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SOLUBILITY ENHANCEMENT OF BOSENTAN MONOHYDRATE USING MIXED HYDROTROPY

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ABSTRACT

Low aqueous solubility is a major problem faced during formulation development of new drug molecules. Bosentan Monohydrate is an antihypertensive agent and is a good example of the problems associated with low aqueous solubility. Bosentan Monohydrate is practically insoluble in water. Hence, purpose of this research was to enhance the solubility of Bosentan by using the concept of mixed hydrotropy. Initially, solubility of Bosentan was determined individually in sodium acetate, sodium citrate, urea and sodium benzoate at concentration of 10, 20, 30 and 40% w/v solutions using purified water as a solvent. Highest solubility was obtained in 40% sodium benzoate solution. In order to decrease the individual hydrotrope concentration mixed hydrotropic agents were used. Highest solubility was obtained in 10:10:20 ratio of urea + sodium acetate + sodium benzoate. This optimized combination was utilized in the preparation of solid dispersions by using distilled water as a solvent. Solid dispersions were evaluated for differential scanning calorimetry and Fourier-transform infrared to show no drug-hydrotropes interaction has occurred. This solid dispersion was compressed to form tablets. Dissolution studies of prepared tablets were done using USP Type II apparatus. The batch F5 tablets show 72.81% cumulative drug release within 60 min. It was concluded that the concept of mixed hydrotropic solid dispersion is novel, safe and cost-effective technique for enhancing the bioavailability of poorly water-soluble drugs. The miraculous enhancement in solubility and bioavailability of Bosentan Monohydrate was clear indication of the potential of mixed hydrotropy to be used in future for other poorly water-soluble drugs in which low bioavailability is a major concern.

INTRODUCTION

About 45% of new chemical entities coming from the discovery are poorly bioavailable. This exerts strong limits to the performance of a drug by necessitating administering a much higher dose than strictly required from the pharmacological point of view. This can induce harmful side-effects or create problems related to cost of treatment. Due to poor bioavailability the formulator may have to select the injection route instead of the oral route.[1] For a better oral bioavailability drug must be soluble in gastro-intestinal fluids that is, drug should be soluble in an aqueous medium and also possess permeability properties for good membrane diffusion in order to reach the bloodstream.[2,3] Hydrotropy is a solubilization process where addition of a large amount of second solute exerts an increase in the aqueous solubility of another solute. The other solute can be a poorly soluble drug. Hydrotropes may be cationic, anionic or a neutral molecule, and possesses a hydrophobic as well as a hydrophilic group.[4]Finding the right hydrotropic agent for a poorly soluble drug requires screening of a large number of hydrotropic agents. However, significant solubility enhancement of drug can be easily achieved by selecting correct hydrotropic agent.[5] Hydrotropic solubilization technique is a promising approach with great potential for poorly soluble drugs. In this method, chemical modification of the drug, use of organic solvents and preparation of emulsion systems is not required.[6, 7]

Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelin-B (ET-B) receptors. Under normal conditions, endothelin-1 binding of ET-A or ET-B receptors causes constriction of the pulmonary blood vessels. By blocking this interaction, bosentan decreases pulmonary vascular resistance. Bosentan has a slightly higher affinity for ET-A than ET-B [14, 15.]

The objective of this study was to increase the solubility of Bosentan monohydrate in water using hydrotropes and their combinations so that oral bioavailability can be increased and to prepare tablets of the same.

MATERIALS AND METHODS

Materials

Bosentan Monohydrate was gifted by Mylan Laboratories, Hyderabad, Sodium acetate, Sodium Benzoate, Tri-sodium citrate (TSC), Lactose, Urea, and Mannitol were purchased by Research-lab Fine Chem Industries, Mumbai.

Determination of solubility

Saturation solubility of Bosentan Monohydrate was determined in distilled water, Methanolic water solution. All media were prepared and excess of Bosentan Monohydrate was added to each of them and kept in an incubator shaker at a speed of 200 rpm for 24 h at 37°C. After 24 h, solution was centrifuged at 2000 rpm for 15 min. Supernatants were diluted with the respective solution (i.e., distilled water, Methanolic water). Absorbance was measured at 270 nm using ultraviolet (UV) visible spectrophotometer (JASCO V-630), and solubility was calculated.

