Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 1
ISSN: 2321–3647(online)

Calcium Alginates Microbeads for Oral Delivery of Clonidine Hydrochloride

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ABSTRACT

In present work calcium alginate (CA) microbeads encapsulated clonidine hydrochloride was prepared by ionotropic external gelation method in which calcium chloride used as cross-linking agent. Clonidine hydrochloride is a sympatholytic centrally acting α_2 adrenergic agonist and imidazoline receptor agonist used to treat high blood pressure, attention deficit hyperactivity disorder, anxiety disorders, migraine, and menopausal flushing but it have very short half life which required some new approach which help to retain it in the body for longer period of time. Calcium alginate microbeads represent a useful tool for oral sustained/ controlled drug delivery. The prepared alginate beads were studied for percent loading efficiency, average particles size, shape and surface morphology, Differential Scanning calorimeter (DSC) and drug release behavior. Chitosan and HPMC incorporated in addition to overcome rapid drug release at higher pH due to its high porosity. Prepared optimized alginate microbeads formulation found to be 356.22±8.1nm average in size, 86.46±2.6% entrapment efficiency. DSC and FT-IR confirm no interaction with the polymers used in the formulation. High concentration of polymer and $CaCl_2$ increase the drug loading efficiency while high concentration of $CaCl_2$ also retard the release of clonidine hydrochloride from the beads. The shape and surface morphology was determined by scanning electron microscopy (SEM) and found the beads in smooth and spherical in shape. In-vitro release study showed that microbeads release 97.85 % drug in 7 hr which was pH dependent and also due to swelling and surface erosion. Microbead release the drug which follow Higuchi matrix diffusion release kinetic. Finally it is concluded that alginate, HPMC and chitosan in combination can be used to prepare microbead to encapsulate clonidine hydrochloride in higher amount. Biodegradable and non toxic nature of polymer make the prepared microbeads suitable for oral administration.

Keywords: Microbeads, alginates, chitosan, HPMC, sustained release.

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Received 11 December 2015, Accepted 28 December 2015

Please cite this article as: Lodhi D *et al*., Calcium Alginates Microbeads for Oral Delivery of Clonidine Hydrochloride. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Some potent drug having low bioavailability due to poor solubility or permeability, low stability in GIT need to research to discover alternate approach to formulate these type of drug with overcoming above problem. Hence, research continuously keeps on searching for ways to deliver drugs over an extended period of time. With. The objective of the present research work was to develop a clonidine sulphate loaded micro particulate oral sustained release dosage form, to reduce dosing frequency, to eliminate the dose related adverse effects and manage anxiety and hypertensive disorder [1-3].

There are some serious problem of these conventional dosage forms is that, instead of delivering minimum effective concentration of drug at the site or organ, it often produces higher concentration which also resulting in untoward adverse reactions. The newer forms of drug delivery systems have emerged largely to overcome the problems experienced with the conventional dosage forms. An ideal drug delivery system, in addition being therapeutically effective and non-toxic, should achieve and maintain effective therapeutic plasma concentration for an extended period [4-7]. This can be solely achieved by the use of modern and sophisticated techniques universally known as "novel drug delivery systems. The goals in designing novel drug delivery systems are spatial placement and temporal delivery of drug. Spatial placement relates to targeting drug to its site of action, while temporal delivery refers to controlling the rate of drug delivery to the target tissue [3,8-10].

Sodium alginate, a hydrophilic biopolymer obtained from brown seaweeds has been found to be highly promising with respect to drug delivery because of its high biological safety. Chemically, it is a polysaccharide composed of varying proportions of D-mannuronic acid (M) and L-guluronic acid (G) residues which are arranged in MM or GG blocks interspersed with MG blocks [5,11, 12, 13]. In addition to its use as a thickening, gel forming and colloidal stabilizing agent in the food and beverage industries, it is also used as a binder in tablet formulation. Its unique property of forming water insoluble calcium alginate gel through ionotropic gelation with Ca+2 ions under simple and mild conditions has made it possible to encapsulate both macromolecular agents and low molecular weight therapeutic agents. The current uses of alginate-based devices are mainly related to encapsulation of various classes of therapeutic agents [1, 14-16].

