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Formulation and Characterization of Alginate Microbeads of Clonidine Hydrochloride for the Treatment of Anxiety and Hypertensive Disorder

Harshwardhan Dubey *, Bharti Patel and Nazneen Dubey

Shri Ram Institute of Technology- Pharmacy, Jabalpur, (M.P.) - India

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Abstract

The objective of this study was to prepare and evaluate sodium alginate microbeads with calcium chloride as cross-linking agent for Clonidinehydrochloride by ionotropic gelation method. Clonidine hydrochloride a centrally acting sympatholytic and imidazoline-derivative hypotensiveagent; selective α2-adrenergic agonist. It stimulates alpha2-adrenergic receptors in the brainstem to decrease sympathetic nervous systemoutflow. It is also administered epidurally to treat pain. Microbeads offer numerous advantages for releasing one of the drugs or part of the samedrug immediately while remaining drug or parts of the same can be sustained release. Prepared microbeads were evaluated for particle size,polydispersity index,zeta potential, particle shape, surface morphology, entrapment efficiency and In-vitro drug release. The prepared beadswere free flowing and white in colour. The drug loaded beads showed 72.9±2.4% to 94.6±2.6 % drug entrapment, which was found to increasewith increase in alginate concentration. In vitro drug release study of these microbeads indicated controlled release for Clonidine hydrochloride83.46% release after 48 hours. Hence the observations of all results of the different batches, MBD 11 showed controlled release action and improved drug availability. From this study it could be concluded that the free flowing micro beads of Clonidine hydro chloride could be successfully prepared by ionotropic gelation technique with high entrapment efficiency and prolonged release characteristics.

Key words: Clonidine hydrochloride, Microbeads, Sodium alginate, Calcium chloride, Ionotropic gelation method

Introduction

Controlled drug delivery technique presents front line part of today's developed technique, in this includes many scientific approaches, serving for individual care[1]. The drug deliverance technique having abundant advantages than existing conventional type of dosage, it involves enhanced effectiveness, minimized poisoning, enhanced consumer conformity also ease[2, 3]. This type of drug deliverance technique utilizes micro molecules, for caring drugs. As the varieties of forms for dosage are invented like microparticle as well as nanoparticles shown more significance [4, 5]. An ideal and advanced oral drug delivery system is that, which exactly controls speed, time as well as site of release of medicament separately of normal physiological variables such as gastrointestinal tract pH, digestive condition of the gastrointestinal tract, peristalsis movement and circadian rhythm. Advance in polymer science and technology outcome in pick up the pace research and developmental activity in the design of drug delivery devices [6, 7].

***Corresponding Author**

Clonidine hydrochloride a centrally acting sympatholytic and imidazoline-derivative hypotensive agent; selective α_2- adrenergic agonist. It stimulates alpha2-adrenergic receptors in the brainstem to decrease sympathetic nervous system outflow [8]. 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[9]. It acts 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 [10]. The aim of the present study, which was to develop sustained release oral product namely microbeads of Clonidine hydrochloride using sodium alginate as the hydrophilic carrier in combination with calcium chloride as drug release modifiers in various proportions to overcome the drug related adverse effects, improve drug bioavailability.

Material and Method

Material

Clonidine was received as a gift sample from Kalindi MedicurePvt. Ltd, Vapi (India). Sodium alginate (Himedia chemicals, Mumbai), Calcium chloride (Unichem chemicals, Mumbai), All other reagents and chemicals used were of analytical grade. Triple distilled water was generated in house**.**

Methods

Method of Preparation of Micro-beads

The microbeads were prepared by ionotropic gelation technique in which sodium alginate (1-4%w/v) was accurately weighed and dissolved in slightly warmed distilled water. The sodium alginate solution was homogenized by stirring on magnetic stirrer for 45 min before formulation. Drug (10-40 %w/v) was accurately weighed and added or disperses in alginate solution during homogenization. After complete the homogenization process, solution was kept stand for 15 min without stirring and then sonicate for 10 min using bath sonicator to remove the air bubbles formed during homogenization. In another beaker 100 ml of 3-6 % w/v calcium chloride solution was prepared in which sodium alginate solution containing drug was dropped with the help of 29-gauge hypodermic needlefitted with a 10ml syringe into previously prepared calciumchloridesolution.10cmdistancewasmaintainedduringdropping the alginates solution. Beads were incubated for 30min and after complete incubation beads were separated by filtering the solution. Obtained beads were washed three times with distilled water and dried at 40 ^{o}C. Prepared beads were stored in very tight container before further use in their characterization[11-13].

