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Research Article

# FLOATING DRUG DELIVERY SYSTEM AS AN APPROACH TO INCREASE THE GASTRIC RETENTION OF METHOTREXATE: FORMULATION AND EVALUATION

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**ABSTRACT**

The objective of the present study is to develop and characterize floating microspheres of methotrexate, which after oral administration could prolong the gastric residence time and increase the bioavailability of the drug, in order to provide the sustained release to minimize the dose dependent side effects as well as to improve patient compliance. Another aim is to investigate drug release at slight higher pH, which is due to frequent administration of water to make dosage form floating. The porous microspheres containing (1) methotrexate – antineoplastic agent, (2) casein – emulsifier which incorporate air bubbles at interface and (3) pectin – polymer, by emulsification extraction method and to evaluate gastro retentive and controlled release properties at pH 4.0. The effects of various process variables on the particle size, % buoyancy and % drug entrapment were assessed by $3^2$ full factorial design and concluded by using two way ANOVA and polynomial regression methods. Process variables had considerable effect on all dependent variables. The microspheres were found to be regular in shape with rough surface. Microsphere formulation M9 showed particle size 59.60±0.95µm, buoyancy 82.0±0.27%, and %drug entrapment 97.54±0.53%. In vitro drug release study was done results calculated by PCP disso *vi* software revealed best fitted model for M9 as Korsmayer Peppas model and drug release in Fickian manner. In vitro cytotoxicity study on KATO III gastric cell line revealed methotrexate microspheres had greater cytotoxic effects on cell line in comparison to pure drug solution. In vivo gamma scintigraphy studies were done using albino mice showed more than 8 hrs retention in upper gastro intestinal tract.

**Keywords:** Floating microspheres, Methotrexate, Emulsification extraction method.

## INTRODUCTION

Oral sustained drug delivery system is complicated by limited gastric residence time. Rapid gastrointestinal transit can prevent complete drug release in absorption zone and reduce the efficacy of administered dose since the majority of drugs are absorbed in stomach or upper part of small intestine. So to develop oral drug delivery systems, it is necessary to optimize both the residence time of system within the gastrointestinal tract and release of drug from the system[1]. Dosage forms that can be retained in stomach are called gastro retentive drug delivery systems (GRDDS)[2]. Gastro retentive floating drug delivery systems have bulk density lower than gastric fluids and thus remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time[3]. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GIT is to control the gastric residence time (GRT) using gastro retentive dosage forms. The aim of the present study is to prepare the floating microspheres of methotrexate which after oral administration could prolong the gastric residence time and increase the bioavailability of the drug and to investigate effect of slight high pH on drug release.

The concept of floating or porous microspheres can also be utilized to minimize the irritant effect of weakly acidic drugs on stomach by avoiding direct contact with the mucosa and providing a mean of getting low dosage for prolonged periods [4, 5].

Methotrexate (MTX) is an antineoplastic antibiotic whose mechanism is similar to alkylating agents. It is a highly toxic drug with a very low therapeutic index. It causes toxicities like stomatitis, gingivitis, glossitis, ulceration, and bleeding of the mucous membrane when given orally and hematological effects like leucopenia, thrombocytopenia, anemia, hemorrhage from various sites in single-dose intravenous administrations, and also some hepatic toxicities by administering as conventional dosage forms. Sustained and targeted delivery of MTX will reduce these toxicities considerably by maintaining a low and constant level of drug in the blood. [6, 7, 8]

Therefore, floating microspheres (GRDFs) have emerged as an efficient means of prolonging gastric residence time, targeting stomach mucosa, and enhancing the bioavailability. Floating microspheres remain buoyant due to lower density than the gastric and intestinal fluids. They are not subjected to 'all or nothing' gastric emptying nature of single unit system and releases the drug in a controlled fashion. The present investigation describes the formulation development of an intragastric floating drug delivery system of MTX and in vitro-in vivo release study at slight high pH [9, 10].

## MATERIALS

Methotrexate was purchased from Micromax Exports, Ahmadabad, Gujarat (India). Casein was purchased from Loba chemicals and Pectin from Southern Citrus Products Pvt. Ltd, Gudur, AP (India). All other chemicals used were of analytical reagent grade.

## METHODS

### Experimental design

A $3^2$ randomized full factorial design was adopted to optimize the variables. In the design two factors were evaluated, each at 3 levels, and experimental trials were performed at all nine possible combinations. In the present investigation, temperature (X1) and stirring speed (X2) were selected as independent variables. The particle size, % drug entrapment and % Buoyancy were selected as dependent variables[11, 12].

