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INCREASING THE BIOAVALILABILITY OF MEBENDAZOLE II. INFLUENCE OF CROSCARMELLOSE ON DISSOLUTION RATE, EXTENT AND MECHANISM IN SIMULATED INTESTINAL MEDIUM

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ABSTRACT: Objective of the research was the developing of tablets with increased rate and extent of release of mebendazole in intestine in order to improve its bioavailability and pharmacokinetic profile. Three solid formulations containing 1%, 2% and 3 % croscarmellose sodium, with superdisintegrant properties were prepared. For the quantitative assay of mebendazole a HPLC-UV method was developed and validated. Bioavailability was estimated by means of the *in-vitro* release kinetics using USP Apparatus 2, in Fasting State Simulated Intestinal Fluid (FaSSIF). Release of mebendazole in FaSSIF was poor (less than 10 % within 2 hours). Dissolution could be described by Higuchi law and Peppas law in both the swelling phase and post-errosion-disintegration phase, suggesting a diffusion controlled release. Both rate and extent of release increased with concentration of croscarmellose predicting a better bioavailability of mebendazole from the croscarmellose containing tablets.

Keywords: mebendazole, croscarmellose, in vitro release, FaSSIF, intestinal suprasaturation

INTRODUCTION:

Mebendazole is a synthetic unsymmetrically substituted benzimidazole, with a broad-spectrum anthelmintic activity, which is orally effective against a number of cestodes and nematodes. Oral mebendazole therapy is the election alternative to surgery in the treatatment of alvolar and cystic echinococcosis (hydatide disease), one of the most lethal of all human helminth infections, caused by infection with the larval stage of the cestodes Echinococcus multilocularis and E. granulosus (Liu et al, 2012). However, mebendazole bioavailability is less than 2% (Dawson et al., 1985). The clinical response in patients treated with mebendazole is higly variable, and is directly correlated to its low and variable bioavailability (Ortan et al., 2015) which is most likely due to a combination of a very low solubility (leading to a absorption from the gastrointestinal tract not exceeding 5–10%), and extensive first pass metabolism of the drug (Briceson et al., 1982; Rivera et al., 2007).

Different studies includes mebendazole in either Class II or Class IV based on the Biopharmaceutics Classification System (Amidon et al., 1995; Lindinberdg et al., 2004). Whatever its classification, mebendazole is a highly problematic active pharmaceutical ingredient, with very low and pH dependent solubility, tendency to precipitate on passage from gastric to intestinal media, highly variable and subject to food-effect in-vivo pharmacokinetic profile (Dayan, 2003). For that type of drugs, USP compendial dissolution methods (generally using acidic media) generally ignore the specific supersaturating phenomenon in intestinal medium and therefore fails to provide significant information on their in vivo behavior.

Amongst the quality control tests, only the study of drug release kinetics has the potential to offer some information about how the active substance will behave in-vivo (Savu et al., 2016; Mircioiu et al 2013). But this tool has its limitations, as for obtaining relevant information is imperative that the drug substance is subject to the same transformations as in-vivo, and most of the compendial dissolution tests fail in that aspect. The intestinal absorption of mebendazole is controlled by many factors, including the extent of supersaturation, pH, fluid volume, viscosity, and bile salts concentration (Dinu-Pirvu et al., 2013; Anuta et al., 2014), therefore biorelevant simulation of gastrointestinal conditions during in vitro dissolution testing is essential in order to obtain significant information in terms of *in-vivo* behaviour.

In this context, the main objective of the present research was the developing of tablets with increased rate and extent of release of mebendazole in intestine in order to improve its bioavailability and pharmacokinetic profile. A better understanding of the release mechanism from the experimental tablets was considered an essential aspect of formulating optimized mebendazole formulations.

