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In-vitro Characterization of Floating Granules of Furosemide

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Abstract

The objective of the study is to formulate and optimize Furosemide based controlled release system for increasing the bioavailability by influencing the residence time in the stomach while remaining detached from the mucosa. This was achieved by successful preparation of floating granules by exploiting melt granulation technique. The formulations F1 to F6 were developed and evaluated for dependent variable (in vitro floating ability) and formulations F4 to F6 were selected as preliminary optimized formulations. The preliminary optimized formulation F4 to F6 were evaluated for micromeritic properties, drug content and percentage yield, in-vitro drug release, percentage in-vitro floating ability. The formulation F4 was selected as optimized formulation exhibiting good floating ability and zero order drug release (85.95 %) at the end of 8 hours. Aging effect on storage was evaluated using In-vitro drug release studies. The In-vitro drug release study of the aged sample showed increase in release behavior, which can be attributed to the phase transformation of Gelucire. In conclusion, hydrophobic lipid, Gelucire 43/01 can be considered as an effective carrier for designing of a multi-unit floating drug delivery system of Furosemide.

Keywords: *Furosemide; Floating granules; Gelucire; In-vitro release study*

Introduction

Among different modes of drug administration, the drug intake by mouth or oral route is one of the most widely used ways of administration of drug. This preference to the oral route of administration can be attributed to their administration, flexibility in formulation, non-invasive nature, of low cost of therapy, patient compliance etc. In the last few decades researchers are showing special interest in developing drug reservoir based oral delivery system which can release the active compound in a controlled fashion for a specific period [1]. Recent scientific and patent literatures supports the claim that extensive research is going-on to develop novel oral controlled release dosage forms which are capable of being retained in the gastrointestinal tract (GIT) for a prolonged and predictable period of time [2]. Several novel approaches which are currently being exploited to achieve this goal are development of floating drug delivery systems (FDDS) [3-5], low- density systems [6], raft systems incorporating alginate gels [7], bio-adhesive or mucoadhesive systems [8], high-density systems [9], super-porous hydrogels [10] and magnetic systems [11]. The current research focuses on the development of Furosemide based floating drug delivery system to achieve gastric retention and prolonged and controlled release. The developed formulations are characterized for different in vivo properties. Furosemide is an anthranilic acid derivative

which is chemically 4-chloro-N-furfuryl-5¬sulfamoylanthranilic acid. It has diuretic property. It is a white to slightly yellow crystalline powder. Furosemide is practically insoluble in water, sparingly soluble in alcohol, freely soluble in dilute alkali solutions and insoluble in dilute acids.

Materials and Methods

Materials and equipment's used

Furosemide (Sanofi Aventis Pharma Mumbai), Gelucire 43/01 (Gattefosse (St Priest,Cedex, France). Acetone (Sd fine chemicals). Potassium chloride (Sd fine chemicals). Hydrochloric acid, Potassium dihydrogen phosphate, Sodium hydroxide pellets, Ethanol (Sd fine-chemicals), Dissolution rate test apparatus(Electrolab Pvt. Ltd. Mumbai), pH /mill voltmeter(Century instrument Pvt. Ltd.), UV-VIS spectrophotometer (Shimadzu Corp. Japan), Standard test sieves(HICON, Grover Enterprises, Delhi), Digital oven(Science tech Pvt. Ltd. India), Digital Electronic Balance (Shinko Denshi corp.Japan), Digital M. P. apparatus(Jindal Scientific instruments, Ambala), Single Pan Electronic Balance(Contech instrument pvt. Ltd. Mumbai), Magnetic Stirrer with Hot Plate (B.D. Scientific Industries, Delhi).

Calibration curve

Selection of media

Fasting state pH is usually remained near about 2 and food, buffers, counter act stomach acid, hence enhancing the pH up to 6.5. Buoyant drug release systems are usually administered in fed state, after taking food starting of MMC is late hence decrease of stomach evacuation rate. Therefore, pH values in fed state conditions for preparation of calibration curves was selected.

Scanning for λmax

The solutions having a concentration of 10 µg/ml in pH 5.8 phosphate buffer was scanned in 200-400 nm in spectrum basic mode and as the results are tabulated in Table 1.

