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Research Article

Timolol Maleate Microspheres: An Ingenious Carrier for Sustained Release Antihypertensive Formulation

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Abstract

Background: Timolol maleate is classified as a BCS Class I drug and functions as a non-selective β-adrenergic receptor blocker. Its ability to lower heart rate and cardiac output has led to its widespread use in the treatment of hypertension.

Objective: Timolol maleate has a short half-life and is rapidly cleared from the body, which limits its therapeutic effectiveness, requiring frequent dosing and potentially affecting patient adherence. To overcome these challenges, sustained-release microspheres of Timolol maleate were developed using the ion gelation method.

Method: The ion gelation technique was employed to create the microspheres due to its various advantages, including ease of use, scalability and gentle processing conditions.

Results: All batches exhibited a comparatively lower swelling index in 0.1M HCl (pH 1.2) than in SIF (pH 6.8). It was observed that increasing the concentration of sodium alginate resulted in higher drug content. The microspheres were sized between 400 and 900 µm and demonstrated excellent flow characteristics. An optimized batch achieved an entrapment efficiency of 88.83% and released 92.15% of the drug over 7 hrs. Furthermore, stability studies conducted according to ICH Q1A(R2) for 3 months at 5±3°C and 25±2°C/60±5% RH indicated no significant changes in evaluation parameters.

Conclusion: The optimized Timolol maleate-loaded microspheres effectively provided sustained drug release through the membrane over 7 hrs. This study contributes to the development of improved drug delivery systems for better hypertension management, addressing the unmet needs in patient compliance.

Keywords: Timolol Maleate, Microspheres, Ion gelation method, Sodium alginate, Calcium chloride, Sustained release, Hypertension

INTRODUCTION

About 82% of the world's population has hypertension, with a majority residing in low- and middle-income countries. In India, it is estimated that roughly 220 million adults are impacted by this condition.[1]

Hypertension, often called high blood pressure, is a medical condition defined by consistently high blood pressure levels. At this time, most antihypertensive medications are provided in conventional dosage forms.[2] Challenges associated with conventional dosage forms, such as patient non-compliance, frequent dosing and complex dosing regimens, hinder their effectiveness.[3] To tackle the issues associated with traditional dosage forms, alternatives like sustained-release medications have been developed. Creating sustained-release drug delivery systems for these medications is essential for enhancing treatment efficacy and maximizing benefits. One approach involves using polymeric microspheres as carriers for sustained drug delivery.[4]

Timolol maleate (TM) is recognized as a BCS Class I drug and is frequently used as a non-selective β-adrenergic receptor blocker, primarily for the treatment of glaucoma, migraine and hypertension.[5] Timolol maleate (TM) works by diminishing the positive chronotropic, positive inotropic, bronchodilator and vasodilator effects initiated by β-adrenergic receptor agonists. For managing hypertension, the usual initial dose of Timolol maleate (TM) for oral use is generally 10 mg, administered once or twice a day. The dosage may be adjusted based on individual response, but the maximum recommended daily intake is typically 40 mg.[6]

Timolol maleate (TM) possesses a half-life of 4 to 5 hrs and exhibits a bioavailability of 60% upon oral administration.[7] Due to its short half-life and bioavailability, Timolol maleate (TM) is an ideal candidate for sustained-release formulations aimed at enhancing patient compliance and minimizing adverse effects. Among various delivery systems, microspheres are a promising choice because of their capacity for

sustained drug release and the potential for prolonged therapeutic benefits.[8]

Microspheres are multiparticulate delivery systems designed for sustained drug release, enhancing drug bioavailability, stability and targeting to specific sites. Sodium alginate, a naturally occurring non-toxic polysaccharide derived from brown algae, is commonly used in drug delivery applications. This linear copolymer features a polysaccharide backbone. Sodium alginate can undergo cross-linking through external gelation methods, enabling the alginate-drug solution to be extruded into microsphere droplets within a $CaCl_2$ solution. Gelling takes place when divalent cations facilitate interchain bonding, creating a three-dimensional network that forms a gel. This study aims to formulate and assess Timolol maleate (TM) loaded sustained-release microspheres using the ion gelation method, with sodium alginate as the polymer and calcium chloride as the cross-linking agent. This approach provides precise control over the characteristics of the microspheres, including their size, drug loading capacity and release kinetics.[9]

