Online Available at www.thepharmaresearch.info

THE PHARMA RESEARCH, A JOURNAL

The Pharma Research (T. Ph. Res.), (2011), 5(1); 62-69.
Published on- 15 Jun 2011

Original Article

ISSN 0975-8216

DESIGN, DEVELOPMENT AND *INVITRO* EVALUATION OF METADOXINE MICROBEADS: IONIC GELATION METHOD

Singh ajay[*1], Kumar pradeep[2], Malik anuj[2], Bharali kumar rajib[3], M.d Alam- imtiyaz[4]

Affiliated to:

[1*] Department of Pharmaceutics, PES College of Pharmacy, Bangalore, Karnataka, (INDIA)
[2] Department of Pharmaceutical Analysis, SGCP, Baghpat, Uttar Pradesh, (INDIA)
[3] Department of Pharmacy, Al-Ameen College of Pharmacy, Bangalore, Karnataka, (INDIA)

Email Click Here

ABSTRACT

The ionic gelatine method proved to be a simple, cost effective and extended the release of the metadoxine from prepared microbeads. The metadoxine is a water soluble drug, having the biological half life less than 1 hr. the microbeads prepared using sodium alginate and pectin as polymers formulation F_4 showed 92% release up to 8 hrs. Among the various evaluation parameters calculated for microbeads the stability studies observed stable (91.13%).

Keywords: Ionic Gelatine, Metadoxine, microbeads, in-vitro dissolution, stability.

INTRODUCTION

Metadoxine is a hepatoprotective drug and mainly used in liver disorder and alcoholic liver diseases. Metadoxine addresses the multiple causes and mechanisms involved in the liver disorder and improves alcohol metabolism and accelerates the elimination of alcohol from the blood. Metadoxine reduces the toxic effects of alcohol. In hepatic stellate cells, Metadoxine prevents the collagen synthesis & reduces fibrosis. Metadoxine acts as an ant-fibrotic agent and also an antioxidant. Metadoxine prevents the redox imbalance &TNF-α indication, one of the earliest events in hepatic damage. The vast potential of Metadoxine in the treatment of alcoholic liver disorders, although there are many marketed product of Metadoxine as conventional dosage form are available but still there is an interesting demand for reducing the dosing frequency because of small biological half life, by controlling the release of the Metadoxine from microbeads.

Material and Method:

Metadoxine was gift samples obtained from Micro Labs, Bangalore. Sodium alginate and pectin were purchased from, S. D. fine chem. Ltd, Mumbai. All other reagents were used of analytical grade.

Preparation of standard plot:

The stock solution was prepared by accurately weighed 10 mg of drug and placed in 100 ml predried volumetric flask. Small volume of distilled water was added and agitated until the drug dissolved completely and made up the volume up to 100ml. From the standard stock solution 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0 ml was pipette out and made up to 10 ml with distilled water to prepare the concentrations from 4 to 40 µg/ml. The prepared concentrations were analyzed at 292 nm. Absorbance mean of thee determinations was taken to check the reproducibility. The observed absorbance was subjected to regression analysis, to study the linearity and other optical characteristics.

Preparation of microbeads:

Microbeads were prepared by ionic Gelatine method[1] that involves 2 %w/v of drug was added to a 1.75 %w/v aqueous solution of sodium alginate in F_1 formulation and also 0.3 %w/v, 0.5 %w/v and 1.0 %w/v of pectin in F_2, F_3 and F_4 formulations respectively (Table 1).

