Preparation and evaluation of zinc sulphate matrices for the individual clinical therapy of Wilson’s disease

Ph.D. thesis

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SUMMARY

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. The prevention of severe permanent damage depends upon early recognition and diagnosis by the physician, followed by appropriate anticopper treatment. Zinc is now one of the recommended therapies for the long-term management of the disease. 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. Long-acting sustained and controlled release preparations make a once-a-day dose treatment possible, thus improving the patients compliance. The rate and extent of drug release from most controlled release wax matrices are influenced by the drug loading/embedding excipient ratio of the systems. The purpose of my thesis was to prepare and evaluate hydrophobic wax zinc sulphate matrices of different drug loadings for the therapy of Wilson’s disease. Wax zinc sulphate matrices were prepared by hot melt technology. The drug release parameters, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and diffuse reflectance spectroscopy of the samples were analysed. The drug release from matrices was tested by the rotating paddle method of USP and the dissolution data were analysed assuming different kinetic models. Both the dissolution rate and kinetic profile can be controlled by alteration the quantity of embedding material. Matrices of 75% zinc sulphate loadings showed steady state diffusion-controlled matrix release with good correlation in vitro. As a result of the steady state diffusion-controlled matrix release, the matrices containing 75% drug loadings were selected for the in vivo examinations. Good absorption of zinc sulphate from the gastrointestinal tract was proven by significant elevation of serum zinc level in patients with Wilson’s disease. No side effect was registered and clinical symptoms of Wilson’s disease remained as stable as they were during the previous D-penicillamin treatment. The abdominal discomfort complaints of patients treated previously zinc sulphate in powder form disappeared when the therapy was changed to wax matrices.
I. 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. The prevention of severe permanent damage depends upon early recognition and diagnosis by the physician, followed by appropriate anticopper treatment. Selection of the drug or drugs to use for a particular patient depends on the stage of the disease (initial acutely ill patient versus chronic maintenance patient) and the type of presentation (neurologic / psychiatric versus hepatic). Zinc is now the recommended therapy for long-term management of the disease. Zinc has shown clinical efficacy at doses of 50 mg three times daily in the stimulation of metallothionein synthesis and reduction of copper absorption. Absorption of dietary zinc occurs over the duodenal and jejunal regions of the gastrointestinal tract. Active transport of zinc into portal blood is mediated by metallothionein. Zinc competes with other metals for absorption, and absorption is believed greatly retarded by ingestion of fiber and phytates.

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. Long-acting sustained and controlled release preparations make a once-a-day dose treatment possible, thus improving the patients compliance. Waxy-type excipients were successfully applied as release-controlling agents. The advantages of the hot-melt technology are that it is a solvent-free process, and is therefore environment-friendly, and it is a time- and cost-saving process, because the particles are produced in one step and be filled into capsules directly and applied in oral therapy.

II. OBJECTIVES
In the literature review of my thesis I intend to summarize those references which are in close connection with different aspects of Wilson’s disease. The primary aim of the first part of the literature review was to give an overview of the metabolic aspects of Wilson’s disease, its genetic background, pathogenesis and different therapeutic possibilities. The secondary purpose of the literature review was to illustrate the formulation aspects of slow and extended release dosage forms focusing on the different mathematical models describing the drug release from matrices.
The objectives of the *experimental part* of my thesis were:

- to formulate zinc sulphate wax matrices of different drug loadings,
- to *in vitro* characterize the zinc sulphate release from the prepared matrices,
- to evaluate the applicability and reliability of some mathematical kinetic models to describe the drug release from non-disintegrating, lipophilic matrix systems,
- to analyze the morphology of the matrices as a function of drug-wall ratios,
- to compare the drug release and the corresponding morphology of the samples,
- to *in process* evaluate the matrices of required drug loadings for the prolonged drug release by the non-invasive diffuse reflectance spectroscopy,
- to *in vivo* evaluate the zinc absorption from the selected matrices.

### III. EXPERIMENTAL PART

#### Materials

Zinc sulphate of Ph.Eur. 4 (M\(_w\)=287.5) was selected as a highly water soluble model drug. 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.

#### 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.

#### Scanning electronmicroscopy

The surface characteristics of matrices were examined by means of a scanning electronmicroscope (Philips XL 30). The specimens were mounted to aluminium stubs
with double adhesive tape. To reduce the charging, the specimens were vacuum coated with gold. Examination was carried out at 12 kV and 25 kV accelerating voltage and 100-1000 times magnifications were used. Magnifications of scanning electronmicroscopic images are signed by the micrometer-line on the pictures; the accuracy of these magnifications was ±2%.

**Energy dispersive X-ray spectroscopy (EDS)**
Samples for the energy-dispersive spectroscopic investigations were in their original (not cleaned) form. Accuracy of EDS-investigations – in the lack of pure element standards – is assumed to be ±2% in the concentration range of 10-20%, while it is assumed to be ±0,3% in the range below 1%. Sensitivity of EDS-measurements is about 0,2-0,3%. Chemical analysis results are therefore qualitative ones.

