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Formulation development and evaluation of controlled release gastro-retentive advance delivery system of furosemide

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Abstract

The most important use of furosemide is in the treatment of hypertension or pulmonary edema because no other group of diuretics is more effective than the loop diuretics in this situation. But there are certain major drawbacks with the conventional therapy of furosemide.Optimized formula was selected by desirability (numerical optimization) technique. Three formulation batches of the predicted optimized formulation of gastroretentive bilayer tablet were prepared and experimentally validated by comparision method. Comparison of the observed and software predicted responses showed a good correlation. This demonstrated that the factorial design optimization technique was successful in the formulation development of gastroretentive layer of furosemide. Finally bilayer tablets were prepared using optimized solid dispersion and optimized floating layer. The final bilayer tablet was evaluated and was found to be within the limits.

Keywords: furosemide, immediate release layer, controlled release gastro-retentive advance delivery system

Introduction

Conventional dosage forms offer only limited advantage for drugs that have an absorption window in the upper small intestine e.g. furosemide because once emptied from the stomach the passage through upper small intestine is rapid, thus limiting the extent of absorption at this site. So, in order to increase the bioavailability of this type of drug the residence time of controlled release dosage form in the upper g.i.t. needs to be prolonged. Following approaches can be used to prolong the residence time of the dosage form in the upper part of gastro intestinal tract.

- High density systems.
- Expandable systems.
- Mucoadhesive systems.
- Floating system

Hydrotropy

The term hydrotropy refers to solubilization process whereby addition of large amount of second solute results in increase in aqueous solubility of poorly soluble compound [1]. The term was first introduced by Neuberg [2]. (1916) to designate anionic organic salts. According to Neuberg, hydrotropic agents are metal salts of organic acids, which at fairly high concentration considerably increase the aqueous solubility of organic substances normally slightly soluble in water. However the term has been used in the literature to designate non- micelle forming substances either liquids or solids, organic or inorganic capable of solubilizing insoluble compounds [3-6].

The mechanism by which the hydrotropic effect occurs is not clear. Some workers have speculated that hydrotropy is simply another type of solubilization, with the solute dissolved in oriented clusters of the hydrotropic agents [4]. Hydrotropic solutions do not show colloidal properties, however[5] others feel that this phenomenon is more closely related to complexation involving a weak interaction between the hydrotropic agent and the solute. Still other reason that the phenomenon must be due to a change in solvent character because of the large amount of additive needed to bring about the increase in solubility.

The influence of large concentration of sodium benzoate on the solubility of caffeine is a classic example of this phenomenon applied to a pharmaceutical system [7]. Other examples include the solubilization of benzoic acid with sodium benzoate.

The chemical structure of the conventinal Neuberg’s hydrotropic salts (prototype, sodium benzoate) consists generally of two essential parts, an anionic group and a hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is a prerequisite for a hydrotropic substance. The type of anion or metal ion appeared to have a minor effect on the phenomenon (Neuberg 1916). On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization [8]. This should imply that hydrotropic agents are molecules having a planar hydrophobic structure brought into solution by a polar group. Hence, it seems rational to propose that molecules with a planar hydrophobic part and a polar group, which is not necessarily anionic, can act as hydrotropic agent [9]. Hydrotropic compounds are compounds that at high concentration solubilize poorly soluble molecule in water [2]. At concentrations higher than minimum concentrations in water, hydrotropic agents self-associate and form non-covalent assemblies of lower polarity i.e. non-polar microdomains which solubilize hydrophobic solutes. The self- aggregation of hydrotropic agents is different from the surfactant self-assemblies in that, hydrotropes form planar or open layer self-assemblies instead of compact spheroid assemblies. Hydrotropic agents are characterized by a short, bulky compact moiety while surfactants have long hydrocarbon chain. In general, hydrotropic agents have a shorter hydrophobic segment, leading to high water solubility than surfactants. The hydrotropy is suggested to be superior to other solubilization methods such as micellar solubilization, cosolvency, miscibility and salting in, because the solvent character is independent of pH, has high selectivity and does not require emulsification.

Each hydrotropic agent is effective in increasing solubility of certain hydrophobic drugs. No universal hydrotropic agents have been found to be effective to solubilize all hydrophobic drugs. Thus, finding the right hydrotropic agent for a poorly soluble drug requires screening of large number of hydrotropic candidates. However, once the correct hydrotropic agent is identified for a series of structurally different drugs, the structure activity relationship can be estabilished. Of the various approaches used, hydrotropic approach is the promising approach with great potential for pooly soluble drugs. For example, if the solubility of poorly soluble compound increases by 2-4 folds in presence of hydrotropic agent, the oral absorption and bioavailability is also expected to increase in the similar extent.

In conclusion, hydrotropy is one of the easiest ways to solubilize the poorly soluble drug, since it only requires the mixing of poorly water-soluble drug in water. This approach does not require chemical modification of the drug, neither use of organic solvents nor preparation of emulsion systems.

