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FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERE LOADED WITH KETOPROFEN FOR COLON TARGETTING

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ABSTRACT

The main purpose of present work is to design a colon-specific drug delivery of Ketoprofen multi particulate systems by using sodium alginate and pectin polymers. Ketoprofen a non steroidal anti inflammatory drug was formulated as microsphere to provide the controlled action and to minimize the local side effects of Ketoprofen by avoiding the drug release in upper gastrointestinal part. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs also known as microparticles. These microspheres are spherical in shape and having rough surface. The microsphere is prepared by ionotropic gelation method by using calcium chloride the cross-linking agent and coated with eudragit polymer for achieving colon specific by combining both pH and polymer based drug delivery. The coating is carried out by oil in oil solvent evaporation method. The prepared microsphere was subjected to various evaluation test such as particle size analysis, entrapment efficiency, swelling studies and in -vitro dissolution studies. The % drug release was highest for the formulation F1 and it also shows the controlled release. Microspheres revealed the absence of drug-polymer interactions. In this present study concluded that eudragit coated microsphere are controlled release for colon targeted delivery of Ketoprofen.

Keywords: Pectin, Sodium alginate, Colon targeting, Ketoprofen.

INTRODUCTION

Microspheres are multi-particulate drug delivery system prepared to obtain extended and controlled drug delivery, to improve bioavailability or stability and to target specific sites. The microsphere is sometimes referred to as microparticles and these a small spherical particle with the diameter in the micrometer range.(0-1000µm).microspheres can be manufactured from various natural and synthetic material and have several applications. The Oral route is the most convenient and preferred route but other routes such as rectal are also used for colon-specific drug delivery system. Rectal administration offers the shortest route for targeting drugs to the colon. However reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also uncomfortable for patients. Microencapsulation is widely used in the pharmaceutical and other sciences to mask tastes or odors, prolong release, impart stability to drug molecule, improve bioavailability, and as multi-particulate dosage forms to produce controlled or targeted delivery. it is, therefore a rapidly expanding technology for achieving controlled release dosage form [1]. Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drugs to the colon. (ie,pectin, chitosan, cyclodextrin, and dextran). pectins are polysaccharides components of plant cell walls and are a family of complex polysaccharides that contain 1,4-linked alpha D-galactosyl uronic resideus. The ability of pectin to rapidly form viscous solutions and gels on contact with aqueous media has been exploited by the pharmaceutical industry in its wide application as a carrier in oral controlled release dosage forms. Sodium alginate a water-soluble salt of alginic acid is a natural polysaccharide extracted from marine brown algae. Sodium alginate has been used a matrix for entrapment of drugs and macromolecules. A mixture of pectin with other polysaccharides such as alginate has combined that good gels are formed from high methoxy pectin and guluronic rich alginates. In ionic cross-linking method dropping or spraying a sodium alginate solution into a calcium or barium chloride solution produce microcapsule. The divalent calcium or barium ions crosslink the alginate form gelled droplets [2]. Ketoprofen is poorly water soluble and one of the most widely used therapeutic substances due to its analgesic, antipyretic, and anti-inflammatory properties. Ketoprofen inhibits the inflammation by acting on

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cycloxygenase. It also inhibits the lipoxygenase pathway leading to decrease in the production of leukotrienes by the leukocyte and the synovial cells. It also makes T cell suppressing the production of rheumatoid factor. Despite the proliferation a development of new non-steroidal anti-inflammatory drugs (NSAIDS), Ketoprofen remains one of the most effective 'over-the-counter' drugs in the treatment of rheumatoid arthritis disease. Use of Ketoprofen with two major limitations first, gastrointestinal side effects including ulceration, gastrointestinal bleeding, and hemorrhage especially in elderly and second, poor water solubility. Successful targeted delivery of drugs to the colon requires the protection of a drug from degradation, release and or absorption in the stomach and small intestine and then ensure controlled release in the proximal colon. This might be achieved by the use of a specially designed drug delivery system that can protect the drug during its transfer the colon. In this work multi-particulate system of Ketoprofen was developed by utilizing the Ph –dependent solubility of eudragit polymers and microbial degradability of pectin polymers [3]. Drug loaded pectin microsphere was prepared and then coated with the eudragit polymer. This polymer shows the solubility at or above pH 7.

MATERIALS AND METHODS

Materials used included Ketoprofen, pectin, sodium alginate was kindly provided as a gift sample by Yarrow Chem Products Mumbai-421201(India). All other excipients and chemicals were used from analytical grade.

