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Formulation and Evaluation of Mucoadhesive Microspheres Containing Timolol Maleate

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ABSTRACT: The present research works describes the development and evaluation of sustained release mucoadhesive microspheres containing Timolol maleate using hydroxypropyl methylcellulose as a polymer.The choice of the timolol maleate is due to design it as novel mucoadhesive formulations include that because of its relatively short biological half-life of about 2–8 h, and has a specific window for the drug absorption at an active, by saturable absorption process and small absorption rate constants. Bio erodible bio adhesive microspheres has been reported to enhance the per-oral bioavailability of certain drugs like dicoumarol, insulin which have been investigated for peroral gene delivery. The increase pharmacokinetic activity of insulin and the plasmid DNA is used as evidence to design the mucoadhesive microspheres uptake of by cells lining the GI epithelium.In this work, an attempt was made to formulate and evaluate drug loaded sustained release Timolol maleate using statistical optimization technique. The main objective of formulating the dosage form was to prolong the drug release time, reduce the frequency of administration and to improve patient compliance. The optimized formulation developed by constrained showed 90.78 % yield, 83.45% drug entrapment efficiency and 167.6 average particle size respectively. The mechanism of drug release was characterized by Higuchi diffusion model. The optimized formulation obtained was evaluated for the responses, % yield, %drug entrapment efficiency and particle size (µm). The actual response values were in accordance with those predicted by mathematical models.

Key words: Mucoadhesive microspheres, timolol maleate, hydroxy propyl methyl cellulose, absorption window, patient compliance.

I. INTRODUCTION:

Mucoadhesive drug delivery systems are the systems which utilize the property of bioadhesion of certain polymers which become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time.Bioadhesion is an interfacial phenomenon in which two materialsat least one of which is biological are held together by means of interfacial forces. The attachment could be between an artificial material and biological substrate, such as adhesion between a polymer and a biological membrane. In the case of polymer attached to the mucin layer of a mucosal tissue, the term "mucoadhesion" is used. The mucosal layer lines a number of regions of the body including the gastrointestinal tract, the urogenital tract, the airways, the ear, nose and eye. These represent potential sites for attachment of bio adhesive system and hence the mucoadhesive drug delivery systems could be designed for buccal, oral, vaginal, rectal, nasal and ocular routes of administration[1,2,3,4].

The more sophisticated a delivery system, the greater is the complexity of these various disciplines involved in the design and optimization of the system. In any case, the scientific framework required for the successful development of an oral drug delivery system consists of a basic understanding of physicochemical, pharmacokinetic and pharmacodynamics characteristics of the drug, the anatomic and the physiologic characteristics of the GIT, physicochemical characteristics and drug delivery mode of the dosage form to be designed[5,6].

Mucoadhesive microspheres are the microparticles having a diameter of 1-1000µm. Microspheres in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages.

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Mucoadhesive microspheres can be tailored to adhere to the mucosal layer of gastrointestinal, nasal cavity and urinary tract thus offering the possibilities of controlled release for localized as well as systemic effect of drugs. The properties of the mucoadhesive microspheres like surface characteristics, force of mucoadhesion, release pattern of the drug and clearance are affected by the type of mucoadhesive polymers used to prepare them[7,8,9].

Mucoadhesive polymers are water soluble or water insoluble polymers with swellable networks. The polymer should possess optimal polarity to make sure it is sufficiently wetted by the mucus and should have optimal fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place. Mucoadhesive polymers can localize in specified regions to improve and enhance bioavailability of drugs, optimum contact with the absorbing surface to permit modification of tissue permeability, which is especially important in the case of peptides, proteins, ionized species and prolonged residence time to permit once-daily dosing, thus improving patient compliance[10].

Advantages of Mucoadhesive Systems

- A prolonged residence time at the site of drug action or absorption.
- A localization of drug action at a given target site.
- An increase in the drug concentration gradient due to the intense contact of particles with the mucosa.
- A significant reduction in dose can be achieved there by reducing dose related side effects.
- Less dosing frequency & shorter treatment period.
- Increased safety margin of high potencydrugs due to better control of plasma levels
- Improved patient convenience and compliance due to less frequent drug administration.
- Reduction in health care costs through improved therapy, shorter treatment period, less frequency of dosing and reduction in personnel time to dispense, administer and monitoring of patients.
- These dosage forms facilitate intimate contact of the formulation with underlying absorption surface. This allows modification of tissue permeability for absorption of macromolecules, such as peptides and proteins.
- Inclusion of penetration enhancers such as sodium glycocholate, sodium taurocholate andlysophosphatidic cholineand protease inhibitors in the mucoadhesive dosage forms resulted in the better absorption of peptides and proteins[11].
- Limitations of Mucoadhesive Systems
- Decreased systemic availability in comparison to immediate release conventional dosage forms; this may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site specific absorption, pH dependent solubility, dose dumping etc. Poor in-vitro, in-vivo correlation & higher cost of formulation[11]

The aim of the present research work is to formulate and evaluate mucoadhesive microspheres of Timolol maleate using Hydroxypropyl methylcellulose K4 for sustain release to prolong drug release, reduce the frequency of administration associated with conventional therapy in order to improve the patient compliance[12].

