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Formulation and Evaluation of Microspheres of Isoniazid for Treatment of Tuberculosis

[1]Abhishek Kumar Jain, [2]Neha Jain, [2]M.L. Kori and [1]U.K. Jain

[1]Bhopal Institute of Technology & Science - Pharmacy, Bhopal, M.P., India
[2]Vedica College of B. Pharmacy, RKDF University, Bhopal, M.P., India

Abstract: *Objective*: The present study involves isoniazid as a model drug for the development and evaluation of microspheres for the treatment of tuberculosis disease. *Material and Methods*: The microspheres were prepared by the solvent evaporation method using variability in a variation of polymers, drug-polymer concentration ratio and stirring rate during the manufacturing process. The size or average diameter of prepared microspheres was characterized by the method prescribed in the text. Scanning electron microscopy was carried out as one of the characteristic methods for the identification of surface characteristics (SEM) of microspheres. *Result*: The result showed, prepared microspheres were spherical with a smooth surface (Characterization of flow property of microspheres indicated as the polymer concentration increases the size of microspheres increase, this could change the flow of microspheres). Variability of stirring rate during the preparation of microspheres change the size as well as flow property of microspheres and this was also observed by *in vitro* drug release kinetic studies. The prepared microspheres exhibited prolonged drug release up to 12 h. The cumulative release of isoniazid significantly increases with increasing polymer concentration ($p<0.05$) and increase the stirring rate ($p<0.05$). The increased density of the polymer matrix at higher concentrations resulted in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. In vitro studies demonstrated diffusion-controlled drug release from the microspheres. *Conclusion*: It is concluded that the controlled release drug delivery system can be prepared at a large scale in the form of microspheres with these polymers for the treatment of tuberculosis.

Key words: Microspheres · Isoniazid · Tuberculosis · Tubercular Bacilli

INTRODUCION

Controlled drug delivery is used to reduce the frequency of dosing, to reduce side effects and thereby obtain a maximum therapeutic effect. One of the methods of controlling the rate of drug release is by microencapsulation [1]. The range of techniques for the preparation of microspheres offers a variety of opportunities to control aspects of drug administration. The term control may be used to broadly and includes phenomena such as protection and masking, reduced dissolution rate and spatial targeting of active ingredient [2]. Microspheres can be defined as solid spherical particles ranging from 1 to 1000 μm. Microspheres are small in size therefore, have a large surface to volume ratios. At the lower end of their size range, they have the colloidal properties [3, 4]. These particles consist of core material isoniazid as a model drug and coating material. The coating materials may be various type ranging from natural polymers such as albumin, gelatin [5, 6], Ethyl cellulose [7], Calcium alginate [8], Chitosan [9] and synthetic such as poly (vinyl alcohol) [10], poly (lactide-co-glycolide) [11] and a combination of two polymers such as chitosan-sodium CMC [11] gelatin-chitosan etc [12]. Ethyl cellulose is used as melt coating; this polymer may be produced by the reaction of soda cellulose and alkyl chloride. Since these alkoxy pendant groups reduce the hydrogen bonding forces, the partially reacted products are water-soluble. Egg albumin is also soluble in water, so this can be used as a coating material for microspheres preparation. This approach may offer the additional specific advantages of accurate delivery of small quantities of protection of labile as a model drug before and after administration and prior to an

Corresponding Author: Dr. Neha Jain, Vedica College of B. Pharmacy, RKDF University, Bhopal, M.P., India.
Tel: 8827892434.

appearance at the site of action. The principle of microsphere manufacture depends on the creation of an interfacial area, involving a polymeric material that will form an interfacial boundary and a method of cross licking the polymer in such a way that the microspheres process a degree of permanency. Isoniazid is considered to the primary drug for chemotherapy of tuberculosis and all patients with the disease caused by isoniazid sensitive strains of tubercle bacillus should receive the drug, if they can tolerate it by Goodman Gilman [13]. It was chosen as a model drug for present study.