PREFORMULATION STUDIES

Preformulation is the first step in the rational development of dosage forms.it can be defined as an investigation of physical and chemical properties of drug substance alone and when combined with the excipient.

Identification by FTIR spectroscopy

Ketoprofen KBr discs were prepared by pressing the Ketoprofen with potassium bromide in the ratio of 1:99%/w and the spectra were recorded between 400-1-4000-1cm-1 using FTIR spectrophotometer.(SHIMADZU 84500, Tokyo, Japan).

Identification of melting point

The melting point of the drug was determined by an open capillary method. The melting point temperature was noted when the drug just starts melting until it completely melts.

Organoleptic properties

The Ketoprofen sample was studied for organoleptic properties such as color, odor, and appearance.

Formulation

The pectin sodium alginate microsphere was prepared by ionotropic gelation technique. First, required amount of pectin was dispersed in a specified volume of distilled water containing the drug and allowed to swell for 2 hours. In another beaker suitable amount of sodium alginate was taken and mixed well with 10 ml of water. The pectin solution containing the drug was added to sodium alginate solution with stirring for 15-20 minutes to produce a viscous form. Then the polymer-drug solution was added drop wise by using syringe having the needle of 26 G into a beaker containing different concentrations of the solution of calcium chloride from a specific height. So the gelatinous precipitate is formed by chemical reaction between sodium alginate and calcium chloride. The prepared microsphere then removed by filtration and washed with distilled water and dried and stored in the well closed container [4].

Coating Procedure

Pectin sodium alginate microsphere was coated with eudragit S-100 using oil-in-oil solvent evaporation method. Pectin sodium alginate microsphere (50 mg) were dispersed in 10 mL of coating solution prepared by dissolving 500 mg of Eudragit S in ethanol: acetone (2:1) to give 5:1(coat: core ratio).This organic phase was then poured into 70 mL of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of the solvent. Finally, the coated microsphere was filtered, washed with n-hexane, and dried in desiccator.

EVALUATION OF PECTIN SODIUM ALGINATE MICROSPHERE

Percentage Yield

The percentage yield of various batches of Ketoprofen microsphere was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for the preparation of microspheres and % yield was calculated as per the formula mentioned below [5].

Percentage yield = (Total weight of microparticle / Total weight of drug and polymer) x100

Entrapment efficiency

Microspheres containing the equivalent to 100mg of the drug was allowed to equilibrate in 25ml of phosphate buffer ph 7.4 for 24hr.The solution was filtered using Whatman filter paper. The resulting solution was analyzed using UV spectrophotometric method at 276nm in the presence of a blank prepared from microspheres containing all materials except the drug.

% Entrapment = (Actual content / Theoretical content) x 100

Particle size analysis

The particle size analysis was used to found the particle size of microspheres. The particle size analysis was performed by optical microscopy [6].

Micromeritic Properties of microsphere

Bulk density

Bulk density largely depends on particle shape, as the particles become more spherical in shape, bulk density increases. In addition as granules size increase, bulk density decrease. Bulk density was determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup. A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit. The bulk density was calculated in gm per ml gm/cc, by the formula [7].

Bulk Density = Mass of Powder/ Bulk Volume

Tapped density

It is the ratio of total mass to the tapped volume of the microsphere. The tapped volume was measured by tapping the microsphere to constant volume. It is expressed in gram/ml and is given by

Tapped Density = Mass of powder/ Tapped Volume

Compressibility index (Carr's index)

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities.

Carr's index a material having values of less than 20% to 30% is defined as the free flowing material.It can be calculated as per given formula

Carr's index = (Tapped density – Bulk density / Tapped density)x100

Hausner ratio

It indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density.

Hausner ratio = Tapped density / Bulk Density

Flow properties

The angle of repose is a relatively simple technique for estimating the flowability of a powder through a funnel and fall freely onto a surface. The height and diameter of the resulting cone are measured and using the following equation, the angle of repose can be calculated.

$\tan \theta = h/r$

Where h, r is the relative height and radius of the powder cone. For most pharmaceutical powders, the angle of repose values ranges from 25 to 45, with lower values indicating better flow characteristics. Values of angle of repose $\leq$ 30 usually indicate a free flowing material and angle $\geq$40 suggest a poorly flowing material.

