Research Article

In vitro Evaluation and Characterization Methods of Antifungal Agent as Microspheres

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ABSTRACT

The main objective is to develop and evaluate controlled release microspheres of the antifungal drug as ketoconazole. The absorption of antifungal drug ketoconazole is enhanced by gastric acidity, because it is more soluble at acidic pH and hence it was an idea to prepare a controlled release multiple unit improved oral dosage form which could prevail over the problem associated with normal conventional tablets. Short t1/2 (half-life) and low molecular weight favored pharmacokinetic importance for development of a novel drug delivery system. Drug containing antifungal agent was prepared using one of the most popular method i.e solvent evaporation method using ethyl cellulose and methyl cellulose as release rate controlling polymer. Optimization Technique: 22 factorial design was selected for optimization of the amount of polymers to be used. Scanning Electron Microscopy: The surface images of the sustained release microspheres were characterized by scanning electron microscopy. Characterization: The prepared microspheres were characterized by their micromeritics properties such as particle shape and size, interquartile coefficient of skewness and kurtosis. Dissolution data obtained from in vitro release studies fitted Higuchi and Peppas model with a diffusion exponent value of 0.67 thus suggesting non fickian diffusion as the mechanism of drug release. The release profile of KTZ could be represented by Jander's equation, exhibiting pH- dependency and therefore the release of KTZ was governed by the diffusion within the microspheres and the solubility. Microspheres prepared by solvent evaporation method using antifungal agent i.e Ketoconazole exhibit controlled release behavior, which was based on pH as diffusion controlled release behavior without matrix erosion.

Keywords: Ketoconazole, Antifungal agents, Optimization, Baker Lonsdale model, Peppas model, Higuchi plot

INTRODUCTION

The main objective was to design diffusion controlled drug delivery system of ketoconazole in order to control or sustain the delivery of the drug and thereby reduce the gastrointestinal disturbances and dose related adverse effects like hepatic dysfunction and allergic reactions as observed with conventional oral dosage form of ketoconazole (tablet). Microspheres of ketoconazole was prepared by solvent evaporation method by using solvent which falls under the category of ICH guidelines (Q3C) to be used in the pharmaceutical industry because of their minimum toxic effect, as the coacervating agent to decrease the remaining solvent.

MATERIALS AND METHODS

Materials

Gift sample of antifungal drug was obtained as gift sample from Torrent pharmaceuticals ltd (Indrad) Gujarat, India. Cellulose derivatives were purchased from S.D fine chemicals ltd, Mumbai, India. Double distilled water was used throughout the study.

Preparation and optimization of drug free microspheres

Drug free microspheres were prepared by one of the most popular technique for the preparation of microspheres i.e solvent evaporation method which has wide acceptance all around the world[1] using ethyl cellulose as a film forming agent around the drug which can retard the release of drug and methylcellulose to provide the spherical shape and integrity to microspheres. 2^2 factorial design was used to calculate the amount of polymers to be used for the preparations of microspheres (Table 1). 2 gm of film forming polymer i.e Ethyl cellulose was taken with 20 ml of organic solvent such as dichloromethane which was considered safe as per ICH guidelines[2]. The polymer phase of ethyl cellulose was then added to 250 ml of 0.25% w/v methylcellulose aqueous solution (over night dispersion). Rotation speed was maintained at 350 round per minute which helps in complete removal of dichloromethane. Microspheres were taken, filtered, washed with distilled water at least three times to remove any traces of residual solvent and then microspheres were stored in desiccators under reduced pressure for one night. Additionally, optimization of microspheres was done with suitable stirring element i.e. mechanical stirrer (which can maintain high rotation speed) Vs magnetic stirrer (slow in speed) in order to keep an eye on the regular shape and % yield of drug free microspheres. t- test was applied for final selection of the stirring element based on 95% confidence interval[3].

Table 1: Factorial design for the optimization of the polymers.

Factors	Methyl cellulose Concentration	Ethyl cellulose Concentration
A1	Low	Low
A2	Higher	Low
A3	Lower	Higher
A4	Higher	Higher

Key (+) - Higher concentration

(-) - Low concentration

Characterization of Microspheres without Drug

Micromeritic Studies

The Micromeritics studies were carried out using optical compound microscope of A1 to A4 by taking the microspheres on a glass slide under 100X magnification. Average diameter of more than 600 microspheres were calculated and noted[4]. Micromeritics properties were calculated by using the noted data and statistical test was applied on the computed data.

