Asian Journal of Chemistry Vol. 19, No. 6 (2007), 4711-4718

Enhancement of the Dissolution and Diuretic Effect of Furosemide Through a Novel Complexation with Humic Acid Extracted from Shilajit

SURAJ P. AGARWAL*, MOHD. AQIL and MD. KHALID ANWER
Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (University)
New Delhi-110062, India
E-mail: agarwal_sp@yahoo.com

Furosemide, a thiazide diuretic exhibits extremely low aqueous solubility. This study investigated the effects of complexation of furosaemide with humic acid extracted from shilajit on release rate and *in vivo* diuretic effect on male Wistar rats. Solid complexes of furosemide with humic acid extracted from shilajit were prepared by solvent evaporation and freeze drying methods in the molar ratio 1:1 and 1:2 (furosemide: humic acid). The complexes were characterized by differential scanning calorimetry, fourier transform infrared spectroscopy and scanning electron microscopy. The comparative release study of furosemide and complexes were carried out in phosphate buffer of pH 5.8. Solvent evaporated and freeze dried complex showed significant improvement in release rate as compared to pure furosemide. Maximum release was observed by the freeze dried complex in the molar ratio 1:2. The optimized complex (1:2 freeze dried) showed significant increase in diuresis in male Wistar rats as compared to pure furosemide. This study confirms that humic acid have a potential to increase the bioavailability of low bioavailable drugs.

Key Words: Furosemide, Shilajit, Humic acid, Complexation, Dissolution enhancement.

INTRODUCTION

Furosemide (FRM) is a potent loop diuretic that is used to adjust the volume and /or composition of body fluid in a variety of situations, including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis[1]. FRM is practically insoluble in water[2]. The oral bioavailability of FRM is very poor due to insufficient aqueous solubility at gastrointestinal pH, making solubility the rate-determining step in the gastric absorption of FRM[3]. Improvement of its dissolution properties is essential because the *in vitro* dissolution behaviour of FRM is closely related to its bioavailability[4].

Shilajit, also known as salajit, shilajatu, mumie or mummiyo is a pale-brown to blackish-brown exudation, of variable consistency, coming out from layer of rocks in many mountain ranges of the world, especially the Himalayan ranges of the Indian subcontinent[5,6].

Shilajit has been reported to contain a number of components including resins, fatty acids, sterols, triterpenes, aromatic carboxylic acid, 3,4-benzocoumarins and α-amino acids[7]. The biological effects of shilajit have been ascribed to two distinct classes of compounds[8] low molecular weight bioactive organic compounds such as oxygenated dibenzo-α-pyrones and the medium molecular weight fulvic and humic acid. While the benzopyrones act as the active principle, fulvic and humic acid act as carrier molecules for *in vivo* transportation of these bioactive substances. Humic acid have a microporous structure[8] and capable of forming complex with non-polar solutes and drug molecules with low bioavailability. These drug molecules can be entrapped in the micropore so as to increase their solubility and dissolution rate, thereby enhancing their bioavailability.

EXPERIMENTAL

An aqueous extract of shilajit was obtained from commercial supplier from Pioneer Enterprises, Mumbai, India. Furosemide was kindly provided as a gift sample by Modi-Mundi Pharma Ltd., India. All other chemicals and reagents used in the study were AR grade.

Extraction of humic acid from shilajit: Finely powdered shilajit (200 g) was successively extracted[9] with 500 mL each of hot organic solvents of increasing polarity *e.g.*, chloroform, ethyl acetate and methanol to remove the bioactive components specifically, oxygenated dibenzo-α-pyrones. Extracted shilajit so obtained was taken and dispersed in 0.1 N aqueous sodium hydroxide with intermittent shaking in the presence of nitrogen at room temperature for 24 h. The suspension was filtered to remove humin (insoluble at all pH in water) and the filtrate was acidified with dilute HCl to a pH less than 3. The solution was allowed to stand at room temperature (25ºC) overnight. Humic acid, which separated out as coagulate, was filtered, dried and pulverized.

Preparation of complexes: Complexes of furosemide with humic acid were prepared in the molar ratio 1:1 and 1:2 (Furosemide:Humic acid) by using following two methods.

Freeze-drying: Solid-state furosemide complexes with humic acid were prepared. The required amount of furosemide and humic acid was accurately weighed and dispersed in water with one drop of ammonia (27 %) and sonicated for 2 h to get a clear solution. The solution was frozen in ultra freezer at a temperature of -30°C by keeping for 24 h and freeze dried over 12 h in Lyph-lock apparatus. The resulting amorphous material was pulverized in a glass mortar and pestle and passed through 100-mesh sieve to obtain a uniform size fine powder.

