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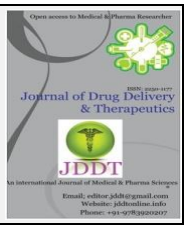

Open Access Research Article

# Formulation and Characterization of Alginate Microbeads of Clonidine Hydrochloride by Ionotropic Gelation Technique

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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 Clonidine hydrochloride by ionotropic gelation method. Clonidine hydrochloride 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. Microbeads offer 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. Prepared microbeads were evaluated for particle size, polydispersity index, zeta potential, particle shape, surface morphology, entrapment efficiency and In vitro drug release. The prepared beads were free flowing and white in color. The drug loaded beads showed 72.9±2.4% to 94.6±2.6 % drug entrapment, which was found to increase with increase in alginate concentration. In vitro drug release study of these microbeads indicated controlled release for Clonidine hydrochloride 83.46% 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 microbeads of Clonidine hydrochloride could be successfully prepared by ionotropic gelation technique with high entrapment efficiency and prolonged release characteristics.

**Keywords:** Clonidine hydrochloride, Microbeads, Sodium alginate, Calcium chloride, Ionotropic gelation method.

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## 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]. Clonidine hydrochloride 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 [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 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 [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 Medicure Pvt. 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 Microbeads

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 homoginaization. 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 homoginization. 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 needle fitted with a 10ml syringe into previously prepared calcium chloride solution. 10 cm distance was maintained during dropping the alginates solution. Beads were incubated for 30 min and after complete incubation beads were separated by filtering the solution. Obtained beads were washed three times with distilled water and dried at 40 °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.

**Table 1: Optimization of polymer in the microbeads 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 interaction mechanism. It stabilize the polymer droplets so it is necessary to optimize the calcium chloride concentration to get a high stable microbeads formulation. Concentration of Calcium chloride was optimized for microbeads formulation by taking different concentration of calcium chloride and other parameter was kept constant. Microbeads were optimized on the basis of average particle size and drug entrapment and their shape and surface morphology.

**Table 2: Optimization of calcium chloride in the microbeads formulation**

| Formulation Code | Sodium Alginate (%w/v) | Calcium Chloride (%w/v) | Drug | Particle size (µm) | Drug 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 microbeads 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 |
| MBD 10 | 3 | 4 | 20 | 162.4±2.2 | 91.6±1.3 |
| **MBD 11** | **3** | **4** | **30** | **163.5±2.6** | **94.6±2.6** |
| MBD 12 | 3 | 4 | 40 | 164.3±2.5 | 94.3±3.4 |

**(n=3)**

### Method of Characterization of Microbeads

#### Particle size, polydispersity index and zeta potential

Average particle size of microbeads was determined by optical microscopy. The microbeads were suspended in methanol and then dispersed on the glass slide. Slide was observed under microscope to determine the size of beads using ocular micrometer. More than 150 beads were observed for their size and the size was presented as their average. Measurement of surface charge was based on the zeta potential (e) that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of surface charge, zetasizer with a field strength of 20 V/cm on a large bore measures cell was used and samples were analyse after diluted with 0.9 % NaCl to adjust a conductivity of 50 lS/cm.

#### Particle shape and surface morphology

Scanning Electron Microscopy (SEM) was used to examine surface morphology of microbeads. Samples were prepared by sprinkling lyophilized microbeads on double adhesive tape adhere on aluminum stub. Then gold coating (thickness about 300A°) was carried out using a sputter coater. Samples were examined and photomicrographs were taken under scanning electron microscope (LEO 435 VP, Eindhoven, Netherlands) at an acceleration voltage of 30 kV SEM image performed at the Indian Institute of Science Education and Research (IISER), BHOPAL, MP, India.

