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Formulation and Evaluation of Gastroretentive Floating Microspheres of Amiloride Hydrochloride

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Abstract

Background: Amiloride, a potassium-sparing diuretic, is widely prescribed for the management of hypertension, congestive heart failure, and various renal disorders. However, its low oral bioavailability due to extensive first-pass metabolism and short half-life necessitates frequent dosing, leading to patient inconvenience and compromised therapeutic outcomes. To address these limitations, the development of novel drug delivery systems capable of enhanced gastrointestinal retention of amiloride has garnered significant attention. Among these, gastroretentive microspheres represent a promising approach, offering prolonged drug release and improved absorption. **Objectives:** The present work by formulation and evaluation of gastroretentive floating microsphere (FM) plays a highly significant role as a 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, micro-balloons, 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. **Discussion:** Hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone, and ethylcellulose were used in varying concentrations to give the FMs of amiloride HCl release-controlling properties by increasing their bioavailability. Lactose was used as a diluent and sodium bicarbonate served as an effervescent agent. Using a solvent evaporation method approach, the gastroretentive FM of amiloride HCl was created. The generated microsphere indicated good floating strength and remained buoyant in the sustained released medium for 24 h. For systemic delivery of amiloride, a potassium-sparing diuretic and antihypertensive medication, through the oral route, a gastroretentive FMs drug delivery system was developed. The different ratios of ethylcellulose and HPMC K-100, sodium lauryl sulfate, sodium bicarbonate, and ethanol are used in formulation. 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. **Conclusion:** The formulation and evaluation of gastroretentive FMs represent a significant advancement in particulate drug delivery systems. These microspheres, with particle sizes ranging from 0.1 to 200 μm, offer versatility in administration.

Keywords: Amiloride HCL, Floating microspheres, Hydroxypropyl methylcellulose k-100

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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. 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, which divides active pharmaceutical ingredients for oral administration into four groups and is also illustrated in Figure 1:

- Class I: High solubility and high permeability
- Class II: Low solubility and high permeability
- Class III: High solubility and low permeability
- Class IV: Low solubility and low permeability.

Introduction to Microspheres

Microspheres are tiny 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

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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. Methods are also shown in figure 2.

Advantages of microspheres as follows:

- 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 flavor
- Reduce the core's reactivity in regard to its surroundings outside of it
- Protects the gastrointestinal tract 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.[2]

Materials and Methods

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. Name of materials and their sources are mentioned in Table 1.

Instruments/Equipment

Standard calibrated and validated laboratory equipments were used to prepare and analyze the microspheres. Homogenizer was used for dispersion and solvent was evaporated after proper homogenization. Different instruments used for evaluation and analysis are mentioned in Table 2.

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 should be established before 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.

Table 1: 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	
9	Sodium phosphate dibasic	
10	Ethylcellulose	

Table 2: Instruments used in this study with their supplier's name

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	Spectralab
10	Fourier transfer infrared spectroscopy	Perkin Elmer technologies
11	Ultraviolet spectroscopy	Perkin Elmer lambda 360
13	Bulk density apparatus	Simtornics

Table 3: The physical properties of drug and identification

S. No.	*Specification*	*Confirm to specification*
1.	Color	Yellow
2.	Odor	Unpleasant
3.	Appearance	Pale yellow to greenish yellow
4.	Taste	Bitter

Table 4: Melting points of drug

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

Table 5: Solubility profile

S. No.	*Descriptive term*	*Parts of solvent required for one part of solute*
1.	Very soluble	<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

Table 6: Design of expert for various formulations

Formulation No.	*HPMC K-100*	*Ethylcellulose*	*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

Table 7: 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
Ethylcellulose (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
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	120.4 9	120.49	120.49	120.49	120.49	120.49
Average filled wt. of caps. (mg)	602.47	602.47	602.47	602.47	602.47	602.47	602.47	602.47

Table 8: The absorption of calibration curve

S. No.	*Conc. mcg/mL*	*Abs. of 1st reading*	*Abs. of 2nd reading*	*Abs. of 3rd reading*	*Mean value of all reading*	*SD*
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

Table 9: 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.