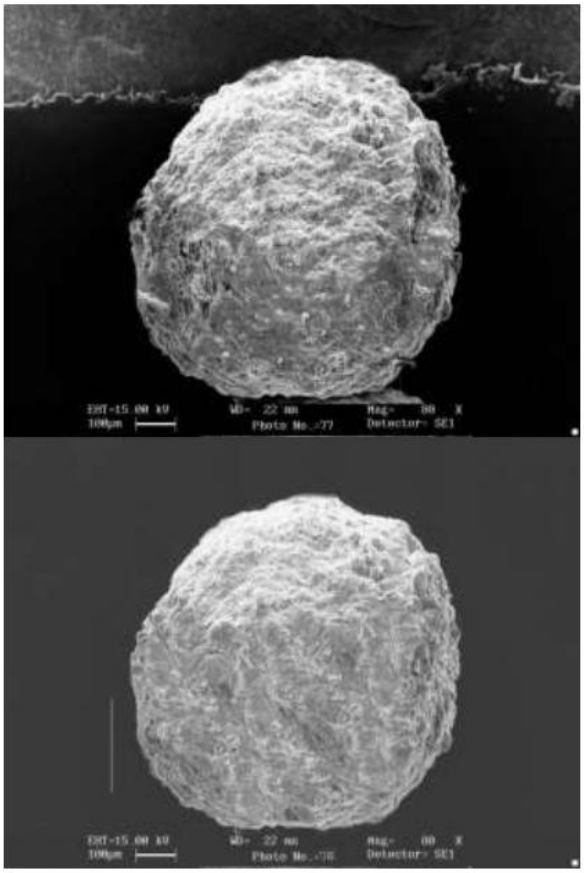
Table 1: Composition of prepared microbeads

Formulations	Formulation ratio		
	Drug (% w/v)	Sodium alginate (% w/v)	Pectin (% w/v)
F_1	2	1.75	-
F_2	2	1.75	0.3
F_3	2	1.75	0.5
F_4	2	1.75	1.0

This solution was dropped manually though a needle size no. 26 G from a hypodermic syringe in to a 2 %w/v solution of $CaCl_2$. The gel microbeads formed were allowed to harden in gelling bath for at least 30 min. After wash with distilled water, they were dried in air at room temperature until constant weight was achieved and then transferred to desiccators under vacuum.

Evaluation Parameters:

Particle size, shape and surface morphology:



SEM Photograph of formulation F1 and F4 formulation

All microbeads were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 500 microbeads were measured randomly by optical microscope. The average particle was determined by using the Edmondson's equation D mean=Σnd/Σn, where n= number of microbeads observed and d= mean size range.

The shape and surface morphology of microbeads was studied by using a Joel JSM-T330A scanning electron microscope.

Drug content and entrapment efficiency:

To determine Drug content and entrapment efficiency, 100 mg of microbeads were crushed in glass mortar and triplicate samples of 10 mg of the crushed microbeads were dissolved in 10 ml of distilled water, vortexed for 5 min and filtered though whatman filter paper no 1. The filtered samples were diluted 50 times with distilled water; drug content was assayed by UV spectrophotometer at 292 nm. Percent of total entrapment efficiency was determined by the formula;

$$\text{Total percentage entrapment} \quad \frac{\text{Practical drug}}{\text{Theoretical drug}} \times 100$$

Tapped bulk density, Percentage compressibility index, Hausner ratio and Dynamic angle of repose:

1g of each pure drug and prepared microbeads were subjected into 10 ml graduated measuring cylinder separately and the initial volume and poured density were noted down. The graduated cylinder was dropped on to a tapped density apparatus until no further changes in volume was noted. The tapped density was then obtained by dividing the weight of sample in gram by the final volume in cm^3 of the material contained in the cylinder. Percentage compressibility index and Hauser ratio were also calculated, using the formula[2].

Dynamic angle of repose of pure drug and prepared microbeads were determined by placing 1 g of pure drug and prepared microbeads separately in rotating cylinder in lab fabricated equipment and allowed to rotate at 25 rpm for 5 min. The angle made by the bulk of the pure drug and prepared microbeads against the horizontal tangent, was recorded and dynamic angle of repose was calculated[2].

***In vitro* drug release:**

In vitro drug release studies were carried out for pure drug and prepared microbeads (F_1, F_2, F_3 and F_4) by using USP dissolution rate test apparatus 1 (basket type, rotating speed 75 rpm at 37±0.5°C). An accurately weighed amount of pure drug (250 mg) and microbeads equivalent to 250 mg of drug were filled in to hard gelatin colorless capsules and placed in basket separately. The dissolution medium was distilled water 900 ml, for the 8 h study. 5 ml samples were withdrawn at specified time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h) and equal volume of fresh medium was replaced immediately that was maintained at same condition. After a suitable dilution (10 times), samples were analyzed by UVspectrophotometry. From the absorbance value, percentage drug released was calculated and compared with dissolution of pure drug in similar condition. All the studies were carried out in triplicate.

