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FORMULATION AND EVALUATION OF ATOMOXETINE HCL MUCOADHESIVE MICROSPHERES

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ABSTRACT

Atomoxetine HCl is a potent inhibitor of the presynaptic norepinephrine transporter with minimal affinity for other monoamine transporters or receptors and is the first non-stimulant medication approved for the management of attention deficit/hyperactivity disorder (ADHD) in children, adolescents and adults. Drug formulating into mucoadhesive microspheres is a modern and a more innovative approach used widely to prolong the drug release pattern and also for targeted drug release.The plasma half of Atomoxetine range is 5.2hrs and the dosage regimen according to FDA dosing guide is start at 40 mg, target at 80mg, maximum 100mg twice a day. Hence, Atomoxetine was chosen as a model drug for prolong action and to improve patient compliance. Atomoxetine HCl mucoadhesive microspheres were prepared by ionotropic gelation technique. The mucoadhesive microspheres were prepared with different polymers, i.e, Sodium alginate, pectin and karaya gum with different combinations. From the studies conducted in this work, it can be concluded that the release of Atomoxetine Hydrochloride can be retarded by encapsulating it in calcium alginate spheres. The optimum formulation, Drug–Sodium alginate–Kharaya gum (1:0.75:0.75) mucoadhesive spheres demonstrated controlled release of the drug for 10hrs and exhibited good mucoadhesive property. The FTIR studies revealed the absence of the drug –polymer interaction in the solid state. The formulated mucoadhesive microspheres of Atomoxetine Hydrochloride can control the drug release; it has good mucoadhesive property and can improve the bioavailability of the drug.

Key words: Atomoxetine Hydrochloride, Sodium Alginate, Pectin, Kharaya gum, Calcium Chloride, Ionotropic gelation method.

INTRODUCTION

The most desirable and convenient method of drug administration is the oral route due to the ease of administration and patient compliance. One limitation for oral delivery is poor bioavailability and for the drug candidates who show absorption window in the proximal gut and is the major obstacle to the development of controlled release formulation. A number of approaches have been developed to increase the time of dug formulation. One of the approaches the formulation of Gastro retentive dosage forms in the form of Mucoadhesive microspheres. Microsphere carrier systems, made from natural polymers are attracting considerable attentions for several years, for sustained drug delivery. Today, those dosage forms which can control the release rates and which are target specific have a great impact in development of novel drug delivery systems. Microspheres are part of such novel delivery systems [1-3]. The success of these microspheres is limited because due to short residence time at the site of absorption. Therefore, it would be advantageous to provide an intimate contact of the drug delivery systems with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and formulating bioadhesive microspheres. These microspheres provide advantages such as efficient absorption and increased bioavailability of drugs owing to high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absporption site [4-7]. Atomoxetine HCl is a potent inhibitor of the presynaptic norepinephrine transporter with minimal affinity for othe rmonoamine transporters or receptors and is the first non-stimulant

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medication approved for the management of attention deficit/hyperactivity disorder (ADHD) in children, adolescents and adults.Atomoxetine Hcl is well absorbed after oral administration with peal plasma concentration of 1 to 2 hrs after which the dose bioavailability is 63% in extensive metabolisers. The logP value is 3.65 which is sufficient to cross the oral mucosa. The plasma half of Atomoxetine range is 5.2hrs and the dosage regimen according to FDA dosing guide is start at 40 mg, target at 80mg, maximum 100mg twice a day. Hence, Atomoxetine was chosen as a model drug for prolong action and to improve patient compliance.

MATERIALS AND METHODS

Materials

Atomoxetine hydrochloride was obtained from Dr Reddys Laboratories Ltd, Sodium Alginate, Pectin & Kharaya gum from Dow chemicals and Calcium chloride & Glacial acetic acid from SD Fine chemicals

Methods

Preformulation Studies

Colour, Odour, and appearance

The colour, odour and appearance of the drug were recorded using descriptive terminology.

Melting point determination

Melting point of the drug sample was determined by capillary method by using melting point apparatus.

Determination of solubility:

The solubility of the Atomoxetine Hcl was determined by adding excess amount of drug in the solvent and equilibrium solubility was determined by taking supernatant and analyzing it on Lab India, double beam spectrophotometer.

