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FORMULATION AND EVALUATION OF CHLORZOXAZONE MICROSPHERES

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ABSTRACT

Microspheres are characteristically free flowing powders having particle size ranging from 1-1000µm consisting of proteins or synthetic polymers. Chlorzoxazone is a centrally acting muscle relaxant used to treat muscle spasm and the resulting pain a discomfort. It is a BCS class II drug having a shorter half-life (1.1 hour) with the dose administration of 3-4 times a day leads to decrease patient compliance. The present study is aimed to improve the patient compliance, reduce adverse effect, protect the drug from enzymatic degradation, to increase the solubility by preparing sustained release microsphere using different polymers like HPMC and carbopol by varying concentration using ionotropic gelation method. Optimization study have been done by Design Expert Software using central composite design and 13 formulations were prepared. All the formulations were evaluated for micrometric properties, percentage yield, mean particle size, drug content, entrapment efficiency, SEM studies and *in vitro* drug release. Depending upon the concentration, the percentage yield is found between 62.5% to 86.33% in all formulations. The mean particle size of microspheres significantly increases with increasing polymer concentration and the range between 141.2µm to 198.6µm. Among all the formulations, F8 showed high drug content (97.3%) and entrapment efficiency 68.56. The surface morphology of microspheres was characterized by SEM and it was discrete and spherical in shape .The *in vitro* drug release studies revealed that F8 formulation exhibits maximum drug release and hence was selected as the optimized formulation .The mechanism of drug release was found to be first order kinetics and kosmeyer – peppas model with fickian diffusion mechanism. From the stability studies microspheres showed no significant change in the physical appearance and drug content.

KEYWORDS: Chlorzoxazone, Microspheres, Muscle relaxant, HPMC, Carbopol, Optimization.

1. INTRODUCTION

A Novel Drug Delivery System (NDDS) can be defined as a new approach that combines innovative development, formulations, new technologies, novel methodologies for delivering pharmaceutical compounds in the body as needed to safely achieve its desired pharmacological effects. Various drug delivery systems have been developed and some of them under development with an aim to minimize drug degradation or loss, to prevent harmful side effects and to improve drug bioavailability and also to favor and facilitate the accumulation of the drug in the required site. There are no. of novel carries which have been established and documented to be useful for controlled and targeted drug delivery. Sustained- or controlled- drug delivery systems provide drug action at a predetermined rate by providing a prolonged or constant release respectively, at the therapeutically effective levels in the circulation.[1]

Chlorzoxazone (5-chloro-2,3-dichloro-1,3benzoxazol-2-one) is a centrally acting muscle relaxant used to treat muscle spasm and the resulting pain a discomfort .It is a BCS class II drug having a shorter half-life(1.1 hour) with the dose administration of 3-4 times a day leads to decrease patient compliance.[2]

In order to decrease the frequency and improve patient compliance a sustained release formulation of Chlorzoxazone is desirable. Microsphere is a carrier for drug in one approach which can be used in a sustained controlled release fashion in order to improve the patient compliance. The present study is aimed at to improve the patient compliance, reduce adverse effect, protect the drug from enzymatic degradation, to increase the solubility by preparing sustained release microsphere using different polymer by ionotropic gelation method.[3]

2. MATERIALS AND METHODS

2.1. Chemicals used: Chlorzoxazone (Yarrow Chem, Mumbai), Sodium alginate (Finar, Ahmedabad), Calcium chloride (oxford lab fine chem LLP, Maharashtra), HPMC (Kemphasol, Mumbai), Carbapol (Kemphasol, Mumbai).

2.2. Instruments used: Electronic weighing balance (Prince Scale Industries, Ahmedabad), Double beam UV spectrophotometer (Systronics, Japan), FT-IR (Jasco model FT/IR 4100 Optical microscope (Blisco, Haryana), Magnetic stirrer (Rotek equipments, Kerala), Scanning Electron Microscopy (JOEL JSM 6701F, Japan), USP dissolution apparatus (Electrolab TDT-06T).

2.3. Formulation of chlorzoxazone microspheres

The Microspheres were prepared according to the ionotropic gelation method using different concentration of HPMC and Carbopol 934. Sodium alginate, Carbopol 934, HPMC were dissolved in distilled water to form a homogeneous polymer solution. The active core material CLZ (0.5g) was dissolved in ethanol and added to the polymer solution and mixed thoroughly with a stirrer to form a smooth viscous dispersion. The resulting dispersion was then added drop wise into calcium chloride (5% w/v) solution through a syringe with a needle of size no: 22. With stirring speed of 100rpm. The added droplets were retained in the calcium chloride solution for 30min to complete the curing reaction and to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried in hot air oven at 60^0c for 2 hrs. and subsequently stored in desiccators. Same procedures were repeated for all batches of formulation.[4]

Design Expert Stat Ease Software was used to design formulations. Thirteen formulations with different concentration of polymers ie, HPMC and carbopol were suggested by the software.

The formulation is shown in table no.1

Table 1: Formulation9table9of9chlorzoxazone.

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
chlorzoxazone	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Sodium alginate	4g	4g	4g	4g	4g	4g	4g	4g	4g	4g	4g	4g	4g
Calcium chloride	3g	3g	3g	3g	3g	3g	3g	3g	3g	3g	3g	3g	3g
HPMC	250 mg	250 mg	250 mg	400 mg	100 mg	250 mg	100 mg	400 mg	250 mg	100 mg	400 mg	250 mg	250 mg
carbopol	250 mg	100 mg	400 mg	250 mg	400 mg	250 mg	100 mg	400 mg	250 mg	250 mg	100 mg	250 mg	250 mg
Ethanol	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
water	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml	200 ml

2.4: ANALYTICAL METHODS

2.4.1: Determination of uv λmax

Dissolve accurately weighed 0.5g of chlorzoxazone in 100ml of phosphate buffer pH6.8 in 100ml standard flask to get 1000mg/ml from the stock solution of chlorzoxazone, 10ml is pipetted out and diluted to 100ml with phosphate buffer pH6.8 to get 100µg/ml. The absorption maximum of standard solution of chlorzoxazone is determined by scanning the resulting stock solution in UV spectrometer at 200 - 400 nm. The absorption maxima obtained is compared with reference standard for the value.