II. MATERIALS AND METHODS:

Timolol maleate and Hydroxypropyl methylcellulose K4 was generously supplied as a gift sample by FDC Limited (Mumbai). Dichloromethane purchase from Qualigens fine chemicals and Ethanol purchase from Karnataka fine chemicals. All other chemicals and reagents used were of analytical grade.

PREFORMULATION STUDIES:

Identification: Infrared absorption spectrophotometryAppearance of solution: A 2.0 per cent w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution.

Specific optical rotation:5.7º to –6.2ºC, determined in a 10.0 per cent w/v solution in M hydrochloric acid[13].

Fourier Transform Infrared Spectroscopy (FT-IR)

In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained using Varian FT-IR 8400 spectrophotometer were compared with the individual spectra of pure drug and excipients variation in the peak were carefully studied.

UV analysis:

Determination of λmax

Stock solution (100µg/ml) of Timolol maleate was prepared in HCl buffer pH-1.2. This

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solution was appropriately diluted to obtain a concentration of 10µg/ml. The resultant solution was scanned in the range of 200nm to 400nm on Shimadzu UV-1800 spectrophotometer[14]. The drug exhibited a λmax at 294.0 nm in HCl buffer pH-1.2. Beers range: 4-36 µg/ml.

Preparation of standard calibration curve of Timolol maleate in HCl buffer

- 100mg of Timolol maleate was accurately weighed and dissolved in 100ml of HCl buffer pH 1.2 (Stock 1) to get a concentration of 1000 µg/ml.
- From the stock solution (stock 1)aliquots were taken and suitably diluted with HCl buffer pH 1.2 to get concentrations in the range of 2 to 16µg/ml. The absorbance of these samples was analyzed by using Shimadzu UV-1800 Spectrophotometer at 294.0 nm against reference solution HCl buffer pH 1.2. The absorbance values were recorded in triplicate and are reported.
- **Method of preparation of Mucoadhesive microspheres:** Microspheres containing Timolol maleate were prepared by a non-aqueous solvent evaporation method. Drug and polymer were dissolved in dichloromethane and ethanol to prepare a slurry. The slurry was slowly injected into the external phase containing liquid paraffin and span 80, while being stirred by a mechanical stirrer equipped with a three-blade propeller at room temperature. The solution was stirred until the solvent evaporated completely. The microspheres formed were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40-60^{0}C) to remove excess of oil. The collected microspheres were dried for 1 hour at room temperature and subsequently stored in a desiccator over fused calcium chloride[15,16,17,18].

Formulation code	Drug polymer ratio	Stirring time	Curing time	Concentration of organic solvent	Concentration of surfactant
F1	1:1	600	1	10	6
F2	1:4	600	1	10	3
F3	1:1	1200	1	10	6
F4	1:4	1200	1	10	3
F5	1:1	600	3	10	6
F6	1:4	600	3	10	3
F7	1:1	1200	3	10	6
F8	1:4	1200	3	10	3

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F9	1:1	600	1	30	6
F10	1:4	600	1	30	3
F11	1:1	1200	1	30	6
F12	1:4	1200	1	30	3
F13	1:1	600	3	30	6
F14	1:4	600	3	30	3
F15	1:1	1200	3	30	6
F16	1:4	1200	3	30	3

Table 1: list of ingredients and variables in formulation of microsphers

Evaluation of microspheres:

Percentage yield:

The practical percentage yield was calculated from the weight of dried microspheres recovered from each batch in relation to the sum of the initial weight of starting materials[19]. The percentage yield was calculated using the following formula

Drug entrapment efficiency

Microspheres equivalent to 10mg of timolol maleate were crushed in a glass mortar and pestle and the powdered microspheres were suspended in 25ml of HCl buffer pH 1.2. And this mixture was vortexed for 5 min. The supernatant was collected and filtered, 1ml of the filtrate was pipetted out and diluted to 10ml and analysed for the drug content using Shimadzu UV-1800 spectrophotometer at 294.0 nm[20].

The drug entrapment efficiency was calculated using the following formula:

Particle size analysis

Particle size of the prepared microspheres was determined by optical microscopy. The optical microscope was fitted with an ocular micrometre and a stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of more than 300 microspheres were measured randomly by optical microscope[21]

Shape and Surface morphology

The shape and surface characteristics of the prepared microspheres were evaluated by means of scanning electron microscopy (ZEISS – IISC Bangalore). The samples for scanning electron microscopy were prepared by gently sprinkling the microspheres on a double adhesive tape, which is stuck to an aluminium stub. The stubs were then coated with gold using a sputter coater (JEOL Fine coat JFC 1100E, ion sputtering device) under high vacuum and high voltage to achieve a film thickness of 30nm. The samples were then imaged using a 20KV electron beam[22].

