AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and *In vitro / In vivo* evaluation of Sustained oral delivery system of Mefenamic acid from floating in situ gelling formulations

Nidhal Khazaal Marie[1*]

1. College of Pharmacy, University of Al-Mustansiriya, Baghdad-Iraq

ABSTRACT

The aim of this work is to prepare oral liquid formulation of the non-steroidal anti-inflammatory drug mefenamic acid with sustained release property using floating in situ gelling technique. Gellan gum and sodium alginate separately were used to form the gels. Results showed the release from gellan gum was slower than that from sodium alginate in concentration dependent manner; where the drug release decreased as the amount of the gelling agent increased. Results showed that increasing the concentration of gellan gum (0.25,0.5 and 1%w/v) caused a decrease in gelation time and floating lag time with floating duration for more than 8 hours and significant decrease ($p<0.05$) in drug release. Increasing the concentration of sodium alginate (1, 1.5 and 2%w/v) also results in significant decrease ($p<0.05$) in gelation time, floating lag time and drug release with long floating duration. On the other hand increasing gas generating agent calcium carbonate (0.5, 1 and 1.5%w/v) reduces significantly floating lag time and drug release but no significant effect on gelation time. Increasing drug loading showed significant increase in drug release. Sorbitol 5%w/v was added to the selected formula as sweetening agent, which leads to decrease in the viscosity with no significant effect on drug release. Overall results indicate that drug release followed diffusion control mechanism, and were used for optimizing the liquid formulation of mefenamic acid with sustained release property, minimizing side effects and improving the bioavailability as which may reduce the dose and dosing rate with optimum effectiveness.

Keywords: Mefenamic acid, floating in situ sol gel formulation, sustained release liquid oral dosage form

*Corresponding Author Email: dr_nidhal_khazaal@yahoo.com

Received 11 May 2013, Accepted 27 May 2013

INTRODUCTION

The goal in designing controlled and sustained drug delivery systems is to reduce the frequency of dosing or to increase effectiveness of the drug by localization at the site of action, decreasing the dose required for providing uniform drug delivery[1]. Many polymers have been employed as drug retarding agents including hydro gels which are three-dimensional polymeric networks that can absorb and retain large amounts of water and biological fluids and swell[2]. In-situ gel forming drug delivery systems are a revolution in oral drug delivery systems. The gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form three dimensional network complexes with cations and hydrogen bonding [3]. The sol-gel transition process is induced by the presence of divalent ions. Gelation was delayed until the orally administered solution reached the stomach by complexing the calcium with sodium citrate, then acidic environment of the stomach causes break down of the complex releasing free calcium ions and causing instantaneous gelation [4]. Floating drug delivery system is also called the hydro dynamically balanced system. Floating drug delivery systems have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system [5]. Floating in- situ gelling system is a promising potential in developing drug delivery system that prolongs the residence time of the formulation[6] .

Mefenamic acid is a non-steroidal anti-inflammatory drug used to treat pain including menstrual pain, decreases inflammation (swelling) and uterine contractions and for premenstrual migraine headache prophylaxis. Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity, the symptoms of pain are temporarily reduced. BUT this drug may cause serious bleeding from the stomach or intestines. This bleeding can occur without warning symptoms at any time during treatment. It is selected in this study for the nature of its ionizable charge[7,8].

Gellan gum is anionic deacetylated polysaccharide with a tetrasaccharide repeating unit of one α-L-rhamnose, one β- D-glucuronic acid and two β-D-glucose residues. It has the characteristic property of temperature dependent and cation-induced gelation. This gelation involves the formation of double helical junction zones followed by gum regation of the double helical

segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water[9,10]. It is approved as a gelling stabilizing and suspending agent in food products [11]. Gellan gum is a multi-functional gelling agent can be used alone or in combination with other products to produce a wide variety of interesting textures, it acts as a thickening or gelling agent and can produce textures in the final product that vary from hard, non-elastic, brittle gels to fluid gels[11,12]. Sodium salt of alginic acid (sodium alginate) is a linear block copolymer polysaccharide, the dilute aqueous solutions of alginates form firm gels on the addition of di and tri-valent metal ions[13]. Calcium carbonate present in the gelling formulation released carbon dioxide in gastric environment thereby making the formulation porous and buoyant and prolonging the residence time. This floating in stomach provides the potential to sustain the drug release over a long period of time[14].

