https://doi.org/ 10.48047/AFJBS.6.5.2024. 8613-8626

African Journal of Biological Sciences

Formulation and *in-vitro* characterization of oseltamivir microspheres

Mamatha Kola*,V. Dedeepya, Mohammad Bakhatwar, Boddu prathyusha

Gokaraju Rangaraju College of Pharmacy,
Department of Pharmaceutics, Osmania University,
Hyderabad, Telangana-500090, India

*Corresponding author-Mamatha kola
mamathak84@gmail.com
phone:9441188329

Article History
Volume 6, Issue 5, 2024
Received: 15 May 2024
Accepted: 02 Jun 2024
doi: *10.48047/AFJBS.6.5.2024. 8613-8626*

Abstract: The goal of this work was to create Oseltamivir (Osm) microspheres through the combination of various polymers, including xanthan gum (XG), sodium alginate (SA), and karaya gum (KG). The ionotropic gelation process was used for producing the Osm microspheres. Oseltamivir acts as sialidase inhibitor with antiviral qualities . Osv prevents the virus's surface-resident enzyme, viral neuraminidase, from executing its task. Thirteen formulations of Oseltamivir microspheres were prepared. OS11 was found to be a target formulation among them. Pre-formulation tests and evaluation characteristics like yield (%), drug content (%), particle size, buoyancy, entrapment (%) efficiency, surface morphology, and dissolution studies were assessed for each formulation. Kinetic models were fitted with the results. FM11 microspheres were spherical in form and stiff. Osv was properly dispersed throughout the microspheres. After taking into account all the factors, such as the particle size (439.92±0.47µm to 636.8±0.91µm), yield (%) (73.24±0.81 to 89.27±2.18%), buoyancy (%) (81.09±0.46 to 97.89±0.08 %), entrapment (%) efficiency (93.28±0.27 to 98.69±0.38%), and other factors, FM11 was identified as the target formulation. This formulation was developed by SA:KG:XG in a ratio of 1:1.5:1.5, and the release showed zero-order with an anomalous transport behavior.

Keywords–microspheres, Oseltamivir, sodium alginate, ionotropic gelation, karaya gum, microspheres xanthan gum, controlled release.

I. INTRODUCTION

Oral administration is the most popular method to provide medication. It's important since it makes patient administration possible without the aid of qualified staff. Recently, the pharmaceutical industry has demonstrated an increasing interest in controlled-release drug delivery in an effort to improve therapeutic benefits such as patient compliance, convenience of dose administration and formulation flexibility.[1]

Compared to immediate-release dosage forms, time-controlled oral drug delivery systems have a number of benefits like reduction in drug concentration fluctuations in plasma and at the site of action over time, which improves therapeutic efficacy and minimises side effects; a reduction in the total dose administered; and an increase in patient compliance due to frequentadministration.[2] The two guiding concepts of GRDS are polymer adherence to the intended local site and floating, which depends on the density of the polymer material utilised. GRDDS significantly extends the gastric retention time (GRT) of medications at the site of absorption and has a prolonged half-life in the stomach (3).

The creation of novel delivery strategies to avoid residence duration in physiological systems is the consequence of cutting edge scientific and technological research. Numerous scientific methods, such as pro adhesive systems, swelling systems, floating systems, and gastro-retentive dose forms, are used to improve and lengthen the gastric residence period. Gastro-retentive drug administration is an approach to increase gastric residence duration and target site-specific medicine release in the upper gastrointestinal tract (GIT) for both local and systemic effects.

Gastro-retentive dosage forms have the ability to remain in the gastrointestinal tract for prolonged periods of time, significantly extending the gastric retention times (GRT) of drugs. Adhesion (4), flotation (5), sedimentation [6], expansion [7], altered shape systems, or concurrent administration of pharmacological drugs [8] that prolong stomach emptying are some methods used for developing gastro-retentive dosage forms. Over the past few decades, a number of gastro-retentive drug delivery techniques have been developed. These include unfoldable, extendible, or swellable systems that limit the amount of dosage forms that can be emptied through the pyloric sphincter, low density (floating) systems that cause buoyancy in gastric fluid, and high-density (sinking) systems that are retained in the stomach's bottom[9].

