Development and Characterization of Doxofylline Hydrogel Beads by Using Single Emulsion Technique

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Abstract

the present work was to development the controlled release Doxofylline hydrogel beads using polymers in various formulations. Doxofylline used to treatment of asthma. These hydrogel beads prepared by using single emulsion technique. Doxofylline causes an irritation in the gastrointestinal mucous membrane and possesses a bitter taste and aftertaste. The half-life in plasma is about 1-2hrs. This makes doxofylline a very good candidate for the formulation of controlled release dosage forms. The formulated were examined by in vitro drug release studies and further perform the stability data for selected or optimized formulation. The work was aimed to develop the controlled release Doxofylline hydrogel beads. Prepared hydrogel beads optimized by considering the drug entrapment efficiency, particle size and in vitro drug release studies. Release of doxofylline was found to be in a controlled manner with increasing polymer content in hydrogel beads and higher release as observed in PH 7.4 medium than that of PH 1.2. In vitro release kinetics of doxofylline from the polymeric beads followed Higuchi kinetics model.

Keywords

Doxofylline, polymers, single emulsion technique, drug entrapment efficiency, in vitro drug release studies.

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1.INTRODUCTION

Hydrogels are water swell able, yet insoluble, cross linked polymer networks synthesized from a variety of hydrophilic monomers.[1] Hydrogels are three-dimensional hydrated network formed by cross linking polymers through either covalent bonds or non-covalent interactions.[2] The high-water content of hydrogels renders them biocompatible to living systems and their soft nature can minimize damages to the surrounding tissues. Due to these reasons, hydrogels have received significant attentions in recent years for biomedical applications, such as drug delivery and tissue engineering. [3,4] Doxofylline, an anti-Asthmatic agent, belongs BCS class-III agent. Asthma is disease of lung airways (bronchi) characterized by hyper active responsiveness to a variety of stimuli and in this condition the airways constricts and becomes inflamed with excess mucus lining the passage. [5]The objective of the present study was to Formulation and evaluate controlled release dosage form of hydrogel beads of polymeric matrix films with suitable polymers containing doxofylline to increase rate of drug release and improve the therapeutic efficacy of the drug and

mainly improve patient compliance with hydrogel beads.[6]

2. MATERIALS AND METHOD
2.1 MATERIALS
Doxofylline was collected as a gift sample from Dr. Reddy's laboratories Hyderabad, HPMC k 5M,

sodium alginate and other excipients were purchased from AR chemicals.

2.2 METHODODOLOGY [7,8,9]
Compatibility study (IR spectroscopy)
The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Formulation development

Table-1: Preparation of hydrogel beads

F.Code	Drug (gm)	HPMC (gm)	Sodium Alginate (gm)	CaCo$_3$ (gm)	Liquid paraffin oil (ml)	Glacial acetic acid(ml)	Tween 20(ml)	CaCl$_2$ (gm)
F1	0.5	0.5	--	0.2	50	10	2	0.5
F2	0.5	1.0	--	0.2	50	10	2	0.5
F3	0.5	0.5	--	0.2	50	10	2	0.5
F4	0.5	1.0	--	0.2	50	10	2	0.5
F5	0.5	--	0.5	0.2	50	10	2	0.5
F6	0.5	0.5	0.5	0.2	50	10	2	0.5
F7	0.5	--	0.5	0.2	50	10	2	0.5
F8	0.5	0.25	0.75	0.2	50	10	2	0.5

Preparation of Doxofylline hydrogel beads
Different concentrations of polymer solutions were prepared by dissolving the specified amount of polymer in 30 ml of hot water, and then drug was dispersed in this polymer solution using magnetic stirrer for 10 min. A suspension of Caco$_3$ was dispersed in to drug polymer solution. After homogenization, the mixture was added into liquid paraffin oil containing different concentrations of emulsifying agent and emulsified at 200 rpm. After emulsification liquid paraffin containing glacial acetic acid mixture was added to w/o emulsion and stirring was continued to permit calcium carbonate solubilization. A solution of Cacl$_2$ containing wetting agent was added to the partition to recover the gelled beads from oily phase by decantation. Hydrogel beads were washed with Cacl$_2$ containing emulsifier to remove residual oil. Hydrogel beads were removed from oily phase by using an acetate buffer at pH 4.5 and successively washed with this buffer until no more oil was detected by optical microscope observation. A sample of prepared beads for formulae is examined under optical microscope to detect the presence of oil droplets. Furthermore, a sample of the prepared beads is pressed between two filter papers to detect the presence of any oily droplets and finally hydrogel beads were dried for 48 hrs. at room temperature.

