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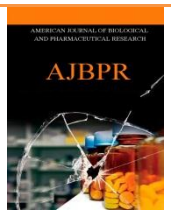

DEVELOPMENT AND EVALUATION OF ALPRAZOLAM IMMEDIATE RELEASE AND PROPRANOLOL SUSTAINED RELEASE FORMULATION USING CAP IN CAP TECHNOLOGY.

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ABSTRACT

In the present research work, a novel cap-in-cap technology is used for the development of Alprazolam Immediate Release and Propranolol Sustained Release formulation. To the best of our knowledge very less in formulation is available on this type of formulations. The advantages of fast releasing liquid-filled-capsules and slow release microspheres-filled-capsules were combined to meet the optimized requirements of our drug delivery system. Propranolol sustained release microspheres were prepared by emulsion solvent evaporation method using ethyl cellulose as polymer and were filled into a smaller capsule. Alprazolam fast is releasing liquid was prepared by using a simple dilution method using olive oil as a medium. This fast releasing liquid and slow releasing microsphere-filled-capsule was further inserted into a bigger capsule body and closed with the cap by sealing. The various formulation batches were subjected to physicochemical studies, entrapment efficiency, drug content, *in vitro* drug release and stability studies. Interaction studies reveal that there was no interaction between drug and polymers employed in this study. The optimized capsule-in-a-capsule formulation, released 95.90% of Alprazolam at the end of 2 hours and 49.41 % of Propranolol at the end of 12h. The drug release profile of Propranolol microsphere fits well with Higuchi model followed by zero order, first order and Korsemeyer-Peppa's model. Korsmeyer-Peppas model analysis indicated that the drug release followed non-Fickian transport mechanism.

INTRODUCTION

Capsule in Capsule formulation which is also known as Duo Cap is a single, oral-dosage unit that comprises a capsule-in-a-capsule and offers broad therapeutic applications. Output profiles for multiple dosing unit that provides internal and external capsules contain the same active drug.

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For example, an immediate release formulation from the outer capsule and a controlled-release formulation from the inner capsule. In addition to modifying the release profiles it is also possible to target the inner and outer capsule into two different areas of the GI tract (small intestine or colon), with the appropriate coating such as enteric coating.

The internal capsule can contain a liquid, semi - solid, powder or granular formulations and the external capsule contains liquid formulations or semi solid combination of drugs. By this method a smaller prefilled capsule can be easily inserted into a larger liquid filled

capsule. Multiple release profiles can also be easily achieved by filling immediate release formulation in an outer larger capsule and sustained or controlled release formulation in inner smaller capsules. Capsule in a capsule formulation consists of two phases
A) Immediate releasing phases
B) Sustained releasing phases [1]

MATERIALS AND METHODS

Drugs and chemicals

Alprazolam was obtained as a gift sample from LAKE CHEMICALS PVT.LTD, Bangalore, India. Propranolol Hydrochloride and hard gelatine capsule were purchased from Yarrow Chem., Mumbai.Olive oil and Tween 80 were purchased from Nice Chemicals Pvt Ltd, Cochin. Ethyl cellulose was purchased from S D Fine CHem Ltd, Mumbai. Dichloro methane was purchased from Spectrum reagents & chemicals Pvt Ltd Edayar, Cochin. All other materials used were of analytical grade.

Drug-polymer interaction studies

Drug and polymers used under the experimental condition were studied for compatibility studies. The study was carried out by taking 1mg sample in 100mg KBr (Perkin Elmer, spectrum-100, Japan). The scanning range was 400 to 4000 cm^{-1} and the resolution was $1cm^{-1}$ [2].

Formulation of cap-in-cap formulation

Cap-in-cap formulation consists of two phases; immediate and sustained release phases. The immediate releasing dose of Alprazolam and sustained release dose of Propranolol was found to be 0.5mg and 60mg, respectively, which are known from already existing literature [3].

Preparation of immediate releasing phase

Alprazolam fast is releasing liquid was prepared using olive oil and drug by simple dilution method [4].