MATERIAL AND METHODS:

Materials

Mebendazole was a gift sample from Iraqi Pharmaceutical Industry (IPI) Company (Baghdad – Iraq). Composition of tablets included Croscarmellose sodium (CC) - an internally cross-linked sodium carboxymethylcellulose, superdisintegrant in pharmaceutical formulations, Ludiflash (BASF) - combination containing manitol 90 % as filler, Kollidon CL-SF 5% disintegrant and Kollicoat SR 30

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D as water insoluble binder which accelerates disintegration (Swabrick et al, 1990), magnesium stearate and talc.

Three different mebendazole formulations were prepared (F1-F3), the difference consisting in the CC concentration: 1% for F1, 2% for F2 and 3% for F3.

Methods

Tablets were controlled using pharmacopeia methods it concerns content in active substance and physico-chemical properties. For assay of mebendazole was developed and validated an HPLC method.

Quantitative analysis of mebendazole

Quantitative analysis of mebendazole was performed by using a Waters HPLC system (Waters, Milford, MA, USA). The chromatographic separation was achieved on a Hypersil Gold, 5-µm 150 x 4 mm column (Thermo Scientific). The mobile phase consisted of an isocratic mixture of 0.1% trifluoroacetic acid: acetonitrile (63:37 v/v) delivered at 1.0 mL/min flow rate. 5 microliters of each sample was injected onto the chromatographic column. The detection wavelength was set at 254 nm.

The HPLC method was subjected to validation in accordance with the International Conference on Harmonization (ICH) regulations Q2(R1) (ICH, 2005) in terms of specificity, linearity, precision (repeatability and intermediate precision) and accuracy. The linearity assessment was performed by using seven mebendazole concentration levels, in the range 0.25-100 µg/mL.

Evaluation of experimental thermodynamic solubility

Thermodynamic (equilibrium) solubility of mebendazole in different simulated intestinal media (FaSSIF and blank FaSSIF) was performed by using the saturation shake-flask method. For each experiment, excess of substance was carefully added to 1.5 ml of medium, in 2 ml Eppendorf polypropylene microtubes. The vials were capped, stirred for 30 seconds at 2500 rpm on an IKA Genius 3 vortex mixer (IKA Werke GmbH & Co. KG, Staufen, Germany) and maintained under mild agitation (250 rpm) for 24 hours at 25ºC on a IKA HS 260 orbital shaker (IKA Werke GmbH & Co. KG, Staufen, Germany). The resulting samples were centrifuged at 12000 rpm, for 10 minutes on a Hettich Mikro 220R centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). Aliquots of 0.2 mL supernatant were collected and diluted with methanol to a final volume of 1 mL. A volume of 5 µL from the resulting sample was injected into the HPLC system. All experiments were performed in triplicate.

In vitro release kinetics experiments

In vitro release kinetics of mebendazole from the experimental tablets under simulated intestinal conditions was studied in 500 mL Fasted State Simulated Intestinal Fluid (FaSSIF), using USP Apparatus 2 (75 rpm). The amount of mebendazole released from the experimental tablet products was determined by sample withdrawal at different times. Samples (2 ml) were withdrawn after 5,10, 15, 30, 45, 60, 90 and 120 min and an equivalent amount of the same media were immediately introduced. The samples were filtered and suitably diluted with methanol. The drug content in the resulting samples was determined by HPLC. The dissolution medium (FaSSIF), with a pH value of 6.5 and containing physiologically relevant surface active agents (3mM sodium taurocholate and 0.75mM phosphatidylcholine) was prepared using a slightly modified version of the fluid described by Galia et al. (Galia et al., 1998), using high-speed stirring instead of methylene chloride for the dispersion of phosphatidylcholine.

RESULTS:

In vitro drug release

Two phases release of mebendazole

For all CC concentrations, the release kinetics of mebendazole from the experimental formulations was characterized by two different phases: pre- and post - disintegration. In the first phase of the dissolution experiment tablets were swollen by the dissolution medium up to approximately twice their initial volume (Figure 1a), followed by the disintegration phase.