Swelling index

swellability of Mucoadhesive microspheres in physiological media was determined by swelling them in the PBS pH 7.4. Accurately weighed 100 mg of microspheres were immersed in the little excess of PBS pH 7.4 for 24 hrs and washed. The degree of swelling was calculated using following formula:

$\alpha = (Ws-Wo) / Wo$

α is the degree of swelling; Wo is the weight of microspheres before swelling; Ws is the weight of microspheres after swelling [8].

***In-vitro* dissolution studies**

Drug release was performed using USP dissolution rate test apparatus(Apparatus 1, 100 rpm, 37±0.5°C) for first 2h in 0.1 N HCL (900ml). Then, 1.7g of KH2PO4 and 2.225g of Na2HPO4.2H2O were added, adjusting the pH to 4.5 by adding 1.0 M NaOH. A release study was continued for another 2h. at predetermined time intervals 2 ml samples were withdrawn and replaced by an equal volume of fresh medium. After 4h, the pH of the dissolution medium was adjusted to 7.4 and maintained for 24 hrs. Samples were filtered, diluted and assayed at each interval for Ketoprofen content released at λmax of 277 nm using double beam UV-spectrophotometer [9].

Release kinetics and mechanism of drug release

The rate and mechanism of drug release from the prepared microspheres were analysed by fitting the dissolution data into various kinetic models like zero order , first order, korsemeyer peppas, Higuchi's model and coefficient of determination r^2 values were calculated for their linear curves by regession analysis of the above plot.

RESULTS AND DISCUSSION

Melting point

The melting point of the Ketoprofen was found to be 93.50c.

Organoleptic property

The organoleptic properties shows that Ketoprofen is an white or off-white, odorless, nonhygroscopic,fine to granular powder with slightly soluble in water.

Fourier transform infrared (FT-IR) study

An IR spectrum of pure ketoprofen showed the peaks 2625cm1 (alkyl C-H Strech), 1648cm1 (Ketone C=O strech), 1730 cm1 (carboilic acid C=O strech). The polymer pectin shows the peak 1740cm-1 (C=O stretch) and polymer sodium alginate shows the peak 2926cm-1. These characteristic peaks of Ketoprofen were not affected with polymers.This indicated that there was no interaction between Ketoprofen and polymers sodium alginate , pectin and eudragit.

Particle size and percentage yield

Several batches of Ketoprofen microspheres prepared using different polymer: drug ratios yielded microspheres in the size range between 410µ - 810µ. The particle size of the formulation was determined by optical microscopy and the Particle size analysis values were tabulated in Table 2. By increasing the calcium chloride concentration, the degree of crosslinking in the particles

increases which in turn reduces the size of the microsphere while percentage yield increases with increasing concentration of polymer and the cross-linking agent.

Flow property and entrapment efficiency

The angle of repose value ranges from 25.45 - 30.11 which indicates that good flow property to the microsphere. The entrapment efficiency of the controlled release microsphere was also studied and the values were found to be in the range b/w 51.4 -70.5. The higher value of entrapment efficiency for the F1 formulation.

Swelling index

The swelling ability of the microsphere on the physiological media was determined and was found to be in the range of 0.10-0.32 and result shown in Table 4. As a result of increasing the polymer concentration and high degree of cross-linking produced particle that reduces the permeability of the particle and the overall swelling of polymer decreased significantly.

In vitro dissolution studies

It was seen from the observation data that the cumulative percentage release from all the drug loaded batches of microspheres within the range of 37.23% to 47.34% in 12 hours study and it was found that the % cumulative release in microspheres was maximum for the batch F1 is 47.34%. The drug release is greatly influenced by the ratio of polymer and the % of calcium chloride..the drug release rate is decreased as the concentration of pectin increased. It was also concluded that the drug release rate was decreased crosslinking increased. Increasing calcium concentration led to a greater degree of cross-linking and limiting swelling patterns and subsequent drug release from the microsphere. Moreover, greater the amount of polymer, thicker the layer of polymer was formed around the drug particles and more effectively the polymer would hold the drug with itself. Which caused the slow release of drug from microsphere. The results show in table 5 which shows that the drug release decreases with increasing polymer concentration.

Release kinetics

In order to determine the mechanism of drug release from the formulation, the invitro dissolution data was fitted to zero order, first order, Higuchi plot and korsemeyer Peppa's plot. The drug release from the formulation fits well with Higuchi model followed by zero order, first order and kors eme yer-Peppa's model. The invitro release data multi-particulate to Korsmeyer Peppa's model which is generally used to analyze the release mechanism. The value of release exponent ‘n' obtained for the best formulation was found to be 0.988 suggesting probable release by super case II transport. The correlation coefficient of F1 is high for zero order model suggesting that follows zero order kinetics which indicates that the best formulation was found to be controlled release.

