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Research Article

Formulation and Evaluation of Micro Beads of Clonidine for the Treatment of Anxiety and Hypertensive Disorder

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Abstract

A chronic medical condition known as hypertension (HT) is characterised by persistently high blood pressure (BP) in the arteries. Although HT is initially asymptomatic, it eventually develops into a significant risk factor for renal, cardiovascular, and cerebrovascular conditions, which in turn are major contributors to morbidity and mortality in industrialised nations. A complicated disorder called HT is thought to affect more than one-fourth of adult humans worldwide. Both its pathophysiology (primary and secondary HT) and the measurements of the resting blood pressure (elevated systolic, diastolic, and pulse pressure) are used to categories it. It results from a complex interplay between genes and a number of environmental risk factors, including as ageing, smoking, inactivity, being overweight or obese, eating too much salt, stress, depression, and anxiety. Millions of people are affected by anxiety and depressive disorders each year, which are the most often diagnosed mental illnesses. These conditions are both characterised by emotional, cognitive, psychomotor, and neurovegetative symptoms. Additionally, stress at work has been identified as a significant risk factor for heart disease (CVD) and HT. Despite the fact that numerous writers have looked into and proposed connections between HT, stress, anxiety, and depression over the past few decades, a complete knowledge of the underlying pathophysiological mechanisms has not yet been attained, particularly with regards to young people. The purpose of this study was to examine the role that stress and anxiety from the workplace play in the HT development of young students of health care professions as well as any potential links to early CVDs.

Keywords: Blood pressure, Anxiety, Work-related stress, Students, Health care professions, Health promotion, Workplace, Occupational medicine

INTRODUCTION

One of the most prevalent diseases in the world, hypertension is thought to affect one-fourth of all people. It is also the third leading cause of disability-adjusted life years and the top cause of mortality globally. In 2025, it was estimated that 1.56 billion persons globally will have hypertension, according to a report by Kearney et al. For both clinical medicine and public health, identifying and describing modifiable risk factors for hypertension is still crucial[1-4]. Genetic, psychological, and environmental factors all seem to play a role in the multifactorial aetiology of hypertension. However, there are intricate physiological processes at play, and it is unclear how psychosocial variables relate to hypertension. One of the most prevalent psychiatric conditions in adults, anxiety affects a person's health and quality of life and is a serious public health issue in many nations. The relationship between anxiety and hypertension has recently received attention because both disorders pose serious risks to public health[5-7]. Numerous epidemiological studies have been carried out to look into this link, with varying degrees of success reported. According to several studies, anxiety and hypertension are linked, with those who experience anxiety having a higher chance of developing hypertension than those who do not[8]. Additionally, those with hypertension are more likely to experience anxiety than people without the condition. However, several experts disagree with the notion that anxiety feelings contribute to the emergence of hypertension. In other instances, worry has even been linked to a drop in blood pressure[9]. A sympatholytic drug with a central action is clonidine hydrochloride. It functions as a selective 2-adrenergic agonist and an Imidazoline derivative. In the brainstem, it activates alpha 2-adrenergic receptors to reduce sympathetic nervous system discharge. Additionally, it is given as an epidural to relieve pain. When pain from cancer persists despite intraspinal opiate therapy, it is used alone or in conjunction with other medications to lower high blood pressure. Alpha-adrenergic receptors in the CNS are stimulated, sympathetic outflow is decreased, vasoconstriction is inhibited, and blood pressure is finally reduced. Reduce the impulses sent by the brain's pain pathways to delay the transmission of pain[10-14]. The work's main goal was to develop a microbeads-based drug delivery system that can effectively treat anxiety and hypertension by encasing large amounts of medication, releasing it slowly and steadily over an extended period of time, and maintaining its therapeutic concentration in blood. With microbeads, one drug or portion of a drug can be released right away while the other drug or portion of the same drug can be released over a longer period of time. These are helpful when interactions between drugs and excipients and between drugs can be predicted using a single type of dosage form. The microbeads of a suitable biodegradable polymer will be synthesized and loaded with clonidine hydrochloride in this dissertation effort, it is hypothesized. Ionic gelation processes will be used to prepare the beads.
After administration, beads' sustained and regulated drug rele

ase will aid to keep the medicine at a therapeutic level in the b ody. Prepared polymeric beads will unquestionably control the hypertensive issue and lower the dosage and unwanted eff ects of the medication.