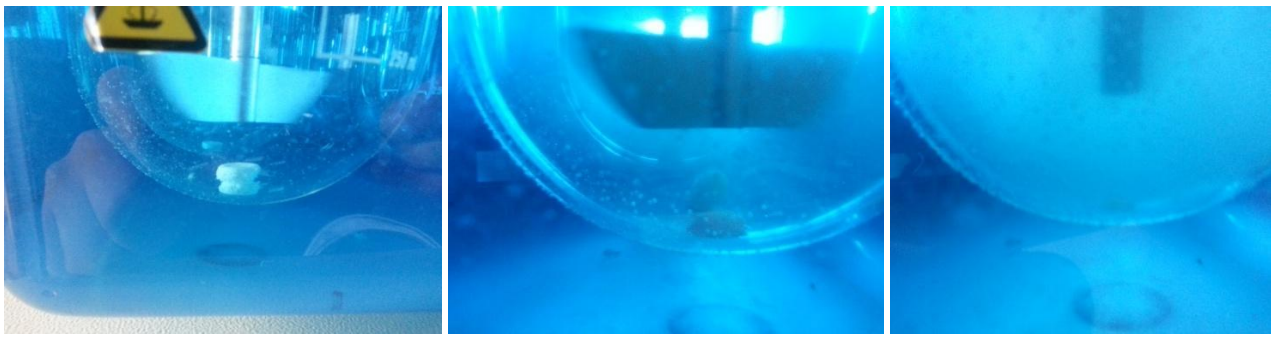

Fig. 1. Physical state of the mebendazole formulations during release test: a.swelled , b. eroding swelled and c. disintegrated

In fact the evolution of concomitant swelling and release is a much more complex phenomenon (Mircioiu et al., 2012; Preda et al., 2012). Alternative mechanism implying swelling and erosion could appear as intermediary phase before total disintegration, as could be interpreted by examining of photo 1b. Last but not least, formation of a supersaturated solution and precipitation were also suggested by evaluation of successive images.

Since the solubility of mebendazole decreases significantly with increases of pH (Carlert et al., 2012) (Figure 2) a possible precipitation of mebendazole in intestine and, consequently, an increased biorelevance of the *in vitro* release kinetics in simulated intestinal medium (Cristofoletti et al., 2016) compared to the USP compendial *in vitro* dissolution test, using as medium 0.1N HCl containing 1% sodium lauryl sulfate.

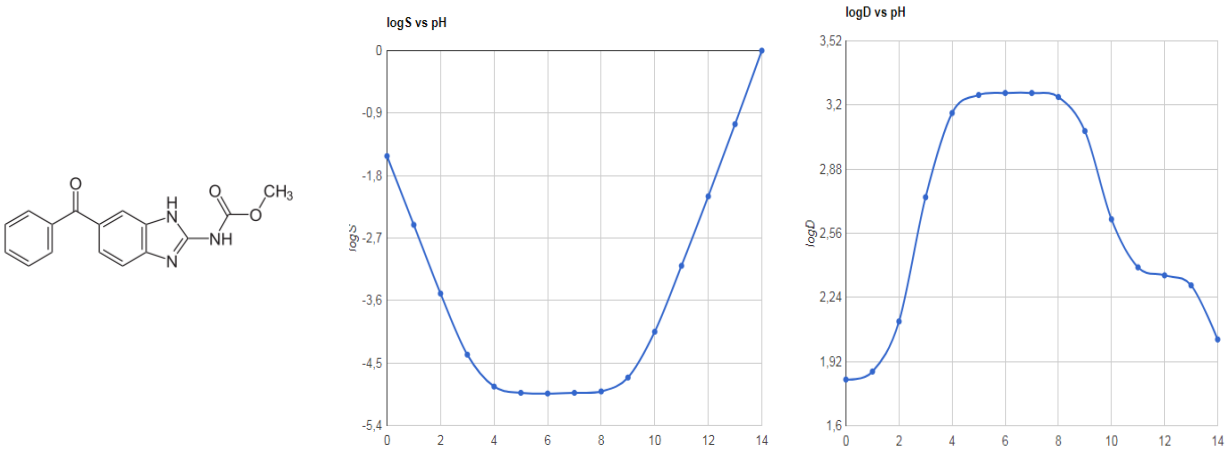

Fig. 2. Simulated solubility and partitioning vs. pH profiles of mebendazole, using ChemAxon Marvin Suite software app.