II. MATERIALS AND METHODS

Materials: Osv was received as a gift sample from Granules india Pvt Ltd. Karaya gum and sodium alginate from S D Fine Chemicals Ltd. in Mumbai. xanthan gum was procured from Mumbai-based Loba Chemie Pvt Ltd.

FTIR and DSC compatibility studies: These techniques were used to ascertain the drug's compatibility with the different polymers that were used.

Formulation of Osv floating microspheres: Osv hollow microspheres for controlled drug delivery were prepared using the ionotropic gelation process. The polymers employed were sodium alginate, Xanthan gum, and Karaya gum, with calcium chloride functioning as the cross-linking agent. To produce a homogenous polymeric mixture, sodium alginate, sodium bicarbonate, and water that had been purified were combined. After adding Osv to create a homogenous dispersion, the mixture was quickly agitated. The gelation media was prepared by dissolving calcium chloride in distilled water. A homogenous alginate solution was extruded into the medium using a needle syringe (gauge 21). The microspheres were stirred in the solution for 30 minutes at room temperature in order to increase their mechanical strength. After that, the microspheres were collected, washed twice with waterand allowed to dry for one day at room temperature.

Table 1: Formulation of microspheres

Ingredients (mg)	**OST 1**	**OST 2**	**OST 3**	**OST 4**	**OST 5**	**OST 6**	**OST 7**	**OST 8**	**OST 9**	**OST 10**	**OST 11**	**OST 12**	**OST 13**
Oseltamivir	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	500.0	**500.0**	500.0	500.0
Sodium Alginate	500.0	500	500.0	500.0	500.0	500	500	500	500	500	**500**	500	500
Xanthan gum	500	750	1000	1250	-	-	-	-	250	500	**750**	750	750
Karaya gum	-	-	-	-	500	750	1000	1250	250	500	**750**	750	750
Drug: polymer	1:1	1:1.5	1:2	1:2.5	1:1	1:1.5	1:2	1:2.5	1:0.5:0.5	1:1:1	**1:1.5:1.5**	1:1.5:1.5	1:1.5:1.5

$NaHCO_3$(mg)	50	50	50	50	50	50	50	50	50	50	**50**	50	50
Calcium chloride (%)	1	1	1	1	1	1	1	1	1	1	**1**	2	3

Characterization of microspheres:

Determination of particle size: An optical microscope was used to ascertain thaverage particle size. After the microspheres were equally spaced out on the slide, the particle size was determined by closely examining them under a calibrated optical microscope. For every formulation, the diameter of 100 microspheres was measured, and the outcomes were noted. [10].

$$\textbf{Percentage Yield (\%)} = \frac{\textbf{Practical yield}}{\textbf{Theoretical yield}} \textbf{ x 100}$$

Yield (%): The total weight of Osv, SA, KG, and XG utilised in each formulation was divided by the dried microspheres weight recovered from each formulation [11].

Yield (%)= (Practical yield) / (Theoretical yield) x 100

Drug Content: To determine the Osv content of hollow microspheres, 75 mg (equivalent) of the microsphere formulation were dispersed into 50 mL of HCl (0.1N), and the polymers were dissolved over a duration of twelve hours with a stirrer[12]. After filtration, absorbance at 221.4 nm was used to determine the percentage of Osv concentration.

$$\% \text{ Drug content} = \frac{\text{Calculated amount of drug}}{\text{Total amount of floating microspheres}} \text{ x } 100$$