#### Entrapment efficiency

Entrapment efficiency of microbeads for clonidine was determined according to the method described by Fry (1978)[14] taking drug loaded microbeads equivalent to 100 mg of clonidnesulphate with 5.0 mL of phosphate buffer pH 7.4 in a beaker. The microbeads were kept for swell and allow for macerates for 24 hr then they were triturate with the help of pestle and morter. The mixture was centrifuged at 4000 rpm for 30 min to settle down the polymeric material and allow the drug in supernatant solution. The 1.0 ml of sample from supernatant solution was taken in a volumetric flask and diluted upto 10 ml. The sample was analyzed for drug concentration using UV spectrophotometer.

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

#### In vitro drug release

The drug release was performed in PBS (pH 7.4) for clonidine loaded microbeads using dialysis bag technique. In this study microbeads equivalent to 100 mg of drug was taken in dialysis tubing (MWCO, 15 KDa, Himedia) and placed in a beaker containing 100 ml of PBS pH 7.4. The dialysis bag retains microbeads and allows passing of free drug into the dissolution media. Temperature was maintained at 37 ± $1^0$ C throughout the study. 2 ml of samples 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 the same volume of fresh PBS pH 7.4 and analyzed for drug concentration by using UV spectrophotometer.

## RESULT AND DISCUSSION

Procured drug was odorless and white crystalline in nature. In solubility study it was found that drug was soluble in water, ethanol, methanol and slightly soluble in chloroform and phosphate buffer pH 7.4 and sparingly soluble in 0.1 N NaOH and 0.1N HCl. Melting point of drug was found 128ºC-134ºC while it was 130 ºC reported in standard monograph. The partition coefficient (log p) value was found to be 1.59 and 1.57 in n-Octanol:PBS pH 7.4 and n-Octanol:0.1 N HCl respectively. The obtained FT-IR characteristic peaks of drug was matched with the peaks of drug given in standard monograph was revealed similar. The drug solution was scan on UV-spectrophotometer at 200-400 nm in web length range to determine the maximum absorbance ($\lambda_{max}$) and it was found at 270 nm. The calibration curve was prepared in phosphate buffer pH 7.4 and distilled water. The regression coefficient ($R^2$) was 0.999 which was shows the linearity of curve in both distilled water and phosphate buffer pH 7.4. The line of equation for the standard curve was y = 0.0139x + 0.0038 and y = 0.0069x+0.0023. The drug excipient interaction study was performed to check in interaction between drug and other formulation excipients by spectrophotometricaly. There was no interaction was found between drug and excipients and it was clearly seen and confirmed by UV spectrophotometricaly scan graph of drug solution and mixture of drug and sodium alginate. All the data of preformulation study was found similar as given in standard monograph which confirmed that the drug was authenticate and pure in form and it could be used for formulation development of clonidine hydrochloride loaded microbeads. Clonidine hydrochloride loaded sodium alginate beads were successfully prepared by ionic gelation method. The microbeads formulations were optimized on the basis of average particle size, drug entrapment, shape and surface morphology. The mean diameter of optimized microbeads of Sodium alginate increased from 156.7±2.30 µm to 215.3±5.8 µm with increasing polymer concentration from 1.0 to 4.0 % w/v. In the present investigation a 3.0 % w/v Sodium alginate concentration was found to be optimized which provide the required size of microbeads Fig. 1 and 2.