MATERIALS AND METHODS

Calcium alginate (S.D. fine pvt. Ltd., Mumbai), Calcium chloride (S.D. Fine Pvt. Ltd., Mumbai), Ethyl cellulose (S.D. Fine Pvt. Ltd., Mumbai), Albumin from egg and Isoniazid were obtained as gift sample from Lupin Research Park (Pune, India). Glutaraldehyde (Loba chemical, India) and all other reagents were of LR grade.

Preparation of Microspheres

Calcium Alginate: These microspheres containing calcium alginate polymer was made by dropping or spraying sodium alginate. Sodium into a calcium chloride solution, the divalent calcium ions cross link the alginate forming gelled droplets. These gel droplets can be permanently cross linked by addition to a polylysine solution. Smaller droplets can be formed by using a pump to force until alginate through the pipette.

Ethyl Cellulose: Solvent evaporation is one of the earliest methods of microsphere manufacture. The polymer and drug must be soluble in an organic solvent, frequently used organic solvent is methylene chloride. The solution containing the polymer and the drug may be dispersed in an aqueous phase to form a droplet. Continuous mixing and elevated temperature were employed to evaporate the more volatile organic solvent to leave the solid polymer-drug particles suspended in the aqueous medium. It was filtered, washed and dried.

Egg Albumin: Egg albumin microspheres were prepared in a polymer drug ratio 20:1 by stirring on the magnetic stirrer and then dispersed slowly on the solution containing liquid paraffin 100 ml [on water bath]. Microsphere were prepared after rotation speed of 400 to 500 rpm for 4-5 h. Decanted the unused liquid and microspheres washed with using petroleum ether 50 ml in 3 times each quantity and dried at room temperature. Finally yellowish colored microspheres were collected.

Characterization of Microspheres

Size and Shape of Microspheres: The size of microspheres was determined using a microscope (Olympus NWF 10x, Educational Scientific Stores, India) fitted with an ocular micrometer and stage micrometer. Scanning electron microscopy (SEM) (Philips-XL-20, The Netherlands) was performed to characterize the surface of formed microspheres. Microspheres were mounted directly into the sample stub and coated with gold film (~200 nm) under reduced pressure (0.133 Pa) [14-15].

Flow Properties: It was determined in terms of Carr's index (I_C) and Hauser' ratio (H_R) using the following equation:

$$H_R = \rho_{t/} \rho_b$$
$$I_c = \rho_t - \rho_{b/} \rho_t$$

The angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined the fixed funnel method by using the following equation:

$$\text{Tan } \theta = 2H / D$$

where, 2H / D is the surface area of the free standing height of the microspheres that formed after making the microspheres flow from the glass funnel [16-18].

Estimation of the Amount of Drug Incorporated [19]:

- Calcium alginate microsphere: Drug loaded calcium alginate microspheres were powdered with using glass mortar and dissolved phosphate buffer pH 7.4 and examined specrophotometrically at 266 nm after suitable dilution.
- Ethyl cellulose: Drug loaded ethyl cellulose microspheres 100 mg were freely powdered in a glass mortar then dichloromethane 10 ml were added to dissolve ethyl cellulose coat and phosphate buffer of pH 7.4 was added at 32-34°C temperature to evaporate dichloromethane. Solution was filtered, examined spectrophtometrically for absorbance at 266 nm.
- Egg albumin: 100 mg portion of drug loaded albumin microspheres were incubated with 15 ml of 5% HCl in absolute ethanol at 4°C for 24 h of incubation. The microspheres were separated by high speed centrifugation at 4000 rpm and the drug content was analysed in supernatant by UV spectrophotometrically.