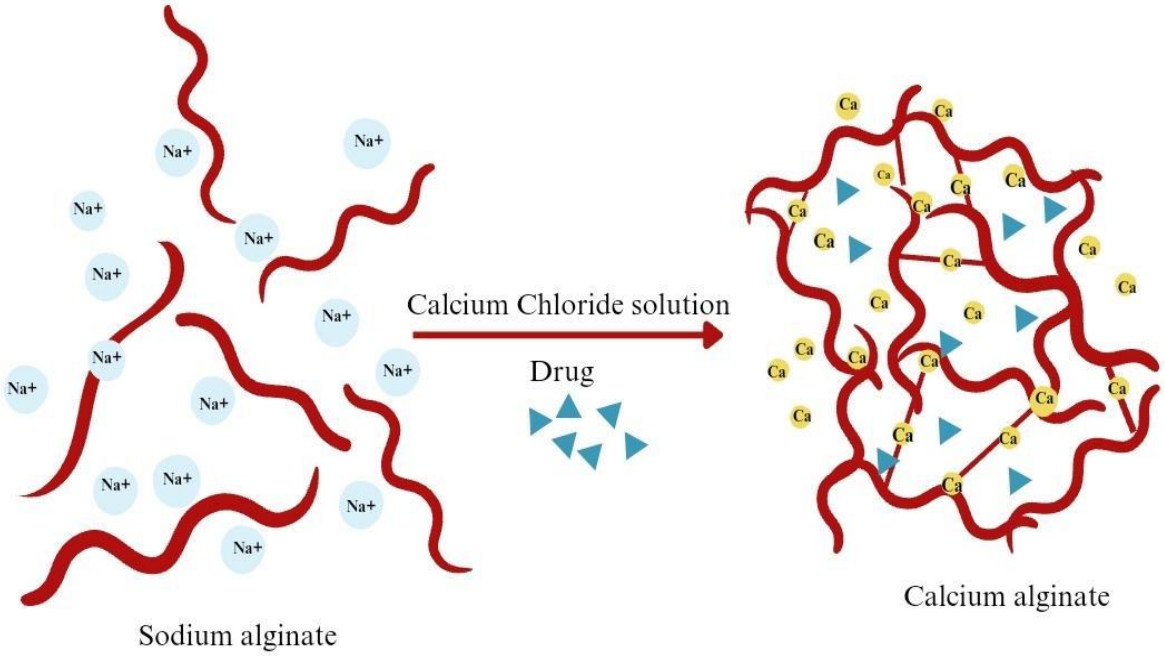

Figure 1: Mechanism of cross linking of Sodium alginate with $CaCl_2$

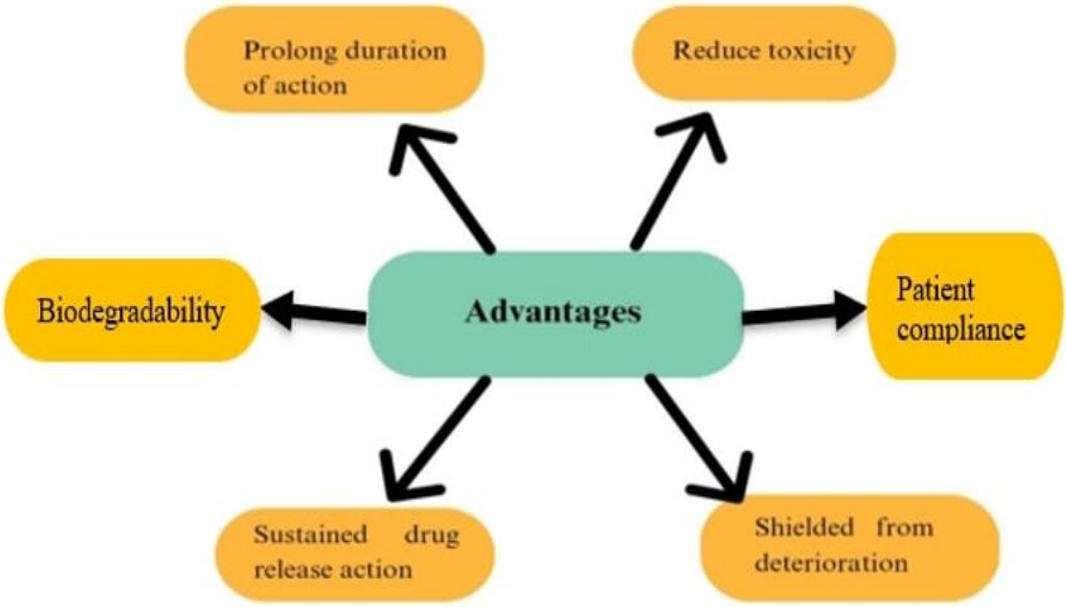

Figure 2: Advantages of Microsphere formulation

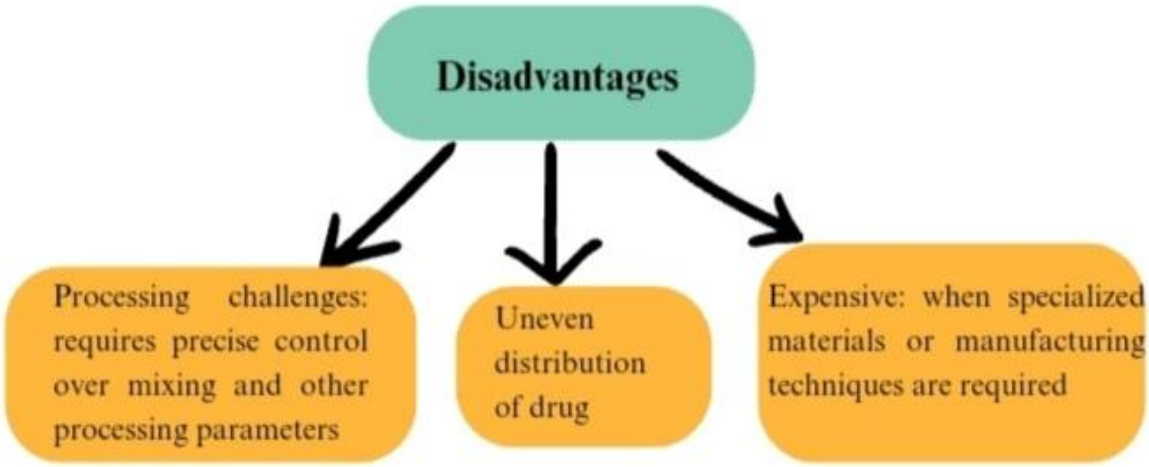

Figure 3: Disadvantages of Microsphere formulation

MATERIALS AND METHODS

Timolol Maleate (TM) was procured from Flax Laboratories Private Limited, Maharashtra and Calcium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate & Sodium lauryl sulphate were procured from S.D Fine Chemicals, Maharashtra. Sodium alginate, hydrochloric acid was procured from Loba chemie Private Limited, Maharashtra

ANALYTICAL METHOD DEVELOPMENT OF TIMOLOL MALEATE (TM):

Standard plot of Timolol Maleate (TM) in 0.1M HCl (pH 1.2)

10 mg of Timolol maleate (TM) was accurately weighed on a milligram balance and transferred into a 10 mL volumetric flask. The volume was then adjusted to 10 mL with 0.1M HCl (pH 1.2), resulting in a stock solution concentration of 1 mg/mL. After removing 10 mL from this stock solution, it was transferred to a 100 mL volumetric flask and the volume was made up with 0.1M HCl (pH 1.2). The concentration of this second stock solution was determined to be 100 µg/mL. The stock solution was diluted with 0.1M HCl (pH 1.2) and seven solutions with concentrations ranging from 10 to 40 µg/mL were prepared for analysis. All seven solutions were made in triplicate. These solutions were analyzed at a fixed wavelength of 295 nm and the absorbance was recorded against 0.1M HCl (pH 1.2) as a blank. A graph of mean absorbance versus concentration (µg/mL) was plotted.

Standard plot of Timolol Maleate (TM) in Simulated Intestinal Fluid (SIF) (pH 6.8)

10 mg of Timolol maleate (TM) was accurately weighed on a milligram balance and transferred into a 10 mL volumetric flask. The volume was then adjusted with Simulated Intestinal Fluid (SIF) (pH 6.8), resulting in a stock solution concentration of 1 mg/mL. From this stock solution, 10 mL was withdrawn and transferred to a 100 mL volumetric flask and the volume was made up with Simulated Intestinal Fluid (SIF) (pH 6.8). The concentration of this second stock solution was 100 µg/mL. The stock solution was diluted with Simulated Intestinal Fluid (SIF) (pH 6.8) and seven solutions with concentrations ranging from 10 to 40 µg/mL were prepared for analysis. All seven solutions were made in triplicate. These solutions were analyzed at a fixed wavelength of 295 nm and the absorbance was recorded against Simulated Intestinal Fluid (SIF) (pH 6.8) as a blank. The mean absorbance for all solutions was calculated and used for further calculations. A graph of mean absorbance versus concentration (µg/mL) was plotted.

Standard plot of Timolol Maleate (TM) in Phosphate Buffer Saline (pH 7.4)

10 mg of Timolol maleate (TM) was accurately weighed and dissolved in 10 mL of phosphate buffer saline (pH 7.4) to generate a stock solution with a concentration of 1 mg/mL. The stock solution (10 mL) was further diluted to 100 mL to produce a standard solution with a concentration of 100 µg/mL. The standard solution was serially diluted with phosphate buffer saline (pH 7.4) to obtain working standard solutions with concentrations ranging from 10 to 40 µg/mL. Using a UV-visible spectrophotometer, the absorbance of the solutions was measured at 295 nm against phosphate buffer saline (pH 7.4) as a blank. A graph of mean absorbance versus concentration (µg/mL) was plotted.

PREPARATION OF TIMOLOL MALEATE (TM) LOADED MICROSPHERES:

The ion gelation method was selected for this investigation due to its simplicity, high yield, and suitability for the current laboratory environment.[10] Timolol maleate (TM) was dissolved in distilled water, while sodium alginate was incorporated into the drug dispersion. The resulting polymeric solution was then added dropwise to a crosslinking solution consisting of a 5% (w/v) aqueous calcium chloride solution, using a 26G needle and stirring at 100 rpm. The mixture was allowed to stand in the crosslinking solution for 20 minutes. To eliminate excess crosslinking agents, the microspheres were filtered and rinsed with distilled water, after which they were permitted to air dry. Different batches were prepared by varying the concentrations of sodium alginate and calcium chloride to investigate changes in their properties.[11]

Table 1: Formulation of Timolol Maleate (TM) loaded sustained-release microspheres

Sr. No.	Batch Code	Sodium alginate (%w/v)	Drug (mg)	Calcium Chloride (%w/v)
1	F1	1%	20	5%
2	F2	1.5%	20	5%
3	F3	2%	20	5%
4	F4	2%	20	3%

