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# Formulation and Evaluation of Floating Microsphere of Esomeprazole

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**Abstract:** With an aim to formulate and evaluate sustained release floating microspheres of Esomeprazole, it was observed that these remain 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. Thereby with hydrophilic polymers the GI retention can be enhanced and reduce frequency of dosing, thereby minimizing the occurrence of side effects, site specificity, increase the effectiveness of the drug and better patient compliance Hence it is concluded that sustained release floating microspheres of Esomeprazole may provide a convenient dosage form for achieving best performance and release and show good bioavailability.

**Index Terms:** Floating Microspheres, Esomeprazole, Sustain release

## 1. INTRODUCTION

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, biodegradable polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones.

## 2. MATERIALS AN METHODS

### 2.1. Pre-formulation studies

#### 2.1.1 Organoleptic properties

Small quantity of the sample was taken to observe its colour, nature, taste and odour.

#### 2.1.2 Melting point

Melting point of drug was determined by Open capillary method. It is one of the parameters to judge the purity of crude drug.

### 2.1.3. Solubility

Solubility of drug was determined in water and methanol, ethanol, chloroform and ethyl acetate.A semi quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute of vice versa. After each addition, the system is vigorously shaken and examined visually for any undissolved solute particles. The solubility is expressed in terms of ratio of solute and solvent. The results are shown in results and discussion.

### 2.1.4. Determination of λmax

A solution of microspheres was prepared in HCl and the solution was scanned in the range of 200 – 400 nm UV spectrum using Systronic double beam spectrophotometer

### 2.1.5. Development of standard curve of Esomeprazole

**Preparation of stock solution**

100mg of drug was weighed accurately and transferred it to 100ml volumetric flask, then ethanol was added and the volume was made up to 100ml with ethanol**.**

**Preparation of standard solution**

1ml of solution was pipette out from above solution in a 10ml volumetric flask and the volume was made up with ethanol 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 and make up the volume with water to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10 µg/ml concentration.

The absorbance of the above prepared standard solution was then measured at 301 nm plotted a graph of concentration (in µg/ml)on X axis and absorbance (in nm) on Y axis

### 2.1.6. Drug excipient compatibility by FTIR

Pure drug was mixed with IR grade potassium bromide in a ratio of (1:100) and pellets were prepared by applying 10 metric ton of pressure in hydrophilic press. The pellets were then scanned over range of 4000-400 cm1 in FTIR spectrometer.

### 2.2 Preparation of of Esomeprazole Floating Microspheres

Floating microspheres were prepared by the solvent evaporation method using 20 mg of Esomeprazole

**Table No.1: Formulations**

| Ingredients in mg | FORMULATION BATCHES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
| Ezomeprazole | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| HPMC K4M | 100 | - | - | - | 50 | 50 | 50 | - | - | - |
| Sodium alginate | - | 100 | - | - | 50 | - | - | 50 | 50 | - |
| Sodium CMC | - | - | 100 | - | - | 50 | - | 50 | - | 50 |
| HPMC K100M | - | - | - | 100 | - | - | 50 | - | 50 | 50 |
| Methanol | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Dichloromethane | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

**Figure 1** *: Preparation of Microsphere* **Figure 2** *: after drying microspheres*

### 2.3. Evaluation of Microsphere:

The prepared floating microspheres were evaluated for Percentage yield, *Buoyancy studies*, Entrapment efficiency, Particle size analysis , SEM. *In vitro* dissolution studies, Kinetics of *In vitro* drug release.

## 3. RESULTS AND DISCUSSION

### 3.1. Pre-formulation studies:

### 3.1.1: Organoleptic properties:

**Table No.2: Organoleptic properties**

| Test | Specifications/limits | Observations |
|---|---|---|
| Color | Light blue | Light blue |
| Odour | Odorless | Odorless |
| Taste | Bitter | Bitter |
| Appearance | Powder | Powder |

### 3.1.2. Melting point:

The melting point was determined to be $185^0$C

### 3.1.3. Solubility :

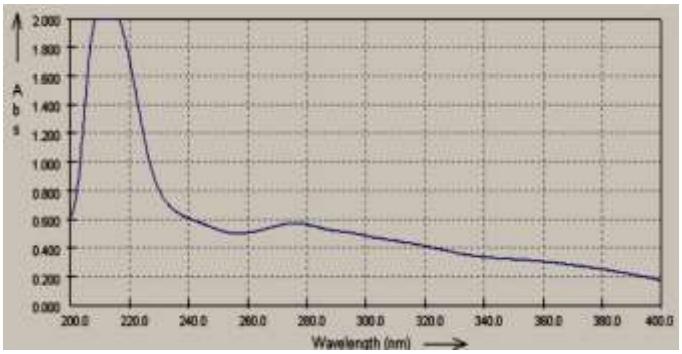
The solubility of Esomeprazole drug is seen in ethanol, dimethyl formaide and DMSO which are organic solvents and it is slightly soluble in water

**Table No.3: Solubility**

| Specification | Result |
|---|---|
| Ethanol, | ++++ |
| Dimethyl Formaide | ++++ |
| DMSO | ++++ |
| Water | +--- |