Although the oral route constitutes the preferred route for drug delivery, however, mefenamic acid remains poorly available when administered by this route due to its limited site for absorption, therefore, the aim of the present work is to develop floating stomach specific sustained release delivery system for mefenamic acid(using ion-triggered in-situ gelling formulations)to increase the residence of mefenamic acid at/ or above the absorption window to improve its bioavailability and minimizing the local irritant effect of the drug. To achieve this purpose, different factors had been studied to optimize the selected formula since no sustained release oral liquid dosage form for mefenamic acid available in the market and study the bioavailability of optimized formula in comparison to the conventional oral liquid dosage form of the drug available in the market.

MATERIALS AND METHODS

Materials

Mefenamic acid, methyl paraben , propyl paraben (Samara drug industry company-Iraq), Ponstan suspension (mefenamic acid suspension)(Park Davis, England), Calcium chloride, disodium hydrogen phosphate ,sodium carbonate, potassium dihydrogen phosphate (BDH chemical Ltd, England) , acid ,sorbitol (Riedel-dehaen Hannover ,Germany), gellan gum and sodium alginate (Hopkins and Williams Ltd ,England).

Preparation of in-situ gelling solutions

Sodium alginate of concentration 1.0, 1.5 and 2.0 %(w/v) were prepared by adding the polymer to distilled water containing 0.25% (w/v) sodium citrate and 0.075% (w/v) calcium chloride with heating to 60 ºC while stirring. After cooling to below 40 ºC various amount of calcium

carbonate and 10%w/v mefenamic acid were added with continuous stirring to form uniform dispersion [15,16]. Gellan gum solutions of concentrations 0.25, 0.5 and 1.0% (w/v) were prepared by adding the gum to distilled water containing 0.17% w/v sodium citrate and 0.016% (w/v) calcium chloride then heating to 90 ºC while stirring. After cooling to below 40 ºC various amounts of calcium carbonate and 10% (w/v) mefenamic acid were added with continuous stirring to form uniform dispersion[14].

Gelation study

The gelation study was carried out by applying the prepared solutions drop wise to simulated gastric fluid pH1.2. Gelation was deducted by visual examination.

In vitro floating study

The in vitro floating study was carried out by measuring floating lag time and duration of floating using simulated gastric fluid pH 1.2 as dissolution medium. Ten milliliters of the formulation was placed in 100 ml dissolution medium. The medium was kept in a stagnant condition and 37^{o}C temperature. The time the formulation took to rise to the medium surface (lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were determined visually[17]

In vitro drug release study

The release of mefenamic acid from the prepared formulations was studied using USP dissolution apparatus with a paddle stirrer at 50 rpm. The dissolution medium used was 900 ml of 0.1 N HCl (pH 1.2) at 37^{o}C. Ten milliliters of the formulation was placed in the dissolution medium and 5 ml of aliquot was withdrawn at every time interval and replenished with preheated (37^{o}C) fresh dissolution medium. The samples were filtered and analyzed using U.V. spectrophotometer at 285nm[18,19,20]. Sols of sodium alginate (1% w/v) containing different concentrations of mefenamic acid (5 and 10 % w/v) were prepared to study the effect of drug concentration on the release profile.

In vivo study for the prepared formula

Ten healthy rabbits ranging in body weight from 1.5 –3.0 Kg were maintained under similar feeding and management conditions. The protocol for the experiment was approved by the Appropriate Animal Care committee in Al-Mustansiriya University. The optimized formula (formula 8) was administered as a single dose of 50mg/Kg body weight(to ten healthy rabbits group) through the feeding cannula orally in comparison to same dose of Ponstan suspension (Parke Davis & Co. Ltd)[21]. Two milliliters samples were drawn from the jugular vein of the rabbits just before and at 0.5, 1.0, 1.5, 2.5, 3.0, 4.0, 6.0, 8.0, 9.0, 10.0, 12.0, 14.0, 16.0 and 18.0

hours after the drug administration. Plasma was separated from each sample and used for the analysis. The plasma was deproteinized with acetonitrile and the supernatant fraction was evaporated to dryness and reconstituted with the mobile phase (phosphoric acid: acetonitrile 40:60 v/v) using reverse phase HPLC with 4.6 mmx15 cm, LC-18ultrasphere-I.P.column.The drug was detected at 285 nm and the retention time for mefenamic acid was 6.8 minutes[22,23]. The concentration of mefenamic acid was determined by comparing the area under the curve (using trapezoidal rule) to that of standard curve. The pharmacokinetic parameters of mefenamic and the bioavailability were obtained applying one–compartment model of analysis[21,24]. Mean values (± SD) were calculated for each parameter. A paired t–test for statistical analysis and a value of $P<0.05$ was taken as being statistically significant

Statistical analysis

Results are given as a mean ±S.D. for triplicate samples .The results were statistically analyzed by using ANOVA the difference of ($P< 0.05$) were considered to be significant. The correlation coefficient (r^2) was determined to examine the linear relationship between two parameters.

