Research Article

FORMULATION AND EVALUATION OF DEXTROMETHORPHAN HYDROBROMIDE CONTROLLED RELEASE HOLLOW MICROSPHERES USING NATURAL POLYMER

Sai Sathavahana C, Ravi Teja Allena*, Anil Getyala, Gangadharappa H V

Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Mysore- 570015

Submitted: 10-12-2013
Revised: 12-01-2014
Accepted: 13-03-2014

*Corresponding author
Ravi Teja Allena,

Email :
raviallena@gmail.com

ABSTRACT

The objective of this study was to formulate and evaluate gastric floating dosage forms of hollow microspheres for controlled delivery of dextromethorphan hydrobromide (DBM). Hollow microspheres were prepared by emulsion polymerization method using natural polymer gelatin as release retardant and coating agent. Hollow microspheres were characterized by FT-IR, DSC, SEM, and micromeritic properties. FT-IR and DSC studies showed no chemical interaction between the drug and polymers. Hollow microspheres were evaluated for percentage yield, encapsulation efficiency and *in vitro* release studies. The obtained angle of repose, % Carr's index, Hausner ratio, and tapped density values were within the limits indicating good flow properties. The surface morphology revealed that microspheres were spherical with minute pores and invert dents on the surface. Among all the formulations, F-3 of hollow microspheres showed highest drug release of 79.69±0.12% at the end of 12h which was considered as the best formulation. The release data was fitted to various mathematical models such as, Higuchi, Korsmeyer-Peppas, First-order, and Zero order to evaluate the release kinetics and mechanism of the drug release. The release kinetics indicated that drug release followed non-fickian diffusion mechanism. Results of the stability studies showed that there were no significant changes in the drug content and physical appearance.

Key words: Dextromethorphan hydrobromide, Hollow microspheres, Gelatin, emulsion polymerization.

INTRODUCTION

The design of oral controlled drug delivery systems (DDS) primarily aimed to achieve more predictable and increased bioavailability. (Garima *et al.,* 2003). Now-a-days most of the pharmaceutical scientists are involved in developing the ideal DDS. This ideal system should have advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Controlled release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose. (Mathiowitz 1999; Shivakumar *et al.,* 2004).

Floating microspheres are gastroretentive drug delivery system based on non-effervescent approach. They are low density

systems, which have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period of time. When microspheres come in contact with gastric fluid the gel formers, polysaccharides and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydro-colloidal layer. The air trapped by swollen polymer lowers the density and confers buoyancy to the microspheres. (Deshpande *et al.,* 1997; Mathiowitz *et al.,* 1987).

Dextromethorphan hydrobromide, a centrally acting non-opioid antitussive drug is an effective and safe for the control of cough & asthma in patients. It is readily absorbed from the upper GIT. Dose is 10-30mg daily in divided dose. Due to its short biological half-life of

Table I. Formulation chart of DBM hollow microspheres

Sl No	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Dextromethorphan hydrobromide (mg)	45	45	45	45	45
2	Gelatin (mg)	100	150	200	250	300
3	Liquid paraffin (mL)	10	10	10	10	10
4	Span 80 (mL)	2	4	_	_	2
5	Tween 80 (mL)	_	_	2	4	2
6	Glutaraldehyde (%)	1	1	1	1	1
7	Distilled water (mL)	5	5	5	5	5

Preformulation studies
The prepared formulations were characterized by Fourier Transform Infra-red Spectroscopy, Differential Scanning Calorimetry, Scanning Electron Microscopy, and X-ray Diffraction analysis.

1.4-3.9h, it requires multiple dosing (2-3 times a day). Multiple dosing leads to fluctuation in the drug blood level and often dose related adverse effects. Multiple dosing also often results in poor compliance and inefficient therapy. To increase therapeutic efficacy, reduce frequency of administration and for better patient compliance twice daily controlled release dextromethorphan hydrobromide gastric floating dosage forms are prepared to prolong the residence time in absorption region for desired period of time.

