RESEARCH PAPER

Received 28 October 1992
Indian J. Pharm. Sci., 1995, 57(5), pp. 189-193

Preparation and Evaluation of sustained Release Preparations of Ferrous Sulphate

MALAY KUMAR SAMANTA*, T. UDAYKUMAR AND B. SURESH
J.S.S. College of Pharmacy, Elkhill Road, Ootacamund - 643 001.

Microspheres of Ferrous Sulphate were prepared by congealable disperse-phase encapsulation technique with Agar, Agar and Hydroxy propyl methyl cellulose mixture, and Ethylcellulose. Suspension of Agar and Hydroxy propyl methyl cellulose mixture showed a better sustained release than Agar and Ethylcellulose. Sustained release iron preparations may not cause gastric irritation and other adverse effects and thereby are expected to possess a better therapeutic efficacy especially during long administration periods.

FERROUS Sulphate, a widely used drug for anaemic patients throughout the world, has been associated with different adverse effects that include gastric and intesinal tract irritation, diarrhoea, vomiting[1], Pulmonary congestion, cerebral Oedema due to overdose[2] and erythropoietic photoporphyria due to photosensitivity[3]. Reduction of the recommended doses of iron salt would reduce almost entirely all the side efffects[4]. A suspension of the drug loaded microspheres may provide a convenient alternative to the currently marketed capsule preparation, (Spansule) for long duration of treatment, for easy swallowing by children and elderely patients. An attempt has been made here to achieve a better therapeutic profile through suspension[5] of microspheres with Agar (I), Agar and Hydroxypropyl methyl cellulose (I + II) and Ethyl cellulose (III).

EXPERIMENTAL

MATERIALS

Ferrous sulphate (E. Merck); Agar, bacteriological grade (Sarabhai Chemicals); Hydroxypropyl methyl cellulose (Wilson Laboratories); Ethylcellulose, 14cp; Light liquid paraffin; Petroleum ether (60-80°C) L.R.; Acetone, A.R; Sodium Benzoate; Methyl cellulose, 400 c.p.; Glycerol (all s. d. Fine Chemicals); Tween 80 (Loba); Ctric acid (E. Merck) and sugar (marketed) were used as received.

METHODS

PREPARATION OF FERROUS SULPHATE MICROSPHERES

The technique used in the present work is congeablable disperse phase encapsulation[6]. The fixed ratio of different polymers [Drug : I = 1:1, 1:2, 1:3, 1:4, Drug: III = 1:1, 1:2, 1:3, 1:4; Drug : I : II = 1:1:0.1, 1:1:0.2, 1:1:0.3, 1:1:0.4] were dissolved in the solvents. Mixtures of drug, I and II were dissolved in water maintained at 90°C. III was dissolved in acetone and the specific quantity of aqueous drug solution was thoroghly dispersed in it. These mixtures were extruded as tiny droplets using a plastic syringe into ice cold liquid paraffin and dispersed thoroughly with a mechanical stirrer (300 rpm). The strong cohesive forces among the drug molecules with polymers in the hydrophobic surrounding medium, converted the droplets into small spheres. After 30 minutes of constant stirring, the microspheres were washed with chilled petroleum ether (60 - 80°C) for four times, collected by cacuum filtration and then

*For Correspondence

dried in the vacuum dryer for two days. Sieving of the mirospheres was carried out with 80, 100 and 120 mesh screens and amounts collected were weighed.

ESTIMATION OF FERROUS SULPHATE CONTENT

Analysis of ferrous sulphate was done by colorimetric method at 515nm[7]. A weighted quantity of the powdered microspheres was transferred to a 100 ml volumetric flask with 60 ml of pH 1.2 buffer. After stirring for 45 minutes, volume was made upto 100 ml. To 1 ml of the filtered solution, 1 ml of 0.2 M sodium acetate solution, 0.4 ml each of hydroquinone and 1, 10- phenanthroline solutions were added. Volume was made upto 5ml with distilled water and mixed well. After allowing to stand for about 1 hour, the intensity of colour was determined in the spectrophotometer at 515nm along with standards similarly prepared, and a blank. Agar microspheres containing 80.6 (A), 89.36 (B), 94.1 (C), 96.3 (D)% of ferrous sulphate; ethyl cellulose microspheres containing 59.3(A), 72.1(B), 87.5(C), 91.2(D)% of ferrous sulphate and Agar + HPMC microspheres containing 99.3(A), 96.3(B), 96.1(C) and 99.5(D)% of ferrous sulphate were used for drug release study.

DISSOLUTION

A USP XX dissolution apparatus II (paddle method) was used to determine **in vitro** release profiles of drug from microspheres. The microspheres equivalent to 50 mg of the drug was dispersed in 900 ml of pH 1.2 hydrochloric acid buffer medium at 37°C and 100 rpm rotation was maintained throughout the study. The sample was pippeted out at different time intervals throughout a period of 24 hours.