Release kinetics in FaSSIF

The swelling of tablets by FaSSIF was a slow process (much slower than the similar process in acidic medium). After this time interval the disintegration into many large diameter particles appeared process easily observable even by direct visual analysis which further persisted for a minimum of 1 other hour.

The set of all individual release curves is presented in Figure 3. It can be observed that these were homogeneously distributed in an apparently large space. A further examination revealed that only a small part of mebendazole was dissolved within two hours, and the difference between the extreme dissolution curves was less than 7 % of mebendazole content.

All curves present two clearly distinct portions: a very slow release for 30 minutes followed by an acceleration. In the neighborhood of the 30 minutes point there is a sudden change in rate and possible in mechanism. Keeping in mind what was globally observed by visual analysis, this change is most probable associated with disintegration of tablets.

Influence of CC concentration can better be understood from examination of Figure 4, where mean mebendazole release curves for the 1, 2, and 3 % CC tablets are presented. Mean curves appear also to be closely related, differing maybe only by random. However, some doubts can arise in this respect from the fact that there is a greater intra-group than inter-groups variability. Curves belonging to the same group are “outliers”. It seems that disintegration appears more quickly in some cases, leading to a significantly faster release.

A more in depth evaluation of the differences was based on the calculation of areas under mean release curves (AURCs).

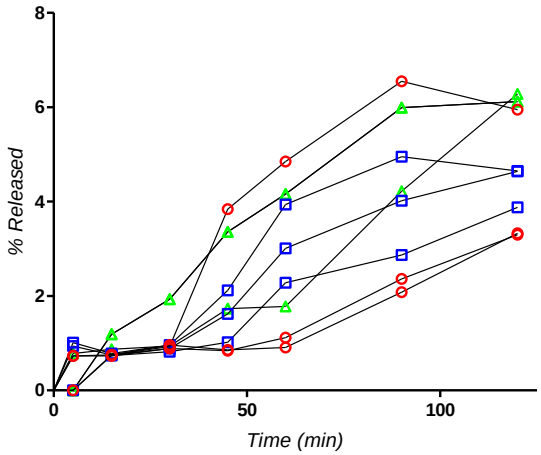

Fig. 3. The individual release curves of mebendazole as function of time

Release in FASSIF was dramatically lower than in simulated gastric fluid.

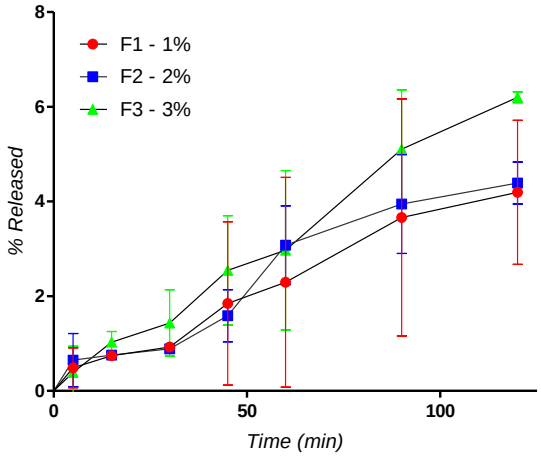

Fig. 4. Mean curves of release in FaSSIF for F1, F2 and F3

The amount released in two hours represents less than 6 % of the mebendazole embedded in tablets.