Clonidine Hydrochloride is a centrally acting sympatholytic and Imidazoline-derivative hypotensive agent; selective α2-adrenergic agonist. It stimulates alpha2-adrenergic receptors in the brainstem to decrease sympathetic nervous system outflow. It is also administered epidurally

to treat pain. It is prescribed alone or in combination for the reduction of high blood pressure and is an adjunct for the treatment of cancer pain when pain persists during intraspinal opiate treatments. It act by stimulating alpha-adrenergic receptors in CNS, decreasing sympathetic outflow, inhibiting vasoconstriction, and ultimately reducing blood pressure. Also prevents transmission of pain impulses by inhibiting pain pathway signals in brain. Since clonidine sulphate have very short half life and envisage to design a sustained drug delivery formulation in a form of microbeads which release the loaded drug in sustained manner for a longer period of time and effectively manage anxiety and hypertensive disorder. Microbeads offers numerous advantages for releasing one of the drugs or part of the same drug immediately while remaining drug or parts of the same can be sustained release. The objective of the present work is design to formulate and evaluate micro beads of clonidine for the treatment of anxiety and hypertensive disorder [17-18].

MATERIALS AND METHOD

Material:

Clonidine hydrochloride purchased from Neon Laboratories Ltd. Mumbai, Sodium alginate and Calcium chloride, HPMC purchased from Himedia Laboratory, Mumbai. All other chemical and regent used are AR grade.

Methods:

Preparation of clonidine hydrochloride loaded microbeads:

Clonidine hydrochloride loaded alginate bead were prepared according to Mandal et al., 2010[18] with slightly modification in which 2.0 % W/V solution of sodium alginate, chitosan and HPMC in different ration was prepared in de-ionized water and sonicate with probe sonicator for 90 sec to get homogenize solution. In 50ml of sodium alginate solution, 200 mg of Clonidine hydrochloride was dispersed uniformly then bubble free dispersion was dropped through a syringe with a needle (0.18mm) into 100 ml aqueous calcium chloride solution (concentration ranging from 1.0-5.0% w/v) and stirred continuously with magnetic stirrer on 500rpm. After 30 minutes of interaction upon stirring, the microbeads were separated from the counter ion solution by filtration, then they were washed three times with distilled water and finely air dried overnight or at 70°C for 3 h in hot air oven [19].

Table 1. Formulation of alginate beads

Formulation code	Sodium Alginate % (w/v)	Calcium Chloride % (w/v)	HPMC % (w/v)	Chitosan % (w/v)
F1	2	1.25	0.5	0
F2	2	2.5	1	0.5
F3	2	5	1.5	1
F4	2	1.25	0.5	0
F5	2	2.5	1	1
F6	2	5	1.5	1.5

Characterization of prepared alginate beads

Determination of drive properties of dried beads

It is ratio of weight by volume. It was resolute by utilizing mark off cylinder, the precisely measured quantity of product microbeads inserted to cylinder and three times tapped. Noted the level, and calculated bulk density using formula [20].

$$\rho b = M/V$$

Where, m = mass of sample, v = volume of sample, ρb =Density

Tapped Density

The sample of about 10 cm3 of powder was carefully introduced in 25 ml glass cylinder. The cylinder tapped at 1 inch height, with intervals of 2-3 second on a rough wood surface three- four times. Density of Bulkiness calculated by using equation Below:

$$Do = M / Vp$$

Where, Do = Tap density ,M = samples wt (gm), Vp = final material volumes (cm^3)

Angle of repose

It was carried out, using funnel, at sufficient height funnel was fixed and, microcapsules were added through it until the pile touched at bottom of funnel. Pile height as well as radius measured and using formula angle of repose calculated [20].

$$\tan \Theta = h/r$$

Where, r = height, and r = is radius of pile

Percentage yield

It was determined by weighing after drying. The précised mass of produced microbeads were divided with mass of total non–volatile components utilized for the microbeads preparation, which gave the total percentage yield of microbeads [21].

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100$$

Particle size determination

The particle size of Clonidine loaded calcium alginate beads was carried out using an optical microscope along with a ocular and stage micrometer having an accuracy of 0.01 mm. Firstly small quantity of beads were suspended in liquid paraffin in a separate beaker and then a drop of suspension was placed on a glass slide and covered with a cover slip. The average sizes of more than 100 beads were determined for each formulation using the calibration factor. The average diameter of the beads was calculated using the following formula:

$$X = \frac{\sum(xi)}{n}$$

X = Average particle diameter, xi = Individual diameter of beads, n = Number of beads.