Research Envizaged

1. Objective

Furosemide is used as a diuretic for the treatment of edema that may accompany C.H.F., cirrhosis of liver and nephrotic syndrome. But it is of special interest to note that the most important use of furosemide is in the treatment of pulmonary edema because no other group of diuretics is more effective than the loop diuretics in this situation and usually i.v. therapy of furosemide is used for such emergency clinical situations so that fast onset of action can be obtained within 5 minutes [10].

But there are several limitations with the parental therapy for ex. unavailability of trained personnel to inject i.v. dose in emergency conditions. On the other hand, onset on action with conventional tablet is slow because of poor solubility of furosemide in aqueous contents of stomach. Thus objective of the project was to develop such an oral dosage form which can provide immediate release of orally administered furosemide so that fast onset of diuretic action can be achieved within few minutes and thus parental therapy of furosemide can be replaced by oral therapy and at the same time retaining the advantage of parental therapy.

2. Objective

Secondly when furosemide is employed in treatment of pulmonary edema it is of paramount importance to inject furosemide at a controlled rate of not exceeding 4 mg/min[71], because rapid injection may result in acute reduction in plasma volume, decreased venous return and cardiac output and an exacerbation of heart failure. Also reports have been published indicating furosemide ototoxicity with rapid injection.

So, for a 10 mg injection it requires at least 2-3 minutes for administration of bolus injection, which is very inconvenient to the patient and also requires a lot of technical skills while injecting. So the objective of the project was also to avoid the risk of heart failure & ototoxicity by rapid injection, but at the same time providing quick onset of action (few mintues) by increasing dissolution quickly using hydrotropic agents. After oral administration, the immediate dissolution of furosemide in *unionized form* in the aqueous contents of stomach can increase the solubility as well as permeability of furosemide which is a class IV drug according to B.C.S. classification and can also provide tremendous patient compliance by avoiding the need of injection for 2-3 min.

3. Objective

Orally administered furosemide has poor bioavailability (35-60%) due to the presence of biological window comprised of the upper gastrointestinal tract. Once emptied from stomach the passage through the upper small intestine is rapid thus limiting the extent of absorption at duodenum. Thus only a fraction of dose is absorbed by duodenum from conventional tablets and the remaining unabsorbed drug is wasted. Also $t_{½}$ of orally administered furosemide is short i.e. 2-3 hrs, thus requiring repetitive administration of 2-4 times a day (depending upon the extent of diuresis required). This leads to increase and decrease of furosemide in blood level and therefore the total amount of drug administered is significantly higher.

So the third objective of the project was to retain the prolonged release layer in the stomach from which furosemide can be released for long duration of time. The controlled amount of furosemide that will be released in the stomach will empty together with fluid and will have the whole surface area of duodenum for absorption thus the extent of absorption i.e. bioavailability will automatically increased. Apart from this the duration of action will also be prolonged because of the dosage form in the stomach will be retained for longer duration of time. Thus the fluctuation in the blood level will also decrease and a constant blood level can be achieved.

In brief the major objective of the project is to develop such a formulation which can

- Provide immediate onset of diuretic action by oral route.
- Avoid the side effects of rapid injection
- Increase the oral bioavailability of furosemide.
- Prolong the duration of diuretic action thus improving the patient compliance.

Material and Method

Following Drugs & materials are used in this method. Furosemide

Drug Identification Tests as per I.P 1996

6.1.1 U.V. Absorption [11].

The light absorption in the range 220 to 360 nm of a 0.0005% w/v (5 µg/ml) solution in 0.1 M sodium hydroxide was determined and the ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 228 nm was noted. The procedure was repeated thrice and results are shown in Table 6.1.

Table 1: UV absorbances of furosemide

S. No.	Absorbance			Ratio of Absorbances
	228 nm	271 nm	333 nm	
1.	0.555	0.312	0.064	0.562
2.	0.554	0.314	0.060	0.567
3.	0554	0.314	0.060	0.567

6.1.2 Melting Point Test [12].

The melting point of furosemide was determined using open capillary method. Furosemide was packed into capillary and the filled capillary was placed in Thiel’s tube filled with paraffin oil. Temperature was noted (Table 6.2) when last particle of furosemide disappeared. The average of three values was considered as the melting point of drug.

Table 2: Melting point determination

S. No	Melting Point (°C)	Average
1	208.0	207.3
2	207.6	
3	206.4	

6.2 Prepration of Calibration Curve of Furosemide

Accurately weighed 50 mg of furosemide was dissolved in 900 ml of D.M. water in a 1000 ml volumetric flask and then volume was made upto 1000 ml with D.M. water. Appropriate dilutions were made with D.M. water so as to obtain a series of solutions in concentration range of 10 – 50 µg/ml. The absorbances of dilutions were measured on Shimadzu U.V. – 1700 A double beam spectrophotometer at λ_{max} of 333 nm against respective reagent blanks. This procedure was done in triplicate and the average was calculated. The absorbance data obtained are shown in Table 6.3.