Bosentan Monohydrate hydrotropic agent interference study

Ultraviolet spectrophotometric study

For determination of interference of hydrotropic agents in the spectrophotometric estimation of Bosentan Monohydrate, the absorbances of the standard solutions of Bosentan were determined in distilled water alone and in the presence of the hydrotropic blend employed for formulation purpose. The absorbances were recorded at appropriate wavelengths. A UV-visible recording spectrophotometer (JASCO V-630) with 1 cm matched silica cells were employed for spectrophotometric determinations.

Fourier-transform infrared study

Fourier-transform infrared spectrum (FTIR) of Bosentan and its physical mixture with hydrotropic agents was recorded over a range 4000-400 cm^{-1} to study principal peaks using FTIR spectrophotometer.

Equilibrium solubility studies in different hydrotropic agents

10% w/v, 20% w/v, 30% w/v and 40%w/v solutions of each hydrotropic agent viz., urea (U), sodium benzoate (B), sodium acetate (A), and tri-sodium citrate were prepared in water. For determination of solubility accurately measured 5 ml of above particular solution of hydrotropic agent was taken in a 10 ml vial and excess amount of drug (Bosentan) was added and mechanically shaken until saturated solution was formed. Each vial was shaken on the mechanical shaker for 12 h and hence that equilibrium solubility can be achieved, and the solution was allowed to equilibrate for 24 h. The solution was further centrifuged at 2000 r.p.m. for 10 min in ultra-centrifuge and further filtered through Whatman grade 41 filter paper. Aliquot was suitably diluted with distilled water and analyzed using UV spectrophotometer at 270 nm. Enhancement ratios in solubility were calculated by the following formula:

$$\text{Solubility enhancement ratio} = \frac{solubility\ of\ drug\ in\ hydrotropic\ agents}{solubility\ of\ drug\ in\ water}$$

Equilibrium solubility studies in mixed hydrotropic blends

Initially 2-3 hydrotropic agents were mixed in 1:1 ratio and dissolved in water to get clear solution, excess amount of drug (Bosentan) was added in above solution and mechanically shaken until saturated solution was formed and solubility in water was determined as shown in Table 1. Further ratio of mixed hydrotropic agent was optimized as shown in Table 2 to achieve maximum solubility of Bosentan in water.

Table 1: Equilibrium solubility of Bosentan Monohydrate in mixed hydrotropic blends

Sr. No.	Hydrotropic Combination	Total Concentration (% w/v)	Individual concentration (% w/v)	Solubility enhancement Ratio
1.	U + A	40	20	2.3127
2.	U + B	40	20	11.8687
3.	U + C	40	20	8.4936
4.	A + B	40	20	16.8282
5.	A + C	40	20	5.7717
6.	B + C	40	20	14.5401
7.	U + A + B	40	13.33	28.5225
8.	U + A + C	40	13.33	17.7911
9.	A + B + C	40	13.33	17.1047
10.	U + B + C	40	13.33	22.3674

U-Urea, A-Sodium Acetate, B- Sodium Benzoate, C- Sodium Citrate.

Table 2: Equilibrium solubility of Bosentan monohydrate in mixed of hydrotropic blends

Sr. No.	Hydrotropic Combination	Total Concentration (% w/v)	Ratio	Solubility enhancement Ratio
1.	U + A + B	40	10:20:10	7.1695
2.	**U + A + B**	**40**	**10:10:20**	**29.1083**
3.	U + A + B	40	15:20:05	5.5063
4.	U + A + B	40	05:20:15	9.7503
5.	U + A + B + C	40	10:10:10:10	8.2383
6.	U + A + B + C	40	05:05:10:10	7.5436
7.	U + A + B + C	40	05:20:10:05	5.9793
8.	U + A + B + C	40	20:05:10:05	6.4329
9.	U + A + B + C	40	10:05:20:05	10.8237
10.	U + A + B + C	40	15:05:15:05	9.0259

U-Urea, A-Sodium Acetate, B- Sodium Benzoate, C- Sodium Citrate.