Determination of drug content

For determination of drug content, 10 mg of beads were suspended in 20 mL of phosphate buffer pH 7.4 and kept for 24 h to extract encapsulated clonidine sulphate. Resultant extract analysed for Clonidine hydrochloride using UV spectrophotometer (ELICO-164, double beam UV Spectrophotometer) at λ_{max} 265 nm [21]. The drug encapsulation or loading capacity of the beads was calculated using following equation.

$$Drug\ Loading = \frac{Total\ amount\ of\ drug\ in\ particles}{Weight\ of\ particles\ taken} \times 100$$

Morphological study using SEM

Surface morphology of drug-loaded alginate beads were determined using Scanning Electron Microscope (FEI-Philips Analytical Electron microscope (Diya labs, Mumbai). Beads mounted directly on scotch double adhesive tape which was adhere on the stub then it was coated with carbon and gold (100 and 50 Å thickness respectively) sputter module in a vacuum evaporator in an argon atmosphere. The coated samples were then observed under a scanning electron microscope operated at 15 KV.

Drug-Polymer Compatibility analysis

The IR analysis of pure drug, and drug loaded beads or polymer were analyzed with FT-IR spectrophotometer (Shimadzu FTIR-8400, Japan) to determine authenticity of drug interaction with polymer. Drug and drug loaded beads were mixed and crushed with KBr and pressed with applying 600kg cm^{-2} to get pellets. Then pellets were analyzed with spectral scanning in the range of 400-4000 cm^{-1}. FTIR and DSC study were performed to identify the drug polymer interaction and it was determined by differential scanning calorimetry (DSC) analysis. This study

was carried out to detect any change on chemical constitution of the drug after combination with the excipients in the ration (1:1).

***In-vitro* drug release profile of formulated microbeads**

The dissolution studies executed utilizing (type II) XXIV USP dissolution rate test apparatus in 0.1 N HCl (900 mL) for 2 hrs followed by phosphate buffer pH 7.4 (900 mL) dissolution media, at 100 rpm and 37±1°C temperature upto 12 hr. At predefined time interval, 5ml of aliquots was withdrawal from apparatus and same volume was replaced with fresh volume of dissolution medium to maintain sink condition. Withdrawn aliquots after adequate dilution analyzed by UV spectrophotometer (ELICO-164, double beam UV Spectrophotometer) at λ_{max} 265 nm. The percent release of Clonidine hydrochloride calculated and graph plotted against time [21,22].

Drug Release Kinetic Studies

The drug dissolution data were subjected to release kinetic studies to find out means of drug release from the prepared microbeads.

Accelerated Stability Studies of the optimized batch

The microbeads from the selected and optimized batch were studied for stability and kept under the accelerated conditions of temperature and humidity (40°C and 75% RH) for the period of six months. Every sample separately weighed and enclosed by aluminum foils and sealed in black PVC bottle and kept in specified conditions at humidity chamber for six months. The formulation was checked for physical changes and drug content [22].

RESULTS AND DISCUSSION

Preformulation Study

Preformulation testing is the first step in the rational development of dosage forms of a drug substance. Preformulation study was done initially and results were directed for the further course of formulation. In solubility and partition coefficient study of drug was determined and it was found that drug is lipophilic in nature. Identification and authentication of drug sample was done by ultraviolet spectroscopy and infrared spectroscopy. The spectra and λ_{max} were found similar which was reported in standard monograph confirm the drug sample was authenticate.

Drug Polymer Compatibility study

DSC thermogram showed endothermic and exothermic peaks. Drug and polymer displayed their characteristic individual melting trends without any appreciable deviation. From this it is observed that there is no interaction between drug and polymer (figure 1).