Dextromethorphan hydrobromide belongs to the class II of biopharmaceutics classification system (BCS), exhibits low solubility and high permeability. Hence, enhanced gastric retention time of dextromethorphan hydrobromide controlled release dosage form will increase its absorption. Therefore, dextromethorphan hydrobromide was selected as a suitable drug for the design of a gastric floating drug delivery system (GDDS) with a view to improve its oral bioavailability.

MATERIAL AND METHODS
Dextromethorphan Hydrobromide was purchased from Divis Laboratories Pvt Ltd, Hyderabad. Gelatin was procured from Loba Chemie Pvt Ltd, Mumbai. Liquid paraffin, Span 80, Tween 80, Glutaraldehyde were purchased from Sigma Aldrich, Bangalore. All other chemicals and reagents used were of analytical grade.

Emulsion polymerization
This method is widely used in microencapsulation process. The microspheres are prepared by emulsion polymerization method. The polymer gelatin is dissolved in water with little heating maintains the solution at 60°C. Drug is dispersed in polymer solution with continuous stirring for about 3min. Then take 10mL of liquid paraffin and add emulsifying agent (span 80 or tween 80) along with 1% glutaraldehyde which was a cross linking agent. The aqueous phase was emulsified into the oily phase by stirring the system with constant stirring at 1000rpm using mechanical stirrer. Stirring was continued for 3h until the aqueous phase was completely removed by evaporation. The light oil was decanted and microspheres were collected by filtering through Whatmann filter paper, dried in an oven at 40ºC for 2h and stored in a desiccator at room temperature. Composition of hollow microspheres is given in table I.

Preformulation studies
The prepared formulations were characterized by Fourier Transform Infra-red Spectroscopy, Differential Scanning Calorimetry, Scanning Electron Microscopy, and X-ray Diffraction analysis.

FT-IR
The FTIR (Fourier Transform Infra-Red Spectroscopy) spectra of the prepared formula-tions were recorded over the range of 400-

4000cm^{-1} by KBr pellet method using FTIR spectrophotometer. The compatibility between the drug and the polymers were compared by FT-IR spectra.

DSC

Differential scanning calorimetry (DSC) was performed on pure sample of drug and its formulation using DSC-Q200 apparatus. Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10°C min^{-1}. The energy was measured as joules per kilocalorie.

SEM

Scanning Electron Microscopy (SEM) The surface morphology of formulations was determined using a scanning electron microscope. Samples were mounted on aluminum mount, using double sided adhesive tape and sputtered by gold under vacuum and were scanned at an accelerating voltage of 15KV before observation.

Angle of repose (Aulton, 1996; Ansel *et al.,* 2000; Brahmankar and Jaiswal 1998)

Angle of repose is the maximum angle possible between the surface of a pile and the horizontal plane. Angle of repose (θ) was assessed to know the flowability of microspheres, by a fixed funnel method using the formula

Tan (θ) = h/r

Where 'h' is height of heap and 'r' is radius of the heap. Angle of repose represents whether the given sample was free flowing or not.

Compressibility index

Compressibility index was measured for the property of powder to be compressed, as such they are measured for relative importance of inter particulate interactions. Compressibility index and was calculated by the following equation, (Rama *et al.,* 1998)

$$\text{Compressibility index} = \frac{[(Dt - Db)]}{Dt \times 100}$$

Where, Dt= tapped density; Db= bulk density;

Drug content

Formulation equivalent to 45 mg of Dextromethorphan hydrobromide was taken and transferred to 100 ml volumetric flask, dissolved and diluted with pH 1.2 HCl buffer. The absorbance of the resulting solution was measured at 279nm using a UV spectrophotometer after filtration through Whatmann filter paper.

