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FORMULATION AND *IN-VITRO* EVALUATION OF MOLNUPIRAVIR GASTRORETENTIVE MICROSPHERES

Y. Ramulu
Sree Dattha Institute of Pharmacy, Sheriguda, Hyderabad, Telangana, India.

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Abstract:
The present study involves formulation and evaluation of sustained release floating microspheres of Molnupiravir sulphate. Endeavour"s with respect to floating mechanism are inculcated in the formulation to achieve longer stay of microsphere in stomach which happen to better site of absorption for the selected drug. Preformulation studies involving organoleptic bulk density, tapped density, angle of repose, compressibility of index, hausner ratio, melting point range, pH, solubility were carried out as per IP specification. Drug excipient compatibilities were carried out and evaluation and FT-IR, SEM. This showed no significant change in any way to the Mixture. Different polymers like sodium alginate, sodium carboxy methyl cellulose, HPMC K4M, HPMC K100M were utilized in the trials. All the physical evaluations are carried in preformulation studies were carried out on all the three different polymers utililized. All the formulations exhibited values withinthe acceptable range. Microspheres were evaluated for buoyancy studies, drug entrapment efficient. Release studies were carried out in 0.1N HCL for 12 hours. Evaluated samples for all the four polymer system. Results indicated that formulation F7, gave 90.12 % release up to 12 hrs which is formulated with HPMC K100M and HPMCK4M combination. Assay was carried out for formulation F7 and was found to be 88.69 %. The mechanism of drug release from microspheresfollows Non-Ficknian release.Remaining formulations gave fluctuating release profiles. The formulation F7 was considered to be better among the trails accomplished.

Keywords: *Formulation Design, Development, In- Vitro Evaluation, Molnupiravir, Gastroretentive, Microspheres*

Corresponding author:
Y. Ramulu,
Assistant Professor
Department of pharmaceutics
Sree dattha institute of pharmacy
Sheriguda, Hyderabad, Telangana.
India. E-mail: ramupharma846@gmail.com

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INTRODUCTION:

The primary aim of oral controlled drug delivery is the most preferable route of drug delivery system is to achieve better bioavailability and release of drug from the system which should be predictable and reproducible, easy for administration, patient compliances and flexibility in formulation for effective therapy or to improve therapeutic efficiency of the drug through improved bioavailability [1, 2, 3]. Gastro retentive dosage forms significantly extend for the period of time, over which drug may be released and thus prolong dosing intervals and increase patient compliance. Gastric retention can be achieved by the mechanism of mucoadhesive or bioadhesion systems, expansion system, high density systems, magnetic systems, super porous hydrogels, raft forming systems, low density system and floating ion exchange resins. Floating drug delivery systems or hydro dynamically balance systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time without affecting the gastric emptying rate. The drug is released slowly at a desired rate from the system and drug residual systems are emptied from the stomach. This results in increase in the gastric residence time and a better control of qualification in plasma drug concentration. [4] Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material [5]. Hollow microspheres, microballoons or floating microparticles are terms used synonymously for floating microspheres. Floating microspheres are, in a strict sense, spherical empty particles without a core. These are free-flowing particles, with size ranging from 1 to 1000µm. Kawashima et al [6]. (1992) have developed non-effervescent hollow polycarbonate microspheres by using an emulsion solvent evaporation method. This gastrointestinal transit-controlled preparation is designed to float on gastric juice with a specific density of less than one. This property results in delayed transit through the stomach. The drug is released slowly at desired rate, resulting in increased gastric retention with reduced fluctuations in plasma drug concentration. Various attempts have been done to retain the dosage form in the stomach as a way of increasing retention time. Floating systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased gastro retention time and reduces fluctuation in plasma drug concentration [7-10].

Molnupiravir (EIDD-2801, MK-4482) is the isopropylester prodrug of N4-hydroxycytidine, With improved oral bioavailability in non-human primates, it is hydrolyzed in vivo, and distributes into tissues where it becomes the active 5'-triphosphate form. The active drug incorporates into the genome of RNA viruses, leading to an accumulation of mutations known as viral error catastrophe. Recent studies have shown molnupiravir inhibits replication of human and bat coronaviruses, including SARS-CoV-2, in mice and human airway epithelial cells. A remdesivir resistant mutant mouse hepatitis virus has also been shown to have increased sensitivity to N4-hydroxycytidine.

The aim of the present study is formulation and evaluation of sustained release floating microspheres of Molnupiravir sulphate.

MATERIALS AND INSTRUMENTS:

Materials: Molnupiravir sulfate purchased from Molecules India Pvt Ltd,Hyderabad. Sodium alginate, Sodium carboxy methyl cellulose are from s.d.fine chem. Ltd. Mumbai. Hydroxy propyl methyl cellulose K4M, Hydroxy propyl methyl cellulose K100M are from Otto chem. Laboratories Pvt.Ltd. Sodium phosphate dibasic dehydrate, Potassium dihydrogen ortho phosphate are from RANKEM,RFCL Ltd, New Delhi.