Optimization of Drug Loaded Microbeads

Optimization of polymer concentration

Optimization of polymer in the microbeads formulation was carried by taking different concentration of polymer and other parameter was remaining constant. Microbeads were optimized on the basis of average particle size and drug entrapment. The stirring speed was kept remain constant i.e. 400-500 rpm.

Table1: Optimization of polymer in the micro-beads formulation

Formulation Code	Sodium Alginate(%w/v)	Calcium Chloride(%)	Drug	Particle size(µm)	Drug Entrapment
MBD 1	1	3	10	156.7±2.30	72.9±2.4
MBD 2	2	3	10	159.4±4.25	76.5±1.9
MBD 3	3	3	10	173.4±3.7	82.2±2.4
MBD 4	4	3	10	215.3±5.8	83.8±2.3

(n=3)

Optimization of Calcium chloride concentration

Calcium chloride worked as gelling agent by ionic interactionmechanism.Itstabilizesthepolymerdropletssoitisnecessary to optimize the calcium chloride concentration to get a high stable micro beads formulation. Concentration of Calcium chloride was optimized for micro-beads formulation by taking different concentration of calcium chloride and other parameter was kept constant. Micro-beads were optimized on the basis of average particle size and drug entrapment and their shape and surface morphology.

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Table 2: Optimization of calcium chloride in the micro beads formulation

Formulation Code	Sodium Alginate(%w/v)	Calcium Chloride(%w/v)	Drug	icle size(µm)	Dru Entrapment	Shape
MBD 5	3	3	10	173.9±2.3	83.2±3.3	Spherical
MBD 6	3	4	10	168.4±4.5	84.3±1.8	Spherical
MBD 7	3	5	10	163.7±2.7	86.4±2.5	Spherical
MBD 8	3	6	10	158.2±3.3	89.5±2.8	Irregular

(n=3)

Optimization of drug concentration

Microbeads were optimized on the basis of average particle size, drug entrapment efficiency and their shape and surface morphology. The entrapment efficiency of drug depends on concentration of drug used. Entrapment efficiency was optimized by taking different concentration of drug and the other parameter was kept constant.

Table 3: Optimization of drug concentration in the micro beads formulation

Formulation Code	Sodium Alginate(%w/v)	Calcium Chloride (%)	Drug	Particle size(µm)	Drug Entrapment
MBD 9	3	4	10	160.7±3.1	88.9±2.1
MBD10	3	4	20	162.4±2.2	91.6±1.3
MBD11	**3**	**4**	**30**	**163.5±2.6**	**94.6±2.6**
MBD12	3	4	40	164.3±2.5	94.3±3.4

(n=3)

Method of Characterization of Micro-beads

Particlesize, polydispersity index and zeta potential

Average particle size of micro beads was determined by optical microscopy.The micro beads were suspended in methanol and then dispersed on the glass slide. Slide was observed under microscope to determine the size of beadsusingocularmicrometer.Morethan150beadswereobserved for their size and the size was presented as their average. Measurement of surface charge was based on thezetapotential(e)thatwascalculatedaccordingtoHelmholtz–Smoluchowskyfromtheirelectrophoreticmobility.Formeasurementofsurfacecharge,zetasizerwitha field strength of 20 V/cm on a large bore measures cell wasused and samples were analyze after diluted with 0.9 % NaClto adjust a conductivityof50lS/cm.

Particle shape and surface morphology

Scanning Electron Microscopy (SEM) was used to examinesurface morphology of microbeads. Samples were preparedbysprinklinglyophilizedmicrobeadsondoubleadhesivetape adhere on aluminium stub. Then gold coating (thicknessabout 300A°) was carried out using a sputter coater. Sampleswereexaminedandphotomicrographsweretakenunderscanningelectronmicroscope(LEO435VP,Eindhoven,Netherlands) at an acceleration voltage of 30 kVSEM imageperformed at the Indian Institute of Science Education andResearch(IISER), BHOPAL, MP,India

Entrapment efficiency

Entrapmentefficiencyofmicrobeadsforclonidinewasdeterminedaccordingtothemethoddescribedby Fry (1978)[14] taking drug loaded microbeads equivalent to 100mgofclonidinesulphatewith5.0mLofphosphate bufferpH7.4inabeaker.Themicrobeadswerekeptforswellandallow for macerates for 24 hr then they were triturate withthehelpofpestleandmortar.Themixturewascentrifugedat4000rpmfor30mintosettledownthepolymericmaterial and allow the drug in supernatant solution. The 1.0mlofsamplefromsupernatantsolutionwastakeninavolumetric flaskanddilutedup to10ml.Thesamplewasanalyzedfordrugconcentrationusing UVspectrophotometer.

$$\text{Drug Entrapment} = \frac{\text{Amount of drug in microbeads}}{\text{Intial amount of drug taken for loading}} \times 100$$

In vitro drug release

ThedrugreleasewasperformedinPBS(pH7.4)forclonidine loaded microbeads using dialysis bag technique.