### Preparation of microspheres

In preliminary batches 10 ml of 15% w/v (in different ratio) casein and pectin solution were added to 60ml Soya oil. Both oil and polymer solution was preheated separately up to 60˚C. Drug was added to the polymer emulsifier solution in two different quantities (50mg and 100mg) as shown in table 1. The mixture was mechanically stirred at 1000rpm to form o/w emulsion, after 5 min the solution was rapidly cooled at 15°C. 250ml of acetone was added to dehydrate & flocculate coacervate droplets. The microspheres were isolated by filtration through sintered glass filter. In factorial design batches M1 to M9, the polymer to emulsifier ratio (1:1) and quantity of drug (100mg) was kept constant which was selected from preliminary batches. The temperature and stirring speed were varied in batches as shown in table 2. All other variables were used as mentioned in preliminary trial batch. The microspheres were isolated by filtration through sintered glass filter. Residual oil over the microspheres was removed by washing with 250ml of acetone. After preparation of microspheres they were stored at room temperature in a air tight container. The effect of formulation variables on characteristics of the microspheres is summarized in Table 2 and in Figure 1(a, b, c) [10,13].

**Table 1: Results of preliminary trial batches.**

| Batch Code | Polymer to Emulsifier Ratio (mg) | Drug Quantity(mg) | Drug Entrapment(%) | Bouyancy(%) | Particle Size and Sphericity |
|---|---|---|---|---|---|
| MP1 | 1250:250 | 50 | 95.32±0.89 | 50.0±0.79 | Small |
| MP2 | 1000:500 | 50 | 94.29±0.57 | 59.0±0.63 | Small |
| MP3 | 750:750 | 50 | 94.01±1.34 | 74.0±0.95 | Small/Spherical/Free flowing |
| MP4 | 500:1000 | 50 | 78.94±2.01 | 75.0±0.88 | Large/Irregular |
| MP5 | 250:1250 | 50 | 72.38±0.99 | 76.0±0.92 | Large/Irregular |
| MP6 | 1250:250 | 100 | 97.75±1.24 | 59.0±0.80 | Small |
| MP7 | 1000:500 | 100 | 96.26±0.73 | 67.0±0.69 | Small |
| MP8 | 750:750 | 100 | 96.10±1.10 | 83.0±1.22 | Small/Spherical/Free flowing |
| MP9 | 500:1000 | 100 | 82.01±0.83 | 84.0±0.96 | Large |
| MP10 | 250:1250 | 100 | 75.33±1.39 | 86.0±0.87 | Large/Irregular |

*** n = 3, all values ± standard deviation, statistically significant at 0.05 level.**

**Table 2: Formulation characteristics of batches in a $3^2$ full factorial design*.**

| Batch Code | Coded value | | Particle size(µm) | Buoyancy (%) | Drug Entrapment (%) |
|---|---|---|---|---|---|
| | X1 | X2 | | | |
| M1 | -1 | -1 | 107.00±0.23 | 74.2±0.23 | 95.40±0.89 |
| M2 | -1 | 0 | 86.00±0.45 | 78.0±0.34 | 95.45±0.48 |
| M3 | -1 | +1 | 76.66±0.35 | 80.3±0.66 | 95.89±0.67 |
| M4 | 0 | -1 | 98.30±0.26 | 75.2±0.34 | 96.68±0.29 |
| M5 | 0 | 0 | 79.40±0.39 | 78.4±0.25 | 96.64±0.88 |
| M6 | 0 | +1 | 64.00±0.98 | 81.0±0.65 | 96.66±0.64 |
| M7 | +1 | -1 | 88.00±0.23 | 76.0±0.37 | 96.69±0.97 |
| M8 | +1 | 0 | 74.80±0.48 | 79.5±0.42 | 97.16±0.48 |
| M9 | +1 | +1 | 59.60±0.95 | 82.0±0.27 | 97.54±0.53 |

| Coaded Values | Actual Values | | Variable levels |
|---|---|---|---|
| | X1 | X2 | |
| –1 | 40 | 500 | Low |
| 0 | 50 | 100 | Medium |
| 1 | 60 | 1500 | High |

*** n = 3, all values ± standard deviation, statistically significant at 0.05 level. $X_1$ is temperature of both phases (ºC), and $X_2$ is stirring speed (rpm). All batches contained 200 mg methotrexate, 1.5% pectin and casein in ratio 1:1.**

*** n = 3, all values ± standard deviation, statistically significant at 0.05 level.**

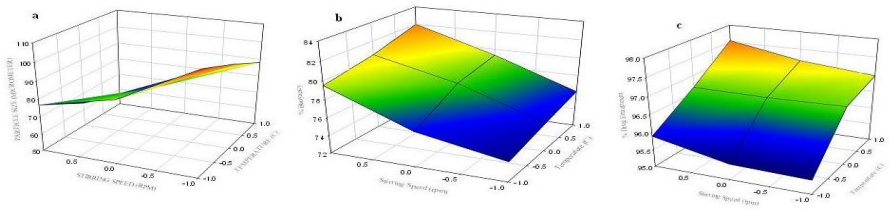

FIG 1. Response Surface Plot Of Methotraxate Microsphere (a) for particle size (b) % buoyancy and (c) % drug entrapment

**Analytical estimation of methotrexate**

Methotrexate was estimated by UV- Vis spectrophotometric method (Shimadzu UV 1601, Kyato, Japan). Solutions of drug were prepared in distilled water and buffers; the absorbance was measured at 256.4nm spectrophotometrically from 2.0 to 20.0µg concentration[14].