Table 10: 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

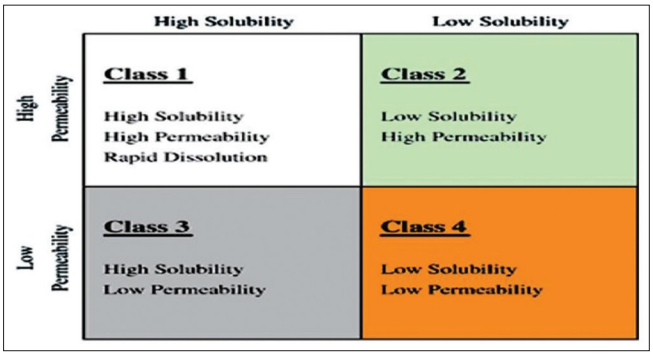

Figure 1: Biopharmaceutical classification system

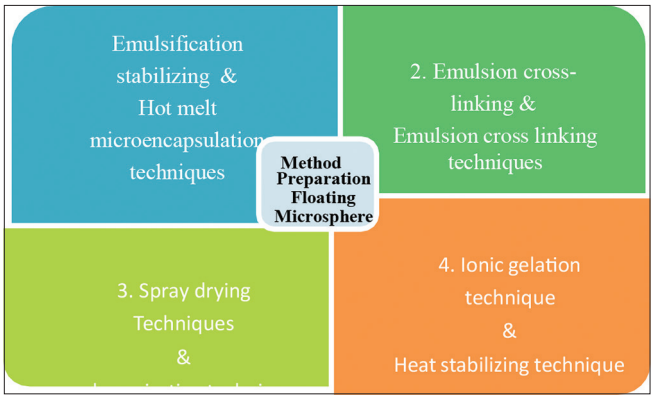

Figure 2: Various methods of preparation of floating microspheres

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 mentioned in Table 3.

Determination of Melting Point

Determination of melting point is a method applied to 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 2–3 mm with an internal diameter of capillary 1 mm 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 is determined and obtained result is depicted on table 4.

Solubility

In aqueous buffers, amiloride (hydrochloride) (hydrate) is only weakly soluble. Amiloride (hydrochloride) (hydrate) should be first dissolved in dimethyl sulfoxide (DMSO) and then diluted with the preferred aqueous buffer for maximum solubility in

Table 11: 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	NH2 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
11.	1205–1124	1114.41	Strong	C-O Stretching	Tertiary Alcohol
12.	1205–1124	1071.21	Strong	C-O Stretching	Tertiary Alcohol
13.	980–935	950.35	Strong	C-C bending	Alkene
14.	850–550	895.25	Strong	C-Cl Stretching	Halo Compound
15.	850–550	815.89	Strong	C-Cl Stretching	Halo Compound
16.	800–550	816.72	Strong	C-Cl Stretching	Halo Compound
17.	790–665	781.43	Strong	C=C Stretching	Alkene

Table 12: 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

Table 13: FTIR functional group of HPMC K-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

Table 14: FTIR functional group of sodium bicarbonate

S. No.	Range	Result	Appearance	Group	Compound
1.	1660–1566	1654.84	Medium	C-C Stretching	Cyclic Alkene
2.	1310–1280	1271.32	Strong	C-O Stretching	Aromatic ester
3.	990–960	985.74	Strong	C-C bending	Alkene
4.	850–550	829.82	Strong	C-Cl Stretching	Halo compound
5.	730–665	678.94	Strong	C=C Stretching	Alkene

Table 15: FTIR functional group of sodium lauryl sulfate

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
7.	1310–1250	1276.88	Strong	C-O Stretching	Aromatic ester
8.	1205–1124	1159.22	Strong	C-O Stretching	Tertiary Alcohol
9.	980–960	970.19	Strong	C-C bending	Alkene
10.	850–550	795.10	Strong	C-Cl Stretching	Halo compound
11.	730–665	678.94	Strong	C=C Stretching	Alkene

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. Solubility profile is mentioned in table 6.