RESULTS AND DISCUSSION

Gelation study

All the prepared formulations (table1) showed instantaneous gelation when added as drop wise to simulated gastric fluid pH1.2. Addition of sodium citrate to the formulation will form a complex with the calcium ions present in the formulation, the complex will be broken in the acidic environment of the stomach and releasing calcium ions which will cause gelation[25].

The prepared formulas were in liquid state before introduced in the simulated gastric fluid; this will allow easy swallowing as a liquid dosage form which then undergoes a rapid sol-gel transition due to ionic interaction after it reaches the stomach. It was observed that formulations containing higher concentrations of sodium alginate underwent instant gelation with significantly stronger gel in simulated gastric fluid than those containing lower concentrations due to internal ionotropic gelation effect on sodium alginate[14]**.** Same results were observed for gellan gum[19]**.**

It was observed that the formulations containing calcium carbonate(gas generating agent) gelled more instantaneously than those not containing calcium carbonate after contact with simulated gastric fluid and the gel formed had adequate strength (when pressed with fine forceps) indicating that calcium carbonate gave a strong gel in short time of delivery into the stomach and will with stand the shear forces likely to be encountered in the stomach thus it will have longer residence time than conventional oral solutions[26]**.**

Floating study

The floating ability of the prepared formulations was studied in simulated gastric fluid (pH 1.2) where the floating lag time and floating duration are shown in (table 1). Formulations did not contain calcium carbonate gave gel that settled at the bottom of the container containing simulated gastric fluid while those containing calcium carbonate gave gel that floated on the simulated gastric fluid with floating lag time less than one minute and long floating duration. This is due to the fact that the presence of calcium carbonate in the formulations lead to generation of carbon dioxide gas which will be entrapped in the gel producing buoyant formulation with a short floating lag time, then calcium ion reacted with the polymers(sodium alginate and gellan gum) producing a cross linked three dimensional gel network that might further restrict diffusion of carbon dioxide gas and drug molecules so resulted in extended period of floating and drug release respectively[16,27].

Three different concentrations of calcium carbonate were used (0.5, 1 and 1.5% w/v) and it was found that as the concentration of calcium carbonate increased; the floating lag time was reduced and duration of floating was increased due to increase in the amount of calcium ion and carbon dioxide gas. It was found also that as the polymer concentrations increased (for both sodium alginate and gellan gum separately) there will be reduction in floating lag time with increase in floating duration and this agreed with reported data[14], while increasing drug loading had no significant effect on floating properties.

Table 1: Content of the prepared formulations and their evaluations

Form. no.	Mefenamic acid (% w/v)	Sodium alginate (% w/v)	Gellan gum (% w/v)	Sodium citrate (% w/v)	Calcium chloride (% w/v)	Calcium carbonate (% w/v)	Gelation (Sec.)	Floating lag time (Sec.)	Floating Duration (hr)	% Cumulative release
F1	10	1	-	0.25	0.075	0.5	38	80	> 8	70
F2	10	1.5	-	0.25	0.075	0.5	22	61	> 8	58
F3	10	2	-	0.25	0.075	0.5	14	77	> 8	50
F4	10	-	0.25	0.17	0.016	0.5	24	30	7	60
F5	10	-	0.5	0.17	0.016	0.5	14	27	> 8	33
F6	10	-	1	0.17	0.016	0.5	12	21	> 8	34
F7	10	1	-	0.25	0.075	-	50	-	-	80
F8	10	1	-	0.25	0.075	1	35	58	> 8	65
F9	10	1	-	0.25	0.075	1.5	37	41	> 8	60
F10	10	-	0.5	0.17	0.016	-	40	-	-	40
F11	10	-	0.5	0.17	0.016	1	10	14	> 8	30
F12	10	-	0.5	0.17	0.016	1.5	8	12	> 8	35
F 13	5	1	-	0.25	0.075	1	36	50	8 >	45

%cumulative release= cumulative % release after 8 hrs. , Sec. =second, hr. =hour

***In vitro* drug release study**

Effect of polymers concentrations and types:

Figure 1 and 2 showed the release of mefenamic acid from the prepared formulations using sodium alginate and gellan gum respectively, and it is found that the rate and extent of drug release is significantly ($p \leq 0.05$) decreased as the polymer concentrations increased and it is attributed to increase in the density of the polymer matrix and also increase in the diffusion path length which the drug have to traverse and it is possible that at higher polymer concentration, drug is trapped in smaller polymer cells and is structured by its close proximity to the polymer molecules, so increasing amount of the polymer lead to increase in the time needed for the drug to leave the formulation (retarded drug release)[26].