In-vitro mucoadhesion studies

The in-vitromucoadhesion study of microspheres was analysed using eggshell

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membranes. A egg shell membrane was obtained from chicken egg. After emptying the egg of its content, the external shell was removed and rinsed with physiological saline and the underlying membrane was mounted on a glass slide. Accurately weighed 50mg of microspheres were placed on the egg shell membrane. This glass slide was incubated for 30min in a desiccator at 93% relative humidity to allow the polymer to interact with the membrane and finally placed on the stand at an angle of 45^0. HCl buffered saline pH-1.2 previously warmed to 37± 0.5^0Cwas circulated all over the microspheres and membrane at the rate of 22ml/min for 5 minutes with the help of a burette. At the end of this process, the detached particles were collected and weighed [23].
Percentage mucoadhesion was calculated from the following formula:

In-vitro drug dissolution studies

Dissolution studies were carried out for all the formulation, employing USP XXIII apparatus (Basket method) at 37± 0.5^0 C rotated at constant speed of 50 rpm using 900ml pH 1.2 HCl buffer medium as the dissolution medium. Microspheres equivalent to 100 mg of Timolol maleate was used for the study. An aliquot of the sample was periodically withdrawn at suitable time interval and the volumes were replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analysed spectrophotometrically at 294.0nm and the concentration of drug was calculated as per the linear regression equation[24].

In-vitro drug release kinetics

The release data obtained was fitted into various mathematical models using PCP Disso – V2.08 software. The parameters 'n' and time component 'k', the release rate constant and 'R', the regression co-efficient were determined by Korsmeyer – Peppas equation to understand the release mechanism. To examine the release mechanism of Timolol maleate from the microsphere formulations, the release data was fitted into Peppa's equation,

$$\mathbf{M_t / M_\infty = Kt^n}$$

Where, M_t/M_∞ is the fractional release of drug, 't' denotes the release time, 'K' represents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusional exponent and characterize the type of release mechanism during the release process.

Release Exponent 'n'	Drug Transport Mechanism	Rate as a function of time
0.5	Fickian Diffusion	$_t$n-0.5
0.5<n<1.0	Non-Fickian Diffusion	$_t$n-1
1.0	Case – II Transport	Zero Order Release
Higher Release	Super Case – II Transport	$_t$n-1

Table 2: n values for different kinetic models

If n<0.5, the polymer relaxation does not affect the molecular transport, hence diffusion is Fickian. If n > 0.5, the solid transport will be non – Fickian and will be relaxation controlled

Other Equations for to study drug release kinetics from dosage forms

a. Zero Order

$$\mathbf{\% R = kt}$$

This model represents an ideal release in order to achieve prolonged pharmacological action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as matrix tablets containing low soluble drugs.

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b. First Order

Log (fraction unreleased) = kt/2.303

The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

c. Matrix (Higuchi Matrix)

% R = $kt^{0.5}$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

d. PeppasKorsmeyer Equation

% R = kt^n

log % R = logk + nlogt

This model is widely used when release mechanism is well known or when more than one type of release phenomenon could be involved. The 'n' values could be used to characterize different release mechanisms as:

Value of 'n'	Mechanism
0.5	Fickian Diffusion (Higuchi Matrix)
0.5<n<1	Anomalous Transport
1	Case – II transport (Zero Order Release)
n>1	Super Case Transport

Table 3: kinetic models

e. Hixson - Crowell Equation

(Fraction unreleased) $^{1/3}$ = 1 – kt

This equation applies to pharmaceutical dosage forms like tablets where dissolution occurs in planes that are parallel to drug surface if the tablet dimension diminishes proportionally, in such a manner that the initial geometric form keeps constant all the time. When this model is used, it is assumed that the release rate is limited by the drug particles dissolution rate and not by the diffusion that might occur through polymer matrix[25].

Stability studies

The selected formulations were packed in their final (amber coloured glass) containers and are tightly closed with the cap. They were stored at temperature and RH as per ICH guidelines for 2 months. Samples were analysed after 0, 30 and 60 days and they were evaluated for % Drug entrapment efficiency and in-vitro drug release studies[26].