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 an ultraviolet (UV) spectrophotometer between 200

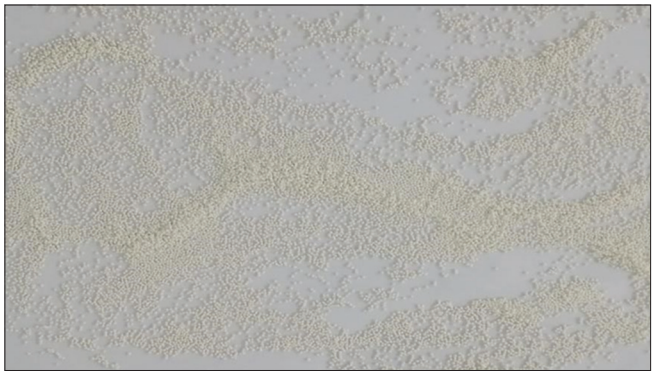

Figure 3: Amiloride HCl with hydroxypropyl methyl cellulose K-100 drug floating microspheres

Figure 4: Amiloride HCl drug with floating microspheres

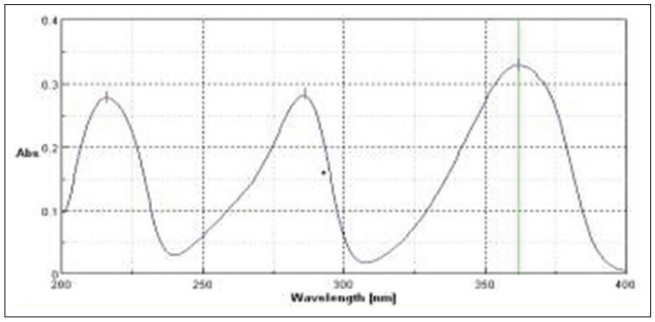

Figure 5: Ultraviolet absorption spectrum of amiloride RS in methanol with absorption maximum at 362 nm

Figure 6: Bulk and tapped density apparatus

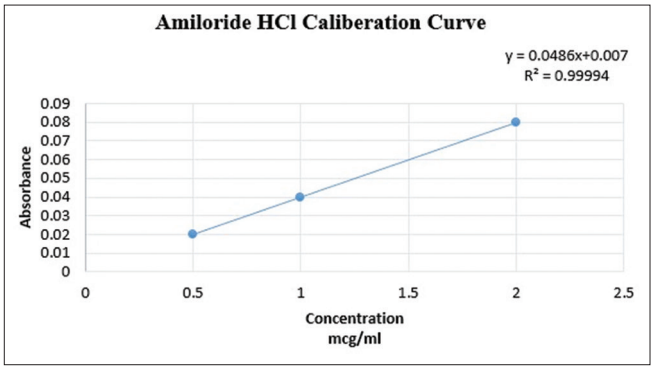

Figure 7: Linearity curve of amiloride HCl

Figure 8: Dissolution apparatus USP (Paddle method)

Table 16: Entrapment efficacy of the drug entrapment of the drug

Formulation	*Abs. (supernatant)*	*Conc.*	*% Entrapment Efficacy*
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 17: Loading capacity of the drug

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

and 600 nm. Amiloride HCl's maximum absorbance in methanol was measured at a wavelength of 362 nm (Bhowmick *et al*., 2012).[3]

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 (Bhowmick*et al*., 2019).

