FORMULATION AND EVALUATION OF GASTRO - RETENTIVE FLOATING MICROSPHERES OF AMILORIDE HCl

Suraj Kumar Sah*, Ms. Anupriya Adhikari and Dr. Shivanand Patil

Department of Pharmacy, Shree Dev Bhoomi Institute of Education Science and Technology, Uttarakhand Technical University, Dehradun, India.

*Corresponding Author
Suraj Sah Suraj

Department of Pharmacy, Shree Dev Bhoomi Institute of Education Science and Technology.

ABSTRACT

The present work by Formulation and Evaluation of Gastro retentive Floating Microsphere plays a highly significant role as particulate drug delivery method. Particle sizes for microspheres range from 0.1 to 200 µm, and they can be administered orally, parenterally, nasally, ophthalmologically, transdermal, colonically, etc. Site-specific targeting and enhanced release kinetics are just two of the issues that have been solved through recent advances in microspheres, including those that are mucoadhesive, hollow, floating, microballoons, and magnetic. Microspheres will play a key role in novel drug delivery in the future by fusing different new methods, particularly sick cell sorting, genetic materials, safe, targeted, and effective drug delivery. Hydroxypropyl Methyl Cellulose, polyvinyl pyrrolidone, and ethyl cellulose were used in varying concentrations to give the floating microspheres of amiloride HCl release-controlling properties by increasing their bioavailability. Lactose was used as a diluent and sodium bicarbonate served as an effervescent agent. By using a solvent evaporation method approach, the gastro-retentive Floating Microsphere of Amiloride HCl was created. The generated microsphere indicated good Floating Strength and remained buoyant in the sustained released medium for 24 hours. For systemic delivery of amiloride, a potassium-sparing diuretic and antihypertensive medication, through the oral route, a gastro retentive floating microspheres drug delivery system was developed. The different ratios of ethyl cellulose and hydroxy propyl methyl cellulose K-100, sodium lauryl sulfate, sodium bicarbonate, and ethanol. The weight, thickness, percentage of moisture absorbed and lost, surface pH, folding resistance, content homogeneity, in vitro residence time, in vitro release, and ex vivo penetration of the microspheres were all assessed.

KEYWORDS: Amiloride HCL, floating Microspheres, Hydroxypropyl methyl Cellulose k-100.

INTRODUCTION

Solubility

A medicine must first be dissolved in aqueous body fluids for effective transport before it can be administered in any dose form and reach its target site and begin to exert its therapeutic effects. In order to reach its target site if a dosage form is supplied in a solid state, it must first dissolve in an aqueous environment. The poor bioavailability of oral dosage forms, which is dependent on factors such water solubility, dissolution rate, drug permeability, first pass metabolism, and sensitivity to efflux mechanisms, poses the biggest obstacle to their design.

High solubility and poor permeability are the causes of a drug's oral bioavailability. To correlate in vitro drug dissolution with in vitro bioavailability, many researchers are using the Biopharmaceutical Classification System (BCS), which divides active pharmaceutical ingredients (APIs) for oral administration into four groups: class I (high solubility and high permeability), class II (low solubility and high permeability), class III (high solubility and low permeability), and class IV (low solubility and low permeability).

Figure: Biopharmaceutical Classification System.
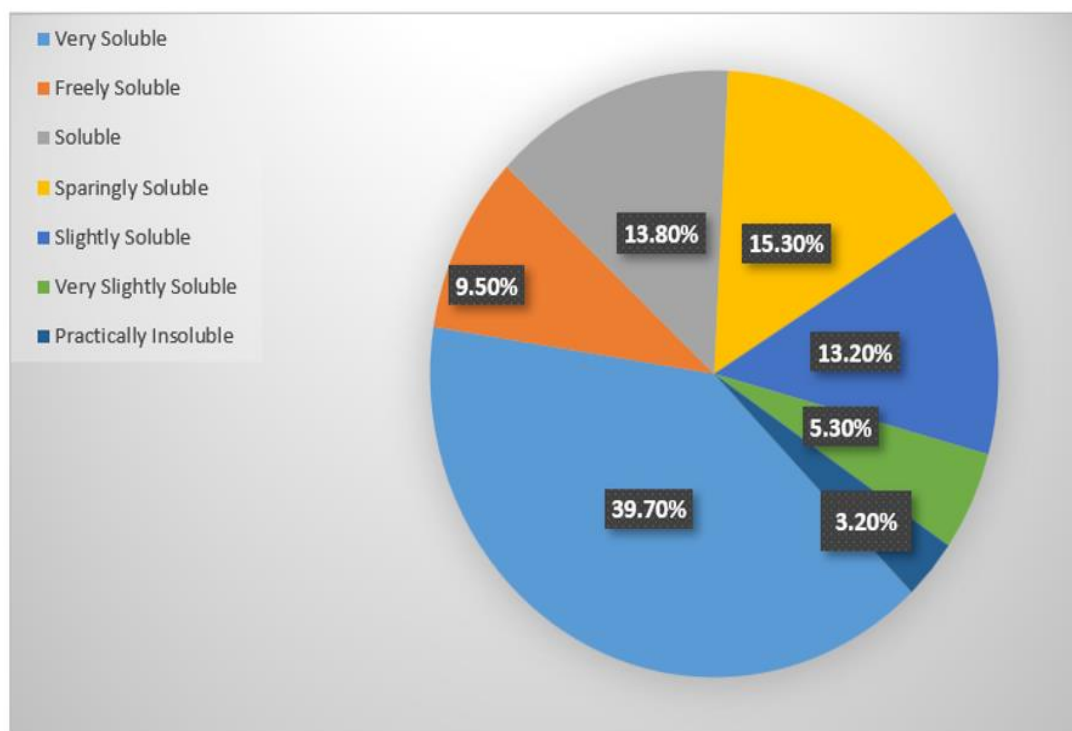
Solubility Distribution of Top 200 marketed oral Drug

Result, the treatment in vivo may fail due to insufficient drug concentrations at the site of action. Micronizing the surface area of medicines with low water solubility is a useful way to increase their bioavailability. The Noyes-Whitney equation (Noyes and Whitney, 1897), which shows that the dissolution rate is directly related to the surface area of the medication, supports this. The Ostwald-Freundlich equation (Ostwald, 1900), and others. Therefore, it can be seen that solubility increases exponentially with particle size (Abujar et al., 2018).

The research and development of novel formulations (such as amorphous systems, nanoparticles, crystal engineering, and solid dispersions), as well as the use of new technologies (such as hot-melt extrusion and spray-drying), have all been actively pursued over the past few decades in an effort to overcome the solubility, dissolution rate, and bioavailability issues associated with poorly water-soluble drugs. Solvents are also used in conventional techniques for processing solids. However, the Q3C standards for residual solvents from the International Conference for Harmonization (ICH) advise against using too many solvents in pharmaceutical applications.

Because it is simple, patient compliance is high, and it has a cheap production cost, the oral route is the most recommended method of medication delivery. For the medicine to be absorbed through the GIT, it must be in solution form. The pharmaceutical business faces

significant challenges due to poor aqueous drug solubility. Drugs with an aqueous solubility of less than 100 g/ML have reduced absorption due to dissolution, which prevents complete absorption from the human GIT.Based on BCS, 40% of the medicine is poorly water-soluble, and 60% of the drug is poorly soluble in GI fluid. To improve the drug delivery system, scientists have created a variety of dosage forms. With the use of several cutting-edge drug delivery system strategies, the danger of dose dumping, intra- and inter-subject variability can be decreased.

INTRODUCTION TO MICROSPHERES

Microspheres are tiny are naturally biodegradable, free-flowing powders with particle sizes smaller than 200 nm that are made of protein or synthetic polymers. In the case of chronic patients, the medication must be administered over an extended length of time, and numerous medications must be taken at the same time. When a medicine's half-life is shorter and patient compliance declines as a result, frequent drug administration is required. Different types of controlled release dosage forms are created and modified to address the aforementioned issues, increasing patient compliance through delayed effects and reducing undesirable effects by lowering peak plasma concentration.

One such strategy of controlled release dosage form in innovative drug delivery system is in the microsphere as drug carriers. "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" is how microspheres are described. It can be characterized as a structure made up of a continuous phase of one or more miscible polymers in which drug particles are distributed at the macroscopic or molecular level. Its particles range in size from 1 to 1000 nm. Advantages of microspheres:

➢ Enhance bioavailability.

➢ Increase the medication release and the reactive core's separation from the other material.

➢ Increase patient compliance by carrying out this.

➢ Change a liquid into a solid and cover up the bitter flavour.

➢ Reduce the core's reactivity in regard to its surroundings outside of it.

➢ Protects the GIT from the drug's irritative effects.

➢ Microsphere characteristics such as size, surface charge, and surface hydrophilicity have been discovered to have a significant impact on how particles behave in vivo.

➢ Biodegradable microspheres offer the benefit of not requiring surgery for insertion or removal as compared to bigger polymer implants.

➢ Offer a sustained and ongoing therapeutic impact.

➢ They allow for medication release under control. such as narcotics, antagonists, and steroid hormones.

➢ Reduce toxicity and dosage.

MATERIAL AND METHOD

Materials: The components utilized to develop the formulation were from commercial sources. Since all of the compounds were of the analytical quality, no additional purification or modification was necessary.

Table: The table below lists the chemicals utilized in this investigation along with their manufacturers.

S.No.	Chemicals	Manufacturer
1	Amiloride HCl	National healthcare pvt. ltd.
2	HPMCK-100	Keva pharmaceuticals pvt. ltd.
3	PVPK-30	Keva pharmaceuticals pvt. ltd.
4	Ethanol	Keva pharmaceuticals pvt. ltd.
5	Hydrochloric acid	Keva pharmaceuticals pvt. ltd.
6	Sodium Lauryl Sulfate	Keva pharmaceuticals pvt. ltd.
7	Sodium Bicarbonate	Keva pharmaceuticals pvt. ltd.
8	Disodium hydrogen phosphate	Keva pharmaceuticals pvt. ltd.
9	Sodium phosphate dibasic	Keva pharmaceuticals pvt. ltd.
10	Ethyl Cellulose	Keva pharmaceuticals pvt. ltd.

Instruments/ Equipment

Table: The instruments used in this study and their suppliers are.