Preparation of sustained releasing phase

Emulsion solvent evaporation method is the widely used method. Calculated quantity of polymer was dissolved in Dichloromethane to form a homogeneous polymer solution. The calculated quantity of drug was added to distilled water and stirred for dissolving the drug and both solutions were mixed thoroughly to form oil in water emulsion. The resulting mixture was then added in a thin stream of 300ml of aqueous solution containing 1% (v/v) tween80 forming oil-water-oil emulsion, while stirring at 1000rpm to emulsify the added dispersion as fine droplets. The solvent, dichloromethane was then removed by evaporation during continuous stirring at room temperature for 3 hours to produce spherical microspheres. Here dichloromethane was used as polymer solvent, aqueous solution as the microencapsulating vehicle, tween80 as the dispersing agent. During 3hours stirring period, dichloromethane was completely removed by evaporation. The microspheres were collected by vacuum filtration and washed repeatedly and dried at room temperature over a night to get free flowing microspheres. By varying this drug: polymer ratio, 6 batches of microspheres were prepared as indicated in the table 1 [5, 6].

Preparation of cap-in-cap formulation

Special leak proof capsules for both smaller and bigger size was used in this formulation. To prepare a novel cap-in-cap technology the prepared optimized sustained release microspheres equivalent to 60 mg of Propranolol were filled in size 3 hard gelatine capsule and was sealed with 15% (*m/m*) warm gelatine solution. This prepared sustained release smaller capsule was filled into a bigger capsule body size 00 which was further filled with the Alprazolam in olive oil equivalent to 0.5mg using medicine droppers. After closing with cap the bigger capsule was also sealed with 15% (*m/m*) warm gelatine solution. The filled capsules were stored at room temperature until testing [5-7].

EVALUATION PARAMETERS

Flow property

Angle of repose method was employed to assess the flow ability of the microspheres. Microspheres were allowed to fall freely through the funnel fixed at 2cm above the horizontal flat surface until the apex of conical pile just touched the tip of the funnel. The angle of repose (θ) was determined by the formula,

$\theta = \tan^{-1} (h/r)$

Where, 'h' is the cone height of beads, 'r' is the radius of the circular base formed by the microspheres on the ground [4-7].

Drug content and Entrapment efficiency

Accurately weighed quantities of microspheres equivalent to 100mg of Propranolol hydrochloride were placed in 25mL of 6.8 PBS. The solution was shaked for about 12 hrs; the supernatant layer of the liquid was assayed by UV-spectroscopy at 290nm. The drug content and encapsulation efficiency were determined by the following equation [7].

Drug content = (concentration × volume of dilution medium ×dilution factor) /1000

Encapsulation efficiency = actual drug content / theoretical drug content ×100

Percentage yield

The dried microspheres were weighed and percentage yield of the prepared microspheres was calculated by using the following formula [7].

Percentage yield = practical yield/theoretical yield ×100

Particle size analysis

Size distribution plays a very important role in determining the release characteristics of the microsphere. Particle size distribution analysis was done by optical microscopy method, using calibrated eye piece micrometer, nearly 100 particles were measured and the results were determined.

***In-vitro* release studies**

Immediate release Alprazolam solution

Dissolution studies were carried out using USP XXIII dissolution test apparatus II basket type (Electrolab TDL- 08L) at a rotation speed of 50rpm and at 37 ± 0.5° using 900 ml of 0.1N HCl (pH 1.2) for two hours.A 1 ml sample was withdrawn at 15 minutes time intervals for 2 hour and replaced by an equal volume of 0.1N HCl (pH 1.2) . Samples withdrawn were filtered through whatsmann filter paper (0.45µm) The amount of Alprazolam was analysed using a Shimadzu UV spectrophotometer at 260nm. The studies were carried out in triplicate and the mean values plotted verses time with standard error of mean, indicating the reproducibility of the results [2, 5]