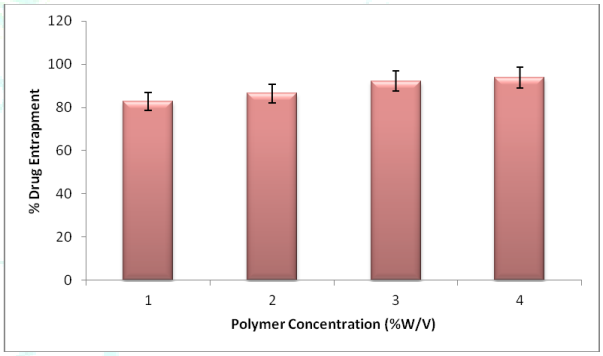


**Figure 1: Effect of polymer concentration on entrapment efficiency of microbeads**

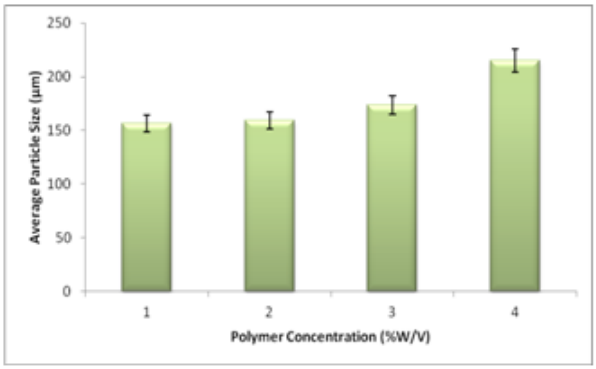


**Figure 2: Effect of polymer concentration on average particle size of microbeads**

The average particle size of microbeads increased with increasing polymer concentration, since at higher concentrations the polymer solution dispersed into larger droplets due to increasing the viscosity of polymer solution

and it was the reason behind the enhancement of average particle size of microbeads. In the case of entrapment efficiency, it was found increase on increasing the sodium alginate concentration it was due to the increasing the entrapment of drug molecules in the molecules of polymer and high dense or high concentration of polymer have more number of polymer molecule network to trap the drug molecules. In the case of optimization of calcium chloride concentration. The particles size found slightly decrease with increasing the calcium chloride concentration. Optimum concentration of calcium chloride is requiring creating complete gelation by ionic interaction of sodium alginate in the microbeads. The complete gelation is directly proportional to high stability and structural integrity for microbeads. There was no major difference was found in case of increasing drug concentration in the formulation but as increase the drug concentration from 10 to 30 %, the drug entrapment efficiency was found increase from 88.9±2.1 to 95.6±2.6% Fig 3 and 4.

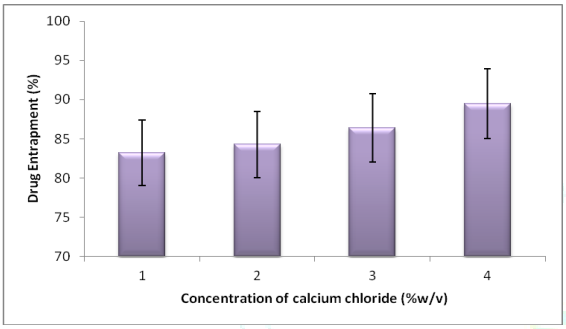


**Figure 3: Effect of calcium chloride on entrapment efficiency of microbeads**

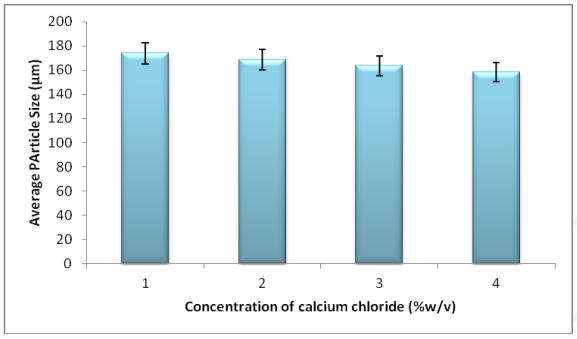


**Figure 4: Effect of calcium chloride on average particle size of microbeads**

Further increasing of drug concentration from 30 to 40 was not found any significant difference in drug entrapment efficiency**.** Formulation coding with MBD 11 consist of 3.0% w/v sodium alginate, 4.0% w/v calcium chloride and 30 % w/v drug concentration was selected as optimized formulation that was shown 94.3±3.4% drug entrapment and163.5±2.6 µm in average particle size Fig 5 and 6.