The drug content was calculated using the following equation:

$$\% \text{ drug content} = \frac{con(^g/_{mL}) \times dilution\ factor}{label\ claim\ (mg)} \times 100$$

Percentage yield (% yield)

The yield was determined by weighing the hollow microspheres and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and polymers used. The formula for calculation of % yield is as follows;

Drug entrapment efficiency

Percentage drug entrapment was determined by UV spectrophotometric method. Drug was extracted from the microballoons using 1N HCl and the absorbance was measured using UV-visible spectrophotometer at 279nm. The drug entrapment efficiency (DEE) was calculated by the equation,

DEE = (Pc / Tc) X 100

Pc is practical content, Tc is the theoretical content.

Particle size analysis

The particle sizes of drug loaded formulations were measured by an optical microscope fitted with an ocular and stage micrometer and particle size distribution was calculated. About 100 microspheres were selected randomly and their size was determined.

Xg=10 x [(ni x log Xi) / N]

Xg is geometric mean diameter, ni is number of particle in range, Xi is the midpoint of range and N is the total number of particles.

Percentage of moisture loss

The dextromethorphan hydrobromide loaded hollow microspheres were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature. The microspheres weighed initially and kept in desiccators containing calcium chloride at 37°C for 24 h.

When no further change in weight of sample was observed, the final weight was noted down.

$$\% \text{ of moisture loss} = \frac{\textit{Initial weight - Final weight}}{\textit{Initial weight}} \times 100$$

Floating lag time and *in vitro* buoyancy

Floating lag time is the time taken by the tablet or hollow microspheres to emerge onto the surface of the dissolution medium after adding to simulated gastric fluid without pepsin (500 ml o pH 1.2 HCl buffer). The time taken by the tablet or hollow microspheres to rise to the surface of the dissolution media and time taken for it to sink was noted, the difference of which gives the duration of buoyancy.

In vitro release studies (Ninam, *et al.,* 2008)

In vitro drug release of dextromethorphan hydrobromide was evaluated in triplicate at 37 ± 0.5°C using a USP XXIV dissolution testing apparatus type 2 (paddle method) at a rotation speed of 50rpm in 250mL pH 1.2 HCl buffer for 12h. At regular time intervals, 10mL of the dissolution medium were withdrawn, replaced with an equivalent volume of fresh dissolution fluid and analyzed for the drug content using a UV-Vis spectrophotometer at 279nm.

Mathematical model fitting

The *in vitro* release data were fitted into various mathematical models using PCP. Disso-V2.08 software to know which mathematical model will best fit the obtained release profile. The parameters like 'n' the diffusion exponent and 'R' the regression co-efficient were determined to know the release mechanisms. The model with the highest correlation coefficient values or determination coefficient (R^2) was considered as the best fit model. If the value of 'n' determined from Korsmeyer-Peppas equation was below 0.45, it indicate that the drug release from the formulation follows fickian diffusion, if 'n' value was between 0.5-0.85, it indicate Non-Fickian diffusion or anomalous mechanism (relaxation controlled) and if 'n' value was above 0.89, it indicate super case II transport.

Stability studies

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and relative humidity (RH) conditions. Optimized formulation was selected and packed in a screw capped bottle and studies were carried out for 90 days by keeping at 40 ± 2°C and 75 ± 5 RH.

Samples were withdrawn on 30th day, 60th day and 90th day and were analyzed for drug content spectrophotometrically at 279nm as per ICH Q1A(R2) guidelines.