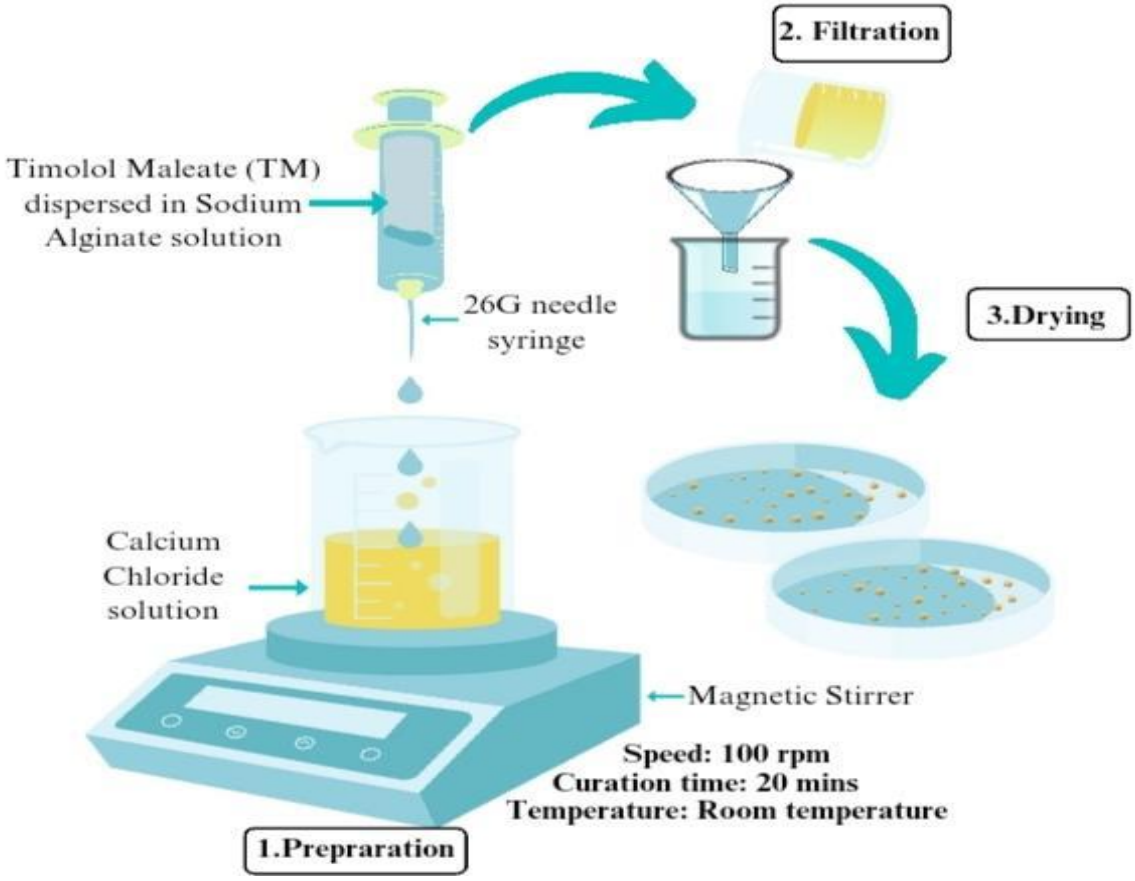

Figure 4: Preparation of Timolol Maleate (TM) loaded microspheres

CHARACTERIZATION OF TIMOLOL MALEATE LOADED MICROSPHERES:

a) Appearance, colour and odour of microspheres

The prepared formulations of Timolol maleate (TM) loaded microspheres were visually inspected.

b) Swelling Index

After accurately weighing the microspheres and soaking them in 0.1 M HCl (pH 1.2) and Simulated Intestinal Fluid (SIF) (pH 6.8) for 2 hrs, the swelling index was determined. After 2 hrs, the microspheres were appropriately filtered and weighed. The weight change was then measured.[12,13]

$$\text{Swelling Index} = (w_2 - w_1)/(w_1) \times 100$$

where,

w_2 = Final weight after 2 hrs (mg)

w_1 = Initial weight (mg)

c) Drug content

The drug content of Timolol maleate (TM) loaded microspheres was estimated by accurately weighing 10 mg of microspheres in a glass mortar and grinding them with a glass pestle. The volume was then made up to 10 mL with Simulated Intestinal Fluid (SIF) (pH 6.8) in a volumetric flask. The mixture was subjected to sonication for 2 hrs and left overnight. Afterward, it was filtered, and the filtrate was analyzed using a UV spectrophotometer at a wavelength of 295 nm. Dilution was performed whenever necessary using Simulated Intestinal Fluid (SIF) (pH 6.8) and the corresponding drug concentrations in the samples were calculated from the standard plot equation.[14,15]

d) Entrapment efficiency

10 mg of Timolol maleate (TM) microspheres were placed in a dialysis bag and 1 mL of Simulated Intestinal Fluid (SIF) (pH 6.8) was added to the bag. The dialysis

bag was then immersed in 10 mL of Simulated Intestinal Fluid (SIF) (pH 6.8) and placed over a water bath for stirring. The rotation speed and temperature were set at 50 rpm and 37°C ± 2°C, respectively. A 1 mL sample solution was withdrawn from the medium at 5, 10, 15, 20, 25 and 30 minutes; the aliquots were then filtered and analyzed using a UV spectrophotometer at 295 nm. An equal volume of fresh medium was added after each withdrawal. The entrapment efficiency was calculated using the formula below.[16,17]

$$\% \text{ Entrapment Efficiency} = (w_1 - w_2 / w_1) \times 100$$

where,

w_1= Total drug (mg)

w_2= Free drug (mg)

e) Particle size

The size of the microspheres was measured using an optical microscope. In this procedure, a stage micrometer was used to calibrate the eyepiece micrometer. The following formula was employed to determine the average diameter.[18,19]

$$\text{Average particle size} = (\sum nd/n) \times C.F$$

where,

n = Number of microspheres

d = Diameter of microspheres

C.F = Calibration Factor

CHARACTERIZATION OF OPTIMISED TIMOLOL MALEATE (TM) LOADED MICROSPHERES:

1. Micromeritic property

a) Angle of repose: The angle of repose of microspheres measures the particle flow properties and is calculated using the fixed funnel standing cone method.[20] It is given by the following equation:

$$\tan\theta = h/r$$

where,

θ = Angle of repose

h = Powder heap

r =Radius of the powder cone

b) Bulk Density: It was determined by placing the microspheres into a 10 mL measuring cylinder and the initial volume was recorded.[21] It is given by the equation:

$$\rho_B = M / V$$

where,

ρ_B= Bulk density (gm/cm^3)

M= Weight of the full container (gm)

V= Container volume (cm^3)

c) Tapped density: The 10 mL measuring cylinder containing microspheres was subjected to 100 taps in the tap density apparatus.[22] According to the USP, tapped density is defined by:

$$\rho_T = m/V_f$$

where,

ρ_T= Tapped density (gm/cm^3)

m = Mass of the powder (gm)

V_f = Tapped volume of the powder (cm^3)

d) Carr's Index: The tendency of a powder to be compressed can be determined by Carr's Index.[23] The Carr Index or compressibility index is calculated from the bulk and tapped density values using the following equation:

$$\text{Carr's Index} = [(\rho_T - \rho_B)/\rho_B] \times 100$$

where,

ρ_T = Tapped Density (gm/cm^3)

ρ_B = Bulk Density (gm/cm^3)

e) Hausner's Ratio: It measures the powder's frictional resistance and is determined by the ratio of tapped density to bulk density.[24]

$$\text{Hausner's ratio} = \rho_T/\rho_B$$

where,

ρ_T = Tapped density (gm/cm^3)

ρ_B = Bulk density (gm/cm^3)

2. *In-vitro* drug release study

The *In-vitro* drug release study of Timolol Maleate (TM) microspheres and the marketed equivalent drug was conducted using the USP Type II dissolution apparatus. A weighed quantity of microspheres equivalent to 25 mg of Timolol Maleate (TM) was added to the dissolution medium. For the first two hrs, 0.1 M HCl (pH 1.2) was used as the dissolution medium, which was stirred at 50 rpm and maintained at 37 ± 2°C. Five millilitres of aliquots were taken every fifteen minutes. The amount of drug in the dissolution medium was then measured by UV spectrophotometry at 295 nm after the aliquots had been filtered through Whatman filter paper. After each withdrawal, 5 mL of fresh dissolution medium was added to maintain the starting volume. The dissolution study continued with 900 mL of Simulated Intestinal Fluid (SIF) at a pH of 6.8 for the following five hrs. The *in vitro* drug release data were subjected to kinetic analysis to establish the drug release mechanism. To determine the drug release kinetics from the microspheres, the release data were analyzed using plots based on the Higuchi, First Order, Zero Order, Hixson–Crowell and Korsmeyer–Peppas models.[25–27]

