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Release Kinetic Study of Controlled-Release Methotrexate Beads by Mathematical Modeling

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ABSTRACT

This paper presents the development and application of an in-vitro drug release mathematical model useful in predicting the in vivo bioavailability of the drug, methotrexate. The model is based on a novel dosage form designed to deliver a drug into the gastrointestinal tract in a controlled manner. Chitosan beads containing methotrexate were prepared using ionotropic gelation technique by dropping methotrexate containing solution of positively charged, chitosan, into Tripolyphosphate solution which forms gelled spheres by Ionotropic Gelation. Beads were characterized by particle size (710 µm to 1000 µm), practical yield (80.66 ± 0.22 - 86.66 ± 0.77 %), drug content (58.56 ± 0.52 - 65.12 ± 0.87 %), and surface morphology by scanning electron microscopy and in-vitro release by USP paddle apparatus. Model equations with R2 = 0.9728 and F = 23.8381, intended to elucidate the drug release mechanism, were fitted to the release data. Mathematical modeling of in vitro dissolution data indicated the best-fit release kinetics was achieved with First Order Release Kinetics with t50 of 4.83 h and t70 of 6.0 h which evidenced that the formulations are useful for a controlled release of methotrexate.

Keywords: Methotrexate beads, Ionotropic gelation, Mathematical Model, First order kinetics.

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INTRODUCTION

Common goal in the development of novel controlled-release dosage forms is the ability to accurately predict the plasma concentration time profile in humans. By reaching such a goal, the development process can be accelerated and products introduced more rapidly than if such predictions are unavailable. Application of a comprehensive bioavailability model facilitates screening of potential drug candidates for controlled release, optimizing formulation design, and interpreting bioavailability data. Efforts to use mathematical modeling for the purpose of predicting dissolution and subsequent in vivo bioavailability of oral delivery systems have been reported in the literature [1-4].

As a matter of fact, Control Release Systems (CRS) bring engineers and pharmacists to work together with the common aim of realizing more and more effective CRS. For this purpose, the use of mathematical modeling turns out to be very useful as this approach enables, in the best case, the prediction of CRS release kinetics before the release systems are realized. More often, it allows the measurement of some important physical parameters, such as the drug diffusion coefficient, resorting to model fitting on experimental release data. Thus, mathematical modeling whose development requires the comprehension of all the phenomena affecting drug release kinetics [5], has a very important value in CRS optimization.

Many controlled-release products are designed on the principle of embedding the drug in a porous matrix. Liquid penetrates the matrix and dissolves the drug, which then diffuses into the exterior liquid. A group of researcher showed that the percentage of drug released versus time data for many controlled-release preparations reported in the literature show a linear apparent first-order rate [6, 7].

Oral controlled-release dosage forms have been developed and studied to restrict these systems to specific regions of the gastrointestinal tract as well as to improve the pharmacological activity and to reduce toxic effects [8]. This paper presents the development and application of a mathematical model useful in predicting the *in vivo* bioavailability of the methotrexate, a model drug. The model is based on a novel dosage form designed to deliver a drug into the gastrointestinal (GI) tract in a sustained manner.

MATERIALS AND METHODS

Methotrexate was procured as gratis sample from Sun Pharmaceuticals Ind. Ltd., Mumbai. Chitosan purchased from Central Institute of Fisheries Technology, Cochin. Tri-polyphosphate was purchased from Sigma, Aldrich Chemical, USA. All other chemicals were A.R. grade and used as received.

Preparation of Microspheres

Different formulations of methotrexate beads were prepared as shown in Table 1, using the Ionotropic gelation with some modifications [9-11]. The methotrexate was dispersed in 1 % wt / vol chitosan solution (disperse phase). The beads were formed by dropping the bubble-free dispersion of disperse phase through a disposable syringe (10 mL) onto a 1 % wt / vol TPP solution containing 0.5 % vol / vol span-80 (continuous phase) with continuous stirring using magnetic stirrer (2-bladed propeller-type agitator (Remi, Mumbai, India). The chitosan microspheres were separated by filtration and rinsed with distilled water; then they were dried by different drying processes viz. air drying for 24 h, oven drying for 6 h at 60^0C (Vacuum Oven - Rectangular Acm-22068-I, India) and freeze drying for 6 h (Freeze Drier Acm-78097 S, India). Dried beads were stored for their characterization.