% solubility = sample absorbance /standard absorbance × dilution factor ×100

Drug-polymer compatibility studies

The interaction study between the drug and excipients in different formulations were performed using FTIR spectrophotometer. The pellets were prepared on KBr press. The spectra were recorded over the wave number range of 4000 to 400 cm-1.

Formulation development

Ionotropic Gelation Method

All the formulations were prepared by Ionotropic Gelation Method. Batches of microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate.

The compositions of different formulations are given in table 1. The alginate spheres were prepared as per the procedure given below: Atomoxetine hydrochloride and all other polymers were individually passed through sieve #60. Sodium alginate (As per Qty mentioned in composition table) and the mucoadhesive polymers (As per Qty mentioned in composition table) were dissolved in purified water (100mL) to form a homogeneous polymer solution. The active substance, Atomoxetine hydrochloride (As per Qty mentioned in composition table), was added to polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion solution was then added manually drop wise into calcium chloride (2.5%w/v) solution (25mL) through syringe with a needle size no #26. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The spheres were collected by decantation, and the product thus separated was washed repeatedly with water to remove excess calcium impurity deposited on the surface of microspheres and dried at 45°C for 12 hours.

Evaluation of Microspheres

Micromeritic properties

Determination of angle of repose

Angle of repose of the microspheres was determined bypassing microspheres through the glass funnel on a horizontal surface. The height (h) of the heap formed was measured and the radius (r) of the cone base was also observed and calculated. The angle of repose (θ) was calculated as follows:

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

Determination of bulk density

Bulk density of the formulations was determined by using the following formula,

$$Bulk\ density = \frac{Sample\ weight}{Sample\ volume}$$

Determination of tapped density

Tapped density is used to investigate packing properties of microcapsules into capsules. The tapped density was measured by employing the conventional tapping method using a 10mL measuring cylinder and the number of tappings was 100 as sufficient to bring a plateau condition. Tapped density was calculated using the following formula:

$$Tapped\ density = \frac{Weight\ of\ the\ microspheres}{Volume\ of\ microspheres\ after\ 100\ tapings}$$

Determination of Hausner's ratio

It is another parameter for measuring flowability of the microcapsules. It is calculated using the following formula,

$$Hausner's\ ratio = \frac{Volume\ before\ tapping}{Volume\ after\ tapping}$$

Determination of compressibility index

It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence

the consolidation index. It is also called as compressibility index. It is denoted by Ci and is calculated using the formula below.

$$Ci = \frac{Tapped\ density - Bulk\ density}{Bulk\ density} X100$$

Drug content

Preparations equivalent to 50mg of drug was weighed accurately and transferred to a 100mL volumetric flask and dissolved in 20mL of alcohol. The volume was adjusted with pH 7.4 Phosphate buffer. The sample was filtered and after suitable dilution, the absorbance of the above solution was measured at 270nm. The drug content was calculated using the calibrated curve.

Particle Size and Shape Analysis

All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The average particle size was determined Mean size of 300 particles was considered as size of the microspheres. Particle size distribution analysis was carried out results are given in table 2.

Drug Entrapment Efficiency

To determine entrapment efficiency, 100mg accurately weighted microspheres were washed and crushed and dissolved in 100 ml with phosphate buffer pH 7.4 solution. The microspheres were kept to soak for overnight. After 12 hrs the solution was filtered through 0.45m membrane filter. The volume was made up to 100 ml with phosphate buffer pH 7.4 and analyzed for drug content spetrophotometrically at 270 nm. Corresponding drug concentrations in samples was calculated from calibration plot.

$$\text{Entrapment efficiency} = \frac{\text{Estimated \% drug content in microspheres}}{\text{Theoretical \% drug content in microspheres}} \times 100$$

Percentage Yield Value

The percentage yield value is defined as the quantity of beads produced as a function of loaded drug and polymer. Results are given in table 6.6.

$$\text{Percent yield} = \frac{\text{Weight of microspheres}}{\text{Total weigth of drug and polymer taken}} \times 100$$

Scanning Electron Microscopy

The shape and surface morphology of the microspheres were examined using scanning electronmicroscopy (JSM-6390, Japan). Microspheres were dusted onto double-sided carbon dust, which was placed onto a sample carrier in the shape of cylinder. After fixing the samples on the stubs, capture a photomicrograph.