Selection of Optimized microspheres

Micromeritics properties of the drug free microspheres were the basis of selection of optimized microspheres. Other parameters such as diameter, % yield and uniformity of shape, were also taken into account for the selection of microspheres. Statistical parameter such as t-Test was applied for the selection of suitable stirring element which was based on % yield and decline in standard deviation of average diameter. The selected formulation was used for preparation of microspheres with drug.

Preparation of Ketoconazole Microspheres

Ketoconazole drug was used for the preparation of microspheres by using one of the most popular method i.e solvent evaporation methods. 2gm of film forming polymer such as Ethyl cellulose was taken in 20 ml of dichloromethane and drug was added in the polymer solution, in different amounts, corresponding to theoretical initial loading range from 5 to 40%w/w (F_1-F_7), Table 2. Ethyl cellulose polymer phase was added to 250 ml of 0.25% w/v aqueous methylcellulose solution (which was kept overnight to form a clear dispersion). Rotation was maintained at 350 rpm (RPM maintained by speed regulator) until complete evaporation of dichloromethane were achieved which was ensured by placing the microspheres in the desiccator. After washing the microspheres with double distilled water, microspheres were again exposed to 0.1N HCl and absorbance of filtered extract was determined by double beam UV spectrophotometer. It was found to that negative absorbance up to 0.001 was found and thin layer chromatography of the filtered extract did not show any spot w.r.t ketoconazole.

Table 2: Formulation codes with % w/w amount of drug

S.NO.	Formulation Codes	% amount of drug (w/w)
1	F_1	5
2	F_2	10
3	F_3	15
4	F_4	20
5	F_5	25
6	F_6	30
7	F_7	40

Evaluation of Ketoconazole Microspheres

Drug entrapment efficiency

10 mg of the Ketoconazole microspheres were taken with 0.1 N Hydrochloric acid buffer and it was mixed with a vortex mixture for near about 15 minutes and centrifuged at 350 round per minute (RPM) and the F_1-F_5 formulations were investigated for further studies.

Micromeritic studies

Ketoconazole microspheres (f1 to f5) were observed under compound microscope using optical microscopy technique for determination of particle size and its distribution under 10X magnification. The mean diameter of approximate 600 microspheres were calculated and the data which was obtained plotted on log-probability scale and data was used for calculations of average microsphere size, standard deviation, coefficient of kurtosis and Intra quartile coefficient of skewness (IQCS).

In vitro dissolution study

200 mg of ketoconazole microspheres were filled in transparent, gelatin capsule having zero size were investigated for drug release study. F_1-F_5 formulations were taken for the in vitro drug release studies which was carried out with the help of dissolution apparatus (USPXXIV Type I rotating basket type) (Hicon, New Delhi) using simulated conditions by using 900 ml of 0.1 N hydrochloric acid buffer (HCl) for 2 hrs (gastric emptying time) followed by dissolution in alkaline phosphate buffer for next 6 hrs at 50 round per minute as per USP NF 2004. 200 mesh size muslin cloth was tied over the rotating basket to prevent the exit of ketoconazole microspheres from the rotating basket[5]. Hard gelatin capsule filled with microspheres was placed in the rotating basket and 10 ml samples were withdrawn at regular time intervals replacing with an equal amount of fresh dissolution medium such as acidic buffer and alkaline buffer respectively (37±0.5º C) immediately after withdrawal of test samples in order to maintain the sink condition. All samples were collected in a test tube which was filtered, diluted suitably (if required) and analyzed by double beam UV spectrophotometer (Pharma Spec1800, Shimadzu Japan) at 269.0 nm for samples in 0.1N hydrochloric acid buffer (HCl) and 280.5 nm for samples in alkaline phosphate buffer. The % amount of drug

dissolved at different time intervals was calculated by using the formula. Dissolution studies were performed in triplicate to check the reproducibility for each batch and the data obtained was plotted against time.

Model fitting

Dissolution data obtained from drug release studies was fixed to various kinetic equations to find out the exact process of drug release from film forming polymers as ethyl cellulose microspheres. Various kinetic models were used such as zero order[6], first order[7], Higuchi[8], Peppas model[9].