Entrapment efficiency (EE): Precisely weighed and ground 75 mg (equivalent) Osv hollow microspheres were placed in 50 mL of HCl (0.1 N) for a duration of 12 hours[13]. The solution was filtered using a Millipore filterand 1 mL of the filtrate was pipetted out and suitably diluted using a fresh solvent. After that, samples that were previously prepared were examined using a spectrophotometer, and the Osv content was calculated and estimated using the following formula:

$$EE(\%) = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \text{ x } 100$$

In vitro buoyancy studies: A dissolution apparatus (Type II–USP) was used to assess the buoyant ability of microspheres. A 75 mg (equivalent) Osv hollow microsphere was incorporated to 900 mL of 0.1 N HCl. After that, the solution was agitated at 50 rpm while maintained at 37 ± 5 °C [14]. Floating time is defined as the amount of time needed for the batch to float to the top (also known as the floating lag time). The amount of time that takes between introducing microspheres to the dissolving medium and observing them rise towards the top of the HCl (0.1N)-filled jar. After 12 hours, the layer of hollow microspheres and the settled microspheres were collected, dried separately, and the percentage of hollow microspheres floating was calculated using equation:

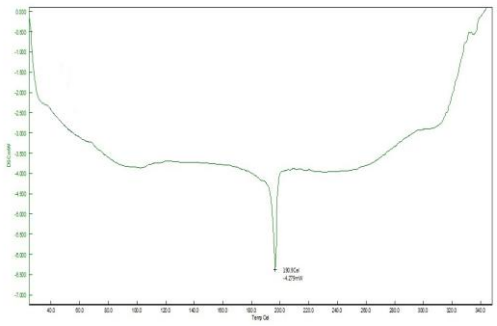
$$\% \text{ Buoyancy} = \frac{Weight\ of\ floating\ \ microspheres\ after\ 12\ h}{Initial\ \ weight\ of\ floating\ \ microspheres} \text{ x } 100$$

SEM:SEM is used for assessing surface topography, texture, and to determine whether a surface is sectioned or broken. The surface characterisation was conducted using the optimized formulation. SEM was used to visualise the microspheres (Zeiss, EVO-MA, Germany). The samples were placed on a brass stub in an electron microscope and covered in an ion sputter while under vacuum [15]. Surface features of floating microspheres were captured via photomicrographs and random stub scanning.

Drug release (In-vitro study): The drug release was studied (in-vitro) using Type-II (USP) dissolving apparatus. The medium utilised was 900 mL of 0.1 N HCl at 50 rpm. The temperature was kept at 37±5℃. 75 mg (equivalent) of OSV microspheres were used. At various time intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, the sample solution (5 mL) was withdrawn. after replacing the medium with an identical volume for a maximum of twelve hours, the absorbances at 221.4 nm weremeasured.[16]
Drug release kinetics: Various models of drug release from microsphere formulations were fitted.

III. RESULTS AND DISCUSSION

DifferentialScanningCalorimetry



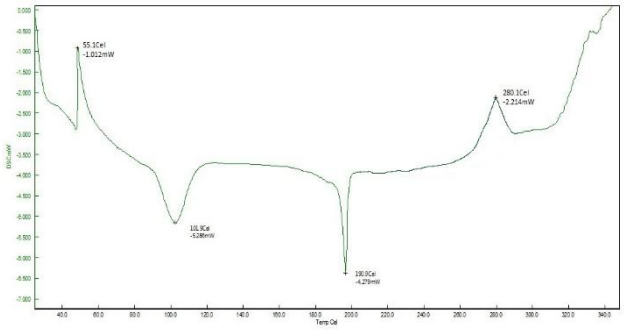


Figure1: DSCofOsv ***Figure 2:DSC of OSV, Xanthan gum and karaya gum***

An exact value for the melting temperature is provided by DSC. Figures 1 and 2 depicted the DSC thermograms. There was no difference found in the DSC comparisons of the pure forms and the mixture containing the excipients. The Osv MP was represented by a peak in the DSC data of 190.9 °C. The typical peak was observed once more at 190.9 °C for a physical mixture (SA, KG, XG), indicating that the Osv and the polymers do not interact. Fourier Transform Infrared Spectroscopy: FTIR investigations were conducted using solid samples of the Osv and excipient.

.