Table 1: **Table for scanned λmax of Furosemide in phosphate buffer which have 5.8 pH.**

S.No.	Solvents	Experimental λmax(nm)
1	10 µg / ml solution of Furosemide in phosphate buffer which have 5.8 pH	271

Preparation of calibration curves

Calibration curves of Furosemide were prepared in phosphate buffer which have 5.8 pH at λmax 271 nm the absorbance data (mean of three reading) with their S.D. at various concentrations of 2–10 µg/ml are tabulated and represented. The Furosemide was found to obey Beer–Lambert's law in the concentration 2–10 µg/ml with regression coefficient (r^2) values 0.9999 in pH 5.8 phosphate buffer. The regression equations were calculated as y=0.088+0.999x (Table 2 and Figure 1).

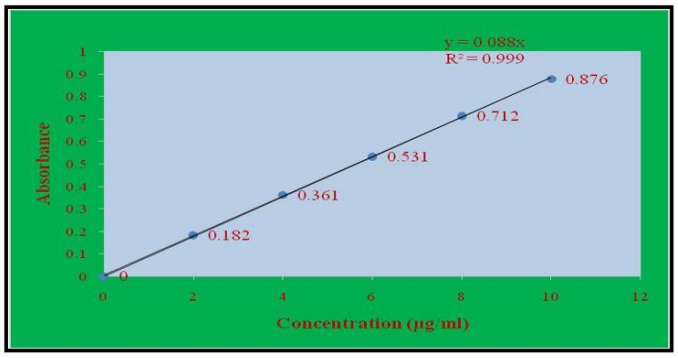

Figure 1: **Calibration curve of Furosemide in pH 5.8 phosphate buffer.**

Table 2: **Calibration curves data of Furosemide using pH 5.8 phosphate buffers.**

S.No.	Concentration (µg /ml)	Absorbance
		pH 5.8 phosphate buffer
1	2	0.182
2	4	0.361
3	6	0.531
4	8	0.712
5	10	0.876

Assay validation of calibration curves

Validation is an analytical procedure by which laboratory studies was done that the performance characteristics of the method obtain the need for the analytical applications. Assay must demonstrate that the analysis method capable to predict the concentrations of unknown samples accurately and precisely. The calibration curves have thus been validated for the assay of active constituent i.e. Furosemide, using the following discussed parameters.

Precision

For the precision of an analytical procedure is used. If the procedure is used repeatedly to multiple homogenous sampling analysis accuracy achieved the intra and interday variation. Calibration curve of Furosemide was prepared in pH 5.8 phosphate buffers. Intraday precision calibration curves prepared in 5.8 pH phosphate buffer were run in triplicate in same day for 3 times and for the inter day precision, calibration curves were prepared in pH 5.8 phosphate buffer were run for three days and % RSD were calculated for both the cases which should be less than 2%. Table 3 and figure 2 shows the intraday precision studies and Table 4 and figure 3 shows the inter day precision studies for calibration curves prepared in pH 5.8 phosphate buffer. For precision of calibration curve prepared in pH 5.8 phosphate buffer, the range of S.D. was 0.0015–0.0045 for the intraday and 0.0030–0.0050 for the interday.

Table 3: **Calibration curve was prepared for intraday precision study in 5.8 pH phosphate buffer.**

S. No	Concentration (µg /ml)	pH 5.8 phosphate buffer				
		Absorbance			Mean	S.D
1	2	0.177	0.182	0.184	0.181	0.0036
2	4	0.354	0.35	0.361	0.355	0.0055
3	6	0.532	0.535	0.541	0.536	0.0045
4	8	0.702	0.706	707	0.705	0.0026
5	10	0.883	0.874	0.88	0.879	0.0045