MATERIAL AND METHODS

Characterization for physiochemical properties of drug

Determination of partition coefficient

In a system of n-Octanol: Phosphate Buffer pH 7.4, n-Octanol: water, the partition coefficient of the medication was investigated. A 50:50 mixture of n-Octanol and Phophate Buffer pH 7.4 was added to three glass bottles containing 5 mg of the medication each to determine the results, it was. The mixture-containing glass bottle was shaken for 24 hours at room temperature in a wrist motion shaker. After proper dilution with the necessary buffer, the mixture was transferred into a separating funnel, two phases were separated, and the amount of drug in the aqueous phase was spectrophotometrically measured using a UV spectrophotometer at a maximum wavelength of 285 nm. The following formula[15] was used to compute the drug's partition coefficient.

$$Partition\ Coefficient\ (K) = \frac{Amount\ of\ drug\ in\ organic\ phase}{Amount\ of\ drug\ in\ aqueous\ phase}$$

Table1: Partition coefficient of clonidine hydrochloride

S.N.	MEDIUM	Log P
2.	n-Octanol: PBS pH 7.4	1.59
3.	n-Octanol:0.1 N HCl	1.57

FT-IR spectroscopy

The information in an FT-IR spectrum is adequate to reveal the structure of a medication or other chemical. Near IR and Far IR are the infrared wavelength ranges between 0.8 and 2.5 and 15 and 200. To prepare the pallet for the FT-IR spectroscopy, approximately 5 mg of the medication was triturated into powder and combined with powdered KBr. The FT-IR spectrophotometer (Bruker, USA) was used to evaluate the pallet[16].

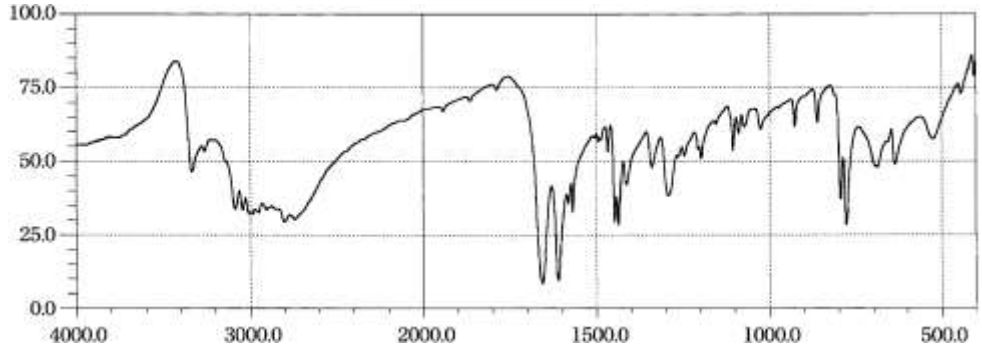


Figure: 1 FT-IR Spectrum of clonidine hydrochloride (Sample)

Determination of λ max of clonidine hydrochloride

The medication solution was examined using a double beam U V spectrophotometer to determine the maximum concentratio n of clonidine hydrochloride. 10 mg of the medication, which was precisely weighed, were dissolved in 10 ml of buffer solution with a pH of 6.8 in a volumetric flask. The final solution had a strength of 1000 g/ml, and 1 ml was pipetted from it into a volumetric flask with a capacity of 10 ml, with 6. 8 pH buffer solution added to get the volume up to 10 ml. On a UV spectrophotometer, this solution was scanned at a wavele ngth of 400–200 nm. The drug's maximum absorbance was measured at 284 nm[17], which was also the greater absorption peak.