Formulation of hydrotropic solid dispersions of Bosentan Monohydrate

For preparation of hydrotropic solid dispersion, accurately weighed 1.0 g sodium benzoate, 1.0g of sodium acetate, 2.0 g of urea were taken in a 100 ml beaker and properly mixed. Further, minimum quantity of warm distilled water sufficient to dissolve the above hydrotropic blend was added, If minimum amount of water (approximately 5 ml) is used lesser will be the time required to evaporate it and chemical stability of drug may not be affected adversely during removal of the 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 above hydrotropic mixture, 1 g of Bosentan (drug to carrier ratio was 1:4) was dissolved in the above solution and temperature was maintained in the range of 55-60°C so as to facilitate the water evaporation. As soon as evaporation of water increases speed of rice magnetic bead automatically decreased due to increased viscosity and it stopped stirring when most of the water was evaporated, this indicates the formation of hydrotropic solid dispersion (wet). 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°C ± 2°C 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, hydrotropic solid dispersions were crushed using a glass pestle mortar and passed through sieve no. 60 and were finally stored in an air tight glass bottle.[15]

Evaluation of hydrotropic solid dispersion of Bosentan Monohydrate

Differential scanning Calorimetry analysis

Thermogram of the Bosentan Monohydrate was recorded by using differential scanning calorimetry (DSC) 60 Shimadzu, Japan. An empty aluminum pan was used as a reference. DSC measurements were performed at a heating rate of 1000°C/min from 30°C to 300°C.

Preparation of tablets by direct compression technique

Three batches of tablets were prepared as shown in Table 3. All the ingredients were passed through 60 mesh sieve separately. Solid dispersion equivalent to 62.5 mg of Bosentan and microcrystalline cellulose were mixed in geometric proportion to get a uniform mixture. Then the other ingredients were weighed and mixed in geometrical order and tablets were compressed using flat round punch of 8 mm sizes on a karnatvati mini Compression Machine.

Table 3: Formulation of Bosentan Monohydrate tablets prepared by direct compression method

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ingredient	Mg								
HSD (≈62.5 mg bosentan monohydrate)	136	136	136	136	136	136	136	136	136
Crosscarmellose Sodium	15	20	25	15	20	25	15	20	25
Microcrystalline cellulose	60	60	60	75	75	75	90	90	90
Aerosil-200	05	05	05	05	05	05	05	05	05
Lactose	81	76	71	66	61	56	51	46	41
Magnesium stearate	02	02	02	02	02	02	02	02	02
Talc	01	01	01	01	01	01	01	01	01

EVALUATION OF TABLETS

Postcompression parameters

Hardness test

The hardness of the tablets was determined using Monsanto Hardness tester. Its unit is expressed in kg/cm^2. Three tablets were randomly picked from each formulation and hardness was determined, the mean and standard deviation value was calculated.[18]

Friability

The friability of tablets was determined by using Roche Friabilator. It is expressed in percentage (%). Twenty tablets were initially weighed (Winitial) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 min or run up to 100 revolutions. The tablets were weighed again (Wfinal). The percentage friability was then calculated by,

$$F = W\ initial\text{-}W\ final/W\ initial \times 100$$

(% Friability of tablets <1% is considered acceptable) [19]

Drug content uniformity

Twenty tablets were weighed and crushed in a mortar then powder containing equivalent to 136 mg of Bosentan was dissolved in 100 ml of methanol to achieve a solution that has a concentration of 1000 µg/ml. 10 ml from this stock solution was taken and diluted to 100 ml using methanol, to get concentration 100 µg/ml. Further, 20 µg/ml solution was prepared by taking 2 ml from the stock solution and diluting to 10 ml. Absorbance was measured at 270 nm.[20]

In vitro dispersion time

Tablet was added to 10 ml of 1% SLS in water at 37°C ± 0.5°C. Time required for complete dispersion of a tablet was measured.

In vitro dissolution studies

Dissolution rate was studied by using USP type-II apparatus (USP XXIII Dissolution Test Apparatus at 50 rpm) using 900 ml of 1% SLS in water as dissolution medium. Temperature of the dissolution medium was maintained at 37°C ± 0.5°C, 10 ml aliquot of dissolution medium was withdrawn at every 2 min interval and filtered and the absorbance of filtered solution was measured by UV spectrophotometric method at 270 nm and concentration of the drug was determined from standard calibration curve.

