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

Ph.D. thesis

Judit Nagy

Tutor: Dr. Romána Zelkó, Ph.D.

University Pharmacy Department of Pharmacy Administration
Semmelweis University

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Szigorlati Bizottság:

Elnök: **Dr. Mátyus Péter, D.Sc.**

Tagok: **Dr. Kata Mihály, D.Sc.**

Dr. Tekes Kornélia, Ph.D.

Hivatalos bírálók:

CONTENTS **Page**

ÖSSZEFOGLALÁS

A Wilson-kór a rézanyagcsere genetikai rendellenessége, számos szövetben rézfelhalmozódáshoz vezet, így a májban és az agyban, valamint progresszív máj és idegrendszeri elváltozásokat okoz. A maradandó károsodások megelozése a betegség korai felismerésétol, diagnosztizálásától, majd a hatékony terápia alkalmazásától függ. Napjainkban a cink ajánlott a Wilson-kóros betegek hosszú távú kezelésére, szokásos dózisa naponta háromszor 50 mg. Hatásmechanizmusa a metallothionein szintézis serkentése, a rézabszorpció csökkentése. Az átlagos plazma eliminációs felezési ideje a legtöbb vízoldékony gyógyszernek, így a cink-szulfátnak is viszonylag rövid (2-4,5 óra), ami naponta többszöri alkalmazást tesz szükségessé. Nyújtott és szabályozott hatóanyag-felszabadulást biztosító készítmények formulálásával napi egyszeri adagolás válik lehetové, ami a betegek compliance-ét növeli. Szabályozott hatóanyag-leadású viasz mátrixokból történo hatóanyag-felszabadulás sebességét és mértékét a beágyazott hatóanyag és a mátrixképzo viasz aránya határozza meg. Értekezésem célja különbözo cink-szulfát/méhviasz tömegarányú mátrixok eloállítása volt Wilson-kóros betegek individuális kórházi kezeléséhez. A mátrixokat olvasztásos technológiával állítottam elo. A minták *in vitro* hatóanyag-felszabadulását forgólapátos módszerrel, morfológiáját és homogenitását pásztázó elektronmikroszkópiával (SEM), energia-diszperzív röntgenanalízissel, valamint diffúz reflektancia spektroszkópiával vizsgáltam. A hatóanyag-felszabadulás kinetikáját különbözo matematikai modellekkel jellemeztem. Az eredmények alapján megállapítottam, hogy a 75% hatóanyagot tartalmazó mátrixok diffúzió-kontrollált két-fázisú hatóanyag-leadást mutatnak. A kioldódás elso fázisa (elso 30 perc, kevesebb, mint 20% hatóanyag szabadul fel), jól jellemezheto elsorendu kinetikai modellel, míg a második fázis nulladrendu kinetika szerint játszódik le. Az *in vitro* vizsgálatok alapján a fenti összetételu kapszulákat választottam ki *in vivo* vizsgálatra. Az *in vivo* eredmények alapján megállapítható, hogy a Wilson-kóros betegek gyomor-béltraktusából a cink-szulfát

abszorpciója az eloállított kapszula napi egyszeri adagolása esetén megfelelo. Mellékhatást nem tapasztaltak, és a Wilson-kóros betegek klinikai tünetei ugyanolyan jók maradtak, mint a korábbi D-penicillamin kezelés során. A porkapszulás kezeléshez viszonyítva a cink-szulfát mátrix-kapszulás kezelésben részesülo betegek hasi diszkomfort tünetei megszuntek.

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 in 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.

A 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 accepted 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.

B OBJECTIVES

In the literature review of my thesis I summarized those references which are in close connection with different aspects of Wilson's disease. I gave an overview of the metabolic aspects of Wilson's disease, its genetic background, pathogenesis, different therapeutic possibilities and I illustrated 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, as individual prescriptions, exclusively from those excipients which are official in the Hungarian Pharmacopoeia,
- 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.

C LITERATURE REVIEW

C.1 METABOLIC ASPECTS OF WILSON'S DISEASE

Copper is an essential trace element. The body contains 50 to 120 mg of copper, and high concentrations are found in the liver, brain, heart, spleen, kidney and blood. Unlike iron, absorption does not appear to be tightly regulated. The U. S. RDA is 1.5 to 3 mg of copper intake per day, although World Health Organization recommendations are somewhat lower. Dietary sources of copper include shellfish, liver, nuts, legumes, bran and organ meats, whereas milk is a very poor source.

Copper is absorbed in the proximal small intestine, and 90% of circulating copper is bound to ceruloplasmin. Copper absorption can be reduced by zinc, because zinc induces the production of the cysteine-rich protein metallothionein, which retains copper in intestinal mucosal cells. Copper so bound is poorly absorbed and lost when the cells slough. Albumin and other proteins transport copper from the mucosal cell to the liver, which takes up most absorbed copper. Copper may be stored in the liver, bound in part to metallothionein, may be secreted into plasma bound to *ceruloplasmin*, which transports 80% of plasma copper, or may be excreted in bile, perhaps bound to ceruloplasmin fragments. Copper is primarily excreted in the feces, and small amounts are also excreted in urine. Copper plays a role in iron metabolism, melanin synthesis and central nervous system function, the synthesis and cross-linking of elastin and collagen, and the scavenging of superoxide radicals. Copper is essential for several enzyme systems, including

superoxide dismutase, which detoxifies free radicals.

Large doses of copper consumed accidentally or intentionally can cause severe hemolysis and acute liver and kidney failure [1, 2].

Since features of zinc deficiency include growth retardation and defects of rapidly-dividing tissues such as the skin, the immune system, and the intestinal mucosa, zinc salts are used as supplements to correct zinc deficiency.

Zinc sulphate and zinc gluconate are given by mouth in doses of up to 220 mg three times daily in the treatment of conditions associated with zinc deficiency such as acrodermatitis enteropathica [3].

Wilson's disease (WD), or hepatolenticular degeneration is a genetic disorder in which there is excessive accumulation of copper in the liver and brain because of an inherited defect in the biliary excretion of copper. WD is transmitted from generation to generation by autosomal recessive inheritance [4].

C.2 GENETIC BACKGROUND OF WILSON'S DISEASE

The genetic defect accounting for defective copper excretion in Wilson's disease has been localized to chromosome 13 (13q14,3-q21,1), and codes for a copper transporting P-type ATPase, ATP7B, as it has been well-known since 1993. About two hundred mutations occurring throughout the whole gene have been documented so far. The most common in Central-Europe is the His1069Gln point mutation (60-74%) [5, 6].

Only homozygotes for this disorder who inherit disease-specific mutations of both alleles of the Wilson's disease gene may go on to manifest clinical evidence of the condition. The disease occurs in every ethnic and geographic population, with a worldwide prevalence of 1 in 30.000, and a heterozygous carrier frequency of 1 in 90.

Particular mutations are found more frequently in specific populations or ethnic groups with varying phenotypic expression in certain of these mutations. However, it is known that heterozygotes with a mutation of a single allele do not develop

disease although they may show varying degrees of abnormality in serum copper markers.

The gene responsible for Wilson-disease (ATP7B) is predicted to encode a putative copper-transporting P-type ATPase. An important feature of this ATPase is the presence of a large N-terminal domain that contains six repeats of a copper-binding motif which is thought to be responsible for binding this metal prior to its transport across the membrane. Sarkar B. et. al. cloned, expressed and purified the N-terminal domain (approximately 70 kD) of Wilson-disease ATPase. Metal-binding properties of the domain showed the protein to bind several metals besides copper, however, copper has a higher affinity for the domain. The copper is bound to the domain in Cu(I) form with a copper: protein ratio of 6.5:1. X-ray absorption studies strongly suggest that Cu(I) atoms are ligated to cysteine residues. Circular dichroism spectral analyses suggest both secondary and tertiary structural changes upon copper binding to the domain. These studies as well as detailed structural information of the copper-binding domain are crucial in determining the specific role played by the copper-transporting ATPase in the homeostatic control of copper in the body and how the transport of copper in the body and how the transport of copper is interrupted by mutations in the ATPase gene [7-14].

The protein ATP7B is important in the vesicular pathway of hepatic copper transport into bile. The WD gene mutation leads to the absence or diminished function of ATP7B, resulting in a decrease in biliary copper excretion and ultimately in the hepatic accumulation of copper. Along with this failure of biliary excretion, there is also reduced incorporation of copper into ceruloplasmin, which is a serum glycoprotein that contains six copper atoms per molecule and is synthesized predominantly in the liver. The process of copper incorporation into apoceruloplasmin is also dependent on ATP7B, and this process is absent or diminished in most patients with WD, leading to a reduced circulating level of serum ceruloplasmin in most patients [15-19].

C.3 PATHOGENESIS

Signs and symptoms of organ disfunction do not appear before 6 years of age, but two thirds of patients have hepatic and/or brain dysfunction between 8 and 20 years. Delay of symptoms until the age of 60 has been described. When copper accumulates beyond the normal safe storage capacity of the liver, hepatocellular injury results. Furthermore, when the storage capacity of the liver for copper is exceeded or when additional cellular copper is released because of hepatocellular damage, levels of non-ceruloplasmin-bound copper in the circulation are elevated and copper accumulates in multiple extrahepatic sites, in particular in the brain. As copper "spills" over to other organs from the liver, pathologic manifestations become evident in the brain, kidneys, eyes, and joints. Organ dysfunction appears to result from copper-induced hepatic inflammation and destruction or from the abnormal release of hepatic copper into the circulation, with toxic effects in many extrahepatic organs.

C.3.1 Copper-induced liver disease

Copper-induced liver disease may present like acute viral hepatitis, chronic active hepatitis or postnecrotic cirrhosis. In patients with Wilson's disease, copper accumulates in the liver, reaching a mean level of about 1000 µg/g dry weight - 40 times the normal. Fatty infiltration of the hepatic parenchyma and nuclear glycogen deposits are the earliest findings by light microscopy (Figure 1).

Figure 1 – Macro- and microvesicular fatty changes, glycogen deposits in nuclei and cellular infiltrates in a hematoxylin- and eosin-stained section of liver from an asymptomatic boy with Wilson's disease [1]

With electronmicroscopy, characteristic mitochondrial abnormalities appear to be specific for Wilson's disease. Later, necrosis, inflammation, fibrosis, bile duct proliferation and cirrhosis ensue. Abnormalities in liver chemistries, particularly elevations in aminotransferases, may be seen at any stage. The capacity of hepatocytes to store copper is eventually exceeded, and copper is released into blood and taken up into extrahepatic tissues [20-22].