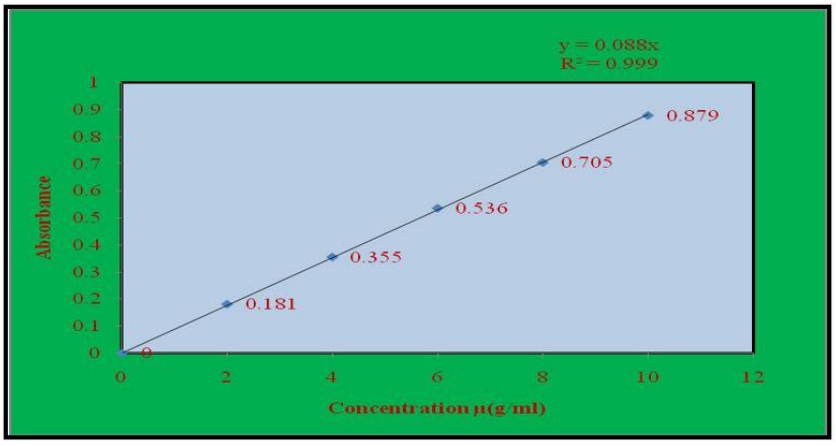

Figure 2: **Calibration curve of Furosemide for Intraday variation in pH 5.8 phosphate buffer.**

Table 4: **Calibration curves was prepared for Interday precision study in 5.8 pH phosphate buffer.**

S.No	Concentration (mcg /ml)	pH 5.8 phosphate buffer				
		Absorbance			Mean	S.D
		A	B	C		
1	2	0.175	0.18	0.182	0.179	0.0036
2	4	0.35	0.351	0.355	0.352	0.0026
3	6	0.532	0.533	0.537	0.534	0.0026
4	8	0.712	0.713	0.717	0.714	0.0045
5	10	0.871	0.875	0.876	0.874	0.0038

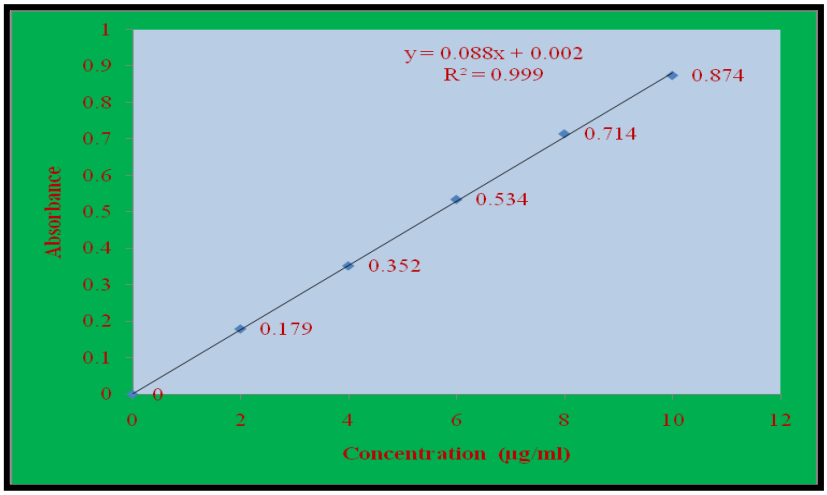

Figure 3: **Calibration curve of Furosemide for Interday variation in pH 5.8 phosphate buffer.**

Linearity

In analytical method Linearity is capability to bring out test proceed that are mathematical alteration, proportional to the concentration of analysis in samples within a provide range. Reading from the reversion line is helpful to given mathematical determination of the length of distance. The distance and range data for calibration curves making in various buffers.

Limit of detection and limit of quantitation

Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantities, under a stated experimental conditions and the limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. These two parameters are required for assay validation as per ICH Q2A guidelines. Limit of detection and limit of quantitation of calibration curves were calculated (Siddiqui et al 2006; Cartensen and Rhodes 2000) which was based on the standard deviation of y–intercept of regression line (SD) and the slope (S) of the calibration curves at levels approximating the LOD and LOQ, LOD=3.3 (SD /S) and LOQ=10(SD /S). LOD and LOQ of calibration curve of Furosemide prepared in pH 5.8 phosphate buffer are shown (Table 5).