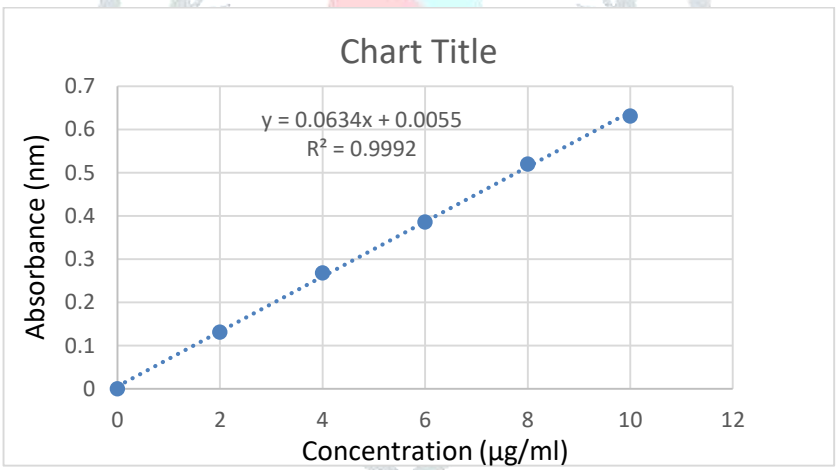
++++ Soluble +---- Slightly Soluble

### 3.1.4. Determination of λmax




### 3.1.5. Development of standard curve of Esomeprazole.

| S. No. | Concentration (µg/ml) | Absorbance (nm) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 2 | 0.131 |
| 3 | 4 | 0.268 |
| 4 | 6 | 0.386 |
| 5 | 8 | 0.520 |
| 6 | 10 | 0.631 |




### 3.1.6. Drug excipient compatibility by FTIR

**Table No.5: Drug – Excipient Compatibility Study Results**

| Drug + Excipient | Initial | After 1 month at | | Compatible |
|---|---|---|---|---|
| | | $40^0$C/75%RH | $60^0$C | |
| 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 + HPMC K100M | White powder | No change | No change | Yes |
| Drug + HPMC K4M | White powder | No change | No change | Yes |
| Drug + Dichloro methane | White powder | No change | No change | Yes |