PREFORMULATION

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms.
Preformulation studies relate to pharmaceutical and analytical investigation carried out in supporting formulation development efforts of the dosage forms. The following preformulation studies were performed for obtained sample of drug.

UV-SPECTROSCOPIC METHOD FOR ANALYSIS OFMOLNUPIRAVIR SULFATE

Preparation of stock solution

Weighed accurately 100mg of drug and transferred it to 100mlvolumetric flask, then add water and the volume was made up to 100ml with water.

Preparation of standard solution

Then pipette out 1ml from above solution in a 10ml volumetric flask and made the volume with water to 10ml. From standard stock solution 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml solution was pipette out in five 10ml volumetric flasks

CALIBRATION CURVE OF MOLNUPIRAVIR SULFATE

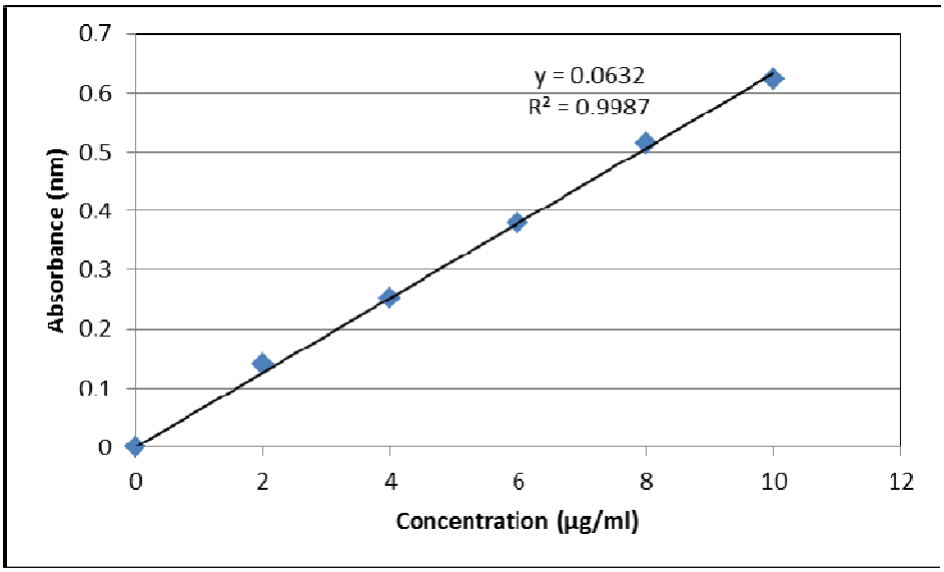
Measured the absorbance of the above prepared standard solution at

296 nm. plotted a graph of concentration on X axis andabsorbance (in nm) on Y axis

Table 1. Calibration curve for Molnupiravir sulfate

S.No.	Concentration(g/ml)	Absorbance(nm)
1	0	0
2	2	0.152
3	4	0.279
4	6	0.392
5	8	0.514
6	10	0.623
Slope	0.0632	
R^2	0.9987	

Fig 1. Calibration curve for Molnupiravir sulfate



FORMULATIONS OF MOLNUPIRAVIR SULFATE FLOATINGMICROSPHERES

Table 2. FORMULATIONS

INGREDIENTS in mg	FORMULATION BATCHES									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Molnupiravirsulfate	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
HPMC K4M	1000	-	-	-	500	500	500	-	-	-
Sodium alginate	-	1000	-	-	500	-	-	500	500	-
Sodium CMC	-	-	1000	-	-	500	-	500	-	500
HPMC K100M	-	-	-	1000	-	-	500	-	500	500
Methanol	5	5	5	5	5	5	5	5	5	5
Dichloromethane	5	5	5	5	5	5	5	5	5	5

Floating microspheres were prepared by the solvent evaporation method using 1000 mg of Molnupiravir sulfate and with different polymer as shown in Table, were dissolved in methanol (5 ml), dichloromethane (5 ml) with vigorous agitation to form uniform drug polymer dispersion. This wasslowly poured into the dispersion medium consisting of light liquid paraffin (100ml) containing 1.1% tween 80. The system is stirred using propeller at 1000 rpm at room temperature for 1hr 30 min — 2 hr. The liquid paraffin was decanted and the microparticles were separated by filtration through a Whatman filter paper, was head thrice with 180 ml of n-Hexane and air driedfor 6-8 hrs**.**

2.a 2.b

Fig. 2.a : Preparation of Microspheres; 2.b : after drying microspheres

RESULTS AND DISCUSSIONS:

PRE-FORMULATION STUDIES

Organoleptic properties

These tests were performed as procedure given, Preformulation part.
The resultsare illustrated in following table.

Table 3. Organoleptic properties

Test	Specifications/limits	Observations
Color	White to off white	Off White powder
Odour	odorless	odorless

The results complies as per specifications

Angle of repose

It was determined as per procedure Preformulation in material and methodpart.
The results are illustrated in following table**.**

Table 4. Flow properties

Material	Angle of repose
Molnupiravir sulphate	29

The result shows that drug having poor flow

Bulk density and tapped density.