2.4.2: Preparation of standard calibration curve of chlorzoxazone

Preparation of stock solution 1: 0.5g of chlorzoxazone was weighed accurately and dissolves in phosphate buffer pH6.8. The volume was made up to 100ml with phosphate buffer pH6.8 (1000µg/ml).

Preparation of stock solution 2: From stock solution 1 10ml was pipette out in 100ml volumetric flask and made up to 100ml with pH 6.8phosphate buffer (100µg/ml).

Dilutions: From stock solution 2 0.2,0.4,0.6,0.8,1ml was pippete out in 10ml volumetric flask and made up to 10ml with 6.8 phosphate buffer which gives concentration as 2,4,6,8,10µg/ ml. The absorbance of these solutions was measured in UV-visible spectrophotometer at 228 nm against some dilution as blank. A calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis.[5]

2.5. PREFORMULATION STUDIES

2.5.1 Solubility Studies

Solubility of CLZ was observed in different solvents such as distilled water, 95% ethanol, alkali hydroxide, isopropyl alcohol and phosphate buffer pH 6.8.

2.5.2. Organoleptic Properties

Physical appearance of drug was observed and compared with official monographs.

2.5.3. Drug – Excepient Interaction Studies

In order to find out the possible interactions between CLZ, HPMC and carbopol used in formulation of microspheres, Fourier Transform Infra-red Spectroscopy (FT-IR) analysis was carried out on pure substances and their physical mixtures.

FT-IR spectra of pure drug, carbopol, HPMC and their physical mixtures were taken by KBr pellet technique between 600 – 4000 cm-1. This is to ensure that there is no incompatibility between drug and polymers. Once spectra were recorded, the peaks of pure drug, polymers and physical mixtures of polymers, drug were compared for incompatibility.[6]

2.6. EVALUATION OF CHLORZOXAZONE MICROSPHERES

2.6.1 Percentage Yield

The prepared microspheres were collected, dried at room temperature and then weighed. The measured weight of prepared microspheres was divided by the total amount of all excipients and drug used in the preparation of microspheres which will give the total percentage yield of microspheres.

$$\text{Percentage yield (\%)} = \frac{\text{The amount of microspheres obtained (g)}}{\text{Theoretical amount (g)}} \text{ X100}$$

2.6.2 Determination of Particle Size

The size of prepared microspheres was measured by an optical microscopy method fitted with eye piece micrometer which was then calibrated with stage micrometer.

Procedure: Calibrate the eye piece micrometer and transfer the microspheres on clean slide. Add one or two drop of liquid paraffin. Dispense the sample uniformly with the help of a brush. Place the cover slip to avoid entrapment of air bubbles. Drain the excess liquid with a blotting paper. Place the slide on the stage of the microscope. Focus the slide in low magnification (10X), observe the presence of individual particle. Shift to high power (45X) and focus the slide. Measure the size of each particle in terms of eye piece divisions. Tabulate the particle in terms of division of eye piece and number of particles. Multiply the number of eye piece divisions by the calibrated values. Classify the diameters in to size ranges and calculate the number of divisions. The average mean size was calculated by retrieving the size of about 100 microspheres from each batch was determined by the given equation.

$$\text{Calibration factor} = \frac{\text{No. of division on stage micrometer}}{\text{No. of division on eye piece micrometer}} \text{ X 100}$$

Size of individual particle (µm) = Number of division on eyepiece × Calibration factor.

Average particle size (µm) = Sum of individual particle/100

2.6.3. Morphological Studies-Scanning electron microscopy (SEM)

In general, SEM has been used to determine particle size distribution, surface topography, texture, fractured surface/sectioned surface and characterizing drug delivery system, owing in large simplicity of sample preparation and ease of operation. The microspheres were taken for the surface characterization. For the external morphology studies, the microspheres were visualized using SEM operating at 15KV. The samples were mounted on electron microscope brass stub and coated with in an ion sputter, under vacuum. The shape and surface characteristic of microspheres were taken by random scanning of the stub and photographs.[7]

2.6.4. Micromeritic Properties

Bulk Density

Bulk density can be defined as "mass of the powder divided by the bulk volume". The packing characteristics of the powders play an important role in determining the physical properties of dosage forms. According to the standard procedure for obtaining the bulk density, weighed quantities of prepared microspheres were filled in 100ml graduated measuring cylinder. The initial volume was noted after tapping for there are times at two seconds interval and the final volume was noted. The bulk density was calculated as per following formula.

Bulk density = Mass of Microspheres / Bulk volume of Microspheres

Tapped Density

Weighed amount of microspheres was introduced in to a 10ml measuring cylinder and cylinder was then tapped from height of 2cm until the time when there was no more decrease in density and volume of the microspheres. It was calculated by using the following equation.

Tapped density (DT) = M/VT

Where DT= Tapped density. M= Mass of the microspheres. VT= Tapped volume.

Carr's (Compressibility) Index

Compressibility index or Carr's index value of micro particles was computed according to the following equation:

% Compressibility = (Tapped density-Bulk density /Tapped density) ×100.

The value below 15 % indicates a powder with good flow characteristics, whereas above 25 poor flow ability. Hausner's ratio=Bulk density/Tapped density. Value less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (=33 % Carr).

Angle of Repose

The fractional force in the loose powder can be measured by angle of repose. This is the maximum angle possible between the surfaces of the site of the powder to the horizontal plane. Angle of repose was calculated by static method using cone and funnel. Funnel was kept on triangular stand, which was kept on horizontal plane. The sample was passed through the funnel and heap formed on the paper was encircled. From the radius of circle and height of conical heap, angle of repose was calculated. The angle of repose θ is calculated by the following equation :

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

Where, θ= Angle of repose, h = Height of cone, r = radius of the cone.

