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Kinetic study of zinc sulphate release from lipophilic matrices prepared for the therapy of Wilson's disease

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The rate and extent of drug release from the most controlled release wax matrices are influenced by the drug loading/embedding excipient ratio of the systems. In the present study hydrophobic wax – zinc sulphate matrices with different drug loadings were prepared for the therapy of Wilson's disease. The drug release was tested by the paddle method of USP and the dissolution data were analysed. Both the dissolution rate and kinetic profile can be controlled by alteration in the quantity of embedding material. Matrices of 75% zinc sulphate loadings showed steady state diffusion-controlled matrix release with good correlation *in vitro*. Good absorption of zinc sulphate from the gastrointestinal tract was proven by significant elevation of serum zinc level in patients with Wilson's disease.

1. Introduction

Wilson's disease is a genetic disorder of copper transport resulting in the accumulation of copper in organs such as the liver and the brain, which leads to progressive hepatic and neurological damage (Sarkar 2000). D-Penicillamin, a chelating agent, is generally used as standard therapy of Wilson's disease. Zinc is also recommended for long-term management of the disease (Brewer 2000). Zinc has shown clinical efficacy at doses of 50 mg three times daily in the stimulation of metallothionein synthesis and reduction of copper absorption. The mean plasma elimination half-lives of most highly water soluble drugs, like zinc sulphate, are relatively short (2–4.5 h), which necessitates several applications a day (Khan 1995). Long-acting sustained and controlled release preparations make a once-a-day dose treatment possible. Waxy-type excipients were successfully applied as release-controlling agents (Huang et al. 1994; Dredán et al. 1998). The hot-melt technology has several advantages, since it is a solvent-free process, and is therefore an environment-friendly, time- and cost-saving process. The particles are produced in one step and are filled into capsules directly and applied in oral therapy (Pallagi et al. 2004). Dietary zinc is absorbed from the duodenal and jejunal regions of the gastrointestinal tract. Active transport of zinc into the portal blood is mediated by metallothionein. Zinc competes with other metals for absorption, and absorption is believed to be greatly retarded by ingestion of fiber and phytates (Brewer 2000).
The aim of the present study was to formulate zinc sulphate sustained release matrices for the treatment of Wilson's disease and to evaluate the *in vitro* release profiles of the formulated systems and the *in vivo* absorption of the selected systems, as well.

2. Investigations, results and discussion

Fig. 1 shows the effect of various drug loadings on drug release profiles. With higher ratios of the embedding material, the rates of drug release were decreased. Changing the proportion of drug loading/embedding material at the embedding procedure results in dissolution curves of manifold shapes, which can be characterized with different mathematical models. The nonlinear parameter estimation of the different release models was made with the Solver function of the computer package Microsoft Excel 5.0. The correlation coefficients of different kinetic equations are summarized in Table 1.

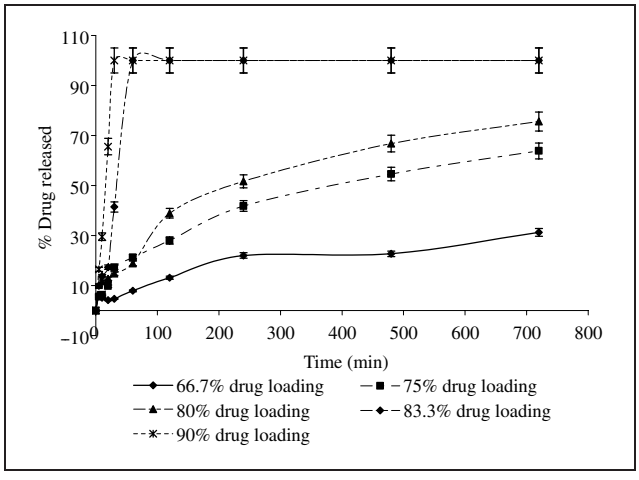

Fig. 1: Influence of the drug loading/embedding excipient ratio on the release of zinc sulphate from wax matrices

Table 1: Goodness of fit of the observed release data to the simulated profiles in case of different zinc sulphate matrices

Drug loading (% w/w)	Correlation coefficients of different models		
	Zero-order (Eq. 1)	First-order (Eq. 2)	Semi-empirical (Eq. 3)
66.7	0.9422	0.9753	0.9777
75.0	0.9446	0.9907	0.9972
80.0	0.9407	0.9920	0.9933
83.3	0.6577	0.9717	0.8678
90.0	0.5223	0.9824	0.8552