III. RESULTS:

UV Analysis

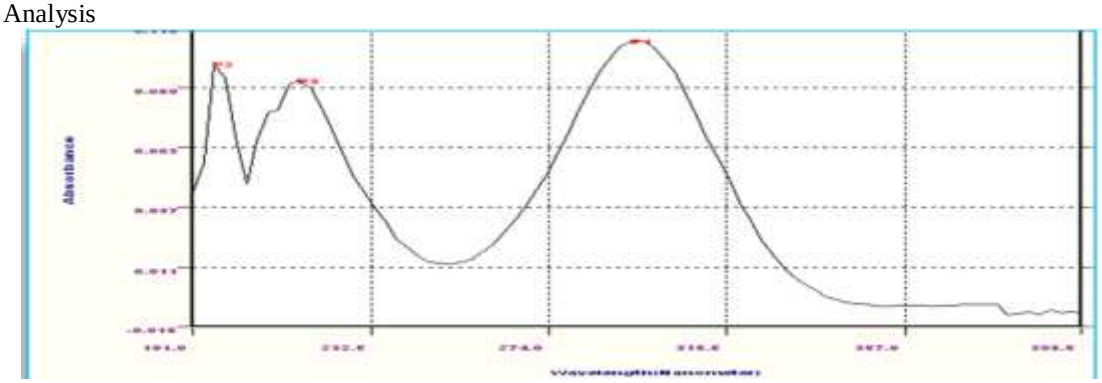

Fig 1: Absorption maxima of timolol maleate

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Sl. No	Conc. (µg/ml)	Absorbance			Average Absorbance	± SEM
		Trial 1	Trial 2	Trial 3		
1	4	0.113	0.111	0.112	0.110	0.0005
2	8	0.210	0.212	0.214	0.212	0.0010
3	12	0.304	0.308	0.309	0.307	0.0015
4	16	0.412	0.416	0.414	0.414	0.0011
5	20	0.510	0.512	0.514	0.512	0.0011
6	24	0.615	0.611	0.613	0.613	0.0011
7	28	0.711	0.710	0.709	0.710	0.0005
8	32	0.811	0.816	0.814	0.814	0.0014
9	36	0.881	0.884	0.881	0.882	0.0010

Table 4: results of UV analysis

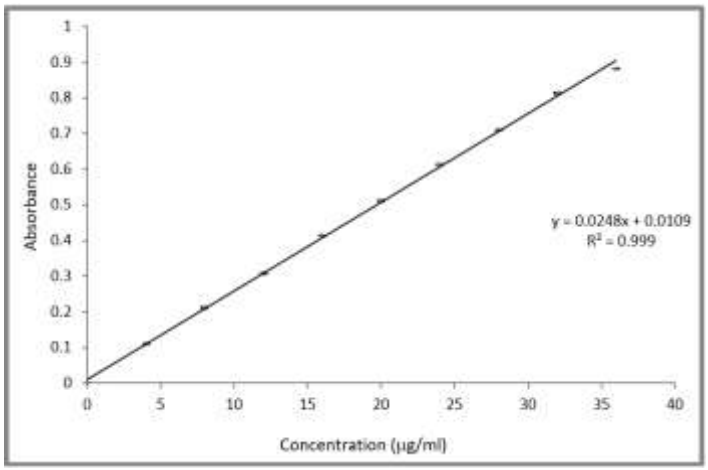

fig 2: UV spectra

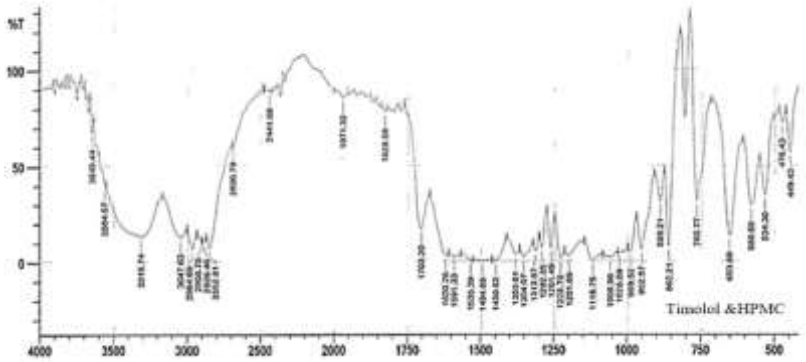

Fig 3: FTIR spectra of drug and polymer

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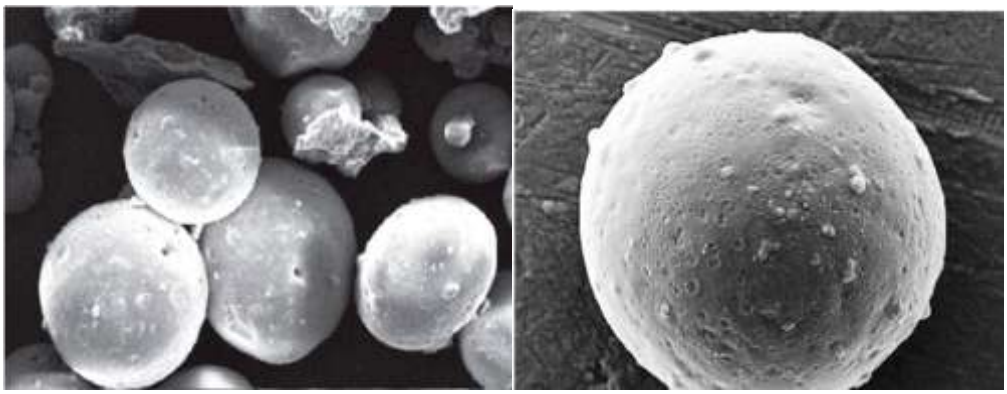

Fig 4: SEM images of microspheres

In Vitro release: The data obtained after dissolution were analysed using first order kinetic equation, Matrix equation, Zero order, Hixson and Peppas equation. The result obtained are shown in the table.