The following plots were made:

- Q_t vs. t (zero order kinetic models);
- log (Q_o-Q_t) vs t (first order kinetic model);
- Q_t vs square root of t (Higuchi model)
- $Q = kt^n$ (peppas model)

Where Q_t is the amount of drug released at time t and Q_o is the initial amount of drug present in the microspheres,

Where k is the constant incorporating geometrical and structural characteristic of the drug release device and Q is total amount of drug released in a given stipulated period of time t and n is the release exponent value which is an indicative of the release mechanism.

Plots were plotted and regression coefficient was calculated and hence the order of release.

Optimized Formulation of Ketoconazole Microspheres

Few parameters were adopted to find out the best formulation in terms of highest drug entrapment efficiency of microspheres, an Intra Quartile Coefficient of Skewness (IQCS) value leads to near zero and best drug controlled release formulation. The F5 formulation was selected as the optimized formulation and was used for further investigational studies.

Scanning Electron Microscopy for Surface Topography

Scanning electron microscopy was used to know about the surface morphology of selected F_5 formulation of microspheres. Sample of drug loaded microspheres were taken on to stubs using double sided tape and vacuum coated with gold film (10A°) by a polaron sputter coater E5100 and analyzed by Scanning electron microscopy JEOL, JSM-T220, Japan).

Stability Studies

20 mg drug loaded microspheres (20 mg) were placed in clear glass vials, sealed and stored at controlled humidity conditions at RT (room temperature) (25°±2°C), Elevated temperature (Oven) (45±2°C) and in a refrigerator temperature maintained up to 5-8°C for a period of 2 months.[10]. Microspheres samples were analysed for % drug content and further investigated for physical and chemical stability at regular interval of 30 days. The withdrawn samples were also subjected to chromatographic analysis technique i.e thin layered chromatography to check out any degradation product in terms of change in retention factor.

RESULTS AND DISCUSSION

Preliminary Trials for Optimization of Microspheres without Drug

Preparation

Solvent evaporation method was used to prepare microspheres by using film forming polymer (ethyl cellulose) and methylcellulose (provides shape and integrity to microspheres). Solvent evaporation method is most popular method for the preparation of drug containing matrix particles from water insoluble polymer for sustained release of drug. The release rate depends upon various factors such as concentration of polymers selected and the nature of polymers used.

Table 3: Various response of Mechanical stirrer Vs magnetic stirrer

S. No.	Parameters	Observation	% response observed
1	% Yield	Increased	2.44
2	Particle size diameter	Decreased	0.13
3	Standard Deviation	Almost same	No change was observed

($p < 0.5$)

Therefore, 2^2 factorial design was used for selecting the concentration of polymers to be used for the preparation of microspheres. On the basis of the factorial design were the basis of selection of microspheres without drug which were prepared (A_1-A_4) using film forming polymer (ethyl cellulose) (5 % as lower limit and 10% by weight as the upper limit) and methyl cellulose which helps in maintaining shape, firmness and integrity by using 0.125% as lower concentration and 0.250% by weight as the higher concentration. Experimental data revealed that higher limits of both polymers i.e ethyl cellulose and methylcellulose gave spherical shape to the drug free microspheres with an average mean diameter of 283.41micron meter (μm). Hence A_4 formulation was selected with high levels of ethyl cellulose and methyl cellulose. In addition to the above, optimization of microspheres was done with suitable stirring element i.e. mechanical stirrer (which can maintain high rotation speed) Vs magnetic stirrer (slow in speed) in order to keep an eye on the regular shape and % yield of drug free microspheres (see table 3). t- test was applied on the data obtained after analysis through both stirring elements and it was found that Mechanical stirrer found superior for final selection of the stirring element based on 95% confidence interval. It was found that there was a significant difference (P> 0.5%) between the methods used, at 95 % confidence interval.