Inthisstudymicrobeadsequivalentto100mgofdrugwastakenindialysistubing(MWCO,15KDa,Himedia) andplaced in a beaker containing 100 ml of PBS

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pH 7.4.Thedialysis bag retains microbeads and allows passing of freedrugintothedissolutionmedia.Temperaturewas maintainedat37±1ºCthroughoutthestudy.2mlofsamples were withdrawn after specified time intervals i.e.0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24 and 48 h and replaced with thesamevolumeoffreshPBSpH7.4andanalyzedfordrugconcentrationbyusingUVspectrophotometer.

Result and Discussion

Procured drug was odorless and white crystalline in nature.Insolubilitystudyitwasfoundthatdrugwassoluble in water, ethanol, methanol and slightly soluble in chloroformand phosphate buffer pH 7.4 and sparingly soluble in 0.1 NNaOH and 0.1N HCl. Melting point of drug was found 128ºC-134ºC while it was 130 ºC reported in standard monograph.Thepartitioncoefficient(logp)valuewas foundtobe1.59 and 1.57 in n-Octanol:PBS pH 7.4 and n-Octanol:0.1 N HClrespectively. The obtained FT-IR characteristic peaks of drugwasmatchedwiththepeaksofdruggiveninstandardmonographwasrevealedsimilar.Thedrugsolution wasscan on UV-spectrophotometer at 200-400 nm in web lengthrange to determine the maximum absorbance (**λ max**) and itwas found at 270 nm. The calibration curve was prepared inphosphate buffer pH 7.4 and distilled water. The regressioncoefficient (R^2) was 0.999 which was shows the linearity ofcurve in both distilled water and phosphate buffer pH 7.4.The line of equation for the standard curve was y = 0.0139x +0.0038andy=0.0069x+0.0023.Thedrugexcipientinteractionstudywasperformedtocheckininteraction betweendrugandotherformulationexcipientsbyspectrophotometrically. There was no interaction was foundbetweendrugandexcipientsanditwasclearlyseenandconfirmed by UV spectrophotometrically scan graph of drugsolutionandmixtureofdrugandsodium alginate. All thedata of preformulation study was found similar as given instandardmonographwhichconfirmedthatthedrug wasauthenticateandpureinformanditcouldbeusedforformulation development of clonidine hydrochloride loadedmicrobeads. Clonidine hydrochloride loaded sodium alginatebeads were successfully prepared by ionic gelation method.The microbeads formulations were optimized on the basis ofaverageparticlesize, drug entrapment,shapeandsurfacemorphology. The mean diameter of optimized microbeads ofSodium alginate increased from 156.7±2.30 µm to 215.3±5.8µm with increasing polymer concentration from 1.0 to 4.0 %w/v.Inthepresentinvestigationa3.0%w/vSodiumalginateconcentrationwasfoundtobeoptimizedwhich provide therequiredsizeofmicrobeadsFig. 1and2.

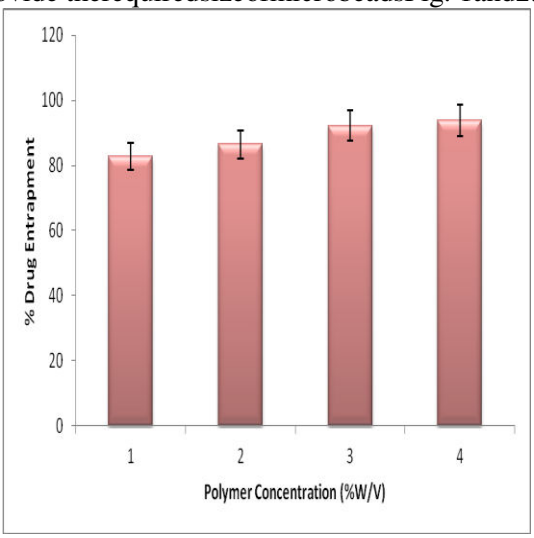

Figure 1: Effect of polymer concentration on entrapmentefficiency ofmicrobeads

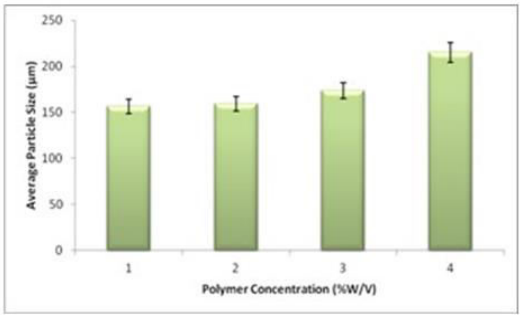

Figure 2: Effect of polymer concentration on averageparticle sizeofmicrobeads

Theaverageparticlesizeofmicrobeadsincreasedwith increasingpolymerconcentration,sinceathigherconcentrationsthepolymersolutiondispersedintolarger dropletsduetoincreasingtheviscosityofpolymersolution and it was the reason behind the enhancement of