Tab. 2.
Calculation of the areas under release curves (AURC)

Time (min)	% released		
	F1 -1%	F2 -2%	F3- 3%
5	0,49	0,65	0,80
15	0,74	0,76	1,00
30	0,93	0,89	1,44
45	1,85	1,59	2,40
60	2,29	3,08	2,71
90	3,67	3,94	4,51
120	4,19	4,39	5,53
AURC (%*minutes)	**278,7**	**303,2**	**353,1**

Considering AURC it is to note the appearance of a linear dependence of the release on the concentration of croscarmellose.

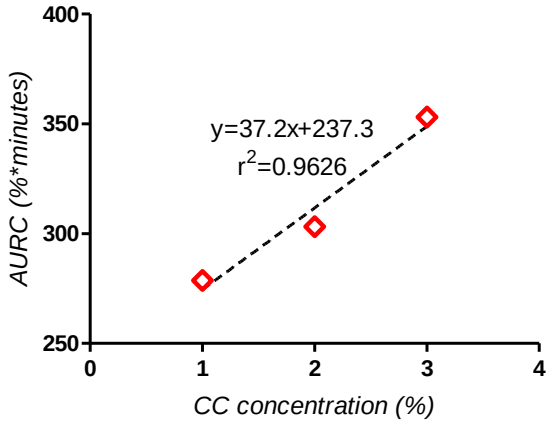

Fig. 5. Dependence of the AURC on the CC-concentration

So that the effect of CC exists and increases with CC concentration, but it is not a large effect. The small bending at the end of the curves suggests a possible saturation due to solubility limitations or to a precipitation after a supersaturation phase. The data are in accordance with the experimental values of the thermodynamic solubility of mebendazole in FaSSIF (6.2 µg/mL) and in FaSSIF blank (1.34 µg/mL).

DISCUSSION:

Dissolution and release mechanism.

Croscarmellose and Ludiflash action mechanism. Ludiflash (BASF) contains manitol 90 % as filler, Kollidon CL-SF 5% disintegrant and Kollicoat SR 30 D as water insoluble binder which accelerates disintegration. The cross-linking increase the matrix swelling and absorb many times its weight in water.

After the swelling phase, a three-dimensional network of hydrophilic cross-linked polymer is formed, which further becomes a hydrogel containing three different domains: “glass” (mostly hydrogel), “tough rubber” (significant proportion of water and hydrogel) and “soft rubber” (mostly water) (Omidian and Park, 2008). Croscarmellose sodium is a very commonly used pharmaceutical additive, approved by FDA. A recent study indicated that association of croscarmellose sodium (7.5%) with pregelatinized starch (6%) as superdisintegrants, increased the dissolution properties of loratadine (5%) from orally fast dispersable tablets (Ciurba et al., 2017).

Later, in the final phase, the further release occurrs across a much higher surface, namely the total surface of particles resulted from disintegration.

As mentioned above before the disintegration phase, concomitant to swelling, erosion of the tablet surface occurs. In the erosion phase the resistance to diffusion in the limit layer is greater than that after disintegration (Desai et al., 2015) resulting in a slight increase of the mebendazole release rate.

Release kinetic modeling

The model considers that the front of solvent is moving slowly inside the tablet determining the the apparition of a network of water channels. Formulations contain both soluble and insoluble polymers. Consequently, a much larger swelling of the insoluble polymer takes place after partial dissolution of polymers and drug. A network of pores, of water channels, or even large cavities full of liquid through which the drug diffuses very quickly appear.

Concomitently mebendazole dissolves in water and diffuses across a limit layer from solid network of the tablet. Since solubility of mebendazole is low, it is to suppose that, at the channel wall the concentration reaches saturation or even supersaturation.

During the swelling of tablet it is to take into consideration at least three different diffusion processes: a) diffusion of mebendazole from tablet surface to dissolution medium, b) dissolution of mebendazole from wall of channel to water (a short way diffusion) and c) diffusion in water along channel and diffusion from channel to the surrounding medium (the long way diffusion).

Initial and boundary conditions differ for the three diffusion domains. Limit diffusion layer is thinner at the surface of tablets due to the stirring of dissolution medium. Diffusion layer in channels is thicker since inside tablet there is no stirring. Water from channels is very different from continuously stirred water from the surface of tablet.