RESULTS AND DISCUSSION

Determination of solubility

The solubility of Bosentan as observed in distilled water, and acetone is presented in Table 4.

Table 4: Solubility data of Bosentan monohydrate

Solvent	Solubility
Water	Very slightly soluble
0.1 N NaOH	Insoluble
Methanol	Slightly soluble
Acetone	Soluble
0.1 N HCl	Insoluble
pH 6.4 phosphate buffer	Insoluble
pH 7.4 phosphate buffer	Insoluble

Bosentan Monohydrate hydrotropic agent interference study

Ultraviolet spectrophotometric study

The UV absorbance spectra of Bosentan was determined in distilled water alone and in the presence of the hydrotropic blend solutions. The results indicate no change in the wavelength of maximum absorbance (λ_{max}) of AZ in any of the solutions. Hence, it was concluded there were no drug-hydrotrope interference.

Fourier-transform infrared study

Fourier-transform infrared was employed to characterize the possible interaction of bosentan and the hydrotropes. All peaks are within the reported range indicating purity of Bosentan. All the major peaks of Bosentan can also be seen in hydrotropic physical mixture. Hence, there were no drug-excipients interactions [Figure 1].

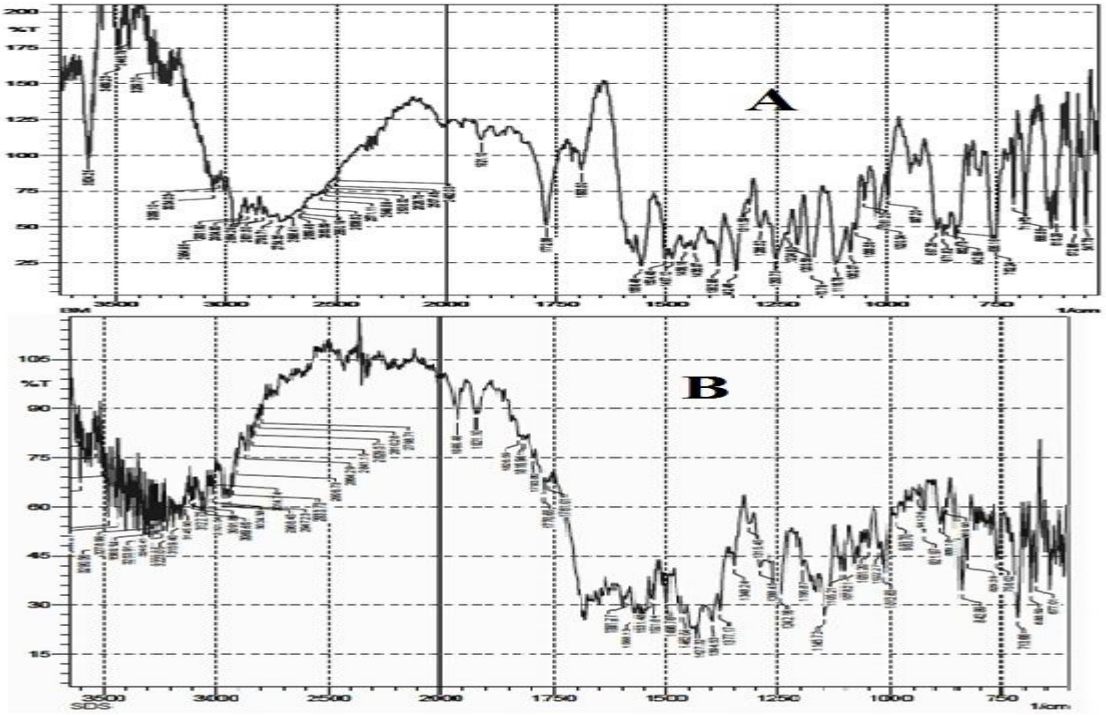


Figure 1.FTIR of Drug and Hydrotropic Solid dispersion

Fourier transform-infrared spectra of (a) Bosentan Monohydrate active pharmaceutical ingredients, (b) Hydrotropic solid dispersion

Equilibrium solubility studies in different hydrotropic agents

Equilibrium solubility of Bosentan in different hydrotropic solutions was evaluated as shown in Table 6. All hydrotropes are able to enhance solubility of Bosentan. Highest solubility enhancement ratio was obtained in 40% sodium benzoate solution. Further, in order to decrease the concentration of sodium benzoate, different combinations of above mentioned four hydrotropic agents in different ratios were tried to determine enhancement in solubility. All blends were also found to increase the solubility of Bosentan as shown in Table 6.