Sustained release Propranolol microsphere

Dissolution studies were carried out using USP XXIII dissolution test apparatus II basket type (Electro lab TDL- 08L) at a rotation speed of 50rpm and at 37 ± 0.5° using 900 ml of 0.1N HCl (pH 1.2) for two hours and the remaining hours in pH 6.8 phosphate buffer. A 1ml sample was withdrawn at 1hr time intervals for 12 hours and replaced by an equal volume of 0.1N HCl (pH 1.2) and phosphate buffer pH 6.8, respectively. Samples withdrawn were filtered through whatman filter paper (0.45µm) and Propranolol released was analyzed using a Shimadzu UV spectrophotometer at 290 nm. The studies were carried out in triplicate and the mean values plotted verses time with standard error of mean, indicating the reproducibility of the results [2, 5]

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Cap-In-Cap formulations

Dissolution studies were carried out using USP XXIII dissolution test apparatus II basket type (Electro lab TDL-08L) at a rotation speed of 50rpm and at 37 ± 0.5°C using 900 ml of 0.1N HCl (pH 1.2) for two hours and the remaining hours in pH 6.8 phosphate buffer. A 1 ml sample was withdrawn at 15 minutes time intervals Alprazolam for the first 2 hours and a 1ml sample was withdrawn at 1hr interval for remaining 10 hrs and replaced by an equal volume of 0.1N HCl (pH 1.2) and phosphate buffer pH 6.8 respectively. Samples withdrawn were filtered through whatsmann filter paper (0.45 micron). The amount of Alprazolam and Propranolol released was simultaneously analyzed using a Shimadzu UV spectrophotometer at 260 and 290 nm respectively. The studies were carried out in triplicate and the mean values plotted verses time with standard error of mean, indicating the reproducibility of the results

Release kinetics studies.

To study the release kinetics in vitro release data was applied to kinetic models such as a zero - order, first order, Higuchi and Korsemeyer-Peppas [5].

RESULTS AND DISCUSSIONS

Alprazolam and Propranolol Cap in Cap technology designed in this study consists of two phases .Immediate release phase of Alprazolam and sustained release phase of Propranolol. This formulation is used for the treatment of Anxiety disorders, Panic disorders, Hypertension, Fast Heartbeat, Migraine and other conditions. The device is formulated into two steps: first Propranolol hydrochloride is prepared into sustained release microspheres using ethyl cellulose as polymer and filled into a size 3 hard gelatine capsule, which was further filled into size 00 hard gelatine capsule body; second Alprazolam was prepared as a oily solution in olive oil using simple dilution method and filled into the bigger capsule body containing smaller capsule filled microspheres

Interaction studies

Propranolol hydrochloride and excipients interaction were analyzed by comparing the IR spectra of the physical mixture, the pure Propranolol and ethyl cellulose and spectra of pure ethyl cellulose as in the fig 1 - 3. Propranolol is given peak in the IR spectrum nearby at 2962 cm^{-1} due to the presence of a secondary amine group, 3286 cm^{-1} due to the secondary hydroxyl group, the aryl alkyl ether displays a stretching band at 1103 cm^{-1} and the peak at 771 cm^{-1} due to α substituted naphthalene. The IR spectra of Propranolol with ethyl cellulose, did not reveal any extra peaks which confirms that there is no interaction between Propranolol and excipients used.

Flow properties, drug content and entrapment efficiency

The angle of repose value indicated in the table 2 ranged from 27˚.196 to 28˚.678, which indicates the good flow properties of the microspheres .The drug entrapment efficiency of the sustained release microspheres was also studied and the values were found to be in the range between 75.011 to 98.76. The drug content of Propranolol microsphere was found to be in the range of 59.26 to 14.166 which is indicated in table 3.

Percentage yield and particle size analysis

The F1 formulation doesn't give any product so it is excluded from evaluation tests. The percentage of the product is found to be in the range of 90% to 97.5%.The particle size of the microspheres was found to be in the range of 542 to 265 as indicated in the table 4.