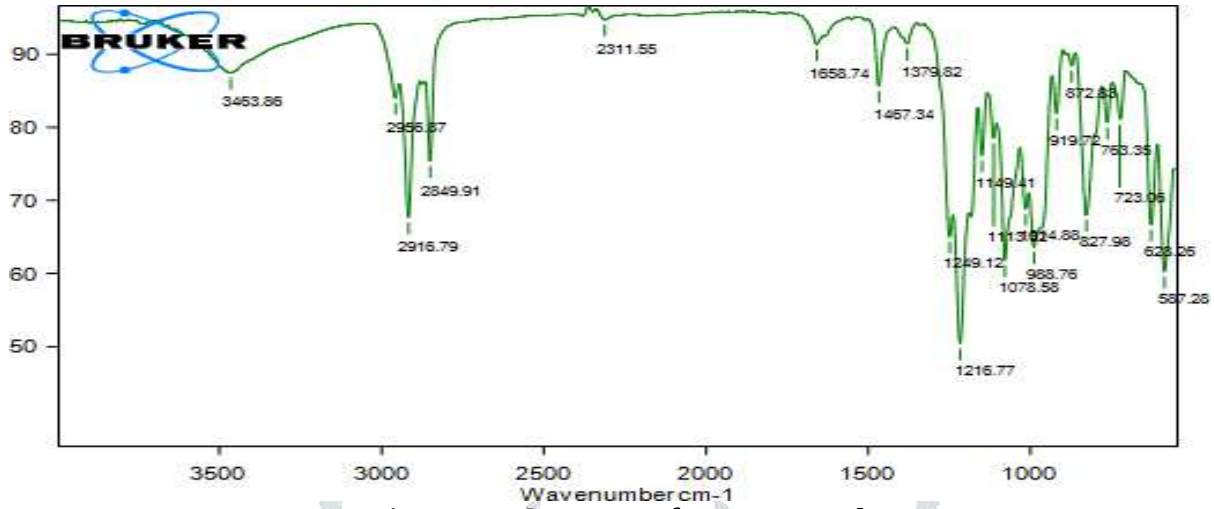



**Figure No. 3: FT-IR of Esomeprazole**

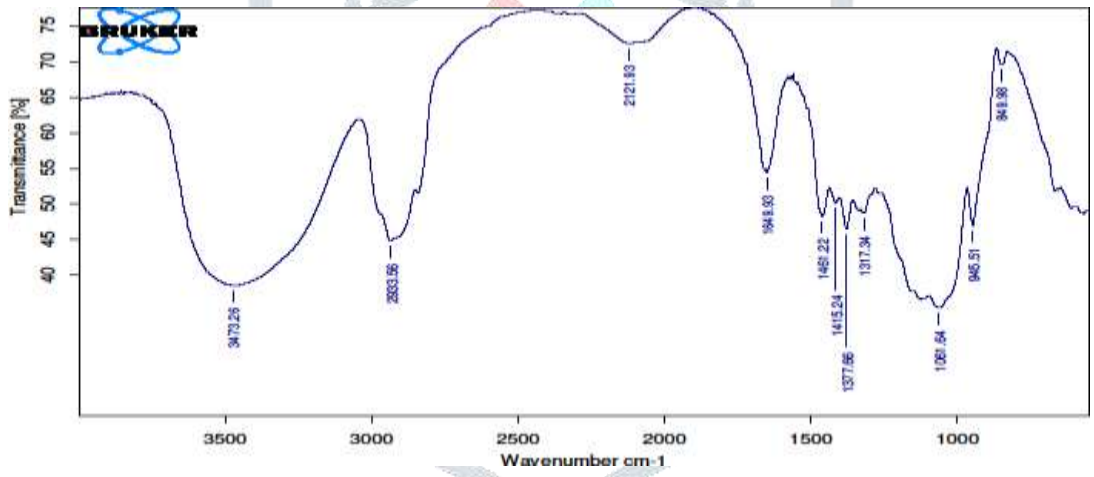



**Figure No. 4: FT-IR Graph of HPMC K4M**

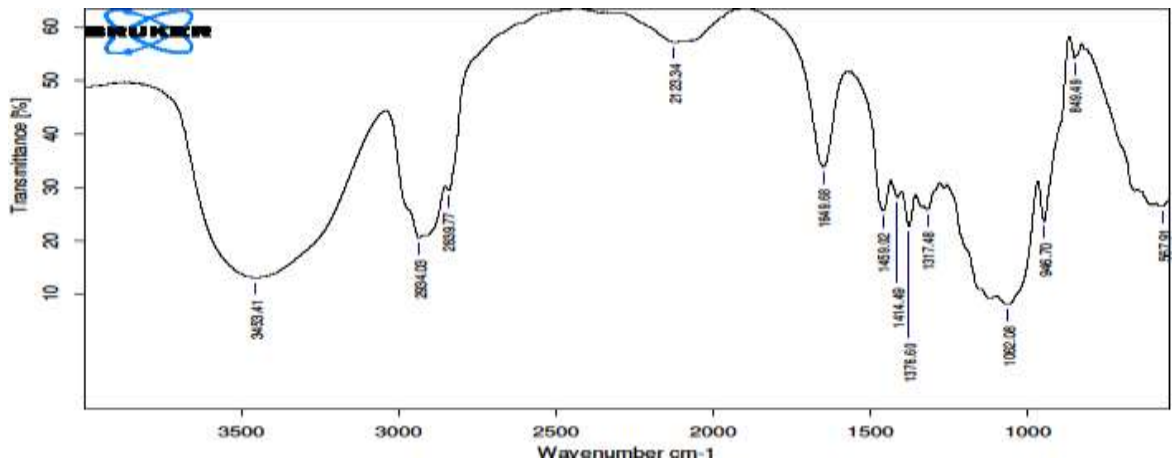



**Figure No. 5: FT-IR Graph of HPMC K100M**

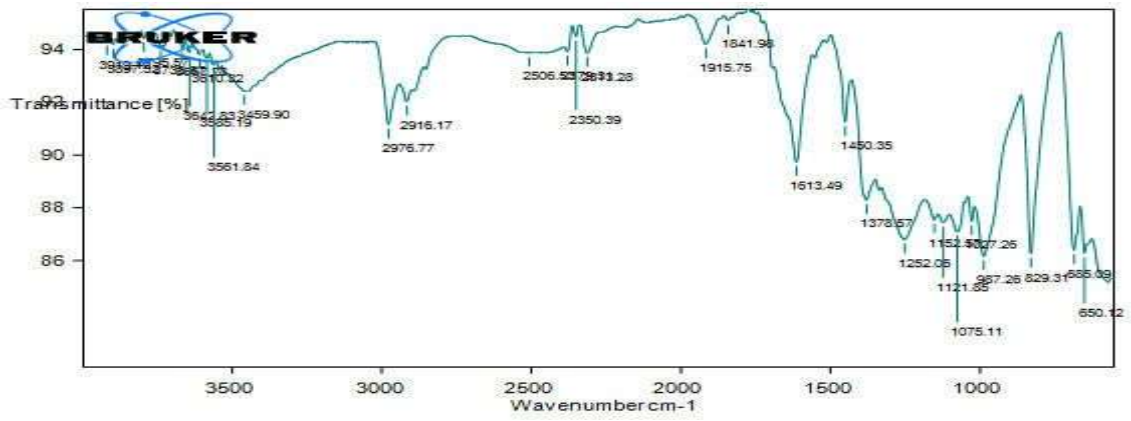



**Figure No.6**: **FT-IR Graph of Mixture of Esomeprazole + HPMC K4M +HPMCK100M**

**Table No.6: Band Assignments for the Infrared Absorption Spectrum of Esomeprazole**

| **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) |

It was observed that there is no chemical interaction between Esomeprazole and the polymers used

### 3.2 Evaluation of Microsphere

#### 3.2.1. Percentage yield

**Table No.7: Percentage yield**

| **Batch No.** | **Percentage yield** | ***In vitro* buoyancy** | **Entrapment efficiency** |
|---|---|---|---|
| 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 |