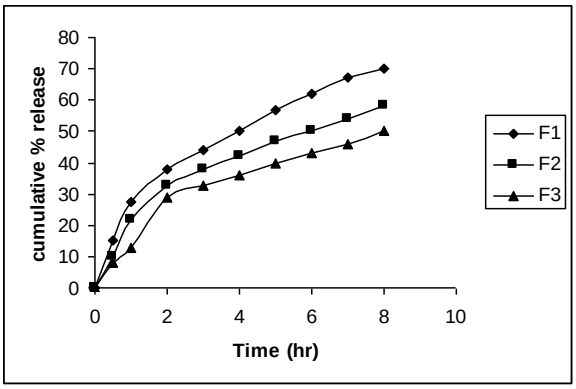

Figure.1- Effect of sodium alginate concentrations on the release of mefenamic acid from floating in situ formulations

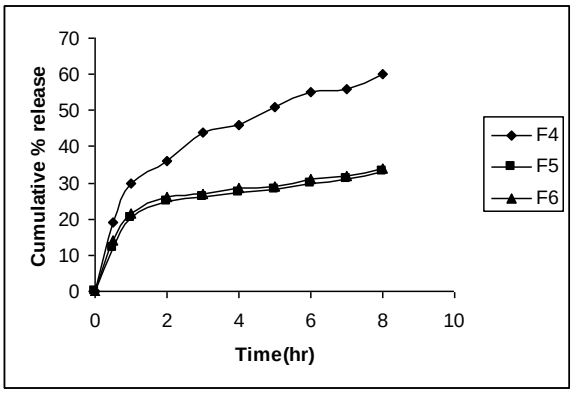

Figure.2- Effect of gellan gum concentrations on the release of mefenamic acid from floating in situ formulations

The release pattern from all the formulations showed high release of the drug at the first 30 minutes followed by slower release which continue to the end of the experimental time (8 hrs) and this can be explained due to incomplete gelation at the beginning and it is evident by a lag time although cross link of the polymer network due to calcium ions occurs instantaneously but

the matrix formed before complete gelation would be in the hydrated state. Then complete gelation and cross linking occurs in addition to low water solubility of the drug at low pH responsible for the retardation of drug release in the second phase, similar bi-phasic drug release was obtained with clarithromycin[14].

Drug release from gellan gum preparations is generally lower than the release from sodium alginate preparations. This is due to the ability of gellan gum to form double helical junction zones followed by aggregation of the double helical segments to form a three dimensional network by complexation with cations and hydrogen bonding with water so it has ability to form strong clear gels at physiological condition which can entrapped the drug firmly and so cause more retardation of drug release[13].

Effect of calcium carbonate concentrations on drug release:

It is shown that the release of the drug from formulations containing calcium carbonate(0.5%, 1% , 1.5% w/v) is lower than those not containing calcium carbonate for both gellan gum and sodium alginate containing preparations (as gelling polymers) as shown in figure 3 and 4. This is because the gelation was predominantly due to the presence of calcium ion while in formulations without calcium carbonate the gelation was mainly due to the presence of monovalent cations (sodium and hydrogen ion) in the formulation. With increase in calcium carbonate concentrations there were decrease in drug release and this agreed with reported data that divalent ions were superior to monovalent cation in promoting the gelation of the polysaccharide[4,25].

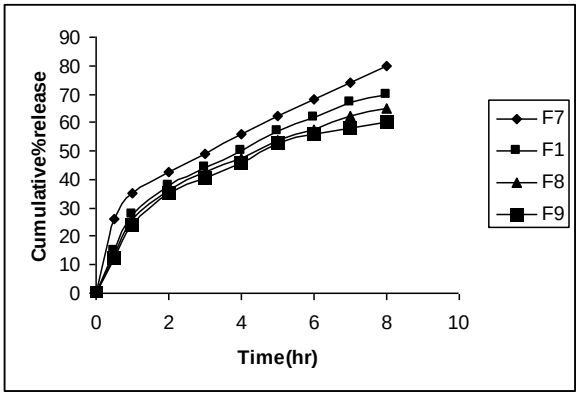

Figure.3- Effect of calcium carbonate concentrations on mefenamic acid release using sodium alginate.