The in-vitro release study of immediate release Alprazolam filled capsule shows 95.5% of release at 30 minutes of dissolution time as indicated in the table 5 and graph is represented in the fig 4.

The in-vitro release study of sustained release microspheres were carried out and optimized formulation shows a release of 45.85% of release at the 12th hour of dissolution as indicated in the table 6 and fig 5.

In order to determine the mechanism of drug release from the formulation, the *in vitro* dissolution data was fitted to Zero order, First order, Higuchi plot and Korsemeyer-peppa's plot (Fig.7). The drug release from capsule-in-a-capsule formulation fits well with Higuchi model followed by zero order, first order and Korsemeyer-peppa's model. The *in vitro* release data was further fitted to Korsmeyer-Peppas model which is generally used to analyze the release mechanism when more than one type of release phenomenon is operational. Good linearity was observed with high 'R^2' value. The value of release exponent 'n' is an indicative of release mechanism. The value of 'n' obtained for the optimized formulation was found to be 0.82 suggesting probable release by non-Fickanian or anamolous diffusion. The analysis of experimental data in the light of the Korsmeyer-Peppas equation, as well as the interpretation of the corresponding values of n, leads to a better understanding of the balance between these mechanisms. The value of 'n' obtained for the optimized formulation was found to be 0.82, which indicates that the release mechanism of propranolol is non-Fickian transport, which suggests that both dissolution and diffusion of the drug in matrices and also its own erosion modulate drug release.

Table 1. Composition of sustained release microsphere

Formulation code	Drug : Ethyl cellulose	Drug (mg)	Ethyl cellulose (mg)
F1	1:0.5	60	30
F2	1:1	60	60
F3	1:1.5	60	90
F4	1:2	60	120
F5	1:2.5	60	150
F6	1:3	60	180

Table 2. Flow property of microspheres

Formulation code	Angle of repose (ɵ)	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility factor (%)	Hauser's ratio	LOD (%)
F2	**27˚.61'**	**0.387**	**0.418**	**7.56**	**1.054**	**3.0**
F3	**28˚.09'**	**0.390**	**0.424**	**6.87**	**1.032**	**2.8**
F4	**27˚.049'**	**0.376**	**0.454**	**7.54**	**1.065**	**2.9**
F5	**28˚.678'**	**0.379**	**0.443**	**8.78**	**1.089**	**2.9**
F6	**27˚.196'**	**0.398**	**0.482**	**8.42**	**1.042**	**3.1**

Table 3. Percentage drug entrapment and drug content

Formulation Code	Percentage Drug Entrapment %	Drug Content (Mg)
F2	**98.76**	**59.26**
F3	89.98	36.73
F4	82.71	35.45
F5	78.42	19.607
f6	75.01	14.166

Table 4. percentage yield and particle size of microspheres

Formulation code	Percentage yield of microsphere (%)	Particle size (µm)
F2	90	542
F3	97.5	510
F4	93.33	350
F5	95.23	290
F6	95.83	265

Table 5. *In-vitro* release study of immediate release Alprazolam filled capsule

Time (min)	Absorbance	Concentration	Drug release	%CR
0	0	0	0	0
15	0.021	0.86	7.745	77.4
30	0.026	1.065	9.55	95.5
45	0.026	1.065	9.55	95.5
60	0.026	1.065	9.55	95.5
75	0.026	1.065	9.55	95.5
90	0.026	1.065	9.55	95.5
105	0.026	1.065	9.55	95.5
120	0.026	1.065	9.55	95.5

Table 6. *In-vitro* release study of sustained release microspheres

Time in hrs	Formulation code				
	F2	F3	F4	F5	F6
0	0	0	0	0	0
1	1.764	1.76	1.76	0.88	0.88
2	4.41	2.64	2.64	2.64	1.76
3	6.17	4.41	3.52	4.41	3.52
4	9.7	6.17	4.41	6.17	4.41
5	13.23	7.94	7.05	8.82	6.17
6	17.64	9.7	9.70	10.58	7.94
7	23.82	13.23	12.35	12.35	8.82
8	27.35	18.52	15	15	10.58
9	30.88	23.35	17.64	17.64	13.23
10	35.29	27.35	20.29	20.29	15.87
11	39.70	30.88	24.7	22.94	18.52
12	45.85	34.41	28.23	26.47	22.94