It was determined as per procedure given Preformulation in materialand method part. The results are illustrated in table**.**

Table 5. Density

Materials	Bulk Density(gm/ml)	Tapped density(gm/ml)
Molnupiravir sulphate	0.19	0.26

Powder compressibility

It was determined as per procedure given in Preformulation inmaterial and method part. The results are illustrated in table**.**

Table 6. Powder compressibility

Material	essibilityindex	Hausner ratio
Molnupiravir sulphate	28.04%	1.34

The results shows that drug having poor flow property

Melting point

It was determined as per procedure given in Preformulation inmaterial and method part. The results are illustrated in following table**.**

Table 7. Melting point

Material	Material pointrange	Result
Molnupiravirsulphate	165 □C	Complies

The result complies as per specification.

SOLUTION PROPERTIES

pH of the solution

It was determined as per procedure given in preformulation inmaterial andmethod part. The results are illustrated in following table**.**

Table 8. pH

Material	Test	Specificityon	Observation
Molnupiravirsulphate	pH	7.5	7.5

The result complies as per specification

Solubility

It was determined as per procedure given in Preformulation inmaterial and method part. The results are illustrated in following table.

Table 9. Solubility

Test	Specification	Result
solubility	Freely soluble in water,Sparingly soluble in DMSO,ethanol,methanol	Complie s

The result complies as per specification.

DRUG-EXCIPIENT COMPATABILITY STUDIES

Discussion:

Drug excipient interactions play a vital role with respect to release of drug from formulation amongst others. FTIR techniques have been used here to studythe physicaland chemical interaction between drug and excipient used.

Table 10. Drug – Excipients Compatibility Study Results

Drug + Excipients	Initial	After 1 month at		Compatible
		40^0C/75%RH	60^0C	
Drug	White powder	No change	No change	Yes
Drug + Methanol	White powder	No change	No change	Yes
Drug + Tween 80	White powder	No change	No change	Yes
Drug + HPMCK100M	White powder	No change	No change	Yes
Drug + HPMC K4M	White powder	No change	No change	Yes
Drug + Dichloromethane	White powder	No change	No change	Yes

IR GRAPHS

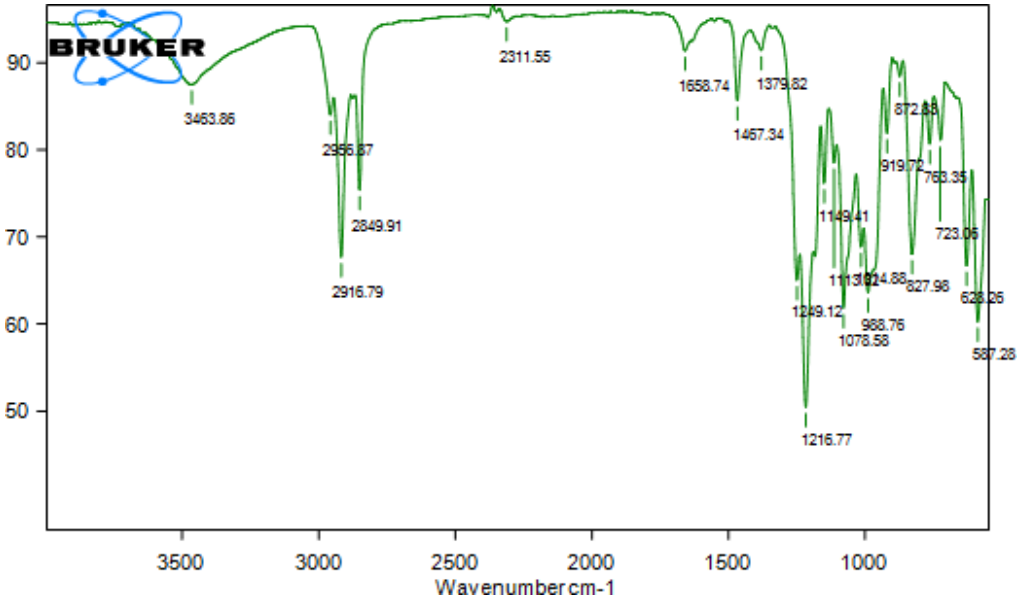


Fig.3 FT-IR of Molnupiravir Sulphate

Table. No 11: Band Assignments for the Infrared Absorption Spectrum of Molnupiravir Sulphate

Band Energy(cm-1)	Assignment
3463.2	tertiary amine hydrochloride (N-H) stretch
2958.6	O-H stretch
2849.7	C-H Stretch
1660.0	Cyclopentene Ring C=C stretch
1451.6	C-H Bending (CH_2 Scissoring)

In the present study, it has been observed that there is no chemical interaction between Molnupiravir and the polymers used. From the figures 11(a),11(b),11(c) and 11(d) it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymer.