RESULTS AND DISCUSSION
FT-IR

The pellets of drug and potassium bromide were prepared by compressing the powders at 20psi for 1min on KBr - press and the spectra were scanned in the wave number range of 4000-450cm^{-1}. FT-IR study was carried on pure drug and final formulations of hollow microspheres. Characteristic peaks were observed at 3075cm^{-1} corresponding to S-H stretch, 793-626cm^{-1} corresponding to C-S stretch, 1338-1316cm^{-1} corresponding to C-N stretch, 1634cm^{-1} (C=O stretch), 1286-1223cm^{-1} corresponding to C-O-C stretching and at 918cm^{-1} corresponding to out of plane bending. In terms of final formulation peaks were obtained at 3407cm^{-1} corresponding to N-H asymmetric stretching, 2924cm^{-1} corresponding to C-H stretching, 1638cm^{-1} corresponding to C=O stretching, 1458cm^{-1} corresponding to C-H bending, 1365cm^{-1} corresponding to OH in plane bending, 1259-1229cm^{-1} corresponding to C-O-C stretching, 1216-1063cm^{-1} corresponding to C-N stretching, 750cm^{-1} corresponding to C-S stretching. All the formulation peaks when compared to pure drug were similar and this implied that there were there no incompatibility issues between the drug and the excipients.

SEM

The scanning electron microscopy (SEM) study was carried to identify the surface morphology of the hollow microspheres which were presented in figures 1a & 1b. SEM photographs showed that the drug loaded hollow microspheres were spherical in nature (mean size of around 285.9μm), having a smooth surface with inward dents and shrinkage due to the collapse of the wall of the microspheres. SEM photograph revealed the
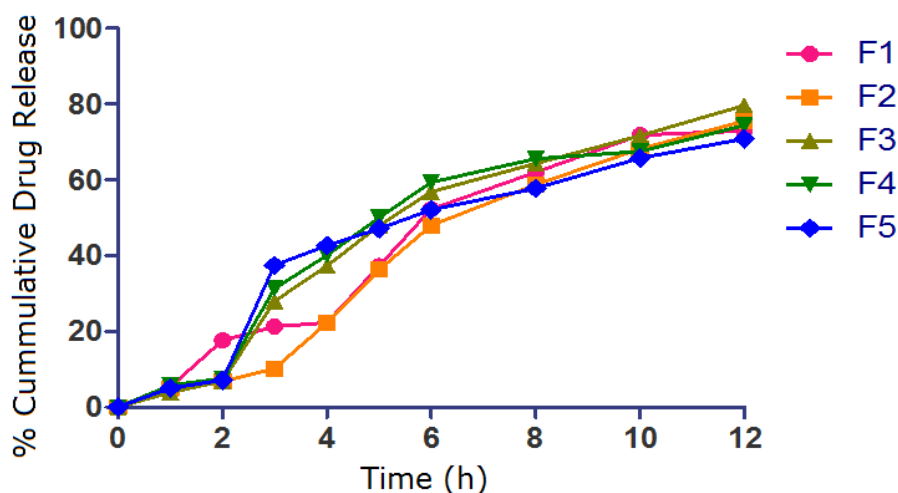
Figure 1. SEM photograph of formulation F3

Table II. Micrometric properties of DBM hollow microspheres

Formulations	Bulkdensity (gm/cm³)	Tapped Density (gm/cm³)	Hausner's ratio	Carr's Index	Angle of Repose (Θ)
F1	0.11±2.01	0.13±1.03	1.12±1.11	10.4±2.21	31.34°±1.01
F2	0.11±1.72	0.12±1.32	1.10±1.28	9.32±1.98	30.00°±1.11
F3	0.12±1.89	0.14±1.49	1.18±1.39	9.5±2.54	35.14°±1.92
F4	0.07±2.63	0.08±1.25	1.13±1.09	11.4±1.89	36.42°±1.78
F5	0.08±1.18	0.09±1.56	1.10±1.35	9.09±2.11	38.27°±1.80

*Mean ± SD, n=3

absence of drug particles on the surface of microspheres indicating the uniform distribution of the drug in the walls of the microspheres. SEM photographs also indicated the presence of minute pores on the surface of the microspheres. It might be due to rapid diffusion of the solvent from the walls of the microspheres and there is a possibility of rupture of microsphere walls.