C.3.2 Effect of copper toxicity on the nervous system

With magnetic resonance imagining, the effects of copper toxicity in the brain are seen most frequently in the lenticular nuclei and less commonly in the pons, medulla, thalamus, cerebellum and cerebral cortex [1].

When copper release from the liver is abrupt and massive, a transient Coomb's negative hemolytic anemia is produced. Prolonged increased release of copper not bound to ceruloplasmin causes basal ganglion and, in some cases, cerebral cortex destruction.

There are patients with WD whose first presenting symptoms are either neurologic or psychiatric, and they are frequently patients in the third decade of life or even older. Most of these patients with central nervous system disease have occult significant liver disease at the time of presentation. The neurologic disease manifests predominantly as motor abnormalities with parkinsonian features of dystonia, hypertonia, and rigidity with tremors and dysarthria. These may cause disabling symptoms that include muscle spasms, dysarthria, dystonia and dysphagia [23, 24].

C.3.3 Effect of copper toxicity on the kidney

Kidney damage may present as nephrolithiasis when renal tubular dysfunction causes hypercalcuria, and some patients develop the Falconi syndrome with aminoaciduria, glucosuria and rickets.

C.3.4 Effect of copper toxicity on the eyes

Ophthalmologic findings include copper deposits at the periphery of the cornea producing the almost diagnostic deposits called Kayser-Fleischer ring and sunflower cataracts. The Kayser-Fleischer ring is most marked at the upper and lower poles of the limbus, the junction between the cornea and sclera, and is due to

the granular deposition of elemental copper on the inner surface of the cornea in Descemet's membranes. The rings have a golden brown or greenish appearance on slit-lamp examination. By the time the neurologic changes occur, usually in the third decade of life, the Kayser-Fleischer rings are almost invariably present although there are exceptions to this rule [25].

C.3.5 Effect of copper toxicity on other organs

In rarer circumstances, WD may present with abnormalities of other organ systems, namely, arthropathy and cardiomyopathy with dysrhythmias.

Bono reported 21 cases of Wilson's disease. The average age at the beginning of the disease was 17.6 years, with a female prevalence (8/13). The signs at first were mostly all neurological (71.4%), then psychiatric (19%) or hepatic (19%). The most common neurological signs were dystonia of members (81%), dysarthria (76%), tremors (76%) or disorders of motoricity (71.4%). Sometimes there were sialorrhea or disorders of the handwriting. The Kayser-Fleischer ring was present in 19 patients. 18 patients had clinical and/or biological hepatic involvement. The diagnosis was confirmed by biochemical examinations, which found a low rate of copper in blood, a sinking rate of ceruloplasmin and a very high rate of urinary copper. The cerebral computer tomography shows a cortical and/or subcortical atrophy (37%), and/or a low density of the central grey cores (35%) [26].

C.4 DIAGNOSIS OF WILSON'S DISEASE

Since many tissues and organs may be affected, Wilson's disease has a broad spectrum of clinical symptoms and many of them are nonspecific.

WD should be considered and excluded in any individual between childhood and the age of 40 years who has unexplained hepatic, neurologic or psychiatric disease. In particular, it should be considered in children or young adults with atypical

extrapyramidal or cerebellar motor dysfunction, neuropsychiatric disease, elevated aminotransferases, or other features of acute or subacute liver disease and with unexplained non-immune-mediated hemolysis. In these circumstances, WD must be considered whether or not there is a family history of liver or neurologic disease. In most cases, the diagnosis can be confirmed on the basis of clinical and biochemical evaluation without the need for liver biopsy.

The diagnosis of Wilson's disease is usually made on the basis of *clinical findings* (Kayser-Fleischer rings, typical neurologic symptoms) and laboratory abnormalities.

In suspected patients, slit lamp examination for Kayser-Fleischer rings (characteristic depositions of copper pigment in the limbus of the iris), 24-hour urine collection for copper, determination of serum copper level and hepatic biopsy are indicated. No *laboratory test* is diagnostic, but complementary results on two or more tests are very helpful (Table 1-2).

Table 1 – Copper levels in different organs [2]

Test	**Levels in Healthy Persons**	**Levels in Wilson's Disease**
Liver copper content (µg Cu/g dry weight)	10-50	100-2000
Serum ceruloplasmin (mg/dl)	20-45	0-20
Serum copper (µg/dl)	70-160	25-70
Urinary copper (µg/day)	3-35	100-1000

Table 2 – Serum ceruloplasmin and hepatic copper concentration values of patients

with Wilson's disease [1]

	Serum ceruloplasmin		Hepatic Copper Concentration	
Group	**No of Patients**	**Mean±SD, mg/dl**	**No of Patients**	**Mean ±SD, g/g Dry Weight**
Wilson's disease Asymptomatic	31	3.6 ± 5.3	36	983.5 ± 368
Wilson's disease Symptomatic	84	5.9 ± 7.1	33	588.3 ± 304
Heterozygous carriers	95*	28.4 ± 8.5	14	117.0 ± 51
Control subjects	180	30.7 ± 3.5	16	31.5 ± 6.8

* 71 parents of patients with Wilson's disease and 24 children, each of whom had one parent with Wilson's disease

Liver biopsies can be assayed for copper content, which is higher in early disease and less elevated in late disease. High hepatic levels do occur in some other liver diseases such as primary biliary cirrhosis. Ceruloplasmin levels are low in 95% of cases, but low levels are not the cause of Wilson's disease. Confusion may arise because ceruloplasmin is an acute phase reactant that is also increased in pregnancy and by estrogens, under such circumstances, apparently normal ceruloplasmin levels may be seen in Wilson's disease. Total serum copper levels may be low but may overlap the normal range. Urinary copper excretion is almost always high, reflecting the use of an ancillary route of excretion. Ultimately, the combination of laboratory tests with a consistent clinical presentation yields a secure diagnosis.

Molecular genetic testing is now the standard for testing asymptomatic siblings. Currently, molecular genetic studies are confined to haplotype analysis of family

members of an affected individual. Such tests involve evaluation of DNA polymorphisms in the nucleotide regions surrounding the ATP7B gene. There have been multiple disease-specific mutations of the WD gene described in probands with the disorder. Even the most common of these mutations account for only 15% to 30% of most WD populations. Newer technologies that utilize molecular genetic testing in newly discovered patients with clinical manifestations of the disease might pinpoint disease-specific mutations in contradistinction to polymorphisms of the gene [4, 27-29].

The diagnosis is confirmed by the demonstration of either:

- a serum ceruloplasmin level <20 mg/dl and Kayser-Fleischer rings or
- a serum ceruloplasmin level <20 mg/dl and a concentration of copper in a liver biopsy sample >250 µg/g dry weight.

Most symptomatic patients excrete >100 µg copper per day in urine and have histologic abnormalities on liver biopsy.

The plasma level of nociceptin was found to be significantly elevated in Wilson's disease patients compared to age-matched healthy controls [30].

C.5 TREATMENT OF WILSON'S DISEASE

Appropriate anticopper therapy for Wilson's disease is the critical element in halting the progression of the disease and allowing patient recovery. Although there are potentially life-saving therapies for Wilson's disease, there is some controversy surrounding the optimal treatments of patients in the various stages of the disease [31].

Low-copper diets are recommended for every patient [2].

Selection of the drug or drugs to use for a particular patient depends on the stage of the disease (i.e. initial acutely ill patient versus chronic maintenance patient) and the type of presentation (i.e. neurologic/psychiatric versus hepatic).

Brewer treated patients initially presenting hepatic disease with a combination of

zinc and triethylene tetramine (trientine), those presenting with neurologic, psychiatric disease with tetrathiomolybdate, and those in the maintenance phase with zinc [32].

Wilson's disease has moved on from being a recognized syndrome that was uniformly fatal to a curative disease for which the genetic basis has been discovered. The prevention of severe permanent damage depends upon early recognition and diagnosis by the physician, followed by appropriate anticopper treatment. Lifelong medical therapy is required and provides life-expectancy near to normal. Interruption of treatment leads to reaccumulation of copper, often resulting in fulminant hepatic failure. Anti-copper treatments have evolved considerably since the days when the only drug available was penicillamine [33-35]. The purpose of the therapy of Wilson's disease is to eliminate the copper by chelators (D-Penicillamine, triethylene tetramine, tetrathiomolybdate) and to inhibit the absorption and accumulation of copper by zinc salts (zinc sulphate, zinc acetate, zinc gluconate.

C.5.1 Application of chelating agents

They induce renal and biliary copper excretion and increased synthesis of metallothionein, which attaches and detoxifies intracellular copper, leading to impaired absorption and the binding of excess intracellular copper [36, 37]. The mobilization of copper from liver stores can result in neurologic worsening during initial treatment with standard chelation regimens [2].

C.5.1.1 D-Penicillamine

D-Penicillamine is generally regarded as the agent of choice for the initial management as it produces a rapid reduction in copper levels.

D-Penicillamine in typical doses of 500 mg twice daily can increase urinary copper to 1500 to 3000 µg/day. D-Penicillamine reduces copper concentrations mainly by chelating copper, which is then excreted in the urine. Two molecules of

penicillamine combine with one atom of copper. Penicillamine also reduces the affinity of copper for proteins and polypeptides thus allowing the removal of copper from tissues and induces the synthesis of metallothionein in the liver, a protein that combines with copper to form a non-toxic product.

Since penicillamine has an antipyridoxine effect, 25 mg/day of pyridoxine is also given. Up to 30% of patients have difficulty in taking penicillamine. About 5 to 10% of patients develop severe penicillamine side effects such as rash, fever, lymphadenopathy, cytopenias, lupus erythematosus, Goodpasture's syndrome and nephrotic syndrome.

Penicillamine is no longer the treatment of choice as there is growing experience with safer and more effective alternatives [38].

C.5.1.2 Triethylene tetramine (Trientine)

Triethylene tetramine (trientine) is a less potent copper chelator than D-Penicillamine which competes for copper bound to serum albumin.

Trientine is usually used in patients intolerant of D-penicillamine.

The dose of trientine is 1 g/day on an empty stomach. Pyridoxine needs not to be given. Although the only reported toxic reaction to trientine is sideroblastic anaemia, the same clinical procedures and laboratory determinations should be performed during its administration as used during penicillamine therapy. Trientine may be more effective when used in combination with zinc [39, 40].