Evaluation [10,11,12]
Particle size
All the prepared batches of Hydrogel beads were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of hydrogel bead on it and average size of Hydrogel beads were determined.

SEM analysis
The morphology of hydrogel beads was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the hydrogel bead was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Drug encapsulation efficiency
Doxofylline 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Doxofylline hydrpgel beads in to the theoretical amount of the drug used in the preparation. The entrapment of the Doxofylline hydrogel beads was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In vitro drug release studies

The release studies were carried out by franz diffusion cell. It is containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken, and one end of the membrane was sealed. After separation of non-entrapped Doxofylline dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug in the patch; Da = The amount of drug released

Drug release kinetics

Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile.

Various mathematical models are:

% drug release = concentration × no.of dilutions × volume of dissolution fluid/1000

Zero Order Release Equation:

The equation for zero order release is

$Q_t = Q_o + K_o t$

Where , Q_o = Initial amount of drug; Q_t = Cumulative amount of drug release at time "t"; K_o= Zero order release constant; T= Time in hours

First Order Release Equation:

The first order release equation is**:**

$\text{Log } Q_t = \text{Log } Q_o + K_t / 2.303$

Where, Q_o = Initial amount of drug; Q_t = Cumulative amount of drug release at time "t"; K= First order release constant= Time in hours

Higuchi Release Equation

The Higuchi release equation is: $Qt = K_H \sqrt{t}$

Where, Q = Cumulative amount of drug release at time "t"; K_H = Higuchi constant; T = Time in hrs

Korsmeyer -Peppas Release Equation

Korsmeyer –Peppas equation is

$F = M_t / M = K_m t^n$

Where, F = fraction of drug released at time 't'; Mt = amount of drug released at time 't'; M = total amount of drug in dosage form; K_m= kinetic constant n = diffusion or release exponent; t = time in hrs; 'n' = Linear regression of log (M_t / M) versus log t

Stability studies

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^0C/60% RH analyzed every month for period of three months.

2. 30^0C/75% RH analyzed every month for period of three months.

3. 40^0C/75% RH analyzed every month for period of three months.

3.RESULTS AND DISCUSSION

FTIR studies

The IR Spectra of drug-sodium alginate did not show much changes. The possibility of interaction was ruled out as there was no major shift in absorption bands of the drug and physical mixture, shows that there is no appearance or disappearance of peaks. It is therefore, expected that the drug and polymer are compatible and free from chemical interactions.
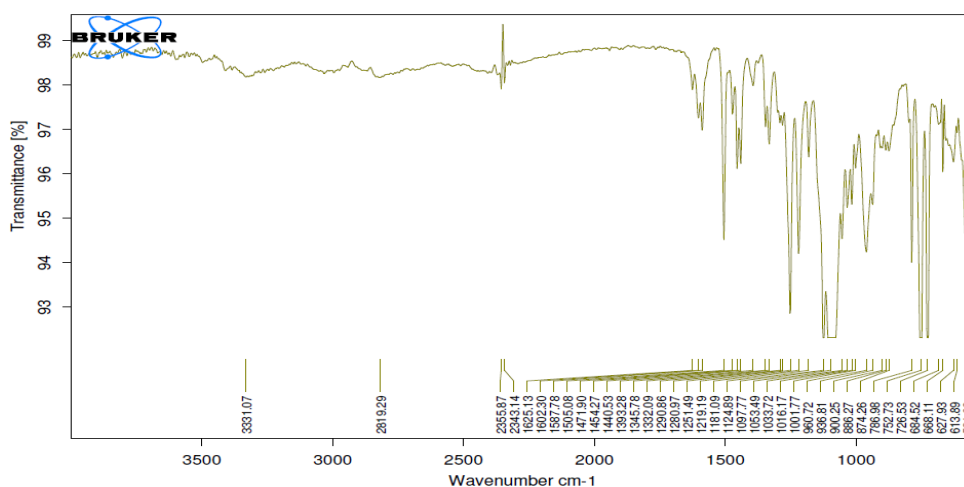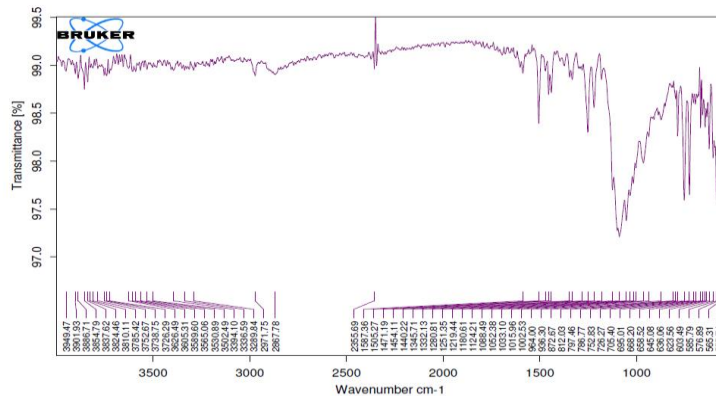
Fig-1: FT-IR spectra of Doxofylline