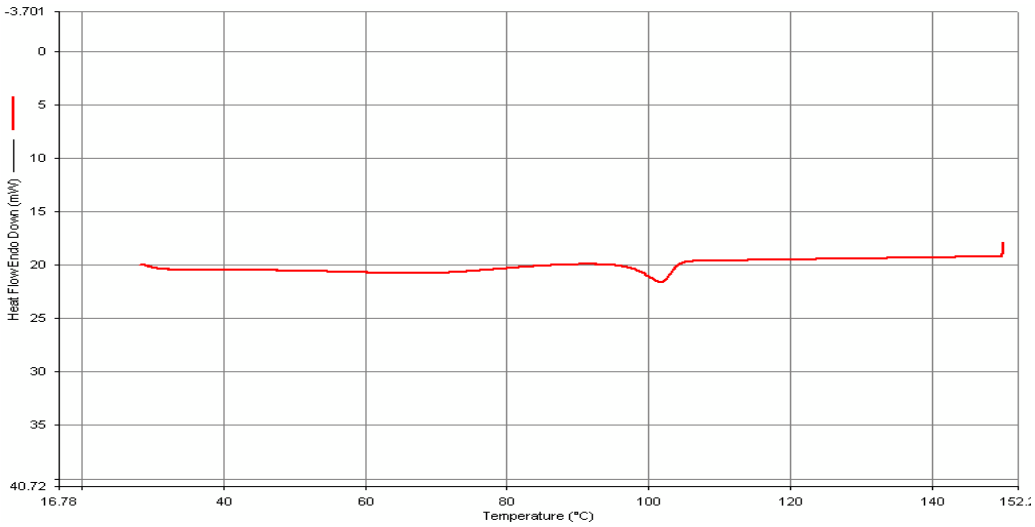

Figure 1: DSC Thermogram of drug sample

Micromeritic Studies

The results of the density of bulkiness and density of tapping were mentioned in table. Bulkiness values were lies in 0.297 to 0.542 g/cm3 and density of tapping values lies in 0.508 to 0.654 g/cm3 i.e. less than 1.2, indicates good packing. The values of Average particle size and angle of repose were lies in between 291.46 ±8.3 to 432.62 ±7.3, and 250-12' to 300-20', respectively indicates acceptable particle size, flow property and also good packing ability (table 2).

Table 2: Micromeritics Studies of Microbeads

Batch	Avg microbeads size	Bulk Density	Tap Density	Angle of Repose
F1	291.46±8.3	0.298	0.522	250-15'
F2	323.44±6.9	0.542	0.654	260 -20'
F3	356.88±8.6	0.526	0.636	250-12'
F4	263.84±8.3	0.430	0.508	300-20'
F5	327.65±7.5	0.482	0.528	250-06'
F6	356.22±8.1	0.516	0.616	310-24'

Estimation of Clonidine hydrochloride.

In this work, clonidine hydrochloride loaded calcium alginate beads prepared by ionic interaction methods according to Mandal et al., 2010 in which crosslinking of sodium alginate was done using CaCl2 and clonidine hydrochloride was loaded within the same. Beads were optimized using different concentration of alginate, drug and calcium chloride and formulate in different formulation (F1-F6)

Particle size was in the range of 291.46±8.3-356.22±8.1µm for the beads obtained using the sequential method. The particle size of the beads increase with increasing the concentration of chitosan and HPMC and slight decreased in size as increase $CaCl_2$ concentrations.

Surface Morphology

The surface morphology of the prepared beads was studied by scanning electron microscopy (SEM) and the SEM photographs at 10000X magnification are showed in figure 2. SEM photographs of the blank beads (figure 2 (a)) compared with drug loaded beads (figure 2 (b)) show a no difference in surface morphology. Surface of both drug loaded and without drug was found smooth in surface and almost spherical in shape.