3. *Ex-vivo* permeation study by non-everted gut technique

The fresh duodenal intestinal segment of the small intestine was collected from a slaughterhouse and transferred to aerated phosphate-buffered saline. It was then carefully cleaned to remove excess fat and undigested food without damaging the internal membrane. One end was tied with a cotton thread and using a syringe, 10 mL of Simulated Intestinal Fluid (SIF) (pH 6.8) was filled. Microspheres equivalent to 25 mg of

Timolol Maleate (TM) were transferred to a sac filled with Simulated Intestinal Fluid (SIF) (pH 6.8) and the other end was also tied with a cotton thread. This prepared sac was placed in 200 mL of phosphate-buffered saline (pH 7.4) at 37 ± 2°C and stirred at 50 rpm with aeration (O_2 - 99% and CO_2 - 1%). The experiment was carried out for 7 hrs and 2 mL aliquots were collected at different time intervals (1, 2, 3, 4, 5, 6 and 7 hrs). [28]

4. *Ex-vivo* mucoadhesion study

A strip of goat intestinal mucosa was collected from a butcher shop. It was carefully washed with Simulated Intestinal Fluid (SIF) (pH 6.8) without damaging the mucosal membrane. Goat intestinal mucosa was used to assess the mucoadhesion property in an *ex vivo* investigation. After collection the tissue sample was sliced to the necessary dimensions (2 cm by 1 cm). After the mucosa had been washed, microspheres from the optimized Timolol maleate (TM) loaded microspheres were applied and the water bath shaker was set to 37 ± 2°C for 20 minutes. Then, microspheres were rinsed thoroughly with Simulated Intestinal Fluid (SIF) (pH 6.8) while placed at an angle of 45°. The number of microspheres adhering to the tissue was calculated and mucoadhesion was expressed as a percentage.[29]

$$\textit{Mucoadhesion (\%)} = \frac{\textit{No. of microspheres adhered} \times 100}{\textit{No. of total microspheres place}}$$

5. Differential Scanning Calorimetry (DSC)

The thermal characteristics of Timolol maleate (TM) were analyzed using Differential Scanning Calorimetry (DSC). About 10 mg of Timolol maleate (TM) loaded microspheres was weighed and placed in a DSC pan which was then sealed properly. This pan was placed in the DSC instrument alongside a reference pan, with the heating range set from 30°C to 300°C. The rate of heating was maintained at 10°C/min. Nitrogen gas was purged at a rate of 20 mL/min during the experiment to maintain an inert environment and the endotherm was recorded. [30]

6. Infrared spectroscopy

Timolol maleate (TM), sodium alginate, placebo and Timolol maleate (TM) loaded microspheres were mixed with KBr to form thin pellets, which were characterized by a Fourier Transform Infrared Spectrophotometer operating in the region from 4000 to 400 cm^{-1}.[31]

7. Scanning Electron Microscopy (SEM)

The surface morphology of the prepared microspheres was examined using an FEI Quanta 200. SEM micrographs of the microspheres were obtained under a high-resolution Scanning Electron Microscope (SEM) equipped with a digital image processor.[32]

8. Stability study

The stability of Timolol maleate (TM) microspheres was checked to assess the long-term viability of the formulation, following the ICH Q1A(R2) guidelines. The stability study provides insight into potential excipient reactions, long-term drug stability and possible drug expulsion from the formulation. It also assesses the stability of the formulation under different environmental and storage conditions. The preparation was divided into two sets and stored at 5 ± 3°C and at room temperature (25 ± 2°C/60 ± 5% RH). Formulations were tested at 0, 30, 60 and 90 days. The formulation was evaluated for its organoleptic properties, swelling index, drug content, entrapment efficiency and average particle size.[33]

RESULT AND DISCUSSION

Analytical method development of Timolol Maleate (TM)

The linearity of the standard plot of Timolol Maleate (TM) in 0.1 M HCl (pH 1.2), Simulated Intestinal Fluid (pH 6.8) and Phosphate Buffer Saline (pH 7.4) was found to be in a concentration range of 10-40µg/ml. The Regression coefficient (r^2) and standard plot equation are shown in the below plotted graph. This equation of standard plot is useful to find out unknown concentration of drug in further study.

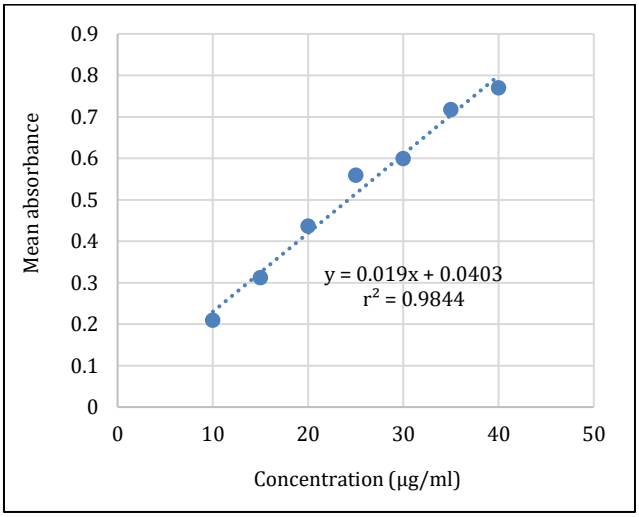

Figure 5: Standard plot of Timolol Maleate (TM) in 0.1M HCl (pH 1.2)

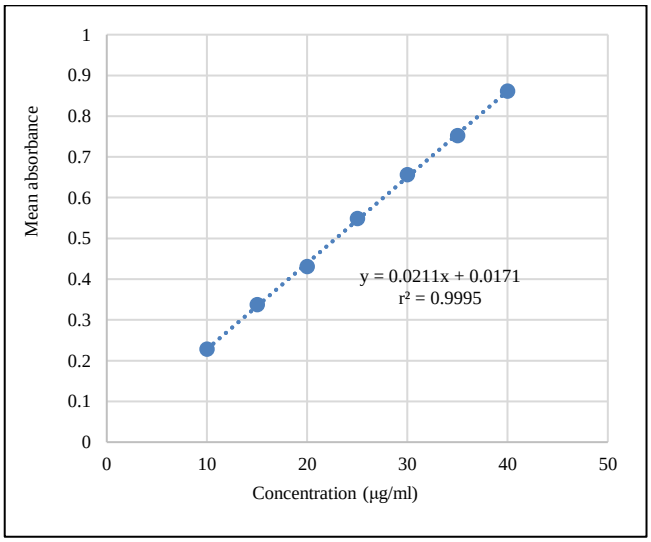

Figure 6: Standard plot of Timolol Maleate (TM) in Simulated Intestinal Fluid (pH 6.8)

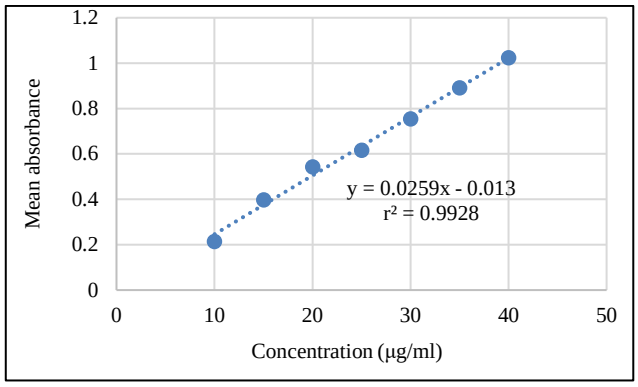

Figure 7: Standard plot of Timolol Maleate (TM) in Phosphate Buffer Saline (pH 7.4)

Preparation of Timolol Maleate (TM) loaded microspheres:

To study the impact of sodium alginate and calcium chloride concentrations on the formulation of Timolol Maleate (TM) loaded microspheres, three different concentrations of sodium alginate and two concentrations of calcium chloride were selected for investigation. Table No. 01 presents the composition of all the formulations developed. Microspheres were successfully formulated by using different concentrations of sodium alginate and calcium chloride under controlled conditions at a stirring speed of 100 rpm at room temperature.