Characterization of elaborated systems

Particle size determination

Particle size determination of the beads was done by sieving method [12] using Indian Standard Sieves # 16, # 22 and # 30. Average particle size was calculated using the formula: - d average = $\sum$ dn / $\sum$ n, where n=frequency weight, d= mean diameter. (Table-2).

Percentage Yield: The beads recovery yield was determined according to:

$$\% \text{ Yield} = \text{weight of beads} / (\text{weight of drug} + \text{weight of polymer}) \times 100$$

Surface Morphology

Scanning electron microscopy was used to evaluate the quality of the microspheres obtained under the various conditions used. After being vacuum-coated with a thin layer of gold, the microspheres were examined by means of a Philips scanning electron microscope at an Acceleration range: 0.2 to 30 kV, using various magnifications to observe the surface morphology.

Determination of Drug content

The MTX-Chitosan Beads were tested for their drug content. The beads containing drug were extracted with 0.1N HCl solution for 36 h. The content was filtered and absorbance of the resulting solution was measured at 303 nm, using 0.1N HCl as the blank by spectrophotometer (UV 1601 Shimandzu Corp., Japan Model) and drug content was determined.

In-vitro Release Studies

The release properties of beads were studied in 0.1N HCl (Scientific USP Std, Model DA). The dissolution medium 500 mL, was maintained at (37 ± 0.5 ^{0}C), & stirring speed was set at 100 rpm (Remi, Mumbai, India). Beads (50 mg) were added to the dissolution medium and samples of 1mL were taken and replaced with fresh medium at predetermined time intervals. The concentration of MTX in each sample was determined by UV spectrophotometer (UV 1601 Shimandzu Corp., Japan Model) at wave length of 303 nm using corresponding blank.

Kinetic analysis of dissolution data (Table 3)

To study the mechanism of drug release from the chitosan beads, the release data were fitted to the following equations [13]: (Time in each case was measured in minutes)

Model 1. Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly follows

$$Q_1 = Q_0 + K_0 t,$$

Where,

Q_1-amount of drug dissolved in time t
Q_0-initial amount of drug in the solution
K_0-zero order release constant

Model 2. First order kinetics

This model has been used to describe absorption and/or elimination of some drugs.

$$\ln Q_t = \ln Q_0 K_1 t$$

Where,

K_1--first order release constant
Q_0-initial amount of drug in the solution
Q_1-amount of drug dissolved in time t

Model 3. Hixson-Crowell model

This model applies to pharmaceutical dosage form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface

$$Q_0^{1/3} - Q_1^{1/3} = K_s t$$

Where,

Ks-constant incorporating the surface volume relation
Q_0-initial amount of drug in the solution
Q_1-amount of drug dissolved in time t

Model 4. Weibull model

This equation can be successfully applied to almost all kinds of dissolution curves and is commonly used in these studies.

$$\text{Log } [-\ln (1-m)] = b \log (t - T_i) - \log a$$

Where,

m- Accumulated fraction of the drug, m, in solution at time, t
a- the scale parameter, which defines the time scale of process
b- Shape parameter, characterizes the curve as exponential, sigmoid or parabolic
Ti- location parameter represents lag time before the onset of the dissolution (usually zero)

Model 5.Highuch model

This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms.

$$Q = \sqrt{tDC_s(2C - C_s)}$$

Where,

Q- amount of drug release in time t
C- inintial drug concentration
Cs- drug solubility in the matrix
D- diffusion constant of the drug molecule in that liquid

Model 6.Korsmeyer-Peppas

This model is generally used to analyze the release of pharmaceutical polymeric dosage from, when more than one type of release phenomena could be involved.

$$\frac{M_t}{M_\infty} = at^n$$

Where,

a- constant incorporating structural and geometric characteristics of the drug dosage form
n- the release exponent (indicative of the drug release mechanism)
Mt/M∞- fractional release of drug

The criterion for selecting the most appropriate model was based on a goodness-of-fit test [14-18].

RESULTS AND DISCUSSION

The mean particle size of Chitosan beads containing methotrexate were found to be in the range of 10 # - 22 # (710 µm to 1000 µm). Methotrexate based Chitosan beads were spherical in shape which could be evident from their SEM photomicrographs (Figure 1).