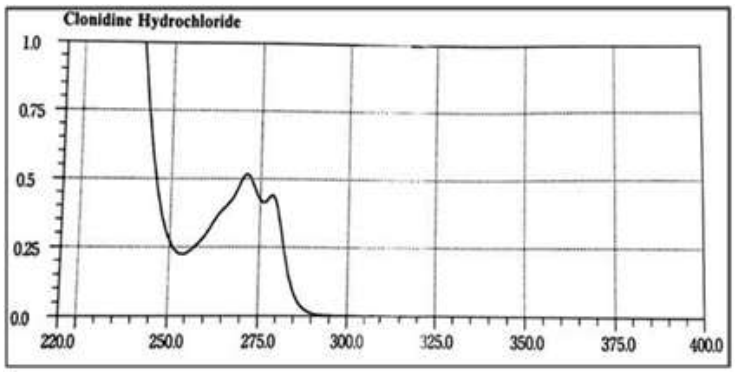


Figure 2: λmax scan graph of clonidine hydrochloride in phosphate buffer pH 6.8

Preparation of calibration curve of clonidine hydrochloride

Clonidine hydrochloride previously created stock solution (10 00 g/ml of strength) was used to create adequate dilution into concentration range of 5–25 g/ml.

To create solutions containing 5, 10, 15, 20 and 25 g/ml, volum etric flasks with capacities of 0.5, 1.0, 1.5, 2.0, and 2.5 ml of sol ution were filled with distilled water up to a 10 mL level. Using a UV spectrophotometer (Labindia-3000 Plus), the absorbance of these solutions was measured at 284 nm. On Microsoft

Excel, a graph between absorbance and concentration was generated using linear regression[18].

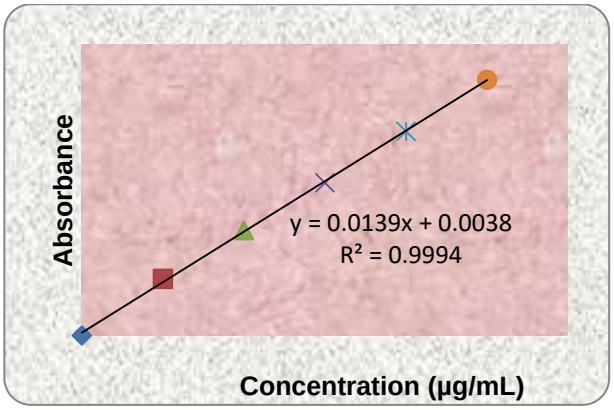


Figure 3: Calibration curve of clonidine hydrochloride in phosphate buffer pH 7.4 at 270 nm

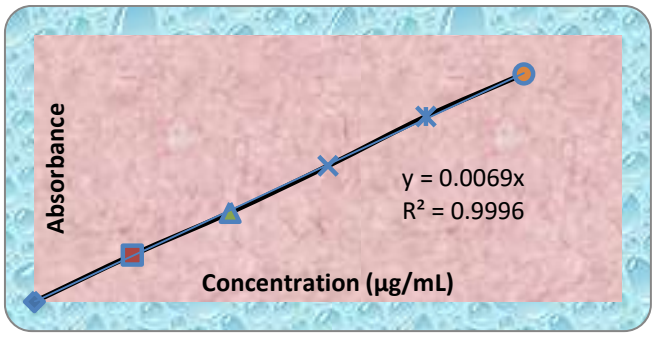


Figure 4: Standard curve of clonidine hydrochloride in distilled water

Stability study

Results of the stability investigation are shown in Table. The clonidine content remained between 90 and 100% of the original content throughout storage at 25°C± 2°C and 60%RH ±5% RH[19]

Study	Storage condition	Minimum time period covered by data at submission	% drug degradation
Long term	25° C ± 2° C 60%RH ± 5%	12 months	0.1 %
Intermediate	30° C ± 2° C 60%RH ± 5%	6 months	0.3 %
Accelerated	40° C ± 2° C 60%RH ± 5%	6 months	3 %

Compatibility studies of drug and excipients

Drug and excipient blends are made in the compatibility testing programme by triturating the drug with several excipients. A mixture of 50 mg of clonidine hydrochloride and 50 mg of excipients was made and put into inert glass vials. Aluminium seal caps were placed on top of rubber closures to seal the vials. The vials were kept at 4°C (the refrigerator) as a control and at 40°C/75%RH for 4 weeks to simulate an accelerated temperature condition. Colour, flow, and sticky visual observations were noted. Using a UV spectrophotometer, the sample was examined for any interactions[20].