S.No.	Instruments	Manufacturer
1	Digital balance	Simtronics
2	Dissolution apparatus	Labindia ds 8000
3	Disintegration apparatus	Labindia DT 1000 auto- sampler
6	Melting point apparatus	Labindia mepa
7	Hot air oven	Hicon
8	pH meter	Spectrlab
10	FTIR spectroscopy	Perkin Elmer technologies
11	UV spectroscopy	Perkin Elmer lambda 360
13	Bulk density apparatus	Simtornics

Preformulation Studies

The majority of medications that have an oral mechanism of action are sold as tablets, capsules, or both. It is crucial that specific fundamental physical and chemical properties of the drug molecule and other derived features of the drug powder be established prior to

creation of the dosage forms with a new drug candidate. Many of the subsequent actions and potential strategies in formulation development will be guided by this knowledge.

The "Preformulation" stage is the first in the deliberate development of a dosage form for a pharmacological substance, both alone and in combination with excipients. When a newly synthesized medicine demonstrates sufficient pharmacologic aptitude in an animal model to warrant evaluation in humans, Preformulation is initiated.

Physical Characterization and Organoleptic Properties of drug
The drug's color, odor, and look were all clearly observed. Three instances of the process were completed, and the mean was given.

Table: shows the physical properties of Drug & identification.

s.no:	Specification	Confirm to specification
1.	Color	Yellow
2.	Odour	Unpleasant
3.	Appearance	Pale yellow to greenish yellow
4.	Taste	Bitter

Determination of Melting Point
Determination of melting point is a method applied of the identification of drug. Open capillary method was used to determine the melting point of Amiloride HCl. The capillary was properly closed at one end by passing it through a flame; the other side which is open is used for filling the drug into it. The drug level filled is 2-3mm with an internal diameter of capillary 1mm and a wall thickness of 0.2 mm. It was then put on a melting point apparatus together with a high accuracy thermometer for complete assembly. The melting point of drug was determined.

Table: Shows the Melting points of Drug.

S.No.	Melting Point	Average
1.	241.0° C	
2.	240.0 °C	241 ±5.5°C
3.	242.0° C	

Solubility

In aqueous buffers, amiloride (hydrochloride) (hydrate) is only weakly soluble. Amiloride (hydrochloride) (hydrate) should be first dissolved in DMSO and then diluted with the preferred aqueous buffer for maximum solubility in aqueous buffers. This approach gives a solubility of amiloride (hydrochloride) (hydrate) of about 0.5 mg/ml in a 1:1 solution of DMSO: Phosphate Buffer (pH 7.2). The aqueous solution should not be kept for longer than a day.

Table: Solubility profile.

S.No.	Descriptive term	Parts of solvent required for one part of solute
1.	Very soluble	Less than 1
2.	Freely soluble	From 1 to 10
3.	Soluble	From 10 to 30
4.	Sparingly soluble	From 30 to 100
5.	Slightly soluble	From 100 to 1000
6.	Very sparingly soluble	From 1000 to 10,000
7.	Practically insoluble or soluble	10,000 and over

Making a standard medication solution and calculating the drug's maximum effective dose (using methanol and a pH 7.4 phosphate buffer): Methanol was used as a blank and an amiloride HCl 5 g/ml solution was scanned in a UV spectrophotometer between 200 and 600 nm. Amiloride HCl's maximum absorbance in methanol was measured at a wavelength of 362 nm. (2018) (Kadam PV et al.).

Stock Solution

Amiloride HCl, 5 mg, was diluted in 50 ml of phosphate buffer, pH 7.4, to create a stock solution with a concentration of 100 g/mL (2018) (Kadam PV et al.).

Standard Solution

1. Stock solutions of (500 or 250 mg/L) were made by using an ultrasonic device (ultrasonicator) to dissolve samples in about 80 mL of deionized water in a volumetric flask measuring 100 ML. The solutions were then diluted to the appropriate concentration with deionized water.

2. After dilution with deionized water, two series of pure single standards pharmaceuticals (0.2-100.0 mg/L) were created.

3. Solutions for binary combinations of common medications Amiloride hydrochloride solutions were made using the following two methods:

The first series of mixture solutions was made using a fixed concentration of amiloride hydrochloride (10 mg/L) with varying concentrations of amiloride hydrochloride (2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, and 40 mg/L). The second series of mixture solutions contains a fixed concentration (20 mg/mL) with varying concentrations of amiloride hydrochloride (2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, and 40 mg/L).

**The drug's calibration curve (Methanol and phosphate buffer 7.4) is plotted**

Pipetting 0.2, 0.4, 0.8, 1.2, 1.6, 2.4, 4.8, and 9.6 ml from the standard stock solution into a 10 ml volumetric flask and filling the remaining space with phosphate buffer produced successively 0.5, 1, 2, 3, 4,5, 6, and 12 and 24 g/mL concentrations (Emami J et al., 2014). Using a UV-visible spectrophotometer, absorbance at 362 nm was measured. The calibration curve was plotted to verify linearity, and the experiment was run in triplicate (Rapalli VK et al., 2020).

The process is the same when using phosphate buffer pH 7.4. The reason why color solutions are colored is because they selectively absorb some light waves while allowing others to pass through. We perceive the light waves that are not absorbed as observers. By calculating We can determine the concentration of solutions based on the amount of light absorbed. It is necessary to identify the wavelength at which absorbance is greatest before performing this kind of spectral analysis. At this wavelength, the spectrophotometer is more sensitive to changes in absorbance. This experiment aims to show how to determine the wavelength with the highest absorbance for any color solution. The procedure entails measuring absorbance between the wavelengths of 200 nm and 600 nm, often at intervals of 25 nm. You may graph the data to see where the highest absorbance is, or you can look at the pairs of data to work out the wavelength. To teach this process, water with food coloring added works really well. Alternatives include the use of colored ion solution, (Panigrahi SK et al., 2019).

Preparation of Gastroretentive Floating Microspheres of Amiloride HCl

Gastroretentive floating microspheres are able to be prepared using a variety of production techniques. However, an important amount of scientific investigators surrounded the world have utilized the solvent evaporation technique and the ionotropic gelation method extensively to investigate the various floating microspheres. The choice of the best method was almost essential for the effective entrapment of active ingredients during the development of floating controlled release microspheres. The nature of the polymer, the drug, and their intended use are typically considered by determining a fabrication technique.
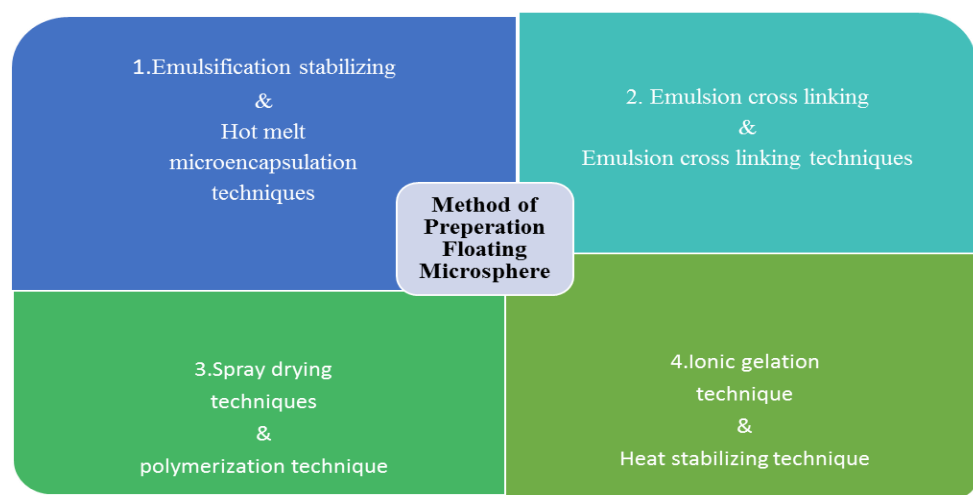
Fig: Shows various Method of preparation of FMs.

Solvent Evaporation Technique: The majority of pharmaceutical industries employ this technique to get substances to release slowly into the body. In this method, an excess of aqueous continuous phase is used to form an emulsion an organic solvent (which is usually methylene chloride) containing dissolved polymer and dissolved/dispersed drug. With the help of an agitator. The shape and size of the particles are influenced by the amount of emulsifier is present in the aqueous phase. When the desired emulsion droplet size has been achieved, the stirring rate is decreased and the organic solvent is evaporated at the proper temperature and atmospheric or reduced pressure. After the solvent from the dispersed phase evaporates and solid polymeric micro particles containing the drug are generated. Filtration, centrifugation, or lyophilization are used for extracting the solid micro particles from the suspension.
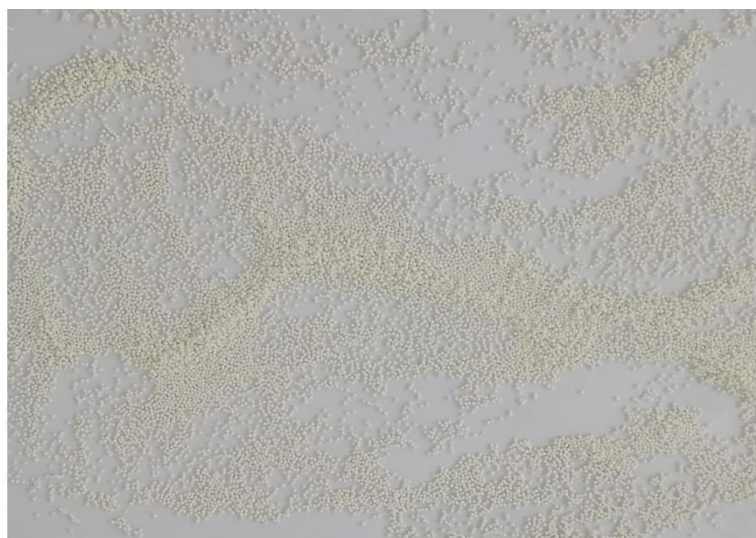
Fig: Shows Amiloride HCl with HPMC K-100 drug Floating microspheres.

**Fig: Shows Amiloride HCl drug Floating microspheres.**

Shows design of expert for various formulation

Formulation (S.No.)	HPMC K-100	Ethyl cellulose	Stirring speed
f-1	0.7	0.7	750
f-2	1.2	0.7	1000
f-3	**1.2**	**1.2**	**750**
f-4	0.7	0.7	750
f-5	0.7	0.7	750
f-6	0.7	1.2	1000
f-7	0.8	0.7	750
f-8	0.5	0.7	1000

Characterization and evaluation of Microspheres and blend

UV-visible spectroscopy is used to determine the inclusion complex's maximum wavelength.