Table 1. Formulation table of microsphere

Formulation code	Concentration of pectin(%w/v)	Concentration of Na alginate(%w/v)	Concentration of calcium chloride (%w/v)
F1	1	2	5
F2	2	2	5
F3	3	2	5
F4	1	2	10
F5	2	2	10
F6	3	2	10
F7	1	2	15
F8	2	2	15
F9	3	2	15

Table 2. Particle size distribution and percentage yield of drug loaded microsphere.

Formulation code	Mean diameter (µm)	Percentage yield(%)
F1	650	85
F2	732	75
F3	810	74
F4	530	91
F5	680	79
F6	765	81.25
F7	410	87.5
F8	520	89
F9	635	91.6

Table 3. Flow properties of drug loaded microsphere.

Formulation code	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner's Ratio
F1	25.45	0.302	0.357	15.40	1.18
F2	26.56	0.280	0.318	11.94	1.13
F3	25.96	0.247	0.327	16.20	1.19
F4	27.47	0.295	0.347	14.98	1.17
F5	28.05	0.302	0.368	17.93	1.21
F6	27.05	0.347	0.421	17.57	1.21
F7	28.81	0.280	0.327	14.37	1.16
F8	30.11	0.287	0.337	14.83	1.17
F9	27.47	0.370	0.368	15.78	1.18

Table 4. degree of swelling and % entrapment efficiency of drug loaded microsphere

Formulation code	% Entrapment efficiency	Degree of swelling
F1	70.5	0.32
F2	60.06	0.30
F3	60.86	0.27
F4	64.53	0.25
F5	58.82	0.22
F6	51.60	0.19
F7	61.6	0.15
F8	53.76	0.12
F9	51.4	0.10

Table 5. Invitro release profile of Ketoprofen loaded microsphere for colon targeting

Cumulative % drug release									
Time(hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	1.12	1.2	1.35	3.37	1.23	1.12	0.33	0.45	3.71
2	3.935	5.7	2.36	5.06	1.68	2.91	3.03	5.67	4.95
3	8.1	8.775	4.38	6.63	6.86	4.27	8.1	7.2	8.4
4	10.8	12.9	6.86	10.8	8.88	8.1	11.02	12.82	11.02
5	13.05	14.17	9.56	14.06	9.11	10.68	13.83	14.7	13.46
6	20.47	21.93	17.1	27.78	22.5	14.85	22.27	25.87	23.62
7	22.5	29.02	20.58	31.61	24.75	18.25	26.66	29.8	24.75
8	27.67	33.52	23.62	34.31	27	21.71	31.16	33.6	27.33
9	32.71	38.36	27.78	37.23	30.46	27.67	34.87	36.22	29.47
10	35.1	41.06	32.71	41.51	33.84	32.17	37.68	38.7	33.18
11	42.16	43.96	36.55	42.5	40.83	36.11	39.2	41.25	35.43
12	47.34	45.55	39.45	44.77	43.08	37.23	44.25	41.51	38.1

Table 6. Release kinetics of drug loaded microsphere

Formulation code	Zero order	First order	Higuchi model	Korsmeyer peppas model
F1	0.984	0.960	0.943	0.988
F2	0.983	0.977	0.961	0.959
F3	0.975	0.961	0.932	0.973
F4	0.961	0.964	0.940	0.898
F5	0.969	0.958	0.935	0.912
F6	0.976	0.960	0.931	0.994
F6	0.987	0.981	0.971	0.963
F8	0.963	0.973	0.966	0.919
F9	0.973	0.982	0.959	0.935