Table 18: 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

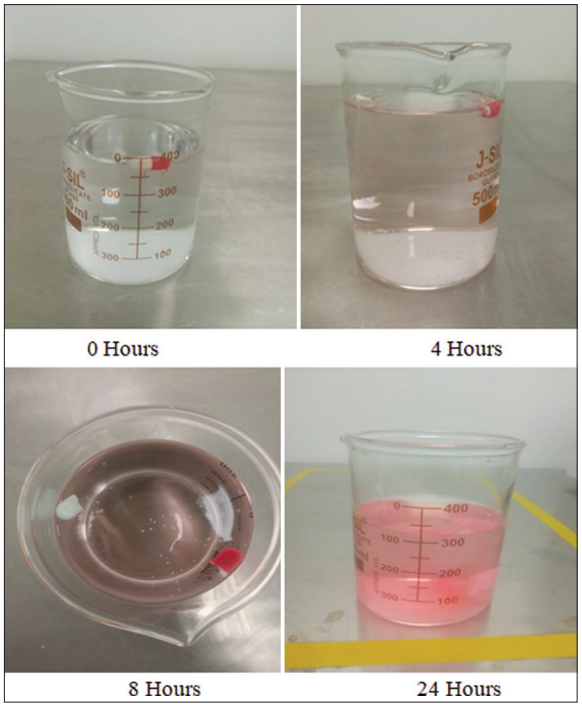

Figure 9: *In vitro* buoyancy study of amiloride hydrochloride floating

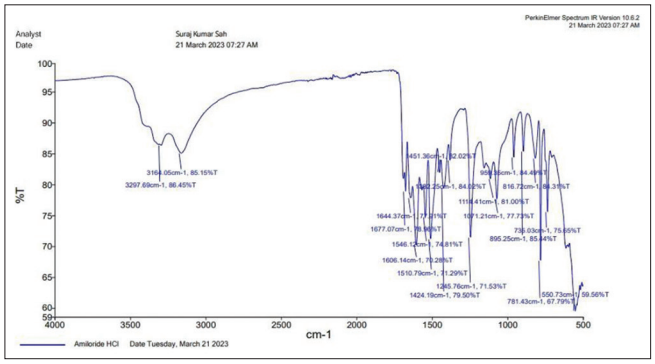

Figure 10: Fourier transfer infrared spectrum of pure drug

Standard Solution

1. Stock solutions of 500 or 250 mg/L were made 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-standard 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).