Fig- 2: FT-IR spectra of optimized formulation

Scanning electron microscopy analysis (SEM) for hydrogel beads

The shape and surface characteristics were determined by scanning electron microscopy using gold sputter technique. The hydrogel Beads were Vacuum dried, coated to 200 A° thicknesses with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15 kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.
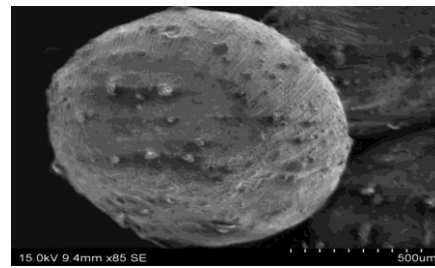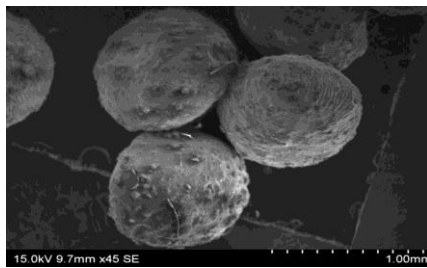

Fig-3: Scanning electron microscopy analysis (SEM) for Optimized formula

The optimized formulation F6 was evaluated for its surface morphology by using SEM analysis. The particle size was found to be 500μm. The beads were found to be smooth and spherical in shape.

Evaluation of hydrogel beads

Determination of drug entrapments efficiency, drug loading, and yield, swelling ratio

Table-2: Evaluation of percentage yield, drug entrapment efficiency, drug content

F. code	Particle size(μm)	% yield	Entrapment efficiency	Drug content
F1	515	55	81.25	97.56
F2	525.5	70	85.78	94.52
F3	512.5	75	83.80	96.72
F4	550.2	82	89.65	97.25
F5	528.1	82	93.88	98.75
F6	546.2	78	85.58	96.20
F7	515.9	75	90.20	97.38
F8	520.5	72	88.80	92.15

DISCUSSION

Particle Size

The particle size values ranged from 512μm to 550.2 μm for all formulations. Increase in the polymer concentrations an increase in the particle size of the beads was observed.

Percent yield

The percentage (%) yield values ranged from 55 to 82 for all the formulations.

Entrapment efficiency

For selected formulation (and sodium alginate) entrapment was found to be more in F5. So it is indicated only optimum concentration is suggestable. From the above result F5 (drug and sodium alginate) was selected as a optimized formulation.

Drug Content

The drug content ranged from 99.99±0.50% to 96.71±0.33% for all the formulations. The drug content for all the formulations was found to be within the limits.