The encapsulation efficiency and percent yield were calculated using the following formula:

% Entrapment efficiency = Mass of incorporated drug / Mass of drug used for microsphere preparation × 100

***In vitro* Release Study:** These 100 mg of the drug loaded microspheres were taken from each of the polymer containing microspheres capsules and using II unit dissolution apparatus in a 900 ml of pH 7.4 buffer solution. 5 ml of drug releasing media was withdrawn at various time intervals of 0, 1, 2 to 12 h. The samples withdrawn were filtered through a membrane filter of pore size 0.4 µm under vaccume. The drug was estimated in the clear filtrate using an UV spectrophotometer at 266 nm [20].

RESULT AND DISCUSSION

The solvent evaporation method was found suitable for the preparation of microspheres. The yield obtained in all batches was good. The prepared microspheres showed variability in size, which is increasing with increasing polymeric concentration of same polymer. The average particle size (average diameter) was also having variability with variations of coating polymeric material as calcium alginate, ethyl cellulose and egg albumin. The microspheres prepared of egg albumin as coating material and stirring rate 500 rpm showed good flow property because finer droplets were prepared with the increasing stirring rate (Table 1). All the micromeritics properties of prepared microspheres showed good to

Table 1: Various parameters of characterization of formulations

S.No.	Drug as core	Polymers	Code	Core: Coat ratio
1	Isoniazid	Calcium alginate	F1**	1:02
2	Isoniazid	Calcium alginate	F2*	1:02
3	Isoniazid	Calcium alginate	F3*	1:01
4	Isoniazid	Ethyl cellulose	F4**	1:02
5	Isoniazid	Ethyl cellulose	F5*	1:02
6	Isoniazid	Ethyl cellulose	F6*	1:01
7	Isoniazid	Egg albumin	F7**	1:02
8	Isoniazid	Egg albumin	F8*	1:02
9	Isoniazid	Egg albumin	F9*	1:01

Stirring rate = * 300 rpm, ** 500 rpm

Table 2: Angle of Repose, Carr's Index and Haussner's Ratio as an indication of powder flow properties

Angle of Repose	Carr's Index	Hausner's Ratio	Type of flow
>20°	5-15 %	-	Excellent
20-30°	12-16 %	<1.25	Good
30-40°	18-21 %	-	Fair to passable
––	23-35 %	<1.25	Poor
––	33-38 %	1.25-1.5	Very poor
>40°	>40 %	-	Extremely poor

Table 3: Various evaluation parameters of microspheres

Code	Angle of Repose[a] (θ)	Carr's Index[a] (%)	Hausner's Ratio[a]	Particle size in d_{avg} (µm) [a]	Percent entrapment efficiency [b]
F1**	21.390+0.671	13.253+0.624	1.245+0.013	36.45±0.540	92.24±1.2%
F2*	23.410+0.035	14.285+0.345	1.160+0.023	37.86±0.436	98.46±1.8%
F3*	22.820+0.553	13.496+0.413	1.142+0.072	37.15±0.495	100.24±1.6%
F4**	25.390+0.308	13.812+0.823	1.265+0.062	37.10±0.512	95.43±1.3%
F5*	29.810+0.071	12.972+0.316	1.132+0.012	38.12±0.436	96.65±1.7%
F6*	22.520+0.351	12.359+0.749	1.134+0.039	37.95±0.378	97.56±1.5%
F7**	23.460+0.421	10.054+0.613	1.267+0.013	38.17±0.435	95.64±1.8%
F8*	24.310+0.312	11.053+0.931	1.148+0.027	39.86±0.532	96.83±1.6%
F9*	25.310+0.412	12.053+0.721	1.145+0.028	38.24±0435	97.43±1.4%

[a]Mean ± SD, n = 10, [b] Mean ± SD, n = 3, Stirring rate = * 300 rpm, ** 500 rpm