PREPARATION OF SUSPENSION

The suspensions of microspheres of ferrous sulphate were prepared using base ingredients of sugar (30 to 60%), methyl cellulose (0 to 1%), sodium benzoate (0.5%), glycerin (40%); tween 80 (0.5%), citric acid (0.1%) and purified water q.s. to 25ml. 1.575 gm of microspheres containing Drug : (I):(II) = 1:1:0.1, were dispersed in 25ml base vehicle and were evaluated for sedimentation volume, according to the Hu/Ho method[8]. Suspensions made with various base vehicles were found to be physically stable. There was a moderate separation in suspension containing no methyl cellulose.

IN VITRO DRUG RELEASE FROM SUSPENSION

A USP XX dissolution apparatus II (Paddle method) was used to determine **in vitro** release profiles of drug from suspension formulation. 1.7 ml of suspension was dispersed in 900 ml of pH 1.2 hydrochloric acid bufer at 37°C and 50 rpm. The samples of one ml were withdrawn at various time intervals throughout 24hrs. The cumulative drug release was observed for 1st, 5th, 10th, 15th, 20th, 25th and 30th day of preparation and was found in almost uniform results during those periods.

IN VIVO STUDY

Six healthy rabbits (1.4 to 1.5kg each) were used in the study. A single dose of 0.6 ml of the prepared suspension along with positive and negative controls were given orally for all the rabbits after a 12 hours fast. Two ml of blood was collected through ear vein at 0, 4, 12 and 24 hours (0.1 ml heparin used as an anticoagulant). Clear plasma was obtained by centrifugation (2000 rpm). The plasma taken in a cellophane bag, immersed into normal saline solution at 1.2 pH (37° ± 1°C), rotated with mechanical stirrer at 60rpm for two hours. One ml of the normal saline was analysed. In all the cases the peak plasma drug concentration. Tmax was 24 hours. Between 4 hrs and 12 hrs there was about two fold increase in the drug concentration and between 12hrs and 24hrs the drug concentration was found almost same. The mean peak plasma concentration, Cmax of the drug was found to be 4.9 mcg/ml. The value of AUC_o^{24} as per trapezoidal rule was found to be 67.09 mcg-hr/ml.

Table-I: Dissolution of Ferrous Sulphate in various Microsphere preparations (Fraction 100/120 Mesh Screen)

Polymer Used	Batch Ratio Drug : Polymer (S)	% of ferrous Sulphate content	Cumulative % release during 24hrs.	
Agar[I]	1:1	80.6	63.5	
	1:2	89.3	55.5	
	1:3	94.1	24	
	1:4	96.3	13.5	
Ethyl Cellulose [III]	1:1	59.3	58.5	
	1:2	72.1	29	
	1:3	87.5	24	
	1:4	91.2	13.5	
Agar + HPMC	1:1:0.1	99.3	97.5	
[I + II]	1:1:0.2	96.3	95.5	<20 hrs
	1:1:0.3	96.1	95.5	<16 hrs
	1:1:0.4	99.5	98	<12 hrs

Drug : Agar : HPMC (1:1:0.1) suspension showed cumulative drug release of 96.5% in 1.2 pH after 24 hrs.

The average *in vivo* drug concentrations in plasma of rabbits were 2.69, 4.31 and 4.9 mcg/ml after 4, 12 and 24 hrs intervals respectively.

EVALUATION OF GASTRIC MUCOSAL DAMAGE CAUSED BY DIFFERENT FERROUS SULPHATE FORMULATIONS

The effect of ferrous sulphate and its different formulations were studied for its gastric mucosal damage in albino rats. 48 hrs fasting rats (200 to 225gm each), six in each group were taken for the study.

1. 1sr group:Received ferrous sulphate raw powder at a dose of 300mg/kg, suspended in 0.5% methyl cellulose.
2. 2nd group:Received Powdered ferrous sulphate tablet equivalent to 300mg/kg of drug in 0.5% methyl cellulose suspension.
3. 3rd group:Received ferrous sulphate marketed capsule preparation (spansules), 300mg/kg of drug in 0.5% methyl cellulose suspension.
4. 4th group:Received ferrous sulphate microspheres suspension equivalent to 300mg/kg of drug (1st day preparation, I-batch)
5. 5th group:Received ferrous sulphate microspheres suspension equivalent to 300mg/kg of drug (10th day preparation, II-batch)
6. 6th group:Received ferrous sulphate microspheres suspension equivalent to 300mg/kg of drug (20th day preparation, III-batch)

Table-II : Evaluation of Gastric mucosal damage and Distribution pattern of different ferrous sulphate formulations (Dose 300mg/kg) in albino rats.