Formulation code	% yield	% DEE	Particle size	% mucoadhesion	% CDR at end of 8 hours
F1	81.25	80.40	200.8	87.51	**87.24**
F2	88.50	85.91	235.2	90.90	**83.78**
F3	79.02	72.64	179.4	80.28	**93.03**
F4	76.57	77.90	191.2	85.54	**84.23**
F5	91.28	82.23	171.6	86.34	**81.83**
F6	78.50	86.91	183.6	80.04	**76.88**
F7	85.51	71.02	163.6	77.44	**95.05**
F8	91.03	77.30	179.4	81.14	**89.54**
F9	71.51	74.40	163.8	84.12	**91.13**
F10	86.42	81.20	180.0	88.34	**79.73**
F11	64.75	73.06	154.2	75.08	**92.34**
F12	89.42	77.30	173.6	73.36	**88.44**
F13	82.75	74.80	130.6	78.84	**90.55**
F14	91.57	78.50	140.4	84.34	**85.52**
F15	79.75	70.80	95.52	72.28	**95.32**
F16	88.28	74.09	107.3	76.72	**90.32**

Table 5: results of characterisation of F1-F16 Formulations

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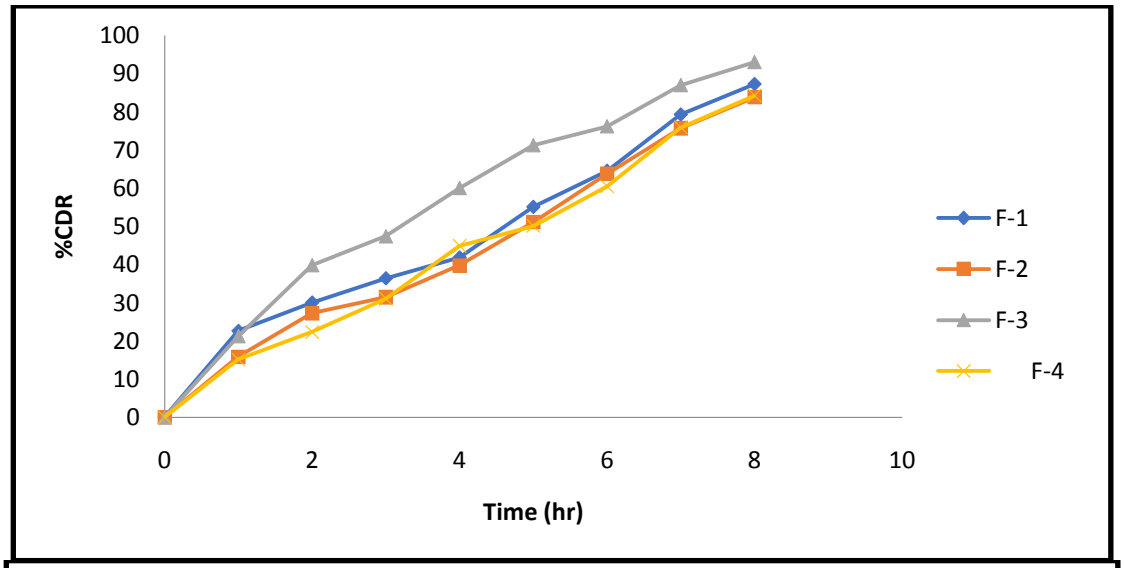