Evaluation

Micromeritic studies

The Micromeritics studies were carried out using optical compound microscope of A1 to A4 by taking the microspheres on a glass slide under 100X magnification. Average diameter of more than 600 microspheres were calculated and noted. Micromeritics properties were calculated by using the noted data and statistical test was applied on the computed data (Table 4). Formulation A_4 without drug with higher concentration of ethyl cellulose and higher concentration of methyl cellulose (selected on the basis of factorial design) was selected for further studies for drug loading because it resulted in an mean diameter of 283.41 micrometer (μm) with minimum value of standard deviation 1.58 (closer to zero) and an inter quartile coefficient of skewness value of – 0.121 which is very close to zero thus we can estimate symmetrical distribution between the different quartile points. The symmetry of distribution is based on the comparison of the height and thickness of the tail and sharpness of peak with those of normal distribution. A downbeat value for kurtosis coefficient suggest a thin tailed and blunt peaked curves of particle size distribution which suggest platykurtic distribution. A_4 formulation also exhibits platykurtic distribution suggesting higher occurrence of fine size of microspheres. This means that a maximum percentage of drug free microspheres be positioned in the range of 281.83 - 283.41μm.

Table 4: Various factors of micromeritics studies of the hollow microspheres

Formulation code	Shape	Kurtosis	% Yield	Mean Diameter (micrometer)	Standard Deviation	IQCS
A1	Irregular	Leptokurtic	41.67	191.82	1.98	0.311
A2	Spherical	Platykurtic	60.49	244.80	1.61	-0.0769
A3	Spherical	leptokurtic	42.67	150.22	2.83	0.125
A4	Spherical	Platykurtic	60.80	283.41	1.58	-0.121

Preparation of ketoconazole microspheres

Ketoconazole drug was used for the preparation of microspheres by using one of the most popular method i.e solvent evaporation methods. 2gm of film forming polymer such as Ethyl cellulose was taken in 20 ml of dichloromethane and drug was added in the polymer solution, in different amounts, corresponding to theoretical initial loading range from 5 to 40%w/w (F_1-F_7). Mechanical stirrer was selected as stirring element after statistical analysis. Microspheres prepared by solvent evaporation technique were investigated for the various below mentioned parameters.

Evaluation of Ketoconazole Microspheres

Drug entrapment efficiency

The drug entrapment efficiency of F_1-F_7 shown in (Fig.1.) clearly indicates As we are increasing the drug loading from 5 – 25 % w/w, the drug entrapment efficiency also increased linearly up to F5 formulation afterward a quick decline was seen in drug entrapment efficiency with values of 30 –40% w/w.[11] Drug release study of Formulation F_5 – F_7 (25% w/w, 30%w/w and 40% w/w of drug loading) was carried out in acidic buffer (0.1 N Hydrochloric acid buffer) for initial 2 hrs followed by dissolution studies in alkaline phosphate buffer pH 7.20 for a period of another 6 hrs. The drug release studies showed that, the % drug release studies (Fig 2) follows Baker – Lonsdale model.[12] As per Baker Lonsdale model, for a dispersion containing drug, the portion of drug which was released slowly decreases with an increase in the initial drug loading whereas, for the dissolved drug, the fraction released at any time is independent of initial drug loading. Baker and Lonsdale developed this model in 1974 from Higuchi model and describe the drug released from a spherical matrix by controlled release mechanism. Analysis of variance (One way ANOVA) followed by scheffs pairs wise comparison did not find any significant difference between the sets. F_1-F_5 formulations were selected for the development of formulation for further investigational parameters for selection of optimized microspheres of ketoconazole drug.

Micromeritic studies

F1 to F5 formulations containing ketoconazole (Table 5) were found spherical in shape but the difference in properties helped us for selecting the best formulation to be used as microspheres. The F_5 formulation with an average mean particle size of 353.6μm with a mean standard deviation of 1.43 near to zero was identified as better formulation with highest % of drug content of 4.854 mg / 10 mg of the drug loaded microspheres. After applying statistical tools on particle size distribution data an Intra quartile coefficient of skewness value of – 0.023 was found almost found zero. If value was found near zero, the size distribution is found practically symmetrical between the quartile points and the value of standard deviation shows regularity in standard size of microspheres. Particle size uniformity is prerequisite criteria for sustained drug release of the drug, which is beneficial for maintaining uniform therapeutic response for the required period of time thus changes in the peaks of drug can be avoided in drug levels. Platykurtic distribution further justified the symmetrical distribution of the microspheres and indicates maximum percentage of fine size of microspheres which is desirable for multiple unit sustained release dosage form.[13]