Table 3: Absorbance data for calibration curve of furosemide in D.M. water

S.No	Concentration (µg/ml)	Absorbance (333nm)
1	10	0.148
2	20	0.297
3	30	0.439
4	40	0.591
5	50	0.748

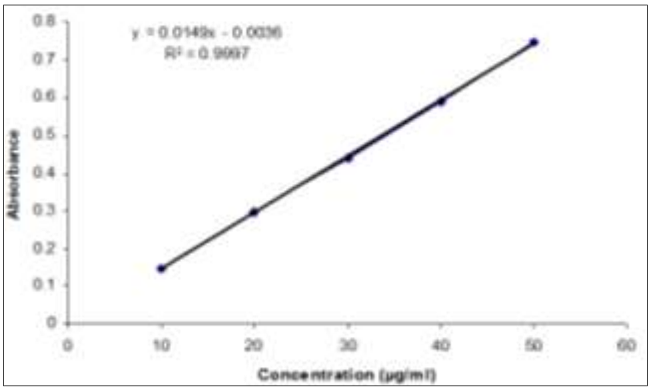

Fig 1: Calibration curve of furosemide in demineralized water

The same procedure was repeated with 0.1 N HCl and their results are shown in

Table 4: Absorbance data for calibration curve of furosemide in 0.1 N HCl

S.No	Concentration (µg/ml)	Absorbance (333nm)
1	10	0.154
2	20	0.309
3	30	0.471
4	40	0.612
5	50	0.749

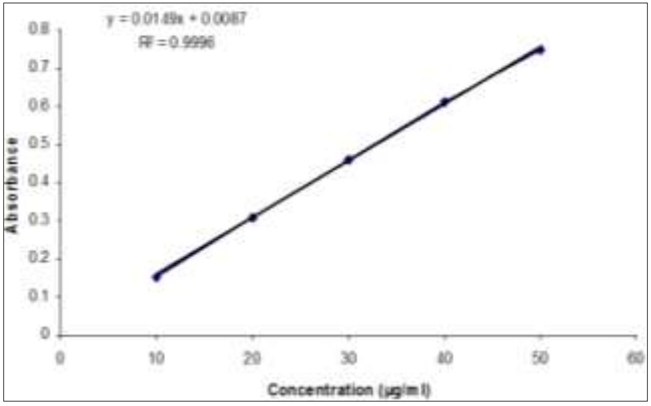

Fig 2: Calibration curve of furosemide in 0.1N HCl

Then, same procedure was repeated using different hydrotropic solutions to obtain regression equation (Table 6.5) for the estimation of furosemide in respective blends of hydrotropic solutions.

Table 5: Regression equations of furosemide in different hydrotrope blends

S. No	Hydrotrope in D.M. water	Regression equation	R^2
1	U	Y=0.0149X+0.0122	0.9950
2	S.A.	Y=0.0151X+0.0173	0.9940
3	S.C.	Y=0.0151X+0.0103	0.9992
4	S.B.	Y=0.0145X+0.0227	0.9984
5	U+A	Y=0.0147X+0.0206	0.9987
6	U+B	Y=0.0157X+0.0064	0.9977
7	U+C	Y=0.0145X+0.0315	0.9955
8	A+B	Y=0.0143X+0.0250	0.9979
9	A+C	Y=0.0147X+0.0162	0.9982
10	B+C	Y=0.0152X+0.005	0.9997
11	U+A+B	Y=0.0149X+0.0102	0.9993
12	U+A+C	Y=0.0153X+0.0061	0.9981
13	A+B+C	Y=0.0147X+0.0171	0.9902
14	U+B+C	Y=0.0149X+0.0086	0.9955
15	U+A+B+C	Y=0.0151X+0.0090	0.9970

Where, U= urea, A= sodium acetate, B = sodium benzoate, C = sodium citrate

6.3 Solubility of Furosemide

Accurately measured 5 ml of D.M. water was taken in a volumetric flask and excess amount of drug was added and shaken for 12 hr in Orbital Flask Shaker (Khera instruments Pvt. Ltd., India). The solution was allowed to equilibrate for 24 hrs. Then, solution was centrifuged at 2000 r.p.m. for 5 minutes in ultra-centrifuge and was filtered through Whatman grade 41 filter. Aliquot was suitably diluted and analyzed using UV spectrophotometer at 333 nm. Same procedure was repeated with 0.1N HCl to determine the solubility of furosemide in 0.1N HCl. Results are shown in Table 6.6.

Table 6: Furosemide solubility

S. No.	Solvent	Solubility of furosemide (µg/ml)
1	D.M. water	82
2	0.1 N HCl	67

6.4 Furosemide Hydrotropic Agent Interference Study

For determination of interference of hydrotropic agents in the spectrophotometric estimation of furosemide, the absorbances of the standard solutions of furosemide were determined in D.M. water alone and in the presence of the hydrotropic blend employed for formulation purpose.

The absorbances were recorded against respective reagent blanks at appropriate wavelengths and results are shown below in Table 6.7. A uv-visible recording spectrophotometer (Shimadzu, U.V. 1700 A Japan) with 1 cm matched silica cells was employed for spectrophotometric determinations.