**Determination of particle size and its size distribution**

The particle size of the microspheres was determined by using optical microscopy method. Approximately 500 particles were counted for particle size using a calibrated optical microscope. The particle size of preliminary batches and factorial batches for individual drug is reported in table 1 and 2[15,16].

**Morphological study of microsphere**

The shape and surface morphology of the microspheres was investigated using scanning electron microscopy (Fig 2). Photomicrographs were observed at 50x magnification operated with an acceleration voltage of 10kV and working distance 9.1mm was maintained[17, 18].

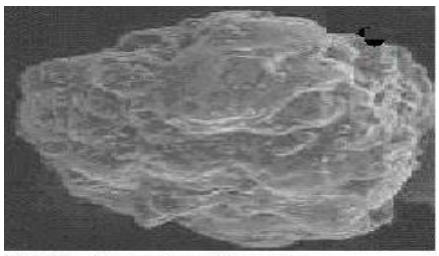

FIG 2. Scanning Electron photomicrograph of Microsphere

**Determination of drug entrapment efficiency**

Microspheres 200mg were crushed in a glass pestle and mortar, and the powdered mixed with distilled water solution then filtered with 0.2mm membrane filter and aliquot of the filtrate was diluted with acetate buffer (pH-4.0). The filtrate was analyzed for drug content and absorbance was measured at 256.4nm using UV spectrophotometer. The drug entrapment efficiency was calculated by the following formula[19, 20] –

$$\text{Percentage drug entrapment} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

**Percentage buoyancy**

Porous microspheres 200mg were spread over the surface of a USP XXIV paddle type dissolution apparatus filled with 900ml of acetate buffer pH 4.0 containing 0.02% v/v of tween 20. The mixture was stirred at 100rpm; particles were pipetted out and separated by filtration. Particles in sinking particulate layer were separated by filtration. Particles of both types were dried in dessicator until constant weight was obtained by both fractions of the microspheres. These were weighed and percentage buoyancy was determined by using following formula[20,21].

Percentage Buoyancy = {[wf/wf + ws] x 100}

**Fourier transforms infrared spectroscopy**

Drug polymer interaction was studied by FT-IR spectroscopy (Shimadzu Affinity I, FT-IR spectrophotometer). The spectrum was recorded for pure MTX, MTX loaded microspheres and unloaded microspheres (placebo). Samples were prepared by triturating 5% of drug or microsphere with 95% of KBr in glass pestle mortar. The scanning range was 4000 $cm^{-1}$ to 400 $cm^{-1}$ and resolution was 2 $cm^{-1}$ [21, 23].

**Flow properties**

Flow properties were determined in terms of Carr's index (Ic) and Hausner's ratio (HR) using the following formula:

$$HR = \rho t / \rho b$$

$$Ic = \rho t - \rho b / \rho t$$

Where, ρt = tapped density
ρb = bulk density

The angle of repose (θ) of the microsphere, which measures the resistance to particle flow, was determined by the fixed funnel method, using the following equation: $\tan \theta = 2H/D$

Where, H the height and D is the diameter of the heap that formed after making the microspheres flow the glass funnel (table 3) [24].

**Table 3: Micromeritic properties of microspheres.**

| Code | Angle of Repose (ɵ) | Carr's Index (%) | Hausner's Ratio |
|---|---|---|---|
| M1 | 27.36±0.690 | 17.17±0.241 | 1.164±0.015 |
| M2 | 24.67±0.508 | 15.14±0.362 | 1.131±0.021 |
| M3 | 22.64±0.321 | 12.45±0.423 | 1.123±0.040 |
| M4 | 25.64±0.423 | 16.46±0.352 | 1.132±0.028 |
| M5 | 22.32±0.034 | 12.78±0.423 | 1.125±0.019 |
| M6 | 20.53±0.540 | 11.03±0.242 | 1.120±0.042 |
| M7 | 24.34±0.231 | 14.95±0.632 | 1.142±0.024 |
| M8 | 23.95±0.321 | 12.67±0.531 | 1.140±0.033 |
| M9 | 20.32±0.432 | 11.01±0.342 | 1.135±0.035 |

**Stability of microsphere at different gastric pH**

Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period where they are exposed to different pH and different enzymatic conditions which can influence their physicochemical properties and drug release behavior and can alter their stability characteristics. To test this hypothesis, drug loaded microspheres were subjected to different pH media where they encountered different ionic strengths and enzymatic conditions and the change in their properties was elucidated by counter checking their particle size. pH dependent stability studies were carried out in following media:

1. pH 1.1: 12 ml HCl (32%) with 1188 ml H2O
2. pH 3.5: 150 ml solution (10.5 g citric acid+100 ml NaOH (1 M)+395.5 ml $H_2O$) with 100 ml HCl
3. Simulated Gastric Fluid (SGF): 0.2% NaCl, Pepsin 0.7% HCl with pH 1.2

Ten milliliters of simulated fluid were added to 10 mg of microspheres. The samples were analyzed after a period of 12hrs in each of the above media. The above time intervals were selected for the study based on expected formulation residence time in stomach. Particle size was determined on the preset time periods. The results are recorded in table 4 [25].