Table 6 Equilibrium solubility of Bosentan Monohydrate in different hydrotropic agents

Sr. No.	Hydrotropic agent	Concentration (% w/v)	Solubility enhancement Ratio
1	Urea (U)	10	1.7665
		20	1.7340
		30	2.3749
		40	1.6772
2	Sodium acetate (A)	10	1.5681
		20	1.4946
		30	2.0793
		40	1.6262

3	Sodium Benzoate (B)	10	1.7065
		20	1.7253
		30	1.7436
		40	1.9223
4	Sodium Citrate (C)	10	1.7566
		20	1.6766
		30	1.7711
		40	1.7351

The blend with maximum solubility enhancement (A+ B + U) was further explored by changing the ratio so that maximum solubility can be obtained with minimum quantity of each hydrotropic agent to decrease their toxic potential .

The blend U + B + A in the ratio of 10:10:20 gave the highest solubility enhancement of 29.1083 when compared to distill water, and therefore, this optimized combination of hydrotropes was selected for the preparation of solid dispersions.

Formulation and evaluation of hydrotropic solid dispersions of Bosentan Monohydrate

Differential scanning calorimetry analysis

The DSC thermogram of Bosentan is shown in Figure 2. The onset temperature was reported in the graph. The melting point of Bosentan monohydrate was 196-198°C and DSC thermogram of Bosentan shows endothermic melting peak at 214.73 °C. DSC thermogram of Bosentan solid dispersion is shown in Figure 3.