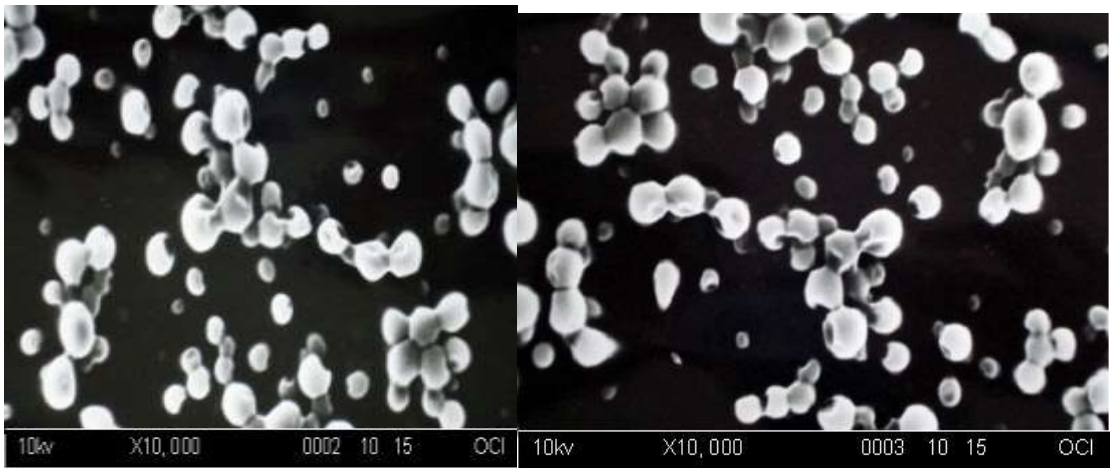

Figure 2: SEM Image of alginate microbeads (a) without drug loaded microbeads (b) Drug loaded microbeads

Drug loading efficiency and percent yield

Drug loading was found to be directly proportional to polymer concentration. From prepared formulation, F6 shows 86.46±2.6 % drug content. It was observed that as increase the concentration of HPMC and chitosan, increase the loading efficiency and it was due to the increase in molecular density of polymers to entrap more drug molecules. The drug loading efficiency further increase with increasing the concentration calcium chloride as cross linking agent. It is due to the greater availability of active calcium binding sites in the polymeric chains and consequently, the greater degree of cross linking as the quantity of sodium alginate, chitosan and HPMC as blend increased. Increase in CaCl2 concentration from 1.25 to 5.0 % and polymer blend concentration from 2.5 to 4.5% resulted in an increase in the drug loading from 82.92±1.4 % to 86.46±2.6 % (figure 3 & table 3)

Table 3: Percentage yield and Percent drug entrapment of microbeads

Batch	% yield	% Drug Entrapment
F1	84.14±0.9	82.92±1.4
F2	86.40±3.6	85.12±2.4
F3	90.25±1.9	86.30±1.1
F4	82.62±2.6	86.48±2.6
F5	82.71±1.4	85.94±1.5
F6	83.50±1.7	86.46±2.6

*All the values represent mean ± standard deviation (n=3)