Table 4: *In-vitro* drug release study of microspheres

Time	F1**	F2*	F3*	F4**	F5*	F6*	F7**	F8*	F9*
0	0	0	0	0	0	0	0	0	0
2	3.01	1.54	4.71	0.322	0.571	1.23	0.781	3.23	4.68
4	8.23	5.43	13.21	2.45	1.76	3.39	3.45	9.23	10.12
6	10.34	9.23	18.68	4.67	5.67	3.39	7.46	15.67	18.34
8	19.87	19.87	35.67	16.46	12.34	14.5	13.23	26.78	27.45
10	31.23	31.25	45.27	26.56	25.67	21.34	26.56	33.24	38.54
12	41.34	44.78	53.25	38.78	36.45	37.56	38.34	44.28	47.65
14	53.37	52.34	66.34	49.87	46.78	58.45	48.34	53.68	59.67
16	65.78	64.21	76.54	59.03	59.04	74.23	58.34	69.76	75.6
18	77.45	74.34	88.74	71.23	69.34	86.46	69.87	79.32	84.34
20	87.32	82.1	95.37	81.23	79.67	93.6	81.26	89.65	91.23
22	97.51	92.1	98.12	91.13	90.23	98.01	91.36	94.78	96.99
24	99.24	99.68	99.99	98.13	99.01	99.34	99.21	99.78	99.98

Stirring rate = * 300 rpm, ** 500 rpm

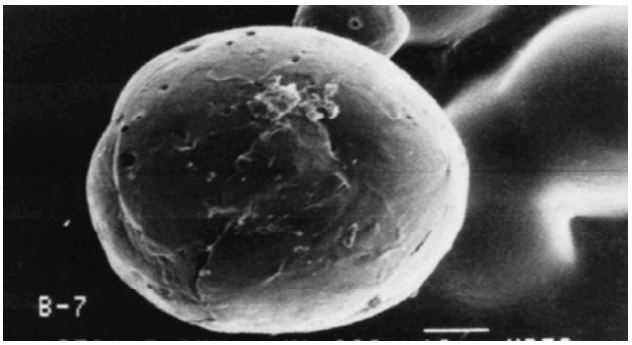

Fig. 1: Scanning electrone microscopic of isoniazid microspheres with calcium alginate 1:2 drug polymer ratio

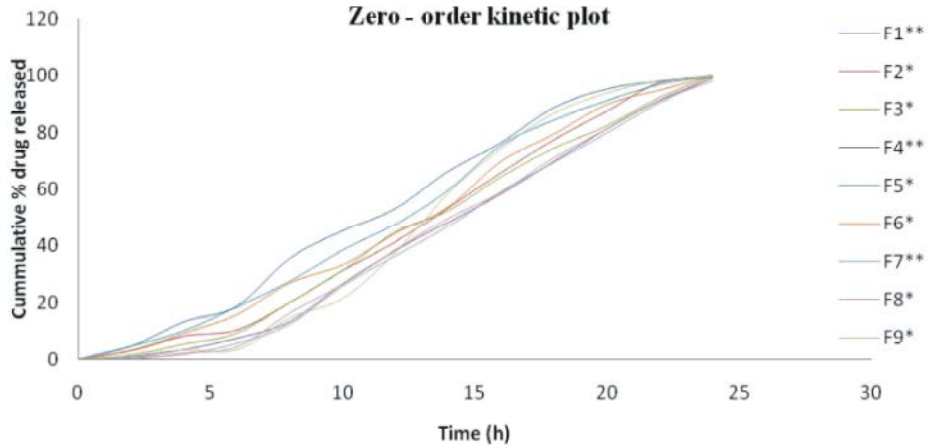

Fig. 2: The effect of different polymers and their concentration on the *in vitro* release of Isoniazid (bars represent mean ± SD; n=3, Zero-order kinetic graph)

excellent flow property of highly viscous polymeric solutions but having large size particles (co-relation between Table 1 and 2). As the stirring rate during the preparation of microspheres was changed, the particle size, surface property and drug content efficiency also changed. The size of microsphere ranged between 36.45 µm to 39.86 µm of all polymer containing microspheres. The particle size distribution of prepared microspheres was uniform and narrows (Table 3). Scanning electron microscopic photograph of calcium alginate containing as polymeric material with 1:1 ratio showed smoother surface than other prepared microspheres with different polymers (Figure 1). Surface of microspheres with containing highly viscous coating