averageparticlesizeofmicrobeads.Inthecaseofentrapmentefficiency, it was found increase on increasing the sodiumalginateconcentrationitwasduetotheincreasingtheentrapment of drug molecules in the molecules of polymerand high dense or high concentration of polymer have morenumberofpolymermoleculenetworktotrapthedrugmolecules.Inthecaseofoptimizationofcalcium chlorideconcentration. The particles size found slightly decrease withincreasingthecalciumchlorideconcentration.Optimumconcentrationofcalciumchlorideisrequiring creatingcomplete gelation by ionic interaction of sodium alginate inthemicrobeads.Thecompletegelationisdirectlyproportionaltohighstabilityandstructuralintegrityfor microbeads.Therewasnomajordifferencewasfound incase of increasing drug concentration in the formulation butas increase the drug concentration from 10 to 30 %, the drugentrapment efficiency was found increase from 88.9±2.1 to95.6±2.6%Fig3and4.

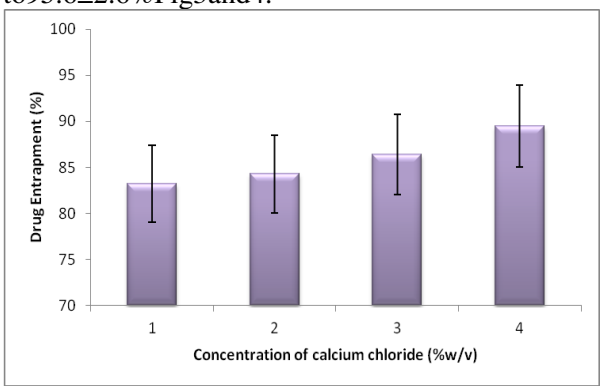

Figure 3: Effect of calcium chloride on entrapmentefficiency ofmicrobeads

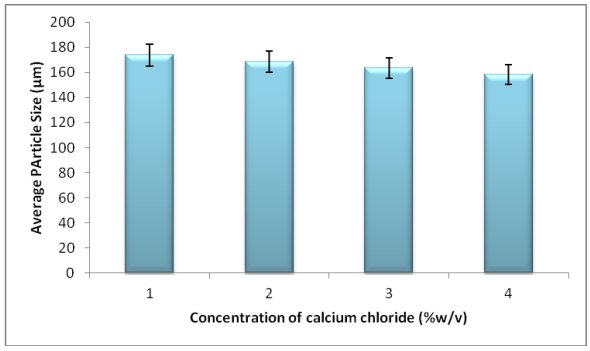

Figure 4: Effect of calcium chloride on average particlesize ofmicrobeads

Further increasing of drug concentration from 30 to 40 wasnotfoundanysignificantdifferenceindrugentrapmentefficiency**.** Formulation coding with MBD 11 consist of 3.0%w/v sodium alginate, 4.0% w/v calcium chloride and 30 %w/vdrugconcentrationwasselectedasoptimizedformulationthatwasshown94.3±3.4%drugentrapment and163.5±2.6µm inaverage particle sizeFig5and6.