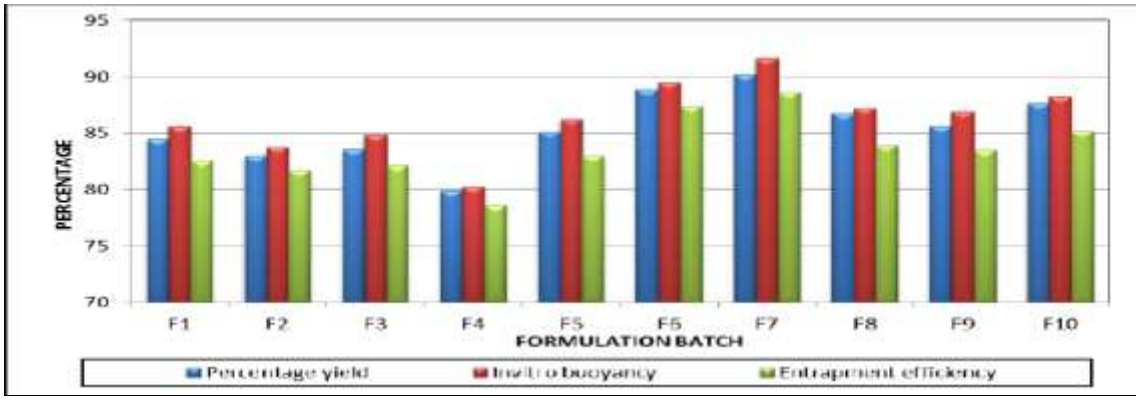



**Figure No. 7: Buoyancy, Entrapment efficiency Study of Floating Microspheres**

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

### 3.2.3. Entrapment efficiency

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 more amount of drug.

### 3.2.4. Particle size analysis

**Table No.8: Particle size analysis**

| S. No | Formulation code | Mean particle size (µm) |
|---|---|---|
| 1 | F1 | 330.12 |
| 2 | F2 | 352.10 |
| 3 | F3 | 419.14 |
| 4 | F4 | 448.46 |
| 5 | F5 | 249.26 |
| 6 | F6 | 358.86 |
| 7 | F7 | 442.78 |
| 8 | F8 | 526.56 |
| 9 | F9 | 516.43 |
| 10 | F10 | 502.26 |

### 3.2.5. Scanning Electron Microscopy (SEM)

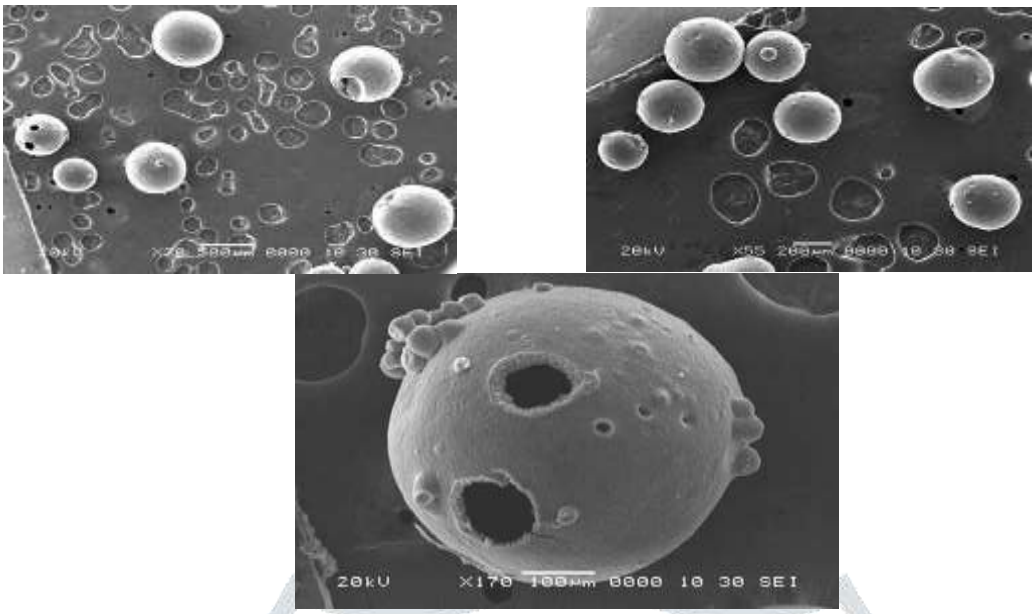



**Figure No. 8: Scanning electron microphotographs of floating microsphere of Esomeprazole**

### 3.3. *In vitro* dissolution studies

**Table No.9: *In Vitro* 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 | | | | | 94.42 | 78.68 | 72.84 | 90.47 | 93.51 | 72.74 |
| 12 | | | | | | 87.04 | 90.12 | | | 85.25 |