***In Vitro* Drug Release Profile**

A USP paddle apparatus has been used to study in vitro drug release from microspheres. In vitro drug release studies were carried out for all batches in USP type II dissolution test apparatus at 100 rpm and the dissolution medium used is 900ml of phosphate buffer pH 7.4. Microspheres containing 500mg of drug was used for dissolution study. Five ml of the aliquot was withdrawn at pre determined intervals. The required dilutions were made with 7.4 pH phosphate buffer and filter the solution and analyzed for the drug content spetrophotometrically (UV 1200, Shimadzu, Japan) at 270nm against suitable blank. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition.

Mucoadhesion testing by *exvivo* wash-off test:

The mucoadhesive properties of the microspheres were evaluated by the *In vitro* wash-off test a 4cm x 4cm piece of goat intestine mucosa was tied onto a glass slide using thread. Microspheres were spread (~100) onto the wet, rinsed, tissue specimen and the prepared slide was hung on to one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beakers containing the simulated gastric fluid USP the pH 7.4 Phosphate buffer. At the end of 30 minutes, 1 hour, and at hourly intervals up to 10 hours, the number of microspheres still adhering on to the tissue was counted. *Mucoadhesion property =No of microspheres adhered/No of microspheres applied X100

Accelerated Stability Studies

Optimized batch microspheres were wrapped in aluminium foil and packed in glass vials. These formulations were then kept in an incubator maintained at 40±0.5°C and 75±5% RH for 3 months. Changes in the particle size, drug content, drug release and appearance of these stored microspheres were investigated at regular intervals (1, 2 and 3 months).

RESULTS AND DISCUSSION

Preformulation study

Physical properties of the Drug

Result:

The results were found as per specifications**.**

Solubility:

These tests were performed as per procedure and the results are illustrated in the following table.

Excipients Compatibility study

From Preformulation study three is no physical change is observed mixture drug and excipients.

The drug shows different peaks at C-H = 3008,

C=C = 1605, 1495, 1466, O-H = 3231, N=N = 1576 and Cl = 1200- 1400cm-1of benzene. FTIR-spectra of Atomoxetine hydrochloride and its physical mixture with excipients are exactly same, and there is no shift of peaks or disappearance of principle peaks or modification of the principle peaks indicating that there is no interaction between the drug and excipients.

The drug loaded spheres were found to be discrete, spherical, free-flowing, and of the monolithic matrix type. All the formulations demonstrated good flow property as the values of angle of repose, Hausner's ratio and the compressibility index of the prepared spheres were very low (Table 5). The values of angle of repose of the different formulations ranged from 16.6 to 23.5°. The values of the bulk density of the different batches ranged from 0.414 to 0.655g/mL. The values of the tapped density of the different formulations ranged from 0.460 to 0.786 g/mL. The values of the Hausner's ratio of the different formulations ranged from 1.08 to 1.21. The values of the carr's index of the different formulations ranged from 10.0 to 16.67%.

The entrapment efficiency was in the range of 45% to 85.5%.Which gives the clear view that as the concentration of the polymer increases the entrapment efficiency of the drug increases which can be seen in the formulation F8 with 87.0%. The spheres were uniform in size, the diameter of the spheres ranged from 211µ to 522µ. The % mucoadhesive properties of the different batches of the spheres are indicated in table. The spheres with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive property in the exvivo wash off test.

The data of physicochemical characterization include percentage yield, drug content, drug entrapment efficiency, mean particle size, of all Atomoxetine hydrochloride Microspheres formulations.

SEM analysis of the F8 formulation showed that the external morphology was found to be uniform in size and discrete, spherical in shape, smooth as in the Figure 3

Dissolution study

Atomoxetine release from the drug loaded spheres was studies in the phosphate buffer (pH 7.4) for 10 hours. The dissolution profiles of the different batches of the spheres are depicted in the Figure 4.Atomoxetine release from the sphere was slow and depends on the composition of the coat. Based on the T_{50} (Hrs) values (time taken for 50% of drug release), the drug release from the different formulations can be arranged as:

F9>F3>F8>F2>F1>F6>F7>F5>F4

Similarly, based on the T75 (hrs) values (time taken for 75% of the drug release), the drug release from the different batches can be arranged as:

F9>F3>F8>F2>F1>F6>F7>F5>F4

The difference in the drug release characteristics of the various spheres are due to the differences in the porosity of the coat formed and its solubility in the dissolution fluid. Among all the batches, the F8 (Drug: Sodium alginate: kharaya gum=1:0.75:0.75) batch considered to be the optimized formulation (T50%=2Hrs, T75%=5hrs, T90%=8Hrs), because among all the batches it shows better extent of the drug release, good entrapment efficiency (87%) and in vitro wash-off test showed good mucoadhesive property. Atomoxetine release from the optimized formulation (F8) was slow and extended over a period of 10hrs and these drug loaded spheres were found suitable for the oral controlled release.

