e-ISSN: 2349-2759 p-ISSN: 2395- 1095

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)

www.jipbs.com

Research article

In-vitro release kinetics, *in-vitro* buoyancy studies and *in-vivo* floating behaviour of gastro-retentive tablets of Ciprofloxacin and Metronidazole

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Keywords: Ciprofloxacin, metronidazole, *in-vivo* release kinetics, buoyancy studies, *in-vivo* floating studies.

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Abstract

An oral sustained release dosage form of a combination of Ciprofloxacin HCl and Metronidazole based on gastric floating matrix tablets was studied. The release of Ciprofloxacin HCl and metronidazole was dependent on the matrix forming polymers HPMC K100M and HPMC E10M and the swelling agent guar gum used in the formulation. The release data of Ciprofloxacin HCl as well as Metronidazole from the matrix tablets were analysed kinetically using zero order, first order, Higuchi model and Krosmeyer Peppas models. The floating lag time, floating duration and drug release data for ciprofloxacin and metronidazole was studied. The overall release mechanism can be explained as a result of rapid hydration of polymer on the surface of the floating tablet and formation of a gel layer surrounding the matrix that controls water penetration into its core matrix. On the basis of *in vitro* release data, the optimised batch F14 comprising of polymers HPMCK100 and HPMC E10M at a ratio of 2:1 and guar gum at a concentration of 3.3 % was subjected to *in-vitro* buoyancy studies in 0.1 N hydrochloric acid and *in-vivo* floating behaviour in dogs. It was concluded that the formulated combination tablet resulted into floating duration of more than 20 hours, which would increase the bioavailability with minimum dosage regimen.

Introduction

Hydrophilic matrices containing swellable polymers are referred to as hydrogel matrices, swellable controlled-release systems or hydrophilic matrix tablets. A number of polymers have been investigated to develop in situ gel-forming systems, due to the ability of these hydrogels to release an entrapped drug in aqueous medium and to regulate the release of drug by control of swelling and cross-linking [1-3].

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 [4]. One method of fabricating controlled-release formulations is by the incorporation of the drug in a matrix containing a hydrophilic, rate-controlling polymer [5, 6]. Hydroxypropyl methyl cellulose (HPMC) is the polymer most widely used as the gel-forming agent in the formulation of solid, liquid, semisolid and even controlled-release dosage forms. Water penetration, polymer swelling, drug dissolution, drug diffusion and matrix erosion from these dosage forms are controlled by the hydration of HPMC, which forms a gel barrier through which the drug diffuses [7,8]. The importance of the diffusion layer for a swollen HPMC matrix was illustrated in a mathematical model [9]. Water-soluble drugs are released primarily by diffusion of dissolved drugs molecules across the gel layer, while poorly soluble drugs are released predominantly by erosion mechanism. The contribution of each mechanism in the overall drug release process is influenced by both, the drug solubility, and also by the physical and mechanical properties of the gel barrier formed [10]. A tablet composed of a polymeric matrix on contact with water builds a gel layer around the tablet, which governs the drug release. In order to establish the mechanism of drug release and swelling kinetics, the experimental data were fitted to zero-order, first order, Higuchi, and Korsmeyer–Peppas [11]. Further, it can be added that the physicochemical properties of the drug as well as polymer and the drug to polymer ratio govern the release of drug from the formulation and thus, modify the release kinetics accordingly [12].

Ciprofloxacin is most widely used in UTI's with a good localized action on the infected sites. Ciprofloxacin produces high urine concentrations because about 50% is excreted in urine in unchanged form [13]. It belongs to quinolones, which have broad spectrum activity against many types of gram-positive as well as gram-negative bacteria to control various diseases [14].
Metronidazole is rapidly and completely absorbed after oral administration of conventional tablet dosage forms [15]. However, antibiotic resistance particularly with metronidazole (MIC > 8 mg/l) frequently causes failure of eradication of H. pylori, [16, 17] which may be due to poor drug concentration at the site of action as after absorption to blood circulation results in distribution of drugs throughout the body[18].
The objective of the research is to study the *in-vitro* release kinetically using zero order, first order, Higuchi model and Korsmeyer Peppas models of the optimized fixed dose combination of Ciprofloxacin and metronidazole gastro-retentive tablets. Further research aimed to analyse the *in-vitro* buoyancy of the optimized tablets in 0.1N HCl and *in-vivo* floating ability of the optimized batch using barium sulfate radiology method in dogs.