Fig 1. FTIR spectrum of Ketoprofen

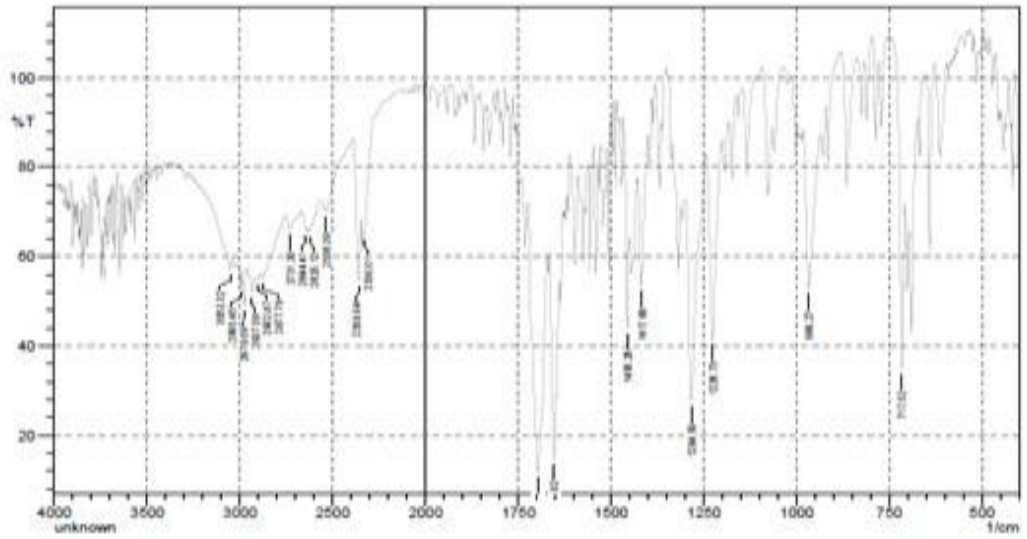


Fig 2. FTIR spectrum of pectin.