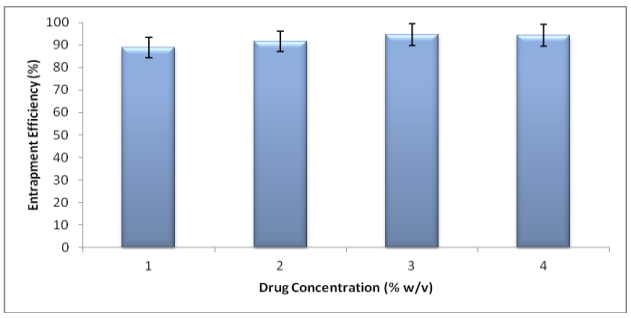


**Figure 5: Effect of drug concentration on entrapment efficiency of microbeads**

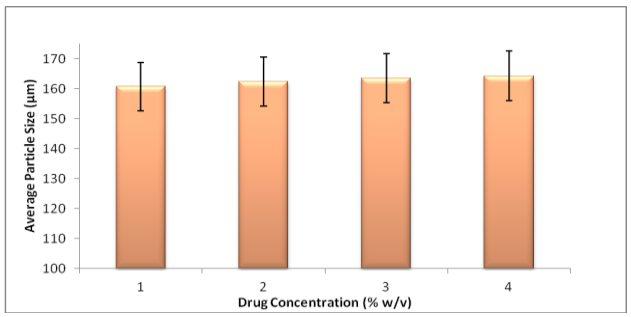


**Figure 6: Effect of drug concentration on average particle size of microbeads**

Scanning electron microscopy (SEM) analysis revealed that the optimized microbeads formulation MBD 11 was found spherical in shape and smooth in surface Fig. 7**.**

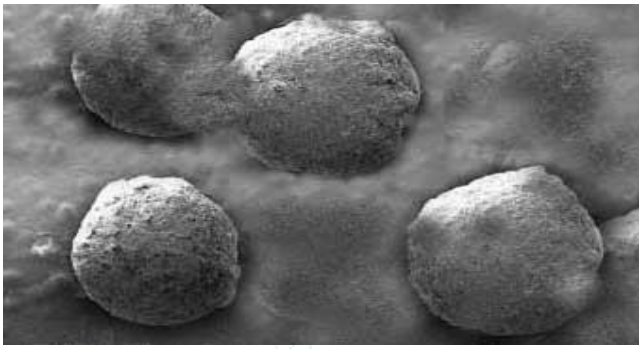

**Figure 7: SEM photomicrograph of drug loaded sodium alginate beads**

In vitro drug release profile of clonidine hydrochloride in PBS pH 7.4 was found 83.46% after 48 hr Table 4 and Fig 8 for optimized formulation (MBD-11) and follows the matrix diffuasion Higuchi release kinetics.

**Table 4: In-vitro drug release of clonidine hydrochloride in phosphate buffer pH 7.4**

| S. No. | Time interval (h) | Plain drug | Clonidine HCL Microbeads |
|---|---|---|---|
| 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 |

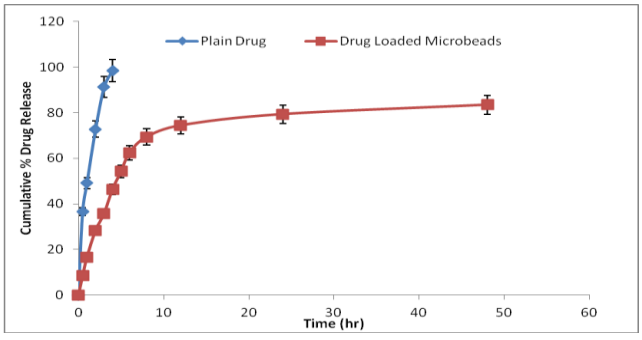


**Figure 8: In-vitro drug release of clonidine hydrochloride from microbeads**

## CONCLUSION

It was concluded that from this study that the microbeads can be prepared from sodium alginate by ionic gelation method and can be encapsulate clonidine hydrochloride without any interaction. It can release drug in very controlled and sustained manner following matrix diffusion Higuchi release kinetic model. The prepared microbeads were optimized for different formulation and process variables and found that microbeads was uniform, spherical and acceptable size range with high drug encapsulation efficiency. The prepared formulation can be use to deliver drugs by oral route for it sustained delivery in GIT system and for maintaining its therapeutic concentration in blood for longer period of time and can be use for the effective management of anxiety and hypertensive disorder.

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