2.6.5. Entrapment Efficiency (EE)

An accurately weighed microsphere (100mg) was taken and drug as extracted from prepared microspheres by digesting for 24hrs with 10ml phosphate buffer Ph6.8. During this period the suspension was agitated. After 24hrs, the solution was filtered and filtrate was analyzed for the drug content. The drug EE was calculated using the following formula.

$$\text{Percentage entrapment Efficiency} = \frac{\text{Estimated percent drug content (amount of drug actually percent)}}{\text{Theoretical percent drug content (Theoretical drug loaded expected)}} \times 100$$

2.6.6. Drug Release Kinetics

The *in vitro* drug release study was carried in USP paddle type II dissolution test apparatus using phosphate buffer (pH 6.8) as dissolution medium (900ml) and temperature was maintained at 37±10c throughout the studies. Paddle speed was adjusted to 50rpm. An interval of each 1hr, 5ml of sample was withdrawn from dissolution apparatus and replacement with 5ml fresh medium followed by analyzed for drug content using UV visible spectrophotometer at 244nm. All the experimental units were analyzed in triplicate (n=3). Cumulative percentage drug release was calculated using an equation obtained from a standard curve.[8]

2.6.7. Optimization of formulation using doe

Statistical design of experiments, a computer-aided optimization technique, was used to identify critical factors, their interactions and ideal process conditions that accomplish the targeted response. The best formulation was determined using Design Expert Stat Ease Software. Central composite design was used for the optimization. In the study, carbopol and HPMC were selected as the two factors and drug content and in vitro drug release were considered as the two responses. Hence, thirteen experimental trials were done. Trials were repeated twice to evaluate experimental errors and increase power ratio. Countor plots were drawn and optimum formulation was selected by optimization criteria.

Key steps of DoE

• Setting objective

• Selection of process variables – Inputs and outputs, ie. factors and responses.

• Selection of experimental design: depends on the objective and the number of factors. In the present study, central composite design was used because 2 factors are considered.

• Executing the design: Screen and optimizing the response.

• Checking whether the data are consistent with the assumptions.

• Analysis and interpretation.[9]

2.6.8. Drug release kinetics

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing Zero order (Q Vs t), First order [Log (Q0-Q) Vs t], Higuchi's square root of time (Q Vs t1/2) and Korsmeyer-Peppa's (Log Q Vs Log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t. In short the results obtained from in vitro release studies were plotted in four kinetic models of data treatment as follows: Cumulative percentage drug release Vs Time (Zero order rate kinetics), Cumulative percentage drug retained Vs Time (First order rate kinetics), Cumulative percentage drug released Vs Square root of Time (Higuchi's classical diffusion), Log cumulative percentage drug release Vs log Time (Korsmeyer-Peppa's exponential equation).

2.6.9. Stability studies

From the prepared microspheres best formulations was selected for the stability studies and were placed in borosilicate screw capped glass containers and stored in different temperatures like room temperature (27±20C, 60 ± 5% RH) and (45±20C, 70 ±5% RH) in stability chamber. At the end of 30, 60, 90 days period, samples were withdrawn and the microspheres are analyzed for their drug content.[10]

3. RESULTS AND DISCUSSION

3.1. Analytical method

3.1.1. Determination of UV λ max

The pure drug of chlorzoxazone in phosphate buffer pH 6.8 was scanned by UV spectroscopy and λ max was found to be 244nm.

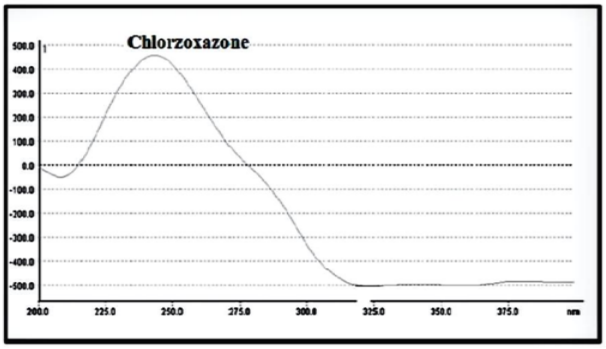

Fig.1: uv spectrum of chlorzoxazone.

3.1.2. Calibration curve of chlorzoxazone

Table 1: Absorbance value of chlorzoxazone.

CONCENTRATION (µg/ml)	ABSORBANCE (at 244 nm)
2	0.221±0.1
4	0.45±0.4
6	0.68±0.3
8	0.901±0.6
10	1.12±0.2

All values are expressed as a mean of ± SD, n = 3

Standard Calibration Curve of Chlorzoxazone

Concentration v/s Absorbance

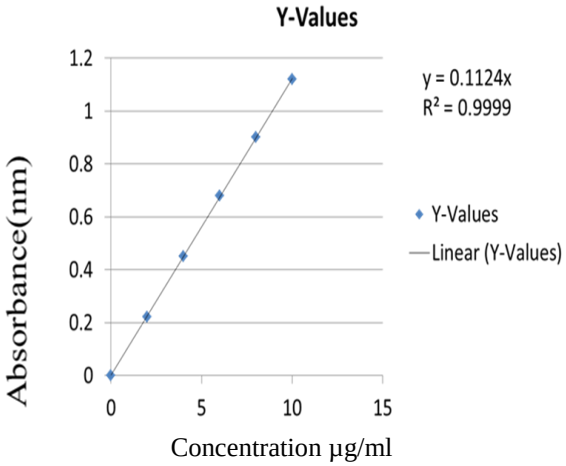

Fig. 2: calibration curve of chlorzoxazone.