In vitro dissolution profiles

Table-3: Dissolution data of prepared hydrogel beads

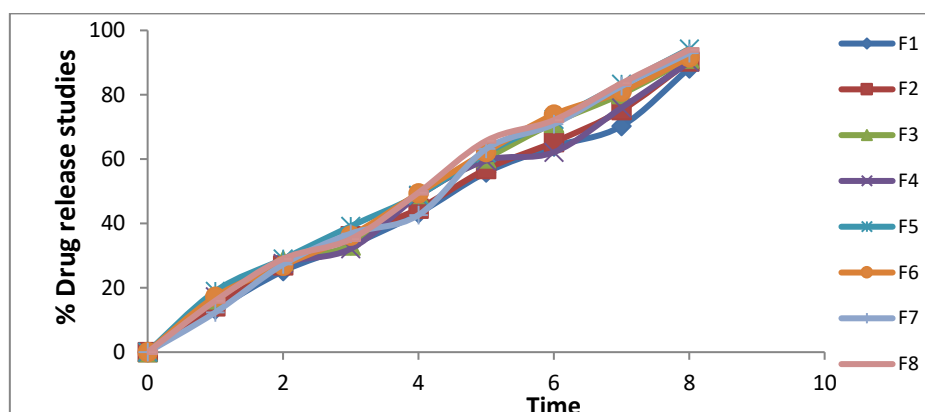| Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	13.23	14.26	16.39	17.22	18.95	17.25	12.36	15.98
2	25.19	26.98	28.44	27.55	28.96	26.93	27.25	28.98
3	33.24	35.99	33.15	32.15	39.15	36.23	36.84	35.15
4	43.19	44.58	49.15	48.56	48.79	49.36	42.63	49.58
5	55.85	56.89	60.23	59.55	61.88	62.23	63.15	65.68
6	63.89	65.28	71.25	62.13	72.32	73.85	70.83	72.11
7	70.28	75.21	80.21	75.96	83.26	80.99	82.65	83.55
8	88.21	90.36	91.25	90.14	94.26	91.62	92.99	93.96

Fig-4: Dissolution profiles of prepared formulations F1 to F8

All the Eight formulations of hydrogel beads were subjected to dissolution studies. Dissolution was carried out in USP type I apparatus at 100 rpm in the volume of 900ml dissolution media of 0.1N HCL for initial 2 hours then in 6.8 pH phosphate buffer for next 6 hours. The drug was released prior to the predetermined lag time (5 hours) in all the four formulations so further work was done by changing the polymer. F5 showed a release rate of 94.26 by end of 8th hour of dissolution study. In the formulation F5 lag time was maintained and drug released after 8 hours. It was observed that a proper lag time of 5 hours was maintained for the sodium alginate.

Kinetic models:

Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations and Korsmayer Peppas equations.

Table-4: Dissolution data for kinetic models

S.NO	Time	log T	Square root of Time	%CR	%Drug remaining	log %CR	LOG% drug retained	cube root of %drug remaining
0	0	0	0	0	100	0	2	4.641589
1	1	0	1	18.95	94.17	0.765669	1.973913	4.549575
2	2	0.30103	1.414214	28.96	92.79	0.857935	1.967501	4.527242
3	3	0.477121	1.732051	39.15	91.5	0.929419	1.961421	4.506164

| S.NO	Time	log T	Square root of Time	%CR	%Drug remaining	log %CR	LOG% drug retained	cube root of %drug remaining
4	4	0.60206	2	48.79	90.28	0.987666	1.955592	4.486047
5	5	0.69897	2.236068	61.88	89.7	1.012837	1.952792	4.47642
6	6	0.778151	2.44949	72.32	27.6	1.859739	1.440909	3.02206
7	7	0.845098	2.645751	83.26	11	1.94939	1.041393	2.22398
8	8	0.90309	2.828427	94.26	0.4	1.998259	-0.39794	0.736806

(a) Zero order kinetics:
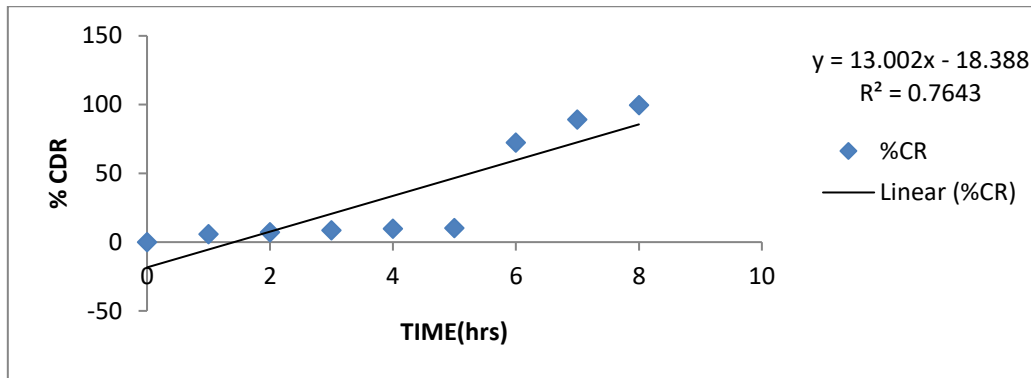
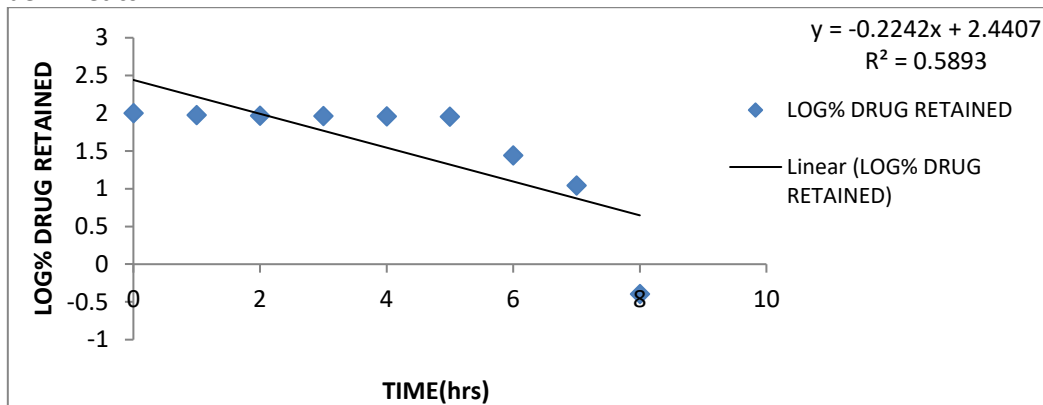Fig-5: Zero order plot for optimized formulation