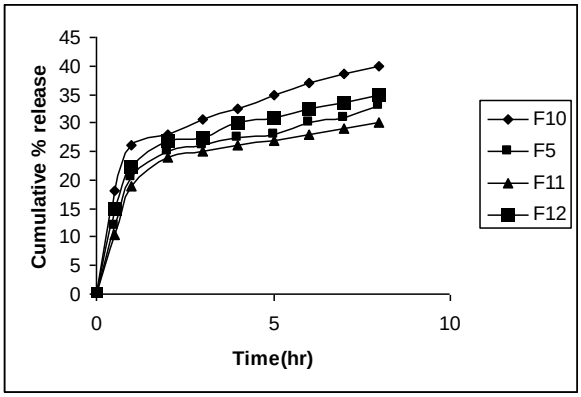

Figure. 4- Effect of calcium carbonate concentrations on mefenamic acid release using gellan gum

Kinetics of drug release

In order to investigate the mode of drug release from floating in situ gels the release data were analyzed with the following mathematical models:

Zero order kinetic; first order kinetic and Higuchi equation (Table 2). The examination of the coefficient of determination (r^2) indicated that drug release followed diffusion controlled mechanism since the (r^2) values for Higuchi model (ranged 0.9988-0.9999) were higher from that of zero order model(ranged 0.8977-0.9556) as well as first order model(ranged 0.7998-0.8966).

Table 2: The coefficient of determination (r^2) for drug release

Formula	Higuchi model	Zero - order	First - order	n- value
1	0.9988	0.8977	0.7998	0.454
2	0.9989	0.8967	0.8341	0.5211
3	0.9990	0.8981	0.7901	0.458
4	0.9998	0.8991	0.8866	0.496
5	0.9992	0.8979	0.8721	0.512
6	0.9993	0.8950	0.8322	0.603
7	0.9991	0.9334	0.8901	0.483
8	0.9931	0.8998	0.8339	0.514
9	0.9990	0.8978	0.8945	0.488
10	0.9959	0.9441	0.8231	0.610
11	0.9996	0.8991	0.8830	0.542
12	0.9997	0.9543	0.8940	0.579
13	0.9999	0.9556	0.8966	0.667

To understand the drug release mechanism the data were fitted to the Korsmeyer –Peppas model ($M_t / M_\infty = k \times t^n$). Where Mt is the amount of released drug at time t, M∞ is the overall amount of the drug, k is the constant incorporating structural and geometric characteristics of the controlled release device, and (n) is the release exponent indicative of the drug release mechanism. The rate constant *k* and the diffusional exponent *n* can be obtained from the intercept and the slope of a plot of *ln*M_t / M_∞ versus *ln* t respectively.

When n ≤0.45 corresponds to a Fickian diffusion, and if 0.45<n<0.89 corresponds to anomalous transport, n ≥ 0.89 indicates to a zero order or case II transport[28,29]. In this study the (n) values were in range of 0.454-0.667 which was further indication that drug release followed diffusion control mechanism mainly anomalous transport which considered swelling of the system in the solvent before the release takes place in addition to polymer relaxation that led to retarding the release with increasing of the concentration of the polymer.

The results showed that the amount of drug released increased significantly ($p < 0.05$) as a function of increasing drug concentration in the prepared sols, this could be due to increase in the thermodynamic activity of the drug and may be due to high availability of the drug on the surface of the gel structure so as drug molecules on the surface leached out in to the dissolution medium it may create empty pores which enable drug molecules in the inner portion of the gel to leach out at a faster rate[30,31].

From the above results the best formula was formula 8 (F8) which showed short lag time and long floating duration with 65 % cumulative drug release after 8 hrs and required lowest amount of calcium carbonate to float .The best formula is liquid in the bottle with acceptable rheological properties (its viscosity 70 cP). To the selected formula 5%w/v sorbitol is added as sweetening agent and it was found that the viscosity of selected formula is reduced to 55 cP with no significant effect on drug release and this could be due to hydrogen bonding of sorbitol molecules and the polymer chain[32]. The lower viscosity of the final formula will improve its ease swallowing and upon contact with simulated gastric fluid it gives instantaneous gelation that gave slow prolonged release.