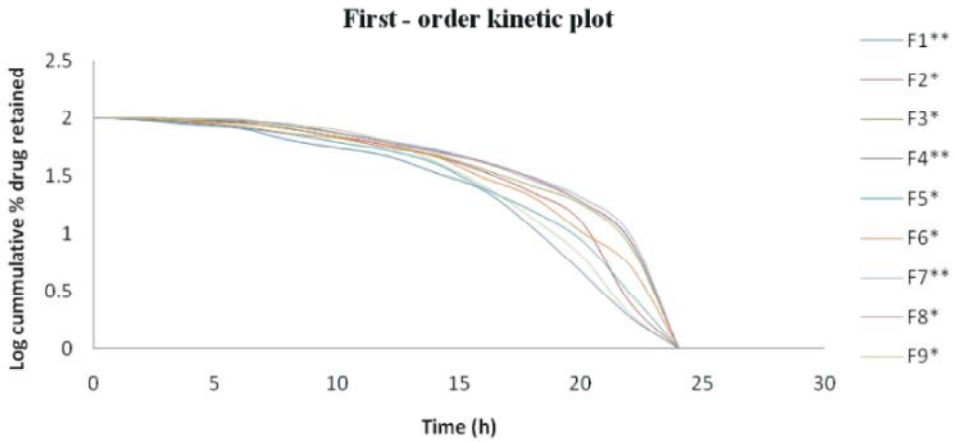

Fig. 3: The effect of different polymers and their concentration on the *in vitro* release of Isoniazid (bars represent mean ± SD; n=3, First-order kinetic graph)

material was changed to rough surface. It was found that percent entrapment of drug was between 92.24-100.24% depending on the different polymers with polymer: drug ratio. Entrapment efficiency of drug in prepared microspheres showed microspheres containing highly viscous coating material decrease but increase their particle size (Table 2 and Figure 3). The in-vitro release profile obtained indicated a biphasic pattern, i.e. initial fast release of the drug called 'burst effect' and later on a sustained-release effect. The initial burst effect may be due to some drug particles on microspheres surface and later slow release due to drug diffusion from polymer matrix, which gets swollen in dissolution media. Drug loaded microspheres containing calcium alginate shows 99.24-99.99% till to 24h but polymer ethyl cellulose containing microspheres release 63.64-69.75% and egg albumin containing only 57.58-61.35% drug release (Table 2 and Figure 2 & 3). It seems drug only release after 12 h about 58.67% from capsular shell. The data obtained for *in-vitro* release were fitted into equations for the zero-order, first-order and Higuchi release models. The in-vitro drug release showed the highest regression coefficient values for Higuchi's model, indicating diffusion to be the predominant mechanism of drug release. All the results indicated that calcium alginate coated prepared microspheres were excellent formulation for treatment of tuberculosis.

CONCLUSION

The ethyl cellulose, calcium alginate and egg albumin microspheres loaded with drugs are successfully prepared by the solvent evaporation method. The method is simple. Microspheres are spherical in shape. The drug loading efficiency is good. The release of drug from the microspheres is good to excellent. There is no interaction between polymers and drugs. Microspheres are stable. It is concluded that the controlled release of the drug delivery system can be prepared at a large scale in microspheres with these polymers, for treatment of tuberculosis with reduced frequency of dosing.