Experimental

Optimization and evaluation of gastro-retentive tablets using combination of ciprofloxacin and metronidazole [19] was carried out. The powder blends obtained were studied for micromeritic properties and the formulated tablets were evaluated for hardness, friability, weight variation, assay, floating lag time, floating duration and drug release profile for ciprofloxacin and metronidazole. The *in-vitro* dissolution of the tablets was carried out using 0.1 N HCl at 37 ±0.5°C (900 ml using USP apparatus II at 50 rpm).

In-vitro drug release kinetics

The *in vitro* drug release mechanism of gastro retentive tablets of ciprofloxacin HCl and Metronidazole from polymeric tablets in 0.1 N HCl can be described by fitting the dissolution data in four different kinetic models as given below [20, 21].

Zero-order equation

$Q_t = Q_0 + K_0t$…… (equation i)

Where, Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant.

First-order equation

$\text{Log}(Q_t) = \log(Q_0) + K_1t/2.303$…(equation ii)

Where, Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K_1 is the first order release rate constant.

Higuchi's equation

$Qt=K_H\sqrt{t}$…….. (equation iii)

Where, K_H is the Higuchi's release constant

Korsmeyer peppas Model

$M_t/M_\infty = K_{KP}t^n$……..(equation iv)

Where M_t / M_∞ is fraction of drug released at time t, K_{KP} is the rate constant and 'n' is the release exponent.
The zero-order rate describes systems where drug release rate is independent of drug concentration [22]. The dissolution data were also fitted according to the well-known exponential equation of Peppas et al [23]. The diffusional exponent, n, is dependent on the geometry of the device as well as the physical mechanism for release. The values of n for a cylindrical shaped device are < 0.43 or 0.43 for Fickian release and 0.85 for case II or zero-order release in this model. For systems exhibiting case II transport, the dominant mechanism for drug transport is due to polymer matrix relaxation. The value of n > 0.43 but < 0.85 is considered as anomalous transport (non-Fickian) and refers to the coupling of Fickian diffusion and polymer matrix relaxation. The value of n > 0.85 is considered as super case II transport [24]. The range for exponent (n) responsible for Fickian diffusion, anomalous transport and case-II transport for thin film, cylinder and sphere is described in table 1 depending on the In Korsmeyer-Peppas kinetic model an (*n*) value which is a diffusional exponent represents the mechanism of drug release from matrix tablets.

In –vitro buoyancy studies

The buoyancy lag time and total floating time [25]: The optimized batch tablets were kept in 0.1N HCl solution at 37±0.5°C and the time taken for tablet to float was noted down as buoyancy lag time and the total time tablet floated was noted down as total floating time. This test was carried out based on visual observations.

In-vivo floating studies

Unlike other formulations, determination of GRT is very important factor for floating drug delivery systems (FDDS). In most cases, it requires an imaging technique that can locate the FDDS in stomach. The following method has been utilized to assess gastroretentivity. Even though there are number of studies reported, the present work makes use of the Radiology study (X-Ray) for the determination of anatomical location and behaviour of floating tablets in the gastrointestinal tract [26].

Table 1. Drug released mechanism and n values for thin film, cylinder and sphere

Exponent (n)			Drug release mechanism
Thin Film	**Cylinder**	**Sphere**	
0.5	0.45	0.43	Fickian diffusion
0.5 < n < 1.0	0.45 < n < 0.89	0.43 < n < 0.85	Anomalous transport
1.0	0.89	0.85	Case-II transport

Preparation of placebo barium sulfate (high density) tablets

The radio-opaque tablets of optimized formulation batch were prepared by the earlier mentioned method, replacing ciprofloxacin and metronidazole with sufficient quantity (10 mg) of barium sulfate and diluent. The other parameters of tablets were kept constant. The *in-vivo* gastro-retention study was carried out by administering a placebo floating tablet using a gastric feed tube to the overnight fasted healthy dogs (n=3) weighing approximately 15 kg. The animals were fasted for 12h and the first X-ray photographed to ensure absence of radio opaque material in the stomach. The dogs were made to swallow one tablet with 100ml of water and monitoring was performed by radiological method (2nd, 12th, and 20th hours) [27]. After ingestion of floating tablets containing barium sulphate, the animal was exposed to X-ray photography in the abdominal region.