C.5.1.3 Tetrathiomolybdate

Treatment by *any modality* can prolong life and interrupt hepatic and brain deterioration.

Ammonium tetrathiomolybdate forms a complex with protein and copper. When it is given with food, it blocks the intestinal absorption of copper, and when taken between meals, it combines with albumin- and ceruloplasmin-bound copper. Ammonium tetrathiomolybdate is under investigation for the initial reduction of

copper levels; it may be particularly suitable for patients with neurological symptoms [41].

C.5.2 Application of zinc salts

Schouwink and Hoogenraad were the first to report studies in Wilson's patients on zinc therapy [42].

Zinc salts are now the recommended therapy for the long-term management of the disease [34]. Zinc salts are completely effective in controlling copper levels and toxicity in Wilson's disease. Zinc's major advantage over other anticopper agents is its extremely low level of toxicity [43].

In 1997, zinc acetate was added to the list of drugs approved by the Food and Drug Administration [44, 45].

Zinc acetate, like other zinc salts is suitable for the maintenance therapy of adult and pediatric Wilson's disease patients but also has efficacy in the treatment of pregnant patients and presymptomatic patients from the beginning.

Zinc is a slowly acting drug in terms of its effect on copper, so zinc is not suitable in those requiring rapid reduction. It takes about 4 to 6 months to control acute copper toxicity. A significant improvement of clinical symptoms and normalization of the parameters of copper metabolism can be expected earliest six months after onset of therapy [42].

Zinc stimulates hepatic and intestinal metallothionein synthesis and reduces copper absorption [46, 47].

Zinc has shown clinical efficacy at doses of 50 mg three times daily.

Shimizu gives a dose of 5 -7,5 mg/kg daily before meals [48].

Brewer gives for pediatric patients of 1 to 5 years of age 25 mg of zinc twice daily, patients of 6 to 15 years of age, if under 125 pounds body weight, 25 mg of zinc three times daily and for patients 16 years of age or older 50 mg of zinc three times daily [49]. Once we have a zinc dose adequate, to induce intestinal cell metallothionein, increasing the zinc dose further is meaningless- that is, there is no

further dose-response beyond this point (a surrogate marker of this state is having an adequate urine zinc- over 2,0 mg/24 hours) [42]. The only side effect is some degree of initial gastric irritation in approximately 10% of patients, which usually decreases and becomes insignificant over time. As with all long-term therapies, compliance is a problem in some patients and dictates regular monitoring with 24 h urine copper and zinc measurements. As with all anticopper therapies, over a long period of time, overtreatment and induction of copper deficiency can occur [43]. Regular examinations of the parameters of copper metabolism are necessary in order to control the therapeutic effect. Free copper serum concentrations and urinary copper excretion should reach values below 10 µg/dl and 80 µg/day, respectively.

C.5.3 Liver transplantation

Fulminant or progressive liver failure has been succesfully treated with liver transplantation by which the genetic defect is phenotypically cured [22].

C.6 FORMULATION ASPECTS OF DIFFERENT DRUGS

Drugs are rarely administered solely as pure chemical substances but are almost always given in formulated preparations. These can vary from relatively simple solutions to complex drug delivery systems, through the use of appropriate additives or excipients in the formulations to provide varied and specialized pharmaceutical functions. It is the formulation additives that, amongst other things, solubilize, suspend, thicken, preserve, emulsify, improve the compressibility and flavour drug substances to form various preparations or dosage forms [52-58].

The principal objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of manufacturing with reproducible product quality. To ensure product quality, numerous features are required – chemical and physical stability, with suitable preservation against microbial contamination if appropriate, uniformity of dose of drug, acceptability to users including both prescriber and patient, as well as suitable packaging and labelling.

Before a drug substance can be successfully formulated into a dosage form, many factors must be considered.

These can be broadly grouped into three categories:

1. biopharmaceutical considerations, including factors affecting the absorption of the drug substance from different administration routes,
2. drug factors, such as the physical and chemical properties of the drug substance, and
3. therapeutic considerations including consideration of the disease to be treated and patient factors.

Appropriate and efficacious dosage forms will be prepared only when all these factors are considered and related to each other [59-62].

Nowadays both hard and soft gelatine capsules are commonly used. Currently hard gelatine capsule manufacturing plants are located in all of the major trading blocs. The two major producers in the world are the American companies, Eli Lilly and Parke Davis, who have both been making hard gelatine capsules since the turn of the century [63].

Gelatine is the major component of the capsule and has been the only material from which they have been successfully made. The reason for this is that gelatine possesses four essential basic properties:

- ? It is non-toxic. It is widely used in foodstuffs and is acceptable for use in every country in the world.
- ? It is readily soluble in biological fluids at body temperature.
- ? It is a good film forming material.

? As a solution in water or a water-glycerol blend, it undergoes a reversible phase change from a sol to a gel at temperatures only a few degrees above ambient. This is in contrast to other films which are produced in pharmacy, where either volatile organic solvents or large quantities of heat are required to effect this change of state. This property enables films of gelatine to be prepared easily.

Gelatine is a substance of natural origin, but does not occur as such in nature. It is prepared by the hydrolysis of collagen, which is the main protein constituent of connective tissues. Thus animal bones and skins are the raw material for the manufacturing. There are two main types of gelatine: type A, which is produced by acid hydrolysis, and type B, which is produced by basic hydrolysis. The choice of manufacturing method depends upon the nature of the raw materials, skins are mainly acid processed whereas bones are usually basic processed. In the USA, most type A gelatin is obtained from pig skins. This material is washed in cold water for a few hours to remove extraneous matter and is then digested in dilute mineral acid (HCl, H_2SO_4, H_2SO_3, or H_3PO_4) at pH 1–3 and 15–20°C until maximum swelling has occurred. This process takes approximately 24 hours. The swollen stock is then washed with water to remove excess acid, and the pH is adjusted to pH 3.5–4.0 for the conversion to gelatin by hot-water extraction.

The hydrolytic extraction is carried out in a batch-type operation using successive portions of hot water at progressively higher temperatures until the maximum yield of gelatin is obtained. The gelatin solution is then chilled to form jelled sheets, which are dried in temperature-controlled ovens. The dried gelatin is ground to the desired particle size.

In the alkali process, demineralized bones (ossein) or cattle skins are usually used. The animal tissue is held in a calcium hydroxide (lime) slurry for a period of 1–3 months at 15–20°C. At the end of the liming, the stock is washed with cold water to remove as much of the lime as possible. The stock solution is then neutralized with acid (HCl, H_2SO_4, H_3PO_4) and the gelatin is extracted with water in an identical manner to that in the acid process.

In the past there has been a significant amount of regulatory activity due to the attention given to bovine sourced gelatin manufacturing processes and the potential transmission of TSE vectors from raw bovine materials into gelatin. In Europe the criteria by which the safety is assured involves controlling the geographical sourcing of animals used; the nature of the tissue used (based on scientific data showing where animal BSE infectivity is located); and the method of production. During the preparation of the bovine bones used in the production of gelatin, specified risk materials that could contain Transmissible Spongiform Encephalopathies (TSEs) vectors are removed. It is a foregone conclusion that TSE infectivity is not present in pharmaceutical grade gelatin [3, 108].

All formulations for filling into hard gelatine capsules must fulfil two basic requirements.

- ? They must be able to be accurately dosed into the capsule shell,
- ? They release their active contents in a form which is available to the patient.

To accomplish this, the formulation is usually a simple blend of the active ingredients together with adjuvants which aid the process, e.g. diluents, glidants, lubricants and surfactants. A variety of materials other than powders can be filled into hard gelatine capsules, e.g. granules, pellets, tablets, semisolids.

C.6.1 Advantages and disadvantages of extended-release products

Extended-release drug products offer several important advantages over immediate-release dosage forms of the same drug. Extended release allows for sustained therapeutic blood levels of the drug, sustained blood levels provide for a prolonged and consistent clinical response in the patient. Moreover, if the drug input rate is constant, the blood levels should not fluctuate between a maximum and a minimum, as in a multiple-dose regimen with an immediate-release drug product. Highly fluctuating blood concentrations of drug may produce unwanted side effects in the patient if the drug level is too high, or may fail to exert the proper

therapeutic effect if the drug level is too low. Another advantage of controlled release is patient convenience, which leads to better patient compliance. If the patient only needs to take the medication once daily, he or she will not have to remember to take additional doses at specified times during the day.

The patient may also derive an economic benefit in using a controlled-release drug product. A single dose of the controlled-release product may cost less than an equivalent drug dose given several times a day in rapid-release tablets.

However there are also a number of disadvantages in using controlled-release medication. If the patient suffers from an adverse drug reaction or becomes accidentally intoxicated, the removal of drug from the system is more difficult with a controlled release drug product. The formulation of controlled-release drug products may not be practical for drugs usually given in large doses (> 500 mg) in conventional dosage forms. As the controlled-release drug product may contain two or more times the dose given at more frequent intervals, the size of the controlled-release drug product would have to be quite large, too large for the patient to swallow easily [64-68].

C.6.2 Different types of slow and extended release dosage forms

Slow and extended release dosage forms may be divided into the following groups:

- ? Coated dosage forms
- ? Matrices
- ? Drugs embedded in hydrophilic polymers
- ? Ion exchangers
- ? Dissolution controlled dosage forms
- ? Erosion dosage forms
- ? Osmotic systems
- ? Pulsed systems

S/ERP is used as abbreviation for slow and/or extended release products. The

following is a description of their release mechanisms and the various possibilities of controlling them.

C.6.2.1 Coated S/ERP

This is the most important of the controlled release dosage forms. The drug is surrounded by a barrier. The slow rate of diffusion through this barrier determines the rate of release and, consequently, the rate of absorption.

Basically, release follows the pattern described below:

- ? Water/gastric or intestinal juice permeates the coating.
- ? The drug dissolves, if the core has a sufficient content of drug, solubility C_s is obtained.
- ? The drug diffuses through the coating.
- ? As long as C_s is maintained in the core, the rate of release remains constant. If C_s is no longer given, the release rate decreases exponentially.

The constant release rate Q/t is described as:

$$Q/t = pAC_s/d \qquad (1)$$

Q=drug released into the sink

t=time

p= permeability of the coating

A = area

d = thickness of the coating

The coating materials are usually organic polymers, above all, ethylcellulose and methacrylic acid methacrylate copolymers, ethylacrylate-methylmethacrylate copolymer and those polymers which also contain another 5% or 10% trimethylammonium ethylmethacrylate. As the surface and the thickness of the

coating may only be varied within certain limits, the permeability coefficients of the coating are of great importance with respect to the control of the release rate.

