. Aulton's Pharmaceutics-The Design and Manufacture of Medicines, Published by Churchill Livingstone Elsevier, 2007.p. 441-482.
10. Brahmarkar D.M, Jaiswal S.B. Biopharmaceutics and Pharmacokinetics, Published by Vallabh Prakashan, Delhi, 2009.p. 399-401.
11. Remington: The Science and Practice of Pharmacy, Published by Wolter Kluwer Health, India, 2006.p. 939-964.
12. The United States Pharmacopeia 31 National Formulary Asian Edition (2006), The United States Pharmacopoeial Convention. 2006.p. 1418-1419.
13. The Indian Pharmacopoeia . Government of India, Ministry of Health and Family Welfare. Published by the Indian Pharmacopoeia Commission, Ghaziabad. 2007.p. 177-183,242,269.
14. Yadav I.K. Formulation, evaluation and optimization of aceclofenac sustained release matrix tablets, of *International Journal PharmTech Research*, 2010; 2(1):592-98
15. Vyas S.P, Khar R.K. Controlled Drug Delivery: Concept and Advances, Delhi, 2012; (2):1-53.
16. Savant S.V. Extended Release Formulation of Metoprolol Succinate Using Ion Exchange Technology, *American Journal of PharmTech Research*, 2012; 2(2):640-651
17. Hemnani M., Patel U. P., Patel G., Daslaniya D., Shah A, Bhimani B. Matrix Tablets: A Tool of Controlled Drug Delivery, *American Journal of Pharmatech Research*, 2011; 1(4):127-143.
18. Nellore R.V. Development of metoprolol tartrate extended-release matrix tablet formulations for regulatory policy consideration, *Journal of Controlled Release*, 1998; 50:247–256.
19. OECD guideline 425(2001) Guidance Document on Acute Oral Toxicity. Up and down procedure.