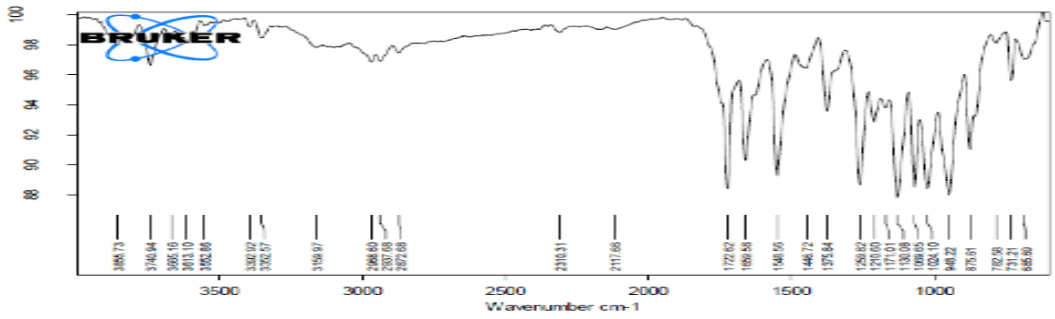


Figure3. FT-IRof Osv

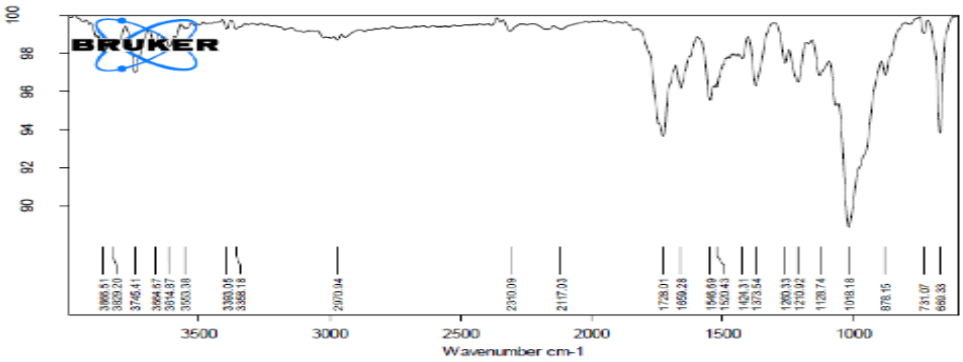


Figure 4. FT-IR of the Drug + Excipients.

The Osv and excipients are compatible with one another, as shown by the infrared spectrum analysis, which also showed no noticeable distinction in Osv peak deviations between pure Osv and mixture form (figs 3 & 4). This suggests that there were no interactions between the excipients and Osv

Development Of Osv Floating Microspheres: The ionotropic gelation technique was employed to efficiently produce Osv microspheres.

Characterization of Osv floating microspheres: As indicated in table 2, the particle size of the prepared floating microspheres ranged from 439.9±0.47 µm to 636.8±0.91 µm. It was found that an increase in polymer concentration led to an improvement in the average particle microsphere size. A larger droplet of the microsphere is produced when the viscosity of the polymer solution increases and the blend flows out of the needle more slowly. The size of the microsphere will increase with a rise in the viscosity of the polymer, increasing the efficiency of drug entrapment. The formulated floating microspheres yielded ranging from 73.24±0.81 to 89.27±2.18%.

Drug content is a crucial component of every pharmacological dosage form in order to reach the greatest plasma concentration of the drug. The formed floating microspheres had an Osv content ranging from 90.10±0.11 to 99.79±0.37%. The Osv formulation' entrapment (%) efficiency varied between 93.28±0.27 and 98.96±0.38%. Increases in polymer concentration resulted in more polymer being accessible to entrap the medication, which improved entrapment efficiency. The research results imply that Osv is dispersed evenly throughout the microspheres.