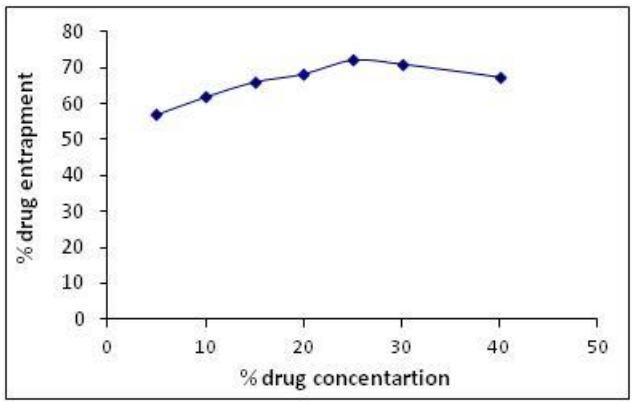

Figure 1: Percentage drug entrapment Vs percentage drug concentration of ketoconazole in microspheres

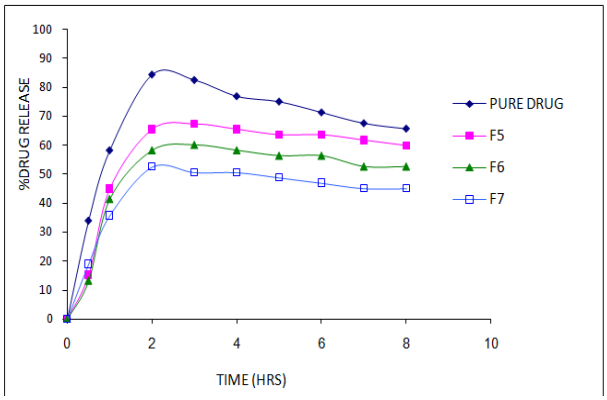

Figure 2: Percentage drug release Vs time (hrs) profiles of pure drug & F_5 to F_7 formulation

Table 5: Drug content and micromeritic properties of F1 to F5 formulations of microspheres.

Factor	Shape	Average diameter	% yield	Drug content	IQCS	Standard	Kurtosis
F1	Spherical	203	71.55	2.199	0.152	2.12	Leptokurtic
F2	Spherical	321.0	77.03	2.323	0.0342	1.68	Leptokurtic
F3	Spherical	252.2	79.03	3.651	0.0322	1.59	Leptokurtic
F4	Spherical	333.6	82.30	3.775	-0.028	1.35	Platykurtic
F5	Spherical	353.6	84.29	4.854	-0.023	1.43	Platykurtic

***Drug Release* study**

In vitro drug release studies were performed with drug loaded microspheres in acidic buffer for 2 hrs & in alkaline buffer for 6 hrs. Data obtained from the study showed biphasic drug release of microspheres (Fig. 3). At Initial stage , a sharp peak effect was observed with F_4 and F_5 formulations in 2 hrs of study with acidic buffer, after this a period of sustained release was observed when microspheres were introduced to alkaline buffer up to 8 hrs. This type of behavior was not found important with the initial formulations having 5 % w/w to 15 % w/w i.e. F_1- F_3. This suggests that the drug release is based on the amount of the drug content present in formulation of microspheres.[14, 15] The alkaline nature of the antifungal drug such as ketoconazole allows the solubility of alkaline drug in the 0.1 N Hydrochloric acid buffer or acidic media used for the test. This will be promoted with quick diffusion of drug through various dissolution media which was supported by filled pores and channels have explanation about the burst effect of drug release in F_4 and F_5 formulations. The slower drug release can be achieved to advanced partition of the drug to microsphere matrix as compared to the various dissolution medium.[16] This was supported by due to alkaline nature of drug, which does not hold its solubility in the tested alkaline medium. Various other factors that can affect the drug release from the microspheres include the size of microsphere, physical state of drug in the polymer, type of polymer and its morphology.[17]