After stabilizing the instrument initially for 30 minutes, blank correction was done using methanol. Then 10μg/mL solution of Amiloride Hydrochloride were scanned separately in UV region ranging from 200nm t0 400nm. The absorption spectra were observed with maximum absorption at 362nm for Furosemide and Amiloride Hydrochloride respectively. The spectra obtained are given fig.
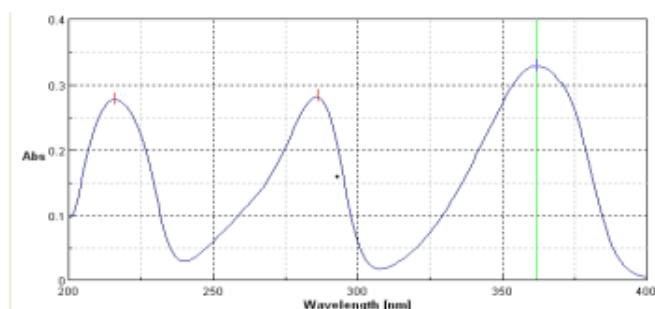
Fig: UV absorption spectrum of Amiloride RS in methanol with absorption maximum at 362 nm.

Density calculation for Bulk (ρo)/Tapped (ρt) Density

To determine bulk/tapped volume, nearly 5.0 g of the microspheres blend was placed in a measuring cylinder with 25 mL capacity. The bulk volume of the NPs was recorded, and then it was tapped, 100 times to obtain the tapped volume. The ρo, as well as ρt of the blend, were determined by using Equations and respectively. (Sreeharsha N et al., 2020).

Fig: Shows Bulk density &Tapped density Apparatus.

MATERIALS AND METHOD

Materials

Amiloride HCl (AB), PVPK-30 and Hydroxypropyl methyl cellulose (HPMC K-100M) were provide by National Health care Pvt. Ltd. All solvents used were of analytical grades and were used as obtained.

Preparation of AB Microspheres

AB microspheres were prepared based on solvent evaporation technique. Different batches of AB microspheres, F1 to F8 were prepared by varying the concentration of ethyl cellulose polymer in the formulation from 1.00 g. respectively(Table).Weighed quantities of drug and polymers were dissolved in mixture of ethanol and dichloromethane (2:2 solvent ratio) at room temperature. This solution was poured in to 50 ml distilled water containing 1.00 % SLS.The resultant emulsion was stirred with a propeller type agitator at 900 rpm for 50 min to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried overnight at room temperature. Concentrations of the ethyl cellulose were optimized based on the % drug release, % entrapment efficiency.

Shows Formulation of Amiloride HCl Microspheres

Table: Formulation of Amlodipine HCl Microsphere.

Ingredients	Formulation Code							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Amiloride Hydrochloride (g)	2	2	2	2	2	2	2	2
Ethyl Cellulose (g)	1	1	1	1	1	1	1	1
HPMC (g)	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Ethanol (g)	39.45	39.45	39.45	39.45	39.45	39.45	39.45	39.45
Water (g)	49.85	49.85	49.85	49.85	49.85	49.85	49.85	49.85
(1%w/v) SLS (g)	1.193	1.193	1.193	1.193	1.193	1.193	1.193	1.193
Sodium Bicarbonate (g)	20	20	20	20	20	20	20	20
Total Quantity (g)	**120.49**	**120.49**	**120.49**	**120.49**	**120.49**	**120.49**	**120.49**	**120.49**
Average filled weight of capsule (mg)	602.47	602.47	602.47	602.47	602.47	602.47	602.47	602.47

Finally, microsphere was prepared by solvent evaporation method, and the label claim i.e. 10 mg per capsule. Which is expressed in the table. So, the average filled weight is 602.47 mg respectively. And filled in size '00' hard Gelatin capsule shell.

RESULT AND DICUSSION

Amiloride Hydrochloride is inhibiting Na channels, thereby preventing the absorption of Na^+ and increasing its excretion along with H_2o, to produce naturesis. In hypernatremia, the plasma and electrochemical forces decreased, which prevents the excretion of k^+ and H^+ in to the lumen. Amiloride HCl used for its potassium -sparing effect in the treatment or prevention of hypokalaemia induced by thiazide or other kaliuretic in patients with congestive heart failure or hypertension. It is a therapeutic drug and pharmacological tool often used in the combination with thiazide diuretics or other kaliuretics-diuretic agents in the congestive heart failure or hypertension. Floating drug delivery microspheres were being formulated and the present study focused on the formulation of FDDS by using different polymers like HPMC K100, ethyl cellulose and Binder PVPK30, Sodium Bicarbonate used as disintegrating agent and to evaluate its efficacy in treat BP and edema. The Floating drug delivery microsphere were characterized for their % Yield of microspheres, Particle Size analysis, Angle of repose, Determination of drug content, Encapsulation efficiency, Swelling studies, In-vitro dissolution studies, Carr's Index IR, floating lag time, swelling studies and erosion Studies. Gastroretentive floating drug delivery system offers simple and practical approaches to achieve increased gastric residence and to modify drug release profile essential for controlled, site specific and localized drug action. IR identification results of drugs

indicate the purity of drug. IR spectra of pure drug and with the excipients are identical and do not show any incompatibility, thus the excipients are compatible with the drug. Lower values of angle of repose below 30 indicate good flow properties of microsphere. All prepared microsphere were found to be in circular shape with no cracks. The drug polymer ratio was found to influence the release of drug and floating characteristics of microsphere. Formulation F3 showed satisfactory results with short buoyancy lag time, long total buoyancy time and controlled drug release up to 24 hrs. The drug release data were explored for the type of release mechanism followed. The best fit with highest determination R^2 coefficient was shown by Zero Order model. Drug content, physical appearance and comparable release profile of floating microsphere F3.

Standard curve of Amiloride Hydrochloride

Table: shows the absorption of calibration curve.

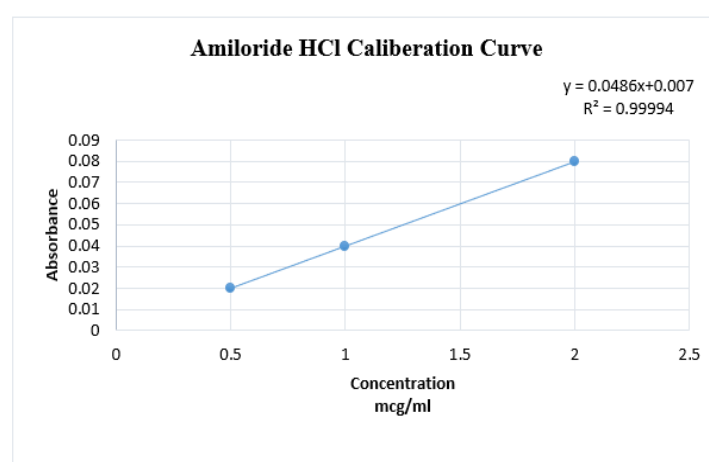| S.No.	Concentration mcg/ml	Absorbance of 1st Reading	Absorbance of 2nd Reading	Absorbance of 3rd Reading	Mean Value of all Reading	S.D.
1.	0.5	0.0218	0.0219	0.0217	0.0218	0.002528
2.	1	0.0454	0.0451	0.0459	0.0454	0.002055
3.	2	0.0847	0.0841	0.0846	0.0844	0.002515
4.	4	0.1641	0.1649	0.0164	0.1151	0.004163
5.	6	0.3238	0.3231	0.0323	0.2264	0.001528
6.	8	0.6447	0.6442	0.0644	0.4511	0.002517

Fig: Shows calibration Curve of Amiloride HCl.

Evaluation Parameters Floating Microspheres

1. Particle size analysis

The sieving method and optical microscopy are used for measuring FM particle size. The mean particle size is measured through the calibrated ocular micrometer.

2. In Vitro dissolution studies

Dissolution investigations for the microspheres were carried out using the USP II equipment (Paddle method) rotated at a constant speed of appropriate rpm with a suitable dissolution liquid. A sample of microspheres containing 100 mg of loaded microspheres was utilized in each test. An aliquot of the sample was repeatedly taken at appropriate intervals and the quantities were replaced with fresh dissolving media in order to maintain the sink condition. At the proper nm, the sample was spectrophotometrically analyzed.
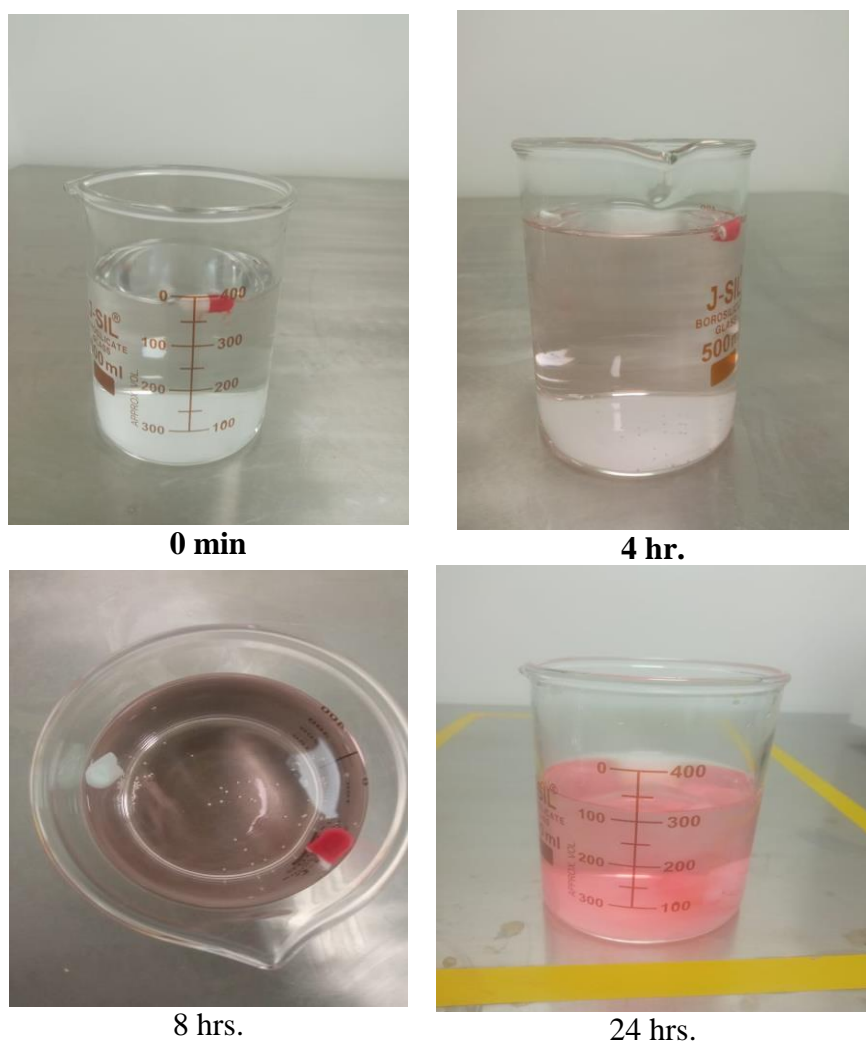

Fig: Shows Dissolution Apparatus USP Ⅱ (Paddle method).