The drug was scanned in UV region (200-400 nm) by preparing 1mg/ml solution using phosphate buffer pH 6.8 to find out wavelength of maximum absorption (λ max). The λ max was found to be 244 nm. So the standard calibration curve of chlorzoxazone was developed at this wavelength. Standard calibration curve of chlorzoxazone was determined in phosphate buffer pH 6.8 by plotting absorbance against concentration at 244 nm. The calculation of drug content, *in vitro* release and stability studies are based on this calibration curve.

3.2. Preformulation studies

3.2.1. Solubility Profile

Solubility studies were carried out in different solvents and it was found that CLZ was slightly soluble in water and soluble in ethanol, isopropyl alcohol, alkali hydroxide solution and phosphate buffer solution pH 6.8 which complies with the pharmacopoeia specifications.

3.2.2. Physical Appearance

Chlorzoxazone occurs a practically white crystalline powder and is odourless.

3.2.3. Identification and compatibility by FTIR studies

FTIR studies were conducted in pure CLZ and their physical mixture.

The FTIR spectrum is shown below.

Table 2: FTIR peaks of (pure drug)chlorzoxazone.

PEAKS cm-1	GROUPS
3869.73	C-H stretching
1616.72	=C-H stretching
1770.52	C=O stretching
843.37	C-C stretching
1297.77	N-H stretching
843.37	C-Clstretching

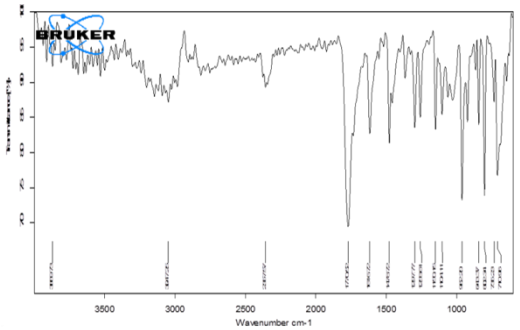

Fig.3: FTIR of pure drug.

Table no.3: FTIR of pure drug + polymer. (chlorzoxazone+sodiumalginate+HPMC+capbopol)

PEAKS(cm-1)	GROUPS
3870.71	C-H stretching
1618.72	=C-H stretching
1775.52	C=O stretching
850.37	C-C stretching
1287.77	N-H stretching
849.37	C-Cl stretching

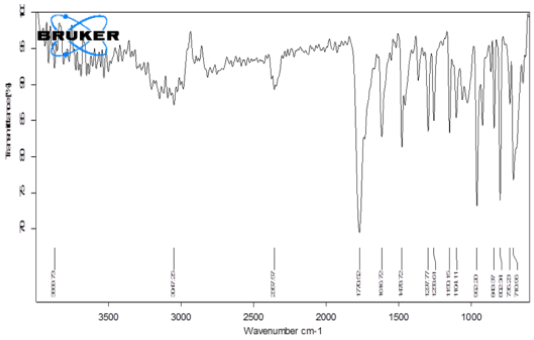

Fig. 4: FTIR of pure drug +polymer.

(chlorzoxazone+sodium alginate+ HPMC+carbopol)

Drug identification is done by FT-IR studies, the peak of chlorzoxazone was obtained at 3869.73 cm-1, 1616.72 cm-1, 1770.52 cm-1, 843.37 cm-1, 129.77 cm-1. There is no significant change in the peak of the pure drug in the FTIR spectrum of physical mixture of pure drug with polymers, ie., carbopol and HPMC. It indicates that there is no chemical interaction between the drug and the polymers. This shows that chlorzoxazone was compatible with both the polymers.

3.3. Characterization of formulated microspheres

3.3.1: Micrometric properties

Micrometric properties studies are conducted and the results are illustrated in the table shown below:

Table 4: Micrometric properties.

Formulation code	Bulk density	Tapped density	Carrs index	Hausner"s ratio	Angle of repose
F1	0.497±0.4	0.543±0.2	13.46±14	1.164±0.01	24.63±0.21
F2	0.464±0.3	0.587±0.3	14.82±13	1.186±0.03	25.73±0.12
F3	0.472±0.5	0.597±0.2	13.96±17	1.169±0.01	24.85±0.62
F4	0.482±0.1	0.573±0.03	15.24±0.13	1.192±0.02	23.77±0.13
F5	0.562±0.1	0.679±0.01	14.74±0.31	1.174±0.01	28.63±0.64
F6	0. 584±0.2	0.696±0.01	15.32±0.21	1.194±0.01	25.63±0.64
F7	0.632±0.1	0.721±0.01	15..96±0.26	1.164±0.04	24.83±0.51
F8	0.683±0.2	0.785±0.02	15.81±0.33	1.182±0.01	28.87±0.86
F9	0.713±0.3	0.832±0.01	14.54±0.16	1.176±0.01	27.64±0.26
F10	0.639±0.3	0.756±0.02	15.79±0.18	1.177±0.01	23.94±0.65
F11	0.6934±0.6	0.746±0.3	12.96±16	1.13±0.02	26.35±0.23
F12	0.784±0.4	0.821±0.4	14.63±17	1.62±0.03	25.62±0.39
F13	0.723±0.3	0.864±0.2	14.96±18	1.176±0.01	26.43±0.462

All values are expressed as a mean ± SD, n = 3

The packing properties of the drug and their formulations are widely depending up on bulk density. It has been stated that bulk density less than 1.2gm/cm3 indicate good flow and values greater than 1.5gm/cm3 indicate poor flow. The result of bulk density, tapped density, Carr's index and Hausner's ratio were mentioned in Table-4. From the results, it was observed that the bulk density and tapped density values were lies in between 0.464 to 0.784 and 0.543 to 0.864g/cm3 i.e. less than 1.2g/cm3, indicating good packing. The Carr's index were lies between 12.96% to 15.96% this indicating excellent and good flow characteristics of the microsphere. The Hausner's ratio were lies between 1.13 to 1.192 i.e., less than 1.25 indicating good flow while greater than 1.5 indicating poor flow.