Drug Treatment	Total Iron content in mcg					Ulcer Scoring	Remarks
	Serum	Heart	Kidney	Liver	Lungs		
Ferrous sulphate raw powder	119	84	60	150	50	4.7 ± 0.618	Severe haemorrhagic lessions observed
Ferrous sulphate Tablet	115	86	62	104	51	4.0 ± 0.312	Severe haemorrhagic lessions observed
Ferrous sulphate Spansules	125	84	61	170	50	1.0 ± 0.212	Mild alternation in rugal pattern
Formulated suspension Batch - I	102	82	65	120	55	0.20 ± 0.068	No abnormalities detected
Formulated suspension Batch - II	105	85	67	150	54	0.20 ± 0.056	No abnormalities detected
Formulated suspension Batch - III	100	81	65	150	50	0.21 ± 0.066	No abnormalities detected
Formulated suspension Batch - IV	108	80	60	120	50	0.20 ± 0.064	No abnormalities detected
Solvent Control (0.5%w/v M.C., 1ml/kg)	68	70	62	90	-	2.32 ± 0.914	Focal haemorrhagic lessions may be due to fasting

7. 7th group:Received ferrous sulphate microspheres suspension equivalent to 300 mg/kg of drug (30th day preparation, IV-batch)

8. 8th group:Received only 0.5% methyl cellulose suspension, 1ml/kg which served as the solvent control.

The different formulations were administered orally, two times daily for 3 days consequetively starting from the last day of fasting. Drinking water was provided truly. One hour after the last treatment, the rats were anaesthetised, their stomach isolated and cut opened along large curvature, washed with saline and careful observations were made for gastric lessions, bleeding changes in normal rugal pattern and weldefined ulcer. The ulcer observed were scored according to the method described by J.W.E. Harrison[9]. The results were tabulated.

EVALUATION OF DISTRIBUTION PATTERN OF FERROUS SULPHATE FROM DIFFERENT FORMULATIONS

The organs such as liver, kidney, lungs, heart and blood were collected from the treated rats. The organs were homogenised seperately in normal saline solution and made into suspension to 5ml. The suspension sealed in cellophane film bag was subjected to dailysis in normal saline by shaking in shaker for one hour. Then the amount of drug dialysed into normal saline solution was analysed.

RESULTS AND DISCUSSION

The pure drug showed more than 90% dissolution in one hour whereas there was a slow release of drug after ecapsulation with I, I +II and III. After analysing all the results, it was also found that the combination [Drug : (I) : (II) = 1:1 : 0.1] gave the better release pattern. Higher percentage of coating materials was found to produce slower release profiles except with I + II.

Stable suspension of ferrous sulphate loaded microspheres (Drug: agar :HPMC = 1:1:o.1, sieve size of 100/120) with 0.5% w/v of methyl cellulose as suspending agent and 40% w/v of glycerin as viscosity imparting agent was found to be the most effective for its sustained action during 24 hours period both in **in vitro** and **in vivo** studies. Prolonged storage did not affect the content uniformity. The **in vivo** bioavailability studies on six batches of rabbits confirmed a sure sustained release activity throughout 24 hours.

The results of gastric damage showed a protection of gastric mucosa with different formulation. The ferrous sulphate powder and tablet showed gastric irritation and gastric haemorrhagic lessions with a high value of ulcer scoring. Whereas the marketed capsule preparations (Spansules) showed a mild alteration in rugal irritation. The sustained release microsphere suspensions showed no such irritation. The changes in the volume of gastric content were also not singinificantly different. The distribution of iron in the organs and serum was not significantly different except in liver. The total iron content in the orgains was also not significantly different with various formulations. With this, we suggest that ferrous sulphate therapy of anaemic patients with sustained release suspension is a better alternative.

REFERENCES

1. Abbarah, T.R., **J. Am. Med. Assoc.** 1976, 236, 2320.

2. Manoguerra, A.S. **Am. J. Hosp. Pharm.** 1976, 33, 1088.

3. Baker, H; **Proc, Roy. Soc. Med.** 1971, 64, 610.

4. Bothwell, T.H. **Med. J. Aust.** 1972, 2, 433.

5. Smith, H.A. Evanson, R.V; and Sperandio, G.J; **J. Am. Pharm. Assoc. Scl. Ed.** 1960, 49, 94.

6. Deasy, P.B; Microsencapsulation and related drug processes; Vol. 20, Marcel Dekker Inc. New York, USA, 1984, 253.

7. Vogel's text book of quantitative inorganic analysis including elementary instrumental analysis; ELBS, 4th edition; William Clowes and Sons Limited, London. 1978, 742.

8. Wared, H.T; and Kammermayer, K; **Ind. Eng. Chem,** 1940, 32, 622.

9. Harrison, J.W.E; **J. Am. Pharm. Assoc.** 1948, 14, 738.