Figure 8: Timolol Maleate (TM) loaded microspheres

Characterization of Timolol Maleate (TM) loaded microspheres:

a) Appearance, colour and odour of microspheres

Timolol Maleate (TM) loaded microspheres are spherical, yellow in colour, and have no odour.

Table 2: Organoleptic properties of Timolol Maleate (TM) loaded microspheres

Sr. No.	Properties	Observation
1	Appearance	Spherical
2	Colour	Yellow
3	Odour	Odourless

b) Swelling Index

It was observed that all formulations exhibited a comparatively lower swelling index in 0.1M HCl (pH 1.2) compared to Simulated Intestinal Fluid (pH 6.8)[34]. The microspheres were found to shrink in acidic pH, which can be explained by the strong interaction between the carboxyl groups of alginates in acidic conditions, leading to the formation of intermolecular and intramolecular hydrogen bonds (polyelectrolyte complex) between the polymers, resulting in reduced swelling. In contrast, the increased swelling of the microspheres in Simulated Intestinal Fluid (pH 6.8) is due to the disruption of these hydrogen bonds, decreasing polyelectrolyte interactions and causing the ionization of the carboxyl groups in alginate, allowing the microspheres to swell as they absorb fluid. Another contributing factor could be the ionization of the cross-linked calcium salts, leading to the exchange of Ca^{+2} ions for Na^{+1} ions in the Simulated Intestinal Fluid. This loosens the dense cross-linked structure, allowing fluid to enter as Ca^{+2} ions are replaced by Na+ ions.

Table 3: Swelling Index of Timolol Maleate (TM) loaded microsphere in 0.1M HCl (pH 1.2)

Batch code	Initial Weight (mg)	Final Weight (mg)	Swelling Index (%)
F1	10.3	10.7	3.883
F2	9.9	11.3	14.14
F3	**9.8**	**11.6**	**13.26**
F4	9.7	10.7	10.30

Table 4: Swelling index of Timolol Maleate (TM) loaded microsphere in Simulated Intestinal Fluid (SIF) (pH 6.8)

Batch code	Initial weight (mg)	Final Weight (mg)	Swelling Index (%)
F1	9.8	80.55	711.93
F2	10.6	98.6	830.18
F3	**10.2**	**100.5**	**885.29**
F4	9.6	89.4	827.08

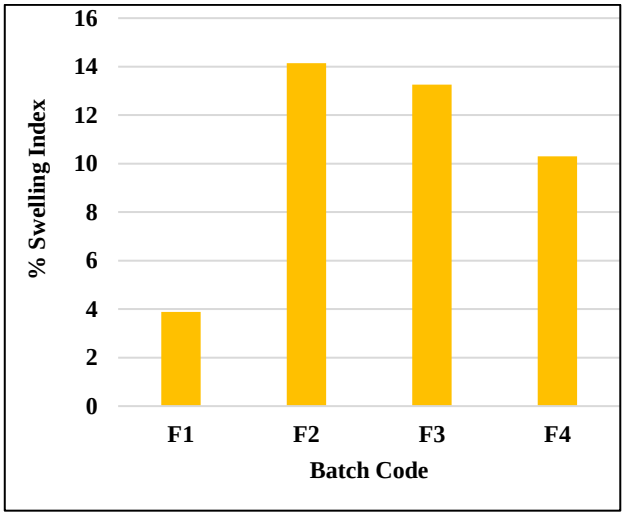

Figure 9: Swelling Index of Timolol Maleate (TM) loaded microspheres in 0.1M HCl (pH 1.2)

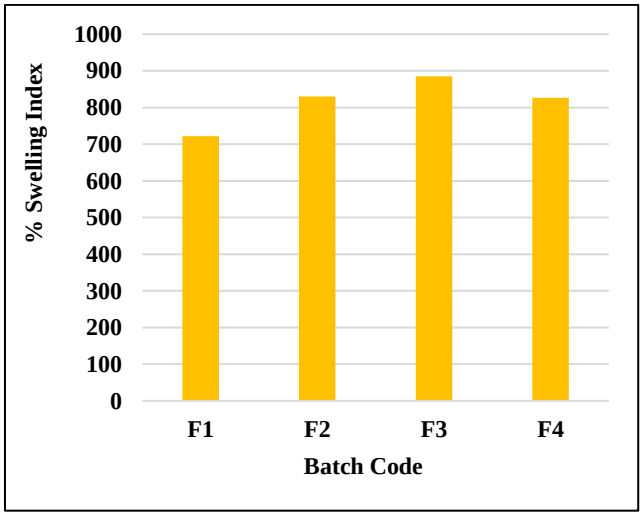

Figure 10: Swelling Index of Timolol Maleate (TM) loaded microspheres in Simulated Intestinal Fluid (SIF) (pH 6.8)

c) Drug content

The drug content was assessed in 10 mg of microspheres and it was observed that an increase in sodium alginate concentration resulted in higher drug content. This can be attributed to the greater availability of active calcium-binding sites within the polymer chains, leading to a higher degree of cross-linking as the amount of sodium alginate increased.[35]

Table 5: Total drug content of Timolol Maleate (TM) loaded microspheres

Batch code	Theoretical drug content mg/10 mg of Microspheres	Actual drug content mg /10mg of Microspheres
F1	1.681 mg	0.918 mg
F2	1.342 mg	0.9067 mg
F3	**0.667 mg**	**0.5745 mg**
F4	0.806 mg	0.610 mg

d) Entrapment efficiency

Entrapment efficiency was evaluated for Timolol Maleate (TM) loaded microspheres using the drug release profile from the dialysis bag. Entrapment efficiency was found be in the range of 71-88%.

Table 6: Entrapment efficiency of Timolol Maleate (TM) loaded microspheres

Batch code	Free drug mg/10mg of Microspheres	Total drug mg/10 mg of Microspheres	Entrapment efficiency (%)
F1	0.2620 mg	0.918 mg	71.45
F2	0.1324 mg	0.9067 mg	76.33
F3	**0.0641 mg**	**0.5745 mg**	**88.83**
F4	0.1443 mg	0.610 mg	83.88

e) Particle size

Particle size of all batches was estimated using an optical microscope. It was observed that as the sodium alginate concentration increases the viscosity of the polymeric solution increases which ultimately result in larger size of the microspheres.[36]

Table No. 7: Average particle size (µm) of Timolol Maleate (TM) loaded microspheres

Batch Code	Particle size (µm)
F1	611.552
F2	693.761
F3	**756.81**
F4	716.648

Characterization of optimised Timolol Maleate (TM) loaded microspheres

1. Micromeritic properties

a. Angle of Repose: Angle of repose was found to be **21.8°**. It indicates an **excellent flow** property of the optimized Timolol Maleate (TM) loaded microsphere.

b. Bulk density: The bulk density of the optimized Timolol Maleate (TM) loaded microsphere was determined by dividing the weight of the sample in grams by the final volume in cm^3 of the sample contained in the 10 ml graduated cylinder. The bulk density value of the optimized Timolol Maleate (TM) loaded microsphere was found to be **0.25gm/cm^3.**

c. Tapped density: Tapped density was determined by the tapping method. The tapped density value of the optimized Timolol Maleate (TM) loaded microsphere was found to be **0.271gm/cm^3.**

d. Hausner Ratio: Hausner ratio was found to be **1.08**, which indicates an **excellent flow** property. Since, the Hausner ratio gives measures of the porosity of a microsphere to be compressed as such they are measures of the relative interparticle interaction. In a free-flowing microsphere such interactions are generally less significant and the bulk and tapped densities will be closer in value resulting in the lower value of the Hausner ratio.[37]

e. Carr's Index: It is determined by using the value of tapped density and bulk density. The percentage Carr's index was found to be **8.4%**