Solvent evaporation: Solvent evaporated complex of furosemide-humic acid was prepared by dissolving the furosemide in acetone and

humic acid in ethanol with 2 drops of ammonia to dissolve humic acid. The humic acid solution was then added to the solution of furosemide with stirring and then solution was sonicated for 2 h. The solution thus obtained was dried in a rotary evaporator under vacuum.

Characterization of complexes: The solid complexes were evaluated by differential scanning calorimetry, fourier transform infrared spectroscopy and scanning electron microscopy.

The infrared spectra of furosemide, human acid and their complexes were recorded on a FTS 40 (BioRad, USA) FTIR instrument and WIN IR software by the KBr pellet technique. Two mg of previously dried sample was mixed with 100 mg KBr and compressed into a pellet on an IR hydraulic press. These pellets were made immediately prior to the recording of the spectrum. Scanning was done from 4000 to 400 cm^{-1}. DSC thermograms (Perkin Elmer Pyris 6 instrument, USA) of the furosemide, human acid and their complexes were recorded. For obtaining the thermogram, 2-3 mg of the sample was accurately weighed and heated in a closed aluminum crimp cell at a rate of 10ºC/min under nitrogen purge with a flow rate 20 mL/min over the temperature range of 50-400ºC.

Scanning Electron Microscopy of samples were performed using Jeol JSM-840 Scanning Microscope with a 10 KV accelerating voltage. The surface of samples for SEM were made electrically conductive in sputtering apparatus (Fine Coat Sputter JFC-1100) by evaporation of gold. A magnification of 1500 and 3000 was used for all samples.

High performance liquid chromatography analysis: Samples were analyzed by a modified method of Below and Burmann[10]. Analysis of all samples was performed with a Waters Breeze HPLC system, Waters (India) Pvt. Ltd. The mobile phase (10 mM potassium hydrogen phosphate/acetonitrile, 60:40 v/v) was pumped at a flow rate of 1.0 mL/min through a Water spherisorb ODS -2, 250 × 4.6 mm column at room temperature. The injected volume was 50 µL and the detection wavelength was 234 nm. Under these conditions, furosemide retention time was 6.6 min.

Release of furosemide from complexes: Dissolution of pure furosemide and inclusion complexes were performed on paddle type USP dissolution apparatus. The dissolution studies were carried out by filling the powder in empty gelatin capsule and suspending in the desolution medium. Conditions used were dissolution medium 900 mL phosphate buffer (pH 5.8), paddle rpm 50 and dissolution temperature 37.5 ± 1ºC. The samples were withdrawn with the help of syringe fitted with needle and filtered through Millipore filter (0.22 µ). Fresh aliquots of the dissolution medium were added to compensate for the quantity of sample withdrawn. The filtered samples were analyzed by HPLC.

***In vivo* study-Diuretic activity in rats:** A method for testing diuretic activity in rats has been described by Lipschitz *et al.*[11]. The test is based on urine excretion and compared with rats treated with a high dose of urea. The Lipschitz value is quotient between excretion by test animals and excretion by the urea control. Male wistar rats weighing between 100-200 g were used in the study. Permission of the institutional animal ethics committee (Jamia Hamdard) was taken before carrying out the studies and their guidelines were followed. Three animals per group were placed in metabolic cages provided with wire mesh bottom and a funnel to collect the urine. Stainless steel sieves were placed in the funnel to retain faeces and to allow the urine to pass. 15 h prior to the experiment food and water were withdrawn. For screening procedure two groups (each group has three animals) were used for FRM alone, FRM-HA (1:2 FD) complex and urea alone. The FRM and FRM- HA (1:2 FD) complex were given orally to two groups of three animals at a dose of 5 mg/kg in 5.0 mL water/kg body weight. Two groups of three animals received orally 1 g/kg urea; additionally 5.0 mL of 0.9 % NaCl solution per 100 g-body weight was given by gavage.

Urine excretion was recorded after 5 and 24 h. Urine volume excreted per 100 g body weight was calculated for each group. Result was expressed as the Lipschitz value.

RESULTS AND DISCUSSION

The yield of human acid after extraction of shilajit was found 7.3 ± 0.6 %. It was little more in yield as compared to reported value[9] 6.8 ± 0.4 %. The complexes of furosemide-human acid were evaluated by DSC, FTIR, SEM and release rate showed that complex formation took place in case of both solvent evaporation and freeze drying method. Maximum complex was formed in case of freeze drying (1:2 freeze dried) method.

The FT-IR spectra of furosemide shows characteristic absorption bands (cm^{-1}) at *ca.* 3390 (N-H stretching, primary sulfonamide), 3300 (N-H stretching), 1690 (C=O stretching), 1550 (C=C stretching, benzene), 1330 (S=O stretching) and 1250 (C-N stretching). FT-IR absorption bands of humic acid extracted from shilajit were found accordance with those reported in literature[12]. The FT-IR spectra of furosemide-human acid complex prepared by solvent evaporation in the molar ratio 1:1 and 1:2 showed reduced peak intensity in the fingerprint region, indicating partial complex formation. While freeze dried complex in the molar ratio 1:1 and 1:2 showed disappearance of characteristic peaks of furosemide and a number of peaks in the fingerprint region were significantly reduced indicating complex formation between the two.