Angle of Repose

Angle of repose less than 40° indicates free flowing properties of microspheres. However angle of repose greater than 400 indicates poor flow of material. It is observed that, the angle of repose for various ratios of the microspheres are found to be less than 300 it indicates free flow properties of the microsphere.

3.3.3: Percentage yield

Percentage yield study was conducted and the results are illustrate in the table shown below.

Table 5: Percentage yield of chlorzoxazone microspheres.

Formulation code	Percentage yield
F1	70.21±0.21
F2	68.12±0.36
F3	82.92±0.42
F4	82.92±0.21
F5	81.96±0.29
F6	70.21±0.38
F7	62.5±0.236
F8	**86.33±0.87**
F9	70.21±0.54
F10	68.12±0.635
F11	81.96±0.498
F12	70.21±0.546
F13	70.21±0.239

All values are expressed as a mean ± SD,n=3.

Fig. 5: Percentage yield of microsphere.

The percentage yield is found between in all formulations 62.5% to 86.33% and compared than other formulations F8 showed maximum percentage yield. From the results, it was observed that, the concentration of polymer increased, the percentage yield of the microspheres was also slightly increased based on polymer proportions.

3.3.4. Mean particle size

Mean particle size study was conducted using optical microscope and the results are illustrated in the table shown below.

Table 6: Mean particle size of chlorzoxazone microsphere.

Formulation code	Mean particle size
F1	185.0±0.43
F2	168.8.±0.2
F3	194.6±0.1
F4	194.6±0.1
F5	188.0±0.071
F6	185.0±0.43
F7	141.2±0.49
F8	**198.6±51**
F9	185.0±0.43
F10	168.8±0.2
F11	188.0±0.071
F12	185.0±0.43
F13	185.0±0.43

All values are expressed as a mean ± SD,n=3.

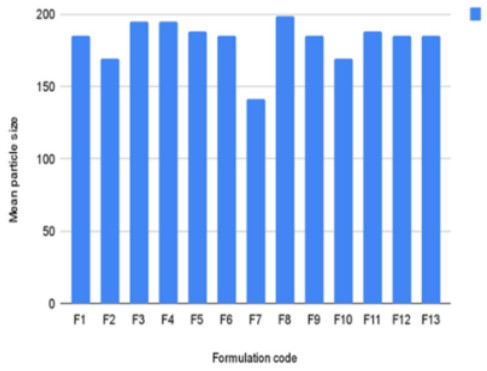

Fig. 6: Mean particle size of microspheres.

The size range of about 100 particles was determined by optical microscopy. The average particle size range of all formulations is done From the results, the particle size of all the formulations was observed in between 141.2 µm to 198.6µm. As the polymer ratio was increased the mean particle size of microspheres were also increased.

3.3.5. Drug content

Drug content of formulated microsphere were determined by UV spectrophotometer at λ max 244 nm and the results of drug content of each formulation was given in the table below.

Table 7: Percentage drug content of microspheres.

Formulation code	Perceentage Drug content
F1	94.6±0.121
F2	89.9±0.06
F3	96.9±0.25
F4	96.8±0.325
F5	96.7±0.369
F6	94.6±0.156
F7	89.5±0.189
F8	**97.3±0.246**
F9	94.6±0.298
F10	93.2±0.358
F11	96.5±0.341
F12	94.6±0.247
F13	94.6±0.239

All values are expressed as a mean ± SD,n= 3.

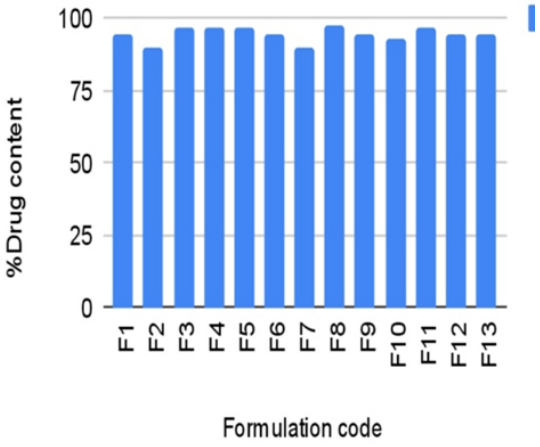

Fig.7: Percentage drug content of microsphere.

Drug content of formulated microsphere were determined by UV spectrophotometer which is in the range of 89.5to97.3. as the polymer concentration increases drug content also increases.

3.3.4. Drug entrapment efficiency

Drug entrapment efficiency was conducted and the results are illustrated in the table shown below.

Table 8: Percentage entrapment efficiency of microsphere.

Formulation code	Percentage Entrapment efficiency
F1	59.6±0.4
F2	56.25±0.7
F3	65.04±0.2
F4	65.04±0.2
F5	63.07±0.3
F6	59.6±0.4
F7	52.6.±0.6
F8	**68.56±0.3**
F9	59.6±0.4
F10	56.25±0.7
F11	63.07±0.3
F12	59.6±0.4
F13	59.6±0.4

All values are expressed as a mean ± SD, n = 3.

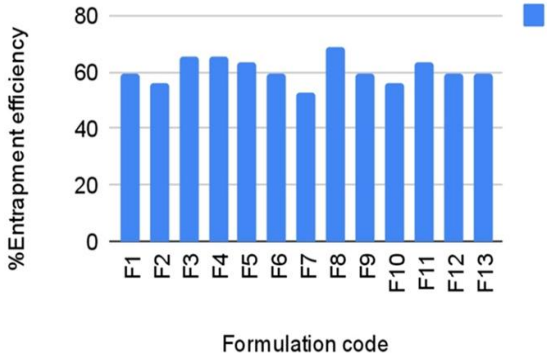

Fig.8: Percentage entrapment efficiency of microspheres.