The following steps can be taken to increase the release rate when using coating of restricted permeability:

? Addition of Plasticizers

The positive effect of plasticizers is due to various mechanisms. First, they lower the T_g of the polymers at temperatures below 37 ^{0}C. Thus the polymers are more elastic and more easily penetrated. Second, they promote a much greater uptake of water in the coating. Both processes result in an increase in the diffusion coefficient and consequently in an increase in the rate of release. Depending on the solubility of the plasticizer in water, it either remains in the coating or is eluted and then forms micro-or macropores.

? Internal Plastification (chemical modification)

Internal plastification is intended to increase the swelling in water.

? Addition of Pore Formers

For example, water-soluble additives such as NaCl, sucrose, cellulose derivates, polyethylene glycols, PEG; additives of this kind produce their own dispersed phase in the coating, either straightaway or in the course of film formation (during the removal of the solvent or dispersing agent). They dissolve on contact with water and form pores, e.g. pigments which are released from the coating at the start of the release process after contact with water.

? Incompleted Coating

C.6.2.2 Drugs embedded in hydrocolloids

Hydrocolloid embeddings may be divided into two groups depending on their release mechanisms. A) those which swell up on contact with water resulting in highly viscous, poorly soluble embeddings and B) those which swell slowly, with a low level of viscosity, tend to dissolve faster (erosion control). The release from

highly viscous embeddings is generally in accordance with the kinetics. The active ingredient gradually diffuses from the gel matrix obtained as a result of swelling. Erosion controlled polymer embeddings yield zero-order release, or thereabouts, provided the surface remains constant. Given an adequate stirring rate, the dissolution/erosion of the polymers is rate determining. Thus, the solubility of the drug is without importance. On the other hand, this means that the release is dependent on the stirring rate.

C.6.2.3 Ion exchangers

Those polymers used most frequently are cross-linked polymers with acid end groups containing bonded basic drugs which are released gradually after forming a hydrogel by swelling. Sulphonic acids are especially suitable for delaying the release of basic drugs. Release from carboxylate resonates is generally too fast. The release kinetics in vivo should be subject to the $\sqrt{t}$ law, i.e. to matrix control. Proper first-order release through control of the film, caused by the liquid adhering to the surface of the ion exchanger particles, occurs less frequently. Thus, it can be seen that the degree of cross-linking in the polymer represents an important parameter for drug release. Increased cross-linking reduces the amount of swelling/ the degree of hydration and thus the rate of release. Further parameters include particle size and bonding affinity between exchangers and drug. In addition to the ion exchange, other co-operative forces may also play an important part, e.g. hydrophobic interactions.

C.6.2.4 Dissolution Controlled S/ERP

The active ingredient is not fast dissolved and therefore cannot sufficiently be absorbed. The dissolution kinetics determines the subsequent absorption. The dissolution process is summarized below:

- ? Disintegration of the crystal lattice by solvation of individual molecules on the crystal surface resulting in the formation of a saturated solution.
- ? Diffusion of the molecules of the drug through the adhering layer into the agitated solvent. This process is rate-determining.

Accordingly, the following expression applies to the release rate dQ/dt under sink conditions:

$$dQ/dt = DAC_s/d \qquad (2)$$

D=diffusion coefficient in the solvent

A=crystal surface, decreasing during dissolution

C_s=saturation concentration

d= thickness of the adhering layer.

Thus, in order to slow down the rate of dissolution, it is necessary to reduce the surface area A (macrocrystals) and decrease the solubility C_s (slightly soluble salts, derivates).

According to the release mechanism, the release rate of dissolution controlled S/ERP is influenced by the stirring rate and the gastrointestinal motility, respectively.

C.6.2.5 Erosion Controlled S/ERP

Erosion controlled release dosage forms may be produced by combining inert lipophilic substances with high dose, soluble binding and filling agents in the absence of disintegrants. Instead of decomposing, they erode following the dissolution/swelling of certain additives on the surface. The most important parameter is the ratio between the non-soluble and the soluble excipients.

The classic erosion dosage forms consist of drug embedded in digestible fats. However, it is difficult to control the release rate of such dosage forms.

C.6.2.6 Osmotic systems

Regardless of whether they are in the form of the usual elementary osmotic pump, the two-chamber push-pull system or the minipump, these controlled release dosage forms are based on the combination of osmotically active substances (drug and/or excipients) and a semipermeable membrane, i.e. one permeable only to water. The resultant osmotic pressure causes the solution of drug to flow out through the given outlet.

C.6.2.7 Pulsatile release

When enough is known about the chronopharmacological and tolerance characteristics of the drug, including other vital parameters, it is sometimes more appropriate to apply it intermittently rather than continually. There are only a few ways of doing so with oral dosage forms as the release of drug and absorption is generally limited to approx. 5 hours. A few of the possibilities, including the basic control principles, are outlined below:

- Combination of Rapidly Available and Enteric Coated Drug (Delayed Release)
- "Explosive" Dosage Forms with Delayed Release of a Given Amount of Drug
- Hydrocolloid Laminates with Intermediate Zones Free From Drug
- Osmotic Systems with Pulsatile Release

When choosing a controlled release system, those aspects which play an important role include ease of manufacturing and reliability (the effect of motility, rate of stirring, pH of the stomach/intestines and food on the rate of release rate is minimal), as well as stability of the release rate during storage. In addition, the aspects of dosing flexibility (lower or higher dose according to demand) and the advantages of multiple units have to be considered. In addition, the extent to which release may be controlled must also be taken into consideration. The essential parameters differ from controlled release form to controlled release form. It is not

even possible to generalize the influence of excipients without greatly affecting the release rate and consequently compromising the reproducibility of the manufacturing procedure, another important factor for the selection of a particular S/ERP [69-73].

C.6.2.8 Matrix Controlled S/ERP

Matrix dosage forms are characterized by their insoluble, possibly porous "skeleton" of indigestible fats and waxes, thermoplastics or inorganic matrix formers such as gypsum. This framework includes the drug and, if necessary, soluble additives. The drug is released by diffusion, however, the excipient does not play a role in this procedure and is left behind as bare framework. Thus, stability problems are less frequent with this type of S/ERP. Matrix controlled S/ERP may be divided into smaller parts, which means dosing flexibility. Even micromatrices (multiple units) may be formed.

The release kinetics frequently corresponds, at least approximately, to the theoretical basis derived by Higuchi ($\sqrt{t}$ -law) as long as $C_s < C_0 e$ (high-load, e = porosity):

$$Q \; ? \; k\sqrt{t} \qquad (3)$$

$$Q \; ? \; A\sqrt{(D?C_s/?)(2C_0 \; ? \; ?C_s)}\sqrt{t} \qquad (4)$$

Q = drug released into the sink

k = constant

D = diffusion coefficient of the drug in the dissolution medium

t = tortuosity (twisting of the capillaries)

C_0 = loading dose in the matrix (concentration)

C_s = solubility of the drug in the dissolution medium

The following factors must be taken into consideration when deciding how best to control the rate of release: the ratio of the drug to the excipient, C_0 , the pore formers e, and the surface area A (minimatrices, extrusion granules). The given solubility of the active ingredient C_s is naturally a very important factor. In order to obtain complete release, the total porosity e must amount to more than approx. 0.25 to ensure that the dissolution medium reaches almost all of the drug particles after penetration (total porosity = the initial porosity by enclosed air plus porosity arising from the dissolved drug and additive). If this is not the case, 100% release is not to be expected.

As with coated S/ERP, the release rate is independent of the hydrodynamics.

A matrix is an inert solid vehicle in which a drug is uniformly suspended. A matrix may be formed simply by compressing or fusing the drug and the matrix material together. Generally, the drug is present in a smaller percentage, so that the matrix provides extended protection against water and the drug diffuses out slowly over time. Most matrix materials are water insoluble, although some may swell slowly in water [74-78].

Matrix type of drug release may be manufactured into a tablet or small beads depending on the formulation composition.

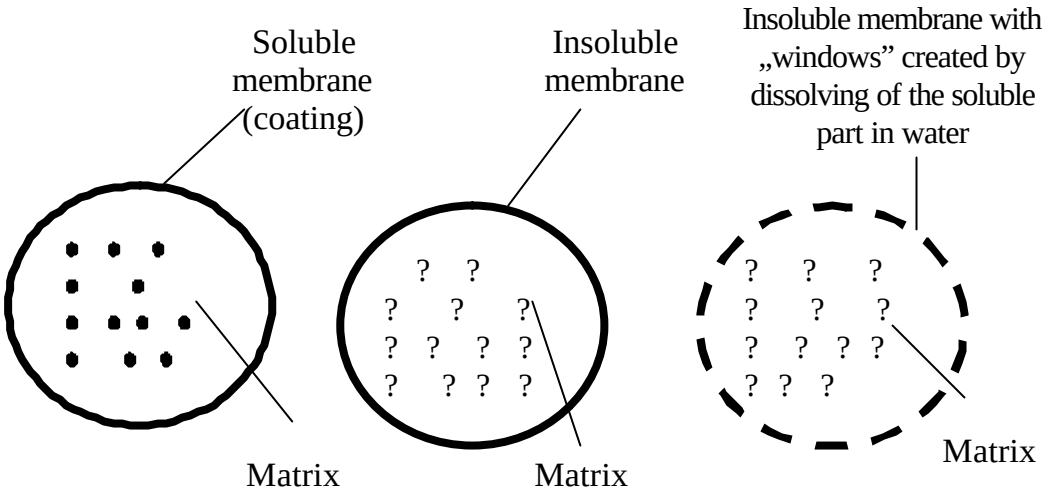


Figure 2 – Examples of three different types of modified matrix-release mechanisms [73]

Figure 2 shows three common approaches by which the matrix mechanisms are employed.

A. The drug is coated with a soluble coating, so drug release relies on the regulation of the matrix material. If the matrix is porous, water penetration would be rapid and the drug would diffuse out rapidly. A less porous matrix may give a longer duration of release. Unfortunately, drug release from a simple matrix tablet is not zero order. The Higuchi equation describes the release rate of a matrix tablet.

B. It represents a matrix enclosed by an insoluble membrane, so that drug release rate is regulated by the permeability of the membrane as well as the matrix.