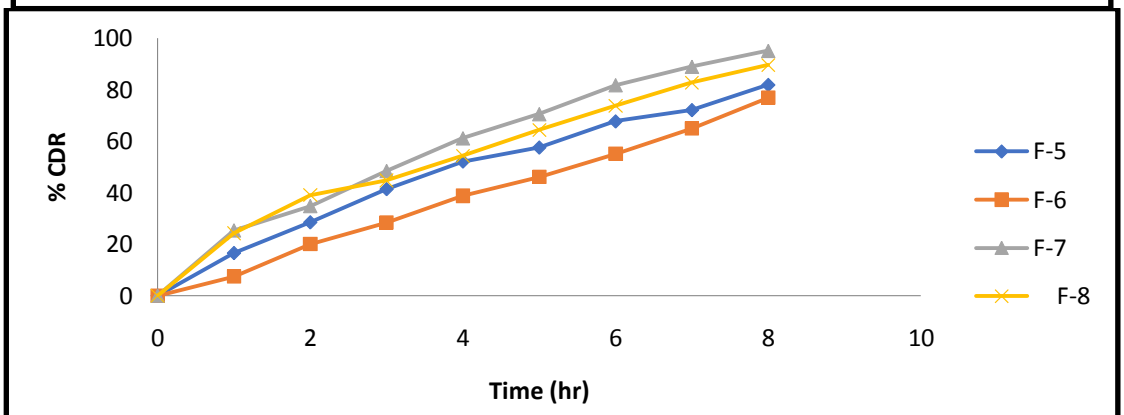

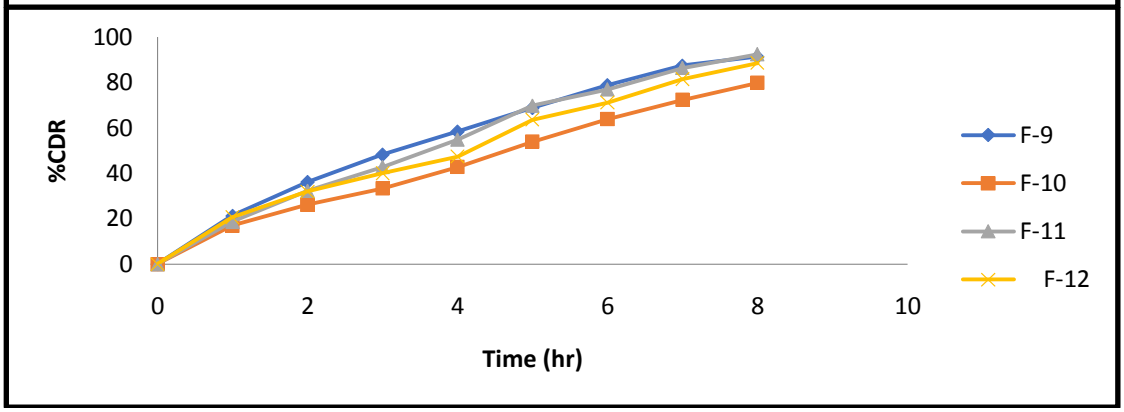

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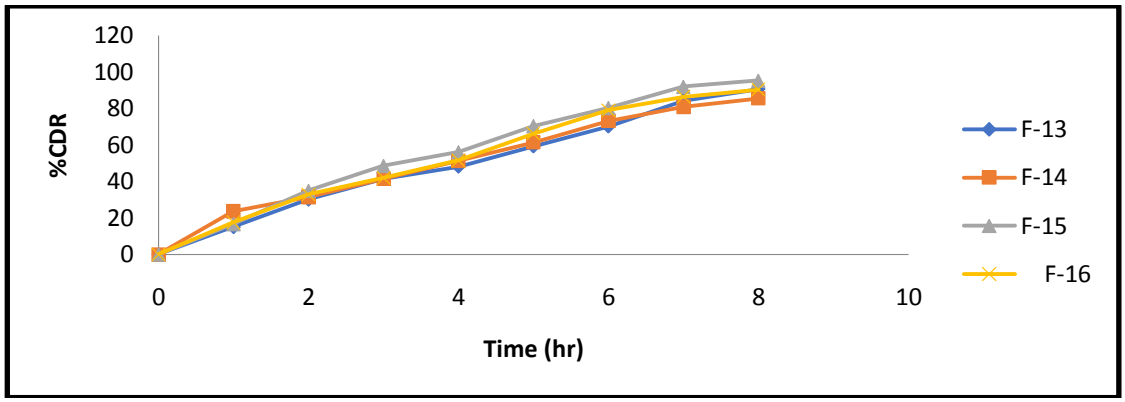

Fig 5,6,7,8 :In Vitro Drug release OF F1-F16 formulations

Optimization formulation:

Formulation's	Drug: polymers ratio	stirring speed (RPM)	curing time (hr)	org solvent (ml)	% surfactant
1	1: 3.73	600	3	24	3.5

Table 6: optimised formulation obtained by software

Dependent variable	Experimental values	Predicted values
% yield	90.78± 5.80	91.53
%DEE	83.45 ± 5.13	87.55
Particle size	167.6 ± 20.32	164.84

Table 7: experiment and predicted values

Time (hr)	%CDR± SD
1	33.36 ± 0.623
2	37.07 ± 1.719
3	40.80 ± 1.770
4	47.86 ± 1.529
5	59.53 ± 1.018

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6	66.90 ± 1.328
7	74.72 ± 1.131
8	85.49 ± 1.153
9	89.27 ± 0.883
10	93.69 ± 1.265