2. *In-vitro* drug release study

In-vitro drug release study was carried out using the USP type II Dissolution apparatus. At the end of 7 hrs study Timolol Maleate (TM) loaded microspheres showed **92.15%** release of drug. Conventional tablet chosen to study the *in-vitro* drug release was Propranolol hydrochloride IP 10 mg. Propranolol hydrochloride belongs to the BCS class I and it is found to be therapeutically equivalent to Timolol Maleate (TM). The data indicated that the TM-loaded microspheres provided a sustained release of the drug. The release kinetics of Timolol Maleate (TM) from the optimized microspheres followed the Hixson-Crowell model (R^2 = 0.9833), suggesting that drug release is influenced by changes in the surface area and diameter of the microspheres.[38]

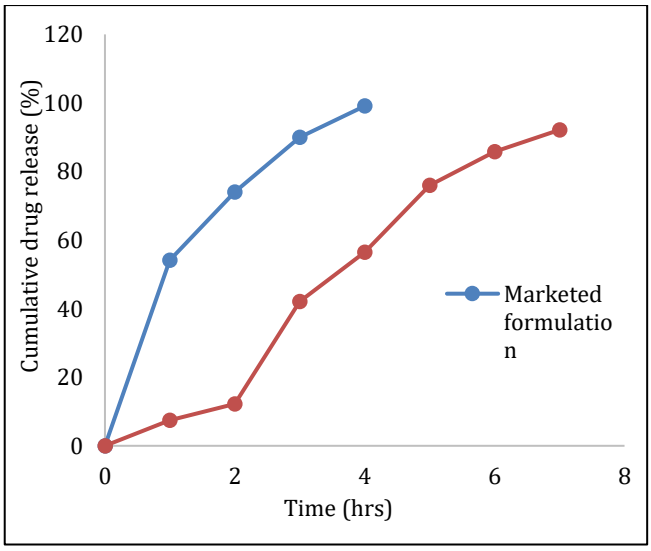

Figure 11: Cumulative drug release (%) versus time (hrs) of Timolol Maleate (TM) loaded microspheres

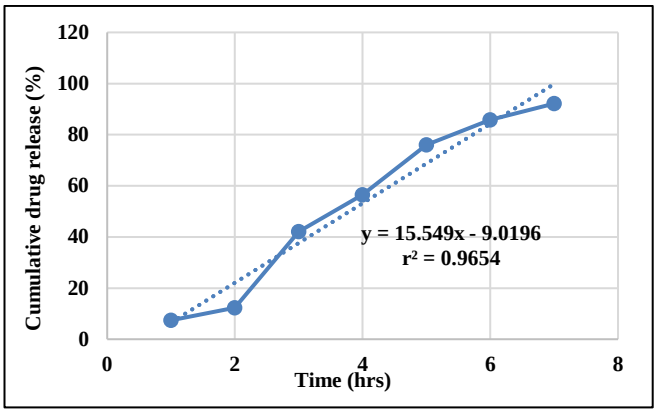

Figure 12: Zero order drug release kinetics of Timolol Maleate (TM) loaded microspheres

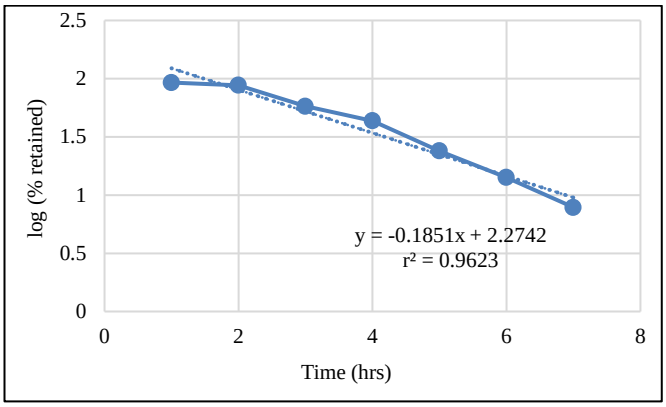

Figure 13: First order drug release kinetics of Timolol Maleate (TM) loaded microspheres

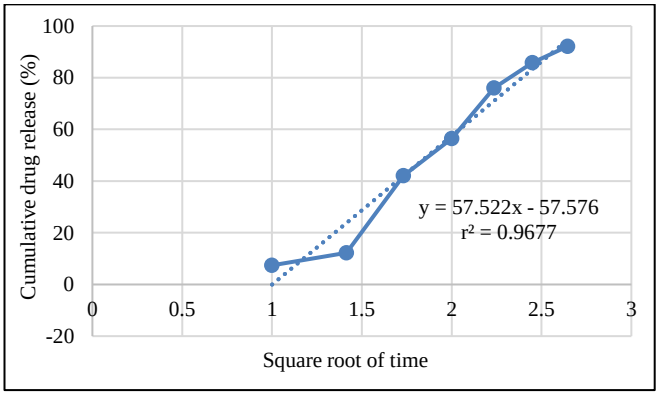

Figure 14: Higuchi release kinetics of Timolol Maleate (TM) loaded microspheres

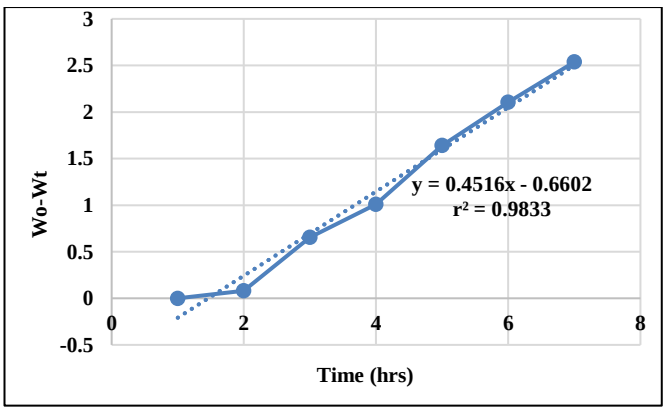

Figure 15: Hixson Crowell release kinetics of Timolol Maleate (TM) loaded microspheres

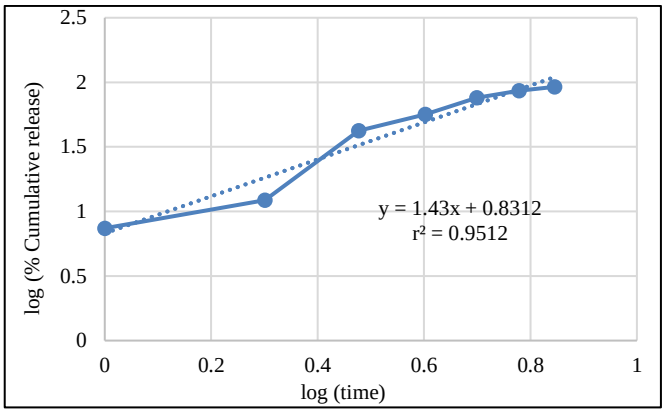

Figure 16: Korsmeyer-Peppas release kinetics of Timolol Maleate (TM) loaded microspheres

Table 8: Correlation Coefficient (r^2) values of optimized Timolol Maleate (TM) loaded microspheres

Release kinetics	Correlation Coefficient (r^2)
Zero Order drug release kinetics	0.9654
First Order drug release kinetics	0.9623
Higuchi release kinetics	0.9677
Hixson-Crowell release kinetics	0.9833
Korsmeyer-Peppas release kinetics	0.9512

3. *Ex-vivo* permeation study by non-everted gut technique

An *ex-vivo* permeation study was conducted on both Timolol Maleate (TM) and the optimized TM-loaded microspheres. It was observed that drug permeation from the microspheres was lower compared to Timolol Maleate (TM) alone. Since TM is a BCS Class I drug, it reached a plateau after 2 hrs. In contrast, the drug permeation from the optimized TM-loaded microspheres exhibited a steady release over 7 hrs. The encapsulation of the drug in the microspheres, prepared by the ion gelation method, facilitated sustained drug release.