3. Buoyancy/Floating Test

Table: Floating time and Buoyancy.

S.No.	Batch No	Buoyancy lag time (sec)	Floating duration (hrs.)
1	F1	50	>24 hrs.
2	F2	55	>24 hrs.
3	F3	45	>24 hrs.
4	F4	60	>24 hrs.
5	F5	70	>24 hrs.
6	F6	80	>24 hrs.
7	F7	90	>24 hrs.
8	F8	55	>24 hrs.

0 min	**4 hr.**

8 hrs.	24 hrs.

Fig: In Vitro buoyancy study of Amiloride Hydrochloride Floating.

4. Swelling ratio

By soaking the known weight of floating microspheres in 0.1 N HCl or phosphate buffer pH 6.8 at 37± 0.5°C for the necessary amount of time in a glass beaker, the swelling property of the microspheres is studied. At various times, the microspheres are allowed to swell before they are removed.

5. Swelling studies

A glass vial containing 10 ml of distilled water and 50 mg of microspheres were put in an incubator set to 37°C with occasional shaking. Up until equilibrium had been reached, the microspheres were periodically removed, blotted with filter paper, and their weight changes were measured. After three hours, the weight of the swollen microspheres was recorded, and the swelling ratio (SR) was calculated using the formula below. The study was done in triplicate.

$$SR= We-Wo/Wo$$

Where,

Wo= Initial weight of the dry microspheres,

We= Weight of the swollen microspheres at equilibrium swelling in the media.

12. Carrs Index: It was measure by using following formula,

$$\text{Carrs Index} = \{(V_b-V_t)/V_b\}*100$$

Where, V_b and V_t are the bulk volume and tapped volume respectively.

Evaluation of blend

The results confirm that all selected parameters (e.g., flow properties) were in the satisfactory range. The flow properties of the blend were confirmed by the Hausner's ratio and are shown in Table. The values obtained for bulk as well as tapped density were observed to be 0.534 and 0.7459, respectively. Compressibility index and Hausner's ratio for powder blend was found to be 23.27% and 1.42. respectively. Eventually, results analysis confirmed good blend flow characteristics of the formulation, which are considered an essential aspect for an ideal blend.

Table: Shows Blend parameters with observed value.

Blend parameters	Observed value
Bulk density	0.4241±0.04
Tapped density	0.6248±0.02
Compressibility index (%)	25.30±0.05
Hauser's ratio	1.43±0.02
Blend flow characteristics	Good

Fourier transfer infrared (FTIR)

The CH-NM was taken and mixed with about 100mg of KBr; the mixture was triturated and put into a cavity for compression. The formed disc was then subjected to FTIR examination which was analyzed within the range of 4000cm^{-1} to 500cm.[-1]
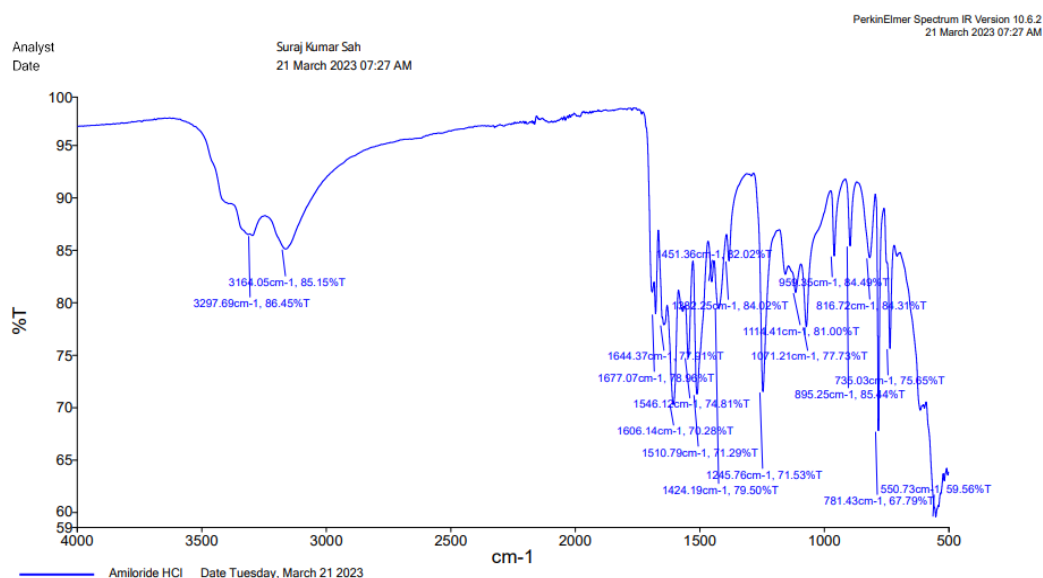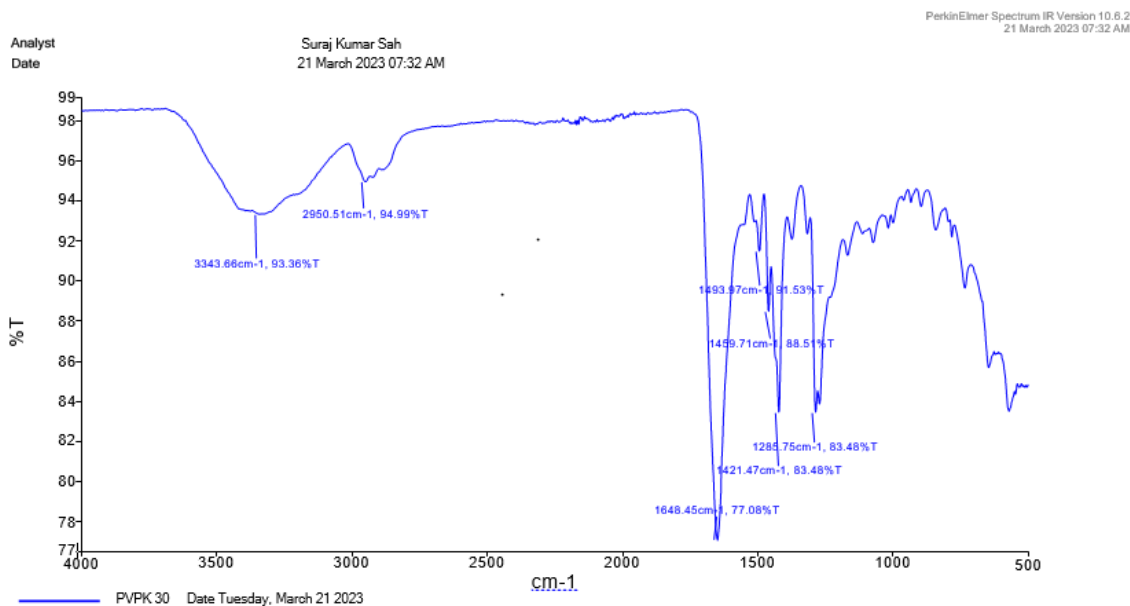
Fig: Shows FTIR Spectra of Pure Drug.

Table: Shows FTIR functional group of Amiloride HCl with their peak.
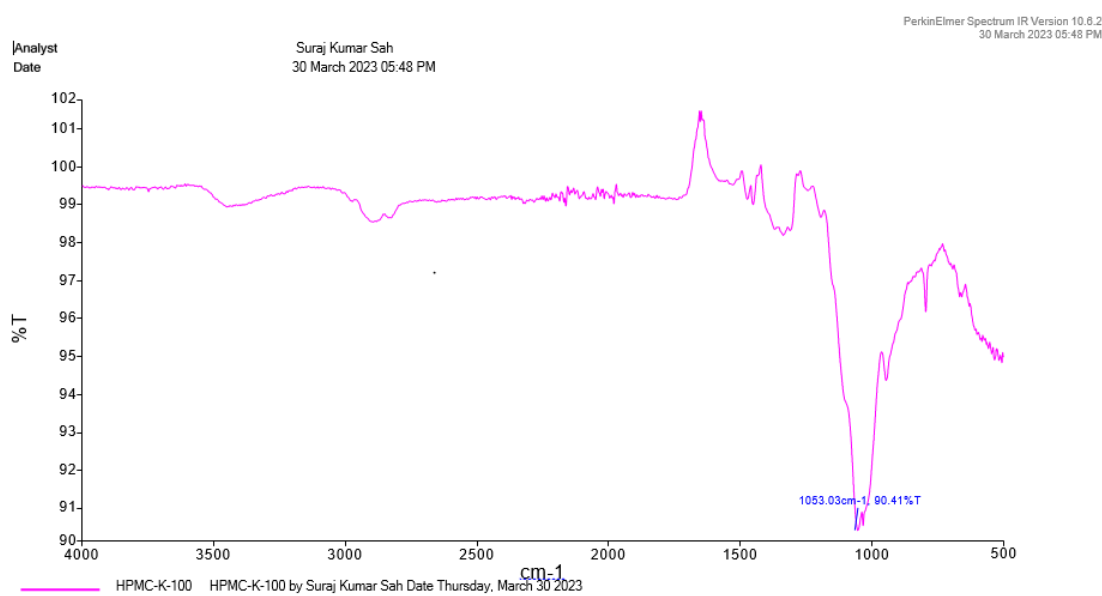
S.No.	Range	Result	Appearance	Group	Compound
1.	3550-3200	3297.69	Medium	N-H Stretching	Amine
2.	3250	3164.05	Medium	NH_2 Stretching	amino acidic
3.	1650-1566	1677.07	Medium	C-C Stretching	Cyclic Alkene
4.	1650-1566	1644.37	Medium	C=C Stretching	Cyclic Alkene
5.	1650-1566	1606.14	Medium	C=C Stretching	Cyclic Alkene
6.	1550-1500	1424.19	Strong	N-O Stretching	Nitro compound
7.	1550-1500	1546.12	Strong	N-O Stretching	Nitro compound
8.	1550-1500	1510.79	Strong	N-O Stretching	Nitro compound
9.	1440-1395	1382.25	Medium	O-H bending	Carboxylic Acid
10.	1310-1250	1245.76	Strong	C-O Stretching	Aromatic ester
12.	1205-1124	1114.41	Strong	C-O Stretching	Tertiary Alcohol
13.	1205-1124	1071.21	Strong	C-O Stretching	Tertiary Alcohol
14.	980-935	950.35	Strong	C-C bending	Alkene
15.	850-550	895.25	Strong	C-Cl Stretching	Halo compound
16.	850-550	815.89	Strong	C-Cl Stretching	Halo compound
17.	800-550	816.72	Strong	C-Cl Stretching	Halo compound
18.	790-665	781.43	Strong	C=C Stretching	Alkene

Fig: Shows FTIR Spectra of PVPK-30 Excipients.