Table 8: %CDR of optimised formulation

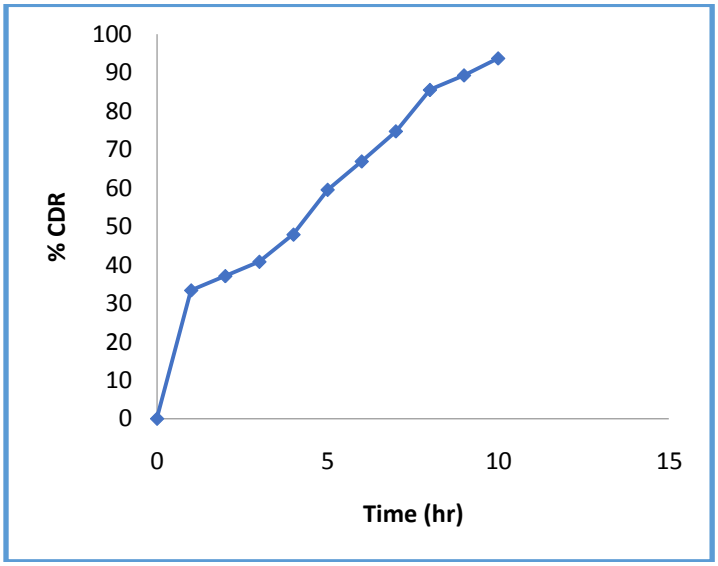

Fig 9: %CDR of optimised formulation

Formulation code	Zero order	First order	higuchi	peppas	hixson	Best fit
1	0.9463	0.9319	0.9882	0.9690	0.8335	Higuchi

n= 0.4982

Table 9: results of kinetic modelling

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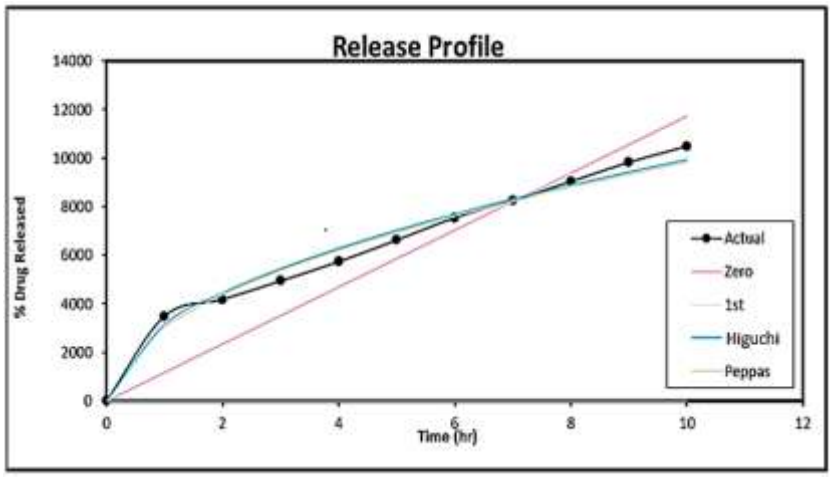


Fig 10: release kinetics of optimised formulation

Stability studies

Morphology: There was no difference observed in appearance of the formulation after stability study.

% Drug entrapment efficiency of the formulations

% Drug entrapment efficiency of the optimized formulation after stability study

Stability condition	Sampling(days)	%DEE
5^0/ Ambient	**30**	**83.4**
	60	**83.4**
25^0/60%RH	**30**	**83.4**
	60	**83.4**
40^0/75%RH	**30**	**83.4**
	60	**83.2**

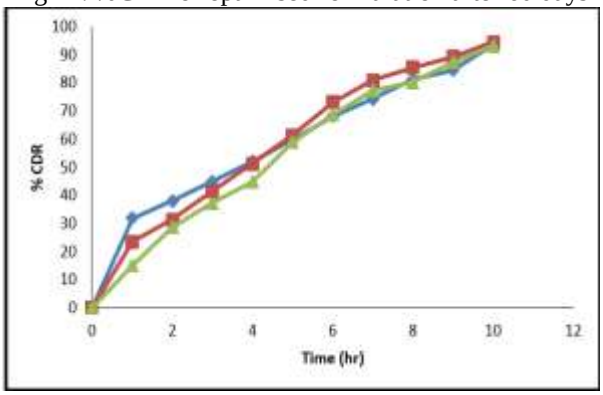
Table 10: results of %DEE after 30 and 60 days of stability studies

In vitro drug release profile of optimised formulation after 60 days

Stability condition	Sampling days	%CDR
5^0/ Ambient	60	93.26
25^0/60%RH	60	94.42
40^0/75%RH	60	93.56

Table 11: results of % CDR of optimised formulation after 60 days

Fig 11: %CDR of optimised formulation after 60 days



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IV. CONCLUSION:

Timolol maleate is an effective antihypertensive drug with good oral bioavailability. Due to its short half-life (2.5-4hrs) and frequent administration, Timolol maleate was selected as candidates for developing sustain release microspheres. A 2^5 half factorial design was employed to produce hydroxypropyl methylcellulose mucoadhesive microspheres of Timolol maleate by non-aqueous solvent evaporation technique. The technique employed was simple and practically viable. By optimizing the levels of drug: polymer ratio, stirring speed, curing time, organic solvent volume and concentration of surfactant a better yield with good entrapment efficiency and sustained release property was obtained. By employing the numerical optimization technique, the number of experimental trials carried out to produce the optimized formulation was considerably reduced thereby substantially cutting down the expenditure on time and money.