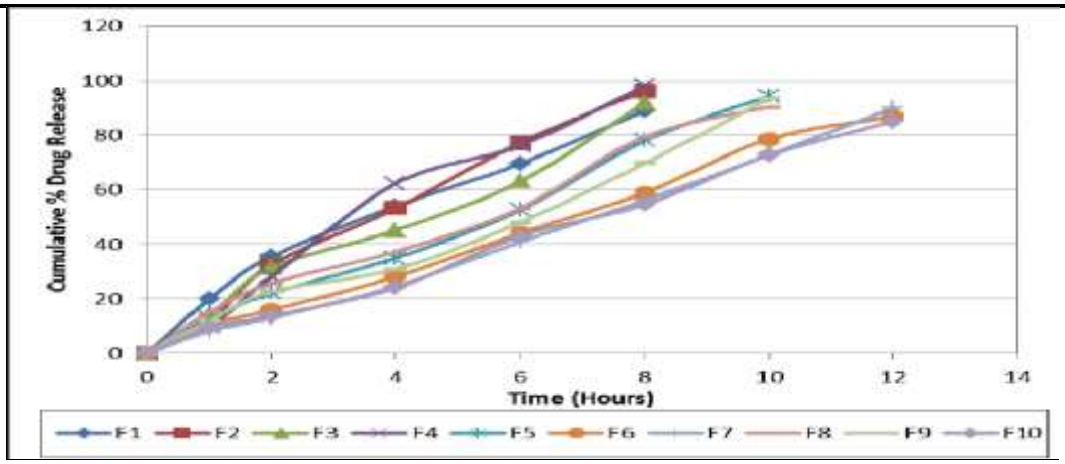



**Figure No. 9: *Invitro* Dissolution Release Profile for F1 – F10 Formulation**

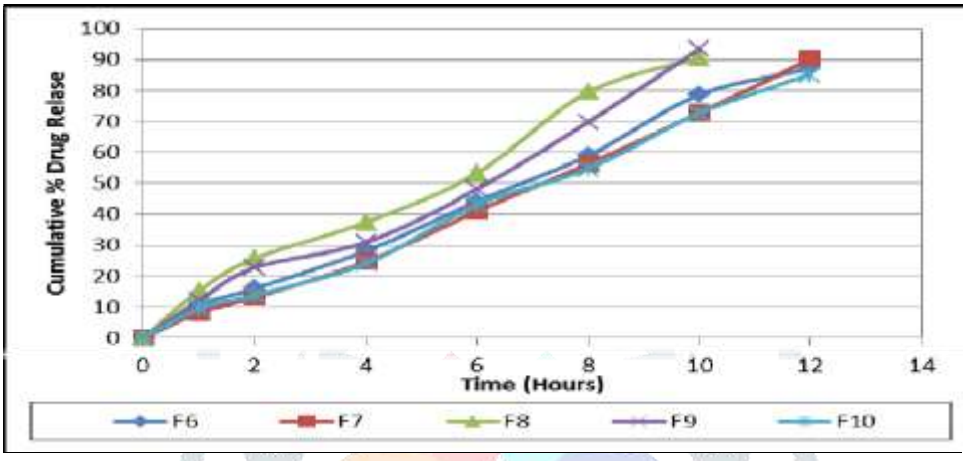



**Figure No. 10: *Invitro* Dissolution Release Profile for F1 – F5 Formulations**

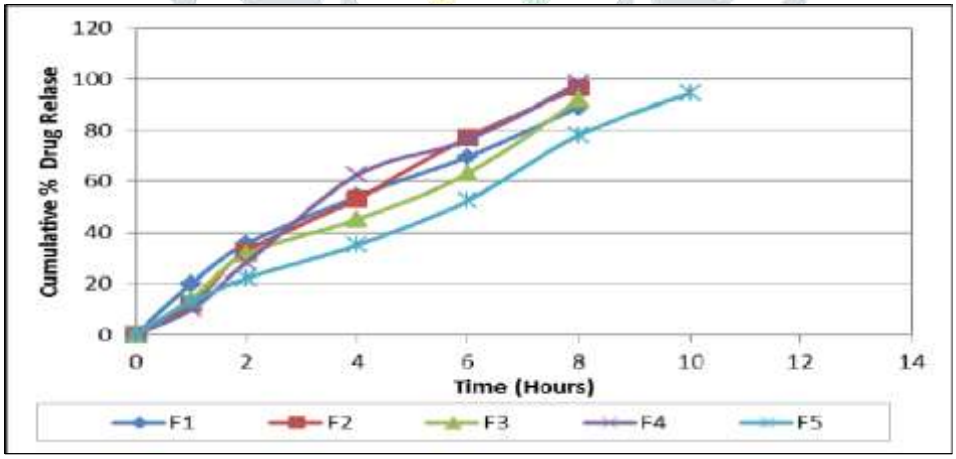



**Figure No.11: *In vitro* Dissolution Release Profile for F6 – F10 Formulations**

### 3.4 Kinetics of *In vitro* drug release

**Table No.10**: **-Drug release kinetics of formulation F7**

| Time (Hr) | Cumulative % drug released | % drug remaining | Square root time | log Cumu % drug remainining | log time | log Cumu % drug released |
|---|---|---|---|---|---|---|
| 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 |