Table 2: Evaluation of microspheres

S. No.	Formulation's	Yield (%) AM±SD*,	Drug content (%)	Entrapment Efficiency(%)	Avg size(µm) AM±SD*, n=3
1	FM1	76.24±1.83	90.10±0.11	94.04±0.25	445.7±0.52
2	FM2	78.42±2.79	92.62±0.23	95.02±0.16	472.3±0.63
3	FM3	80.62±1.57	94.74±0.24	96.33±0.21	528.2±0.79
4	FM4	82.06±1.44	94.96±0.18	98.12±0.30	627.3±0.70
5	FM5	76.82±1.59	94.12±0.34	94.89±0.37	449.5±0.57
6	FM6	73.24±0.81	95.81±0.18	96.22±0.43	483.6±0.74
7	FM7	82.08±0.69	96.75±0.19	97.03±0.34	501.2±0.76
8	FM8	75.25±0.87	97.16±0.21	98.88±0.46	636.8±0.91
9	FM9	80.28±1.58	97.19±0.28	93.28±0.27	439.9±0.47
10	FM10	78.02±0.66	98.54±0.43	94.41±0.09	546.9±0.86
11	FM11	89.27±2.18	99.79±**0.37**	98.96±0.38	**623.3±0.87**
12	FM12	87.15±1.38	98.83±0.31	98.27±0.11	621.2±0.84
13	FM13	86.89±1.26	96.95±0.10	97.96±0.52	619.4±0.62

∗Average of 3 readings

Buoyancy:%.the range of all formulations' percentage buoyancy was 81.09±0.46% to 97.89±0.08%. As illustrated in Table 3, All formulation demonstrated desired floating lag time (27–98 sec) and floating duration (7–12 hours). In vitro, all of the prepared microspheres exhibited rapid floating behaviour that lasts less than two minutes, resulting in buoyant systems. The hgher polymer ratio led to a significant increase in buoyancy by increasing the volume of the cavity inside the microspheres. At 97.89±0.08%, Formulation FM11 displayed the greatest percentage buoyancy value.

Table 3: % Buoyancy, Floating lag time of microspheres

S.no	Formulation's Code	Floating Lag Time (sec)	Total Floating Duration (h)	Percentage buoyancy (%) AM±*SD**, (n=3)
1	FM1	27.02±0.83	7.01±0.52	90.66±0.21
2	FM2	31.67±0.34	9.12±1.01	91.29±0.11
3	FM3	36.69±0.53	10.08±0.54	91.33±0.19

4	FM4	43.66±0.82	11.22±1.14	97.41±0.04
5	FM5	40.38±0.47	10.27±0.71	81.09±0.46
6	FM6	64.02±1.11	12.67±0.46	94.70±0.16
7	FM7	67.15±0.38	9.31±0.85	95.08±0.43
8	FM8	68.12±0.61	10.48±1.32	97.47±0.07
9	FM9	47.32±0.84	11.12±0.83	92.69±0.36
10	FM10	98.32±1.34	12.85±1.63	95.31±0.14
11	**FM11**	**33.21 ±2.01**	**11.01±0.91**	**97.89±0.08**
12	FM12	97.47±1.29	10.18±0.57	96.67±0.34
13	FM13	98.89±2.34	9.24±1.26	95.29±0.27

SEM

SEM was used to evaluate the surface morphology, and the photomicrographs of floating microspheres generated by SEM are displayed in (fig 5). The smooth surface, round shape, and stiff nature of the microspheres were identified by the SEM examination results. The absence of aggregation development in the SEM image suggests that the microspheres are physically stable.

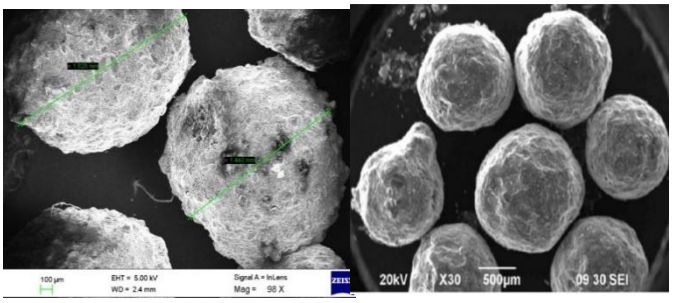


Figure 5. SEMof microspheres.