First order kinetics

Fig-6: First order plot for optimized formulation
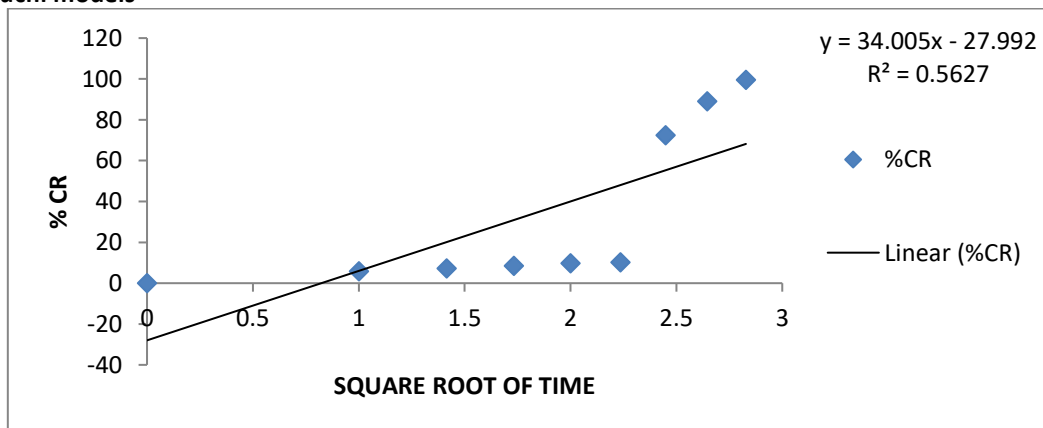
(c) Higuchi models

Fig-7: Higuchi plot for optimized formulation

(d) Korsmayer peppas equations:

$y = 1.4372x + 0.4677$
$R^2 = 0.6677$

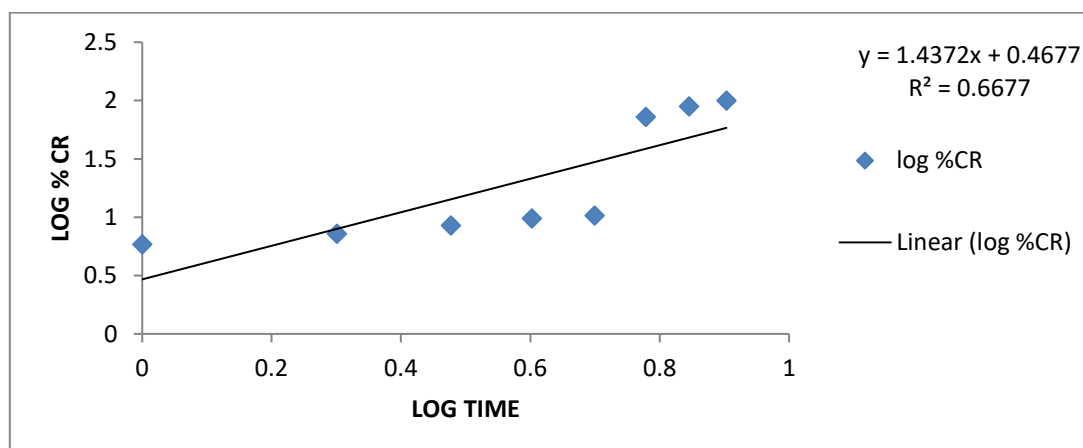◆ log %CR

—— Linear (log %CR)