Method of preparation of microbeads

The ionotropic gelation process was used to create the microb eads, and sodium alginate (1-4%w/v) was precisely weighed and dissolved in mildly warmed distilled water. Prior to formulation, the sodium alginate solution was homogenized by swirling on a magnetic stirrer for 45 minutes. During homogination, the drug, clonidine sulphate (10–40%w/v), was carefully weighed and added or dispersed in the alginate solution. the After the homogenization procedure was finished, the solution was let to remain for 15 minutes without stirring before being sonicated for 10 minutes with a bath soni cator to eliminate any air bubbles that had formed. Another beaker was used to generate 100 ml of 3-6% w/v calcium chloride solution, into which a drug-containing sodium alginate solution was injected using a 29 gauge hypodermic needle attached to a 10 ml syringe. The alginates solution was dropped at a distance of 10 cm. After a 30-minute incubation period, the beads were separated by filtering the solution. The obtained beads were dried at 40°C after being rinsed three times with distilled water. Before being used in their further characterization, prepared beads were kept in a very tight container[21].

Optimization of drug loaded microbeads

Optimization of polymer concentration

By using various polymer concentrations and keeping all other parameters constant, the formulation of the microbeads was o ptimised. On the basis of average particle size and drug entrapment, microbeads were optimised. 400–500 rpm was used as the constant stirring speed.

Table 2: 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	156.6±2.1	72.7±2.2
MBD 2	2	3	10	158.7±4.2	76.4±1.7
MBD 3	3	3	10	172.9±3.5	82.3±2.3
MBD 4	4	3	10	215.3±5.4	83.6±2.5

(n=3)

Optimization of calcium chloride concentration

Calcium chloride used an ionic interaction mechanism to function as a gelling agent. Calcium chloride gives the bead's morphological structure stability and strength. By using various calcium chloride concentrations while keeping all other parameters constant, the concentration was optimised for the manufacturing of microbeads. The average particle size, drug entrapment, shape, and surface morphology of microbeads were all optimised.

Table 3: Optimization of polymer 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

The concentration of the medicine utilised determines how well it is captured. Maximum Entrapment effectiveness moves the formulation closer to the optimal microbeads formulation characteristic. In order to maximise drug concentration, multiple drug concentrations were used during the formation of the microbeads while all other parameters remained constant.

Table 4: Optimization of drug concentration in the microbeads formulation

	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

Optical microscopy was used to measure the average particle size of microbeads. The microbeads were first scattered on the glass slide after being suspended in methanol. To measure the size of the beads, an ocular micrometre was used to look at the slide under a microscope. More than 150 beads were measured for size, and the average size was given. Based on the zeta potential (e), which was determined by Helmholtz-Smoluchowsky from their electrophoretic mobility, surface charge was measured. Samples were analysed after being diluted with 0.9% NaCl to achieve a conductivity of 50 lS/cm for the measurement of surface charge using a zetasizer with field strength of 20 V/cm on a large bore measurements cell.

Particle shape and surface morphology

Scanning electron microscopy was used to examine the surface morphology of microbeads. First, a very little amount of formulation was spread over the counterfoil that had been taped up before. Then a sputter coater was used to apply a 300A° thick layer of gold coating. Under a scanning electron microscope (LEO 435 VP, Eindhoven, Netherlands) with an acceleration voltage of 30 kV, samples were inspected and photomicrographs were taken. The Indian Institute of Science Education and Research (IISER), Bhopal, MP, India, produced this SEM image. Microbeads as seen in SEM and TEM images.