Table 7: Drug-hydrotrope interference study

Drug	Solvent system used	Drug conc. (µg/ml)	Hydrotrope conc. (µg/ml)	Wave-length (nm)	Absorbance against resp. blank
Furosemide	D.M. Water + S.A	20	1000	333	0.301
Furosemide	D.M. Water + S.B	20	1000	333	0.306
Furosemide	D.M. Water +S.C	20	1000	333	0.300
Furosemide	D.M. Water + Urea	20	1000	333	0.303
Furosemide	D.M. Water	20	1000	333	0.301

6.5 Approximate Solubility Determination in Different Blends of Hydrotropic Agents

Initially, weight of a 10 ml volumetric flask filled with 3 ml of a particular blend of hydrotropic solution was determined. Then small aliquot of drug was added and flask was shaken manually so as to dissolve the drug. When drug got solubilized, further small aliquots were added and procedure was repeated till hydrotropic solution got nearly saturated. Then, weight of volumetric flask with saturated solution was determined. Finally, the difference in weight gave the approximate amount of drug dissolved in 3 ml of hydrotropic solution, from which the approximate percentage of drug solubilized was calculated.By this technique, rough estimation of solubility enhancement by a particular hydrotropic blend was determined. Equilibrium solubility was determined only for those blends in which considerable enhancement in solubility was obtained.

6.6 Equilibrium Solubility Studies in Different Blends of Hydrotropic Agents

Accurately measured 3 ml of a particular blend of hydrotropic agent was taken in a 10 ml volumetric flask and excess amount of drug was added and mechanically shaken until saturated solution was formed. The volumetric flask was shaken on mechanical shaker for 12 hrs so that equilibrium solubility can be achieved and solution was allowed to equilibrate for 24 hrs. Then solution was centrifuged at 2000 r.p.m. for 5 minutes in ultra-centrifuge and then solution was filtered through Whatman grade 41 filter. Aliquot was suitably diluted with D.M. water and analyzed using UV spectrophotometer at 333 nm.

Initially solubility of furosemide was determined individually in 4 hydrotropic agents namely urea (U), sodium acetate (A), sodium benzoate (B), sodium citrate (C) at concentration of 10%, 20%, 30% and 40% solutions. (Table 6.8)

From the results of above studies it was concluded that solubility of furosemide was increasing with increasing concentrations of hydrotropic agents, for example solubility in 40 % urea solution was found to be much higher than solubility in 10%, 20% or 30% urea solutions.

Table 8: Equilibrium solubility of furosemide in different hydrotropic blends

S. No.	Hydrotropic Agents	Concentration (w/v)				Solubility enhancement ratio
		10%	20%	30%	40%	
1	Urea	0.067	0.094	0.131	0.191	23.875
2	Sodium Acetate	0.013	0.078	0.142	0.239	29.857
3	Sodium Benzoate	0.283	0.627	1.171	2.157	296.632
4	Sodium Citrate	0.015	0.034	0.060	0.129	16.125

Highest solubility was obtained in 40% sodium benzoate solution. Then, in order to decrease the concentration of sodium benzoate, different combinations of above mentioned 4 hydrotropic agents in different ratios were tried to determine Enhancement in solubility, so that total concentration of hydrotropic agents was always 40% w/v.

So, all possible combinations of 2 hydrotropic agents were taken in such a way that total concentration was always 40% with fixed ratio of 20:20. As shown in Table 6.9.

Table 9: Equilibrium solubility of furosemide in different hydrotropic blends

S. No.	Combination	Total Conc. (%w/v)	Individual conc. (%w/v)	Solubility (%w/v)	Solubility enhancement ratio
1	U + A	40.00	20.00	0.651	81.375
2	U + B	40.00	20.00	2.909	363.625
3	U + C	40.00	20.00	0.943	117.875
4	A + B	40.00	20.00	2.148	268.516
5	A + C	40.00	20.00	0.067	8.375
6	B + C	40.00	20.00	3.005	375.625

Where, U= urea, A= sodium acetate, B = sodium benzoate, C = sodium citrate

Then all possible combinations of 3 hydrotropic agents were used to determine the solubility so that total concentration was again 40 %, but individual concentration was 13.3 % w/v (Table 6.10).

Table 10: Equilibrium solubility of furosemide in different hydrotropic blends

S.No.	Combination	Total Conc. (%w/v)	Individual conc. (%w/v)	Solubility (%w/v)	Solubility enhancement ratio
1	U +A +B	40.00	13.33	1.918	239.756
2	U+A +C	40.00	13.33	0.243	30.375
3	A+B +C	40.00	13.33	0.926	115.754
4	U+B+C	40.00	13.33	3.958	494.752

Where, U= urea, A= sodium acetate, B = sodium benzoate, C = sodium citrate

The blend with maximum solubility enhancement (U+B+C) was further Explored by changing the ratio so that maximum solubility can be obtained (Table 6.11).