Results and Discussion

The micromeritic properties results, tablet evaluation parameters are described in table II and III. The drug release profile of the formulation batches and the target profile of Cifran OD and Flagyl ER for Ciprofloxacin and metronidazole are represented in Figures 1 and 2 respectively.

In-vitro drug release kinetics

To know the mechanism of drug release from the prepared formulations, the data of the optimised batch F14 was treated according to first-order (Log Cumulative Percentage of Drug Remaining Versus Time), Higuchi's (Cumulative Percentage of Drug Released Versus Square Root of Time), korsmeyers peppas Model (log of % drug release versus log of time in hours) and zero order (Cumulative % Of Drug Released Versus Time) pattern. Table IV represents the model Comparison for ciprofloxacin and metronidazole drug release kinetics and various measured parameters like intercept, slope and R^2 for the optimized batch. Graph of zero order release, Cumulative % Drug Release Versus Time resulted in straight line with R^2 of 0.9912 and 0.9097 for Ciprofloxacin and metronidazole respectively. Graph for first order release (log % Drug Remaining Versus Time) resulted in straight line with R^2 of 0.8223 and 0.9966 for Ciprofloxacin and metronidazole respectively.

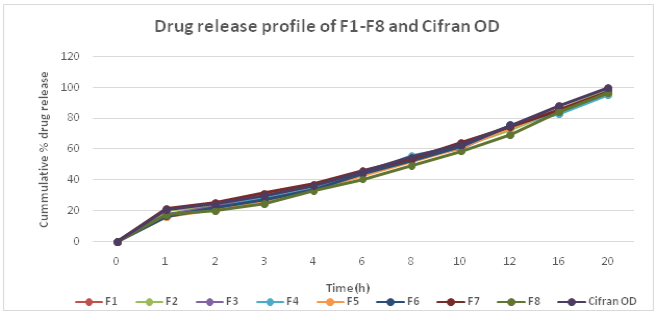

Figure 1. Drug release profile of Ciprofloxacin for batches F1-F8 and Cifran OD

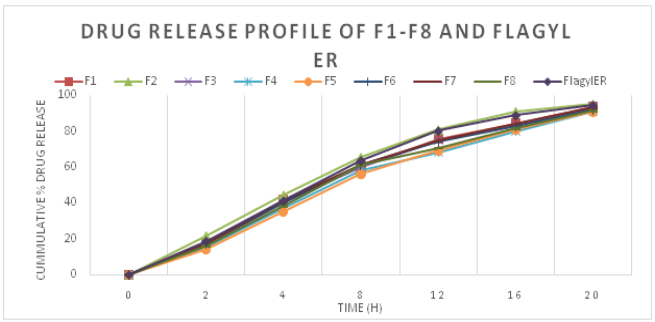

Figure 2. Drug release profile of Metronidazole for batches F1-F8 and Flagyl ER

Table 2. Micromeritic properties of powder blends of batches F1 to F8

Powder Blend	Angle of repose (°)	Loose Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index (%)	Hausner's ratio
F1	28.37	0.679	0.729	16.27	1.25
F2	27.64	0.634	0.714	16.59	1.22
F3	27.29	0.654	0.753	19.51	1.27
F4	25.83	0.613	0.737	17.93	1.21
F5	26.71	0.668	0.768	17.44	1.23
F6	25.68	0.654	0.726	16.28	1.21
F7	27.59	0.689	0.741	17.21	1.18
F8	26.21	0.613	0.792	17.63	1.26

Table 3. Hardness, friability, weight variation, assay, floating lag time and floating time of batches F1 to F8

Batch number	Hardness (Kg/cm²) (n=10)	Friability (%) (n=10)	Weight variation (mg) (avg±%SD) (n=20)	Assay (90-110%) (n=3)	Floating lag Time (S)	Floating Time (h)
F1	4.5±0.19	0.39±0.58	99.7±1.57	97.6±1.8	62	>20
F2	4.7±0.53	0.41±0.91	100.1±0.89	97.1±2.7	75	>20
F3	4.8±0.81	0.48±0.53	99.8±1.15	101.7±0.9	69	>20
F4	4.8±0.46	0.51±0.15	99.8±1.07	98.2±1.3	60	>20
F5	5.0±0.27	0.45±0.47	100.2±2.34	99.3±1.1	79	>20
F6	4.6±0.89	0.51±0.12	100.1±2.5	97.1±2.3	81	>20
F7	4.7±0.29	0.45±0.19	99.9±1.2	98.7±1.4	83	>20
F8	4.8±0.31	0.43±0.21	99.8±1.6	101.5±2.8	69	>20