Application of kinetic models to characterize the *in vitro* drug release from microbeads:

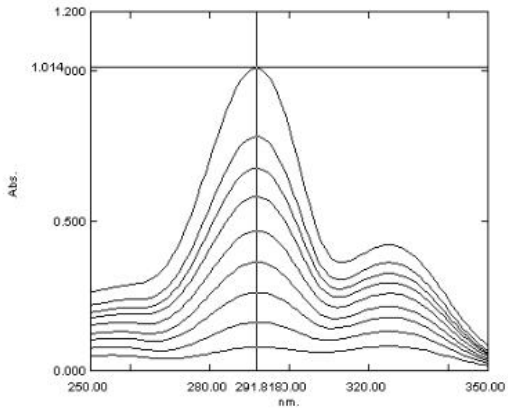
To find out the mechanism of drug release from microbeads, the dissolution data of each batch was fitted to various kinetic equation[3, 4], namely Zero order, First order, Higuchi square root of time and Peppas-korsmeyer. The correlation coefficient were calculated and used to find the fitness of the data.

Stability studies of microbeads:

All the batches of Metadoxine microbeads were tested for stability. The preparations were divided into 3 sets and were stored at 4^0 C (refrigerator), room temperature and 40^0 C (thermostatic oven). After 15, 30 and 60 days drug content of all the formulations was determined by the method discussed previously. In vitro release study was also carried out of the best formulation.

Results and Discussion:

The calibration curve of Metadoxine was prepared in the concentration range of 4-40 µg/ml in distilled water. The calibration curve (figure 1) proves its linearity, followed Beer's–Lambert law with r^2 value 0.9995.



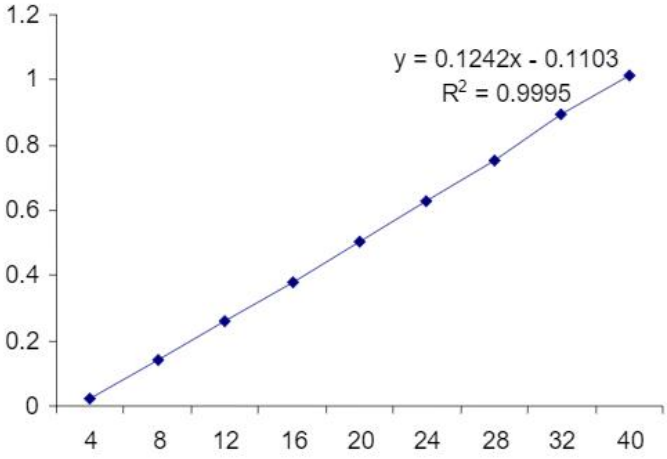


Figure 1: Calibration curve of Metadoxine in distilled water at λ_{max} 292nm

The ionic Gelatine method involved ionic interaction between the negatively charged carboxyl groups of sodium alginate and the positively charged counter ion such as: Ca^{++}. The addition of the divalent ions such as: Ca^{++} produced a partial neutralization of carboxylate groups present on the alginate chain, forming insoluble gelatinous microbeads. Calcium alginate pectinate microbeads were prepared by ionic Gelatine method as previously described, with the exception that 2 %w/v of drug was added to an aqueous solution comprising 1.75 %w/v sodium alginate in all formulations and also 0.0%, 0.3 %w/v, 0.5 %w/v and 1.0 %w/v of pectin in F1, F_2, F_3 and F_4 respectively and microbeads were made as explained previously. Pectin with low degree of esterification (35%) along with sodium alginate forms gel microbeads by ionic Gelatine with divalent calcium ion. Gelatine occurred due to intermolecular cross-linking between the

divalent calcium ions and the negatively charged carboxyl groups of pectin and sodium alginate molecules.