Figure 2. Differential scanning Calorimetry thermogram of Bosentan monohydrate

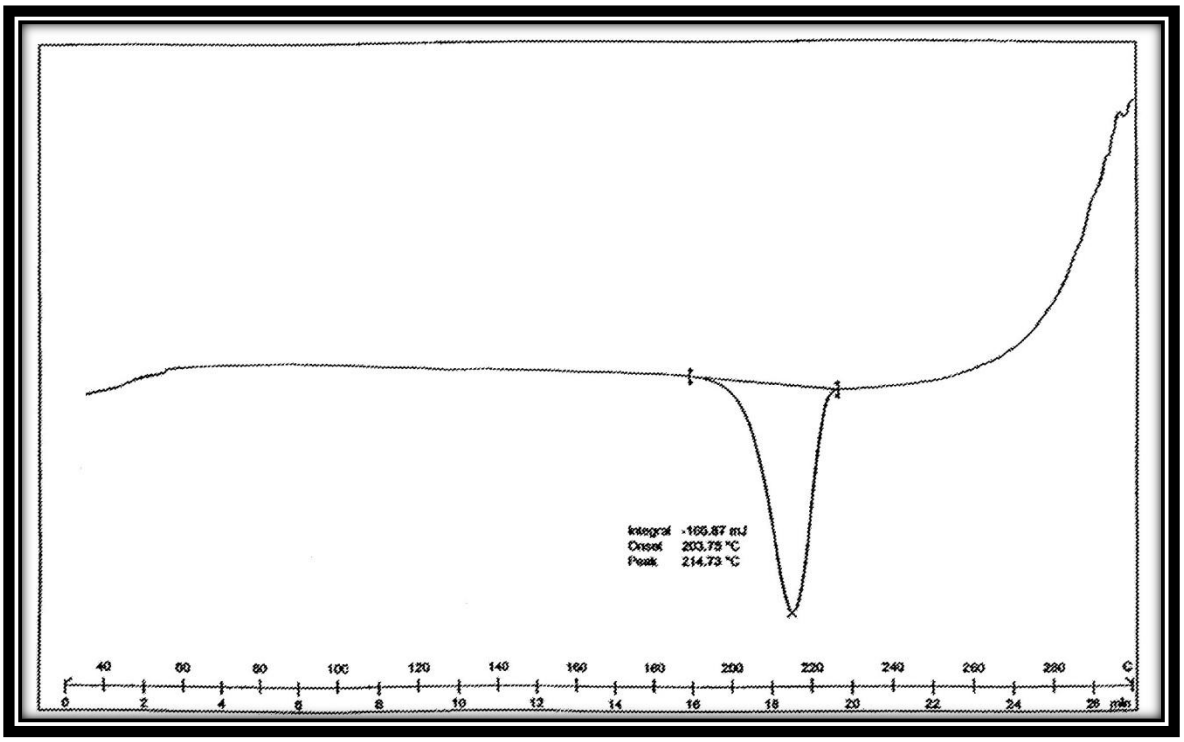
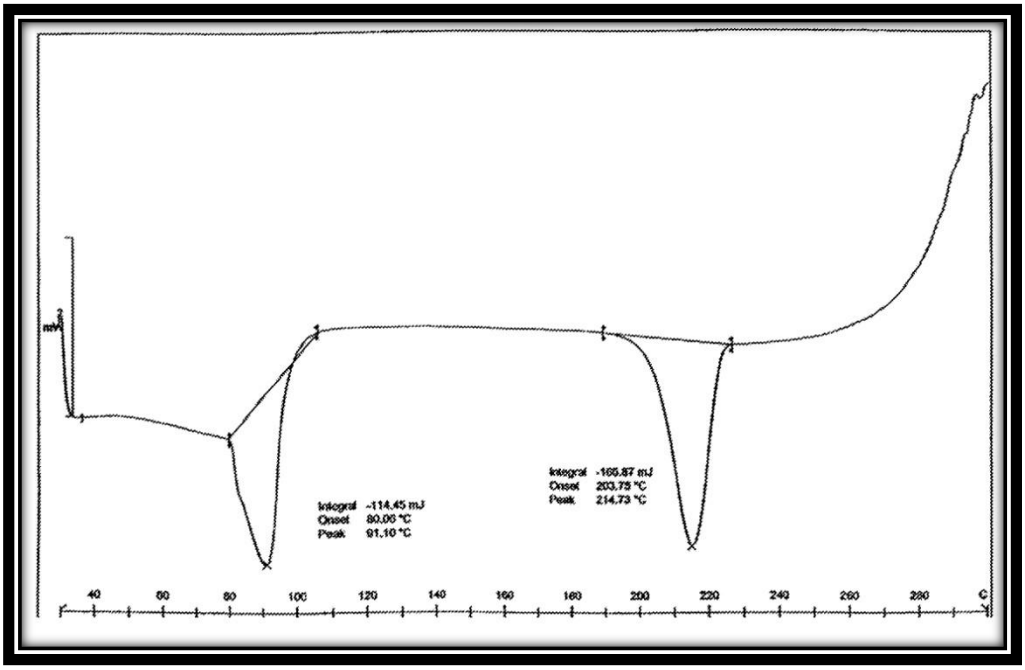


Figure 3: Differential scanning calorimetry of physical mixture of Bosentan Monohydrate and hydrotropic solid dispersion