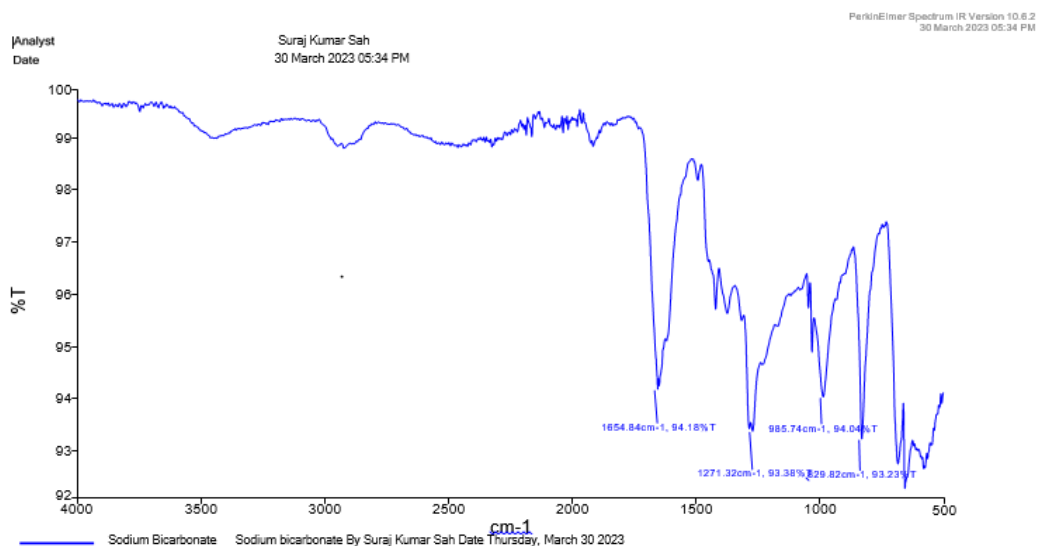**Table: Shows FTIR functional group of PVPK-30 with their peak.**

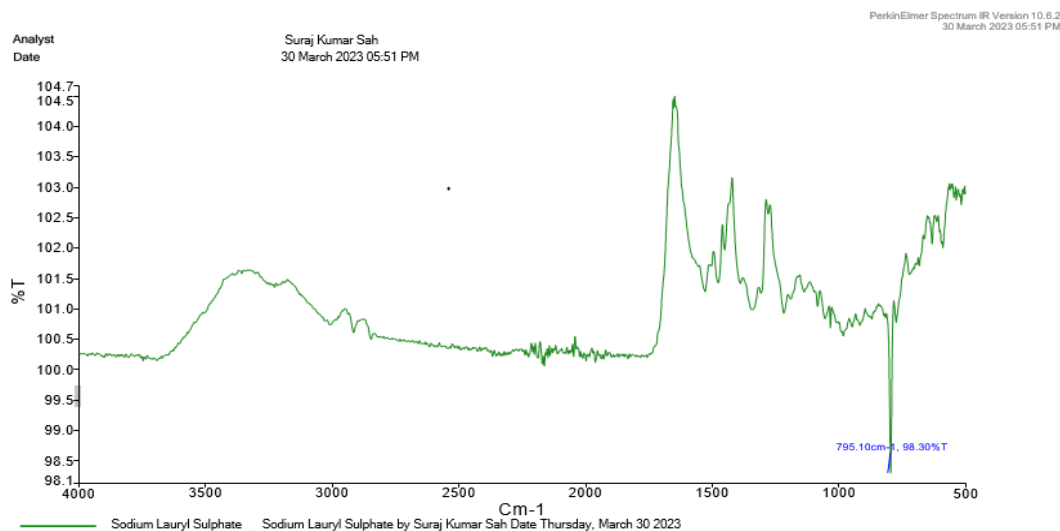| S. No:	Range	Result	Appearance	Group	Compound
1.	3550-3200	3343.66	Medium	N-H Stretching	Amine
2.	3300-2500	2650.51	Strong, broad	O-H Stretching	Carboxylic Acid
3.	1650-1566	1648.45	Medium	C-C Stretching	Cyclic Alkene
4.	1440-1395	1431.18	Medium	O-H bending	Carboxylic Acid
5.	1550-1500	1493.97	Strong	N-O Stretching	Nitro compound
6.	1550-1500	1459.71	Strong	N-O Stretching	Nitro compound
7.	1550-1500	1421.47	Strong	N-O Stretching	Nitro compound
8.	1310-1250	1285.75	Strong	C-O Stretching	Aromatic ester

Fig. Shows FTIR Spectra of HPMCK-100 Polymer.

Table: Shows FTIR functional group of HPMCK-100 with their peak.

S. No:	Range	Result	Appearance	Group	Compound
1.	1124-1055	1053.03	Strong	C-O Stretching	Tertiary Alcohol
2.	980-960	970.19	Strong	C-C bending	Alkene
3.	850-550	815.89	Strong	C-Cl Stretching	Halo compound

Fig: Shows FTIR Spectra of Sodium bicarbonate.

Table: Shows FTIR functional group of Sodium bicarbonate with their peak.

S. No:	Range	Result	Appearance	Group	Compound
3.	1660-1566	1654.84	Medium	C-C Stretching	Cyclic Alkene
2.	1310-1280	1271.32	Strong	C-O Stretching	Aromatic ester
4.	990-960	985.74	Strong	C-C bending	Alkene
5.	850-550	829.82	Strong	C-Cl Stretching	Halo compound
6.	730-665	678.94	Strong	C=C Stretching	Alkene

Fig: Shows FTIR Spectra of Sodium Lauryl Sulphate

Table: Shows FTIR functional group of Sodium Lauryl Sulphate with their peak.

S.No.	Range	Result	Appearance	Group	Compound
1.	3550-3200	3351.37	Medium	N-H Stretching	Amine
2.	3300-2500	2655.98	Strong, broad	O-H Stretching	Carboxylic Acid
3.	1650-1566	1622.13	Medium	C-C Stretching	Cyclic Alkene
4.	1650-1566	1570.06	Medium	C=C Stretching	Cyclic Alkene
5.	1550-1500	1500.62	Strong	N-O Stretching	Nitro compound
6.	1440-1395	1431.18	Medium	O-H bending	Carboxylic Acid
12.	1310-1250	1276.88	Strong	C-O Stretching	Aromatic ester
13.	1205-1124	1159.22	Strong	C-O Stretching	Tertiary Alcohol
14.	980-960	970.19	Strong	C-C bending	Alkene
15.	850-550	795.10	Strong	C-Cl Stretching	Halo compound
16.	730-665	678.94	Strong	C=C Stretching	Alkene

Table: Shows Entrapment Efficacy of the Drug Entrapment of the drug.

formulation	Abs (supernatant)	concentration	%EE
F-1	0.418	8.138614	83.72277
F-2	0.416	8.09901	83.80198
F-3	**0.489**	**9.525168**	**94.71672**
F-4	0.488	9.525168	94.71672
F-5	0.418	8.138614	91.86139
F-6	0.415	8.079208	91.92079
F-7	0.347	6.732673	86.53465
F-8	0.398	7.742574	92.25743

Table: Shows Loading Capacity of the Drug Loading capacity.

| Formulation	Wt. of pellet (mcg)	Abs (pellet)	Conc.	%DL
F-1	90	0.568	11.10891	10.99892
F-2	84	0.716	14.0396	13.69717
F-3	86	0.716	14.0396	13.49962
F-4	91	0.672	13.16832	13.03794
F-5	95	0.516	10.07921	9.833374
F-6	89	0.449	8.752475	8.665817
F-7	84	0.568	11.10891	10.99892
F-8	93	0.615	12.0396	11.74596

In vitro release of drug of different formulation

Throughout the study, the USP dissolution apparatus type I, Basket type, was employed. Using a cyanoacrylate adhesive, one film of each formulation was attached to the central shaft just above the basket. A 900 ml solution of pH 6.6 phosphate buffer (PB) was used as the dissolution medium. At a rotational speed of 50 rpm and a temperature of $37\pm 0.5°C$, the release study was conducted. The release study took place for 8 hours. Every hour, 1 ml of the sample was taken out of each station and replaced with the same volume of the dissolution medium. Each sample that was withdrawn undergo filtering, appropriate dilution,

and spectrophotometric analysis at 362 nm. The information displayed represented the average of three findings.

Table: Shows Drug Release Of all Formulation Drug Release Profile.