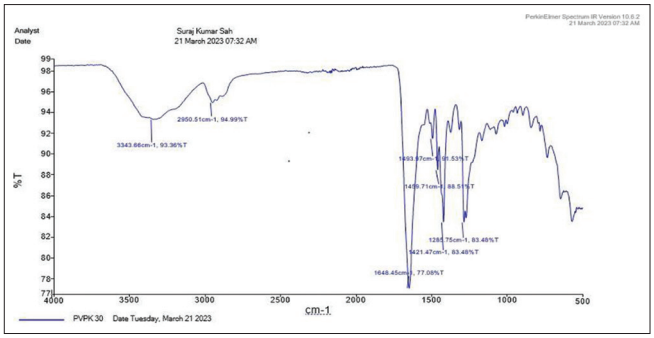

Figure 11: Fourier transfer infrared spectra of PVPK-30 excipients

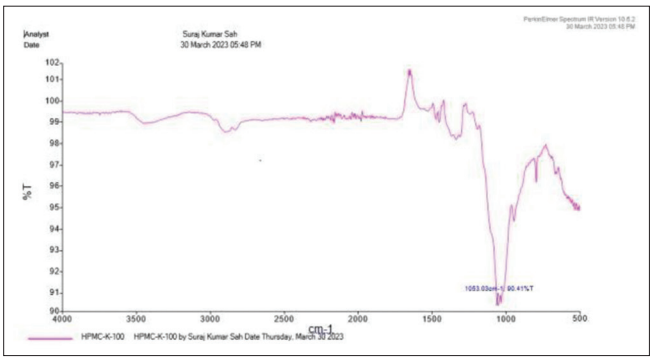

Figure 12: Fourier transfer infrared spectra of HPMC K-100 polymer

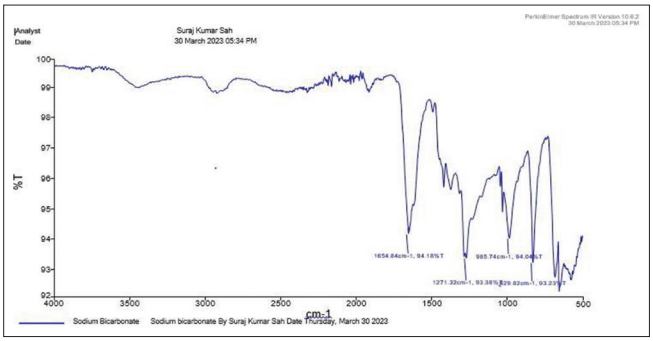

Figure 13: Fourier transfer infrared spectra of sodium bicarbonate

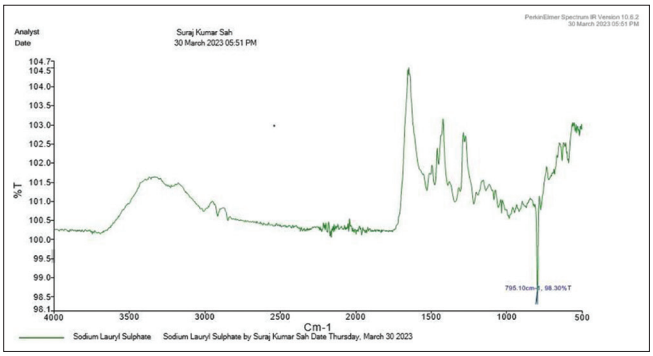

Figure 14: Fourier transfer infrared spectra of sodium lauryl sulfate

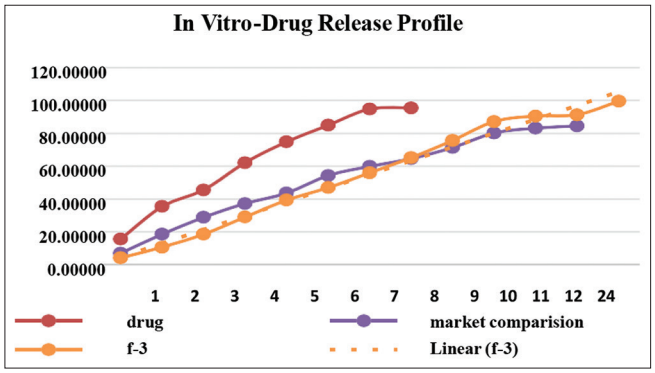

Figure 15: *In vitro* drug release of all

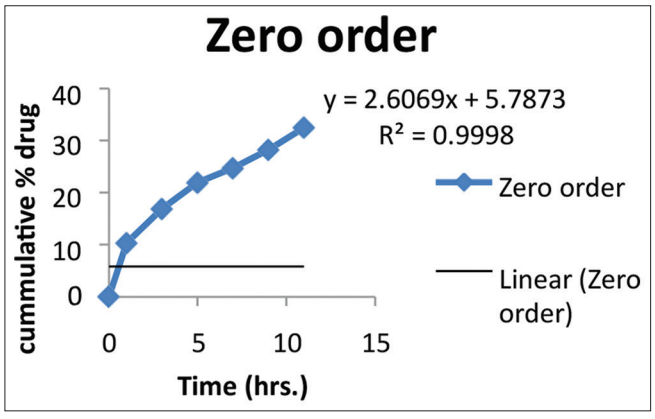