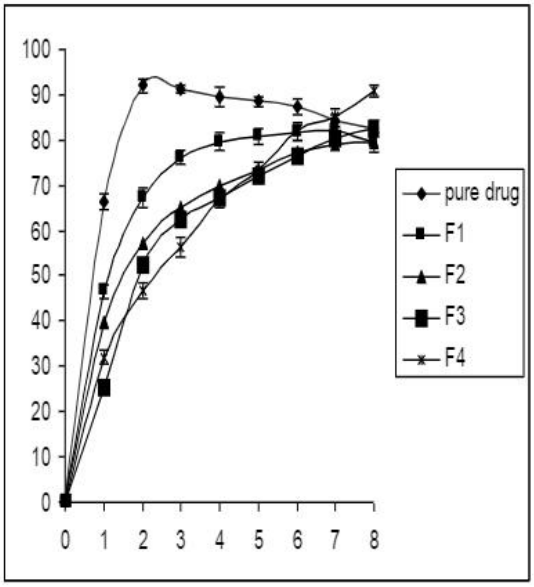
Morphological examination of the calcium alginate microbeads (F_1) and calcium alginate-pectinate microbeads (F_4) were carried out using scanning electron microscope (SEM) that revealed all microbeads prepared were spherical in shape and found to be slightly rough surface and uniform which is suitable for internal administration.

The poured density, tapped density, percentage compressibility index and Hausner's ratio of pure drug and prepared microbeads were determined (Table 2) in order to find out the flow properties. The tapped density and percentage compressibility value of pure drug were found that 0.61 g/cm^3 and 47.62 % respectively which indicating that pure drug exhibited extremely poor flow properties. The tapped density value of all microbeads formulations ranged between 0.74 g/cm^3 to 0.88 g/cm^3. These results are further substantiated by the percentage compressibility index that was found in the range of 11.77 % to 14.61 %, suggesting excellent flow properties of all microbeads formulations. Hausner's ratio is another mean of defining the flow properties. The numerical values of pure drug was found to be 1.94 which was greater than 1.6, this indicates that pure drug having more cohesive and less free flowing powder, whereas, all microbeads formulations were clearly representing the Hausner's ratio less than 1.2 (Table 2), this indicates that microbeads exhibited low interparticle friction and good flow property. Dynamic angle of repose was determined using lab fabricated rotating cylinder and the flow property of pure drug and prepared microbeads (F_1, F_2, F_3 and F_4) is reported in Table 2. The pure drug exhibited very poor flow properties with an angle of repose to be greater than 40°. All the microbeads formulation exhibited good flow property with an angle of repose within the range of 20° to 30°.

Table 2: Analysis against parameters given below for pure drug and F1-F4 formulations

Parameters	Pure drug	F_1	F_2	F_3	F_4
Poured density (g/cm^3)	0.33	0.67	0.69	0.76	0.76
Tapped density (g/cm^3)	0.61	0.74	0.79	0.84	0.88
Compressibility index (%)	47.62	12.16	12.81	11.77	14.61
Hausner ratio	1.94	1.14	1.16	1.14	1.17
Dynamic angle of repose (degree)	76°	25°	24°	21°	22°
Average particle size (µm)	18.41	639.89	667.69	673.89	685.79

In vitro drug release studies for pure drug and prepared microbeads (F_1, F_2, F_3 and F_4) were carried out by using distilled water as dissolution medium. Figure 2 show the comparative percentage drug release profile of pure drug and drug loaded microbeads (F_1, F_2, F_3 and F_4),



It is seen in Figure 2, the pure drug was completely dissolved (94.09 ± 2.48 %) within 2 h but, while the release of drug from calcium alginate microbeads (F_1) was found to be 74.07 ± 2.44 %. This fast release of Metadoxine in distilled water was occurring due to its high solubility in this medium. After 2 h, the calcium alginate microbeads (F_1) disintegrated and lost remaining drug within 3 h. According to literature (George and Abraham), at alkaline pH, the water of environments penetrates in to the chains of alginate to form hydrogen bridges through their available –OH and COO- groups. As a consequence, the microbeads turn in to a hydrogel and have their diameter increased, favoring the drug diffusion.