***In vivo* study:**

Table 3 shows the mean plasma concentration values (and standard deviation) of mefenamic acid at the selected time intervals in 10 rabbits receiving single dose 50 mg/Kg orally using selected formula (F8) and Ponstan suspension (Parke Davis & Co. Ltd). The pharmacokinetic parameters including area under the curve (AUC), maximum plasma concentration(c max), time to reach maximum concentration (t max), absorption rate constant (Ka), elimination rate constant (Ke),elimination half-life (t½),volume of distribution (Vd) and total clearance(Cl) in both cases were obtained and shown in table 4. As shown in figure 5 the data of mefenamic acid follows one-compartment open model and agreed with reported data for mefenamic acid[33]. In the present study, as shown in table 4 and figure 5 the mean time to reach peak plasma concentration (tmax) was found to be 9.1 hr from the selected formula which is significantly different from that for ponstan suspension (2.6 hr) and the absorption rate constant is also significantly slower which

indicates slow continuous prolong release of mefenamic acid from the selected formula prepared using floating stomach specific in situ gelling preparation which increased the residence of the drug at/ or above the absorption window and so took longer time to reach maximum plasma concentration[5]. In addition to that the C max for selected formula is significantly higher from Ponstan suspension (P≤ 0.05) which indicates that the sustained release of the drug from the prepared dosage form lead to availability of significant amount of the drug at the window absorption site for mefenamic acid and this lead to increase the amount of drug absorbed, and hence increases its bioavailability[6,13]**.** The bioavailability of the mefenamic acid from the selected formula was found to be 100% relative to the reference Ponstan suspension which is conventional oral liquid dosages form. While there is no significant changes in elimination half-ife, elimination rate constant, total clearance and volume of distribution

Table – 3: Mean (± SD) plasma concentrations of mefenamic acid at the time intervals, after oral administration of the selected formula (F8) and Ponstan suspension (Parke Davis & Co.Ltd)

	Mean (± SD) plasma concentrations (µg/ml)													
	0.5 hr	1.0 hr	1.5 hr	2.5 hr	3.0 hr	4.0 hr	6.0 hr	8.0 hr	9.0 hr	10.0 hr	12.0 hr	14.0 hr	16.0 hr	18.0 hr
F8	0.60 ± 0.038	0.62 ± 0.065	0.70 ± 0.019	0.81 ± 0.045	0.9 ± 0.048	1.2 ± 0.023	2.6 ± 0.169	3.6 ± 0.230	3.72 ± 0.135	3.70 ± 0.138	3.61 ± 0.05	1.6 ± 0.013	1.2 ± 0.011	0.8 ± 0.015
Ponstan suspension	0.72 ± 0.016	1.8 ± 0.051	2.7 ± 0.113	3.8 ± 0.140	3.1 ± 0.320	1.7 ± 0.053	1.2 ± 0.012	0.5 ± 0.013	0.4 ± 0.068	0.32 ± 0.024	0.2 ± 0.013	0.15 ± 0.011	0.11 ± 0.031	0.1 ± 0.005

Table–4: Mean (±SD) values of the pharmacokinetic parameters following oral administration of the selected formula (F8) and Ponstan suspension

	C max (µg/ml)	T max (hr)	AUC (µg/ml). hr	Ke (hr $^{-1}$)	T ½ elim (hr)	Ka (hr $^{-1}$)	Vd(1/kg)	Cl (ml/hr/kg)
F 8	4.352 ± 0.123	9.1 ± 0.114	16.019 ± 0.581	0.247 ± 0.01	2.805 ± 0.045	0.619 ± 0.083	12.893 ± 0.321	90.997 ± 1.099
Ponstan suspension	3.453 ± 0.221	2.6 ± 0.097	15.994 ± 0.598	0.241 ± 0.02	2.875 ± 0.047	0.891 ± 0.086	12.995 ± 0.334	91.792 ± 1.171