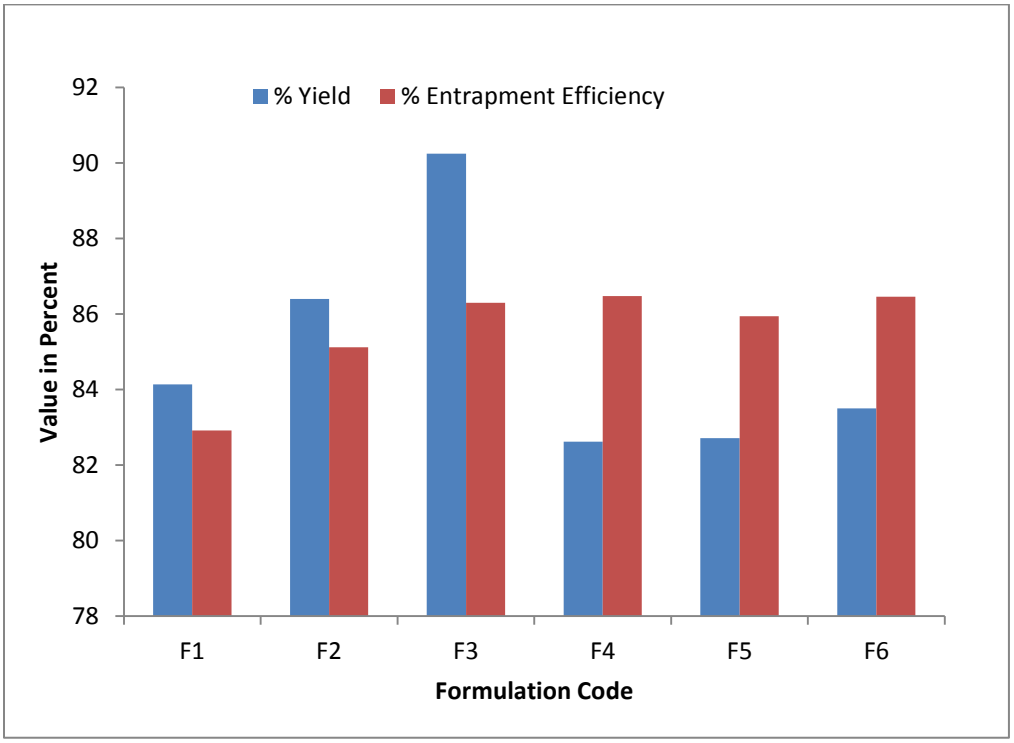

Figure 3: Percent yield and percent entrapment efficiency

***In-vitro* release profile study of formulated microbeads**

The release profile of prepared bead in 0.1 N HCL for first 2 hours and in phosphate buffer for 6 hours from are shown in Figures 4-6. The drug release from the alginate beads depends on the penetration of the dissolution medium into the beads, swelling and dissolution of alginate matrix, and the dissolution of the drug subsequent to leaching through the swollen matrix. Drug release from optimized alginate beads formulation (F6) was found about 7.6 % drug in the first 2 h in 0.1 N HCl. While drug release was found 97.8 % in phosphate buffer pH 7.4 after 7 hr. The less amount release i.e. 7.6% in 0.1% HCl is due to bead remain intact in the acidic media. While it come in contact with higher pH then it become swell and gradually erosion or dissolve and release the drug in higher amount but in controlled manner. The release profile of all formulation is given in table. When the HPMC concentration increase with formulation then drug release was goes to slow in comparison to other formulation which contain low concentration of HPMC. HPMC provide viscous environment in the stagnant layer which retard the release of drug. It was found that the formulation shows Higuchi matrix diffusion release kinetic for optimized formulation. Different release kinetic model shown in figure 5-7.

Table 4. In vitro Cumulative % drug release profile

S. No	Time	% Drug Release					
		F1	F2	F3	F4	F5	F6
1	0	0	0	0	0	0	0
2	1	7.137	8.517	7.594	8.450	6.472	7.650
3	2	12.982	13.271	13.160	13.748	13.532	13.872
4	3	22.874	24.482	25.654	25.759	25.593	24.981
4	4	38.764	37.268	38.147	37.620	38.398	39.760
5	6	62.765	61.734	62.157	65.650	63.564	62.705
6	8	82.726	84.372	85.245	83.746	84.650	85.760
7	10	98.659	98.360	98.590	97.540	98.380	97.853

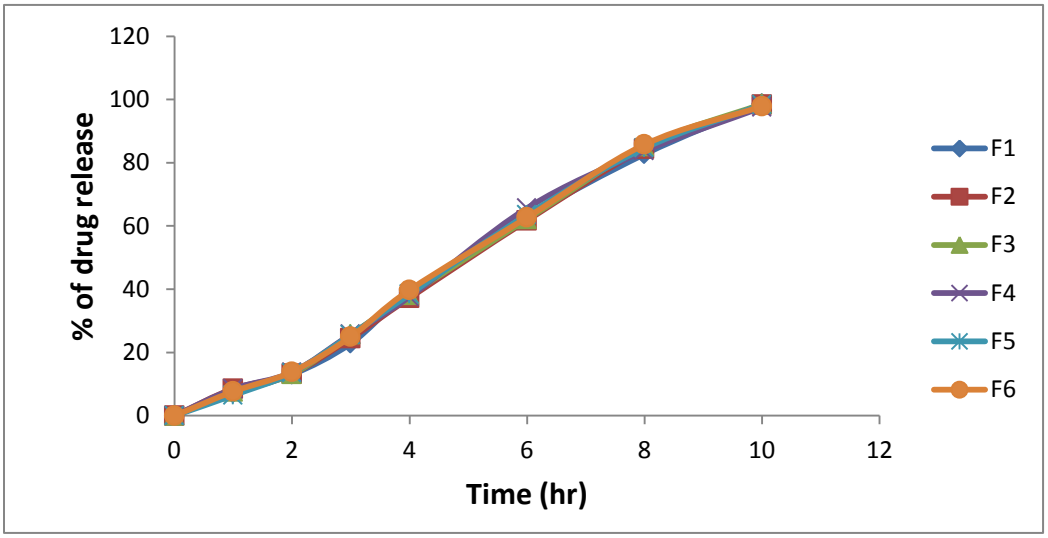

Figure 4: In vitro cumulative % drug release of clonidine hydrochloride from microbeads

***KMP = KorsmeyerPeppas model *HXC = Hixson Crowell model**

The batch F6 followed the zero order and non Fickian drug release except batch F3, it follows the Hixson crowel model non fickian drug release.