The DSC thermogram of furosemide and human acid as well as their complexes in the molar ratio 1:1 and 1:2 prepared by solvent evaporation (on rotary evaporator) and freeze drying techniques. Furosemide exhibits a characteristic, sharp exothermic peak at 219ºC indicating decomposition of the drug[13]. Two endothermic peaks could be observed near 208 and 214°C which could be attributed to degradation of the furosemide. The disappearance or shifting of endo- and exothermic peaks of drug is a strong indication of the formation of complex with β-cyclodextrin[14]. Human acid exhibits a thermogram devoid of any endothermic peak which indicates that it does not have any sharp melting point. An exotherm is observed above 330ºC indicating decomposition of human acid. The thermograms of the differenet complexes prepared by the various techniques showed the complete absence of the characteristic exothermic peak of furosemide indicating complex formation.

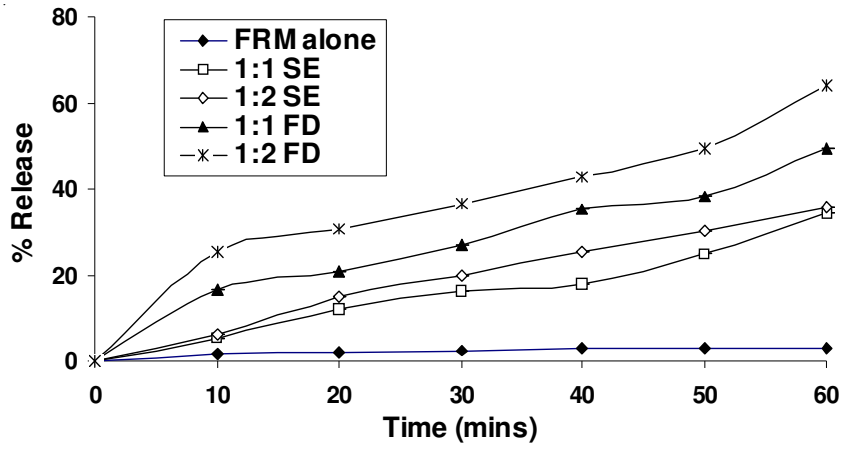

Fig. 3. Release profile of furosemide and furosemide-human acid system in phosphate buffer pH (5.8)

Scanning electron microscopy of furosemide (Fig. 2) showed crystalline particles of regular size, indicating crystalline nature while human acid appears (Fig. 3) as a fluffy material. The 1:2 freeze dried complex appears as a complete homogeneous mass, indicating complete complex formation (Fig. 4).

The release profile of furosemide-human acid systems prepared by solvent evaporation and freeze drying method are shown in Fig. 1. Dissolution of furosemide alone was found to be very slow which was due to insolubility of furosemide in water. Only 3 % release was obtained at 1 h as compared to a release of 64.1 % from 1:2 freeze dried complex of