C. It represents a matrix tablet enclosed with a combined film. The film

becomes porous after the dissolution of the soluble part of the film. An example of this is the combined film formed by ethylcellulose and methylcellulose. Close to zero-order release has been obtained with this type of release mechanism [79-88].

D EXPERIMENTAL PART

D.1 MATERIALS AND METHODS

D.1.1 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 ^{0}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.

D.1.2 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 ^{0}C (? 1^{0}C). The zinc sulphate crystals of 630-800 ?m particle size 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.

D.1.3 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 ^{0}C. Sampling times were the following: 5, 10, 20, 30, 60, 120, 240, 480, 720 min.

D.1.4 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 $ZnSO_4 \cdot 7H_2O$. Introduce the prescribed solution into a 500 ml conical flask and dilute to 200 ml with water R . Add about 50 mg of xylenol orange triturate R and hexamethylenetetramine R until the solution becomes violet-pink. Add 2 g of hexamethylenetetramine R in excess. Titrate with 0.1 M sodium edetate until the violet-pink colour changes to yellow. 1 ml of 0.1 M sodium edetate is equivalent to 6.54 mg of Zn.

D.1.5 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, described by Eq. (5-11), was made with the Solver function of the computer package Microsoft Excel 5.0.

D.1.5.1 Zero-order model

The drug release from the dosage form follows a ‘steady-state release’ [89, 90], running at a constant rate:

$$M_t/M_\infty = kt \qquad (5)$$

where M_t is the amount of drug released at time t,

M_∞ is the maximal amount of the released drug at infinite time,

k is the rate constant of drug release.

D.1.5.2 First-order model

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

$$M_t/M_\infty = 1 - \exp(-kt) \qquad (6)$$

D.1.5.3 Higuchi square root time model

The most widely used model to describe drug release from matrices, derived from Higuchi for a planar matrix, however, it is applicable for systems of different

shapes, too:

$$M_t/M_\infty = K\,t^{1/2} \qquad (7)$$

D.1.5.4 Semiempirical model proposed by Ritger and Peppas

Dissolution data were analyzed using the equation proposed by Ritger and Peppas to describe the mechanism of drug release from matrices.

$$M_t / M_\infty = Kt^n \qquad (8)$$

where M_t corresponds to the amount of drug released in time t, M_∞ is the total amount of drug that must be released at infinite time, K is a constant 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. If n approaches to l, the release mechanism can be zero order and on the other hand, if 0.5 <n <1, non-Fickean transport could be obtained.

The model-independent mean dissolution time (MDT) was calculated as follows [77]:

$$MDT = n/[(n + 1)(K^{(1/n)})] \qquad (9)$$

D.1.5.5 Weibull distribution

The Weibull distribution can be assigned as a generalized form of the exponential function [91], hence it can be widely used for the analyses and characterization of drug dissolution process from different dosage forms [70].

$$M_t/M_\infty = 1 - \{\exp - [(t - t_0)/t]^{\beta}\} \qquad (10)$$

where t_0 is the lag time of the drug dissolution,

t the mean dissolution time, when 63,2% of $M_?$ has been released,

ß shape parameter of the dissolution curve.

The cumulative percentage of released drug versus time data was assessed for zero order release kinetics. The logarithm of the amounts of the remaining drug must be released versus time data were assessed for first order kinetics and the data of cumulative percentage drug release versus square root of time data were used to evaluate for Higuchi model kinetics [76, 89-102].

D.1.6 In vivo test

Zinc absorption was investigated in patients with Wilson's disease, treated and controlled at the Hepatological Outpatient Unit of the 1st Department of Medicine of Semmelweis University. The diagnosis was based on the characteristic clinical symptoms, the low level of serum ceruloplasmin, the Kayser-Fleischer ring positivity and the detection of ATP7B gene mutation, using the international scoring system.

Absorption of zinc sulphate from gastrointestinal tract was investigated by determination of zinc level in five patients with Wilson's disease (mean age 30,8 ± 5 year, male / female = 4 / 1, disease duration: 2-15 year).

Four patients were on D-penicillamine treatment and one was treated with zinc sulphate in the 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.

The clinical tolerance was investigated throughout longer treatment ranged 1 to 6 months. Serum samples were stored at minus 20 °C.

D.1.7 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.

D.1.8 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%.

D.1.9 Energy dispersive X-ray spectroscopy (EDS)

Samples for the energy-dispersive spectroscopic (EDAX) 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.

D.1.10 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.

$$R\% = (I_R/I_0)100 \qquad (11)$$

where I_R is the intensity of the diffusely reflected light collected by the integrating sphere and I_0 is the intensity of the incident light [103].
The intensity of the reflected light in the presence of an absorbing material can be characterized by the Kubelka-Munk equation [100-106]:

$$K/S = (1-R_?)^2/2R_? \qquad (12)$$

where $R_?$ is the intensity of totally reflected light,

K is the absorption coefficient and

S is the scattering coefficient.

S depends on the number, size, shape and refractivity of particles, and K depends on

the absorbing material and the wavelength.

D.2 RESULTS AND DISCUSSION

D.2.1 Comparison of zinc sulphate release and morphology of matrices

Table 3 summarizes the values of *K*, *n* and MDT calculated by the Eq. 10, 11 and obtained for the drug release profiles from each zinc sulphate matrix. As it was expected, along with the increase of the matrix base of samples, the MDT values were also increased. The *n*? 0.5 values refer to the combined mechanism of drug release, thus parallel diffusion occurs through the hydrophobic pores and from the surface of the matrices. 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. (9) can be explained by the diffusion mechanism of the salt from the wax surface. 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 hours. The zinc sulphate release from matrices of 75% and 80% drug loadings can be described with good correlation ($r^2_{75\%}$ = 0.9972; $r^2_{80\%}$ = 0.9933) by the semi-empirical model commonly applied for matrices (Eq.11). To interpret the difference in drug release characteristics, the morphology and the related composition of various matrices were carried out. The SEM photo (*Fig. 3 a, b*) demonstrates the surfaces of matrices of 83.3% drug loading containing zinc sulphate salts without being embedded into the wax matrix base. The morphology of the matrices of 90% drug loading was very similar. The results of energy-dispersive X-ray spectroscopy confirmed the co-location of zinc sulphate at the matrix surface, the absence of C of the EDX spectra (*Fig. 4*) refers to the lack of organic matrix base around the zinc sulphate crystals (*Fig. 5*, *Table 4*) . The diffusion-controlled matrix release is most close to the samples of 75% and 80% zinc sulphate loadings.

Table 3

Analysis of release data (Eq. 9) from zinc sulphate matrices of different drug loadings

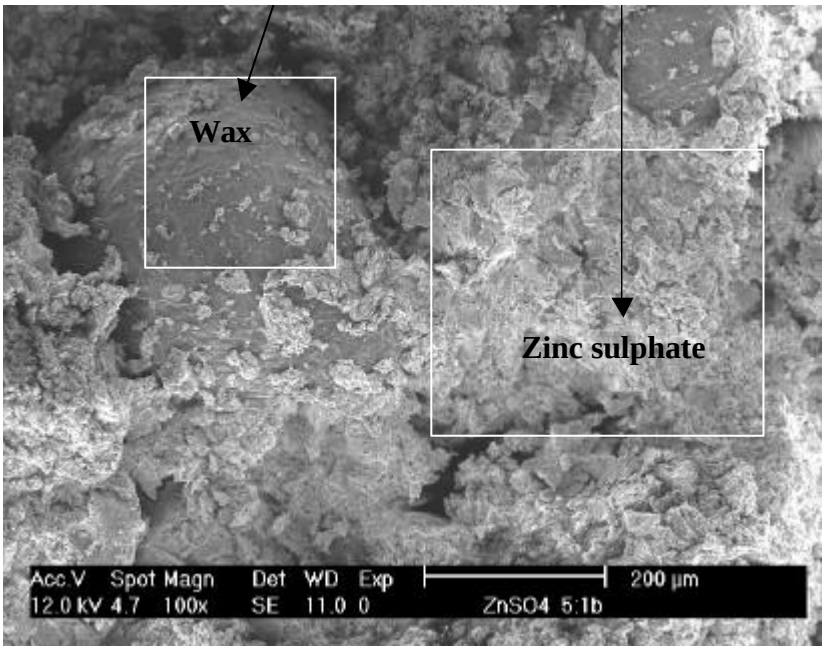
Drug loading (%w/w)	**K** x 100 h^n	***n***	**MDT (h)**	**Correlation coefficient**
66.7	4.81 (±0.21)	0.45 (±0.02)	4.27 (±0.31)	0.9777
75.0	4.40 (±0.17)	0.48 (±0.02)	3.44 (±0.16)	**0.9972**
80.0	4.23 (±0.12)	0.50 (±0.02)	3.22 (±0.15)	**0.9933**
83.3	18.03 (±0.68)	0.29 (±0.01)	1.45 (±0.09)	0.8678
90.0	37.69 (±0.95)	0.17 (±0.01)	0.74 (±0.05)	0.8552

Table 4

Chemical elements of different parts of the matrix, Wt %

Sample	C	O	S	Zn
Spherical wax particle	80,5	10,1	1,8	7,6
Zinc sulphate crystals particle	-	13,6	15,9	70,5

a



b

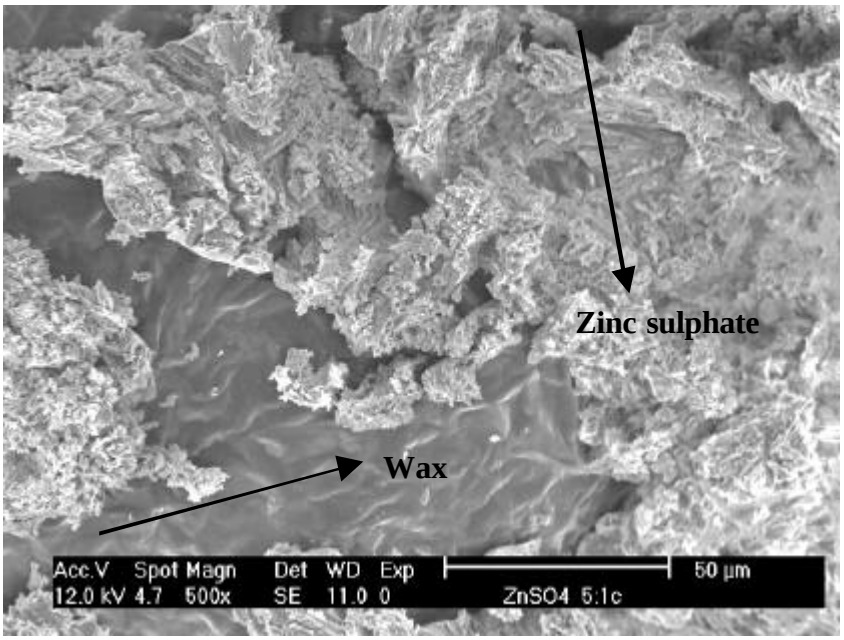


Figure 3 – Scanning electron microscopic photos of matrices of 83.3 %w/w drug loadings