Time	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8
0.5	4.554455	4.554455	**4.356436**	4,752475	2.376238	5.346535	1.980198	3.168317
1.	8.910891	6.336634	**9.90099**	8.7122871	4.950495	6.930693	4.950495	4.752475
2.	13.06931	14.05941	**17.42574**	15.44554	8.910891	12.07921	9.306931	15.24752
3.	18.0198	21.38614	**27.32673**	21.78218	12.07921	19.80198	14.25743	20.19802
4.	23.36634	28.11881	**36.63366**	27.92079	15.44554	29.70297	20.79208	26.53465
5.	28.91089	35.44554	**45.54455**	34.65347	19.80198	36.0396	26.93069	31.88119
6.	34.05941	42.77228	**54.85149**	39.80198	23.76238	43.76238	31.88119	37.62376
7.	39.60396	49.50495	**63.56436**	47.52475	28.51485	51.08911	37.42574	43.36634
8.	45.54455	58.61386	**72.27723**	54.85149	36.63366	59.60396	44.55446	39.60396
9.	50.69307	64.55446	**80.59406**	61.38614	43.56436	68.71287	50.49505	46.33663
10.	55.84158	70.29703	**84.55446**	67.52475	52.87129	78.41584	55.84158	52.47525
11.	62.17822	76.23762	**86.73267**	72.07921	62.9703	83.56436	61.78218	58.41584
12.	71.68317	82.17822	**88.11881**	78.61386	71.48515	85.54455	69.30693	66.33663
24.	85.34653	89.30693	**95.24752**	87.92079	86.73267	92.07921	85.94059	83.36634
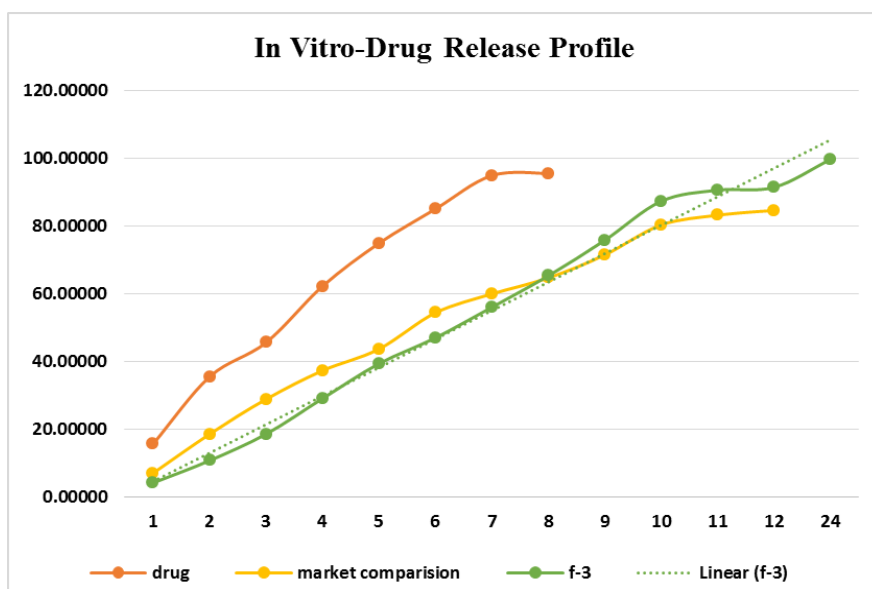
**Fig: Shows in-vitro Drug Release of all Formulation.**

Release kinetics:

The release kinetics data the diffusion profile of the drug from the floating microsphere showed fitted with Hixson Crowell of release kinetics mechanism for the drug from the microsphere the in-vitro release profile was analysed by various kinetic models. The kinetic models used were zero order, first order, Higuchi and Korsmeyer Peppas and Hixson Crowell equation (Table). The releases constant was calculated from the slope of the respective plots.
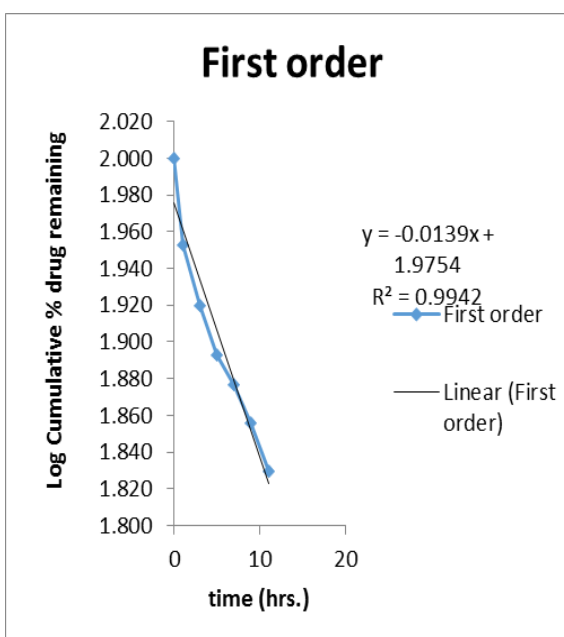
Higher correlation was observed in the Korsmeyer Peppas. The sustained release profile of the drug from the microsphere conformed to Korsmeyer Peppas for the release of the drug from the microsphere. In order to investigate the release mechanism of present drug delivery system, the release data of prepared Amiloride HCl complex forming HPMCK-100 microsphere in phosphate buffer (pH 7.4) were compared to classic drug release kinetics models. The release rates were analysed by least square linear regression method. Release models such as zero order, first order model, Higuchi model and Rigger-Peppas, Hixson Crowell model (Table.) were applied to the release data. The F-3 value of coefficient of determination (R^2) in Hixson Crowell were found to be 0.9443 which indicates the integrity of floating microspheres and Sustained release release. The value of coefficient of determination was found to be 0.9443.

Table: shows the various model with their R^2 values.

S.No.	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer Peppas (R^2)	Hixson-Crowell (R^2)
F-1	0.8872	0.8800	0.9135	0.9107	0.8845
F-2	0.8687	0.8521	0.8945	0.9072	0.8264
F-3	0.9264	0.9114	0.9204	0.8963	0.9443
F-4	0.8845	0.9324	0.9148	0.9246	0.8352
F-5	0.8789	0.8645	0.9126	0.9153	0.8794
F-6	0.9124	0.8745	0.9248	0.8817	0.8871
F-7	0.8652	0.8654	0.8948	0.9451	0.8438
F-8	0.8851	0.8215	0.9115	0.9188	0.8638
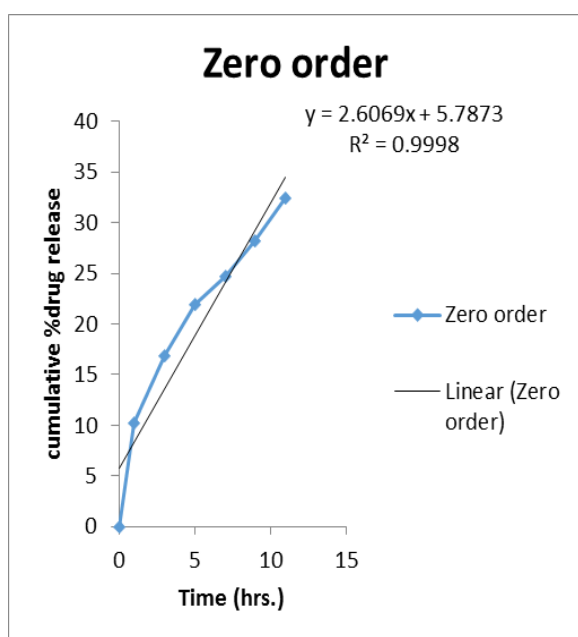
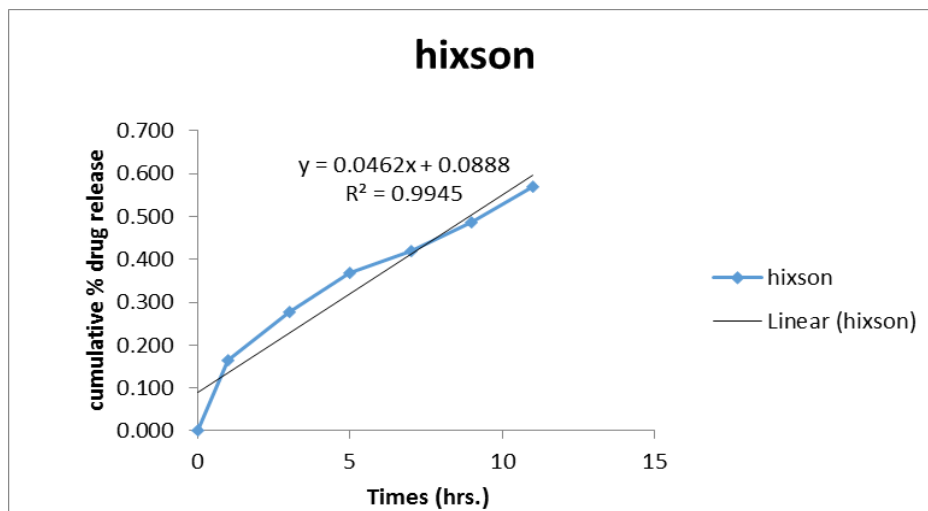Fig: shows zero order model   Fig: first order model
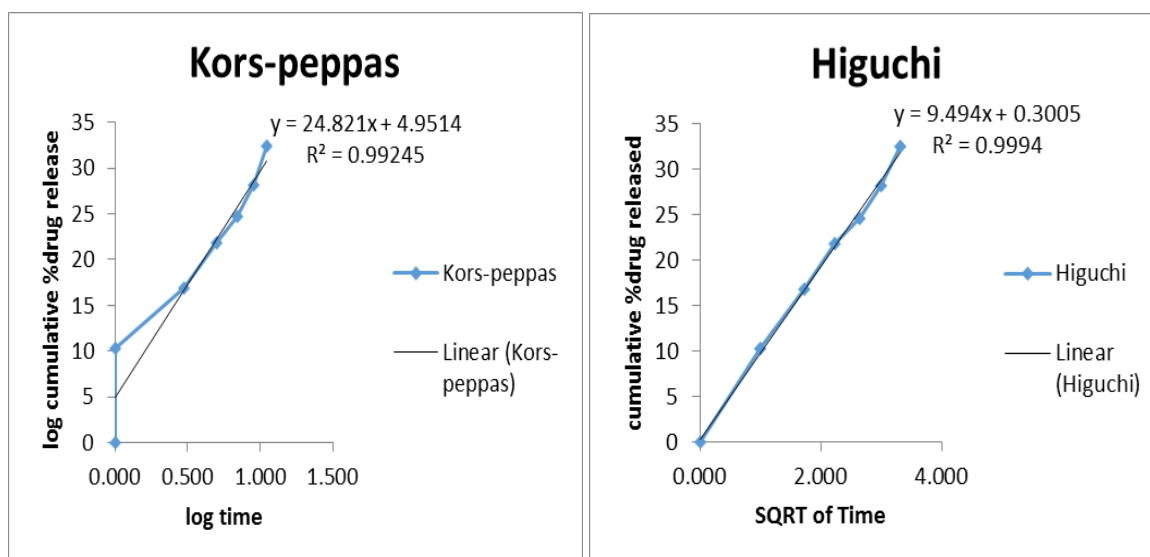
Fig: Hixson Crowell model.

Fig: Korsmeyer-Peppas model.   **Fig: Higuchi model.**

SUMMARY AND CONCLUSION

In the present study, we have successfully developed formulation of Gastroretentive floating microspheres of Amiloride HCl incorporated with sustained release polymer HPMC K-100 & PVPK-30 grade used as excipients for the treatment of hypertension and antidiuretics. We used as our polymer Ethyl cellulose to form the microspheres, HPMC K-100 is used as a formulation in sustained release polymer with ethanol and water are used as a formulation of microspheres were described method prepared by using a methods solvent evaporation method. We performed analytical techniques to evaluate the purify of the drug and by all the results from these techniques, the drug obtained from company was found to be pure. Furthermore, evaluation of microspheres formulation was done; in vitro drug release, drug

entrapment, and kinetic release of microspheres formulation was also performed. All the following works are done during formulation was also performed.