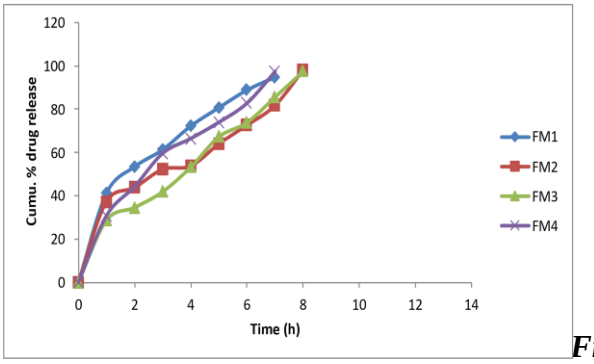


Figure 6. Release (In-vitro) studies of FM1, FM2, FM3and FM4

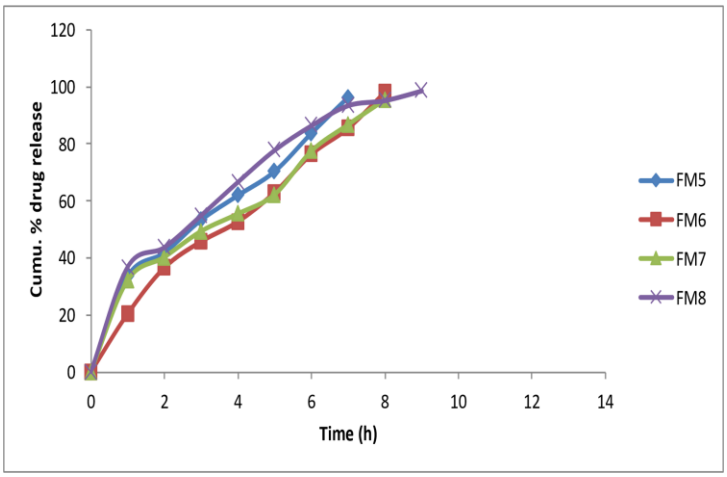


Fig: 7: Release (In-vitro) studies of FM5, FM6, FM7and FM8

Using the ionotropic gelation technique, the aforementioned (in-vitro) dissolving profile was noted for (FM1, FM2, FM3, and FM4) containing SA and XG in the ratios of 1:1, 1:1.5, 1:2, and 1:2.5. Using the ionotropic gelation technique, the FM1 formulation among these four indicated a release of 93.25%±0.86% in 8 hours (fig. 6). The other formulations (FM5, FM6, FM7, and FM8) contained SA and KG in the ratios of 1:1, 1:1.5, 1:2, and 1:2.5. Out of all four formulations, the FM7 formulation had a 9-hour release of 93.02±0.44%. Figure 7 showed the outcomes of the formulations FM5–FM8.

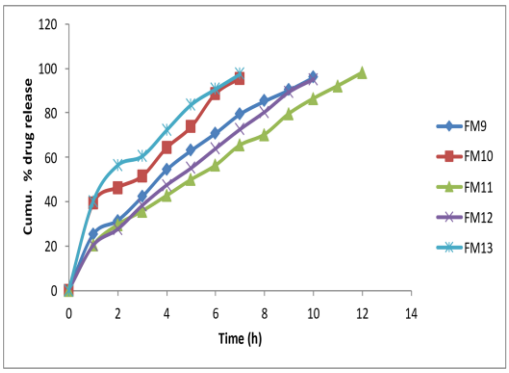


Fig: 8: Release (In-vitro) studies of formulation's FM9, FM10, FM11, FM12 and FM13

The above dissolution profile was noted for (FM9, FM10, FM11, FM12, and FM13) formulations with SA, XG, and KG in ratios of 1:0.5:0.5, 1:1:1, 1:1.5:1.5, 1:1.5:1.5, and 1:1.5:1.5. Out of the four formulations, FM11 exhibited a release rate of 98.89±0.49% after a 12-hour duration. The formulations FM9–FM13's results were displayed in (fig -8).Osv hollow microspheres' (in vitro) behaviour demonstrated controlled and prolonged release. The target formulation was determined to be FM11, which was made using SA, KG, and XG (1:1.5:1.5) respectively, with a concentration of 1% calcium chloride.

were observed. The target formulation of Osv floating microspheres, prepared in a 1:1.5:1.5 ratio with SA, KG, and XG, has been chosen for the investigation. FM11's regression coefficient value was determined. Since the value of regression for the FM11 formulation was found to be highest, 0.993, the optimal fit model is hence (zero-order). The Korsemeyer-Peppas plot further supports the Osv release mechanism. FM11's (n) value is 0.672, which denotes release by Anomalous behaviour.