The regression coefficient (R^2) Values of the optimized formulation are indictaed in above table. Based on the values of R^2, the drug release from the formulation exhibit zero order kinetics. The values of the release rate constants, K (For the zero order and first order) and the diffusion exponent (n) are represented in the above table. The value of the K1(H^{-1}), the first order release rate constant of the optimum batch is 0.528. The value of K_0 (mg/Hr), the zero order release rate constant of the optimum formulation is 8.815. The formulation obeyed Higuchi equation (R^2>0.9), indicating that the drug release mainly depends on diffusion and erosion. The data was fitted to the Korsemeyer-Peppas equation and the values of the diffusion exponent "n" for the optimum batch F8 was 0.4678 which indicates that the drug release shows non-fickian diffusion.

Accelerated Stability Studies

Optimized batch microspheres were wrapped in aluminium foil and packed in glass vials. These formulations were then kept in an incubator maintained at 40±0.5°C and 75±5% RH for 3 months. At different regular intervals (1, 2 and 3 months) the stored microspheres were checked for Changes in the particle size, drug content, drug release and appearance.

Table 1. Formulation composition of Atomoxetine hydrochloride Mucoadhesive Microspheres

S.No:	Batch No.	Coat Composition	Drug to Polymer Ratio	Qty. Per Batch (mg)	Gelling agent
1	F1	Drug : Sodium Alginate	1:1	500:500	Calcium chloride (2.5%w/v)
2	F2	Drug : Sodium Alginate	1:1.5	500:750	
3	F3	Drug : Sodium Alginate	1:2	500:1000	
4	F4	Drug : Sodium Alginate: Pectin	1:0.5:0.5	500:250:250	
5	F5	Drug : Sodium Alginate: Pectin	1:0.75:0.75	500:375:375	
6	F6	Drug : Sodium Alginate: Pectin	1:1:1	500:500:500	
7	F7	Drug : Sodium Alginate: Kharaya gum	1:0.5:0.5	500:250:250	
8	F8	Drug : Sodium Alginate: Kharaya gum	1:0.75:0.75	500:375:375	
9	F9	Drug : Sodium Alginate: Kharaya gum	1:1:1	500:500:500	

Table 2. Description of Atomoxetine Hydrochloride (API)

Test	Description
Colour	white colour amorphous powder
Odour	Odourless

Table 3. Solubility of Atomoxetine Hydrochloride (API) in various solvents

Solvents	Solubility
Water	Soluble
pH6.8Phosphate buffer	Soluble
Methanol	Freely soluble
Ethanol	Freely soluble

Table 4. Melting point determination

Material	Melting Point	Melting Point Range
Atomoxetine	167^0c	167-169°C

Table 5. Micrometric properties of different Atomoxetine hydrochloride Microspheres

Formulation Code	Bulk density (gm/mL)	True density (gm/mL)	Hausner's ratio	Compressibility index	Angle of repose
F1	0.655	0.786	1.20	16.67%	23.5
F2	0.616	0.711	1.15	13.37%	20.4
F3	0.564	0.599	1.06	10.71%	19.5
F4	0.612	0.723	1.18	15.38%	21.2
F5	0.596	0.644	1.08	11.49%	19.8
F6	0.414	0.460	1.11	10.00%	18.2
F7	0.573	0.647	1.12	17.49%	19.4
F8	0.543	0.652	1.20	16.67%	18.2
F9	0.518	0.627	1.21	15.39%	16.6

Table 6. Percentage yield, drug content, drug entrapment efficiency, mean particle size, of all Atomoxetine hydrochloride Microspheres

Formulation Code	Yield (%)	Drug Content (%)		Drug entrapment efficiency (%)	Mean particle size(µm)
		Theoretical	practical		
F1	80.0	50.00	22.50	45.0	211± 14.5
F2	84.5	40.00	28.10	70.2	225 ± 19.9
F3	86.5	33.33	24.40	73.2	307 ± 17.0
F4	87.0	50.00	32.10	64.2	315 ± 22.9
F5	83.5	40.00	27.00	67.5	313 ± 25.0
F6	84.0	33.33	28.50	85.5	375 ± 22.9
F7	85.0	50.00	34.50	69.0	512 ± 13.1
F8	89.0	40.00	34.80	87.0	514 ± 21.8
F9	89.0	33.33	26.00	78.0	522 ± 21.8

All values are mean±SD of all the three determinations.