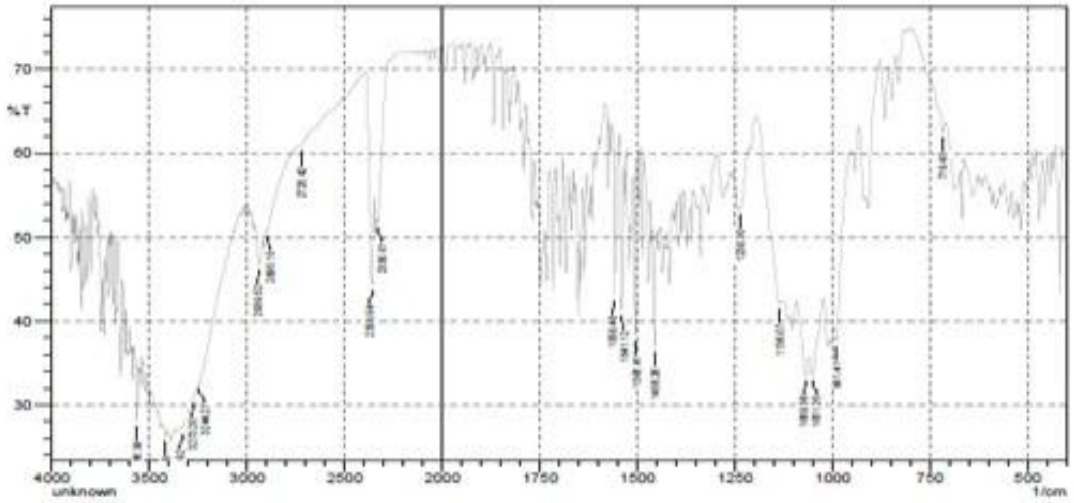


Fig 3. FTIR spectrum of Sodium alginate

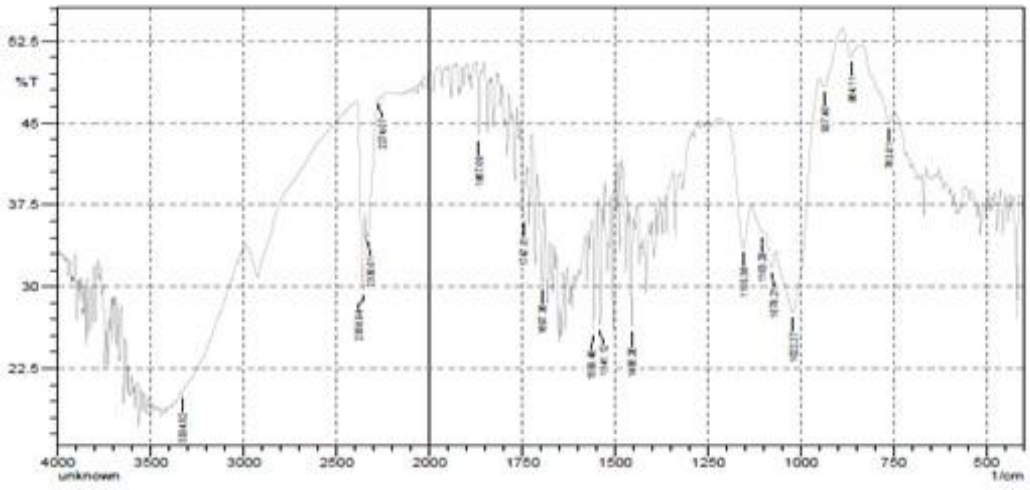


Fig 4. FTIR spectrum of Ketoprofen+pectin+sodium alginate

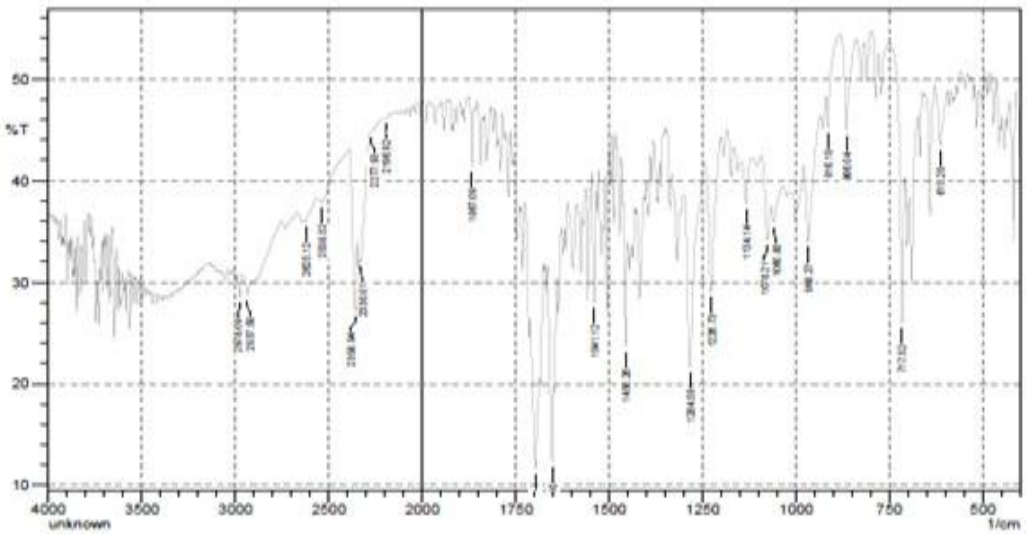
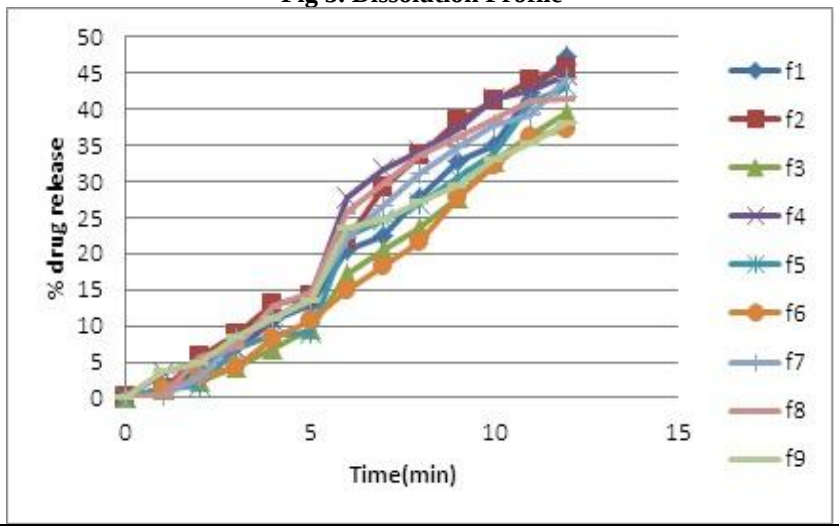


Fig 5. Dissolution Profile



CONCLUSION

Controlled release drug delivery employs drug encapsulating devices from which therapeutic agents may be released at a controlled rate for long period of time, ranging from days to months. Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their ability to encapsulating a variety of drugs. In the present investigation colon, specific enteric coated microspheres of Ketoprofen were prepared by ionotropic gelation method and were evaluated for different parameters. The entrapment efficiency and invitro drug release were found to be greatly influenced by the cross linking agent. Increasing concentration of the counter-ion led to a greater degree of cross-linking of pectin chains and reducing swelling and drug release. It could be concluded that Eudagit coated pectin sodium alginate microsphere can be used for effective and controlled delivery into the colon.

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