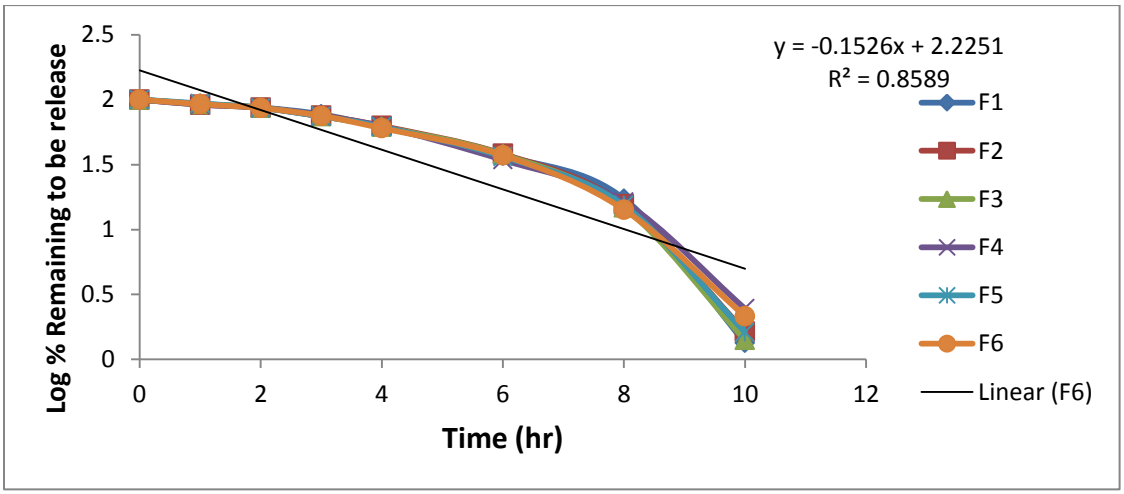

Figure 5: First Order Release Kinetics

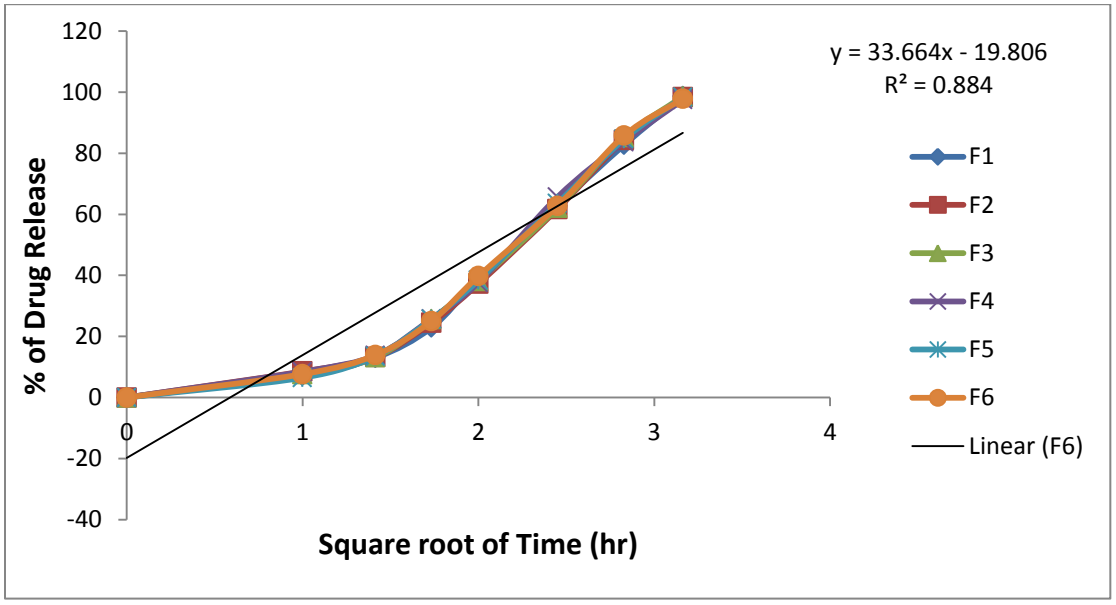

Figure 6: Higuchi Equation of Microbeads

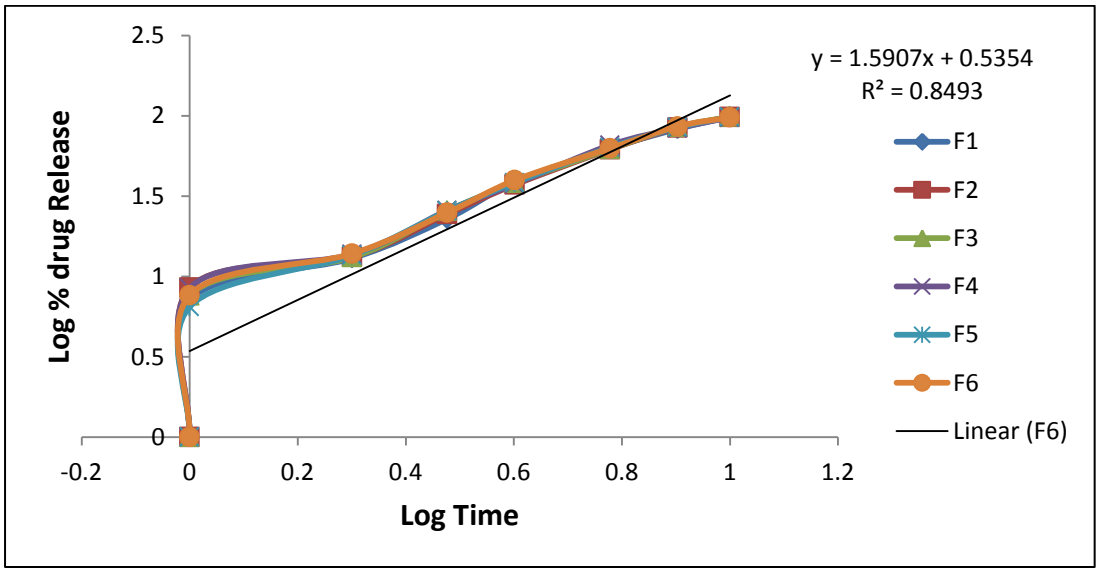

Figure 7: Pappas Equation of micro beads

Accelerated Stability Studies

The microbeads from the selected and optimized batch (FH) were studied for stability and kept under the accelerated conditions like raised temperature and moisture up to period of six months. The results revealed no marked alterations in physical appearance and drug releasing properties.

Table 5. Accelerated stability testing effect of temperature and humidity on *in-vitro* drug release for formulation F6.

Time (Hours)	Cumulative % Drug Released			
	At 0 Month	At 2ndMonth	At 4thMonth	At 6thMonth
0	0	0	0	0
1	20.96	19.69	19.19	18.89

2	27.47	25.87	24.17	23.97
3	33.63	31.83	30.93	30.13
4	42.72	41.12	40.92	40.32
5	54.19	52.87	52.17	51.97
6	61.15	60.13	59.83	59.23
7	73.81	71.89	71.21	70.11
8	82.72	81.26	80.86	80.21
9	88.63	86.93	86.13	85.33
10	90.94	88.24	87.94	87.14
11	94.49	93.94	93.14	92.64
12	97.78	95.81	95.12	94.82

CONCLUSION

Oral sustained release drug delivery using microbeads preparation is potential tool for the methodology expansion and upgrading purpose. Generally clonidine hydrochloride are given into conventional, immediate releasing preparations and the frequency of administration increased up to twise-thrise time for ones a day because of clonidine hydrochloride have shorter biological half life. In such a case, the sustained release formulation will be beneficial than the immediate release dosage form as therapeutic level is maintained for an extended period of time, eliminating maxima in drug concentration commonly associated with multiple doses. Clonidine hydrochloride loaded calcium alginate beads along with using HPMC, chitosan in various ratios successfully prepared by the ionotropic gelation method, using calcium chloride as a cross linking agent. The beads were found with high drug entrapment, suitable average particle size. It shows Higuchi matrix diffusion sustained drug release kinetics in acidic and phosphate buffer media. The drug entrapment is increases with increased calcium chloride and polymer concentration until saturation of polymer with drug. Increase in HPMC concentration retarded the drug release because it produced high viscous environment in stagnant layer. Finally it is concluded that alginate, HPMC and chitosan in combination can be used to prepare microbead to encapsulate clonidine hydrochloride in higher amount. Biodegradable and non toxic nature of polymer make the prepared microbeads suitable for oral administration.

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