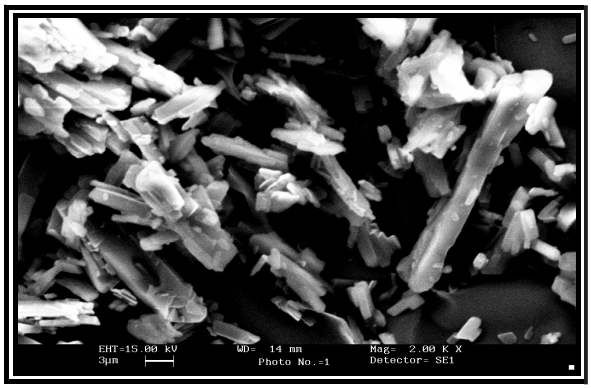

Fig. 2. SEM of furosemide

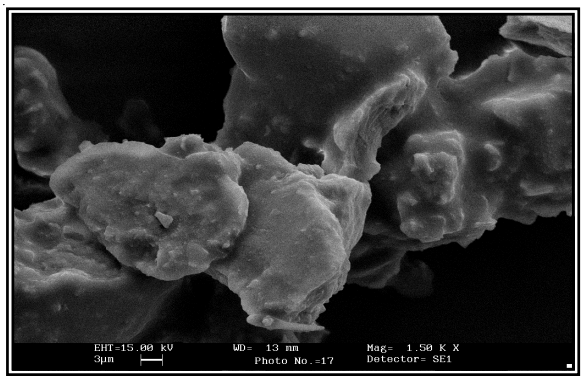

Fig. 3. SEM of human acid

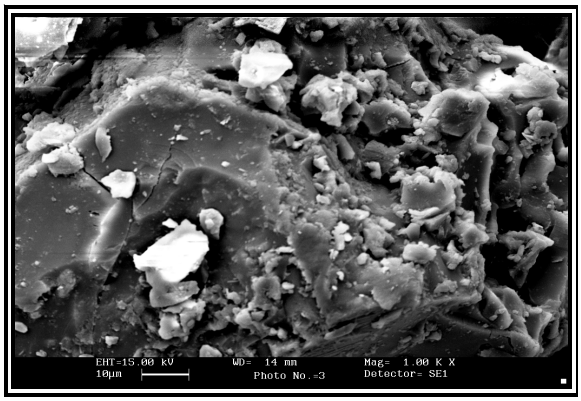

Fig 4. SEM of 1:2 freeze dried complex

furosemide-human acid system. The study clearly demonstrates that when furosemide is complexed with human acid there is a significant increase in the dissolution rate of the drug. Statistical data analysis was performed using one-way analysis of variance (Anova) for comparing the release

between furosemide and their complexes. Dissolution efficiency (0-60 min) showed significant difference between the complexes of furosemide ($F_{(4,25)}$ = 11.295, $p < 0.05$).

The results of diuretic activity studies conducted on male Wistar rats have been documented in Tables 1 and 2. The diuretic effect and LIPSCHITZ value of optimized complex (1:2 freeze dried) showed positive response as compared to the furosemide alone. Hence, it is concluded that with the prepared complex, the bioavailability is increased significantly.

TABLE-1
VOLUME OF URINE COLLECTED (mL/100 g body wt. of rats)

Samples	Urine volume (mL/100 g body weight of rat)	
	After 5 h	After 24 h
Furosemide	1.33	3.16
Urea	0.33	0.66
Furosemide-human acid (1:2 freeze dried)	1.74	3.40

TABLE-2
COMPARATIVE LIPSCHITZ VALUE OF FUROSEMIDE AND 1:2 FREEZE DRIED COMPLEX

Samples	Lipschitz value (T/U)	
	After 5 h	After 24 h
Furosemide	4.03	3.16
Furosemide-human acid (1:2 freeze dried)	5.27[a]	5.15

[a]Excellent diuretic effect.

ACKNOWLEDGEMENTS

One of the authors, S.P. Agarwal is grateful to All India Council of Technical Education for Emeritus fellowship. The authors would like to thank Dr. Rajesh Khanna for critical review of the manuscript.

REFERENCES

1. M.D. Murray, K.M. Haag, P.K. Black, S.D. Hall and D.C. Brater, *Pharmacotherapy*, **17**, 98 (1997).
2. A.M. Al-Obaid, F.J. Al-Shammary, K.A.M. Al-Rashood and M.S. Mina, in ed.: K. Florey, Academic Press New York, Vol. 18, pp. 153-193 (1989).
3. V.S. Chungi, L.W. Dittert and R.B. Smith, *Int. J. Pharm.*, **4**, 27 (1979).
4. H.O. Ammar, M. Ghorab, S.A. El-Nahhas, L.H. Emara and T.S. Makram, *Pharmazie*, **54**, 142 (1999).

5. R.A. Chopra, I.C. Chopra and K.L. Handa, Indegenous Drugs of India, U.N. Dhar & Sons, Calcutta, pp. 457-461 (1958).
6. S. Ghosal, *Indian J. Indeg. Med.*, **9**, 1 (1992).
7. S. Ghosal, J.P. Reddy and V.K. Lal, *J. Pharm. Sci.*, **65**, 772 (1976).
8. S. Ghosal, J. Lal and S.K. Singh, *Soil Biol. Biochem*., **23**, 673 (1991).
9. S. Ghosal, P.C. Dandya and S.B. Vohra, The Facets and Facts of Shilajit, Research and Development of Indigenous Drugs Institute of History of Medicine and Medical Research New Delhi, pp. 72-80 (1989).
10. E. Bleow and M. Burrmann, *J. Liq. Chromatogr.*, **17**, 4131 (1994).
11. W.L. Lipschitz, Z. Hadidlan and A. Kerpcsar, *J. Pharmacol. Exp. Ther.*, **79**, 97 (1943).
12. M. Schnitzer and S.U. Khan, Dekker New York, p. 327 (1972).
13. L.L.B. Ponto and R.D. Schoenwald, *Clin. Pharmacokinet.*, **18**, 381 (1990).
14. Y.L. Loukas, V. Vraka and G. Gregoriades, *J. Pharm. Biomed. Anal.*, **16**, 263 (1997).

(*Received*: 22 July 2006; *Accepted*: 24 April 2007) AJC-5613