Release kinetics

Table 7. Regression analysis of different models of optimised formulation

Model	Regression coefficient (R^2)
Zero order	0.966
First order	0.958
Korsmeyer peppas	0.987
Higuchi	0.994

Fig 1. FTIR spectrum of propranolol

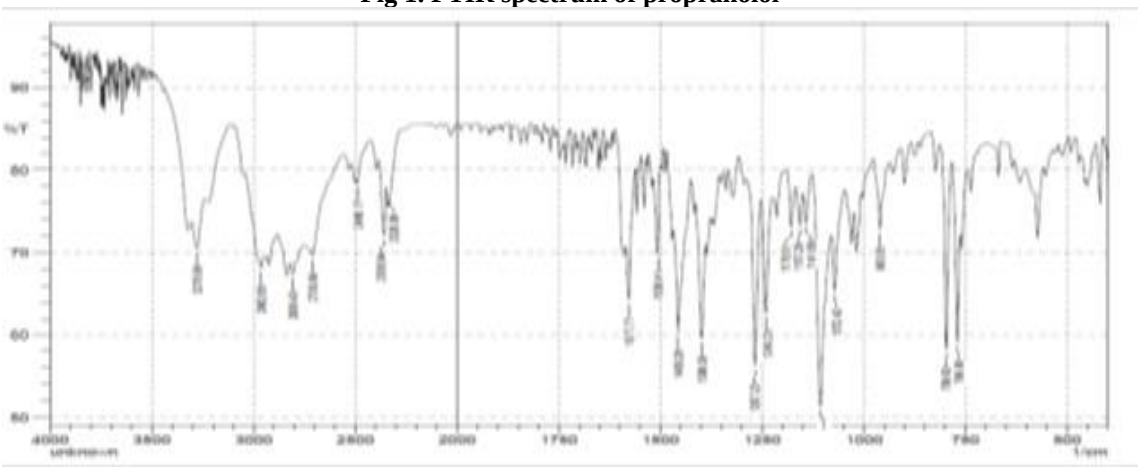


Fig 2. FTIR spectrum of ethyl cellulose

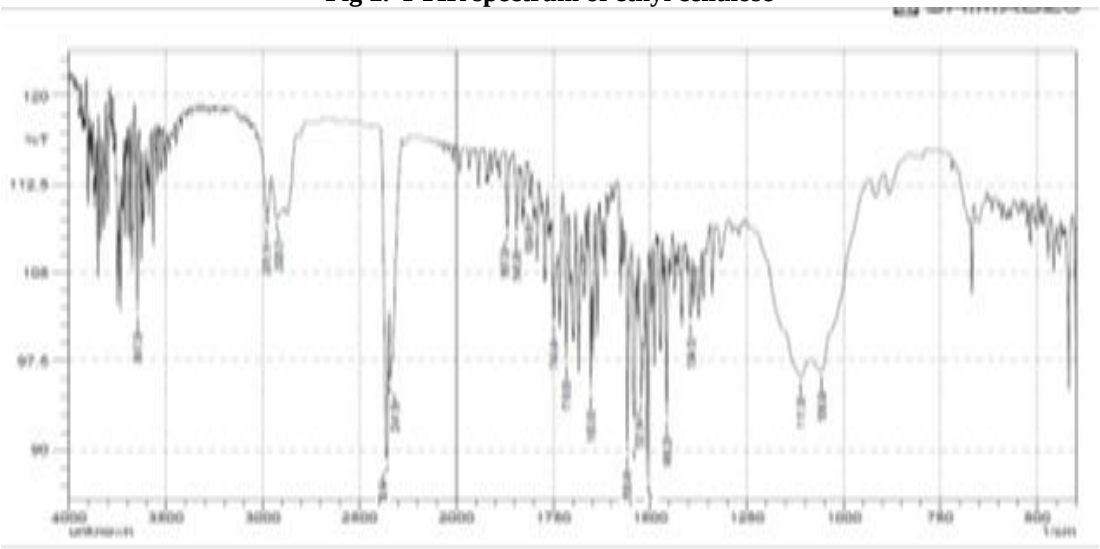