Bulk density

Bulk density may influence capsule filling, dissolution and other properties. The Bulk density refers to a measure used to describe a packing of microspheres. Bulk density largely depends on particle shape. As particles become more spherical in shape bulk density is increased. In addition microspheres size increases, bulk density decreases. Here, the bulk density of dextromethorphan hydro-bromide hollow microspheres formulations F1-F5 are determined and given in table II.

Tapped density

Tapped density largely depends on particle shape. As particles become more spherical in shape tapped density is increased. In addition microspheres size increases, tapped density decreases. Here the tapped density of Dextromethorphan hydrobromide micros-pheres formulations F1-F5 are determined and given in table II.

Angle of repose

The angle of repose for Dextromethorphan hydrobromide hollow microspheres shows good flow characteristics that lead to the uniform filling of dosage form in capsules. It was done as per the procedure and given in table II.

Table III. Evaluation parameters of DBM hollow microspheres

Formulations	Particle size (µm)	% yield	Drug content (%w/w)	% drug entrapment efficiency	% moisture loss	% buoyancy
F1	67.16±3.0	79.5%	94.29±1.32	78.16±1.12	3.57±0.02	80.7±4.8
F2	57.75±3.5	81.7%	95.87±1.28	88.11±1.38	1.69±0.12	83.4±2.1
F3	94.25±7.0	88.8%	96.10±1.40	90.31±1.59	1.63±0.29	94.2±3.2
F4	87.65 ± 4.5	71.3%	89.06±1.59	68.27±1.62	3.97±0.11	85.0±2.3
F5	83.75 8.5	73.28%	82.69±1.87	89.52±2.18	2.92±0.08	92.2±4.6

* Mean ± SD, n=3
In vitro release studies

Figure 3: Prepared hollow microspheres of DBM.

Carr's index

The measurement of free flowing granules can also be done by compressibility with the tapped and untapped values. The compressibility index of Dextromethorphan hydrobromide hollow microspheres is determined and given in table II and it is found to have good flow characteristics.

The Hausner's ratio of dextromethorphan hydrobromide hollow microspheres was calculated and it shows good flow characteristics. The values are given in table II

Particle size analysis

All the hollow microspheres 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 50 microspheres were measured randomly by optical microscope. The microspheres were uniform in size with a mean size range of 57.75±3.5 to 94.25±7.0µm which fall in the arbitrary particle size range of 5-50mm. Influence of surfactants was clearly observed in terms of particle size. As the concentration of surfactants increased particle size decreased. Comparing between the surfactants, Tween 80 showed good particle size than span 80. The particle size ranges are shown in table III.

Percentage Yield

Percentage yield of the formulations was found in the range 71.3-88.8%, and among the five formulations the % yield of formulation F3 is 88.8%.

Drug content

In case of drug content study, drug content for all the formulation was in the range of 82.69±1.87 - 96.1±1.40% w/v.

Drug entrapment efficiency

The amount of dextromethorphan hydrobromide present in the hollow microspheres was determined by extracting the drug into 0.1 N HCl under magnetic stirring for a period of 2h. The solution was filtered through Whatmann filter paper no.5, suitably diluted and estimated for drug content spectrophotometrically at 279 nm and tabulated in table III. Formulation F3 showed highest amount of entrapment efficiency of 90.31±1.59% and this was one of the factor for determining the optimized formulation.

Floating lag time

All the batches of microspheres showed no lag time or a lag time of zero seconds which indicated that all the microspheres formed were hollow.

Percentage of Mmoisture Loss

Percentage of moisture loss of the formulation was found in the range 1.63-3.97 %.

In vitro buoyancy percentage

In vitro buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres. The *in vitro* buoyancy percentage was calculated as per the procedure and tabulated.

$$\% \ Buoyancy = \frac{Microsphere \ remained \ floating}{Total \ mass \ of \ microspheres} \ X \ 100$$

In vitro release studies

The *in vitro* release studies were performed in 250 ml of pH 1.2 HCl buffer thermostatically maintained at 37±0.5°C based on Chinese Pharmacopoeia (2005 Ed.) method II. Paddle rotational speed was set to 50rpm. 10mL samples were withdrawn and replaced with an equal volume of the same fresh medium at predetermined time intervals. The sample solutions were filtered through a 0.45 μm membrane and analyzed using a UV spectrophotometer at 279nm. Release pattern for formulation F1-F5 are shown in the following figure 4.