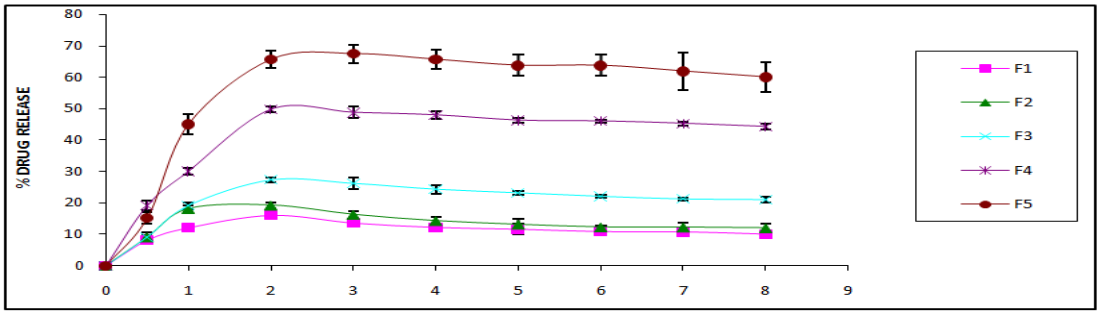

Figure 3: Percentage drug release Vs time (Hrs) profile of F1 to F5 formulations of ketoconazole

Model fitting

In order to have important information for the various release models, the percentage drug release profiles were computed to various kinetic models. Table 6 summarizes the coefficient of correlation for the various release kinetic models of microspheres of ketoconazole. High correlation coefficient value was found to be a more appropriate model for the kinetic dissolution data. The dissolution data obtained fitted in Higuchi and Peppas model gives r^2 values of 0.9216 and 0.9134 respectively

which signify diffusion as the mechanism of release of ketoconazole microspheres. n value (diffusion exponent value) was found to be 0.67, which is more than 0.45 thus we can confirm the non-fickian release.

Table 6: Dissolution profiles of F1 to F5 formulation after various kinetic model treatment

Formulation	Zero order r^2	First order r^2	Higuchi plot r^2	Peppas plot r^2
F1	0.8575	0.8618	0.9183	0.9045
F2	0.7904	0.7850	0.9085	0.8876
F3	0.8927	0.8783	0.8976	0.8962
F4	0.8511	0.8293	0.9116	0.9025
F5	0.8711	0.7942	0.9216	0.9134

Scanning Electron Microscopy for Surface Topography

Scanning electron microscopy was used to know about the surface morphology of selected F_5 formulation of microspheres. It is clear from scanning electron microscopy photomicrograph (Fig. 4) that the microspheres were spherical, uniform, with rough surfaces with an average diameter of 363.33µm ± 1.21 (standard deviation). The irregular surfaces of the microspheres may be certified to rapid solvent evaporation during the preparation method of microspheres.[18] This surface topography is also supported by literature, which reports that microspheres with irregular surfaces are achieved when most popular technique solvent evaporation is used.[19]

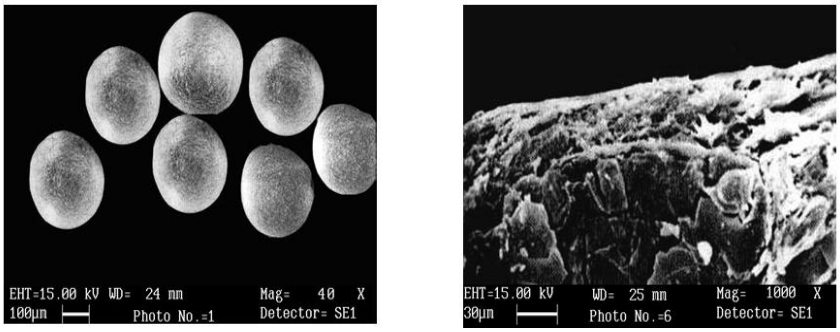

Figure 4: SEM images of ketoconazole microspheres & its surface images

Stability Studies

20 mg drug loaded microspheres (20 mg) were placed in clear glass vials, sealed and stored at controlled humidity conditions at RT (room temperature) (25°±2°C), Elevated temperature (Oven) (45±2°C) and in a refrigerator temperature maintained up to 5-8°C for a period of 2 months (10). Microspheres after subjected to different condition were found stable as indicated in table no. 7

Table 7: Stability studies of ketoconazole microspheres stored under different conditions of temperature

S.No.	Days (months)	Drug content under variable storage conditions		
		Refrigerator (5-8°C)	Room temperature (25°±2°C)	Oven temperature (45±2°C)
1	0	4.854	4.854	4.854
2	30	4.639	4.801	4.813
3	60	4.566	4.799	4.819

CONCLUSION

Microspheres prepared by solvent evaporation method using antifungal agent i.e Ketoconazole exhibit controlled release behavior, which was based on pH as diffusion controlled release behavior without matrix erosion. The formulated microspheres were stable and could be further modified for the development of pH independent oral controlled release microspheres by addition of enteric polymer (anionic poly electrolytes). The enteric coated polymer contributes as matrix forming base in 0.1 N HCl ((hydrochloric acid) acidic media and increased the pore for ketoconazole drug release caused by dissolution of enteric polymer in neutral and alkaline media thus resulting in pH independent release in the GIT (gastrointestinal tract).