The zinc sulphate release from matrices of 75% and 80% drug loadings can be described with good correlation (correlation coefficient$_{75\%;\,80\%} = 0.9972$; 0.9933) by the semi-empirical model commonly applied for matrices Eq. (3). Preceding the release of the zinc sulphate through the pores of the matrices, the zinc sulphate from the matrix surface can be dissolved into the dissolution medium. The zinc sulphate dissolution from the matrix surface is more dominant in the case of samples of 83.3% and 90% drug loadings. The low correlation coefficients ($r^2_{83.3\%} = 0.8678$; $r^2_{90\%} = 0.8552$) between the measured release data and those calculated by Eq. (3) can be explained by the diffusion mechanism of the salt from the wax surface. The release profile of these samples fits mostly to the first order kinetic model. To interpret the difference in drug release characteristics, the morphology and the related composition of various matrices were examined previously. The results demonstrated that the surfaces of matrices of more than 83.3% drug loadings containing zinc sulphate salts without being embedded into the wax matrix base, while in the case of 80% w/w or less zinc sulphate content, the drug is embedded in greater extent into the wax matrix base. Energy-dispersive X-ray spectroscopy confirmed the co-location of zinc sulphate at the matrix surface, the absence of C of the EDX spectra referred to the lack of organic matrix base around the zinc sulphate crystals (Nagy et al. 2004).

In the case of 66.7% drug loading, the higher amount of matrix base decreased the total porosity of the matrix, thus less than 30% of the embedded salt could be released after 12 h (Fig. 1).

The parameters of the Weibull distribution were summarized in Table 2 and demonstrate that Eq. (4) is suitable for the description of the dissolution of zinc sulphate almost independently from the quantity of embedding material.

Along with the increase of the drug loadings of the matrices, the β values significantly increased and the τ_0 values decreased. There were no identified interpretable τ_0 values.

At 75% zinc sulphate loading the dissolution process can be characterized preferably by a two-phase dissolution kinetic. In the first phase (beginning 30 minute-period) less than 20% of drug is released following first order kinetic (correlation coefficient: 0.9742). In the second phase a closely constant rate of drug dissolution can be observed (correlation coefficient: 0.9754) demonstrating zero-order or steady state release. As a result of the steady state diffusion-controlled matrix release, the matrices containing 75% drug loadings were selected for the *in vivo* examinations.

Table 2: Characteristic parameters of Weibull distribution

Drug loading coefficient	β Shape-parameter	τ_d value (min)	Correlation
66.7	0.7491	235.58	0.9727
75.0	0.7556	201.76	0.9942
80.0	0.7990	190.71	0.9913
83.3	2.4609	37.62	0.9962
90.0	1.7608	17.72	0.9957

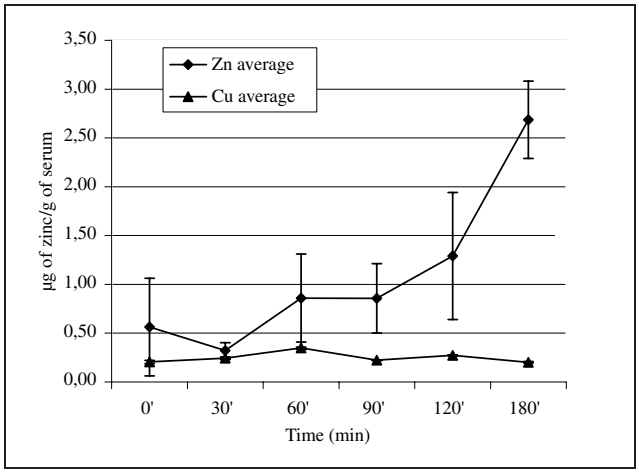

Fig. 2: Serum zinc level in Wilson disease patients before and after the administration of 300 mg zinc sulphate wax matrix capsule

Good absorption of zinc sulphate from gastrointestinal tract was proven by significant elevation of serum zinc level within 180 min in each patient with Wilson disease. The characteristic absorption curve is presented in Fig. 2. The drug was well tolerated throughout the one month treatment with 300 mg zinc sulphate daily. No side effect was registered and clinical symptoms of Wilson disease remained as stable as they were during the previous D-penicillamin treatment. The abdominal discomfort complaints of one patient treated previously with zinc sulphate in powder form disappeared when the therapy was changed to wax matrices.