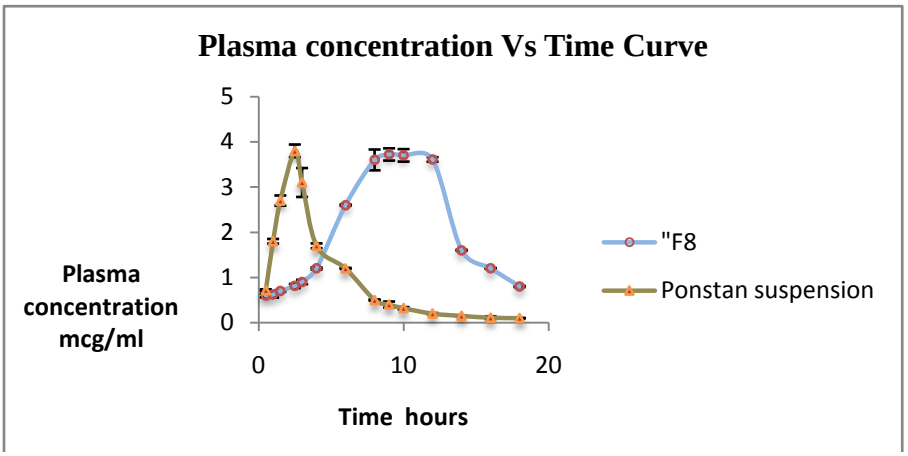

Figure 5- Plasma concentration versus time curve of F8 formulation and the marketed Ponstan suspension.

The results showed that the floating stomach specific sustained release delivery system for mefenamic acid (using ion-triggered in- situ gelling technology) is a promising dosage form to enhance the absorption of the drug from its window site and improves its bioavailability and can be used as a sustained release oral liquid dosage form.

CONCLUSION

Mefenamic acid is poorly available when administered orally due to its limited site for absorption, also it has local irritant effect on the stomach, therefore, this work presented sustained release oral delivery system for mefenamic acid that increased the residence of mefenamic acid at/ or above the absorption window and improved its bioavailability using ion-triggered floating in-situ gelling formulations and evaluate them by in vitro and in vivo study to optimize the selected formula since no sustained release oral liquid dosage form for mefenamic acid available in the market.

ACKNOWLEDGMENTS

Thanks and gratitude for the national institutes for drug monitoring in Iraq for their help and cooperation during all steps of the work. Thanks are extended to Samara drug industry Company-Iraq for supplying the drug and some other ingredients. Gratitude and appreciations for Dr.Jumah Masood (College of Pharmacy/Al-Mustansiriya University) and Enas R. Ibrahim (Yarmook college for pharmacy) for their endless help and support.

REFERENCES

1. Saraswat R., Bhan C.S. and Gaur A., A review on polymers used in in-situ gel drug delivery systems, Int. J. pharm. Innova. 2008; 1: 110-118.

2. Kaparissides C., Alexandridou S., Kotti K., Chaitidou S., Recent advances in novel drug delivery systems, J. Nanotechnology online 2006.
3. Patel R.R., Formulation, evaluation and optimization of stomach specific in-situ gel of clarithromycin and metronidazole benzoate. Int. J. Drug Deliv. 2010; 2: 141-153.
4. Tang J., Tung M. A. and Zeng Y.,Gelling properties of gellan solution containing monovalent and divalent cations, J. Food sci. 1996 ; 62 : 688-692.
5. Sharma N., Agarwal D., Gupta M.K., Mahaveer Pr. and Khinchi A., Comprehensive review on floating drug delivery system, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2: 428-441.
6. Rajinikanth PS, Balasubramanium J, Mishra B., Development and evaluation a novel floating in-situ gelling system of amoxicillin for eradicating of Helicobacter pylori, Int. J. Pharm. 2007; 335: 114-122.
7. Khullar R., Kumal D., Seth N, Saini S., formulation and evaluation of mefenamic acid emulgel for topical delivery, Saudi Pharmaceutical Journal, 2012; 20: 63–67.
8. The pharmaceutical codex, 11th Ed. The pharmaceutical press. London, 1979; pp268.
9. Jay A. J., Colquhoun I. J., Ridout M. J., Brownsey G. J., Morris V. J., Fialho A. M., Leitao J.H. and Sa-Correia I., Analysis of structure and function of gellans with different substitution patterns, Carbohydr. Polym. 1998; 35: 179–188.
10. Manna B., Gambhir A. and Ghosh P., Production and rheological characteristics of the microbial polysaccharide gellan, Lett. Appl. Microbiol., 1996; 23: 141–145.
11. Paulsson M., Hagerstrom H. and Edsman K., Rheological studies of the gelation of deacetylated gellan gum in physiological conditions. Eur. J. Pharm., 1999; 1: 99-105.
12. Donner, L.W. and Douds D.D., Purification of commercial gellan to monovalent cation salts results in acute modification of solution and gel-forming properties, Carbohydrate Research ,1995; 273 : 225-233.
13. Kubo W., Miyazaki S. and Attwood D., Oral sustained delivery of paracetamol from in-situ gelling gellan and sodium alginate formulations, Int. J. Pharm., 2003; 258: 55-64.
14. Mishra B. and Rajinikanth P.S., Floating in situ gelling system for stomach site specific delivery of clarithromycin to eradicate H. pylori, J. Control. Release, 2008; 125: 33-41.
15. Miyazaki S., Kawasaki N., Kubo W., Endo K. and Attwood D., Comparison of in situ gelling formulation for the oral delivery of cimetidine, Int. J. Pharm., 2001; 220:161-168.
16. Miyazaki S., Kubo W. and Attwood D., Oral sustained delivery of theophylline using in-situ gelation of sodium alginate, J. Control. Release, 2000; 67: 275-280.