Table.No. 12. EVALUATION OF MICROSPHERES

BatchNO.	Angle of Repose	Bulk Density(g/ml)	Tapped Density(g/ml)	Carr' s Index	HuasnerRatio
F1	25.08	0.2826	0.3177	10.38	1.11
F2	23.52	0.2671	0.3242	14.07	1.19
F3	24.24	0.2841	0.3318	16.47	1.15
F4	27.62	0.2853	0.3420	16.13	1.19
F5	24.09	0.2965	0.3446	14.47	1.16
F6	27.37	0.2924	0.3321	11.94	1.13
F7	26.64	0.2768	0.3394	13.68	1.22
F8	24.71	0.2891	0.3503	16.04	1.21
F9	26.14	0.2965	0.3446	13.54	1.16
F10	25.50	0.2721	0.3242	15.07	1.19

Discussion:

The angle of repose for the formulations F1-F10 was found to be in the range23.52˚ to 27.62˚ shows good flow property

Compressibility index for the formulations F1-F10 found between10.38% to16.13% indicating the good flow property.

Table 13. PERCENTAGE YIELD, *INVITRO* BUOYANCY, DRUG ENTRAPMENTEFFICIENCY OF FLOATING MICROSPHERES OFMOLNUPIRAVIR SULPHATE

BatchNo.	Percentageyield	*Invitro* buoyancy	Entrapmentefficiency
F1	84.52	85.65	82.60
F2	82.98	83.75	81.73
F3	83.54	84.82	82.17
F4	79.89	80.23	78.61
F5	85.15	86.24	83.04
F6	88.91	89.54	87.39
F7	90.21	91.66	88.69
F8	86.78	87.26	83.92
F9	85.59	86.92	83.47
F10	87.66	88.22	85.21

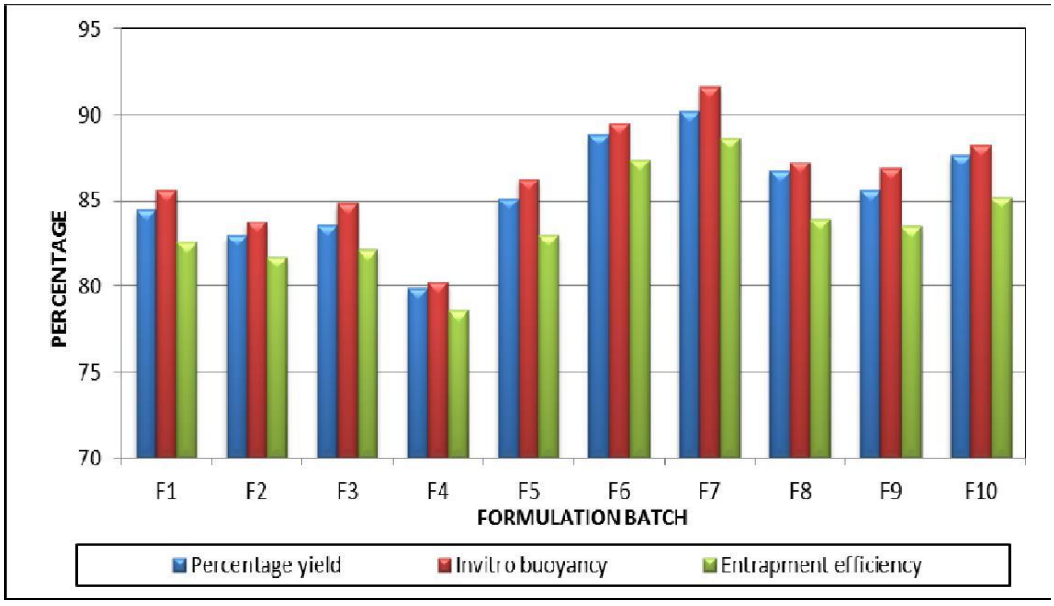


Fig.No. 4: Buoyancy, Entrapment efficiency Study of FloatingMicrospheres

Percentage yield

The maximum percentage yield was found in F7 formulation and wasnoted to be 90.21 % among all formulations.

The floating microspheres were prepared with different and combination of two polymer of HPMC K4M, HPMC K100M, Sodium Alginate and Sodium CMC to investigate the influence of encapsulation efficiency and were used to determine its influence on floating behavior.

***Invitro* Buoyancy Discussion**

The different polymers with same ratios of formulation were selected for optimization of their buoyancy property. The formulations in which combination of HPMC K4M, HPMC K100Mare giving the better results.

The formulations, are selected as the best formulations depending upon their buoyancy, encapsulation efficiency. From the results of all the ten formulations, it is confirmed that the change in polymers of Sodium Alginate, Sodium CMC, HPMC K4M, K100M influences the properties of the formulations. The formulation F7 with drug and combination of two polymer 1:1 ratio, is giving the best result of buoyancy property.

The microspheres, having lower densities (having a hollow core) exhibited buoyancy and are expected to be retained in gastric environment for more than 12 hrs. This may be attributed to a decrease in density of microspheres with an increase in polymer concentration.

Fig.No. 5: Buoyancy Study of Floating Microspheres

Entrapmentefficiency Discussion

The percentage entrapment efficiency of various formulation parameters of the prepared microspheres were shown in table. The entrapment efficiency varied from 78.61 to 88.69.