3. Experimental

Zinc sulphate of Ph.Eur. 4 ($M_w = 287.5$) was used. The chosen matrix base material was white beeswax (melting range of 62–65 °C, Ph.Eur. 4). To prevent the sedimentation of the zinc sulphate, 5% w/w glycerol monostearate 40–55 (Ph.Eur. 4) was added to increase the viscosity.

3.1. Sample preparation

The thermosoftening matrix material in all cases was heated in a double jacketed vessel mixer (Erweka SG 3/W, Erweka, Germany) to 70 °C (± 1 °C). The zinc sulphate crystals were mixed into the molten mass to obtain the following drug loadings: 66.7%, 75%, 80%, 83.3%, 90% w/w. The molten mass was filled into hard gelatine capsules before congealing to form a skeletal sustained release dosage form.

The zinc sulphate content of each capsule was 0.30 ± 0.01 g.

3.2. In vitro drug release studies

For the determination of dissolution profiles of the samples, the rotating paddle method of USP 23 at 100 rpm was used (Erweka DT 6RE, Germany). The study was conducted in 200 ml of pH = 6.8 phosphate buffer solution at 37 ± 0.2 °C. Sampling times were the following: 5, 10, 20, 30, 60, 120, 240, 480, 720 min.

The dissolved zinc sulphate concentrations were measured by complexometric titration according to the Ph.Eur. Monograph.

3.3. In vivo drug release and absorption study

Absorption of zinc sulphate from the gastrointestinal tract was investigated by determination of zinc levels in five patients with Wilson's disease (mean age 30.8 ± 5 years, male/female = 4/1, disease duration: 2–15 years). The

diagnosis was based on an international scoring system. Four patients were on D-penicillamine treatment and one was treated with zinc sulphate in the form of a powder. D-Penicillamine treatment was suspended at least one week prior to the study.

Zinc sulphate was administered after an overnight fast. The blood was withdrawn into vacutainer tubes using indwelling catheter. Serum zinc level was measured before and 30, 60, 90, 120 and 180 min after oral administration of 300 mg zinc sulphate in hydrophobic wax matrices sulphate capsule. Serum samples were stored at −20 °C.

The concentration of inorganic zinc and copper was determined by means of an inductively coupled plasma optical emission spectrometer (ICP-OES, Atom Scan 25, Thermo Jarrell Ash, Merck, Darmstadt, Germany). Sample preparation for the measurement of the element in caraway and fennel oil: the samples (0.5 g oil or 10 ml of evaporated solution) were digested with HNO_3 (5 ml) and H_2O_2 (2 ml). After digestion, the samples (three parallel) were diluted to 10 ml, from which the elements were determined.

3.4. Analysis of the release profiles

The following mathematical models were evaluated considering the dissolution profiles of the non-disintegrating matrices.

3.4.1. Zero-order model

The drug release from the dosage form follows a steady-state release running at a constant rate:

$$M_t/M_\infty = kt \quad (1)$$

where M_t amount of drug released at time t, M_∞ the total amount of the released drug at infinite time, k is the rate constant of drug release.

3.4.2. First-order model

The drug activity within the reservoir is assumed to decline exponentially and the release rate is proportional to the residual activity:

$$M_t/M_\infty = 1 - \exp(-kt) \quad (2)$$

3.4.3. Semi-empirical mathematical model for the assessment of drug release from controlled release devices

Dissolution data were analysed using the equation 3 (Ritger and Peppas 1987; Macheras and Dokoumetzidis 2000; Samani et al. 2003; Rao et al. 2003) to describe the mechanism of drug release from matrices.

$$M_t/M_\infty = Kt^n \quad (3)$$

where K is a kinetic constant characteristic of the drug/polymer system and n is the release exponent indicating the type of drug release mechanism. If n approaches to 0.5 the release mechanism can be Fickean. This specific case is also referred as the Higuchi model. If n approaches to 1 the release mechanism can be zero order and on the other hand if $0.5 < n < 1$ non-Fickean transport could be obtained.

3.4.4. Weibull distribution

The Weibull distribution can be assigned as a generalized form of the exponential function, hence it can be widely used for the analysis and characterisation of drug dissolution process from different dosage forms.

$$M_t/M_\infty = 1 - \{\exp - [(t - t_0)/\tau]^\beta\} \quad (4)$$

where t_0 is the lag time of the drug dissolution, τ the mean dissolution time, the 63.2% of M_∞ has been released, β shape parameter of the dissolution curve.

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