Table 7. Percentage mucoadhesive property of Atomoxetine Hydrochloride in pH 7.4 buffer

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	44	51	48	30	56	52	54	70	80
1	20	36	31	29	38	44	42	54	78
2	-	15	27	-	29	13	34	40	69
3	-	-	-	-	14	-	12	42	60
4	-	-	-	-	-	-	-	38	55
5	-	-	-	-	-	-	-	32	43
6	-	-	-	-	-	-	-	26	39
7	-	-	-	-	-	-	-	10	26
8	-	-	-	-	-	-	-	-	16
9	-	-	-	-	-	-	-	-	8
10	-	-	-	-	-	-	-	-	-

Table 8. Dissolution profiles

Time (Hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	42.25	38.75	30.41	50.84	46.48	39.48	44.58	34.47	30.25
2	60.75	54.29	48.58	75.47	70.95	61.34	65.47	55.58	48.78
3	75.26	70.36	61.47	90.57	88.47	75.58	80.28	64.65	59.47
4	82.48	79.57	72.56	99.26	97.56	90.57	92.34	70.47	60.38
5	94.15	85.64	81.35			99.34	99.58	75.68	69.47
6	99.82	94.27	90.76					83.56	76.58
7		99.38	93.48					90.85	81.68
8			98.68					95.38	90.78
10								99.86	95.47

Table 9. Coefficient of regression (R^2) values

Coefficient of regression (R^2) values of Atomoxetine loaded microspheres				
Formulation	Zero Order	First Order	Higuchi Model	Peppas Model
F8	0.8448	0.8071	0.9852	0.9749

Table 10. Dissolution parameters

Dissolution parameters of mucoadhesive microspheres of Atomoxetine						
Formulation	K0(mg/hr)	K1(H-1)	n	T50 (Hrs)	T75 (Hrs)	T90 (Hrs)
F8	8.815	0.528	0.4678	2.0	5.0	8.0

Table 11. Stability studies

Condition/Tests →	Appearance	Drug Content (%)	Particle size (µm)	Cumulative % Drug release at 10Hrs
Initial	Spherical	34.8	512 ± 21.8	99.86
40°C/75%RH-1Month	Spherical	34.0	522 ± 14.2	98.48
40°C/75%RH-2Months	Spherical	33.7	526 ± 26.8	99.02
40°C/75%RH-3Months	Spherical	33.2	528 ± 16.4	97.5

Fig 1. FT-IR Spectrum of Atomoxetine hydrochloride

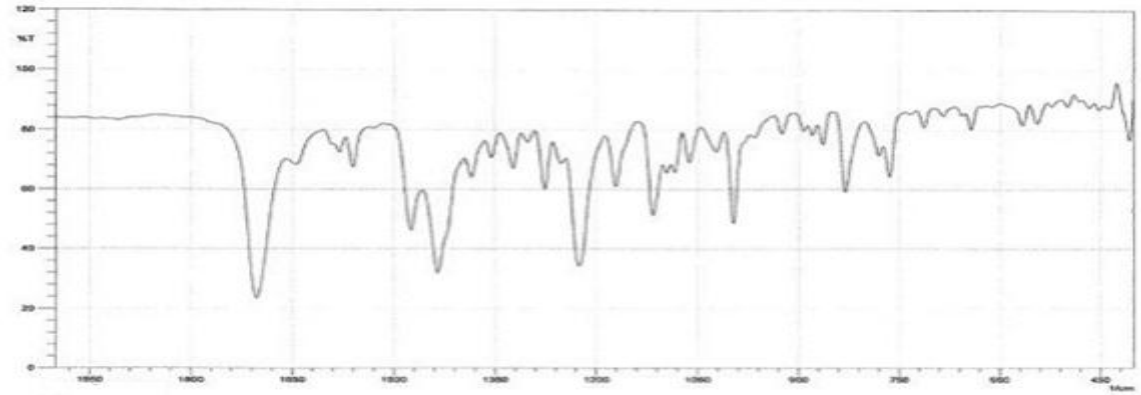


Fig 2. FT-IR Spectrum of Atomoxetine hydrochloride with Physical mixture of Excipients