The formulation F7 is having high encapsulation efficiency of 88.69% and F4 is having low encapsulation efficiency of 78.61%.

The low encapsulation is because of using single polymer of HPMC K100M than the drug concentration where the quantity of HPMC K100M is insufficient to entrap the drug. The high encapsulation efficiency is because of using combination of polymers of HPMC K100M, HPMC K4 where the increase in the HPMC concentration forms larger microspheres encapsulating moreamount of drug.

Table 14. *INVITRO* RELEASE PROFILE

Time (hours)	BATCH NO.									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	20.03	12.05	14.18	10.01	12.91	10.56	8.25	15.23	12.11	9.54
2	35.56	32.56	31.56	28.19	22.12	15.99	13.11	25.76	22.77	13.84
4	54.14	53.29	45.25	62.43	35.12	28.21	24.72	37.45	30.99	24.15
6	69.65	77.38	63.14	76.37	52.64	44.14	40.99	53.28	48.12	43.09
8	89.14	96.38	92.02	98.24	78.11	58.98	56.25	79.54	69.82	54.69
10	69.65	77.38	63.14	76.37	94.42	78.68	72.84	90.47	93.51	72.74
12	89.14	96.38	92.02	98.24	90.47	87.04	90.12	87.04	90.12	85.25

Fig. 6. *Invitro* Dissolution Release Profile for F1 – F10 Formulations

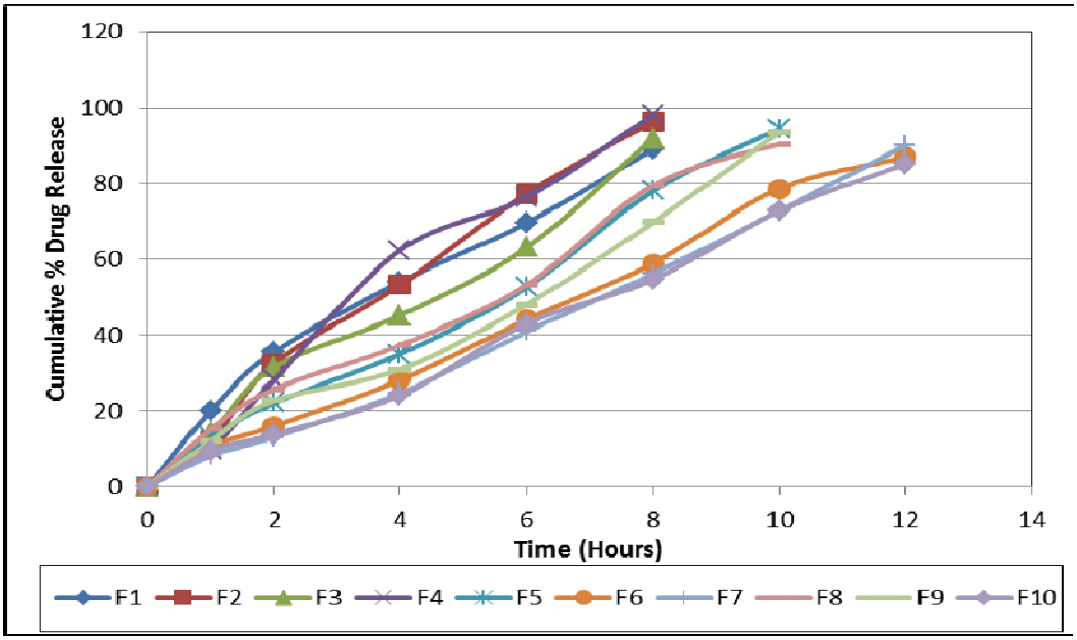


Fig. 7. *Invitro* Dissolution Release Profile for F1 – F5 Formulations

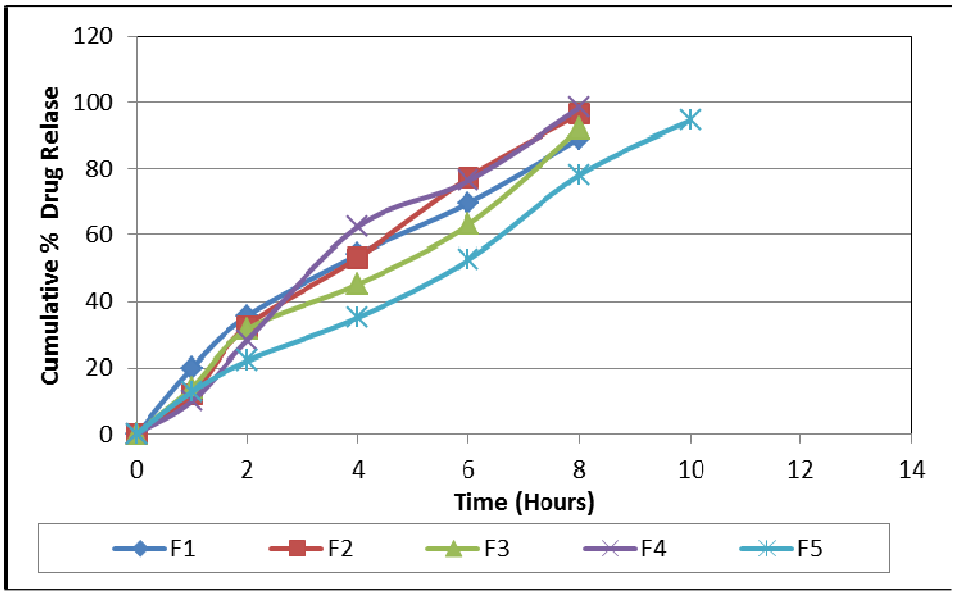


Fig. 8. *Invitro* Dissolution Release Profile for F6 – F10 Formulations

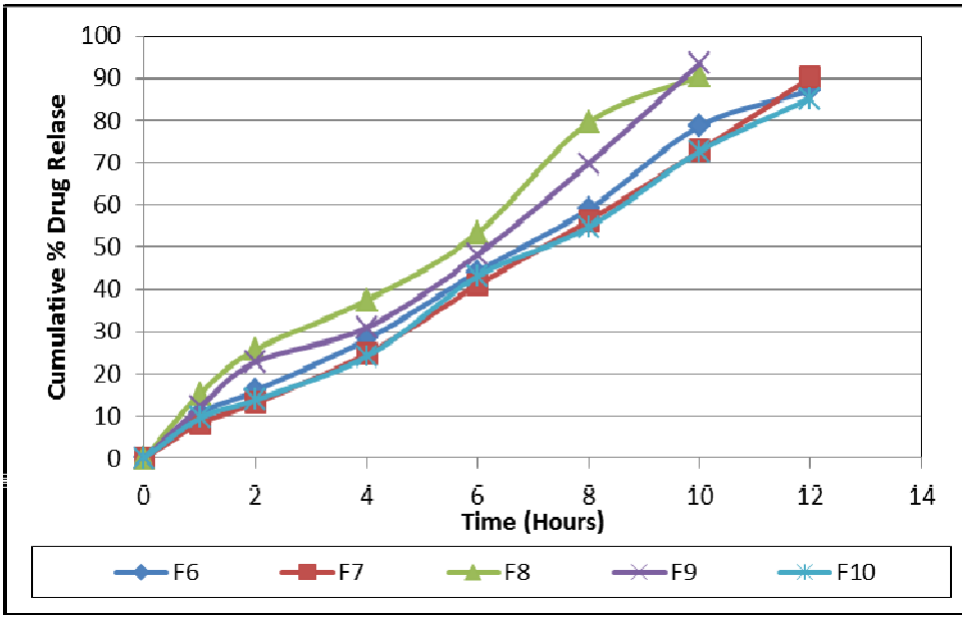
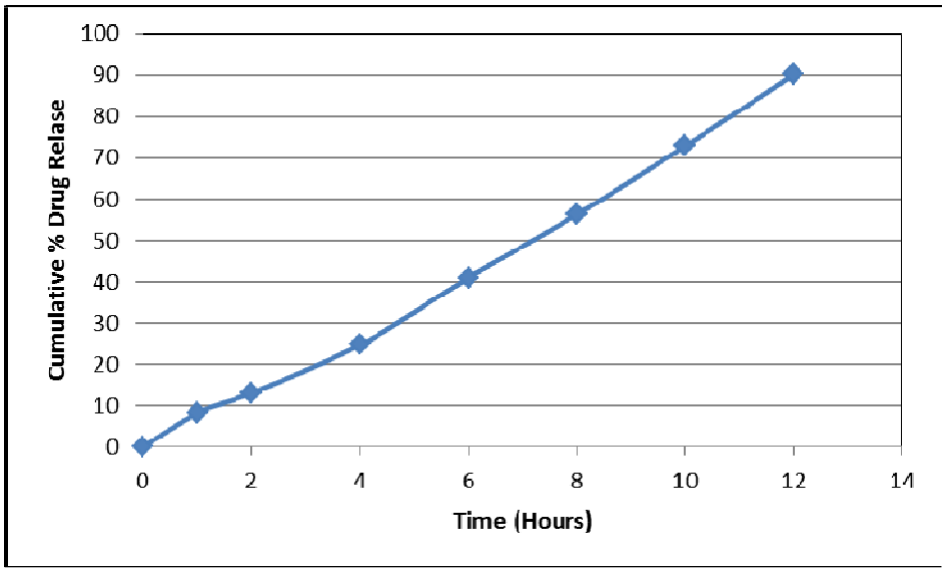


Fig. 9. *Invitro* Dissolution Release Profile for Best Formulation F7



Discussion:

From the *Invitro* dissolution study of all formulations (F1-F10), formulation F7 release around 90.12% of drug at the end of 12 hours for a sustained release. Therefore the F7 formulation chosen as the best formulation from all ten batches.