Table 11: Equilibrium solubility of furosemide in different hydrotropic blends

S.No.	Combination	Total Conc. (%w/v)	Ratio	Solubility (%w/v)	Solubility enhancement ratio
1	U+B+C	40.00	10:20:10	4.782	597.751
2	U+B+C	40.00	10:10:20	1.934	241.759
3	U+B+C	40.00	15:20:5	5.285	660.625
4	U+B+C	40.00	5:20:15	3.405	425.625

Where, U= urea, A= sodium acetate, B = sodium benzoate, C = sodium citrate

Finally, combinations of four hydrotropic agents were used to determine the solubility so that total concentration was again 40 %, but ratio of individual agent was 10: 10: 10: 10. This blend was also further explored by changing the ratio so that blend with maximum solubility can be obtained Table 6.12.

Table 12: Equilibrium solubility of furosemide in different hydrotropic blends

S. No.	Combina-tion	Total Conc. (%w/v)	Ratio	Solubility (%w/v)	Solubility enhancement ratio
1	U+A+B+C	40.00	10:10:10:10	1.183	147.875
2	U+A+B+C	40.00	5: 5: 10: 20	1.953	244.125
3	U+A+B+C	40.00	5: 20: 10: 5	1.132	141.575
4	U+A+B+C	40.00	20: 5: 10: 5	3.085	385.625
5	U+A+B+C	40.00	10: 5: 20: 5	4.524	565.529
6	U+A+B+C	40.00	15: 5: 15: 5	4.247	530.875

Conclusion

The blend U+B+C in the ratio of 15:20:5 gave the highest solubility enhancement, and therefore, this optimized combination of hydrotropes was selected for the preparation of solid dispersions.

Results and Discussion

Formulation of Hydrotropic Solid Dispersions of Furosemide

For preparation of hydrotropic solid dispersion in 1:6 ratio, accurately weighed 2.25 gm urea, 3 gm of sodium benzoate, 0.75 gm of sodium citrate (so that total weight of the mixture was 6 gm) were taken in a 100 ml beaker and were mixed properly. Then, minimum possible quantity of warm, demineralized water sufficient to dissolve the above mixture was added, because lesser the amount of water lesser will be the time required to evaporate it and chemical stability of drug may not be affected adversely (during removal of water). Dissolution of the hydrotropic mixture was facilitated by agitation of a teflon coated magnetic rice bead on a high speed magnetic stirrer. After complete dissolution of hydrotropic mixture, 1 gm of furosemide was dissolved in the above solution and temperature was maintained in the range of 55- 60ºC so as to facilitate the evaporation of water. As evaporation proceeded, speed of rice bead automatically decreased and it stopped stirring when most of the water was evaporated, thus indicating the formation of solid dispersion (wet).

Table 13: Composition of hydrotropic solid dispersion

S. No.	Drug: Hydrotrope blend	Quantity taken (GM)			
		Furosemide	Urea	Sodium Benzoate	Sodium Citrate
1	1:6	1.00	2.25	3.00	0.75
2	1:8	1.00	3.00	4.00	1.00
3	1:10	1.00	3.75	5.00	1.25
4	1:12	1.00	4.50	6.00	1.50

The wet solid dispersion thus obtained were spread on several watch glasses and the watch glasses were kept in hot air dry oven maintained at 50± 2ºC so that remaining moisture could also be evaporated easily and a constant weight with no further weight loss (due to evaporation) could be obtained. After complete drying, solid dispersions were crushed using a glass pestle mortar and passed through sieve # 60 and were finally stored in an air tight glass bottle. Same procedure was utilized to prepare hydrotropic solid dispersion in the ratio of 1:8, 1:10, 1:12, using appropriate quantity of hydrotropic agents (Table 6.13).

6.8 Formulation of Physical Mixture of Furosemide

For preparing physical mixture in 1:6 ratio, accurately weighed 1gm of furosemide, 2.25 gm of urea, 3 gm sodium benzoate and 0.75 gm of sodium citrate were mixed using geometric dilution technique and were intensely triturated using glass pestle mortar. After complete mixing, the powder mass was passed through sieve # 60 and was finally stored in an air tight glass bottle. Same procedure was utilized to prepare physical mixture in the ratio of 1:8, 1:10, and 1:12 using appropriate quantity of hydrotrope Table 6.13).

6.9 Determination of Drug Content in Solid Dispersion and In Physical Mixture

Powered solid dispersion or physical mixture equivalent to 20 mg of furosemide was accurately weighed and transferred to a 100 ml volumetric flask.

Approximately 30-40 ml of demineralized water was added and flask was shaken to dissolve the contents completely and the volume was made upto the mark with demineralized water.

Table 14: Drug content of furosemide in solid dispersions or physical mixture

S. No.	Drug: Hydrotrope blend	Drug content (mg)	
		Solid dispersion	Physical mixture
1	1:6	20.33	20.07
2	1:8	19.60	21.19
3	1:10	20.73	19.60
4	1:12	18.68	20.53

Further 1 ml of the above solution was diluted upto 10 ml with demineralized water and absorbance of this solution was measured at 333 nm against corresponding reagent blank. The analysis was carried out in triplicate and drug contents were determined. Results of the analysis are shown in the Table 6.14.