Table 5: **Preparation of calibration curve of other validation parameters phosphate buffer in 5.8 pH.**

Parameters	Phosphate buffer of pH 5.8
Dimension Correlation coefficient	0.999
y – intercept	0.088x
Slope	0.173
Range	2 -10 µg / ml
LOD	0.322 µg / ml
LOQ	1.298µg / ml

Compatibility studies

Infra-red spectrophotometer is used for performing compatibility analysis. The pure drug and physical mixture of drug and polymer were analysis with the help of infra-red spectroscopy. The drug excipients interaction performs an important role with the respect to deliver of drug from the dosage form from other, Fourier transform infrared procedure have been used here to estimation of the physical and chemical interaction between drug and excipients used. It was watch that there is no incompatibility found between Furosemide and used polymer. It was seen that there was no alteration found in these main peaks in Infra-Red spectroscopy of mixture of drug and polymer, which indicate there were no physical interaction because of them between drug and polymer produced some bond between each other. The peak achieved in the spectra of each dosage form correlate with the peak of drug spectrum. This indicate that the drug was not incompatible with the dosage form components.

Drug delivery profile analysis

The in-vitro drug release profiles of the granules made by melt granulation technique (F4-F6) were compared with unmixed drug. The cumulative percentage release data was shown in Table 6 and Figure 4. In the taken food condition pH of stomach was about pH 2.0 –pH 6.5. Thus pH 5.8 was taken as in the taken food condition stomach. In fed state, at pH 5.8, the pure drug showed 99.79 % while formulation F4, F5 and F6 showed 83.92 %, 79.15 % and 76.84 % drug release after 9 hours, respectively. As the Gelucire 43/01 ratio was increased in the formulations F4 to F6 prepared by melt granulation technique. The release rate was lowered due to water soluble of Gelucire 43/01.

Release kinetics of preliminary optimized formulations

Regression coefficient, n and k values were obtained for zero order, first order, from the values obtained from % of drug deliver. The in-vitro drug deliver profiles were clarified by the data obtained from model fitting. Model dependent parameters indicate zero order models as the best fit model. It was concluded that formulation F4 prepared by melt granulation with Drug: Gelucire 43/01 ratio 1:0.5 followed zero order release kinetics as the best fit model.

Table 6: **In-vitro drug release data of preliminary optimized formulations in pH 5.8 phosphate buffer.**

S. No.	Time intervals (hours)	Cumulative percentage drug release in pH 5.8 phosphate buffer			
		Pure drug	F4	F5	F6
1	0	0	0	0	0
2	1	62.43 ± 0.416	18.74 ± 0.319	22.81 ± 0.517	27.12 ± 0.198
3	2	78.50 ± 0.526	26.71 ± 0.125	28.11 ± 0.317	32.11 ± 0.510
4	3	86.62 ± 0.528	32.61 ± 0.317	37.15 ± 0.318	39.17 ± 0.157
5	4	92.61 ± 0.721	41.51 ± 0.550	41.61 ± 0.518	45.61 ± 0.318
6	5	96.26 ± 0.316	50.81 ± 0.710	51.18 ± 0.314	55.21 ± 0.341
7	6	99.82 ± 0.219	61.61 ± 0.218	61.41 ± 0.218	67.81 ± 0.319
8	7	99.61 ± 0.116	68.81 ± 0.210	77.61 ± 0.110	77.61 ± 0.181
9	8	99.65 ± 0.158	76.84. ± 0.510	79.15 ± 0.516	83.92 ± 0.424