REFERENCES

1. Baker, J.A., 1987. Microencapsulation. In: Lea & Febiger (Ed). The Theory and Practice of Industrial Pharmacy, varghesey Publishing House, Mumbai. Edition III, pp: 412-429.
2. Fujinoto, S., M. Miyajak, F. Endoh, Takanashi and K. Terao, 1985. Cancer, 55: 522-526.
3. Burgess, D.J., 1990. Colloids and colloid drug delivery systems, In. Encyclopedia of pharmaceutical technology, vol. 3, J. Swarbric and J. C. Boylan, eds., Marcel Dekker, Inc. New york, pp: 31-63.
4. Poovi, G., S. Padmapriya, S. Lakshmi, 2015. Review on Magnetic Microsphere, Global Journal of Pharmacology, 9(4): 296-310.
5. Narayani, R. and K.P. Rao, 1994. Controlled release of anticancer drug from biodegradable gelatin microspheres, J. Microencapsulation, 11: 69-77.
6. Samanta, M.K., S.T. Vanwan, K. Babu and B. Suresh, 1997. Formulation and evaluation of chlorpromazine Hcl loaded self cross linked gelatin microsphere, Ind. J. Pharm. Sci., 59: 68-72.
7. Swarbrick, J. and J. Boylan, 1990. Encyclopedia of pharmaceutical technology, vol. 10, Marcel Decker, Inc. Newyork, pp: 1-12.

8. Dhawan, S. and A.K. Singla, 2003. Nifedipine loaded chitosao microspheres prepared by emulsification phase separation, Biotech Histochem, 78: 243-254.
9. Kurkuri, M.D. and T.M. Aminbhavi, 2004. Poly (vinyl alcohol) and poly (acrylic acid) sequential inter penetrating network pH sensitive microspheres for delivery of diclofenac sodium to intestine, J. Control. Rel., 96: 9-20.
10. Chaw, C.S., Y.Y. Yang and T.T. Phau, 2003. Water soluble Beta methasone loaded poly (lactide)-co-glycolidel hollow microparticles as sustained release dosage form. J. Pharm. Sci., 20: 349-359.
11. Bayony, M.A., 2003. Influence of polymers weight ratio and pH of polymers solution on the characteristics of chitosan-carboxyethyl cellulose microspheres containing theophyllin, Bull. Chin. Pharm., 142: 336-342.
12. Pandey, R. and G.K. Khuller, 2004. Chemotherapeutic potential of alginate, chiosan microspheres as antitubercular drug carrier, J. Antimicrobial Chemothr, 53: 635-640.
13. Goodman Gilman, 2001. The Pharmacological basis of Therapeutics, 10 eds, published by Mcgill companies, North America, pp: 1275.
14. Jain, A.K., C.P. Jain, K. Gaur and A. Kakde, 2009. Effect of natural biodegradable and synthetic polymer for gastric disease by floating microspheres. Continental J. Pharm. Sci., 3: 1-6.
15. Jain, A.K., C.P. Jain, Y.S. Tanwar and P.S. Naruka, 2009. Formulation, characterization and in vitro evaluation of floating microspheres of famotidine as a gastro retentive dosage form, Asi J. Pharmac, 3(3): 222-226.
16. Jain, A.K., C.P. Jain, N. Jain and R.S. Bishnoi, 2014. Enhancement the dissolution profile of ciprofloxacin and metronidazole with -cyclodextrin and acidic solubilizing additives. W J P P S, 2(5): 540-551.
17. Jain, N., M.L. Kori and A.K. Jain, 2018. Pectin and Its Combination with Different Polymers for Colon-Targeted Drug Delivery: A Review, Inventi Rapid: NDDS, 1: 1-8.
18. Qadri, M.F., 2015. Effect of Calcium Chloride and Barium Chloride on Release Behavior of Babul (Acacia nilotica) Gum Microspheres, Global Journal of Pharmacology, 9(3): 282-288.
19. Kumar, D.V., A. Mishra and T.S. Easwari, 2013. Formulation and Development of Acrycoat-Coated Chitosan Beads of Ornidazole for Colon Targeting, European Journal of Applied Sciences, 5(2): 47-52.
20. Singh, A., P.K. Sharma and R. Malviya, 2011. Preparation, evaluation and optimization Famotidine-Alginate Microspheres Using $(3)^2$ Full Factorial Design, European Journal of Biological Sciences, 3(2): 52-60.