In practice frequently is followed a power law

$$M_t / M_\infty = kt^n$$

The law met initial in heat transfer theory was introduced later in pharmaceutical literature (Peppas, 1984; Peppas, 1985) for describing concomitant diffusion and erosion and is known as the “Peppas equation”. In case of solvent washing layer by layer the tablet, the square root law (Higuchi, 1961) is more appropiate:

$$M_t / M_\infty = k\sqrt{t}$$

But it is note that law of Higuchi proved to be applicable in much many cases, when the phenomenological model is different that the model of Higuchi. We can apply the f2 criterium for testing similarity of two curves containing n points, one considered as reference - R an another as tested - T

$$f2 = \log \frac{100}{\sqrt{1 + \frac{\sum_{i=1}^{n}(R_i - T_i)^2}{n}}}$$

This metric has as generic threshold between similar and non-similar the 10 % value and comparison of all release curves in FaSSIF proved their similarity. In these conditions, it was considered more appropriate for estimation of the release mechanism to evaluate the mean release data for all nine curves (three for every formulation).

Starting from the results obtained in case of release in simulated gastric medium, it was tried first the fitting of data by a linear regression between released amount and square root of time - Higuchi model.

There is an additional reasons for application of Higuchi square root law, the fact that in FaSSIF the solubility of mebendazole is much smaller and concentration in water swelling tablets is more probable the saturation concentration which fact is fitting the conditions considered in deriving Higuchi law.

On other hand, it is more difficult to define what does mean in this case “release of 60 to 80 % of the embedded active substance” another restriction in the application of law of Higuchi. In this case we could refer to “total available to release amount” based on solubility in release medium, though solubility also is difficult to define due to apparition of supersaturation. It is to observe that the fitting was succesful for entire domain of the second phase, i.e. included the 120 minutes point, as can be seen in figure 6a, release started after a time lag greater that in case

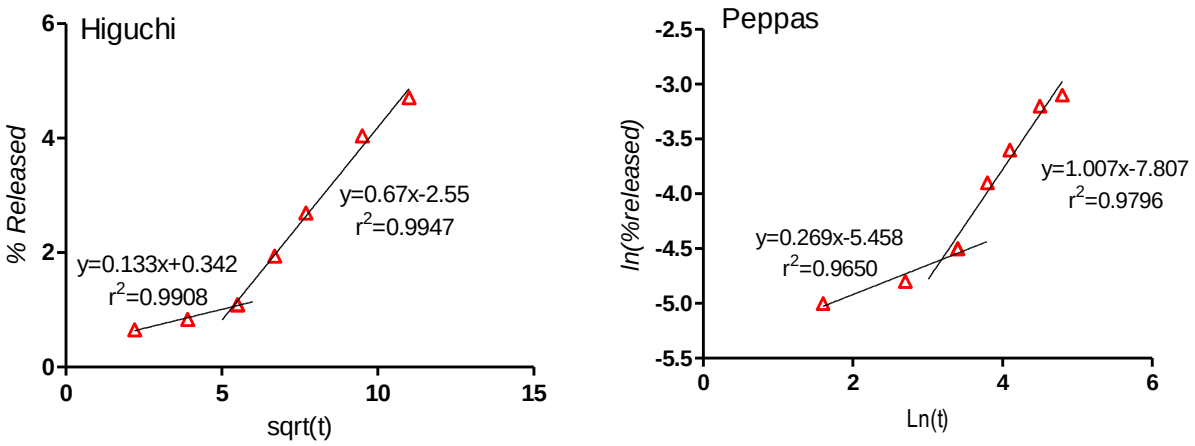

Fig. 6. Two-phase modeling of release of mebendazole using: a. Higuchi model, b. Peppas model of simulated gastric medium, diffusion controled evolution appears to be the principal component of release mechanism.