The obtained consequences evidence revealed that potential utilization of microspheres with effacious delivery of HPMC K-100 microspheres for intervention of various diuretic and hypertension related problem. The floating microsphere drug delivery system which is beneficially for the sustained release vital, physical hindrance to reach targeted cell. Among them various techniques, HPMC K-100 listed as much comfortable for sustained release delivery, with evidence of favourable biological properties of HPMC K-100 & Sodium Bicarbonate used as effervescent. Most of the previous investigation clearly indicated that microspheres filled as pellets in capsules shell travelled in to gastric media more freely as compared to various microsphere preparation. Several studies have shown that microspheres can transport across gastric media more readily than tablets. Therefore, types of this properties of microspheres is extensively advantages for drug, gastric medium, as well as that compound having acid hate properties molecules with low transported capacity in acidic medium in stomach.

Previously HPMC K-100 based microspheres of hypertension and diuretic drug has been reported to deliver the drug to acidic medium successfully. The various formulation treated with Amiloride HCl A microspheres prepared by using sustained polymers had significantly higher acidic and basic medium drug levels than those treated with tablets of Amiloride HCl. Based on these considerations, HPMC K-100 was selected as a polymer for the development of microspheres for the present investigation.

Amiloride HCl provides diuretic and antihypertensive activity (principally due to the hydrochlorothiazide component), while acting through the amiloride components to prevent excessive potassium loss that may occur in patients receiving a thiazide diuretic. Due to this latter component, the urinary excretion of magnesium is less. The onset of the diuretic action of amiloride microsphere is within 1 to 2 hours and this action appears to be sustained release for approximately 24 hours.

The oral application of Amiloride HCl, now commercially available as tablets and oral suspension, is limited by the poor bioavailability. Based on these factors the present study attempted to develop sustained release formulation of HPMC K-100 with ethyl cellulose

Amiloride HCl effective floating microsphere delivery of the drug with improved bioavailability.

Microspheres are prepared using various method, including the solvent evaporation method, which is simple to operate and can be used to optimize the particle size of drug that can cross the gastric medium.

During the preformulation studies of the drug sample was found to be yellow color, with bitter, unpleasant taste and smell like a mildy aromatic. It has an average melting point is 241℃, Drug and excipient study was performed using UV visible spectra. The solubility studies reflect that the drug has good solubility in 0.5 mg/ml in a 1:1 solution of DMSO: phosphate Buffer (PH 7.2), solubility in water 0.52 g/100 ml: in alcohol 1.96 g/100 ml at 25℃, practically insoluble in ether.

The standard curve of Amiloride HCl drug was prepared at UV absorption maximum phosphate buffer 7.4, The method used for the estimation of drug followed Beer Lambert's law in the concentration range 0.5 µg/ml with good accuracy, and this is evident from the regression coefficient obtained from the calibration curve.

The current development of Amiloride HCl microspheres formulations have shown all the pH range with in the acceptable criteria i.e., (6.4-7.4). This pH, ranges indicated that, the formulated product may not proceed any harmful irritation when administered. Investigation also revealed that with increasing the quantity of chitosan, vice versa in pH of formulation.

From the FTIR spectral analysis study, it was found that FTIR spectrum of pure Drug and combination of pure drug with polymer like HPMC K-100 and prepared microspheres showed all the characteristic peaks of Amiloride HCl confirming the physical and chemical compatibility of the pure drug and polymer.
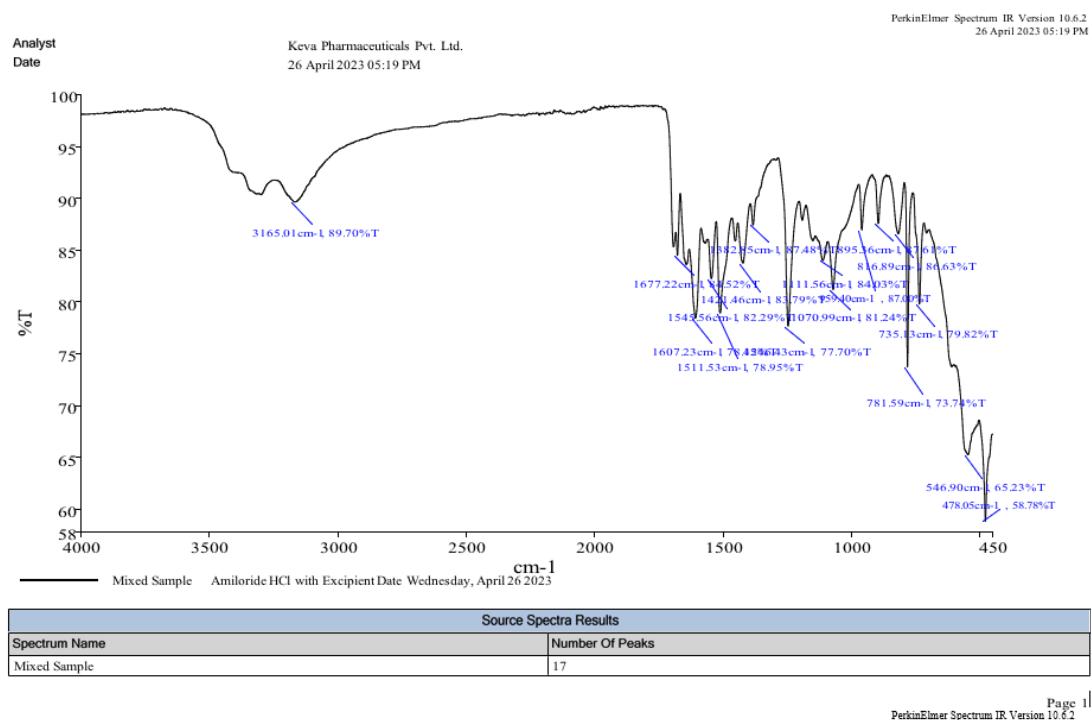
Fig: Shows FTIR Spectra of mixed drug & Excipients.

From FTIR study, it was pure drug Amiloride HCL & combination of HPMC K-100 with polymeric agent such as HPMC-100 sustained release formulation with ethyl cellulose prepared microspheres showed all the characteristic peaks of Amiloride HCl confirming the physical and chemical compatibility of the pure drug and polymer.

The in-vitro diffusion of HPMC K-100 sustained release polymer Amiloride HCl drugs from the Gastroretentive floating microspheres was studied via monitoring drug leakage till 24hrs. Amiloride HCl release from Gastroretentive Floating microspheres have an initial rapid release followed by a sustained release over a period of 24 hours. The sustained release may be due to the release of the drug in Gastroretentive floating the surface of the stomach, and the subsequent slow release of Amiloride HCl from the sustained release forming microsphere may be due to the release of the drug from the microspheres in the floating of the stomach.

The cumulative percentage drug released for f1, F2, F3, f4, F5 up to f8 after 24hrs was 83.72%, 83.80%, 94.71%, 94.71%, up to 92.25% respectively. Maximum drug release was found in F-3 (94.71%) and minimum was found in F-8 (92.25%). Among all the formulations F-3 was selected as an optimized formulation due to its desirable drug release during 24hrs.

In order to develop the release mechanism of present drug delivery system, the release data of prepared Amiloride HCl microsphere forming HPMC K-100 Sustained release polymer in phosphate buffer (pH 7.4) were compared to classic drug release kinetics models. The release rate was analysed at least square linear regression method. Release models such as zero order, first order model, Higuchi model and Ritger-peppas, Hexson Crowell models (Table) were applied to the release data.

The coefficient determination (R^2) for equation for release of Drug from drug forming sustained release microsphere (F-1, F-2, F-3, F-4 up to F-8) in phosphate buffer were 0.9135,0.8945,0.9204,0.9148, and up to 0. 9115 signifying Higuchi order release pattern and the value of coefficient of determination (R^2) in Korsmeyer peppas equation were found to be 0.9107,0.9072,0.8963,0.9246 up to 0.9188 which indicates the integrity of Amiloride HCl microspheres and sustained release polymer HPMC K-100.Substituting the release values in Korsmeyer-peppas equation, the value of coefficient of determination was found to be 0.9135,0.8945,0.9204,0.9148 and 0.9115.

FUTURE PERSPECTIVES

Scientists have tried to study the use of Amiloride and other drugs in treatment of high blood pressure or swelling due to heart failure or cirrhosis of the liver. Amiloride is classified as potassium-sparing diuretic via direct oral drug preparation to the floating microspheres of the G.I. tract. However, repeated administration of Amiloride using this method is a limitation which has to be addressed. It is a duty for researchers to employ the broad knowledge and understanding of nanotechnology in developing a suitable method of administration of Gastro retentive floating microspheres preparation which does not require invasion especially in hypertension and diuretic diseases patients and release the floating microspheres surrounding the G.I. membranes are already compromised. This will directly increase patience compliance; it will improve self-administration which is a measure to reduce hospital visits by the patients.

REFRENCES

1. Himanshu Bhusan Samal, Suddhasattya Dey, Dhiraj kumar, D Sandeep Kumar, S.A. Sreenivas and V. Rahul, "Formulation, Characterization and In-Vitro Evalution of Floating Microspheres of Nateglinide", International Journal of Pharma and Bio Sciences, 2011; 2(1): 147-156.

2. Neha Narang, "An Updated Review On: Floating Drug Delivery System", *Int J App Pharm*, 2011; 3(1): 1-7.

3. Monica Kawatra, Upendra Jain, Jaspreet Ramana, "Recent Advances in Floating Microspheres as Gastro-Retentive Drug Delivery System: A Review", International Journal of Recent Advances in Pharmaceutical Research, 2012; 2(3): 5-23.

4. Pandey Manisha, Singh Bandana, Kanoujia Jovita, Saraf Shubhini A., "Formulation and Evaluation of Floating Microspheres of Famotidine", International Journal of PharmTech Research, 2010; 2(2): 1415-1420.

5. Pavan Kumar Perumandla, Sree Priya, "Formulation And In Vitro Evaluation Of Floating Microspheres Of Dextromethorphan Hydrobromide", International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(4): 206-210.

6. Rajkumar K, Sainath Goud R, Sai Sowjanya P, Anusha P, Lavanya Adavi S, E.R Reddy, "Floating Microspheres: A Novel approach In Drug Delivery", Journal Of Drug Delivery Research, 2012; 1(4): 1-20.