Evaluation of fast dissolving tablets

Post compression parameters

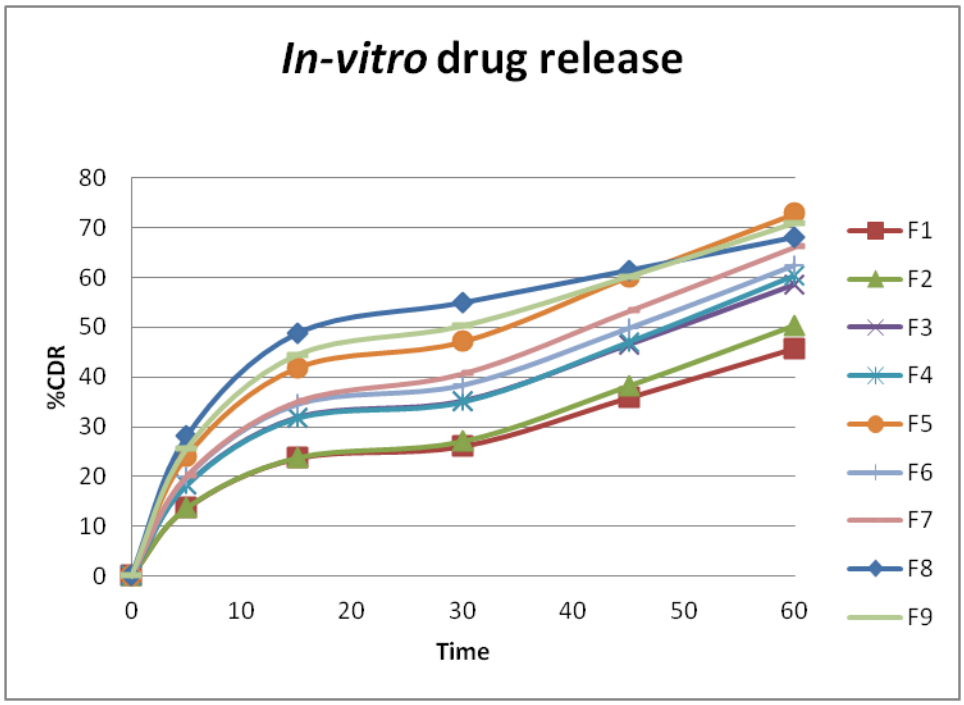
Table 7 shows the hardness and friability of tablets are in an acceptable range. The *in vitro* dispersion time was found to be that is, within a minute hence tablets disintegrate, dissolve fast. Drug uniformity study results show that there was uniform distribution of drug throughout the batch. The F5 batch was found to be best because it showed maximum hardness, less *in vitro* dispersion time and good friability.

Batch No.	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Compressibility index (%)	Hausner's ratio	Angle of repose(ɵ)
F1	0.476 ± 0.001	0.576 ± 0.004	18.45 ± 1.566	1.197 ± 0.06	33.67 ± 0.020
F2	0.495 ± 0.002	0.568 ± 0.003	12.72 ± 0.1625	1.159 ± 0.06	32.76 ± 0.19
F3	0.494 ± 0.002	0.593 ± 0.007	16.64 ± 0.660	1.148 ± 0.03	30.46 ± 0.25
F4	0.506 ± 0.001	0.575 ± 0.003	11.98 ± 0.903	1.34 ± 0.02	29.71 ± 0.65
F5	0.498 ± 0.001	0.591 ± 0.004	15.77 ± 0.641	1.29 ± 0.1	29.72 ± 0.45
F6	0.490 ± 0.001	0.573 ± 0.003	14.53 ± 0.779	1.267 ± 0.09	29.01 ± 0.29
F7	0.536 ± 0.005	0.588 ± 0.005	8.27 ± 0.44	1.079 ± 0.007	29.80 ± 0.60
F8	0.491 ± 0.001	0.568 ± 0.006	15.38 ± 1.3288	1.168 ± 0.01	29.74 ± 0.96
F9	0.500 ± 0.002	0.544 ± 0.004	8.16 ± 0.9825	1.038 ± 0.04	31.20 ± 0.946

In vitro dissolution studies

The F5 batch showed good dissolution profile shown in Figure 4. 72 % of the drug release takes place within 60 min. When the tablet enters into dissolution medium tablet disintegrates, further due to hydrotropic solid dispersion, soluble carrier releases the drug in molecular form due to which the dissolution of tablet increased and drug is released quickly from tablets and absorb rapidly by oral route resulting in increased bioavailability.

Figure 4. *In vitro* dissolution studies of batch F5



CONCLUSION

The present research work concludes that the hydrotropy is a novel, safe and effective way to enhance solubility of poorly aqueous soluble drugs. Immediate dissolution of practically insoluble drug Bosentan monohydrate in aqueous dissolution media indicates its great potential to solubilize 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 the scientific community to assess its efficiency and applicability.

REFERENCES

1. Sikarra D, Shukla V, Kharia AA, Chatterjee DP. Research article techniques for solubility enhancement of poorly soluble drugs: An overview. J Med Pharm Allied Sci. 2012; 1:1–22.
2. Behera AL, Sahoo SK, Patil SV. Enhancement of solubility: A pharmaceutical overview. Pharm Lett. 2010; 2:310–8.