The amount of drug was estimated by crushing the microspheres and extracting with aliquots of phosphate buffer (pH 6.8) repeatedly. The results of the drug entrapped in the microspheres were tabulated in Table no.9 and the histogram. The percentage EE of the formulated microspheres was found in the range between 52.6% to 68.56%. From the results maximum EE is found in F8 formulations and it's due to increase with increasing the polymer concentration. At the highest polymer concentration would be expected to more drug will be bound in the microspheres (due to increase in particle size).

3.3.5: SEM studies

The surface and shape characteristics of microsphere were determined by scanning electron microscopy (SEM). Photographs were taken and recorded at suitable magnification. The photograph shows below.

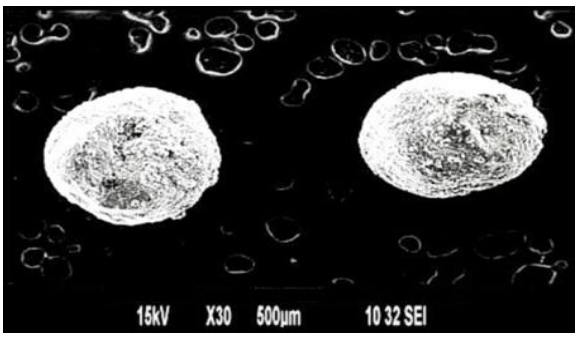

Fig.9: Sem of F8 Formulation.

The surface morphology was determined by SEM for characterization of shape and size of microspheres and the scanned images are shown in the fig.10 and the results showed that the prepared microspheres are that the microspheres were discrete and almost spherical in shape with rough outer surface due to incomplete homogeneity of drug and polymer and also due to higher concentration of drug uniformly dispersed at the molecular level in the polymer matrice.

3.3.6. DRUG RELEASE KINETICS

In vitro drug release of formulations were determined using modified USP type II dissolution apparatus fabricated in our laboratory and the results are given below.

Table 9: Cumulative percentage drug release of formulations.

TIME (hrs)	CUMILATIVE %DRUG RELEASE												
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	11.3 ±0.2	18.2 ±0.3	17.8 ±0.1	16.8 ±0.6	16.2 ±0.4	11.3 ±0.1	15.4 ±0.4	10.2 ±0.2	11.3 ±0.1	12.1 ±0.5	14.1 ±0.1	11.3 ±0.2	11.3 ±0.4
2	26.8 ±0.1	23.2 ±0.5	38.4 ±0.2	37.2 ±0.1	36.4 ±0.4	26.8 ±0.1	18.6 ±0.5	23.4 ±0.1	26.8 ±0.3	28.2 ±0.2	30.2 ±0.1	26.8 ±0.9	26.8 ±0.3
3	45.6 ±0.4	38.1 ±0.1	58.2 ±0.3	55.8 ±0.1	54.9 ±0.2	45.6 ±0.1	32.9 ±0.4	40.6 ±0.4	45.6 ±0.1	48.1 ±0.8	51.4 ±0.1	45.6 ±0.2	45.6 ±0.1
4	56.2 ±0.1	54.6 ±0.4	69.4 ±0.6	66.2 ±0.4	62.5 ±0.1	56.2 ±0.9	45.2 ±0.1	52.8 ±0.4	56.2 ±0.6	59.2 ±0.2	60.2 ±0.1	56.2 ±0.1	56.2 ±0.3
5	69.2 ±0.2	69.5 ±0.6	74.2 ±0.4	79.2 ±0.1	76.3 ±0.2	69.2 ±0.4	53.6 ±0.5	66.7 ±0.6	69.2 ±0.1	70.1 ±0.4	74.3 ±0.1	69.2 ±0.3	69.2 ±0.4
6	86.3 ±0.3	82.2 ±0.5	79.8 ±0.1	81.4 ±0.6	79.5 ±0.9	86.3 ±0.8	79.5 ±0.1	81.9 ±0.2	86.3 ±0.8	88.2 ±0.2	89.1 ±0.1	86.3 ±0.1	86.3 ±0.2
7	89.2 ±0.1	84.7 ±0.2	92.8 ±0.4	92.7 ±0.3	93.2 ±0.5	89.2 ±0.2	82.4 ±0.9	92.1 ±0.4	89.2 ±0.2	90.1 ±0.3	92.8 ±0.6	89.2 ±0.8	89.2 ±0.4
8	90.4 ±0.5	85.6 ±0.6	94.2 ±0.1	93.8 ±0.2	94.3 ±0.9	90.4 ±0.8	86.8 ±0.1	94.8 ±0.6	90.4 ±0.2	92.3 ±0.5	95.2 ±0.6	90.4 ±0.8	90.4 ±0.2
9	93.9 ±0.2	89.6 ±0.5	96.7 ±0.6	96.9 ±0.2	96.2 ±0.3	93.9 ±0.6	89.5 ±0.6	97.4 ±0.1	93.9 ±0.3	93.1 ±0.4	96.8 ±0.9	93.9 ±0.2	93.9 ±0.1
10	95.5 ±0.2	90.8 ±0.5	97.6 ±0.3	97.3 ±0.8	97.4 ±0.1	95.5 ±0.6	90.1 ±0.5	98.7 ±0.6	95.5 ±0.2	94.2 ±0.1	97.2 ±0.2	95.5 ±0.5	95.5 ±0.2

All values are expressed as a mean of ± SD, n = 3

IN VITRO RELEASE STUDY OF FORMULATIONS

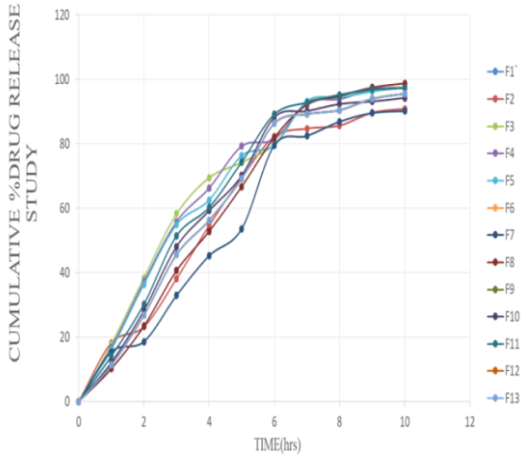

Fig.10: *In vitro* drug release of formulations.