**Table 4: Initial and final particle size after exposure to different gastric pH**

| Medium | Initial Size Size | Final |
|---|---|---|
| pH 1.1 | 59.60±0.95 | 60.20±0.95 |
| pH 3.5 | 59.60±0.95 | 61.30±0.95 |
| SGF | 59.60±0.95 | 60.99±0.95 |

**In vitro dissolution studies**

In Vitro dissolution studies were performed using US Pharmacopoeia XXIII Dissolution apparatus II (paddle type). An accurate weighed sample (50 mg) of optimized porous microsphere (M9) was dropped into 900 ml of hydrochloric acid buffer (pH 2.0) and acetate buffer (pH4.0) maintained at a temperature at 37˚C ± 0.5˚C and stirred at a speed of 50 rpm. At different time intervals, a 10ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at 37˚C. The collected samples were filtered and analyzed at $\lambda_{max}$ 256.4nm using a UV-Visible spectrophotometer against both the buffers taken as blank. Percentage drug dissolved at different time intervals was calculated using Lambert-Beer's equation. The drug release was calculated using various models[26].

**In vitro cytotoxicity analysis of methotrexate loaded microsphere on Kato III Human gastric cancer cell line**

The KATO III human gastric cancer cell line were purchased from National Centre for Cell line Pune and cultured in Deshpandey laboratories, Bhopal. MTT assay was performed, [(3 – (4, 5 – dimethylthiazol] 2] Y) 2, 5 – diphenyl tetra sodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazon product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed using the standard operating procedures. To examine the effects of pure methotrexate and methotrexate loaded Porous microspheres, the cells were treated with 0.001, 0.01, 0.1, 1 and 10 mg/ml of methotrexate and similar concentrations of optimized microspheres (M9). Cell lines maintained in appropriate condition were seeded in 96 well plates treated with different concentration of the test samples and incubated at 37ºC, 5% $CO_2$ for 1-5 days. MTT reagent was added to the wells and incubated for 4 hours; the dark blue formazon product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550nm in Elisa reader. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC50 values[13, 23].

**Gamma scintillography**

The optimized formulation (M9) and nonfloating microspheres (NFM) of 500 mg each, loaded with $SnCl_2$ and methotrexate, were placed in a test tube and soaked in 10 mL of normal saline (0.9% NaCl) for 15 minutes. A small amount of sodium pertechnetate solution equivalent to radioactivity of 40 mBq obtained from a technetium generator and stored in a sterile vial was added to the tube. The suspension was mixed intermittently for 15 minutes using a shaker. The supernatant was removed and the labeled microspheres were recovered by filtration through a Whatman filter paper (No. 41) followed by washing thoroughly with distilled water. The microspheres were then allowed to dry in air for 15 minutes. Six healthy albino mice were used to monitor the in vivo transit behavior of the floating and nonfloating microspheres. These mice were divided into 2 groups (group A and group B). The location of the formulation in the stomach was monitored by keeping the subjects in front of E-CAM gamma camera with SPECT technology (Single-photon emission computed tomography). The gamma

camera has a field view of 40 cm and is fitted with a medium-energy parallel hole collimator. The 140 keV gamma rays emitted by $^{99m}$Tc are imaged. Specific stomach site (anterior) was imaged by E-Cam gamma camera after definite time intervals and activity counts recorded for a 5-minute period to calculate the counts per minute (cpm). The gamma images were recorded using an online computer system, stored on magnetic disk, and analyzed to determine the distribution of activity in the oral cavity and stomach. In between the gamma scanning, the animals were freed and allowed to move and carry out normal activities and allowed to take water frequently until the formulation had emptied the stomach completely to increase the pH of stomach [13, 21].

**RESULT AND DISCUSSION**

Microsphere loaded with drug were prepared using biodegradable polymer like pectin, casein as emulsifying agent by using emulsification extraction method. Pectin was selected as polymer for the preparation of porous microspheres owing to its biodegradable property and stability at lower pH. In preliminary batches different ratio of polymer to emulsifier was used for preparing the polymer solution, the polymer solution was too viscous at ratio 1250:250 and 1000: 500 (pectin: casein) and difficult to pour in oil. As the quantity of polymer increased from 250-1250mg in polymer to emulsifier ratio, percentage entrapment efficiency of microspheres increased with low % buoyancy. On the other hand if quantity of emulsifier was raised from 250 to 1250 in polymer to emulsifier ratio, microspheres having irregular and large size particles with increased buoyancy and with less entrapment efficiency were produced. Therefore 750:750 (1:1) of pectin to casein was found to be optimum concentration of polymer and emulsifier which provide microspheres of small size with good percentage entrapment efficiency and increased percentage buoyancy.