Table 4. Results of statistical data model fitting for optimized formulation batch number F14

Metronidazole	Intercept	Slope	R^2
zero order	25.816	3.913	0.9097
first order	-0.0646	2.021	0.9966
Higuchi	-2.59	22.86	0.9839
Korsmeyer-pappas	1.2071	0.63	0.9611
Ciprofloxacin	Intercept	Slope	R^2
zero order	18.19	4.23	0.9912
first order	-0.0784	2.13	0.8223
Higuchi	-5.35	22.16	0.985
Korsmeyer-pappas	1.24	0.56	0.9838

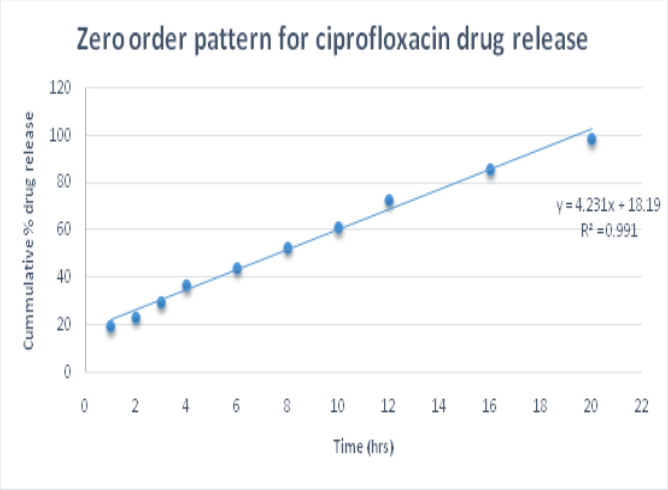

Figure 3. Zero order release graph for Ciprofloxacin

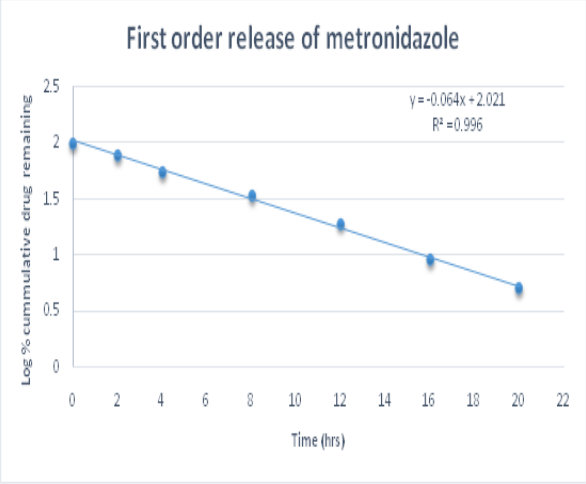

Figure 4. First order release graph for metronidazol

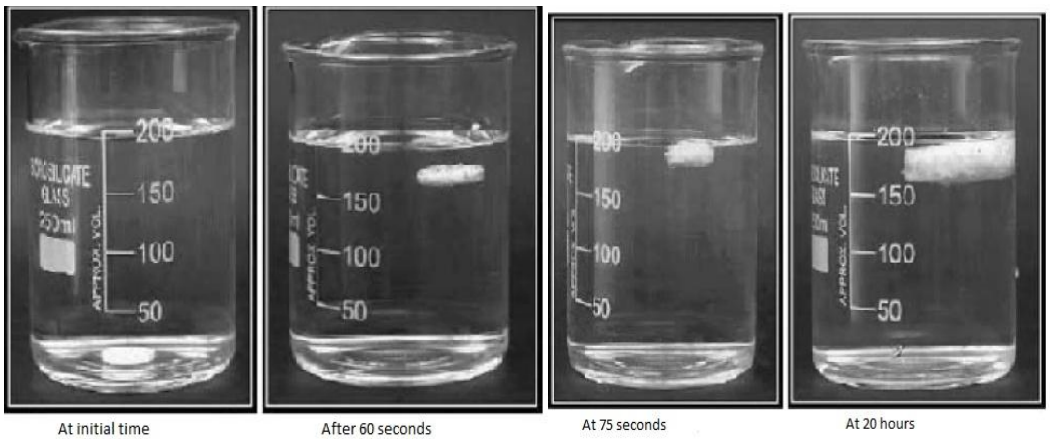

Figure 5. *In-vitro* Buoyancy test of F14 at various time intervals

Figure 6. X-ray images of optimized formulation placebo at various intervals of administration