Entrapment efficiency

According to the procedure outlined by Fry (1978), the effectiveness of microbeads at entrapping clonidine sulphate was assessed by mixing drug-loaded microbeads equivalent to 100 mg of clonidine sulphate with 5.0 ml of phosphate buffer pH 7.4 in a beaker. After being macerated for 24 hours and then being centrifuged for 30 minutes at 4000 rpm, the obtained formulation of microbeads was ground using a pestle and mortar. The sample (1 ml) from the supernatant solution was taken out and put into a volumetric flask with a 10 ml capacity. Phosphate buffer solution with a pH of 7.4 was then added to the volume to make it 10 ml. Using a UV spectrophotometer, the sample's drug concentration was determined.

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

In vitro drug release

For clonidine-loaded microbeads, the drug release was carried out using the dialysis bag technique in PBS (pH 7.4). In this investigation, dialysis tubing (MWCO, 15 KDa, Himedia) microbeads with 100 mg of medication were taken and placed in a beaker with 100 ml of PBS pH 7.4 in order to conduct the experiment. 2.0 ml of the sample was obtained during the research at a temperature of 37°C, and the same amount was then replaced with fresh PBS pH 7.4 at appropriate intervals. The samples were then assessed using a UV spectrophotometer to ascertain the drug concentration.

Table 5: In-vitro drug release of clonidine hydrochloride in phosphate buffer pH 7.4

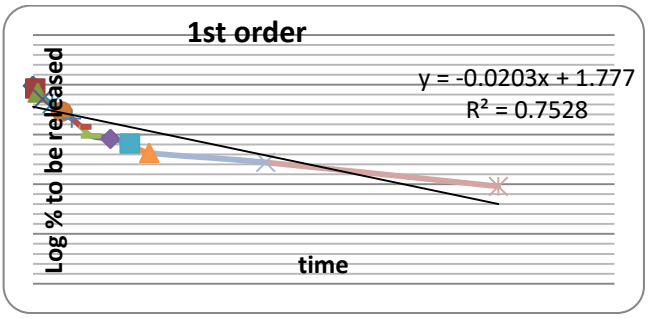
S. No.	Time interval (h)	Plain drug	Clonidine HCL Microbeads
1	0.5	10	8.43
2	1	15	16.53
3	2	20	28.26
4	3	25	35.68
5	4	30	46.35
6	5	35	54.23
7	6	45	62.45
8	8	55	69.38
9	12	75	74.43
10	24	80	79.34
11	48	93	83.46

Data treatment

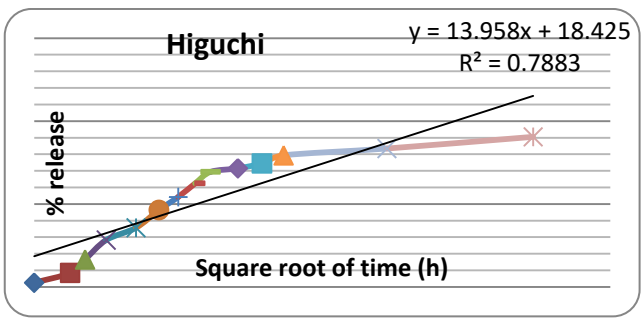
The drug's release rate from microbeads is complicated. They entail interactions, interface movement, and drug diffusion. Correlation coefficient values comparing the lines gathered from the graphical representation of several mathematical models are used to describe the rate of release process. The gathered data were compiled using the mathematical models below in order to identify the drug release mechanism of drug-loaded microbeads:

1. Zero Order (Cumulative percentage of drug release versus time)

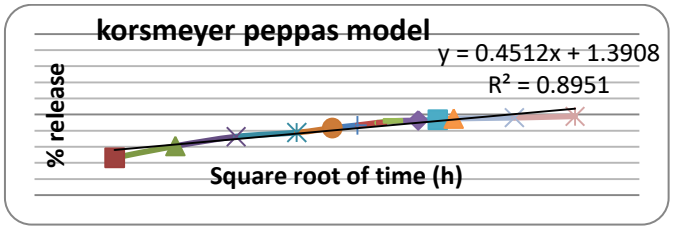
2. First Order (Log percent of drug unreleased versus time)