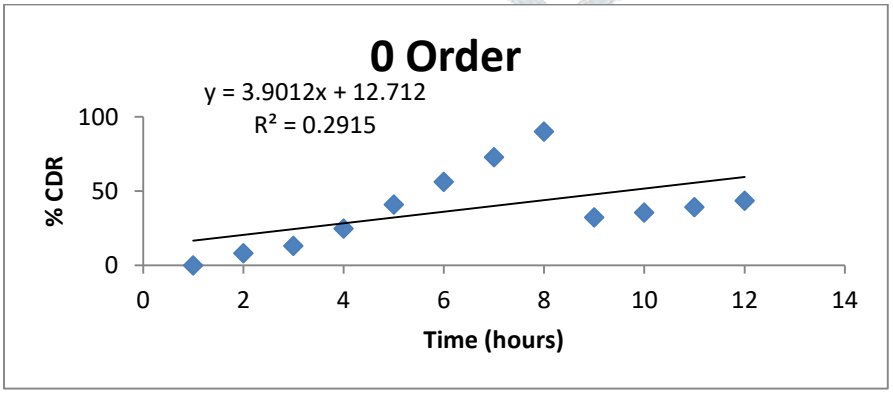



**Figure No.12: Zero order kinetic model**

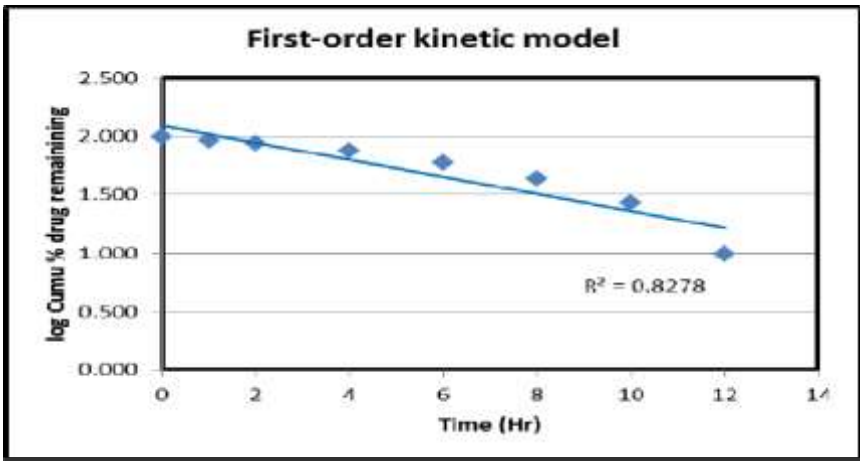



**Figure No. 13: First order kinetic model**

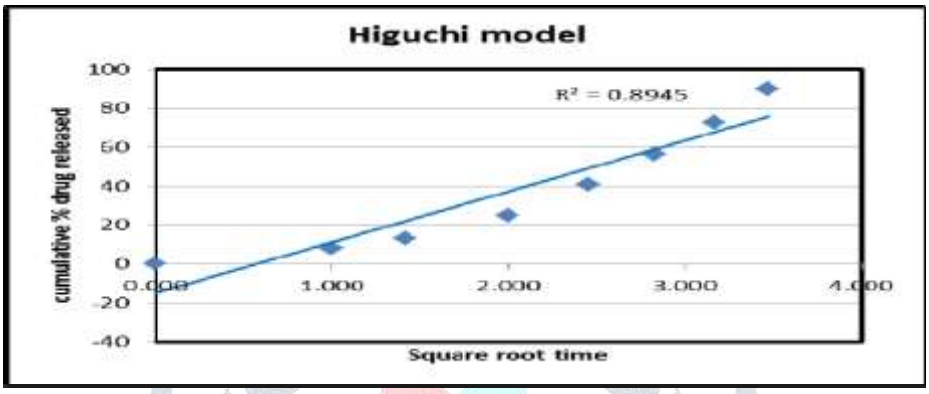



**Figure No.14: Higuchi model**

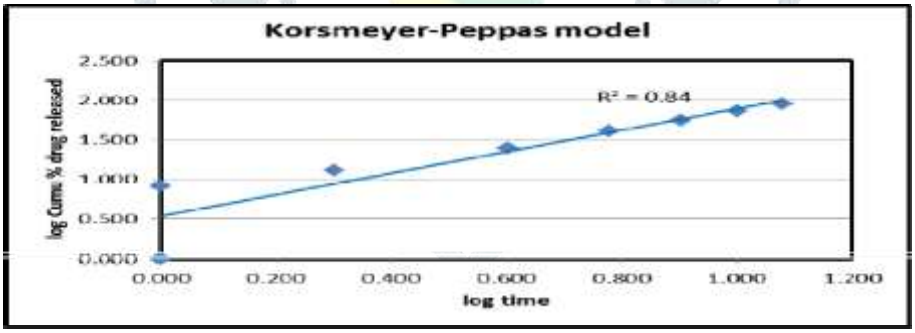



Figure No.15: Korsmeyer-Peppas model

**Table No. 11**: **Regression Coefficient of F7**

| Formulation | Regression coefficient ($R^2$) values | | | |
|---|---|---|---|---|
| | **Zero order** | **First Order** | **Higuchi Model** | **Korsemeyer - peppas** |
| Ezomeprazole Floating Microspheres | 0.2915 | 0.8278 | 0.8945 | 0.8400 |

## 4. CONCLUSION

Sustained release Floating Microsphere approach for Esomeprazole purposes that with hydrophilic polymers the GI retention can be enhanced and reduce frequency of 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. These floating microspheres remain 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 Esomeprazole may provide a convenient dosage form for achieving best performance and release and show good bioavailability.