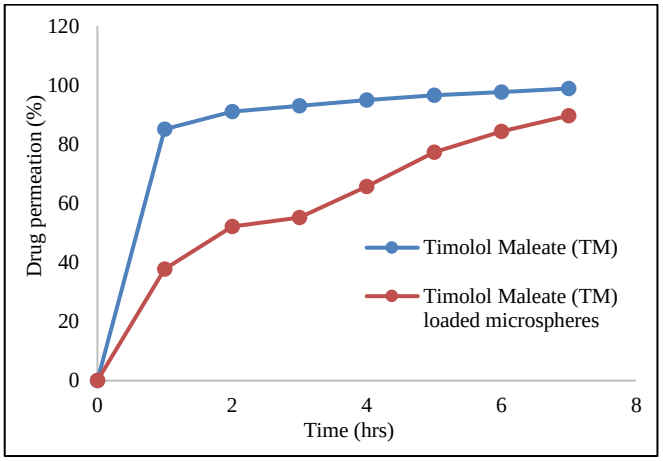

Figure 17: Drug permeation (%) versus time (hrs) of Timolol Maleate (TM) loaded microspheres

4. *Ex-vivo* mucoadhesion study

Mucoadhesion was found to be **55%**. The concentration of sodium alginate, a mucoadhesive polymer had an impact on the mucoadhesion properties.

5. Differential Scanning Calorimetry (DSC)

The DSC thermogram of the Timolol Maleate (TM) showed a sharp melting endothermic peak at 204.02°C.

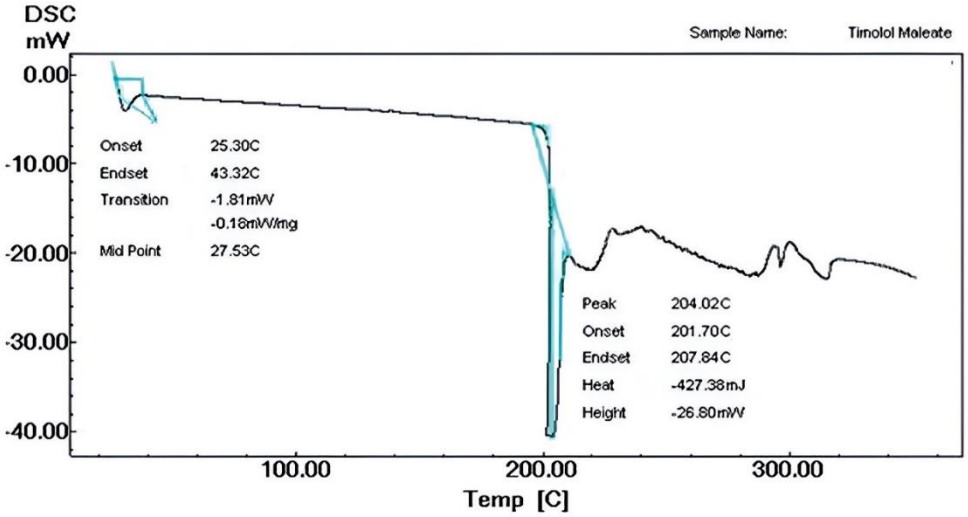

Figure 18: Differential scanning calorimetry (DSC) of Timolol Maleate (TM)

In microspheres, it showed an endothermic peak at 104.58°C indicating the loss of water resulting in the dehydration of the sample. The Timolol Maleate (TM) loaded microsphere showed melting endotherm at 198.05°C. This finding indicates molecular dispersion of drugs within the microsphere. Peak shows a shift in temperature as well as reduction in peak due to the encapsulation of drugs into the polymer.

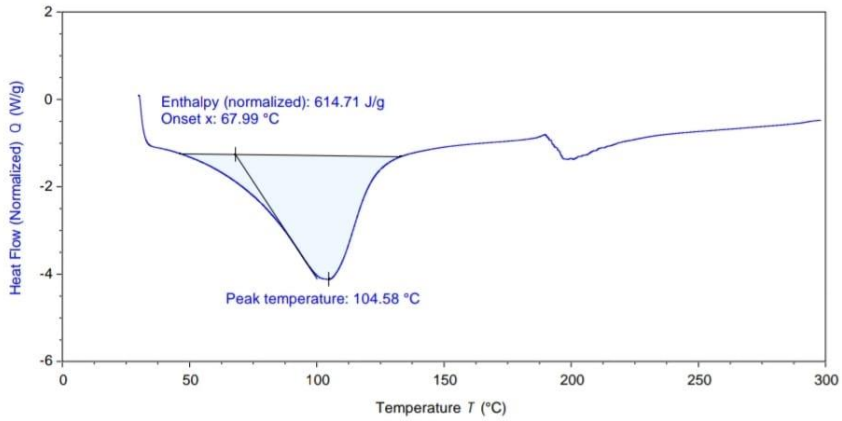

Figure 19: Differential Scanning Calorimetry (DSC) of optimized Timolol Maleate (TM) loaded microspheres

6. Infrared spectroscopy

FTIR spectra of Timolol Maleate (TM), sodium alginate, placebo and TM-loaded microspheres were analyzed and compared to assess the molecular interactions between the components in the microsphere formulation. The Timolol Maleate (TM) spectrum shows the most peaks corresponding at 3535.52cm^{-1} (O-H stretch), 3037.89cm^{-1} (N-H stretch), 2972.31cm^{-1} (C-H stretch), 1305.81cm^{-1} (C-O stretch). A relatively low peak intensity indicates that the O-H groups are unlikely to bond to each other via hydrogen bonds. Sodium alginate, Placebo and Timolol Maleate (TM) loaded microspheres showed peaks at 3251.98 cm^{-1}, 2980.02cm^{-1} and 3344.57cm^{-1} (O-H stretch) respectively.

Asymmetrical and symmetrical C=O stretch has been observed in Sodium alginate (1593.20cm^{-1} and 1408.06cm^{-1}), Placebo (1597.6cm^{-1} and 1421.54cm^{-1}) and Timolol Maleate (TM) loaded microspheres (1606.70cm^{-1} and 1423.47cm^{-1}). In case of Placebo and Timolol Maleate (TM) loaded microsphere, cross linking of sodium alginate by a significant concentration of Ca^{+2} was shown a reduction in the intensity and a slight decrease in the wavenumber of the C=O stretch peak as compared to C=O stretch peaks in sodium alginate. Timolol Maleate (TM) loaded microspheres showed the absence of characteristic Timolol Maleate (TM) peaks, confirming successful encapsulation of Timolol Maleate (TM).[39]