Relaxation of polymer in the first "swelling phase" is visible with naked eye and it is possible to play a part in the second phase also in connection with the structure of particles. Since concomitant relaxation and diffusion are usual better fitted by Peppas power law, it was evaluated the performance of fitting, again in two steps, of this more general model. As can be seen in figure 6b, the Peppas model proved to be applicable also. On other hand, from mathematical considerations (Sandulovici et al., 2009) connected with the stability of predictions, it is to prefer the model with the lower number of parameters, in our case the Higuchi model.

So that, errosion observed by visual analysis seems to have a lower influence on the total release kinetics. In order to obtain a confirmation of this balance between diffusion and relaxation phenomena the Weibull model was aditionnally applied, since it is one of the most general model in biological sciences, being applicable to processes runing in several steps with constant rate of intertransfer s. Time course of % released quantity R could be described by Weibull distribution in the form

$$\ln(-\ln(1-R(t)/100)) = \ln\alpha + \beta \ln t$$

where R is the released percent .

As can be seen in Figure 7 the fitting was again in two steps, which is extremely unusual. Weibull model, as a rule, is fitting well the entire set of dissolution data.

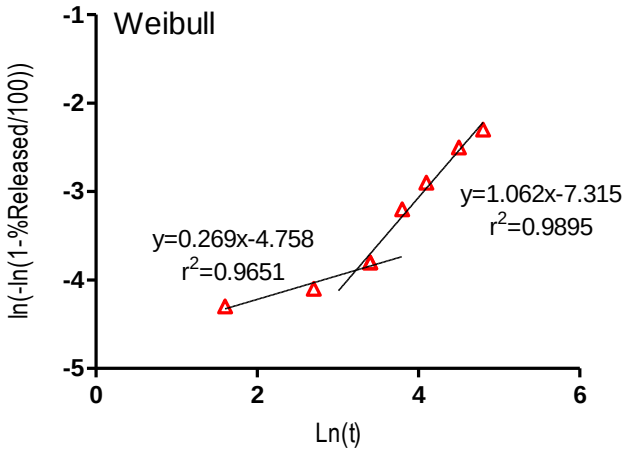

Fig. 7. Weibull biphasic modeling of the release of mebendazole (mean data)

Simulations concerning Weibull function (Kosmidis et al., 2003) and fitting of experimental data (Papadopoulouet al., 2006) concluded that $\beta \leq 0.75$ indicates Fickian diffusion while a combined mechanism (Fickian diffusion and swelling controlled transport) is associated with β values in the range $0.75 < \beta < 1$. Paradoxically, the beta coefficient is greater, in our case, in the second phase, when the swelling is supposed to be ended. Hence, the processes are most likely very complex, including eventual further swelling of the large diameter particles resulting from disintegration.

CONCLUSIONS:

Formulations with mebendazole as active substance, with Ludiflash as disintegrant and croscarmellose as superdisintegrant are feasible as tablets, with characteristics meeting compendial specifications.

Release of mebendazole in FaSSIF was increased by addition of CC. Increase of the area under release curves as function of croscarmellose concentration as metrics of the effect, was linear. However, the dissolution of mebendazole in the dissolution media was limited to approximately 5% of the available mebendaazole concentration, due to dissolution medium saturation, suggesting that solubility is indeed responsible for mebendazole low bioavailability.

Release of mebendazole from tablets followed a two phase time course: a slow release in the first 30 minutes during swelling and erosion of polymeric matrix and a more rapid release from particles resulted after disintegration.

In both phases, in spite of concomitant swelling, diffusion toward inside channels and diffusion at the interfaces with release medium in very different initial and boundary conditions, the release was described very well by square root laws, in both pre and post disintegration phases.

Application of Peppas law for intermediary case between diffusion and swelling controlled mechanism didn't improved the fitting in comparison with square root law fitting. Unusual in release experiments, application of more general Walodi Weibull distribution function lead to two theoretical curves describing different the data into the two phases. The obtained values for β suggested an even more complex mechanism that diffusion and erosion.

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