REFERENCES

1. Dubernet C, Rouland JC, Benoit JP, Ibuprofen loaded ethylcellulose microspheres: analysis of the matrix structure by thermal analysis, Journal of Pharm. Sci, 80, 1991, 1029.
2. Dhanaraju MD, Bhaskar K , Vamsadhara C, A multiple emulsion method to entrap hydrophilic compound into ethylcellulose microspheres, Indian Drugs, 40, 2003, 99.
3. Bolton S, Pharmaceutical statistics practical and clinical application, 2nd ed., revised and expanded, 44, 1990,222.
4. Bayomi AM, El-Sayed MY, Casein microspheres as a controlled parenteral drug delivery system, Drug Dev. Ind. Pharm, 20, 1994, 2607.
5. Gohel MC, Amin FA, Studies in the preparation of diclofenac sodium microspheres by emulsion solvent evaporation technique using response surface analysis, Indian J. Pharm. Sci, 61, 1999,48.
6. Baveja SK, Rao KVR, Devi KP, Zero order release hydrophilic matrix tablets of beta adrenergic blockers, International Journal of Pharmaceutics, 39(1-2), 1987, 39-46.
7. Wagner JG, Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets, J. Pharm. Sci., 58, 1969, 1253.
8. Higuchi WI, Fawzi N, Katdare T, Model for the dissolution of calcium hydroxypatite powder, J. Phys. Chem, 96, 1963, 861.
9. Korsemeyer RW, Gurny R, Doelker E, Peppas NA, Mechanism of solute release from porous hydrophilic polymers, Int. J. Pharm, 15, 1983,25.
10. Guo JH, Preparation methods of biodegradable microspheres of bovine serum albumin loading efficiency and release profiles, Drug Dev. Ind. Pharm, 20(16), 1994, 2535.
11. Jain R, Navnit HS, Waseem M, Rhodes TC, Controlled drug delivery by biodegradable poly (ester) devices: different preparative approaches, Drug Dev. Ind. Pharm, 24, 1998, 703.
12. Costa P, Lobo JM, Modelling and comparison of dissolution profiles, Eur. J. Pharm. Sci, 13, 2001,123.
13. Mahrouk EGM, Al-Meshal MA, Mahrous GM, Preparation and evaluation of sustained release indomethacine nonpareil seeds, Drug Dev. Ind. Pharm, 19 (15), 1993, 1903.
14. Mthushamy K, Shibi KP, Ravi TK, Preparation and evaluation of albumin-chitosan microsphere containing theophylline, Ind. J. Pharm. Sci., 65, 2004, 245.
15. Perugini P, Genta I, Pavanetto F , Baruffini A, Study on glycolic acid delivery by liposomes and microspheres, Int. J. Pharm., 196, 2000, 51.
16. Pothal RK, Sahoo SK, Chatterjee S, Barik BB, Preparation and evaluation of mucoadhesive microcapsules of theophylline, The Ind. Pharm, 12, 2004, 74.
17. Mishra B, Jayanth P, Sankar C, Development of chitosan-alginate microcapsules for colon specific delivery of metronidazole, Indian Drugs,40 (12), 2003, 695.
18. Polk A, Amsden B, Yao de K, Peng T, Goosen MF, Controlled release of albumin from chitosan-alginate microcapsules, J. Pharm. Sci, 83, 1994,178.
19. Puglisi G, Giammona G, Santagati NA, Carlisi B, Villari A, Preparation and biological evaluation of ethylcellulose microspheres containing tolmetin, Drug Dev. Ind. Pharm. 18, 1992, 939.
20. Akiyama Y. Yoshika M, Horibe H, Hirai S, Kitamori N, Toguchi H., pH independent controlled release microspheres using polyglycerol esters of fatty acids, J. Pharm. Sci, 83, 1994, 1600.

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