Fig 3. FTIR spectrum of microsphere

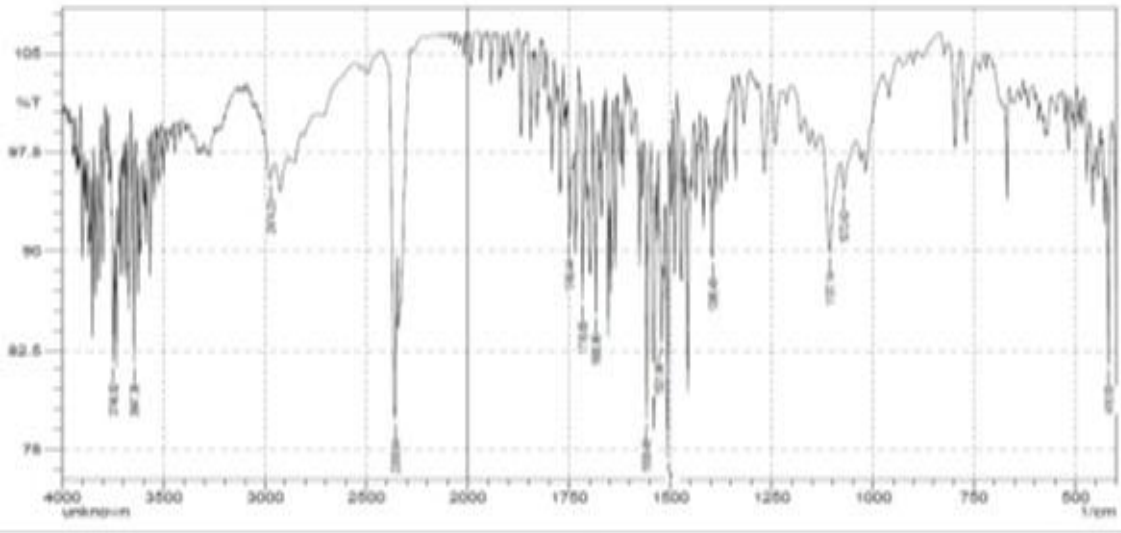


Fig 4. % CR of Alprazolam IR formulation

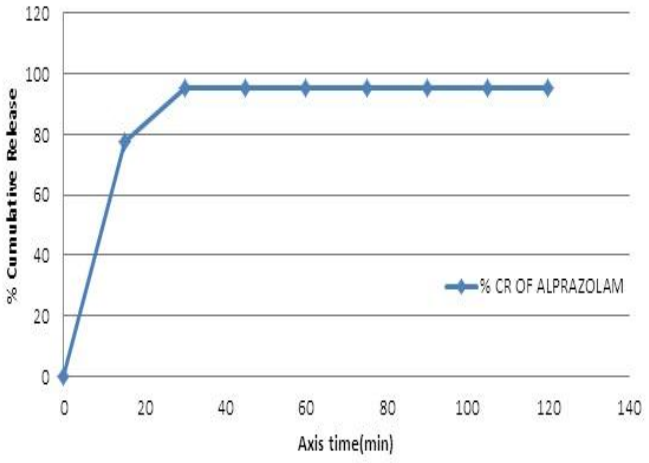


Fig 5. *In-vitro* release kinetics of sustained release microspheres

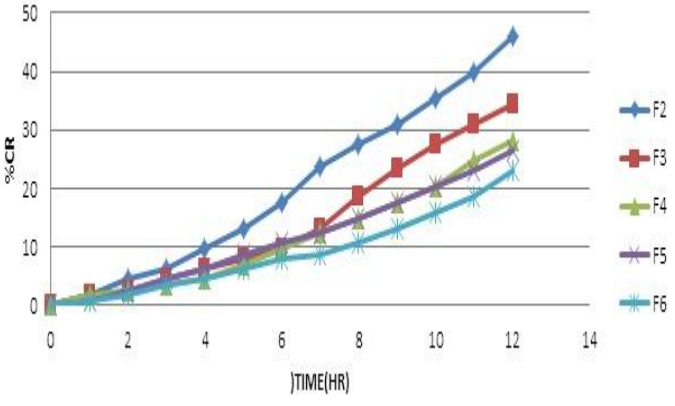


Fig 6. *In-vitro* release of cap in cap formulation

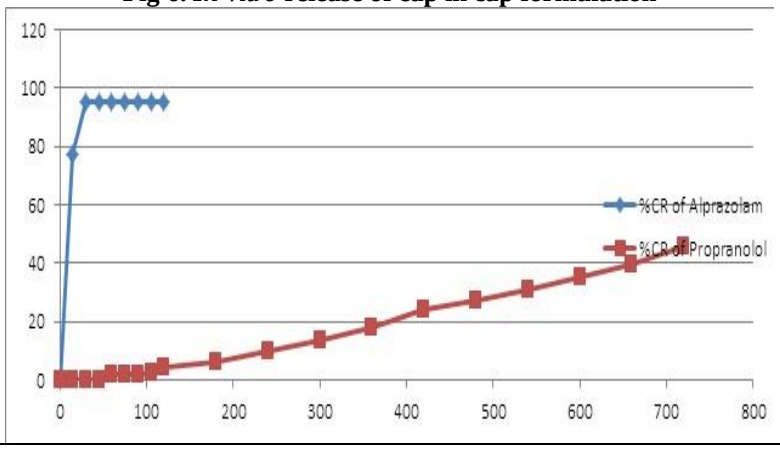
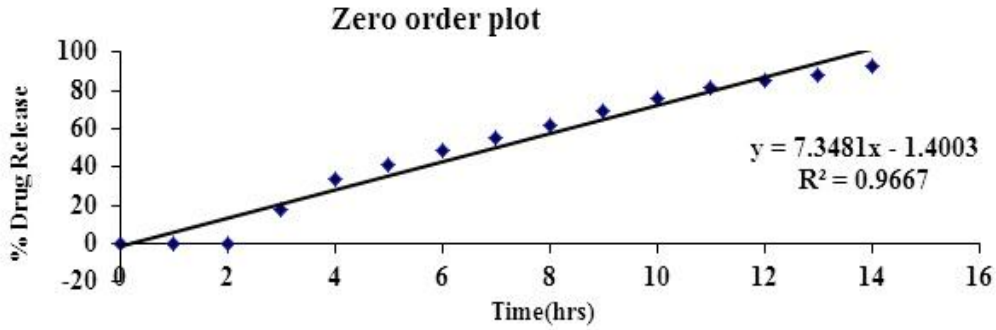