## 5. REFERENCES:


1. Thummar, A. V., Kyada, C. R., Kalyanvat, R., &Shreevastva, B. 2013. A review on mucoadhesive microspheres as a novel drug delivery system. *International Journal for Pharmaceutical Research Scholars*, *2*(2), 188-200.
2. Mukherjee, S., &Bandyopadhyay, P. 2012. Magnetic microspheres: a latest approach in novel drug delivery system. *JPSI*, *1*(5), 21-25.
3. Jamini M., and Rawat S., 2013. A review on microsphere, *Res. j. pharm. boil. chem. sci*.1
4. Patel, N. R., Patel, D. A., Bharadia, P. D., Pandya, V., & Modi, D. 2011. Microsphere as a novel drug delivery. *International Journal of Pharmacy & Life Sciences*, *2*(8).
5. Singh, C., Purohit, S., Singh, M., & Pandey, B. L. 2013. Design and evaluation of microspheres: A Review. *Journal of drug delivery research*, *2*(2), 18-27.
6. Prasanth v.v., Moy A. C., Mathew S. T., Mathapan R., 2011. Microspheres An overview, *Int. J. Res. Pharm. Biomed. Sci.*,
7. Prasad, B. S., Gupta, V. R. M., Devanna, N., &Jayasurya, K. 2014. Microspheres as drug delivery system-a review. *J Glob Trends Pharm Sci*, *5*, 1961-72.
8. Mohan, M. E. N. E. S. H. W. A. R. I., Sujitha, H. A. Z. A. R. I., Rao, V. U. M., Ashok, M., & Kumar, A. B. 2014. A brief review on mucoadhesive microspheres. *IJRRPAS*, *4*(1), 975-86.
9. Kumar, A., Jha, S., Rawal, R., Chauhan, P. S., &Maurya, S. D. 2013. Mucoadhesive microspheres for novel drug delivery system: A Review. *Am. J. PharmTech Res*, *3*(4), 195-213..
10. Batra, D., Kakar, S., Singh, R., &Nautiyal, U. 2012. Magnetic microspheres as a targeted drug delivery system: An overview. *Journal of Drug Delivery Research*, *1*(3), 10-17.
11. Patra, C. N., Dutta, P., Sruti, J., & Rao, M. B. 2011. Floating Microspheres: Recent Trends in the Development of Gastroretentive Floating Drug Delivery System. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *4*(1), 1296-1306.
12. Mukund, J. Y., Kantilal, B. R., &Sudhakar, R. N. 2012. Floating microspheres: a review. *Brazilian Journal of Pharmaceutical Sciences*, *48*, 17-30.
13. Kawatra, M., Jain, U., &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.
14. Ramteke, K. H., Jadhav, V. B., & Dhole, S. N. 2012. Microspheres: as carrieres used for novel drug delivery system. *Iosrphr*, *2*(4), 44-48.
15. Dupinder, K., Seema, S., Gurpreet, S., & Rana, A. C. 2012. Biodegradable microspheres: A review. *International Research Journal of Pharmacy*, *3*(12), 23-28.
16. Saralidze, K., Koole, L. H., &Knetsch, M. L. 2010. Polymeric microspheres for medical applications. *Materials*, *3*(6), 3537-3564.
17. Patel, B., Modi, V., Patel, K., & Patel, M. 2012. Preparation and evaluation of ethyl cellulose microspheres prepared by emulsification-solvent evaporation method. *International journal for research in management and pharmacy*, *1*(1), 82-91.
18. Bansal, H., Kaur, S. P., & Gupta, A. K. 2011. Microspheres: methods of preparation and applications: A comparative study. *Int J Pharm Sci Rev Res*, *10*(1), 69-78.
19. Alagusundaram, M., Chetty, M. S., Umashankari, K., Badarinath, A. V., Lavanya, C., &Ramkanth, S. 2009. Microspheres as a novel drug delivery system-a review. *Int J Chem Tech Res*, *1*(3), 526-534.