3. Higuchi square root (Cumulative percentage of drug release versus square root of time)



4. Koresmeyer's Peppas models (Log of Cumulative percentage of drug release versus log time)



The release data graph was plotted according to following equations,

- Zero order : M = M0 - K0t
- First order : Log C = Log C0 - Kt / 2.303
- Higuchi square root law : Q = Ktn
- Koresmeyer's Peppas models : Mt/M∞ = ktn

Where M, C, and Q represent the drug's release at time t, M, and C represent the drug's total amount, and K and k represent the appropriate rate constants. In the Korsemeyer-Peppas model, Mt/M is the fractional drug release at time t, k is the constant including the property of macromolecular polymeric systems and the drug, and n is a kinetic constant used to distinguish the transport mechanism. For Nanosponges, the correlation coefficient values of various mathematical models were examined, and the results are shown below.

Table 6: r^2 value of optimized formulations

CORRELATION COEFFICIENT	
KINETIC MODELS	**Microbeads**
Zero order plot	$R^2 = 0.486$
First order plot	$R^2 = 0.752$
Higuchi plot	$R^2 = 0.788$
Korsemeyer's Peppas plot	$R^2 = 0.895$

The microbeads are inferred to obey the Korsemeyer-Peppas equation based on the data above since their r2 values are 0.895 and, respectively, which are very close to unity. Since drug release occurs through diffusion from the polymeric matrix of microbeads in the Korsemeyer's Peppas model, it was chosen as the model with the best fit.

RESULT AND DISCUSSIONS

Pre-drug formulation was done, and physiochemical characteristics and other aspects of the drug were researched. This investigation included the determination of physiochemical parameters such as solubility, melting point, partition coefficient, drug-excipient interaction, max scan utilizing UV-spectrophotometry, and FT-IR spectrophotometry. To verify the validity of the purchased medicine, the data from these trials were compared to the information provided in typical monographs. The medicine that was purchased was an odourless, white crystalline substance. According to a

solubility investigation, the medication was minimally soluble in chloroform but completely soluble in polar and semipolar solvents such water, ethanol, and methanol. In 0.1N NaOH and 0.1N HCl, the medication was only weakly soluble. The drug's melting point was determined to be between 128°C and 134°C, but the conventional monograph stated 130°C. The partition coefficient (log p) values for n-Octanol:PBS pH 7.4 and n-Octanol:0.1 N HCl were determined to be 1.59 and 1.57, respectively. The obtained FT-IR characteristic drug peaks were similar to the peaks of the medicine listed in the standard monograph (figure 1). To find the maximum absorbance (max), the drug solution was scanned on a UV-spectrophotometer at 200–400 nm in the web length range (figure 2). The phosphate buffer pH 7.4 and distilled water were used to produce the calibration curve. The regression coefficient (R2) was 0.999, indicating that the curves for both pure water and the pH 7.4 phosphate buffer were linear. The standard curve's equation was y = 0.0139x + 0.0038 and y = 0.0069x. The goal of the drug excipient interaction study was to spectrophotometrically determine whether there was any interaction between the drug and other formulation excipients. No interactions between the drug and excipients were discovered, which was well demonstrated and corroborated by the UV spectrophotometric scan graphs of the drug solution and the drug and sodium alginate mixture. Clonidine hydrochloride's weblength did not change at all. The pre-formulation study's findings were found to be comparable to data from the literature that attests to the validity of the medicine. Ionic gelation was used to successfully create sodium alginate beads that were loaded with clonidine hydrochloride. The average particle size, drug entrapment, shape, and surface morphology of the microbead formulations were all optimized. It was discovered that the average microbead size grew as the sodium alginate concentration increased, measuring 156.7± 2.30 m at 1% w/v and 215.3 ±5.8 m at 4.0% w/v sodium alginate, respectively. 3.0% w/v of sodium alginate was shown to be optimal in the current experiment, providing the necessary size of microbeads.