Kinetics of drug release :

Table 15. Drug release kinetics of formulation F7

Time (Hr)	cumulativee % drug released	% drug remainingg	Square root time	log Cumu % drug remanningg	Log time	logCumu % drug releaseed
0	0	100	0.000	2.000	0.000	0.000
1	8.25	91.75	1.000	1.963	0.000	0.916
2	13.11	86.89	1.414	1.939	0.301	1.118
4	24.72	75.28	2.000	1.877	0.602	1.393
6	40.99	59.01	2.449	1.771	0.778	1.613
8	56.25	43.75	2.828	1.641	0.903	1.750
10	72.84	27.16	3.162	1.434	1.000	1.862
12	90.12	9.88	3.464	0.995	1.079	1.955

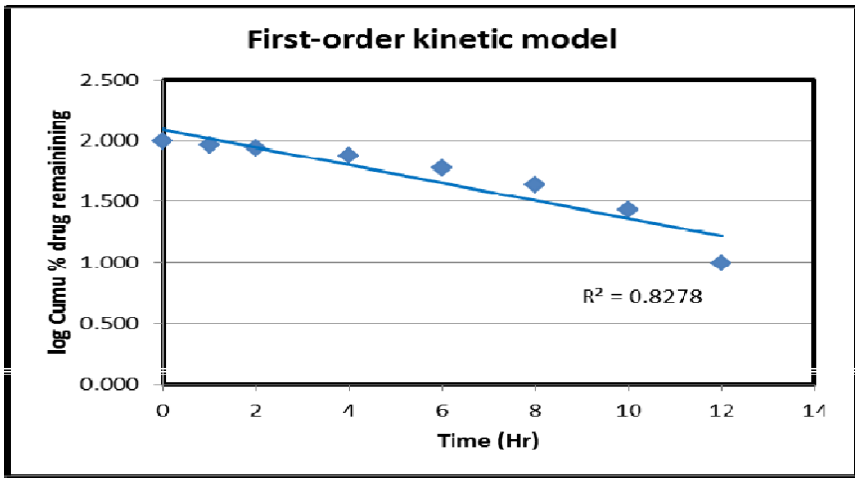


Fig. 11. FIRST ORDER KINETIC MODEL

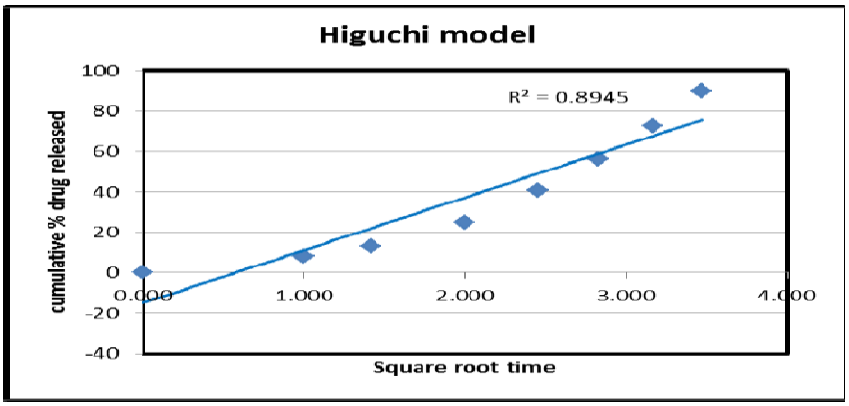


Fig.12.**Higuchi Model**

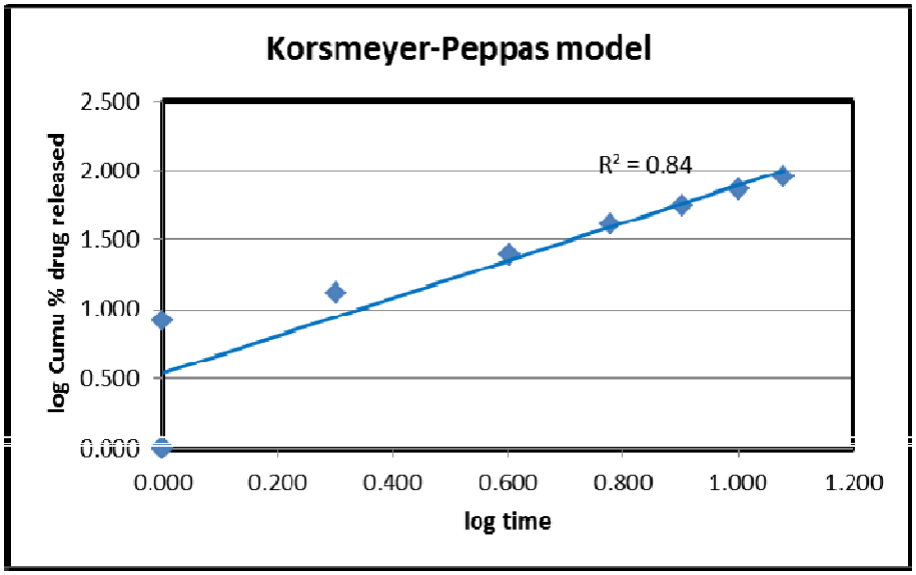


Fig.13. KORSEMEYER PEPPAS MODEL

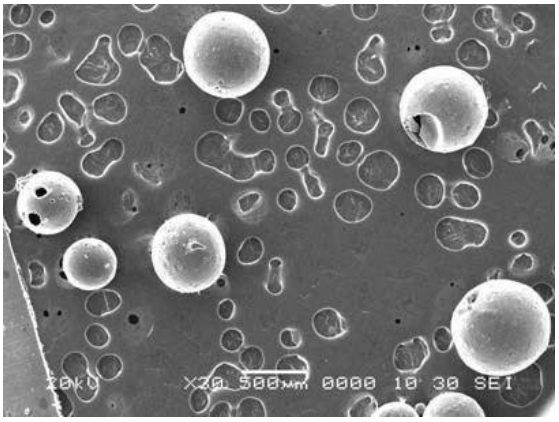
Table 16. Regression Coefficient of F7

Formulation	Regression coefficient (R^2) values			
	Zeroorder	First Order	HiguchiModel	Korsemeyer - peppas
Molnupiravir Floating Microspheres	0.9955	0.8278	0.8945	0.8400

n = 0.9735

The regression coefficient values and n values show that the drugreleases followNon - Fickian release (Diffusion and swelling).

Fig 14. Scanning Electron Microscopy (SEM)



DISCUSSION:

Morphology of floating microspheres was examined by scanning electron microscopy. The view of the microspheres showed hollow structure with a smooth surface morphology exhibited range of sizes within each batch. The outer surface of microspheres was smooth and dense, while the internal surface was porous. The shell of microspheres also showed some porous structure it may be caused by evaporation of solvent entrapped within the shell of microsphere after forming smooth and dense layer.

CONCLUSION:

The ultimate goal for sustained drug release is to maximize therapeutic activity while minimizing the negative side effects of the drug. In this regard, floating microspheres have emerged as a novel drug delivery system to treat HIV with Molnupiravir sulphate.

The type of polymer affects the drug release rate and the mechanism. Polymer swelling is crucial in determining the drug release rate and is also important for flotation. A lesser FLT and a prolonged floating duration could be achieved by using different polymer combinations. In this study sustained release Floating Microsphere approach for Molnupiravir purposes that withhydrophilic polymers the GI retention can be enhanced and reduce frequencyof dosing, thereby minimizing the occurrence of side effects, site specificity, increase the effectiveness of the drug and better patient compliance This gives a signal to extending this approach to similar combinations of drugs used in clinical practice so as to improve bioavailability of poorly absorbed drugs in GIT.