Fig-8: Kors mayer peppas plot for optimized formulation

The *in-vitro* drug release was fitted to the various kinetic models. The regression coefficient (R2) value for zero order, first order, higuchi's, Hixon crowell and pepas were plotted for formulation F5 and (R2) was found to be 0.764, 0.589, 0.562, 0.703 and 0.667 respectively. The drug release kinetics followed the zero-order kinetics.

Stability study
There was no significant change in physical and chemical properties of the Hydrogel beads of Optimized formulation F-5 after 3 Months of stability studies.

Table-5: Results of stability studies of optimized formulation F-5

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-5	25^{0}C/60%RH % Release	94.26	94.25	94.23	94.21	Not less than 85 %
F-5	30^{0}C/75% RH % Release	94.26	94.21	94.22	94.20	Not less than 85 %
F-5	40^{0}C/75% RH % Release	94.26	94.22	94.23	94.21	Not less than 85 %

From the above result it can be concluded that there was no significant change in physical and chemical properties of the Hydrogel beads of formulation F-5 after 3 Months.

4.CONCLUSION
By studying all the experimental results of the prepared Doxofylline, the results suggest that hydrogel beads containing anti asthmatic drug like Doxofylline were successfully formulated by ionotropic gelation technique by using sodium alginate, sodium alginate and combination of sodium alginate as polymers and calcium carbonate as cross linking agent to produce sustained release delivery system. The prepared hydrogel beads were strong spherical with narrow size distributions could be prepared with high yields and good entrapment efficiencies. The mechanism of drug release from Doxofylline exhibited first-order kinetics and the release of the drug from the hydrogel beads was found to be following case-II transport. The data suggest that a promising controlled release micro particulate drug delivery of Doxofylline can be developed. Further detailed investigation is required to establish efficacy of these formulations. Stability studies of the formulations. Further in-vivo investigation is required to correlate in-vitro release studies. Further preclinical and clinical study is necessary for the use of Doxopylline hydrogel beads as oral controlled drug delivery. Bioavailability study in human volunteers is necessary to establish drug product.

5.REFERENCES

1. Gokhle AB, Saraf MN, Recent Development in asthma therapy, Indian Drugs 39(3) MARCH 2002, P-121
2. http://www.medicinenet.com/asthma/page3.htm
3. Moore, R. Y., Circadian rhythms: basic neurobiology and clinical applications. Ann. Rev. Med. 1997 48: 253-266.
4. Tintu Sara James.et.al., Design and characterization of salbutamol sulphate hydrogel beads as Sustained Release. IJPI's Journal of Pharmaceutics and Cosmetology, Vol 2: 9 (2012).
5. Sanjay Kumar Panda.et.al., formulation and evaluation of losartan hydrogel beads IJPSR, June 2013; 2(6):1-8.
6. M. S. Khan, B. K. Sridhar, and A. Srinatha, Review article on Hydrogel beads, Indian J Pharm Sci.2010 Jan-Feb;72(1):18-23.
7. Sivakumar Vellaichamy.et.al., design, prepare and characterization of Salbutamol by ionotropic gelation, IJPAS.
8. M. Vani.et.al., design, prepare and in vitro evaluation of GRDDS beads of ranitidine hydrochloride, Int J Pharm 1(1), 1-4.
9. Debendra Kumar Mohapatra.et.al., development and characterization of domperidone sublingual tablets. Issue 2, Volume 2 (April 2012).
10. Amit Kumar Nayak.et.al., Hydrogel beads of gliclazide: Design, development, and evaluation, Science Asia 36 (2010).
11. Manjanna K.M., Shiva kumar.B, Pramod kumar T.M., Formulation of Oral Sustained Release Aceclofenac Sodium Microbeads, IJPRIF, Vol.1, No.3, pp 940-952, July-Sept 2009.
12. 12.VM. Sherina, K. Santhi and C.I.Sajeeth., Formulation and Evaluation of tragacanth hydrogel beads as a Carrier for the Controlled Release of Nifedipine, Vol. 1 (2) Apr – Jun 2012.