As mentioned above, alginate microbeads had probably insufficient cross-linking density to prevent drug molecules to diffuse out. With the addition of pectin with sodium alginate i.e. calcium alginate-pectinate microbeads (F_2, F_3 and F_4), the release of entrapped drug during first 2 h was significantly reduced. Three different pectin concentrations with sodium alginate were used in order to study the effect of pectin concentration on drug release from microbeads. Accordingly, three batches of calcium alginate-pectinate microbeads containing 1.75 %w/v sodium alginate and three different pectin concentrations of 0.3 %w/v, 0.5 %w/v, and 1.0 %w/v in F_2, F_3 and F_4 respectively were prepared. The results (Figure 2) of *in vitro* dissolution studies indicated that the amount of drug release decreased with increase in pectin concentration. This was expected, since on increasing pectin amount with sodium alginate, interaction between two polymers had increased, forming a closer network, which decreases the diffusion of the drug outwards.

As observed in (Figure 2) F_1, F_2, F_3, and F_4 released 92.0±1.52, 67.2±2.23, 57.2±1.69 and 46.7±1.87 % of Metadoxine respectively within 2h. Only F_4 formulation follows a slower gradually increasing drug release phase extending up to 8 h.

Various release kinetic models were applied by using PCP Disso v2.0 Software to determine the mechanism of drug release from microbeads and the data is summarized in table 4. It was observed that the highest correlation coefficient (r^2) found for Higuchi model, which

indicates the drug release from the microbeads, occurred via diffusion mechanism. Furthermore peppas model revealed a high correlation coefficient, which confirms drug release from microbeads, was diffusion controlled.

Table 3: Drug content and percent entrapment efficiency for formulationF1-F4.

Formulation code	Actual drug content (%)	Entrapment efficiency (%)
F_1	42.96 ± 0.21	61.75 ± 0.94
F_2	44.36 ± 0.52	68.74 ± 1.15
F_3	45.65 ± 0.73	75.73 ± 1.46
F_4	47.24 ± 0.52	88.51 ± 1.29

Table 4: Drug release models

Formulations	Zero order model (r^2)	First order model (r^2)	Higuchi model (r^2)	Peppas model (r^2)
F_1	0.4532	0.3233	0.8624	0.8836
F_2	0.5200	0.5251	0.9399	0.9680
F_3	0.7653	0.7681	0.9390	0.9446
F_4	0.8547	0.8587	0.9824	0.9983

The stability study showed that there was no changes in the appearance of the microbeads indicating that formulations were physically stable at all the conditions to which they were exposed. It was observed that there was slight reduction in the drug content in the microbeads which were stored at 40^0 C after storage for 60 days and no change in the drug content of the formulations stored at room temperature and at 4^0. *In vitro* release studies revealed that the formulation F_4 stored at 40 showed 86.64% release. The one which was stored at 4^0C showed 91.13% and room temperature batch showed 90.77% release after 8h. The Analysis of Variance (ANOVA) applied and showed there was no significant change in the drug release from all the formulations. The formulation F4 exhibited a significant sustained in vitro release of Metadoxine. Further there is potential to improve Metadoxine release, which could be established by in vivo evaluation of microbeds in animal model or human volunteers.

Acknowledgements:

The authors are grateful to M/s. Micro Labs, Hosur for providing gift samples of drugs. The Authors are also Thankful to the I.I.T. Roorkee for permitting SEM.

References:

1. Rodriguez M.L.G., Holgado M.A., Lafuente C.S., Rabasco A.M. and Fini A. Alginate/chitosan particulate systems for sodium diclofenac release. Int. J. Pharm, 2002, 232, 225.
2 Aulton M.E. (2002), Pharmaceutics: The science of dosage form design, Churchill Livingston, New York, Second Edition, pp. 197-210.
3 Costa P., Manuel J. and Lobo S. Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci, 2001, 13, 123.

4 Saravanan M, Dhanaraju M.D., Shridhar S.K., Ramchandran S., Sam S.K.G., Bhasker K. and Rao G.S. Preparation, characterization and *in vitro* release of ibuprofen polystyrene microspheres, Indian J. Pharm. Sci, 2004, 66(3), 287.