6.10 Dissolution Rate Studies

Dissolution tests are one of the most widely used tests in quality control of dosage forms. Dissolution tests become especially important when dissolution is the rate limiting step as in the case of B.C.S. class II or B.C.S. class IV drugs. In order to select the optimum ratio of solid dispersion or physical mixture for final formulation, dissolution rate studies were performed. The ratio of solid dispersion with highest dissolution rate i.e. fastest dissolution was selected for preparing immediate release layer of the final formulation.

6.10.1 Procedure

Solid dispersion or physical mixture equivalent to 20 mg of furosemide were tested in dissolution rate studies using U.S.P. XXIV (type II) dissolution test apparatus (Model TDT6P, Electrolab Mumbai, India) with paddle to rotate at 50 r.p.m. 900 ml of 0.1 N HCl was taken as dissolution media with temperature of 37± 0.5°C. At definite time interval 10 ml of the sample were withdrawn and were analyzed for drug content. Withdrawn samples were also replaced with fresh dissolution media. Calculations for the amount of drug were done using respective regression equations and the results of the dissolution studies are shown from Table 6.15-6.24.

Table 15: Dissolution rate studies of solid dispersion of 1: 6 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.336	22.192	19.972	99.864
2	5	0.334	22.059	19.853	99.268
3	10	0.329	21.728	19.555	97.778
4	20	0.330	21.794	19.615	98.076
5	30	0.329	21.728	19.555	97.778

Table 16: Dissolution rate studies of solid dispersion of 1: 8 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.329	21.7211	19.552	97.775
2	5	0.331	21.860	19.674	98.374
3	10	0.329	21.661	19.493	97.484
4	20	0.328	21.664	19.498	97.489
5	30	0.326	21.259	19.376	96.688

Table 17: Dissolution rate studies of solid dispersion of 1: 10 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.338	22.324	20.092	100.461
2	5	0.334	22.059	19.853	99.268
3	10	0.335	22.125	19.913	99.566
4	20	0.329	21.728	19.555	97.779
5	30	0.331	21.860	19.674	98.374

Table 18: Dissolution rate studies of solid dispersion of 1: 12 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.328	21.663	19.495	97.482
2	5	0.326	21.529	19.375	96.885
3	10	0.326	21.529	19.377	96.883
4	20	0.327	21.596	19.436	97.183
5	30	0.324	21.397	19.257	96.288

Table 19: Dissolution rate studies of physical mixture of 1: 6 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.121	7.953	7.158	35.793
2	5	0.165	10.867	9.780	48.905
3	10	0.198	13.052	11.747	58.738
4	20	0.232	15.301	13.774	68.870
5	30	0.243	16.033	14.429	72.141

Table 20: Dissolution rate studies of physical mixture of 1: 8 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.118	7.754	6.979	34.89
2	5	0.143	9.410	8.469	42.347
3	10	0.207	13.649	12.284	61.420
4	20	0.219	14.44	12.993	64.996
5	30	0.254	16.761	15.085	75.427

Table 21: Dissolution rate studies of physical mixture of 1: 10 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.184	12.125	10.913	54.666
2	5	0.197	12.986	11.688	58.440
3	10	0.234	15.437	13.893	69.466
4	20	0.268	17.688	15.919	79.599
5	30	0.295	19.476	17.529	87.645

Table 22: Dissolution rate studies of physical mixture of 1: 12 ratio

S. No.	Time (min)	Absorbance	Concentration (µg/ml)	Amount (mg)	% Drug Dissolved
1	1	0.159	10.470	9.423	47.11
2	5	0.184	12.125	10.913	54.566
3	10	0.269	17.754	15.979	79.897
4	20	0.273	18.019	16.217	81.089
5	30	0.311	18.152	16.337	81.685

Table 23: Comparative account of different ratios of solid dispersions

S. No.	Time (min)	Cumulative percent Dissolved			
		1:6	1:8	1:10	1:12
1	1	97.48	97.77	100.46	97.48
2	5	96.88	98.37	99.268	96.88
3	10	96.88	97.48	99.566	96.88
4	20	97.18	97.48	97.77	97.18
5	30	96.34	96.688	98.374	96.288

Table 24: Comparitive account of different ratios of physical mixture

S. No.	Time (min)	Cumulative percent Dissolved			
		1:6	1:8	1:10	1:12
1	1	35.79	34.89	54.666	47.11
2	5	48.90	42.347	58.440	54.566
3	10	58.738	61.420	69.466	79.897
4	20	68.870	64.996	79.599	81.089
5	30	72.14	75.427	87.645	81.685

Conclusion

From the above studies, it is evident that all the ratios of solid dispersions were dissolved completely within 1 minute, and when observed visually, they were found to be dissolved only within 10-20 seconds. While, on the other hand, none of the physical mixture dissolved completely even after 30 minutes.Since there was no significant difference in dissolution rate of different ratios of solid dispersions, therefore 1: 6 ratio was considered to be optimum ratio and was used for further studies.