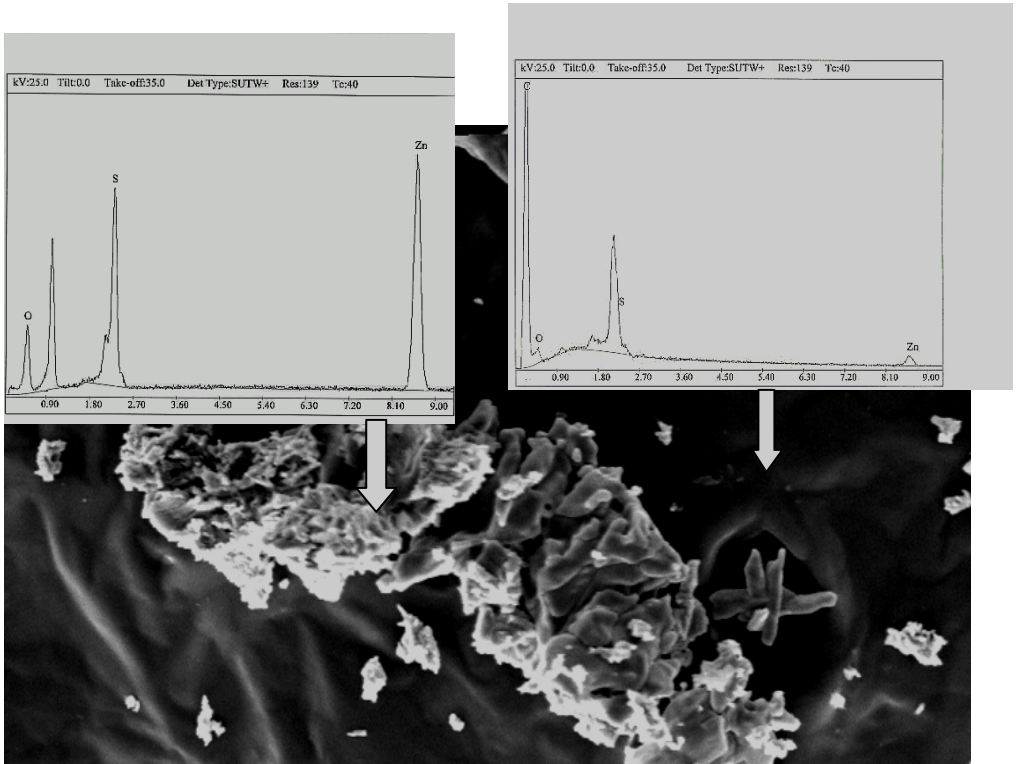


Figure 4– (a) Scanning electron microscopic photo (Magnification: 1000x) and (b) EDX spectra of the matrix surface of 83.3%w/w zinc sulphate loading

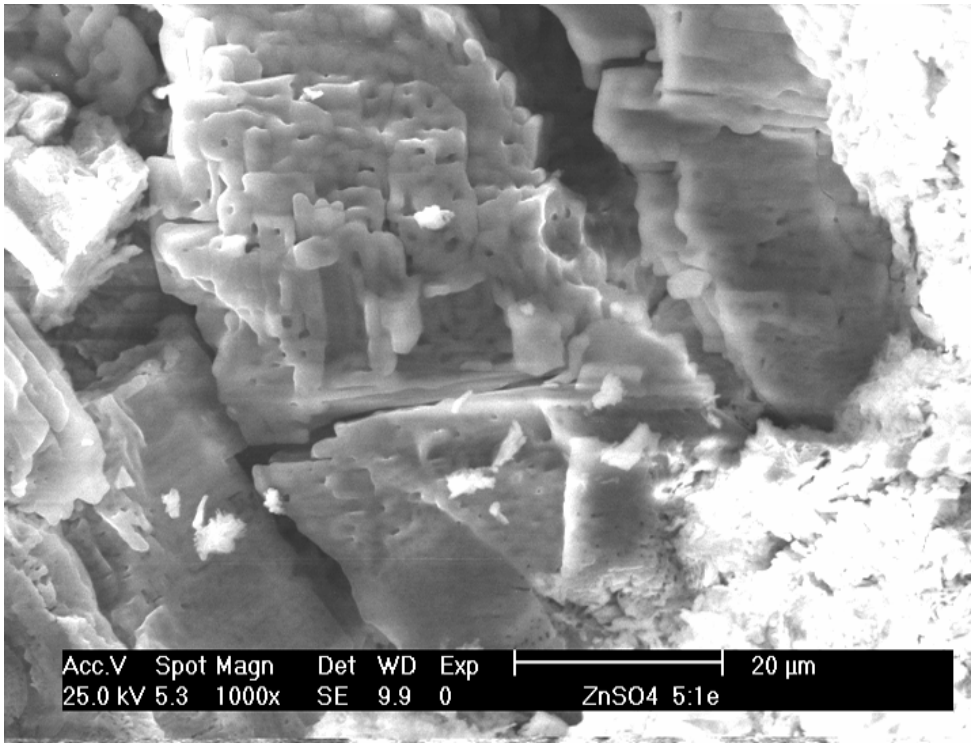


Figure 5 - Scanning electron microscopic photo (Magnification: 1000x) of matrix surface representing zinc sulphate crystals without being embedded into the matrix base

Zinc sulphate loading is 83.3%w/w in the matrix.

D.2.2 Kinetic study of zinc sulphate release from lipophilic matrices

Figure 6 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 loadings/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 5*. 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. (9). 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. (9) can be explained by the diffusion mechanism of the salt from the wax surface, as it was characterized in the *Subsection 8.1*. The release profile of these samples fits mostly to the first order kinetic model. 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 hours (*Fig. 6*).

The parameters of the Weibull distribution were summarized in *Table 6* and demonstrate that the Eq. 11 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 ? values significantly increased and the t values decreased. There were no identified interpretable t values.

At 75% zinc sulphate loading the dissolution process can be characterized preferably by a two-phase dissolution kinetic. In the first phase (beginning 20 minute-period) less than 15% of drug is released following first order kinetic (r^2= 0.9794). In the second phase a closely constant rate of drug dissolution can be observed (r^2= 0.9513) demonstrating zero-order or steady state release (*Fig. 7a, b*). As a result of the steady state diffusion-controlled matrix release, the matrices containing 75% drug loadings were selected for the in vivo examinations.

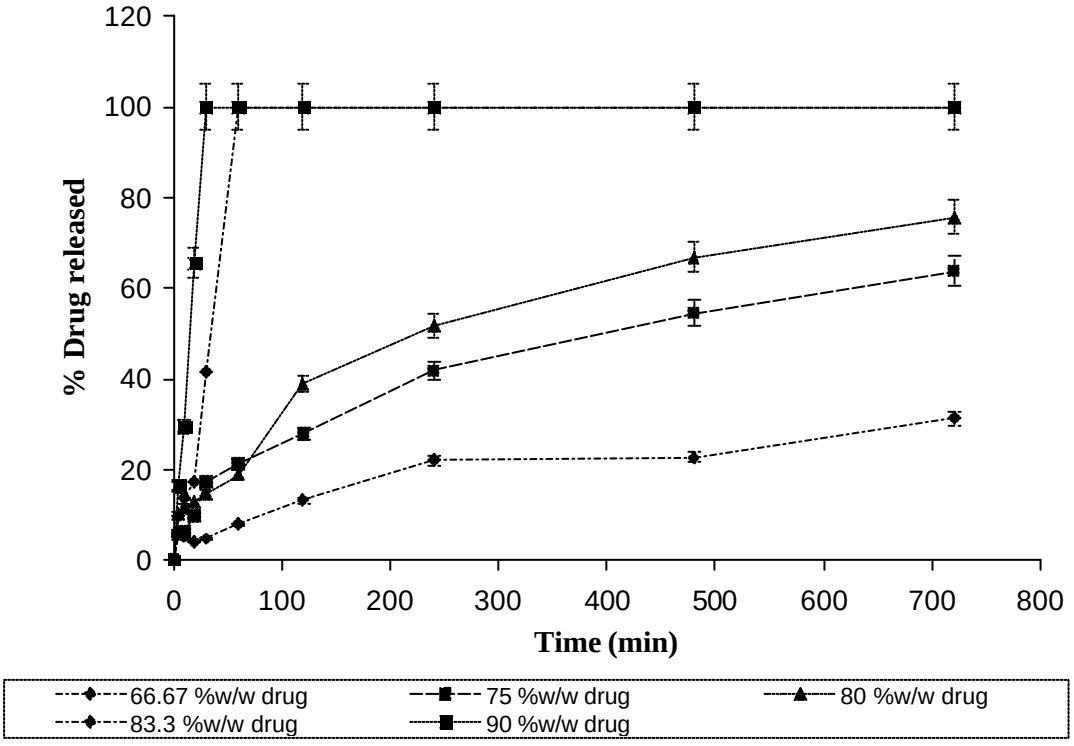


Figure 6 – Zinc sulphate release profiles of matrices of different drug loadings (average values ?S.D., n=5)

Table 5

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

Drug loading	**Correlation coefficients of different models**		
(%w/w)	**Zero-order (Eq. 5)**	**First-order (Eq. 6)**	**Semi-empirical (Eq. 8)**
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

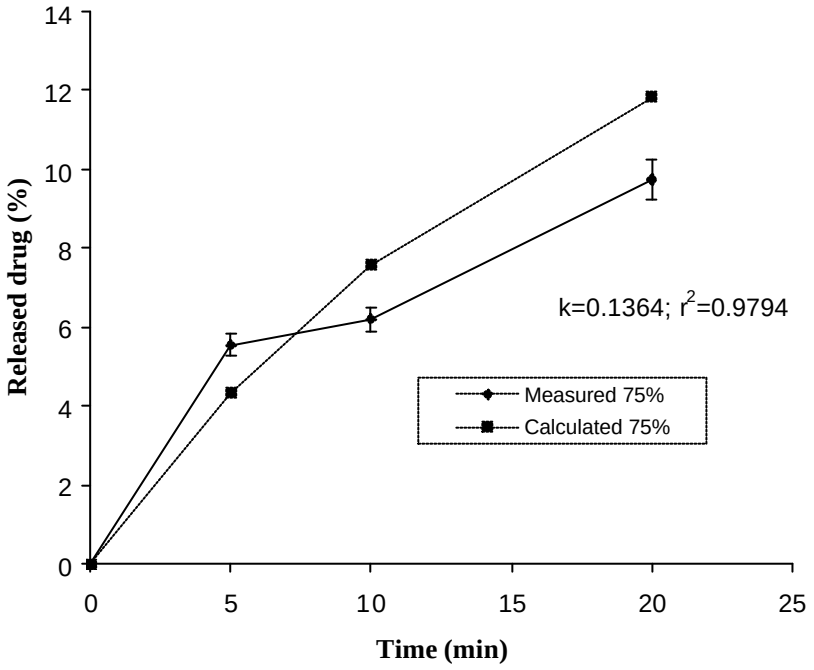
Table 6

Characteristic parameters of Weibull distribution (Eq. 10)

Drug loadings ? shape-parameter		**t value (min)**	**Correlation coefficient**
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

a.



b.