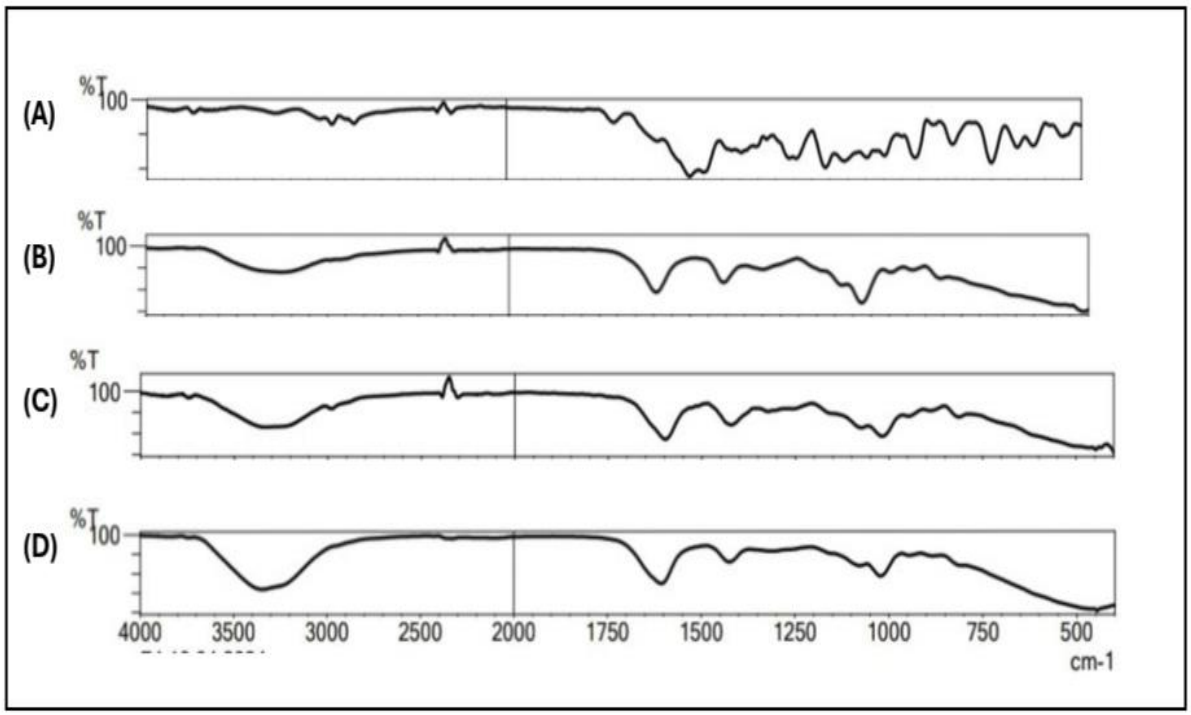

Figure 20: FTIR spectra A) Timolol Maleate (TM) B) Sodium alginate C) Placebo D) Optimised Timolol Maleate (TM) loaded microspheres

7. Scanning Electron Microscopy (SEM)

The SEM pictures revealed that the microspheres had a roughly spherical form, were distinct, freely flowing and ranged in size from 400 to 900µm. It was also discovered that the potential for drug crystals to exist on the surface of the microspheres were the reason for their rough surface.[40]

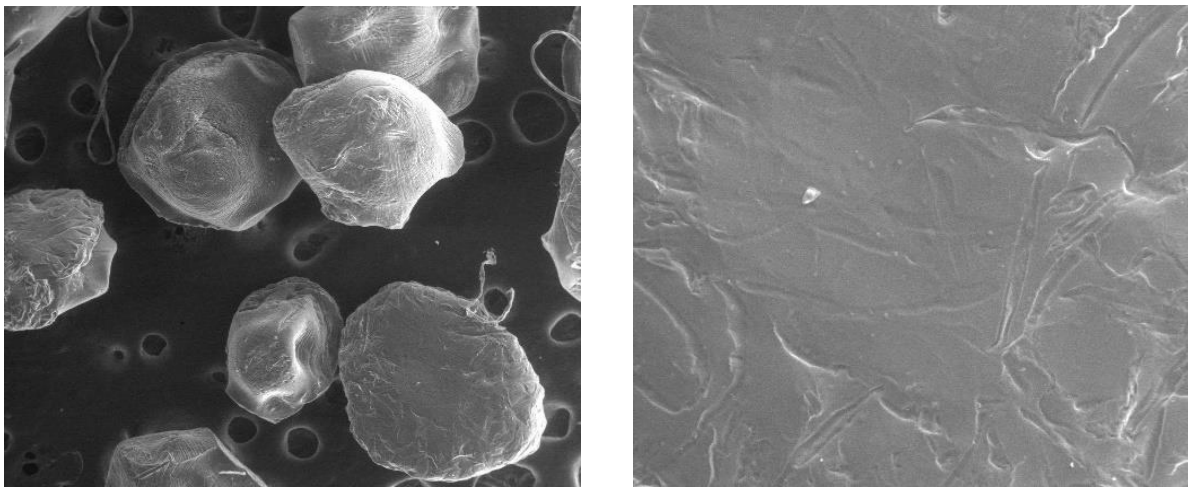

Figure 21: Scanning Electron Microscope (SEM) images of optimized Timolol Maleate (TM) loaded microspheres

8. Stability study

Optimised Timolol Maleate (TM) loaded microspheres had their stability assessed at 5±3°C and 25±2°C/60±5%RH as per the ICH Q1A(R2) guidelines and were assessed on the interval of 30, 60 and 90 days. The microspheres retained their appearance, swelling index, drug content, entrapment efficiency and particle size and did not show significant change in the properties during the three-month storage period at 5±3°C and 25±2°C/60±5%RH.

Table 9: Stability study of optimised Timolol Maleate (TM) loaded microspheres

Parameters	One Month		Two Months		Three Months	
	5±3°C	25±2°C/ 60±5% RH	5±3°C	25±2°C/ 60±5% RH	5±3°C	25±2°C/ 60±5% RH
Appearance in colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Swelling index (%) in HCl (pH 1.2)	12.78%	13.45%	13.34%	13.23%	13.25%	13.71%
Swelling index (%) in SIG (pH 6.8)	799.56%	856.78%	864.45%	897.12%	892%	895.25%
Total drug content (mg/10mg of microspheres)	0.5567 mg	0.5690 mg	0.4868 mg	0.5656 mg	0.5632 mg	0.5851 mg
Entrapment efficiency (%)	87.03%	87.98%	88.56%	87.34%	87.52%	87.61%
Particle size (µm)	659.62 µm	701.56 µm	708.45 µm	723.54 µm	707.83 µm	769.81 µm

CONCLUSION

This study successfully developed sustained release microspheres containing Timolol Maleate (TM) to achieve sustained drug release. The ion gelation method was employed to develop these microspheres, using sodium alginate as the biocompatible polymer and calcium chloride as the cross-linking agent. TM was identified through physical characterization including appearance, colour, odour, solubility, UV spectroscopy, FTIR and melting point determination.

Various batches of TM-loaded microspheres were developed by altering the concentrations of sodium alginate and calcium chloride. These batches were evaluated for drug content, swelling index, entrapment efficiency and particle size distribution to optimize the formulation. The F3 batch showed the most promising results and underwent further evaluations including dissolution, permeation, micromeritic properties, mucoadhesive studies, FTIR, SEM, DSC and stability.

The optimized TM-loaded microspheres exhibited a swelling index of 885.29% in simulated intestinal fluid (pH 6.8) and 13.26% in 0.1M HCl (pH 1.2). The drug content was around 86.13% and the drug entrapment efficiency was 88.83% with a particle size of approximately 756.8 µm. The *In-vitro* drug release was 92.151% over 7 hrs demonstrating sustained release compared to a conventional formulation that released 99.1% in 4 hrs. The microspheres also showed a steady drug permeation over 7 hrs while TM alone peaked after 2 hrs due to its high permeation profile. The mucoadhesion property was found to be 55%.

FTIR analysis indicated successful drug loading within the microspheres, with notable shifts in characteristic peaks. DSC thermograms revealed a shift in the melting point, indicating drug encapsulation within the polymer. Drug release kinetics, best described by Hixson-Crowell release kinetics ($r^2 = 0.9833$) suggested drug release due to matrix erosion or dissolution of drug particles.

Stability studies conducted as per ICH Q1A(R2) guidelines showed that the microspheres remained stable at both 5±3°C and 25±2°C/60±5% RH over three months. TM-loaded microspheres demonstrated a sustained drug release profile over 7 hrs. Future research could focus on developing compatible dosage forms and exploring targeted delivery for enhanced drug efficacy. Additionally, combining TM with other agents for synergistic effects presents a potential avenue for further study.

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Author's Contribution: All authors have contributed equally to this work.

Conflict of Interest: The authors declare no conflict of interest.

Source of Support: Nil

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethics approval: Not applicable.

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