**Diffuse reflectance spectroscopy**
A Hitachi U-2501 UV/VIS/NIR spectrophotometer (Hitachi, Japan) equipped with integrating sphere (d=60 mm) and PbS detector was applied for the comparison of the diffuse reflectance spectra of different samples. The reflectance of samples of 200-800 µm particle size was detected in the 200-2500 nm wavelength range using 5 mm layered cell.

**In vitro drug release studies**
For the determination of dissolution profiles of the samples, the rotating paddle method of USP23 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.

**In vitro determination of the released zinc concentrations**
The dissolved zinc sulphate concentrations were measured by complexometric titration according to the following prescription of the Ph.Eur. Monograph. Complexometric titration of zinc was carried out. 1 ml of 0.1 M sodium edetate is equivalent to 28.75 mg of ZnSO₄·7H₂O. 1 ml of 0.1 M sodium edetate is equivalent to 6.54 mg of Zn.
Analysis of the release profiles

The following mathematical models were evaluated considering the dissolution profiles of the non-disintegrating matrices. The nonlinear parameter estimation of the release model applied for matrices was made with the Solver function of the computer package Microsoft Excel 5.0.

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

\[
\frac{M_t}{M_\infty} = kt \tag{1}
\]

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

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

\[
\frac{M_t}{M_\infty} = 1 - \exp(-kt) \tag{2}
\]

Semi-empirical mathematical model for the assessment of drug release from controlled release devices
Dissolution data were analysed using the Eq (3) to describe the mechanism of drug release from matrices.

\[
\frac{M_t}{M_\infty} = K t^n \tag{3}
\]

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 \( \leq \) \( n \) \( \leq \) 1, non-Fickean transport could be obtained.

The model-independent mean dissolution time (MDT) was calculated as follows:
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.

\[ \text{MDT} = n/[n^2 + 1(K^{1/n})] \]  \hspace{1cm} (4)

\[ \frac{M_t}{M_?} = 1 - \{\exp - [(t-t_0)/t]^\beta\} \]  \hspace{1cm} (5)

where \( t_0 \) is the lag time of the drug dissolution, 
\( t \) the mean dissolution time, when 63.2% of \( M_? \) has been released,
\( \beta \) shape parameter of the dissolution curve.

In vivo test

Absorption of zinc sulphate from gastrointestinal tract was investigated by determination of zinc level in five patients with Wilson disease (mean age 30.8±5 year, male/female=4/1, disease duration: 2-15 year). The diagnosis was based on international scoring system. Four patients were on D-penicillamine treatment and one was treated with zinc sulphate in form of powder. D-penicillamine treatment was suspended at least one week prior to the study. The zinc 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 minutes after oral administration of 300 mg zinc sulphate in hydrophobic wax matrix capsules. Serum samples were stored at minus 20°C.

Determination of element concentration in the serum

The concentration of inorganic zinc and copper was determined with the use 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.
IV. CONCLUSIONS

Hydrophobic wax zinc sulphate matrices of different drug loadings were prepared for the individual hospital therapy of Wilson’s disease. In vitro dissolution data were analysed assuming different kinetic models. Both the dissolution rate and kinetic profile can be controlled by changing the quantity of embedding material.

The release mechanisms from matrices of 75% and 80% w/w zinc sulphate loadings were described with good correlation by the semi-empirical Fickian diffusion based release model.

Besides the zinc sulphate diffusion through the pores of the wax matrices, the parallel diffusion of the zinc sulphate crystals from the matrix surface is dominant in the case of samples of 83.3% and 90% w/w drug loadings.

The combination of SEM and EDS analysis visualize the morphology of the matrices and the related composition thus explaining the differences in the release characteristics.

The Weibull distribution was 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 $t_0$ values significantly increased and the $t_0$ values decreased.

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. In the second phase a closely constant rate of drug dissolution can be observed 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.

Polynomial equation was applied with good correlation to describe the relationship between the wax content of zinc sulphate matrices, their reflectance value measured at the characteristic wavelength of the wax-matrix base and the mean dissolution time of zinc sulphate.

Based on the obtained non-linear model, diffuse reflectance spectroscopy enables rapid in-process control during the formulation of modified-release wax matrices, without destructive sample analysis.
Good absorption of zinc sulphate from gastrointestinal tract was proven. 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 a patient treated previously zinc sulphate in powder form disappeared when the therapy was changed to wax matrices.

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VI. PUBLICATIONS AND LECTURES

Publications

   *Die Pharmazie, 59(4), 327-328, (2004).*  
   IF=0.696

   *Journal of Thermal Analysis and Calorimetry. 68(2), 531-537 (2002).*  
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   *Acta Pharmaceutica Hungarica, 73, 237-241 (2003).*

   *Die Pharmazie, accepted for publication.*  
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Lectures

2. **Nagy Judit**: Wilson-kór individuális terápiájának biztosítása cink-szulfát mátrix tabletával.  


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