3. Limbachiya MI, Agarwal M, Sapariya A, Soni S. Solubility enhancement techniques for poorly soluble drugs: Review. Int J Pharm Sci Rev Res. 2011; 4:71–86.
4. Saleh AM, El-Khordagui LK. Hydrotropic agents: A new definition. Int J Pharm. 1985; 24:231–8.
5. Kapadiya N, Singhvi I, Mehta K, Karwani G, Dhrubo JS. Hydrotropy: A promising tool for solubility enhancement: A review. Int J Drug Dev Res. 2011; 3:26–33.
6. Kim JY, Kim S, Papp M, Park K, Pinal R. Hydrotropic solubilization of poorly water-soluble drugs. J Pharm Sci. 2010; 99: 3953–65.
7. Lee J, Lee SC, Acharya G, Chang CJ, Park K. Hydrotropic solubilization of paclitaxel: Analysis of chemical structures for hydrotropic property. Pharm Res. 2003; 20:1022–30.
8. Maheshwari RK, Indurkhya A. Formulation and evaluation of aceclofenac injection made by mixed hydrotropic solubilization technique. Iran J Pharm Res. 2010; 9:233–42.
9. Badjatya JK, Bodla RK. Enhancement of solubility of fenofibrate by using different solubilization techniques. Asian J Pharm Life Sci. 2011; 1:144–8.
10. Maheshwari RK, Jagwani Y. Mixed hydrotropy: Novel science of solubility enhancement. Indian J Pharm Sci. 2011; 73:179–83.
11. Jayakumar C, Morais AP. Solubility enhancement of theophylline drug using different solubilization techniques. Int J Pharm Pharm Sci. 2012; 2:7–10.
12. Lachman L, Libermann HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd ed. New Delhi: Varghese Publishing House; 1991. pp. 297–301.
13. MC Clure N. Stability studies in overview of ICH guidelines for drug products. Fremont, CA: Matrix Pharmaceutical Inc; 1997. Available from URL: (http://www.mcclurenet.com)
14. Authorized USP pending monograph, 2012, United States Pharmacopoeia.
15. AHFS Drug Information, 2012, Vasodilating Agents, Miscellaneous, page no.1853-1856.
16. USFDA-Recommended Dissolution Medium, URL-http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults_Dissolutions.cfm (Last accessed: March 2015).
17. Costa P, Lobo JM. Modeling and Comparison of Dissolution Profiles. European Journal of Pharmaceutical Sciences. 2001; 13: 123-133.
18. Lachman L., Liberman H.A., Kaning J.L. The Theory and Practice of Industrial Pharmacy. 2nd ed., 4th Indian reprint. Bombay. Verghese publishing house; 1991: 291-342.
19. ICH Harmonized Tripartite Guideline, International Conference on Harmonization. Stability testing of new drug substances and products Q1A (R_2) and Evaluation for stability data Q1E. Current step version, 6 February 2003.
20. Alton's Pharmaceutics: The Design and Manufacturing of Medicines, Churchill Livingstone Elsevier. 3rd edition, 2007: 322-538.
21. Jayakumar C, Morais AB. Solubility Enhancement of Theophylline Drug Using Different Solubilisation Techniques, International Journal of Pharmaceutical And Clinical Science, 2012; 2(1): 7-10.
22. Sajid MA, Choudhary V. Solubility Enhancement Methods with Importance of Hydrotropy, Journal of Drug Delivery and Therapeutics, 2012; 2(6): 96-101.
23. Pillbox-Prototype Pill Identification System, United States National Library of Medicine, National Institutes of Health (NLM), URL-http://pillbox.nlm.nih.gov/pillimage/search_results.php?getimprint=letters+or+numbers+on+either+side+of+the+pill&getingredient=Azilsartan&getshape=&getinactiveingredients=&getfirstcolor=&getauthor=&getsize=&getDEAschedule=&getscore=0&getlabelCode=&getprodCode=&getnorelabel=NULL&hide=1&submit=Search(Last accessed: March 2015).