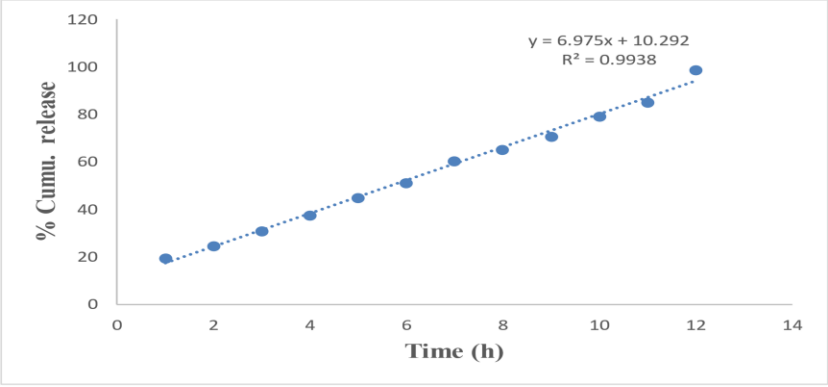


Fig- 9: Release (In- vitro) of Osv in HCl (0.1 N) – Zero ordermodel

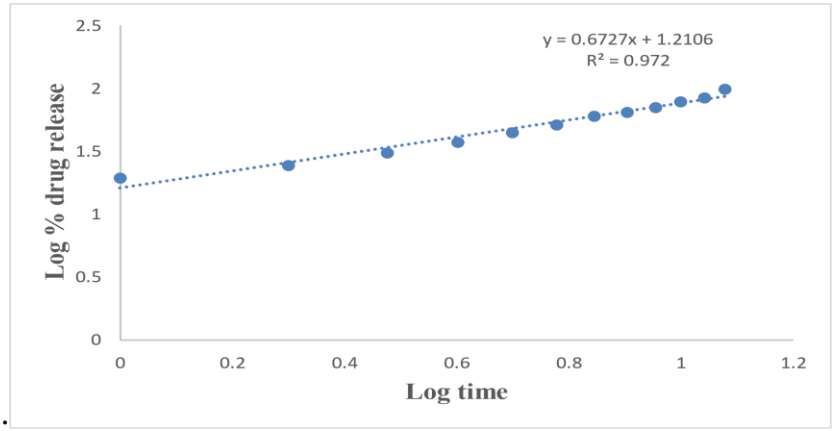


Fig- 10 Release (In- vitro) of Osv in HCl (0.1 N) Korsemeyer peppas model

IV.CONCLUSION

By releasing the medication gradually over time, the concept of making hollow microspheres containing Osv provides a good, practical way to provide a prolonged therapeutic effect. The current study effectively used a range of polymers, such as sodium alginate (SA), xanthan gum (XG), and karaya gum (KG), along with ionotropic gelation to produce hollow Osv microspheres. The FT-IR spectra showed no interaction between oseltamivir and polymers. SEM verified the smooth surface of the floating microspheres, or oseltamivir. FM1-13 particles ranged in size from 439.9±0.47µm to 636.8±0.91 µm.

As the polymer ratio increases, Osv microspheres' mean size increases. This is because a thickening of the dispersion was produced as the amount of the polymer increased, leading to the formation of huge droplets and large microspheres. The yield (%) of oseltamivir hollow microspheres ranged from 73.24±0.81 to 89.27±2.18%. The reported Oseltamivir content ranged from 90.10±0.11 to 99.79±0.37%. The range of oseltamivir%EE was 93.28±.27 to 98.96±.37%. An increase in polymer conc indicated an improvement in entrapment efficiency since the higher conc meant that there was more polymer available to entrap the medication. The outcomes imply that oseltamivir is appropriately distributed and that they were within the accepted bounds. After a 12-hour period, the buoyancy (%) of all formulations ranged from 81.09±0.46% to 97.89±0.08%.