Higuchi model was plotted for optimized batch based on Cumulative % Drug Release versus Square Root of Time, which resulted in a straight line with R^2 of 0.985 and 0.9839 for ciprofloxacin and Metronidazole respectively. Korsmeyer peppas model was analysed by plotting graph between Log % drug releases versus Log of time in hours. The R^2 values obtained were 0.9838 and 0.9611 for Ciprofloxacin and metronidazole respectively. The Correlation coefficients obtained for zero order plot was found to be highest out of all the data models, therefore it is concluded that the release of ciprofloxacin hydrochloride follows zero order. While the correlation coefficient of first order release is found to be highest for metronidazole thus indicating that metronidazole follows first order release. The plots are depicted in Figure 3 and 4 for ciprofloxacin and metronidazole respectively. For tablets of a known geometry (in this case a caplet) n = 0 .5 means Fickian diffusion, 0 .5 < n < 1.0 non-Fickian diffusion, and n = 1 .0 Case II diffusion. The value on n lies between 0.5 and 1 thus indicating that the optimized formulation shows a non-Fickian mechanism which means that the process of diffusion and relaxation run at comparable rates i.e., drug release is by coupling of Fickian diffusion and polymer matrix relaxation - so-called anomalous diffusion - and may indicate that drug release is controlled by more than one process.

In-vitro buoyancy studies

On visual inspection of the *in-vitro* buoyancy test, the optimized batch tablet stays at bottom of the beaker containing 0.1N HCl solution at 0 s. At 75 s, the tablet was found to rise at the surface of the fluid in the beaker. The tablet maintained its buoyancy and kept floating on the surface till 20 hours. The images of the buoyancy test at various time intervals is presented in Figure 5.

In-vivo floating studies

The X-ray images of the optimized placebo batch are represented in Figure 6. The images of the in-vivo studies of the test formulation depict the floating nature of the formulation at various intervals of the study.

Conclusion

A combination of ciprofloxacin with an antimicrobial agent active against anaerobes, such as metronidazole, could prove an efficient way of treating mixed aerobic/ anaerobic infections.

Development of gastro retentive dosage form can be advantageous, that can provide prolong gastric retention and increase efficacy of the dosage form. The research aimed at achieving a floating lag time of 75 seconds and the floating duration of more than 20 hours with not less than 90 % of drug release by 20 hours for the developed floating tablets. The drug release kinetics was studied for the optimized batch F14, to determine the type of drug release. For ciprofloxacin drug release, the correlation coefficient for zero order was found to be highest of all with R^2 of 0.9912, slope of 4.23 and a positive intercept of 18.19 making it the best fitting release model for ciprofloxacin. The intercept for first order and Higuchi model was found to be negative with a lower R^2 value as compared to zero order release.

Korsmeyer-peppas equation showed a slightly lower correlation coefficient of 0.9838 with a slope of 0.56 and a positive intercept of 1.24.

The study of metronidazole drug release, showed that the correlation coefficient for first order was found to be highest of all with R^2 of 0.9966, slope of 2.021 and a negative intercept of -0.0646 making it the best fitting release model for metronidazole. The optimized formulation batch shows a non-Fickian diffusion mechanism as calculated by Korsmeyer-peppas equation which means that the process of diffusion and relaxation run at comparable rates. Further, *in-vitro* buoyancy studies were undertaken for the optimized batch. The *in-vitro* buoyancy studies depicted that the tablet rises on the surface of the floating medium by 75 seconds and maintains the floating behaviour till 20 hours. These floating studies were further carried out *in-vivo* in dogs where it was found that the tablets of the optimized polymer

ratio remained floating as observed in the X-ray images at 2, 12 and 20 hours thus confirming the floating duration of the formulation.

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