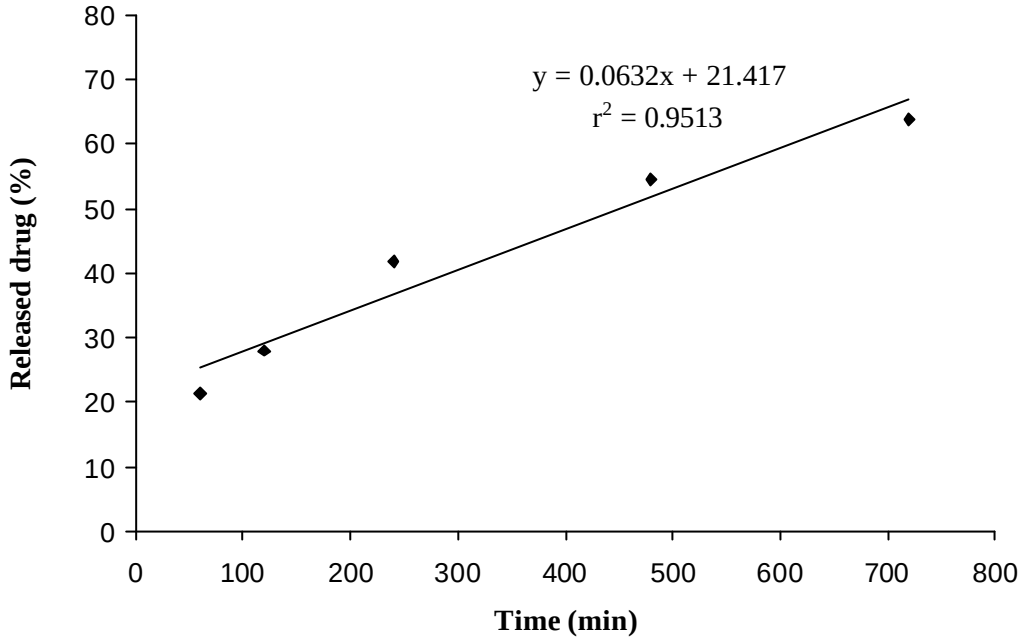


Figure 7 - Two-phase dissolution kinetics – **a**: first order kinetics, **b**: zero-order kinetics (average values ? S.D., n=5)

D.2.3 Non-invasive control of different matrices by diffuse reflectance spectroscopy

The various matrix samples can be characterized by their diffuse reflectance spectra. The C-H peaks (C-H stretch first overtone at 1705 nm) denoted in the *Figure 8* demonstrate the presence of organic matrix base. Along with the increase of the wax content of samples, the reflected light intensity was proportionally decreased. The latter relationship was successfully applied earlier for the non-invasive analysis of coated dosage forms and wax matrix systems [99-101, 104-107]. The results of the present study also enable the selection of matrices of diffusion-controlled drug release based on their in process diffuse reflectance control.

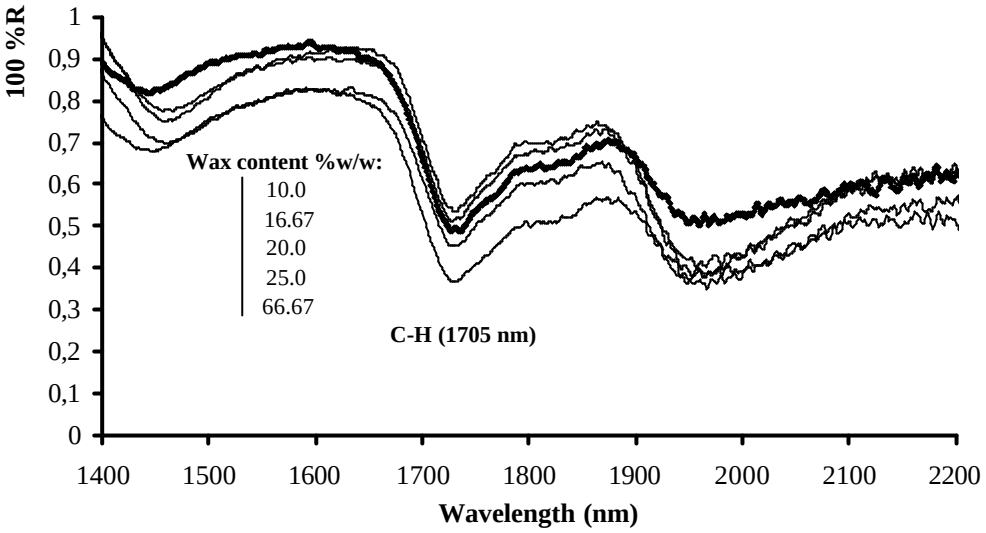


Figure 8 - Diffuse reflectance spectra of zinc sulphate matrices of different wax contents

D.2.4 Influence of the wax content on the characteristic diffuse reflectance and mean dissolution time values of matrices

The following polynomial equation, obtained after significance testing at the 95% confidence level, describes with good correlation (r^2=0.9950) the relationship between the wax content of zinc sulphate matrices (x), their reflectance value measured at the characteristic wavelength of the wax-matrix base (y) and the mean dissolution time of zinc sulphate (z):

$$z = a + bx + c/y + d/y^2 \qquad (13)$$

where a, b, c, d are constants.

The obtained non-linear model enables the prediction of the mean dissolution time of matrices based on their reflected light intensity measured at the characteristic wavelength of wax-matrix base. It is possible to apply near-infrared spectroscopy as a method for rapid in-process control during the formulation of modified-release wax matrices, without destructive sample analysis.

D.2.5 Results of the in vivo absorption

Good absorption of zinc sulphate from gastrointestinal tract was proven by significant elevation of serum zinc level within 180 minutes in each patient with Wilson's disease.

The characteristic absorption curve of a patient previously treated with D-penicillamine is presented on *Figure 9*. The closed circles indicate the mean of three measurements of serum zinc level with SD. The elevation of serum zinc level started 90 min after the administration. The highest value was reached at 180 min proving the good absorption of zinc sulphate from capsules.

The drug was well tolerated throughout the 1 to 6 months treatment with 300 mg

zinc sulphate daily. No side effect was registered and clinical symptoms of Wilson's disease remained as stable as they were during the previous D-penicillamine treatment. The abdominal discomfort complaints of a patient treated previously zinc sulphate in powder form disappeared when the therapy was changed to 300 mg zinc sulphate wax matrix capsule.

During the follow-up of the patients there was no significant change in the serum zinc level. The values varied between 1.0-2.1 µg% not exceeding the normal range. It means that the zinc sulphate wax matrix capsule treatment in a dose of 300 mg daily did not result overdosage of zinc. However, this treatment could provide sufficient amount of zinc in the target tissues.

Figure 10 illustrates the average serum zinc levels in Wilson patients after the daily administration of 300 mg zinc sulphate wax matrix capsule. The results indicate that adequate serum zinc levels could be obtained with the daily administration of 300 mg zinc sulphate matrix capsules.

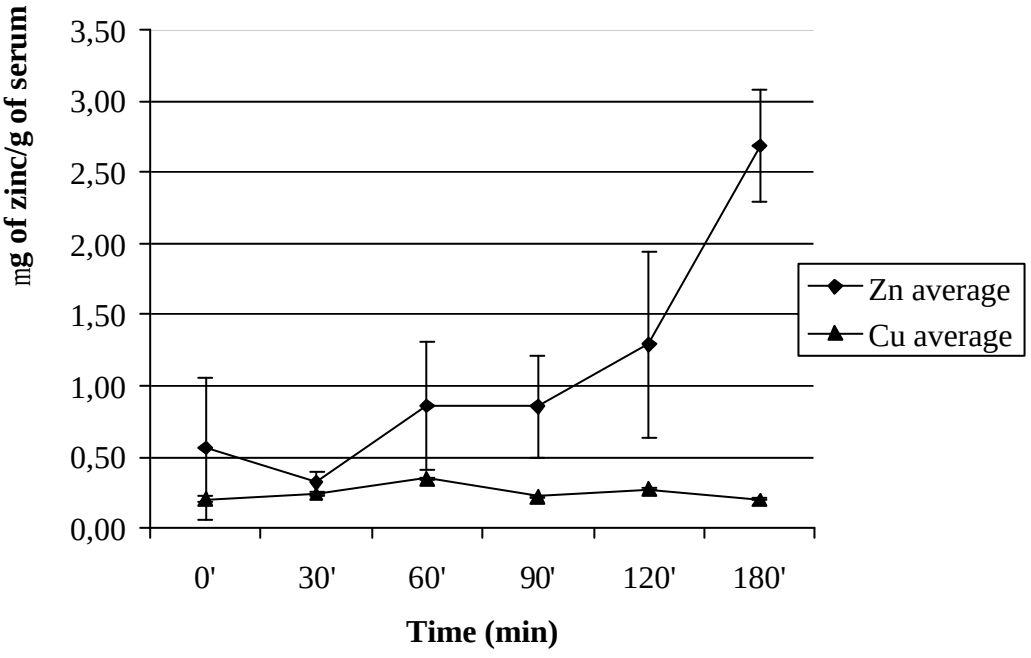


Figure 9 – Serum zinc level in Wilson disease patients before and after

the administration of 300 mg zinc sulphate wax matrix capsule (average values?S.D., n=5)