Figure 16: Zero-order model

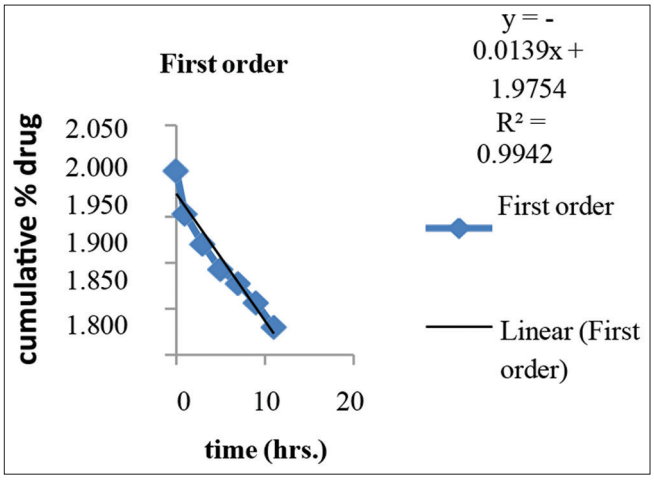

Figure 17: First-order model

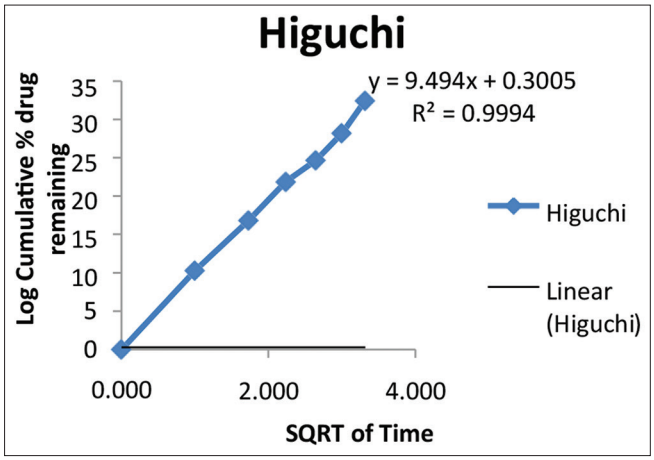

Figure 18: Higuchi model

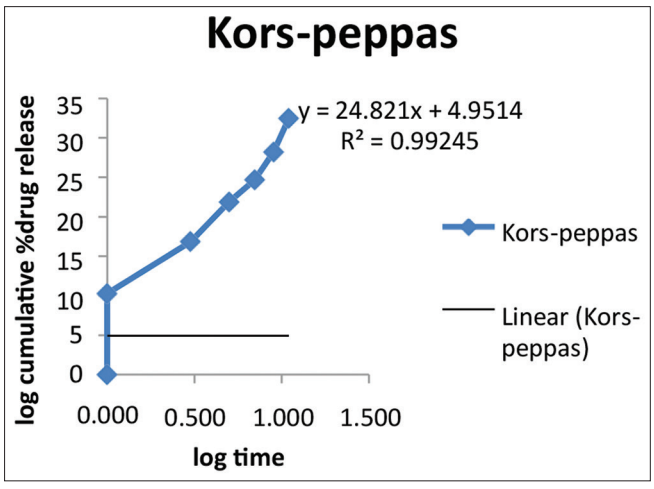

Figure 19: Korsmeyer–Peppas model

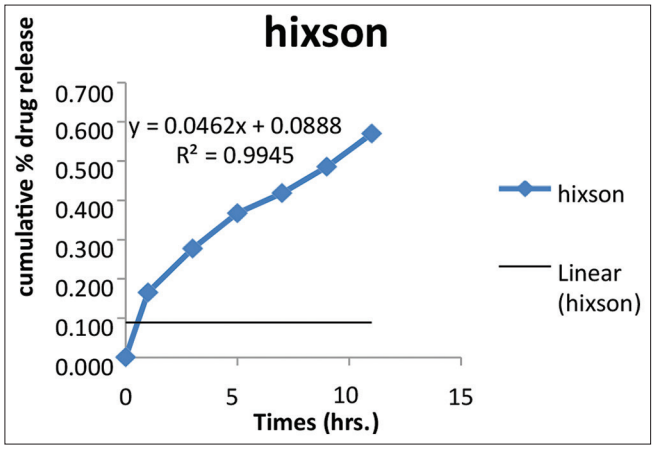

Figure 20: Hixson–Crowell model

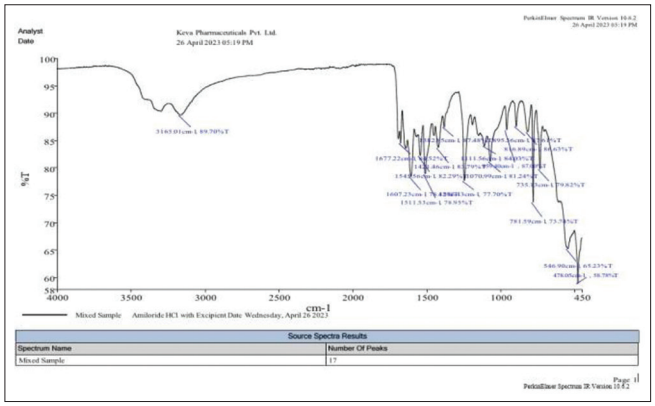

Figure 21: Fourier transfer infrared spectra of mixed drug and excipients

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 *et al*., 2008). 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.