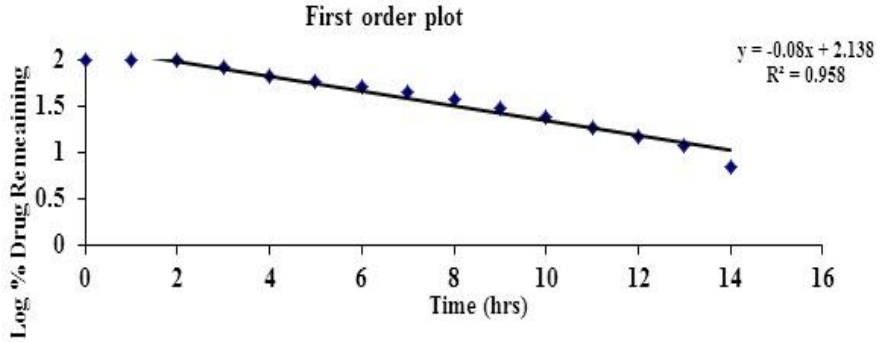
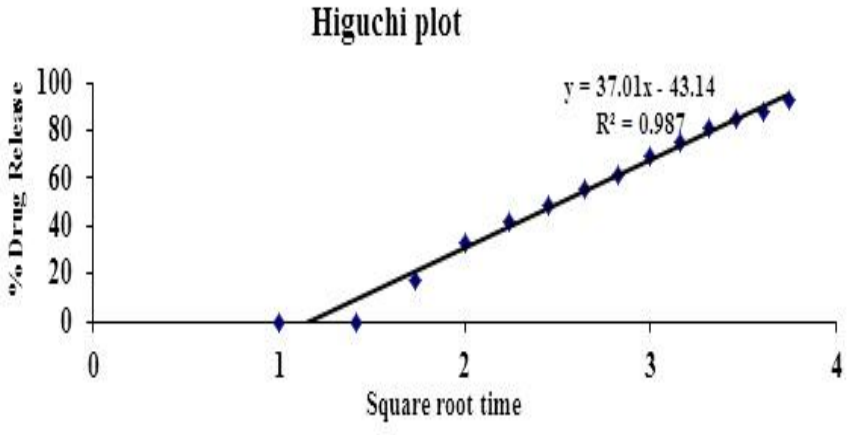
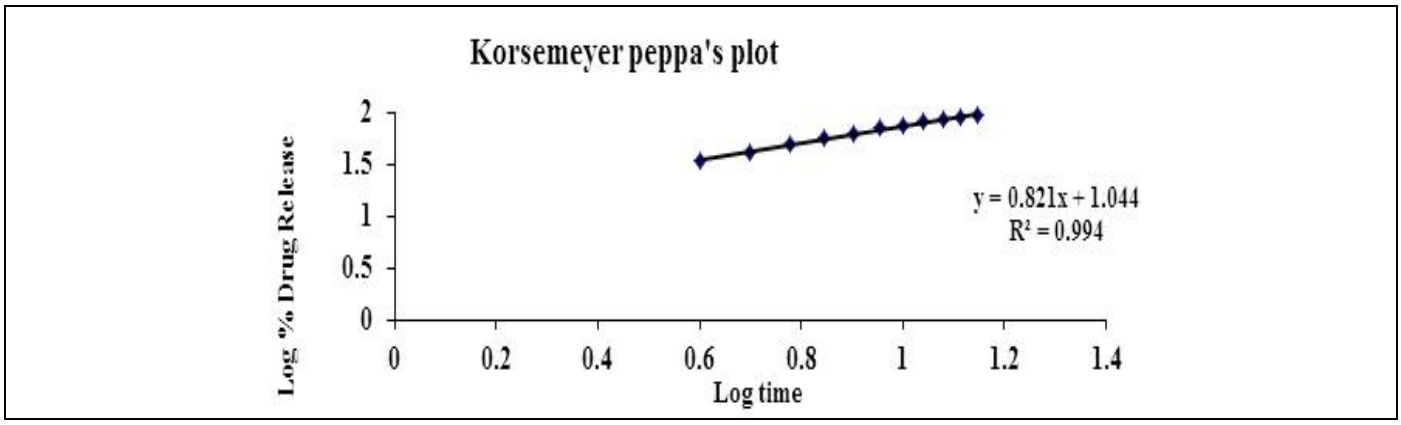


Fig 7. Release kinetics study of microspheres









CONCLUSIONS

A novel capsule in capsule drug delivery system was successfully developed by filling smaller microsphere filled capsule into a bigger liquid filled capsule body. The smaller and bigger capsule body was sealed with 15% (m/m) warm gelatine solution. The optimized formulation of microsphere was selected through in vitro release studies. The optimized microsphere formulation, released 43.235% of drug at the end of 12th hour.

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CONFLICT OF INTEREST

No

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