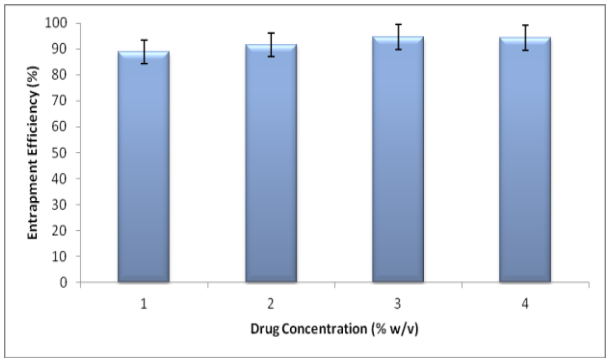

Figure 5: Effect of drug concentration on entrapmentefficiency ofmicrobeads

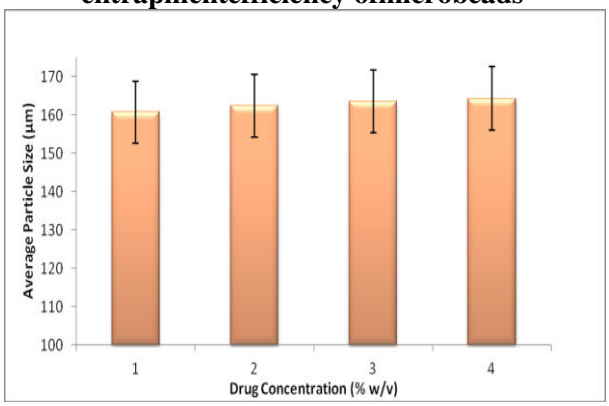

Figure 6: Effect of drug concentration on averageparticle sizeofmicrobeads

Scanning electron microscopy (SEM) analysis revealed thatthe optimized microbeads formulation MBD 11 was foundsphericalinshapeandsmoothinsurfaceFig.7**.**

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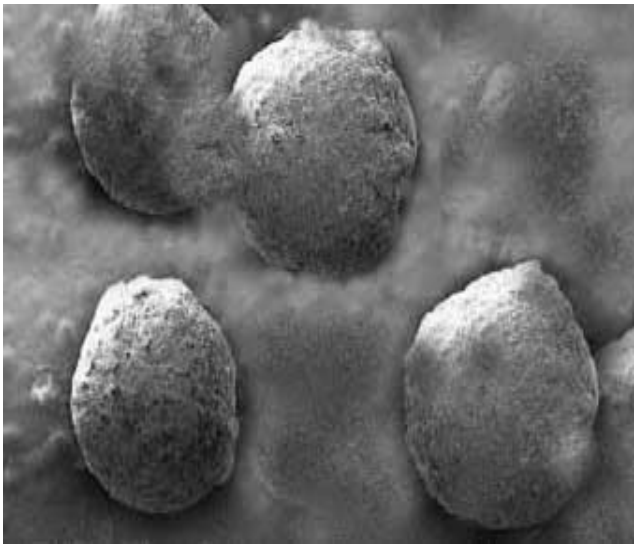

Figure 7: SEM photomicrograph of drug loaded sodiumalginate beads

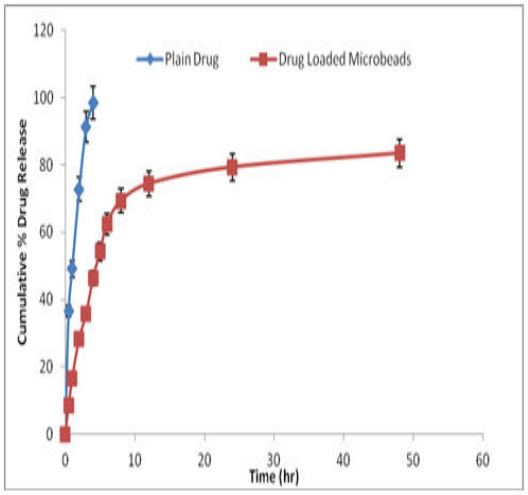

Figure 8: In-vitro drug release of clonidinehydrochloride from microbeads

Invitrodrug releaseprofileofclonidinehydrochlorideinPBS pH 7.4 was found 83.46% after 48 hr Table 4 and Fig 8for optimized formulation (MBD-11) and follows the matrixdiffusionHiguchireleasekinetics.

Table 4: In-vitro drug release of clonidine hydrochlorideinphosphatebufferpH 7.4

S.No.	Timeinterval(h)	Plaindrug	Clonidine HCLMicrobeads
1	0.5	36.59	08.43
2	1	49.15	16.53
3	2	72.79	28.26
4	3	91.38	35.68
5	4	98.49	46.35
6	5		54.23
7	6		62.45
8	8		69.38
9	12		74.43
10	24		79.34
11	48		83.46

Conclusion

It was concluded that from this study thatthe microbeadscanbepreparedfromsodiumalginatebyionicgelationmethodandcanbeencapsulateclonidinehydrochloridewithoutanyinteraction.Itcanreleasedruginverycontrolled and sustained manner following matrix diffusionHiguchireleasekineticmodel.Theprepared microbeadswereoptimizedfordifferentformulation andprocessvariables and found that microbeads were uniform, sphericalandacceptablesizerangewithhighdrugencapsulationefficiency.The prepared formulation can be used to deliverdrugs by oral route for it sustained delivery in GIT systemandformaintaining its therapeuticconcentrationinbloodfor longer period of time and can be used for the effectivemanagementof anxietyandhypertensivedisorder.

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