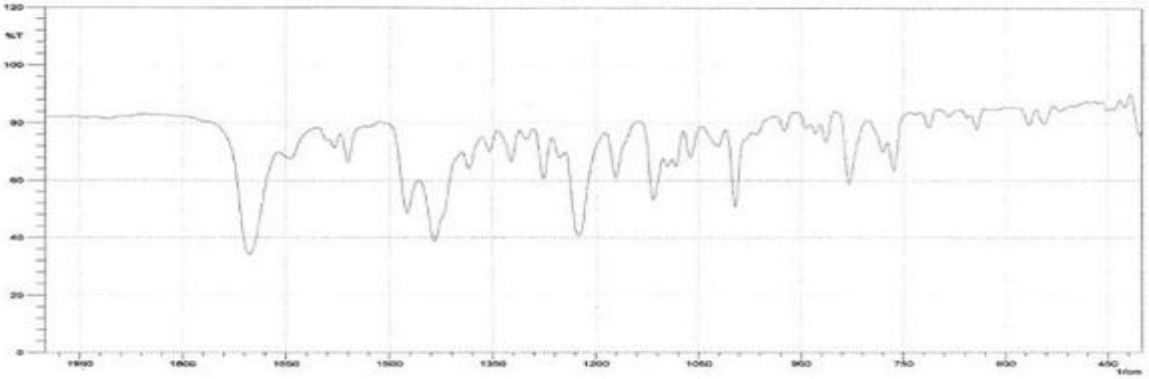


Fig 3. SEM Analysis

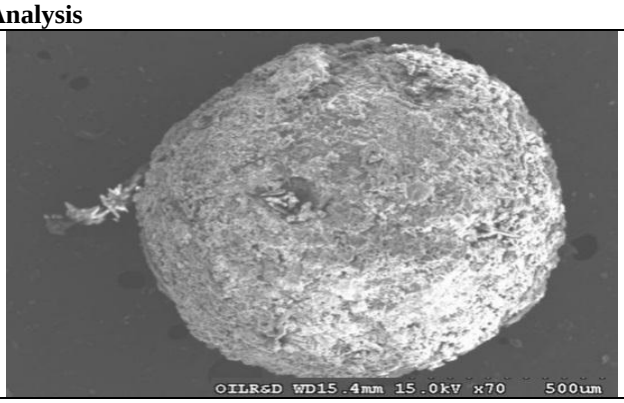


Fig 4. Dissolution profiles

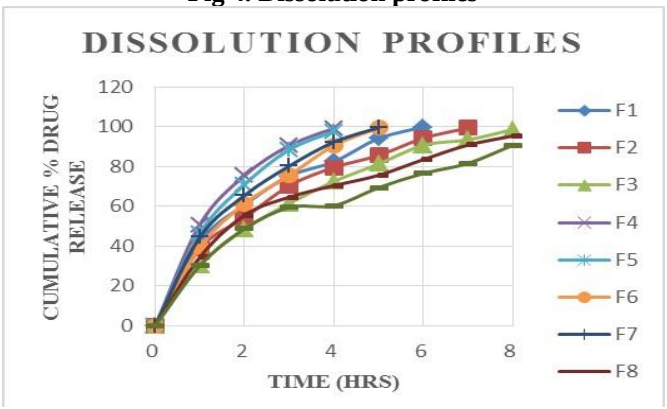


Fig 5. Release Kinetic studies-zero order

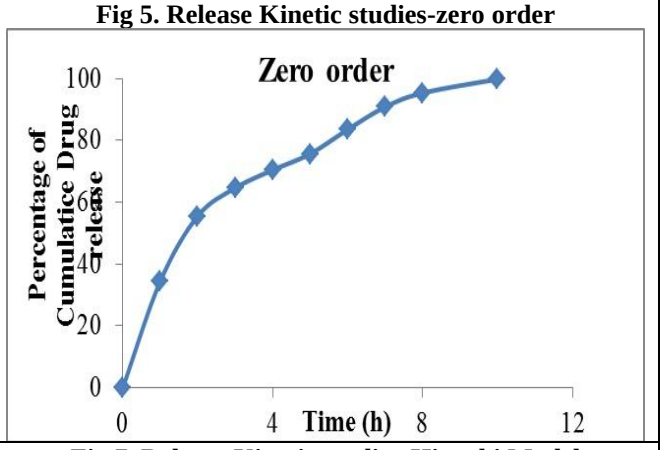


Fig 6. Release Kinetic studies-First Order

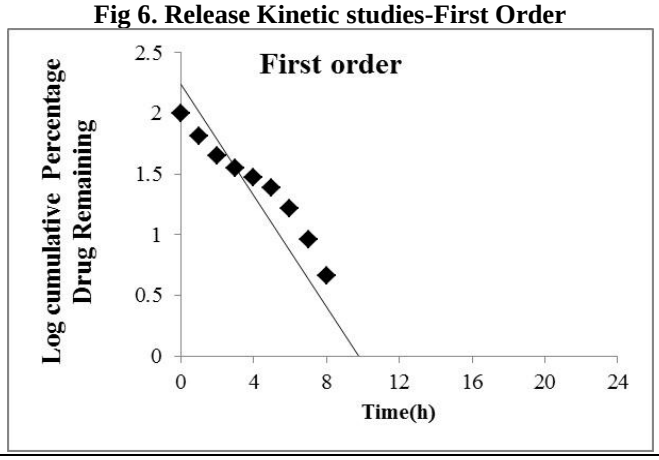


Fig 7. Release Kinetic studies-Higuchi Model

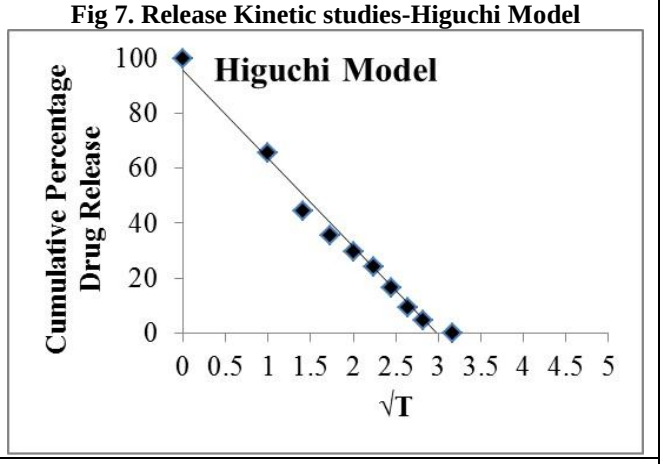
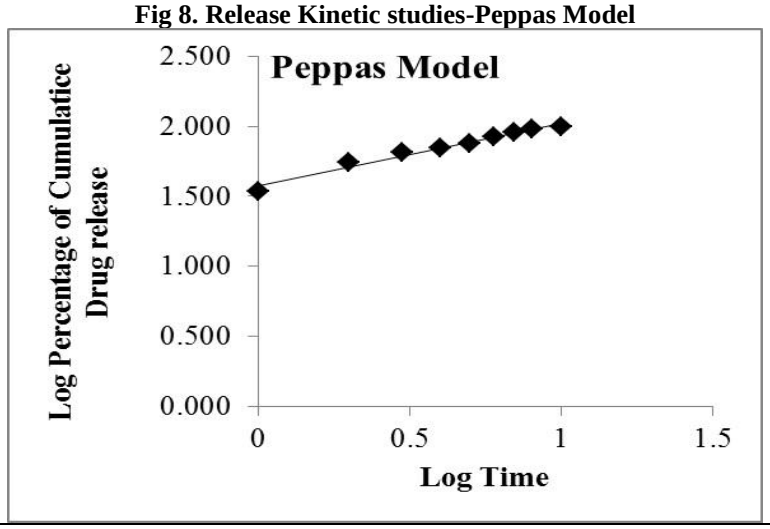


Fig 8. Release Kinetic studies-Peppas Model



CONCLUSION

From the studies conducted in this work, it can be concluded that the release of Atomoxetine Hydrochloride can be retarded by encapsulating it in calcium alginate spheres. The optimum formulation, Drug–Sodium alginate–Kharaya gum (1:0.75:0.75) mucoadhesive spheres demonstrated controlled release of the drug for 10hrs and exhibited good mucoadhesive property. The FTIR studies revealed the absence of the drug –polymer interaction in the solid state. The formulated mucoadhesive microspheres of Atomoxetine Hydrochloride can control the drug release; it has good mucoadhesive property and can improve the bioavailability of the drug.

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