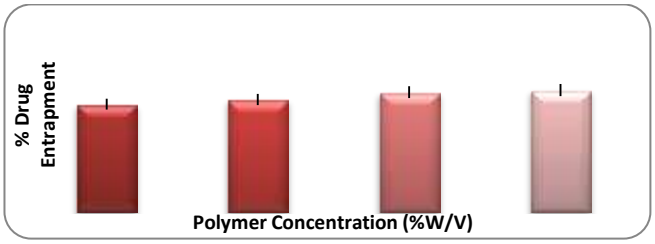


Figure 5: Effect of polymer concentration on entrapment efficiency of microbeads

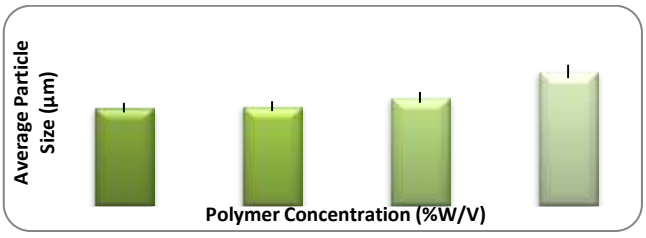


Figure 6: Effect of polymer concentration on average particle size of microbeads

Since the polymer solution dispersed into larger droplets at higher concentrations due to increasing the viscosity of the polymer solution, which in turn caused an increase in microbead size, the average particle size of microbeads rose with increasing polymer concentration. Similar to this, entrapment effectiveness increased as sodium alginate concentration was raised. A polymer molecule network is made available by high concentration in order to trap drug molecules in greater concentration. Drug entrapment rates for formulations MBD 1, MBD 2, MBD 3, and MBD 4 were 72.9±2.4, 76.6±1.9, 82.3±2.4, and 83.8±2.3%, respectively.

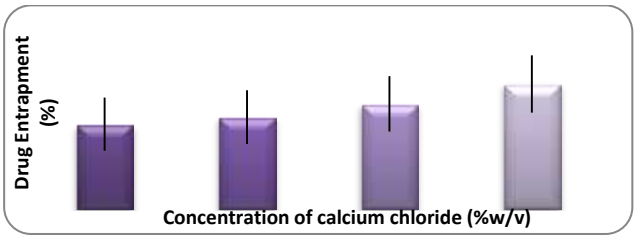


Figure 7: Effect of calcium chloride on entrapment efficiency of microbeads

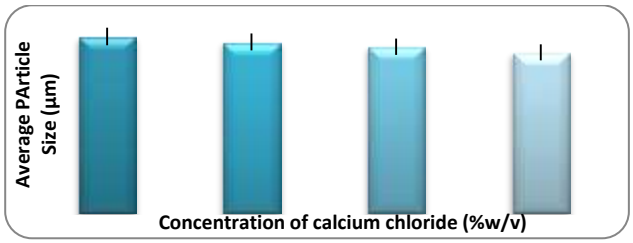


Figure 8: Effect of calcium chloride on average particle size of microbeads

When the concentration of calcium chloride is optimized. With an increase in calcium chloride concentration, smaller particles were detected. For the calcium chloride concentration to be at its ideal level, sodium alginate in the microbeads must completely gel. For microbeads, good structural integrity and stability are directly correlated with complete gelation. When the drug concentration in the formulation was increased from 10% to 30%, no significant differences were discovered, however the drug entrapment efficiency increased from 88.9 ±2.1 to 95.6 ±2.6%.