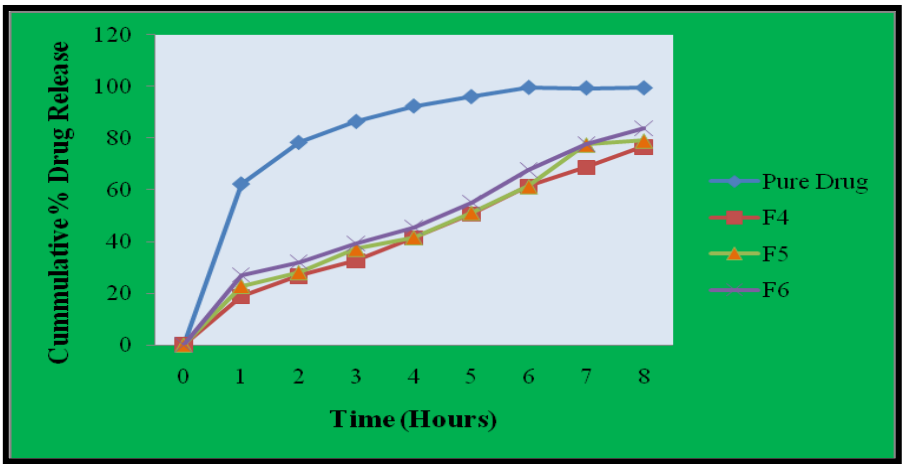

Figure 4: **Comparative %drug release profile in pH 5.8 phosphate buffer.**

Selection of optimized formulation

The formulations F4 to F6 were screened for different evaluation parameters like micromeritic feature, percentage in-vitro unsinkable ability, percentage yield and drug content and in vitro drug deliver. Model dependent parameters were calculated. It was concluded that formulation F4 prepared by melt granulation with Drug: Gelucire 43/01 ratio 1:0.5 followed zero order release kinetics as the best fit model.

Effect of aging

In-vitro drug delivery

In-vitro drug deliver figuration of granules on become older (after 10 and 30 days) were shown on aging

increase the drug deliver, which may be apply for to the phase complete change. The unsinkable capacity of the granules was not influence by become older. The zero-order kinetics shown by aged granule, so the drug deliver kinetics were no influence by become older (Table 7 and Figure 5).

Table 7: **In-vitro drug delivery data of optimized dosage form (F4) in pH 5.8 phosphate buffer after aging.**

S.No.	Time intervals (hr)	Cumulative percentage drug release in pH 5.8 phosphate buffer		
		1st Day	10th Day	30th Day
1	0	0	0	0
2	1	18.74	30.56	31.1
3	2	26.71	36.54	37.12
4	3	32.61	42.96	43.54
5	4	41.51	49.12	50.1
6	5	59.12	60.45	62.15
7	6	69.57	69.98	70.5
8	7	78.73	80.12	82.12
9	8	85.95	86.45	87.49

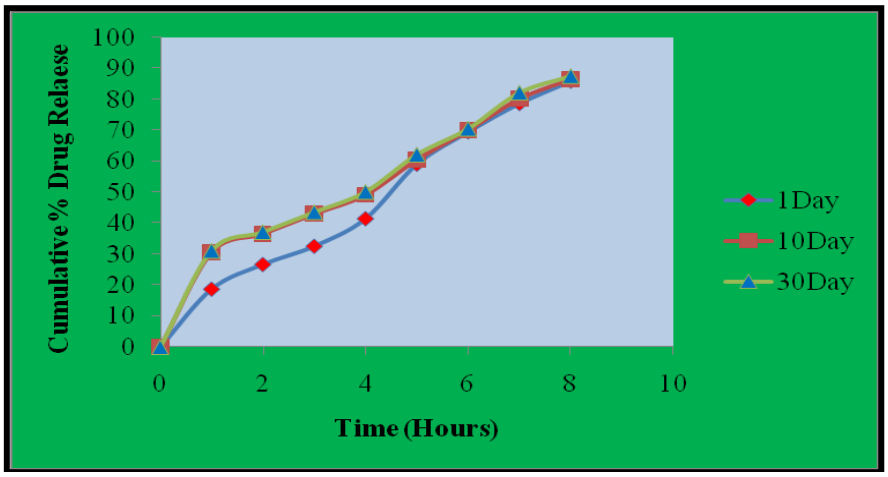

Figure 5: **Release profile of Furosemide from formulation F4 showing effect of aging.**

Conclusion

The aim of the study was to successfully develop and evaluate unsinkable granular release setup with an objective to prevent the delivery ratio of Furosemide. Different developed formulation was evaluated for the quality test and the floating ability of the granules and the deliver ratio of the drug (Furosemide). It was determined that the granules size can be controlled by changing the composition ratio of Gelucire 43/01. Formulation F4 (Drug: Gelucire 43/01 ratio 1:0.5) exhibited good buoyancy and drug release (98%) with zero order release pattern were taken as best formulation for further evaluation. The study demonstrated that water soluble lipid Gelucire 43/01, can be used as a drug transporter for developing multi-unit buoyant drug release setup of Furosemide.

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