6.11 Micromeritic Properties of Solid Dispersions

Any method of measuring powder flow must be practical, useful, reproducible and sensitive, and must yield meaningful results, but actually no simple powder flow method is adequate or complete to characterize the wide range of flow properties experienced in the pharmaceutical industries. Therefore, an appropriate strategy is the use of multiple standardized test methods to characterize the various aspects of powder flow as needed by the pharmaceutical scientist. Following micromeritic properties of the solid dispersions were studied

- Bulk density
- Tapped density
- Compressibility index
- Hausner ratio
- Angle of repose

6.11.1 Bulk Density

Accurately weighed, 5 gm of solid dispersion were filled in a 10 ml graduated cylinder and its unsettled volume, Vo was noted. The bulk density was calculated in gm/cm^3 by the following formula, and recorded in Table 6.27.

Bulk density (D_o) = M/V_o

where, M = Mass of powder taken, V_o= Apparent volume.

6.11.2 Tapped Density[73]

Accurately weighed, 5 gm of solid dispersions were filled in a 10 ml graduated cylinder. The tapping of the cylinder was done on a wooden surface for 500 times and the tapped volume V_i was noted. Tapping was continued further for additional 750 times and the tapped volume, V_f was noted. The difference between two tapping volume was less than 2%, so V_f was considered as a tapped volume. The tapped density was calculated in gm/ cm^3 by following formula, and recorded in Table 6.27.

Tapped density (D_f) = M/V_f

Where, M = weight of sample powder taken, V_f = Final tapped volume

6.11.3 Compressibility index and Hausner ratio [74]

The compressibility index (CI) and Hausner ratio are measures of the propensity of the powder to be compressed. As such they are the measures of inter-particulate interactions. In a freely flowing powder, such interactions are less significant and bulk densities and tapped densities are closer in value. For a poorly flowing powder there are frequently greater inter-particulate interactions and therefore a greater difference in the bulk density and tapped density. These differences are also reflected in the Carr's index and Hausner ratio.

1. **Compressibility index (CI)**

It was calculated using the following formula, and recorded in Table 6.27

C.I. = $\{(V_o - V_f)/V_o\} \times 100$

Where, V_o = Initial volume of untapped powder, V_f = Tapped volume

2. **Hausner ratio**

Tapped density and bulk density were measured and the Hausner ratio was calculated using the following formula, and recorded in Table 6.27.

Hausner ratio = D_f / D_o,

Where, D_o = Bulk density, D_f = Tapped density

Table 25: Relationship of compressibility index and Hausner ratio with powder flow[71]

% compressibility	Hausner Ratio	Flowabilty
<10	1.00-1.11	Excellent
11-15	1.12-1.18	Good
16-20	1.19-1.25	Fair
21-25	1.26-1.34	Passable
26-31	1.35-1.45	Poor
32-37	1.46-1.59	Very poor
>38	1.60	Very, very poor

6.11.4 Angle of repose [74].

The angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane. A glass funnel was held in place with a clamp on a ring support over a plate. The height of the funnel through which the granulation blend was to pass was fixed relative to base. Approximately 25 g of granulation blend was transferred through the funnel. The height of the pile (h) and the radius of the base (r) were measured with the ruler. The angle of repose was calculated using the formula mentioned below and are reported in Table 6.27.

$\tan \theta = h/r$ or $\theta = \tan^{-} h/r$

Where,

h = height of pile,

r = radius of the base of the pile,

θ = angle of repose

Table 26: Relationship between angle of repose and powder flow [71].

Angle of repose (degree)	Flowability
25- 30	Excellent
31 –35	Good
36 -40	Fair
41-45	Passable
46-55	Poor
56-65	Very poor
>66	Very, very poor

Table 27: Results of micromeritic properties of solid dispersions

S. No.	Parameter	Result
1	Bulk Density (gm/cm^3)	0.704
2	Tapped Density (gm/cm^3)	0.847
3	Compressibility Index	16.901
4	Hausner Ratio	1.203
5	Angle of repose	32°

Conclusion

The closeness of values of bulk density and tapped density indicates the free flowing property of solid dispersions. The values of compressibility index, Hausner ratio and angle of repose indicate that the flow character of solid dispersion is fair and no aid is needed to increase the flow properties.

Summary and Conclusion

The most important use of furosemide is in the treatment of hypertension or pulmonary edema because no other group of diuretics is more effective than the loop diuretics in this situation. But there are certain major drawbacks with the conventional therapy of furosemide.1) Oral dosage form has poor bioavailability and slow onset of action because of poor solubility of furosemide in aqueous contents of

Stomach. On the other hand emergency clinical situations such as pulmonary edema require fast onset of action, therefore, only parentral therapy of furosemide is given in such emergency clinical situations.