Two different quantities of drug 50mg and 100mg were used for preparation of microspheres. Hundred mg of drug can be easily incorporated in microspheres, but no significant effect of amount of drug on % entrapment efficiency and on % buoyancy were seen (table 1).

On the basis of preliminary trials $3^2$ full factorial design was employed to study the effect of independent variables temperature and stirring speed on dependent variables particle size, percentage buoyancy and percentage drug entrapment efficiency. The results are depicted in Table 2 and in Figure 1(a, b, c).

A statistical model incorporating interactive polynomial term was used to evaluate the response

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{11} + b_{22}X_{22}$$

Where, Y is the dependent variable, $b_0$ is the arithmetic mean response of nine runs, $b_1$ is the estimated coefficient for the factor $X_1$. The main effects ($X_1$ and $X_2$) represent the average results of changing one factor at a time from its low to high value. The interaction terms ($X_1X_2$) show how the responses change when two factors are simultaneously changed. The polynomial terms ($X_1X_1$ and $X_2X_2$) are included to investigate nonlinearity. The fitted equation relating the responses particle size, % drug entrapment, and % buoyancy to the transformed factor are shown in equation 1, 2 and 3 for methotrexate microspheres.

$$PS = 1.06\ x_1^2 - 0.09\ x_1\ x_2 + 1.81\ x_2^2 - 8.39\ x_1 - 16.02\ x_2 + 79.36 \quad \text{Eq-1}$$

$$\%DE = -0.26\ x_1^2 + 0.0225\ x_1\ x_2 + 0.105\ x_2^2 + 0.82\ x_1 + 0.175\ x_2 + 96.59 \quad \text{Eq-2}$$

$$\%B = -0.2\ x_1^2 + 0.15\ x_1\ x_2 + 0.05\ x_2^2 + 1.3\ x_1 + 2.75\ x_2 + 78 \quad \text{Eq-3}$$

To demonstrate graphically the effect of the temperature and stirring speed, the response surface plots Figure 1(a, b, c) were generated for the dependent variables, particle size, % drug entrapment and %buoyancy using Sigma Plot software. Results of ANOVA for the measured responses are provided in Table 6. The statistical analysis of the factorial design batches was performed by multiple polynomial regression analysis using Microsoft Excel. The data clearly depicts that the Particle size (PS), % drug entrapment (%DE), and % Buoyancy (%B) values are strongly dependent on the selected independent variables. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (positive or negative). The value of the correlation coefficient indicates a good fit (Table 5).

**Table 5: Multiple regression output for dependent variables*.**

| Parameters | Coefficient of Regression Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $B_0$ | $b_1$ | $b_2$ | $B_{12}$ | $b_{11}$ | $b_{22}$ | *r* | *P* |
| *PS* | 79.36 | -8.39 | -16.02 | -0.09 | 1.06 | 1.81 | 0.991 | <0.001 |
| *%EE* | 78.00 | 1.3 | 2.75 | 0.15 | -0.2 | 0.05 | 0.991 | <0.001 |
| *%B* | 96.59 | 0.82 | 0.175 | 0.022 | -0.26 | 0.10 | 0.969 | <0.001 |

***PS*, particle size; %*EE*, % drug entrapment; %*B*, % buoyancy.**

**Table 6: Results of analysis of variance for measured response*.**

| Parameters | df | SS | MS | F | Significance F |
|---|---|---|---|---|---|
| **For PS** | | | | | |
| Regression | 2 | 19063.172 | 981.586 | 163.363 | <0.001 |
| Residual | 6 | 36.052 | 6.009 | | |
| Total | 8 | 1999.223 | 249.903 | | |
| **For %EE** | | | | | |
| Regression | 2 | 4.218 | 2.019 | 46.652 | <0.001 |
| Residual | 6 | 0.271 | 0.0452 | | |
| Total | 8 | 4.489 | 0.561 | | |
| **For %B** | | | | | |
| Regression | 2 | 55.515 | 27.757 | 159.373 | <0.001 |
| Residual | 6 | 1.045 | 0.174 | | |
| Total | 8 | 56.560 | 7.070 | | |

***df indicates degree of freedom; SS, sum of square; MS, mean sum of square; and F, Fischer's ratio**

To evaluate the contribution of different levels of factor ($X_1$) and factor ($X_2$), 2-way ANOVA followed by Tukey test was performed using Sigma Stat software. From the results of Tukey test, it was found that both factor X1 and X2 had significant effect on particle size, % buoyancy and % drug entrapment at different levels. Results of two-way ANOVA for the both factors at different levels are provided in Table 7.