From the above formulations, F8(98.7) was found to be the best formulation. The data obtained from *in vitro* drug release profile was fitted into various kinetic models to study the drug release pattern and formulation F8was used for stability studies as per ICH guidelines.

3.3.7. Optimization by design expert software

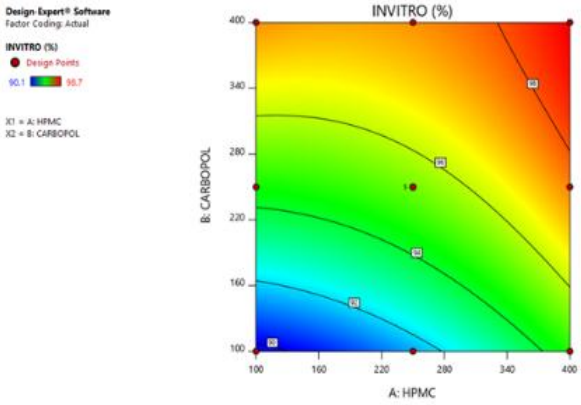

Fig.11: Countour plot showing the effect on carbopol and HPMC in *in vitro* drug release.

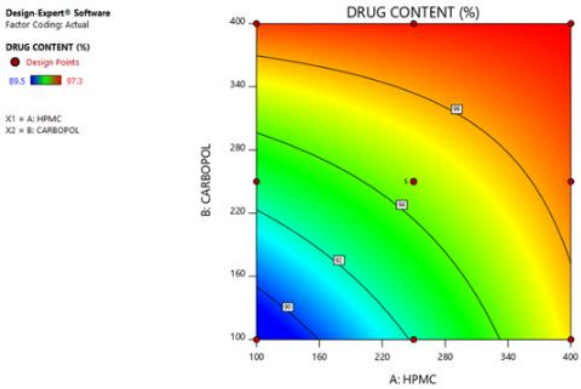

Fig.12: countour plot showing the effect of carbopol and HPMC on drug content.

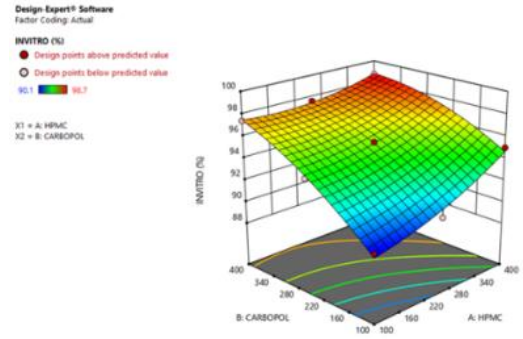

Fig.13: 3 D surface plot showing the effect of carbopol and HPMC in *invitro* drug release.

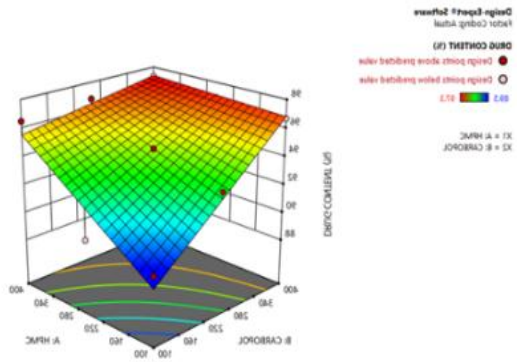

Fig.14::3 D surface plot showing the effect of carbopol and HPMC on drug content.

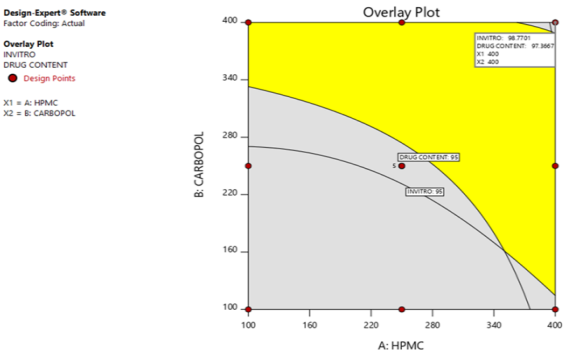

Fig.15: overlay plot.

The formulation is optimized by Design expert Software version 13.0.7.0. central composite design was used to find the optimized formulation.13 formulations were suggested by the software and after optimization of the analyzed data, 6 solutions were obtained. From the 6 solutions, one was selected by considering the drug content and *in vitro* drug release. The batch with carbopol- 400mg, HPMC 400mg with desirability 1 was found to be optimum. From this data, formulation F8 was selected as the optimized formulation. The formulation F8 showed highest values for drug content and *in vitro* drug release. Hence, the data obtained from the *in vitro* drug release was fitted to various kinetic models and stability studies were conducted on selected formulation as per ICH guidelines.

Fig.16: Photograph of best (F8) formulation.

3.3.8 Drug release kinetics

ZERO ORDER RELEASE MODEL OF F8 FORMULATION

A plot on time v/s cumulative percentage drug release

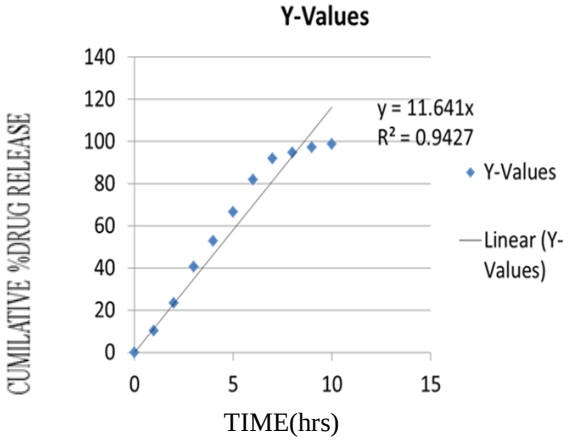

Fig.17: Zero order release model.