Practical yield and Drug content in Chitosan beads were found to be 80.66 % to 86.66 % and 35 to 59%, respectively. As per figure 2, the *in vitro* drug release profile of best batch (CHI01) revealed the t_{50} and t_{70} values 4.83 h and of 6.0 h respectively suggests the sustained release pattern.

Drug release kinetics may be affected by many factors such as polymer swelling, polymer erosion, drug dissolution/diffusion characteristics, drug distribution inside the matrix, drug/polymer ratio and system geometry (cylinder, sphere and so on) [19,20]. The drug release from the chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and erosion of the chitosan matrix, and the dissolution, and dissolution and subsequent diffusion of the drug through the swollen and un-swollen chitosan matrix. The swelling of the chitosan beads was dependent on the pH of the dissolution medium. The beads when wetted by the acidic solution medium swelled extensively.

The model-dependent or curve fitting approach has been successfully used to compare in vitro dissolution profiles of solid dosage forms [21-23]. To explore the release pattern, results of the i*n vitro* dissolution data were fitted to the various models, which characterizes the transport mechanism. Based on various mathematical models, the magnitude of the release exponent "n" indicates the release mechanism (i.e., Fickian diffusion, case II transport, or anomalous transport). In the case of the Fickian release mechanism, the rate of drug release is much less than that of polymer relaxation (erosion). So the drug release is chiefly dependent on the diffusion through the matrix. In the present study, the limits considered were n= 0.45 (indicates a classical Fickian diffusion-controlled drug release) and n= 0.89 (indicates a case II relaxational release transport; non-Fickian, zero-order release). In the non-Fickian (anomalous) case, the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation. Case II release generally refers to the polymer relaxation. Values of n between 0.45 and 0.89 can be regarded as an indicator of both phenomena (drug diffusion in the hydrated matrix and the polymer relaxation) commonly called anomalous transport [24].

Curve fitting of the *in vitro* release data at 37°C in 0.1 N HCl was accomplished by nonlinear regression (Fortran software), and the results are shown in Table 3. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism.

From the release exponent in the Korsmeyer-Peppas model (n = 0.5908), it can be suggested that the mechanism that led to the release of MTX was an anomalous diffusion with constant release rate adequate for a sustained release dosage form. However, the release data analysis applying these mathematical models can be purely empirical, and no definitive conclusion can be drawn concerning the dominating mass transport mechanism.

Figure 1: SEM Photomicrograph of Chitosan beads at 65 x magnification (CHI 01)

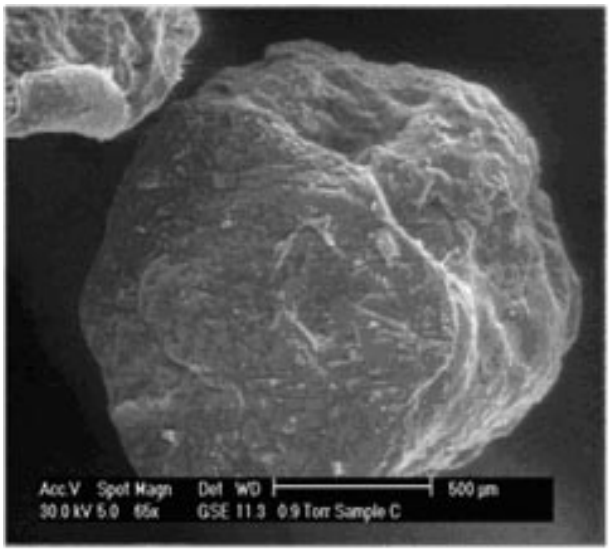

Figure 2: Dissolution profile of MTX release from optimized batch (CHI01) chitosan beads