7. Ramya Shivani B. and Krishna Sailaja A., "Preparation and Evaluation Of Floating Microspheres Of Omeprazole Microspheres By Solvent Evaporation Method", International Journal of Basic and Applied Chemical Sciences, 2015; 5(3): 67-78.

8. A Porwal G Swami, and SA Saraf Preparation and evaluation of sustained release microballoons of propranolol Daru., 2011; 19(3): 193–201.

9. Abhijeet A. Durgavale, Archana R. Dhole,Shrinivas K. Mohite, Chandrakant S. Magdum. Formulation and evaluation of floating microsphere of Captopril using different gas forming agents. Am. J. Pharm. Tech. Res., 2012; 2(2): 56-63.

10. Ali J, Arora S, Khar RK. ; Floating Drug Delivery System: A Review; AAPS Pharm Sci Tech., 2005; 06(03): E372-E390.

11. Ankur raj Sharma, Afroz khan Gastroretentive Drug Delivery System: An Approach to Enhance Gastric Retention for Prolonged Drug Release IJPSR, 2014; 5(4): 1095-1106.

12. Arora S, Javed A, Ahuja A, Khar RK, Baboota S; Floating Drug Delivery System: AReview; AAPS Pharm Sci Tech, 2000; 6(3): 372-390.

13. Aulton, M.E., Pharmaceutics: The science of dosage form design, 3rd Eds., English Language Book Society, Churchill/livingstone, 1990; 610–612.

14. Awasthi R., Kulkarni GT. Development and characterization of amoxicillin loaded floating microballoons for the treatment of Helicobacter pylori induced gastric ulcer. Asian J. of pharma. Sci., 2013; 8: 174-180.

15. Babu V. B. M., Khar R. K., "In vitro and In vivo studies of sustained release floating dosage forms containing salbutamol sulphate", Pharmazie, 1990; 45: 268-270.

16. Badve, S.S., Sher, P., Korde, A., Pawar, A.P. Development of hollow/porous calcium pectinate beads for floating-pulsatile drug delivery. Eur. J. Pharm. Biopharm, 2007; 65: 85–93.

17. Bansal H, Kaur SP, Gupta AK. Microspheres: Methods of preparation and applications: A comparative study. Int. J. Pharm. Sci. Rev. Res., 2011; 10: 69-78.

18. .Banker, G.S., Lachman, L., Libermann, A. The theory and practice of Industrial Pharmacy, Tablets. 3 rd Eds., Bombay, Varghese Publishing House, 1991; 293–295.

19. Caldwell LJ, Gardner CR, Cargill RC. Drug delivery device which can be retained in the stomach for controlled period of time. US Patent, April 5, 1988; 473 5804: 49.

20. Remington LA, Goodwin D. Clinical Anatomy and Physiology of the Visual System E-Book. Elsevier Health Sciences; 2021 Jun 25.

21. DIVYA A. FORMULATION OF NANOEMULSIONS FOR THE ENHANCEMENT OF BIOAVAILABILITY OF SOME DRUGS 2020.

22. Bandamwar KL. Mechanism and clinical significance of superficial micropunctate fluorescein staining of the cornea (Doctoral dissertation, University of New South Wales).

23. Varela-Fernandez R, Diaz-Tomé V, Luaces-Rodríguez A, Conde-Penedo A, García-Otero X, Luzardo-Álvarez A, Fernández-Ferreiro A, Otero-Espinar FJ. Drug delivery to the posterior segment of the eye: Biopharmaceutic and pharmacokinetic considerations. Pharmaceutics, Mar, 2020; 12(3): 269.

24. Manzari MT, Shamay Y, Kiguchi H, Rosen N, Scaltriti M, Heller DA. Targeted drug delivery strategies for precision medicines. Nature Reviews Materials, Apr, 2021; 6(4): 351-70.

25. Tripathi KD. Essential of Medical Pharmacology, Jay Pee brother's medical publishers (P) Ltd., 2008; 479-590.

26. Robertson, RM: Robertson: Harman, JG: Limbird, LE: Molin off, PE: Ruddon, RW and Gilman, LE: Molin off, PE; Ruddon, RW and Gilman, AG, "Goodman and Gilman's The pharmacological Basis of Therapeutics", MeGraw Hill, New York, 1996; 9: 751.

27. Silaja AK, Anusha K, Jyothika M. Biomedical applications of microspheres. J Modern Drug Discovery Drug Delivery Res., 2015; 4: 1-5.

28. Yadav R, Bhowmick M. Rathi V, Rath J, Design and characterization of floating microspheres for rheumatoid arthritis, Journal of Drug Delivery and Therapeutics, 2019; 9(2-S): 76-81.

29. Sreeharsha N, Rajpoot K, Tekade M, Kalyane D, Nair AB, Venugopala KN, Tekade RK. Development of metronidazole loaded chitosan nanoparticles using QbD approach—A novel and potential antibacterial formulation. Pharmaceutics, Oct, 2020; 12(10): 920.

30. Madhusudan A.Reddy B, Venkatesham M, Veerabhadam G. Design and evaluation of efavirenz loaded solid lipid nanoparticles to improve the oral bioavailability, Int J pharm Sci., 2012; 2(4): 84-89.

31. Katat L, Tshweu L, Naidoo S, Kalombo L, Swai H. Design and Formulation of Nano-ionized spray dried efavirenz –part I: Influence of formulation parameters Nanoparticles Res., 2012; 14(11).

32. Sahil K. Akanksha M, Premjeet S. Bilandi A, Kapoor Microsphere: A review.Int.J. Res. Pharm. Chem., 2011; 1(11): 84-98.

33. James S. Encyclopaedia of Pharmaceutical Technology, 3rd edition, 1999; 1325-133.

34. Sinha V.R. and Trehan A. Biodegradable microsphere for protein delivery Control. Res, 2003; **90**: 261-80.

35. Brahamankar D.M, Jaisawal S.B, Biopharmaceutics and Pharmacokinetics a treatise, 1st ed. Vallabh prakasan; New Delhi, 1995; 64-70.

36. Ansel, Pharmaceutical Dosage form and Drug Delivery System, Lippincott, 7th edition, 553.

37. Mitra SB. Sustained –Release Oral Medicinal Delivery Device. US patent, May 1984; 4: 451,260.

38. Kwashima Y, Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharm Sci., 1992; 81: 135-40.

39. Hawang SJ, Shree GJ, Lee KM, Oh KH, Kim CK. Release characteristic of ibuprofen from excipients-loaded alginate gel beads. Int J Pharm., 1995; 116: 125-8.

40. S. Sharma, P. Sher P, S. Badve, P.P. Atmaram, AAPS Pharm SciTech., **2005; 6: E618**.

41. "Targeted and Controlled Drug Delivery Novel Carrier System "by S. P Vyas and R.K. khar, **2002;** 417-457.

42. Patel Samir Kumar, Tara Chand and Talsania Maulik. International Journal of Research in pharmaceutical and Biomedical Sciences, **2013;** 4(2): 568-572**.**

43. Emami J, Tajeddin M, Ahmadi F, preparation & In-Vitro Evaluation of Sustained Release Matrix Tablets of Flutamide using synthetic and natural occurring polymers, IJPSR, 2008; 7: 247-257.

44. Raghavendra rao N.G, Gandhi sagar, patel tarun, Formulation and evaluation of sustained Release Matrix Tablets of Tramadol HCl, IJPPS, 2009; 1: 60-70.

45. Jain Swati, Neeleash Kumar, Singhai A.K et al, Development and Evaluation Sustained Release Matrix tablets using prunus American L. Gum as a release retardant, JAPER, 2013; 3: 206-216.

46. Approved Drug products with Therapeutic Equivalence Evaluations, 6t^h Ed., U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Center for Drugs and Biologics, Rockville, Md., 1985; 1-2.

47. Rathananand Mahalaxmi, Kumar DS, Shirwaikar A, Kumar Ravi, Sampath kumar D, Prasad RS. Preparation of mucoadhesive for nasal delivery by spray drying Intd.J. Pharm, Sciences, 2007; 69,5: 651-65.

48. Food and drug administration (FDA), Division of Biopharmaceutics, Bioavailability protocol guidelines for ANDA and NDA Submission, 1977.

49. Garg, R., Gupta, G.D. Progress in Controlled Gastroretentive Delivery Systems, Trop.J. Pharma. Res., 2008; 7(3): 1055-1066.

50. Stockwell AF, Davis SS, Walker SE. In vitro evaluation of alginate gel systems as sustained release drug delivery systems", J. control, release, 1986; 3: 167-175.

51. Legrand E. Acclofenac in the management of inflammatory pain. Exp Opin Pharmacother, 2004; 5: 1347-57

52. Whitehead H, Fell JT, Collett JH. Development of a Gastroretentive Dosage Form, European Journal of Pharmaceutical Sciences, 1996; 4(1): 182-186.

53. Ravi Kumar, Swati Patil, M. B. Patil, SacD R. Patil, Mahesh S. Paschapur," Design and Invitro Evaluation of Oral Floating Matrix Tablets of Acclofenac", International Journal of ChemTech Research CODEN(USA): IJCRGG ISSN: 0974-4290., Oct-Dec, 2009; 1(4): 815-825.

54. Krogel I, Bodmeier R. Development of a multifunctional matrix drug delivery system surrounded by an impermeable cylinder. J Contr Rel., 1999; 61: 43-50.

55. Mathur P, Saroha K, Syan N, Verma S, Kumar V. Floating drug delivery system: An innovative acceptable approach in Gastroretentive drug delivery, Archives of Applied Science Research, 2010; 2(2): 257-270.

56. Sakhare SS, Yadav AV, Jadhav PD, Design, development and characterization of mucoadhesive gastro spheres of carvedilol. Int J App Pharm., 2016: 8: 37-42.

57. Durga pal S, Mukherjee S, Goswami L., Perpetration, characterization of and Evaluation of floating microparticles of ciprofloxacin J Appl pharm, 2017; 9: 1-8.

58. Baveja K, Ranga Rao KV, Kumar Y. Microencapsulation of soluble pharmaceuticals Microencapsule, 1986; 3: 33-7.

59. Ramo S, Haapalainen M, Latvala S. Development and Validation of a UHPLCMS/MS Method for the Analysis of Fusarium Mycotoxins in Onion. Food analytical methods, Aug, 2021; 14(8): 1524-36

60. www.drug.com

61. www.sciencedirect.com

62. www.google.com