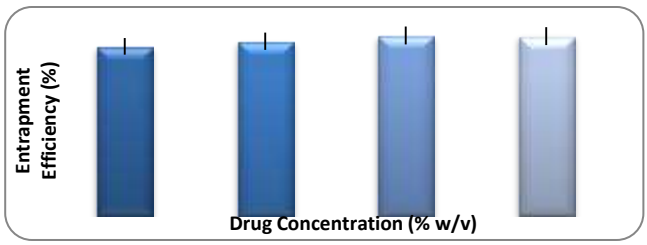


Figure 9: Effect of drug concentration on entrapment efficiency of microbeads

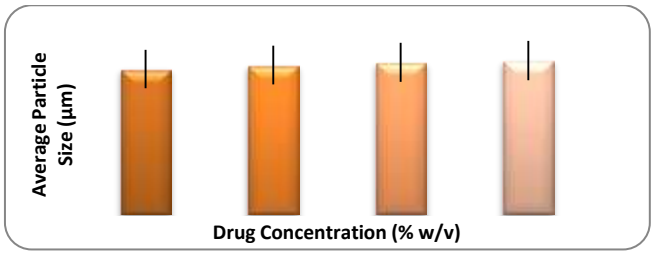


Figure 10: Effect of drug concentration on average particle size of microbeads

There was no discernible difference in drug entrapment effectiveness with an increase in drug concentration from 30 to 40. Formulation coding with MBD 3 was chosen as the optimal formulation because it showed 94.3±3.4% drug entrapment and 163.5±2.6 m in average particle size. It contains 3.0% w/v sodium alginate, 4.0% w/v calcium chloride, and 30% w/v drug concentration. The optimized microbeads formulation was discovered to be smooth and spherical in shape using scanning electron microscopy (SEM) examination. Following the matrix diffusion Higuchi release

kinetics, the in vitro dissolution profile of clonidine hydrochloride in PBS pH 7.4 was found to be 83.46% after 48 hours.

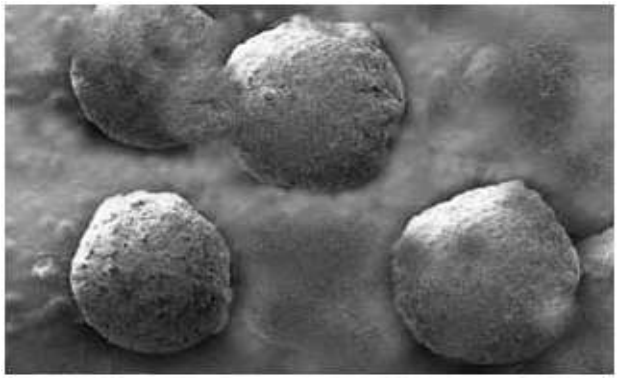


Figure 11: photomicrograph of drug loaded sodium alginate beads

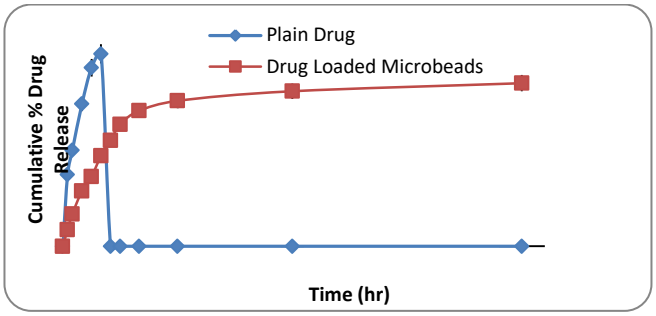


Figure 12: In-vitro drug release of clonidine hydrochloride from microbeads

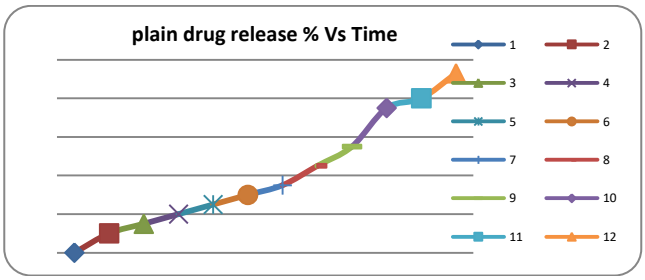


Figure 13: In vitro drug release of plain drug

CONCLUSION

Ionic gelation was used to successfully create sodium alginate microbeads that were loaded with clonidine hydrochloride. It offers clonidine hydrochloride medication release that is controlled and consistent and that adheres to the matrix diffusion Higuchi release kinetic. The produced microbeads had a high degree of drug encapsulation effectiveness and were determined to be homogenous, spherical, and in an acceptable size range. The produced formulation may be used to provide medications orally for extended absorption in the gastrointestinal tract and to maintain a therapeutic blood concentration for a longer period of time. It may also be used to effectively treat anxiety and high blood pressure disorders.

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