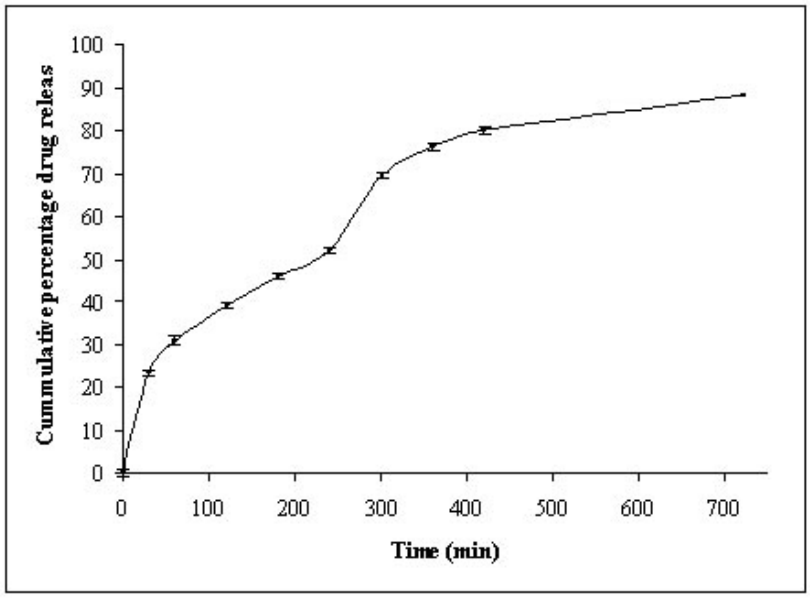

Table 1: Formulations showing the physicochemical attributes

Batch No.	Concentration of Span-80 % vol /vol	Concentration of TPP % wt /vol	Drug content % ± SD	T_{50} (min)	T_{70} (min)
CHI 01	0.5	1.0	59.10 ± 0.66	290	360
CHI 02	1.0	1.0	49.70 ± 0.76	230	380
CHI 03	1.5	1.0	39.48 ± 0.77	125	190
CHI 04	0.5	2.0	42.30 ± 0.84	310	380
CHI 05	0.5	3.0	35.70 ± 0.65	370	450

Table 2: Particle size analysis and drug release after 12 h.

Batch No.	Sieve No.	Size in (μm)	Mean Diameter (μm ± SD)	Drug Release (%) (After 12 h)
CHI01	≤ 16 #	≤ 1000	608.16 ± 0.59	88.02 ± 0.77
CHI02	16-22 #	1000-710	760.89 ± 0.51	88.04 ± 0.65
CHI03	16-22 #	1000-710	782.78 ± 0.36	90.12 ± 0.42
CHI04	≥ 16 #	≥ 1000	947.29 ± 0.54	87.34 ± 0.85
CHI05	≥ 16 #	≥ 1000	913.58 ± 0.72	85.32 ± 0.76

Note- The mean ± SD. n = 6 for mean diameter

In this selected formulation, the calculated regression coefficients for zero order, first order, Higuchi, Hixon Crowell, Korsmeyer and Weibull models were 0.9008, 0.9728, 09652, 0.9618, 0.9661 and 0.9453, respectively. Therefore, the release seems to fit the First order release kinetics. The best fit with the highest correlation coefficient was shown in first order equation. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the first order plot kinetic model where dissolution rate is dependent on the concentration of the dissolving species [25].

The value of r^2 (0.9728) was found to be highest for the First order model. The sum of square residuals (SSR = 190.6542) and F value (23.8381) were lowest for the First order model, which also indicates that the test product follows First order release kinetics. The values of slope and intercept obtained from the nonlinear equation of the First order model were found to be -0.0031 and 4.6073, respectively. Thus, the

pharmaceutical dosage forms following this dissolution profile, such as those containing water soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by the unit of time will be diminish.

Table 3: Results of f-test and regression analysis (Batch CHI 01)

Parameters	Zero Order	First Order	Higuchi	Hixon-Crowell	Korsmeyer	Weibull
SSR	705.4612	190.6542	247.2653	200.3280	216.6537	213.6708
F	88.1827	23.8381	30.9082	25.0410	30.9505	30.5244
Corre. Coefficient	0.9491	-0.9863	0.9825	0.9807	0.9829	0.9723
r^2	0.9008	0.9728	0.9652	0.9618	0.9661	0.9453
Slope	0.1230	-0.0031	3.5815	0.0034	0.5908	0.9035
Intercept	15.1197	4.6073	-4.3099	0.1268	-1.7119	-2.2756

*r^2- square of correlation coefficient, SSR- sum of square residuals

Diffusional Exponent (n) 0.5908

Mechanism of drug release Anomalous Diffusion

The three best models chosen for calculation of F

Min F = 23.83

Med F= 25.04

High F= 30.52

Critical value of F (5%) =3.44 D.F. [8,8]

F (med/min) = 1.05

The difference is insignificant between medium and minimum value of F.

F (high/min) = 1.28

The difference is insignificant between high and minimum value of F.

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