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 (Sunil *et al*., 2020).[5] The calibration curve observed as per table 8 is shown in figure 7.

Preparation of Gastroretentive Floating Microspheres (FMs) of Amiloride HCl

Gastroretentive FMs 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 FMs. 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 (Nagiat *et al*., 2013).[7,8]

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 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 evaporation of the solvent from the dispersed phase, solid polymeric microparticles (containing drug) are generated. Filtration, centrifugation, or lyophilization is used for extracting the solid microparticles from the suspension.

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 min, blank correction was done using methanol. Then, 10 μg/mL solution of amiloride hydrochloride was scanned separately in UV region ranging from 200 nm to 400 nm. The absorption spectra were observed with maximum absorption at 362 nm for furosemide and amiloride hydrochloride, respectively.

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 using equations and respectively (Sreeharsha *et al*., 2020). Figure 6 shows bulk and tapped density apparatus used for this parameter testing.

Table 19: Various models 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

Materials

Amiloride HCl (AB), PVPK-30, and hydroxypropyl methylcellulose (HPMC K-100M) were provided by National Health Care Pvt. Ltd., Nepal. All solvents used were of analytical grades and were used as obtained.

Preparation of AB Microspheres

AB microspheres were prepared based on the solvent evaporation technique. Different batches of AB microspheres, F1 to F8, were prepared by varying the concentration of ethylcellulose polymer in the formulation from 1.00 g. respectively. 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 into 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 ethylcellulose were optimized based on the % drug release and % entrapment efficiency.

Finally, microspheres were 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 (Nagiat *et al*., 2013). Figure 3 and 4 depict the floating microshperes observed after preparation.

Results and Discussion

Amiloride hydrochloride inhibits 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 prevent the excretion of potassium and H+ into the lumen. Amiloride HCl is used for its potassium-sparing effect in the treatment or prevention of hypokalemia 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 kaliuretic-diuretic agents in congestive heart failure or hypertension. Floating drug delivery microspheres were being formulated and the present study focused on the formulation of floating drug delivery system (FDDS) using different polymers such as HPMC K100, ethylcellulose and Binder PVPK30, sodium bicarbonate used as disintegrating agent and to evaluate its efficacy in treat BP and edema. The floating drug delivery microsphere was 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 FDDS 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. Infrared (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, and thus, the excipients are compatible with the drug. Lower values of angle of repose below 30 indicate good flow properties of microsphere. All prepared microspheres 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 h. The drug release data were explored for the type of release mechanism followed. Loading capacity of drug was listed in table 17. The best fit with highest determination R2 coefficient was shown by zero-order model. Drug content, physical appearance, and drug release profile of Formulation F3 was observed good.

Evaluation Parameters FMs

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.

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 to maintain the sink condition. At proper nm, the sample was spectrophotometrically analyzed.

Swelling ratio (SR)

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

Swelling studies

A glass vial containing 10 mL of distilled water and 50 mg of microspheres was put in an incubator set to 37°C with occasional shaking. Up until equilibrium had been reached, the microspheres were periodically removed and blotted with filter paper, and their weight changes were measured. After 3 h, the weight of the

swollen microspheres was recorded, and the 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.

Carrs index

It was measured using following formula,

$$\text{Carrs Index} = \{(Vb-Vt)/Vb\} \times 100$$

Where, Vb and Vt 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 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 were 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.

Fourier Transfer IR (FTIR)

The CH-NM was taken and mixed with about 100 mg 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 4000–500 cm^{-1} are described in table11 to table 16. FTIR observations and spectrums after examinations were illustrated in figure 10 to figure 15 and figure 21.

In vitro Release of Drug of Different Formulation

Throughout the study, the USP dissolution apparatus type I, Basket type, as shown in figure 8, 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 was used as the dissolution medium. At a rotational speed of 50 rpm and a temperature of 37± 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 underwent filtering, appropriate dilution, and spectrophotometric analysis at 362 nm. The information displayed represented the average of three findings. The information displayed represented the average of three findings in figure 9.