REFERENCE:

[1]. Chowdary KPR, Rao YS. Mucoadhesive microspheres for controlled drug delivery. Bio Pharm Bull 2004;27(11):1717-1724.
[2]. Anand S, Surana, Rakhee K, Kotecha. An overview on various approaches to oral controlled drug delivery system via gastro retention. Int J Pharm Sci Rev Res 2010;2(2):68.
[3]. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tract and dosage forms for site specific delivery. Int J Pharm 1996;136(1):117-139.
[4]. Sachin KN, Singh B, Rao RK. Controlled drug delivery through microencapsulation. Malay J Pharma sci 2006;4(1):65-81.
[5]. Andrews G, Thomas P, Jones D. Mucoadhesive polymeric platforms for controlled drug delivery. Eur J Pharm & Bio 2009; 71:505-518.
[6]. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
[7]. Ugwoke MI, Agu RU, Verbeke N, Kinget R. Nasal mucoadhesive drug delivery background applications trends and future perspectives. Adv Drug Del Rev 2005; 57:1640-1665.
[8]. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
[9]. Patil SB, Sawant KK. Mucoadhesive microspheres: a promising tool in drug delivery. Cur Drug Del 2008;5:312-318.
[10]. Chowdary.R, Srinivas.L. Mucoadhesive drug delivery systems: a review of current status. Ind drugs. 2000;37(9):400-405.
[11]. Chowdary KPR, Rao YS. Mucoadhesive microspheres for controlled drug delivery. Bio Pharm Bull 2004;27(11):1717-1724.
[12]. Handbook of pharmaceutical excipients 2009; 6th Edi, Pharmaceutical Press, USA.
[13]. Indian pharmacopoeia 2007, vol.3, Indian Pharmacopoeia Commission, Ghaziabad 2007;398-400.
[14]. Japan Pharmacopoeia, official monographs of part -1, 2002:1483-484.
[15]. Miyazaki Y, Ogihara K, Yakou S, Nagai T, Takayama K. In-vitro and in-vivo evaluation of mucoahesive microspheres consisting of dextran derivatives and cellulose acetate butyrate. Int J Pharm 258;(2003):21-29.
[16]. Obeidat WM, Price JC. Evaluation of enteric matrix microspheres prepared by emulsion-solvent evaporation using scanning electron microscopy. J Microencap 2004;21(1):47-57.
[17]. Obeidat WM, Price JC. Viscosity of polymer solution phase and other factors controlling the dissolution of theophyllene microspheres prepared by the emulsion solvent evaporation method. J microencap 2003;20(1):57-65.
[18]. Kristmundsdottir T, Ingvarsdottir K. Influence of emulsifying agents on the properties of cellulose acetate butyrate and ethyl cellulose microcapsules. J Microencap 1994;11(6):633-9.
[19]. Gungor BE, Cevher E, Bergisadi N. Preparation and in-vitro evaluation of cefadroxil loaded chitosan microspheres. Acta Pharm Sci 2007;49:167-178.
[20]. Tarun K, Mandal, Tenjarla S. Preparation of biodegradable microcapsules of Zidovudine using Solvent evaporation: Effect of the modification of aqueous phase. Int J Pharm 1996; 137:187-197.
[21]. Patel JK, Patel RP, Amin AF, Patel MM. Formulation and evaluation of mucoadhesive Glipizide microspheres. AAPS Pharm Sci Tech 2005:6(1):E49-E54.
[22]. Yadav S, Jain S, Prajapati S, Motwani M, Kumar S. Formulation and in-vitro and in-

vivo characterization of acyclovir loaded Mucoadhesive microspheres. J Pharm Sci Tech 2011;3(1):441-447.

[23]. Tao Y, Lu Y, Sun Y, Gu M, Lu W et al., Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. Int J Pharm 2009;378: 30-36.

[24]. Patil SB and Murthy RSR. Preparation and in-vitro evaluation of mucoadhesive chitosan microspheres of Amlodipine Besylate for nasal administration. Ind J Pharm Sci 2006;68(1):64-67.

[25]. Costa P, Manuel J, Lobo S. Modeling and Comparision of dissolution profiles. Eur J Pharm Sci 2001;13:123-133.

[26]. Tamizharasi S, Rathi JC, Rathi V. Formulation and evaluation of Pentoxifylline-loaded poly (ε-caprolactone) microspheres. Ind J Pharm Sci 2008;70(3):333-337.