2)During parenteral therapy of furosemide, it is of paramount importance to inject furosemide at a controlled rate not exceeding 4 mg/min., because rapid injection may result in acute reduction in plasma volume, decreased venous return and cardiac output and an exacerbation of heart failure. Also, reports have been published indicating furosemide's ototoxicity with rapid injection. So, for a 10 mg injection it requires at least 2-3 minutes for administration of bolus injection, which is very inconvenient to the patient and also requires a lot of technical skills while injecting. Sometimes, unavailability of technically skilled persons for injecting i.v. dose of furosemide in emergency conditions is also a major concern.

3) Half life ($t_{½}$) of orally administered furosemide is short i.e. 2-3 hrs, therefore, repetitive administration of tablets for 2-4 times a day (depending upon the extent of diuresis required) is desired. Also active site of absorption of furosemide is stomach and upper part of small intestine. So, once emptied from stomach the passage through the small intestine is rapid, thus limiting the extent of absorption.

Thus, keeping all these things under consideration, it was planned to formulate a bilayer tablet of furosemide in which one layer could serve as immediate release layer and can immediately dissolve orally administered furosemide in unionized form in aqueous contents of stomach so that fast onset of diuretic action can be achieved within short duration of time and thus, parenteral therapy of furosemide can be replaced by oral therapy (even in emergency clinical situations of pulmonary edema).This immediate release layer could also avoid the risk of heart failure and ototoxicity because of rapid injection, but at the same time can provide quick onset of action. Patient convenience is also an added advantage of this immediate release layer.

On the other hand second layer i.e. floating layer of the proposed bilayer tablet could provide prolonged release in the stomach, which is the active site of absorption. The controlled amount of furosemide that will be released in the stomach will empty together with fluid and will have the whole surface area of duodenum for absorption thus, the extent of absorption i.e. bioavailability will automatically be increased. Apart from this the duration of action will also be prolonged thus avoiding the need of repetitive administration.

In order to prepare immediate release layer of bilayer tablet, initially solubility of furosemide was determined in water and 0.1 N HCl, which was found to be very poor. Therefore, aqueous solubility of furosemide was enhanced with the help of hydrotropic solubilization technique which provided an appreciable 660 fold enhancement in solubility. Then, using the optimized combination of hydrotropic blend, solid dispersions were prepared in different ratios and by using the optimized ratio of solid dispersion, immediate release layer of the bilayer tablet was prepared. Solid dispersions were evaluated for drug content and micromeretic properties. X.R.D. studies, D.S.C. studies, and S.E.M. studies of the prepared solid dispersions were also performed which indicated absence of any complex formation between furosemide and hydrotropic agents. Selection of appropriate blend of hydrotropic agents was a crucial Step for the development of immediate release layer of poorly soluble furosemide.

After optimization of immediate release layer, gastro-retentive floating layer was developed using optimization technique. Initially, pre-optimization studies were performed to select the dependent and independent variables for the 3^2 factorial design. Nine formulation batches were designed for the formulation optimization studies.

Two factors *viz.*

1. Amount of Polyox WSR-N80
2. Amount of HPMC K4 M

Were selected as independent varibles. These independent variables were studied at three levels. For this purpose 3^2 full factorial design was used.

Cumulative percent drug release at 8th hr and floating lag time were considered as dependent response variables for the studies The results obtained from the experiments were statistically analyzed for response variables. Response surface graphs and contour plots were prepared for each response variable.

Optimized formula was selected by desirability (numerical optimization) technique. Three formulation batches of the predicted optimized formulation of gastroretentive bilayer tablet were prepared and experimentally validated by comparision method. Comparison of the observed and software predicted responses showed a good correlation. This demonstrated that the factorial design optimization technique was successful in the formulation development of gastroretentive layer of furosemide.

Finally bilayer tablets were prepared using optimized solid dispersion and optimized floating layer. The final bilayer tablet was evaluated and was found to be within the limits.

Conclusions

From the above studies, following conclusions were derived

1. Conclusion

Hydrotropy is a novel, safe and effective way to enhance solubility of poorly aqueous soluble drugs. Immediate dissolution of practically insoluble drug i.e. furosemide in aqueous dissolution media indicates it's great potential to solublize the drug in biological fluids and thus appreciable enhancement in bioavailability and onset of action can be expected. Thus the concept of mixed hydrotropy is an emerging field which can serve as a milestone for solubility enhancement and therefore deserves an urgent attention of scientific community to asses it's efficiency and applicability.

2. Conclusion

Floating dosage forms can significantly prolong the gastric residence time of drugs and thus can improve bioavailability, reduces drug waste with new therapeutic possibilities and substantial benefit to the patients.

3. Conclusion

There are many advantages of using optimization techniques while developing a formulation because it gives the researcher the ability to study interactions between factors. It is an excellent tool for the developing the best possible formulation under a given set of condition with minimum experimentation, saving considerable time, money and effort. Moreover it is strongly favored by regulatory agencies because it justifies the choice of ranges and finds a robust (optimum) region and thus should be used wherever possible to obtain a product with superior quality.

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