**Table 7: Results of two way ANOVA for factors X1 and X2 at different levels.**

| **Particle size – Factor X1** | | | | | | |
|---|---|---|---|---|---|---|
| **Comparison** | **Diff of Means** | **p** | **q** | **P** | **P<0.050** | |
| -1.000 vs. 1.000 | 16.787 | | 3 | 11.139 | 0.003 | Yes |
| -1.000 vs. 0.000 | 9.453 | | 3 | 6.273 | 0.025 | Yes |
| 0.000 vs. 1.000 | 7.333 | | 3 | 4.866 | 0.056 | No |
| **Factor X2** | | | | | | |
| -1.000 vs. 1.000 | 32.047 | | 3 | 21.265 | <0.001 | Yes |
| -1.000 vs. 0.000 | 17.833 | | 3 | 11.834 | 0.003 | Yes |
| 0.000 vs. 1.000 | 14.213 | | 3 | 9.432 | 0.006 | Yes |
| **% Drug entrapment – Factor X1** | | | | | | |
| 1.000 vs. -1.000 | 1.640 | | 3 | 16.826 | <0.001 | Yes |
| 1.000 vs. 0.000 | 0.560 | | 3 | 5.745 | 0.033 | Yes |
| 0.000 vs. -1.000 | 1.080 | | 3 | 11.081 | 0.003 | Yes |
| **Factor X2** | | | | | | |
| 1.000 vs. -1.000 | 0.350 | | 3 | 3.591 | 0.131 | No |
| 1.000 vs. 0.000 | 0.280 | | 3 | 2.873 | 0.220 | Do Not Test |
| 0.000 vs. -1.000 | 0.0700 | | 3 | 0.718 | 0.872 | Do Not Test |
| **% Bouyancy – Factor X1** | | | | | | |
| 1.000 vs. -1.000 | 2.600 | | 3 | 9.192 | 0.007 | Yes |
| 1.000 vs. 0.000 | 1.100 | | 3 | 3.889 | 0.106 | No |
| 0.000 vs. -1.000 | 1.500 | | 3 | 5.303 | 0.043 | Yes |
| **Factor X2** | | | | | | |
| 1.000 vs. -1.000 | 5.500 | | 3 | 19.445 | <0.001 | Yes |
| 1.000 vs. 0.000 | 2.800 | | 3 | 9.899 | 0.005 | Yes |
| 0.000 vs. -1.000 | 2.700 | | 3 | 9.546 | 0.006 | Yes |

Particle size and surface morphology assessed by scanning electron photomicrographs showed that microspheres are spherical with rough surface (Figure 2). In optical microscopy it was observed that the particle size of microspheres was 107.00±0.23 to 59.60±0.95µm. The maximum percentage drug entrapment was found to be 95.40±0.89 to 97.54±0.53 and percentage buoyancy was found to be 74.2±0.23 to 82.0±0.27 (Table 2).

The peaks which are present in spectra of placebo microspheres are similar to of drug loaded microspheres. FTIR analysis reveals that complete encapsulation of drug occurs in microparticles (Figure 3).

Flow properties of the formulations were determined and it was found that angle of Repose, Housner's Ratio and Car's Index, for microsphere was 20.32±0.432 to 27.36±0.690, 11.01±0.342 to 17.17±0.241, and 1.135±0.035 to 1.164±0.015, respectively (Table 3). The GI stability of the particles was investigated by subjecting the particles to simulated GI fluids and found to be quite stable (Table 4) under the study conditions and duration. This formed an important exercise, as stable particles would remain floating and result in increase in subsequent bioavailability of drug.

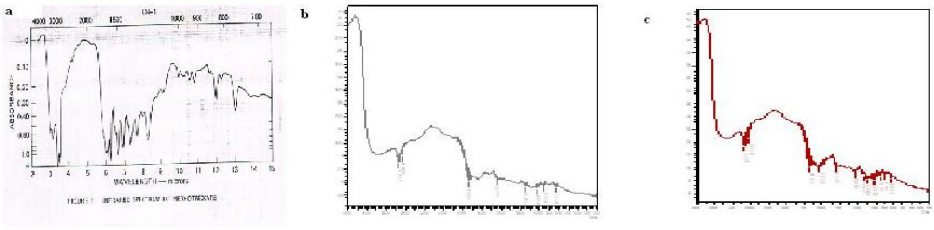

FIG 3. FTIR Spectra of microspheres (a) pure methotrexate (b) plecebo microsphere, (c) methotrexate microsphere.

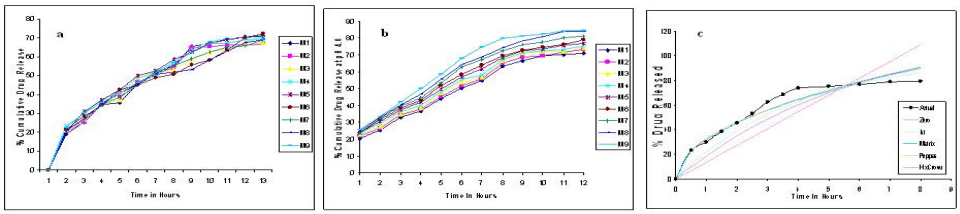

FIG 4. Comparative dissolution profile of methotrexate formulations (a) at pH 2.0 (b) at pH 4.0 and (c) Percentage drug release with model fit curve for M9 at pH 4.0.