Mathematical model fitting

The *in vitro* release studies data was fitted into various mathematical models to determine which one is the best-fit model. The results indicated that the best-fit model of optimized formulation F3 was found to be peppas model while all other formulations were following first order. Graph between log percentage release and log time was plotted and n value was found to be 0.811 indicating the release of drug from all the formulations was found to be by non-fickian diffusion.

Stability studies

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The optimized formulation (F-3) was subjected to stability studies according to ICH guidelines by storing at 25ºC/60 % RH and 40ºC/75 % RH for 30days. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. After analysis, drug content after 30 days was found to be 98.66%±0.82, after 60 days it was 97.90%±1.56 and after 90 days it was 97.55%±0.96. From the data, it was clear that the formulation did not undergo any chemical changes/interaction during the study period explained based on drug content values which was found to be significant.

CONCLUSION

The objective of this study was to prepare and evaluate floating hollow microspheres of Gelatin loaded with Dextromethorphan hydrobromide for controlled release. These microspheres were prepared by emulsion polymerization method. From the results, it can be concluded that the hollow microspheres formulation is easy to administer, simple with increased patient compliance. Formulation F3 was determined as the optimized formula as it exhibited highest drug entrapment efficiency and maximum drug release compared to other formulations. Hence dextromethorphan hydrobromide could be formulated into hollow microspheres as controlled drug release dosage form by emulsion polymerization method.

REFERENCES

Ansel, HC., Allen, Jr., LV., *et al,* 2000, *Pharmaceutical Dosage forms Drug Delivery Systems,* 7th Edn., Lippincott Williams & Wilkins, Philadelphia, PA, p,96-141.

Aulton, ME., 1996, *Pharmaceutics-The science of dosage form design, 1 st (International student)* Edn., Churchill Livingstone , New York, p,113-138.

Brahmankar, DM., Jaiswal, SB., 1998, *Biopharmaceutics and Pharmacokinetics*, 2 nd Edn., VallabhPrakashan, New Delhi, p,23.

Deshpande, A., Shah, N. H., Rhodes, C. T., Malick, W.,1997, Development of a novel controlled release system for gastric retention. Pharm Res, 14, 815-22.

Garima, Piyush., Vishal., Arvind, K., 2003, Gastroretention: A means to address regional variability in intestinal drug absorption. *Pharma Tech.*, 27, 50-68.

ICH Harmonised Tripartite Guideline: Stability testing of New drug substances and products Q1A(R2), http://www.ich.org/ fileadmin/public_web_site/ich_products /guidelines/quality/q1a_r2/step4/q1a_r 2__guideline.pdf

Mathiowitz, E., Langer, R.,1987, Polyanhydride microspheres as drug carriers by hot melt microencapsulation.*J Control Rel.,* 5, 13-22.

Mathiowitz, Edith ed: 1999, *Encyclopedia of controlled drug delivery,* Vol-I, New York Wiley; 9-11.

Ninan, M., Lu, X., Wanga, Q., 2008, Development and evaluation of new sustained-release floating microspheres. *Int J Pharm.,*358, 82-90.

Pharmacopoeia of the People's Republic of China, Chemical Industry Press, 2005.

Rama, Prasad, Y., Krishnaiah, Y., Satyanarayana, S., 1998, In vitro evaluation of guar gumas a carrier for colon-specific drug delivery. *J Controlled Release.,*51, 281-287.

Shivakumar, H. G., Vishakante, D., Pramod, Kumar, T. M.,2004, Floating controlled drug delivery systems for prolong gastric residence. *Indian J Pharm Educ.*, 38, 172-79.