20. Chaudhari, A., Jadhav, K. R., &Kadam, V. J. 2010. An overview: Microspheres as a nasal drug delivery system. *International Journal of Pharmaceutical Sciences Review and Research*, *5*(1).
21. Sahil K., Akanksha M., Premjeet S., Bilandi A., Kapoor B., (2011) Microsphere: A review. *Int. J. Res. Pharm. Chem.*,
22. Kumar, B. P., Chandiran, I. S., Bhavya, B., &Sindhuri, M. 2011. Microparticulate drug delivery system: a review. *Indian journal of pharmaceutical science & research*, *1*(1), 19-37.
23. Dhakar, R. C., Maurya, S. D., Sagar, B. P., Bhagat, S., Prajapati, S. K., & Jain, C. P. 2010. Variables influencing the drug entrapment efficiency of microspheres: A pharmaceutical review. *Der Pharmacia Lettre*, *2*(5), 102-116.
24. Parmar, H., Bakliwal, S., Gujarathi, N., Rane, B., &Pawar, S. 2010. Different methods of formulation and evaluation of mucoadhesive microsphere.
25. Mali, D. S., Talele, S. G., Mogal, R., &Chaudhari, G. 2014. Review on nasal microspheres. *Am. J. Pharm Tech Res*, *4*(1), 97-111.
26. Liu, L., Wu, Q., Ma, X., Xiong, D., Gong, C., Qian, Z., ... & Wei, Y. 2013. Camptothecine encapsulated composite drug delivery system for colorectal peritoneal carcinomatosis therapy: biodegradable microsphere in thermosensitive hydrogel. *Colloids and Surfaces B: Biointerfaces*, *106*, 93-101.
27. Singh, V., & Chaudhary, A. K. 2011. Preparation of Eudragit E100 microspheres by modified solvent evaporation method. *Acta Pol Pharm*, *68*(6), 975-980.
28. Barcia, E., Herrero-Vanrell, R., Díez, A., Alvarez-Santiago, C., López, I., &Calonge, M. (2009). Downregulation of endotoxin-induced uveitis by intravitreal injection of polylactic-glycolic acid (PLGA) microspheres loaded with dexamethasone. *Experimental eye research*, *89*(2), 238-245.
29. Das, M. K., &Senapati, P. C. 2007. Evaluation of furosemide-loaded alginate microspheres prepared by ionotropic external gelation technique. *Acta Pol Pharm*, *64*(3), 253-62.
30. Khandai, M., Chakraborty, S., Sharma, A., Pattnaik, S., Patra, C. N., Dinda, S. C., & Sen, K. K. 2010. Journal of Advanced Pharmaceutical Research. 2010, 1, 48-60. *Journal of Advanced Pharmaceutical Research*, *1*, 48-60.
31. Behera, B. C., Sahoo, S. K., Dhal, S., Barik, B. B., & Gupta, B. K. 2008. Characterization of glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method. *Tropical Journal of Pharmaceutical Research*, *7*(1), 879-885.
32. Sudhamani, T., Reddy, K. N., Kumar, V. R. R., Revathi, R., &Ganesan, V. 2010. Preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. *International Journal of Pharma Research and Development*, *2*(8), 119-125.
33. Kadam, N. R., &Suvarna, V. 2015. Microsphere: a brief review. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *5*(47), 13.
34. Shivhare, U. D., Rathod, H. D., &Mathur, V. B. 2013. Development and evaluation of floating pulsatile microspheres of metaprololtartarate using emulsification-solvent evaporation technique. *Scholars Academic Journal of Pharmacy*, *2*(5), 365-372.
35. Ige, P. P., Agrawal, K., &Patil, U.2015. Enhanced in vitro dissolution of Iloperidone using CaesalpiniaPulcherrimamucoadhesive microspheres. *Beni-Suef University Journal of Basic and Applied Sciences*, *4*(1), 26-32.
36. Senthil A., Narayanaswamy V. B., Ajit I., Deepak S. G.,Bhosale R.S., 2011.Mucoadhesive microspheres. *Int. J. Res. Ayurveda Pharm.*
37. Semalty, M., Yadav, S., &Semalty, A. 2010. Preparation and characterization of gastroretentive floating microspheres of ofloxacin hydrochloride. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *3*(1), 819-823.
38. Selvaraj, S., Karthikeyan, J., &Saravanakumar, N. 2012. Chitosan loaded microspheres as an ocular delivery system for acyclovir. *Int J Pharm PharmSci*, *4*(1), 125-32.
39. Lee, J., Tan, C. Y., Lee, S. K., Kim, Y. H., & Lee, K. Y. 2009. Controlled delivery of heat shock protein using an injectable microsphere/hydrogel combination system for the treatment of myocardial infarction. *Journal of Controlled Release*, *137*(3), 196-202.
40. Li, Z., Li, L., Liu, Y., Zhang, H., Li, X., Luo, F., & Mei, X. 2011. Development of interferon alpha-2b microspheres with constant release. *International journal of pharmaceutics*, *410*(1-2), 48-53.
41. Kang, J., Wu, F., Cai, Y., Xu, M., He, M., & Yuan, W. 2014. Development of Recombinant Human Growth Hormone (rhGH) sustained-release microspheres by a low temperature aqueous phase/aqueous phase emulsion method. *European Journal of Pharmaceutical Sciences*, *62*, 141-147.

42. Zhao, H., Wu, F., Cai, Y., Chen, Y., Wei, L., Liu, Z., & Yuan, W. 2013. Local antitumor effects of intratumoral delivery of rlL-2 loaded sustained-release dextran/PLGA–PLA core/shell microspheres. *International journal of pharmaceutics*, *450*(1-2), 235-240.
43. Martin, M. J., Calpena, A. C., Fernandez, F., Mallandrich, M., Gálvez, P., &Clares, B. (2015). Development of alginate microspheres as nystatin carriers for oral mucosa drug delivery. *Carbohydrate polymers*, *117*, 140-149.
44. Li, R., Feng, F., Wang, Y., Yang, X., Yang, X., & Yang, V. C. 2014. Folic acid-conjugated pH/temperature/redox multi-stimuli responsive polymer microspheres for delivery of anti-cancer drug. *Journal of colloid and interface science*, *429*, 34-44.
45. Ho, M. L., Eswaramoorthy, R., Wu, S. C., Wang, G. J., Chang, J. K., Fu, Y. C., ... & Wang, Y. H. 2014. *U.S. Patent No. 8,883,862*. Washington, DC: U.S. Patent and Trademark Office.
46. Ho, M. L., Wang, G. J., Chang, J. K., Fu, Y. C., Tzeng, C. C., &Rajalakshmanan, E. 2014. *U.S. Patent No. 8,865,220*. Washington, DC: U.S. Patent and Trademark Office.
2011.