Microparticulate drug delivery systems are crucial where the sustained release of drug is desired for a longer time period and chronic illness like cancer forms no exception to this. One of the desired attributes of oral chemotherapy is reduced dosing frequency and accumulation of the dosed drug in the tumor tissues by enhanced permeation retention that can be attained using microparticles.

Figure 4(a) reveals that the microspheres M1, M2, M3, M4, M5, M6, M7, M8 and M9 at pH 2.0 released – 70.98±0.41, 66.93±0.62, 67.48±0.74, 70.86±0.86, 71.8±0.33, 72.06±0.51, 68.96±0.32, 68.77±0.11, 69.26±0.37 percent drug , respectively at the end of 8hrs. Figure 4(b) reveals that the at pH 4.0 M1, M2, M3, M4, M5, M6, M7, M8 and M9 released – 70.98±0.49, 73.24±0.87, 73.97±0.45, 75.33±0.88, 76.93±0.27, 78.9±0.64, 81.2±0.62, 4.18±0.71, 84.52±0.95 percent drug respectively at the end of 8hrs. Figure 4 (a, b) reveal that the drug release at pH 4.0 is more as compared with at pH 2.0. Drug release from formulation M9 was high, sustained for more than 8 hrs and in controlled manner at pH 4.0. To ascertain the drug release mechanism and release rate, the data of formulation M9 (at pH 4.0) were fitted by using PCP Diss V3 dissolution software. The model selected were Zero Order(r=0.6862, k=13.6266), First Order (r=0.9177, k= -0.2480), Higuchi (r=0.9737, k=32.1291), Korsemeyer Peppas (r=0.9790, k=32.9459) and Hixon Crowell (r=0.8665, k=-0.0664) as represented in Figure 4(c). All models passes t test. The result suggests that for formulation M9 best fit model was found to be Korsemeyer Peppas model. The value of

correlation coefficient was 0.9790. The n = 0.4889 value shows Fickian release pattern from formulation M9.

The inhibitory potency of the pure drug on the Kato III cell line was compared by using the $IC_{50}$ value. The $IC_{50}$ represents the concentration of the drug at which 50% of inhibition is produced. Drug loaded microspheres showed very significant results compared with the pure drug solution. Figure 5 (a) and (b) for pure drug and drug loaded microspheres (M9) both showed cytotoxicity against Kato III cells. Hence the formulation can be effectively tested for its anticancer activity.

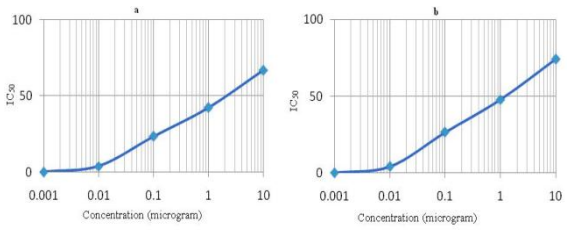


FIG 5. (a) IC50 of Methotrexate Solution (b) IC50 of Methotrexate Microspheres

The optimized formulation (M9) had shown good in vitro buoyancy and controlled release behavior and hence was finally selected for in vivo study (i.e. gamma scintigraphy), and the results were compared with non floating microsphere prepared using same polymer. The scintigrams just after administration were captured at different time intervals and stomach transit of microspheres was found to be more than 8hrs. Due to porous nature of the microspheres it floats on gastric content and remains in stomach. Up to 8hrs, continuous release of drug occurs in stomach. After 8 hrs, the arrival of formulation into other parts of gastrointestinal tract was indicated by a remarkable decrease in radioactivity as observed in stomach in comparison to previous time interval. It may probably be due to the entry of formulation into other parts.

In the present study, the potential of floating microsphere as drug carriers for oral delivery was investigated. The method of preparation of microspheres of methotrexate was found to be simple and reproducible. It was found that porous microspheres showed a desirable high drug content, good stability, flow properties, buoyancy and adequate drug release at pH 4.0 (frequent administration of water is require with floating dosage forms which may lead to increase in git pH); hence, formulation of methotrexate prepared by this method is suitable for controlled and sustained drug delivery. This study shows that floating microsphere could be a useful carrier for methotrexate. Similarly, other drugs can also be incorporated to get the local release of the drug in stomach. Pharmacokinetic studies are required to explore the safety and efficacy of this novel but effective drug system.