17. Rosa M., Zia H. and Rhodes T.,Dosing and testing in-vitro of a bioadhesive and floating drug delivery system for oral application, Int. J. Pharm., 1994; 105: 65-67.
18. Rania A.H., Dhanne A.S. and Mahed D.M., Preparation in vitro and in vivo evaluation of stomach specific metronidazole loaded alginates beads in local anti-Helicobacter pylori therapy, J. Control. Release, 2007; 119: 207-214.
19. Kedzierewicz F., Lombry C., Riosr R., Hoffman M. and Maincent P., Effect of the formulation on the in vitro release of propranolol from gellan beads, Int. J. pharm., 1999; 178: 129-136.
20. Singh H., Kumar R. and Singh P., Development of UV spectrophotometric method for estimation of mefenamic acid in bulk and pharmaceutical dosage forms, Int. J. Pharm. Pharm. Sci., 2011; 3: 237-238.
21. Qamar S., Bukhari N.I., Ahmad M., Jamshaid M. and Muzaffar N.A.,"Effect of dehydration on the pharmacokinetics of mefenamic acid", Tr. J. of Medical Sciences, 1999; 29: 101-104.
22. Ito K., Niida Y., Sato J., Owada E., Ito K. and Umetsu M., Pharmacokinetics of mefenamic acid in preterm infants with patent ductus arteriosus, Pediatrics International, 1994; 36: 387-391.
23. Niopas I. and Mamzoridi K., Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography, Journal of Chromatography, 1994; 656 : 447-450**.**
24. Shinkuma D**.,** Hamaguchi T., Yamanaka Y. and Mizuno N., Correlation between dissolution rate and bioavailability of different commercial mefenamic acid capsules, Int. J. Pharm., 1984; 21: 187-200.
25. Choi B.Y., Park H.J., Hwang S.J. and Park J.B., Biological interactions between divalent cation and polysaccharides, Int. J. pharm. 2002; 239: 81-91.
26. Wamorkar V., Varma M. M. and Manjunath S. Y., Design and optimization of in-situ gel of metoclopramide using 3^2 full factorial design, J. Pharm. Research, 2011 ; 4: 2780-2783
27. Miyazaki S., Aoyama H., Kawasaki N., Kubo W. and Attwood D., In situ gelling gellan formulation as vehicle for oral delivery, J. Control. Release, 1999; 67: 275-280.
28. EI-Samaligy S.A. and Basalious.E.B, Formulation and evaluation of diclofenac sodium buccoadhesive discs, Int. J. pharm. 2004; 286: 27-39
29. Korsmeyer R.W., Gurney R., Doelker E., Buri P. and Peppas N.A., Mechanisms of solute release from porous hydrophilic polymers, J. Pharm. Sci. 1983 ; 15 : 25-35.

30. Paulsson M. and Edsmanl K., Controlled drug release from gels using lipophilic interactions of charged substances with surfactants and polymers, J. Coll. Interface Sci. 2002; 248: 194-200.
31. Zidan A.S., Sammour O.A., Hammed M.A. and Megrab N.A., Formulation of anastrozole microparticles as biodegradable anticancer drug carriers, AAPS Pharm. Sci. Tech. 2006; 7: 61.
32. Miyazaki S., Kubo W, Itoh K. and Konno Y., The effect of taste masking agents on in situ gelling pectin formulations for oral sustained delivery of paracetamol and ambroxol, Int. J. Pharm. 2005; 297: 38-49.