Formulation Release Kinetics

The release kinetic data of the diffusion profile of the drug from the FM showed fitted with Hixson Crowell of release kinetics mechanism for the drug from the microsphere; the *in vitro* release profile was analyzed by various kinetic models. The kinetic models used were zero order, first order, Higuchi, Korsmeyer and Peppas, and Hixson–Crowell equation. The release 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. To investigate the release mechanism of the 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 analyzed by least square linear regression method. Release models such as zero order, first-order model, Higuchi model and Rigger–Peppas, and Hixson–Crowell model were applied to the release data. Graphs from different models were shown on figure 15 to figure 20.

The F-3 value of coefficient of determination (R^2) in Hixson Crowell was found to be 0.9443 which indicates the integrity of FMs and Sustained release. The value of coefficient of determination was found to be 0.9443 (Christophe *et al*., 2008).

Summary and Conclusion

In the present study, we have successfully developed formulation of gastroretentive FMs of amiloride HCl incorporated with sustained release polymer HPMC K-100 & PVPK-30 grade used as excipients for the treatment of hypertension and antidiuretics. Ethyl cellulose is used for formation of polymer along with HPMC K-100 and water by using solvent evaporation method. We performed analytical techniques to evaluate the purity of the drug and by all the results from these techniques, the drug obtained from company was found to be pure. Furthermore, evaluation of microsphere formulation was done; in vitro drug release, drug entrapment, and kinetic release of microsphere formulation were also performed. During formulations, different activities that were performed are discussed below.

The obtained consequence evidence revealed the potential utilization of microspheres with efficacious delivery of HPMC K-100 microspheres for the intervention of various diuretic and hypertension-related problems. The FM drug delivery system is beneficial for the sustained release of vital, physical hindrance to reaching targeted cell. Among them, various techniques, HPMC K-100, listed as much comfortable for sustained release delivery, with evidence of favorable, 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 traveled into gastric media more freely as compared to various microsphere preparations. Several studies have shown that microspheres can transport across gastric media more readily than tablets. Therefore, types of these properties of microspheres are 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 have been reported to deliver the drug to acidic medium successfully. The various formulations treated with amiloride HCl microspheres prepared 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 1–2 h and this action appears to be sustained release for approximately 24 h.

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 ethylcellulose amiloride HCl effective FM delivery of the drug with improved bioavailability.

Microspheres are prepared using various methods, 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, the drug sample was found to be yellow color, with bitter, unpleasant taste and smell like a mildy aromatic. Melting point of the sample is found to be 241°C. 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°C, 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 has shown all the pH range within 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 quantity of Chitosan is proportional to the pH in the 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.

From FTIR study, it was pure drug amiloride HCL and combination of HPMC K-100 with polymeric agent such as HPMC-100 sustained release formulation with ethylcellulose 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 FMs was studied through monitoring drug leakage till 24 h. Amiloride HCl release from gastroretentive FMs has an initial rapid release followed by a sustained release over a period of 24 h. 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 24 h was 83.72%, 83.80%, 94.71%, 94.71%, up to 92.25%, respectively. The details of drug release were described in table 18. 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 24 h.

To develop the release mechanism of the 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 kinetic models. The release rate was analyzed at least square linear regression method. Release models such as zero order, first order model, Higuchi model, and Korsmeyer–Peppas, Hexson–Crowell models (Table 19) were applied to the release data.

The coefficient determination (R^2) of equation for the release of drug from drug forming sustained release microsphere (F-1, F-2, F-3, F-4 up to F-8) in phosphate buffer was 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 was 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 (Sunil *et al*., 2020).

Future Perspectives

Scientists have tried to study the use of amiloride and other drugs in the treatment of high blood pressure or swelling due to heart failure or cirrhosis of the liver. Amiloride is classified as potassium-sparing diuretic through direct oral drug preparation to the FMs 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 their broad knowledge and understanding of nanotechnology in developing a suitable method of preparation and administration for gastroretentive FMs. These formulations should be non-invasive, especially in hypertension and diuretic diseases patients. Along with this, they should also work for better release of the drug around the already compromised gastrointestinal membranes (Monica et al., 2012).[8] This will directly increase patient compliance; it will improve self-administration which is a measure to reduce hospital visits by the patients.

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