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**REFERENCES**

1. Sato Y, Kawashima Y, Takeuchi H, Yamamoto H. *In-vitro* evaluation of floating and drug releasing behavior of hollow microspheres [microballoons] prepared by emulsion solvent diffusion method. Eur J Pharma Biopharm 2004; 57: 235-243.
2. Streubel A, Siepmann J, Bodmeier R. Gastroretentive drug delivery system. Expert Opin Drug Deliv 2006; 3(2): 217-233.
3. Singh B, Kim KH. Floating drug delivery system: An approach to oral controlled drug delivery via gastric retention. J control Release 2000; 63: 235-259.
4. Thanoo BC, Sunny MC, Jayakrishnan A. Oral sustained release drug delivery systems using polycarbonate microspheres capable of floating on the gastric fluid. J Pharma Pharmacol 1993; 45: 21-24.
5. Basavaraj et al. Design and characterization of gastroretentive microspheres of ketoprofen. Am J Pharm Tech Res 2012; 2(3): 600-615.
6. Methotrexate. AHFS Drug information, American Society of Health system Pharmacists American Hospital Formulary Service 2003; 45:1078-85.
7. Chabner BA, Ryan DP, Luize Paz-Ares. Anti neoplastic agents: In Goodman and Gilmans, McGraw-Hill editors. The pharmacological basis of therapeutics. 10th ed. New York p. 1243-1247 and 1758.
8. Indian pharmacopoeia, Ministry of Health and Family Welfare Government of India, 1996. p. 312.
9. Shishu, Gupta N, and Agrawal N. Stomach specific drug delivery of 5-FU using floating alginate beads. AAPS Pharm Sci Tech 2007; 8(2): article 48.
10. Rathor S and Ram A. Porous microsphere of 5-FU: a tool for site specific drug delivery in gastric cancer. Inter J of Current Pharm Research 2011; 3(1): 38-42.
11. Patel JK, and Patel RP. Formulation and evaluation of mucoadhasion glipizide microspheres. AAPS Pharm Sci Tech 2005; 06(01): E49-E55.
12. Patel DM and Patel MM. Optimization of fast dissolving etoriocoxib tablets prepared by sublimation technique. Indian J of Pharm Sci 2006; 68(2): 222-226.
13. Rathor S and Ram A. Formulation and characterization of an intragastric floating drug delivery system of doxorubicin hydrochloride: in vitro in vivo drug release study. AJPCR 2012; 5(1):150-158.
14. Verma JK and Syed HA. Spectrophotometric method for determination of methotrexate, sildenafil citrate and trimetazidine dihydrochloride in pharmaceutical formulations. Journal of Pharmacy Research 2010, 3(3), 615-617.
15. Murata Y, Sasaki N, Miyamoto E, Kawashima S. Use of floating alginate beads for stomach-specific drug delivery. Eur J Pharm Biopharm. 2000; 50: 221-226.
16. Choi BY, Park HJ, Hwang SJ, Park JB. Preparation of alginate beads for floating drug delivery system: effects of $CO_2$ gas-forming agents. Int J Pharm 2002; 239:81-91.
17. Paharia A, Yadav A, Rai G, Jain SK et al. Eudragit coated pectin microspheres of 5-FU for colon targeting. AAPS Pharm Sci Tech 2007; 8(1): article 12.
18. Deshpande AA, Rhodes CT, Shah NH, Malick AW. Controlled-release drug delivery systems for prolonged gastric residence: an overview. Drug Dev Ind Pharm 1996; 22: 531-540.
19. Ichikawa M, Watanabe S, Miyake Y. A new multiple-unit oral floating dosage form, I: preparation and evaluation of floating and sustained-release characteristics. J Pharm Sci 1991; 80: 1062-1066.
20. Singh BM, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000; 63: 235-239.
21. Jain SK, Agrawal GP, and Jain NK. Evaluation of porous carrier based floating orlistat microspheres for gastric delivery. AAPS Pharm Sci Tech 2006; 7(4): article 90.
22. Jain SK, Awasthi AM, Jain NK, Agrawal GP. Calcium silicate based microspheres of repaglinide for gastro-retentive floating drug delivery: preparation and in vitro characterization. J Control Release 2005; 107: 300-309.
23. Kaushik D, Sardana S, and Mishra D. In vitro characterization and cytotoxicity analysis of 5-FU loaded chitosan microsphere for targeting colon cancer. Int J Pharm Edu and Research 2010; 44(3): 267-273.
24. Kale RD, Tayade PT. A multiple unit floating drug delivery system of piroxicam using eudragit polymer. Int J of Pharmaceutical Sci 2007; 69(1): 120-123.
25. Kalaria DR, Sharma G, Beniwal V, and Ravikumar MNV. Design of biodegradable nanoparticles for oral delivery of doxorubicin: In vivo pharmacokinetics and toxicity studies in rats. Pharmaceutical research. 2009; 26(3):492-501.
26. Patel JK, Patel RP, Patel MP. Formulation and evaluation of mucoadhesive glipizide microspheres. AAPS Pharm Sci Tech 2005; 6(1): EE49-EE55.
27. Jain AK, Jain CP, Tanwar YS and Naruka PS. Formulation, characterization and in vitro evaluation of floating microspheres of famotidine as a gastroretentive dosage form. Asian Journal of Pharmaceutics 2009; 3(3): 222-226.