The outcomes also showed that when the polymer concentration rises, the amount of medication released decreases. In vitro studies revealed that OSV hollow microspheres had a controlled and

prolonged release profile. FM11's in-vitro dissolution data was fitted using kinetics. Regression value (0.993) was given by target formulation FM11. It has been confirmed as a release with zero orders. The drug release mechanism is further supported by the Korsemeyer-Peppas plan. FM11's (n-value) of 0.672 indicates that it was released due to abnormal conduct. Based on the examination of Osv hollow microspheres, it was determined that SA, KG, and XG were used in the target formulation at a ratio of 1:1.5:1.5 for regulated drug delivery.

V.ACKNOWLEDGEMENT: The principal and management of Gokaraju Rangaraju College of Pharmacy are appreciated by the authors for their support and provision of all the facilities needed for study.

CONFLICT OF INTEREST: All the authors have no conflicts of interest to declare.

V. REFERENCES

1. Pinto JF. Site-specific drug delivery systems within the gastro-intestinal tract:from the mouth to the colon. International journal of pharmaceutics. 2010 Aug16;395(1-2):44-52.

2. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tractand dosage forms for site-specific delivery. International journal ofpharmaceutics. 1996 Jun 14;136(1-2):117-39.

3. Yin L, Qin C, Chen K, Zhu C, Cao H, Zhou J, He W, Zhang Q. Gastro-floatingtablets of cephalexin: preparation and in vitro/in vivo evaluation. Internationaljournal of pharmaceutics. 2013 Aug 16;452(1-2):241-8.

4.Ponchel G, Irache JM. Specific and non-specific bioadhesive particulate systemsfor oral delivery to the gastrointestinal tract. Advanced drug delivery reviews.1998 Dec 1;34(2-3):191-219.

5. Deshpande AA, Rhodes CT, Shah NH, Malick AW. Controlled-release drugdelivery systems for prolonged gastric residence: an overview. Drugdevelopment and industrial pharmacy. 1996 Jan 1;22(6):531-9.

6. Davis SS, Stockwell AF, Taylor MJ, Hardy JG, Whalley DR, Wilson CG,Bechgaard H, Christensen FN. The effect of density on the gastric emptying ofsingle-and multiple-unit dosage forms. Pharmaceutical research. 1986Aug;3(4):208-13.

7. Urquhart J, Theeuwes F, inventors; Alza Corp, assignee. Drug delivery systemcomprising a reservoir containing a plurality of tiny pills. United States patentUS 4,434,153. 1984 Feb 28.

8. Gröning R, Heun G. Oral dosage forms with controlled gastrointestinal transit.Drug Development and Industrial Pharmacy. 1984 Jan 1;10(4):527-39.

9. Boldhane SP, Kuchekar BS. Development and optimization of metoprololsuccinate gastroretentive drug delivery system. Acta pharmaceutica. 2010 Dec1;60(4):415-25.

10. Wilson CG, Washington N. The stomach: its role in oral drug delivery.

11. Physiological Pharmacetical: Biological Barriers to Drug Absorption. Chichester, UK: Ellis Horwood. 1989:47-70.

11.Tekade BW, Chaudhari YA. Gums and mucilages: Excipients for modified drug delivery system. Journal of Advanced Pharmacy Education & Research. 2013;3(4).

12.Shah AN, Tanveer M, Shahzad B, Yang G, Fahad S, Ali S, Bukhari MA, Tung SA, Hafeez A, Souliyanonh B. Soil compaction effects on soil health and cropproductivity: an overview. Environmental Science and Pollution Research. 2017 Apr;24:10056-67.

13.Jain P, Pradeep T. Potential of silver nanoparticle- coated polyurethane foam as an antibacterial water filter. Biotechnology and bioengineering. 2005 Apr 5;90(1):59-63.

14.Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. New England Journal of Medicine. 2017 Dec 28;377(26):2531-44