When these floating microspheres compared to other floating dosage forms like floating tablets have bulk density less than gastric fluid and soremain buoyant in the stomach for prolonged period of time and these are used as multiunit dosage form and drug release optimization and show efficiency level. So, Sustained release floating microspheres of Molnupiravir may provide a convenient dosage form for achieving best performance and release and show good bioavailability.

REFERENCES:

[1] Basak, S. C., Rahman, J., and Ramalingam, M. (2007). ―Design and invitro testing of a floatable gastro retentive tablet of Metformin hydrochloride‖, Pharmazie, 62, 145-148.

[2] Chadhari, P., and Chaudharis, K. P. (2008). ―Design and evaluation of bilayer floating tablets of tizanidine hydrochloride‖, Indian J. Pharm Educ Res., 42, 36-46.

[3] Shah, D., Shiah, Y., and Rampradhan, M. (1997). ―Development and evaluation of controlled release diltiazem micro particles using crosslinked poly (vinyl alcohol).‖ Drug Dev. Ind. Pharm, 23(6), 567-574.

[4] Arora, S., and Alij, A. A. (2005). ―Floating Drug delivery systems. A review‖, AAPS pharm scitech, 6, 372-390.

[5] Kataria, S., Middha, A., Sandhu, P., Bilandi, A., and Kapoor, B. (2011). ―Microsphere: A review‖, International Journal of Research in Pharmacy and Chemistry, 1(4).

[6] Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T., and Itoh, Y. (1992). ―Hollow Microspheres for Use as a Floating Controlled Drug Delivery System in the Stomach‖ J. Pharm. Sci., 81,135-140.

[7] Kawatra, M., Jain, U., and Ramana, J. (2012). ―Recent Advances In Floating Microspheres As Gastro-Retentive Drug Delivery System: A Review‖, Int J Recent Adv Pharm Res, 2(3), 5-23.

[8] Chawla, G., Gupta, P., Koradia, V., and Bansal, A.K. (2003). Gastroretention- a means to address regional variability in intestinal drug absorption, Pharm. Tech. 27(7): 50-51.

[9] Chickering, D. E., Jacob, J. S., and Matho, W. E. (1995). ―Reactive Polymers‖, (25), 189-206.

[10] Ch'Ng, H. S., Park, H., Kelly, P., and. Robinson, J. R., (1985). ―Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery II—Synthesis and Evaluation of Some Swelling,Water-Insoluble Bioadhesive Polymers,‖ J. Pharm. Sci. 74 (4), 399–405