FIRST ORDER DRUG RELEASE MODEL OF F8 FORMULATION

A plot on time v/s log cumulative percentage drug release

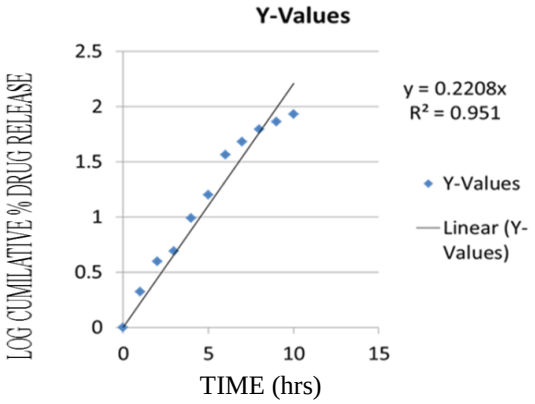

Fig.18 First order release model.

HIGUCHI RELEASE MODEL OF F8 FORMULATION

A plot on square root of time v/s cumulative percentage drug release.

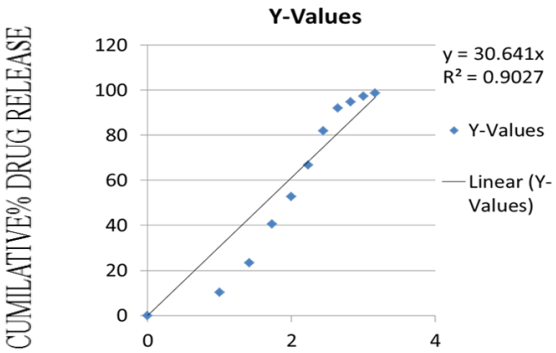

Fig.19: Higuchi release model.

KOSMEYER PEPPAS MODEL OF F8 FORMULATION

A plot on log time Vs log cumulative percentage drug release.

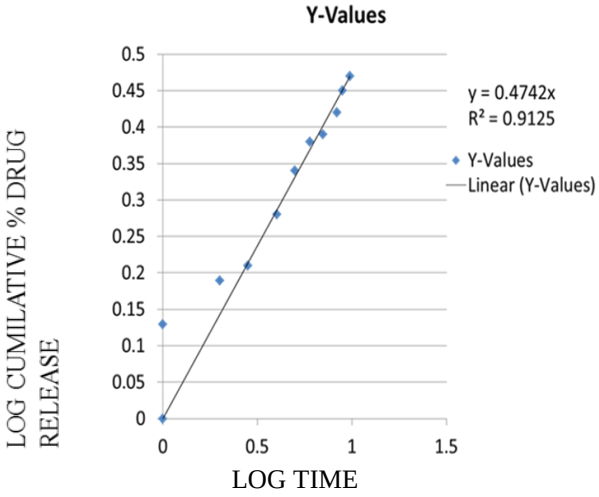

Fig.20: Kosmeyer peppas model.

Drug release kinetics

Table 10. Drug release kinetics

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI	KOSMEYER PEPAS	
F8	R^2	R^2	R^2	R^2	n
	0.9427	0.951	0.9027	0.9125	0.4742

In the formulation F8, the R^2 value of zero order kinetics was found to be 0.9427, first order release kinetic was found to be 0.951, So the co-efficient of determination indicated that the release data was best fitted with first order kinetics. When the drug release data was put in to Higuchi's equation, good correlation coefficient (r) values 0.9027 were obtained, indicating the drug release was diffusion controlled release mechanism. The release data obtained were also put in Korsmeyer-Peppas model in order to find out n values, which describe the drug release mechanism. The n values of F8 formulation was found to 0.4742 with correlation coefficient value 0.9125, indicating Fickian diffusion mechanism. Hence,

the above observations, the release microspheres provide a sustained release for a period of sufficient hours.

3.3.9. Stability study data

The optimized formulation F8, containing carbopol and HPMC was evaluated after storage at 3 different storage conditions. The data obtained is illustrated in table no. 23.

Table 11: Stability study data.

FORMULATION CODE	Storage condition	Sampling interval	Physical apppearence	DRUG CONTENT(%)
F8	$40^0C \pm 2^0C$ at 75%±5%RH	0 days	No change	97.3±0.136
		30 days	No change	96.2.1±0.152
		60 days	No change	95.8±0.159
		90 days	No change	95.2± 0.162
	$25^0C \pm 2^0C$ at 60%±5%RH	0 days	No change	97.3±0.182
		30 days	No change	96.5±0.198
		60 days	No change	96.1.3±0.121
		90 days	N o change	95.3±0.142

4. CONCLUSION

Chlorzoxazone microspheres were prepared by ionotropic gelation method using different polymers with varying concentration. The prepared formulation was evaluated for their physical appearance, micrometric properties, particle size analysis, percentage yield, drug content, entrapment efficiency, SEM study, in vitro release, kinetics study, stability study.

Out of the different formulation prepared, formulation F8 having the same ratio of HPMC and carbopol showed better results, hence selected as optimized formulation.

From the kinetic studies data the rate of drug release follows first order kinetics and shows Korsmeyer-Peppas model with fickian diffusion mechanism.

It was concluded that, prepared CLZ microspheres might be better practical approach to achieve the retarded effect and continuously releasing the medication over extended period of time in the GIT with expected to decreased GI side effects due to the less dosage frequency of administration and all the results data were found to be satisfactory and the formulated microspheres can be selected for the targeted drug delivery system for potential therapeutic uses, there by sustaining and prolonging the systemic absorption of CLZ and improve the patient compliance.

5. ACKNOWLEDGEMENT

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