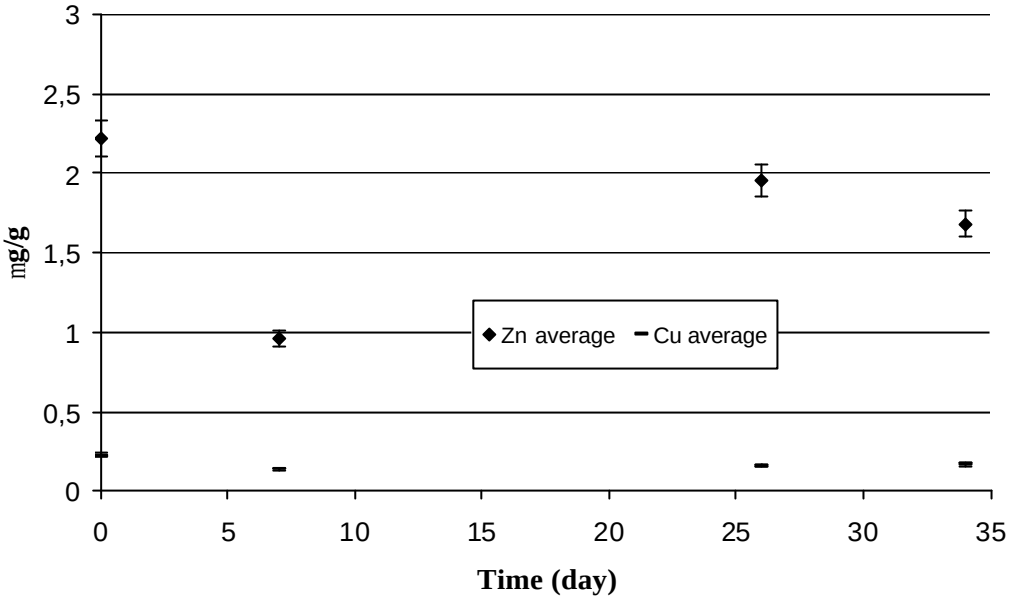


Figure 10 – Average serum zinc levels in Wilson patients before and after the daily administration of 300 mg zinc sulphate wax matrix capsule

E NEW SCIENTIFIC RESULTS AND CONCLUSIONS

? Hydrophobic wax - zinc sulphate matrices of different drug loadings were prepared for the individual clinical 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 ? 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's 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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REFERENCES

1. Braunwald E., Fauci A. S., Kasper D. L., Hauser S. L., Longo D. L., Jameson J. L., Harrison's Principles of Internal Medicine. 15th Edition. McGraw-Hill Medical Publishing Division, 2001, pp. 468-469, 1751-1769, 2274-2275.

2. Andreoli T. E., Carpenter C. C. J., Griggs R. C., Loscalz o J. Cecil Essentials of Medicine. Fifth Edition. WB Saunders, Philadelphia; 2001

3. Martindale, The Extra Pharmacopoeia. Thirty-first Edition. James E F Reynolds. London Royal Pharmaceutical Society, 1996.

4. Ferenci P., Wilson's disease. Ital. J. Gastroenterol. Hepatol. 1999. Jun-Jul, 31(5):416-25.

5. Szalay F., Lakatos P., Firneisz G. et al., His1069Gln mutation in Hungarian

Wilson's patients. Hepatology, 26(4):Pt.2.609A, 1997.

6. Harada M., Sakisaka S., Terada K. et al., A mutation of the Wilson disease protein , ATP7B, is degraded in the proteosomes and forms protein aggregates. Gastroenterology, 120, 967-974, 2001.

7. Sarkar B., Copper transport and its defect in Wilson disease: characterization of the copper-binding domain of Wilson disease ATPase. J. Inorg. Biochem., 79(1-4), 187-191, 2000.

8. Fatemi N., Sarkar B., Molecular Mechanism of Copper Transport in Wilson Disease. Environmental Health Perspectives Supplements, Volume 110, Number 5, 2002.

9. Tanzi R.E., Petrukhin K., Chernov I., et al., The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat. Gen., 5, 344-350, 1993.

10. Petrukhin K.E., Lutsenko S., Chernov I., et al., Characterization of the Wilson disease gene encoding a P-type copper transporting ATPase: genomic organization, alternative splicing, and structure/function predictions. Hum. Mol. Genet., 3,1647-1656, 1994.

11. Shah A.B., Chernov I., Zhang H.T., et al., Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analysis. Am. J. Hum. Genet., 61, 317-328, 1997.

12. Saito T., Okabe M., Hosokawa T., et al., Immunhistochemical determination of the Wilson copper-transporting P-type ATPase in the brain tissues of the rat. Neurosci. Lett., 266,13-16,1999.

13. Petrukhin K., Fischer S.G., Pirastu M., et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. Nat. Genet., 5,338-343,1993.

14. Riordan S.M., Williams R., The Wilson's disease gene and phenotypic diversity. J. Hepatol., 34,165-171, 2001.

15. Sokol R.J., Twedt D., McKim J.M., et al., Oxidant injury to hepatic mitochondria in patients with Wilson's diesease and Bedlington terriers with copper toxicosis. Gastroenterology, 107, 1788-1798,1994.

16. Lutshenko S., Cooper M.J., Localization of the Wilson disease protein product to mitochondria. Proc. Natl. Acad. Sci. USA, 95, 6004-6009, 1998.

17. Schaefer M., Hopkins R., Failla M., et al., Hepatocyte-specific localization and copper-dependent trafficking of the Wilson's disease protein in the liver. Am. J. Physiol., 276, G639-G646, 1999.

18. Sternlieb I., Van den Hamer C.J.A., Morell A.G., Alpert S., Gregoriadis G., Scheinberg I. H., Lysosomal defect of hepatic copper excretion in Wilson's disease (hepatolenticular degeneration). Gastroenterology, 64, 99-105, 1973.

19. Scheinberg I.H., Gitlin D., Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). Science, 116, 484-485, 1952.

20. Wilson D.C., Phillips M.J., Cox D.W., Roberts E.A., Severe hepatic Wilson's disease in preschool-aged children. J. Pediatr. 137, 719-722, 2000.

21. Wilson A.K., Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain, 34, 295-307,1912.

22. Shilsky M.L., Scheinberg I.H., Sternlieb I., Liver transplantation for Wilson's disease: indications and outcome. Hepatology, 19, 583-587, 1994.

23. Angius A., Dessi V., Lovicu M., et al., Early and severe nurological features in a Wilson disease patient compound heterozygous for two frameshift mutations. Eur. J. Pediatr. 157, 128-129, 1998.

24. Firneisz G., Szalay F., Halasz P., Komoly S., Hypersomnia in Wilson's disease: an unusual symptom in an unusual case. Acta Neurol. Scand., 101, 286-288, 2000.

25. Brewer G.J., Fink J.K., Hedera P., Diagnosis and treatment of Wilson's disease. Semin. Neurol., 19(3), 261-270, 1999.

26. Bono W., Moutie O., Benomar A., Aidi S., el Alaoui-Faris M., Yahyaoui M., Chkili T., Wilson's disease. Clinical presentation, treatment and evolution in 21 cases. Rev. Med. Interne, 23(5), 419-431, 2002.

27. Pandit A., Bavdekar A., Bhave S., Wilson's disease. Indian J. Pediatr., 69(9), 785-791, 2002.

28. Cossu P., Pirastu M., Nucaro A., et al, Prenatal diagnosis of Wilson's disease by analysis of DNA polymorphism. N. Engl. J. Med., 327(1), 57, 1992.

29. Sternlieb I., Wilson's disease and pregnancy (editorial). Hepatology, 31, 531-532, 2000.

30. Hantos M. B., Tekes K., Szalay F., Peripheral nociceptin level in Wilson's disease patients. Acta Pharm. Hung., 72, 260-264, 2002.

31. Subramanian I., Vanek Z. F., Bronstein J., Diagnosis and treatment of Wilson's disease. Curr. Neurol. Neurosci. Rep., 2(4), 317-323, 2002.

32. Brewer G.J., Wilson's Disease. Curr. Treat. Options Neurol., 2(3),193-204, 2000.

33. Schilsky M.L., Diagnosis and treatment of Wilson's disease. Pediatr. Transplant., 6(1), 15-19, 2002.

34. Brewer G.J., Recognition, diagnosis, and management of Wilson's disease. Proc. Soc. Exp. Biol. Med., 223(1), 39-46, 2000.

35. Gollan J.L., Gollan T.J., Wilson disease in 1998: genetic, diagnostic and therapeutic aspects. J. Hepatol., 28(Suppl 1), 28-36, 1998.

36. Smolarek C., Stremmel W., Therapy of Wilson disease. Gastroenterol, 37(4), 293-300, 1999.

37. Walshe J.M., Yealland M., Chelation treatment of neurological Wilson's disease. Q. J. Med., 86, 197-204, 1993.

38. Walshe J.M., Penicillamine: A new oral therapy for Wilson's disease. Am. J. Med., 21, 487-495, 1956.

39. Schilsky M.L., Treatment of Wilson's disease: what are the relative roles of

penicillamine, trientine, and zinc supplementation? Curr. Gastroenterol. Rep., 3(1), 54-59, 2001.

40. Scheinberg I.H., Jaffe M.E., Sternlieb I., The use of trientine in preventing the effects of interrupting penicillamine therapy in Wilson's disease. N. Engl. J. Med., 317, 209-213, 1987.

41. Brewer G.J., Johnson V., Dick R.D., et al., Treatment of Wilson disease with ammonium tetrathiomolybdate. II. Initial therapy in 33 neurologically affected patients and follow-up with zinc therapy. Arch. Neurol., 53,1017-1025, 1996.

42. Brewer G.J., Treatment of Wilson' s Disease with zinc. J. Lab. Clin. Med., 134(3), 322-324, 1999.

43. Brewer G.J., Zinc acetate for the treatment of Wilson's disease. Expert Opin. Pharmacother. 2(9), 1473-1477, 2001.

44. Brewer G.J., Johnson V.D., Dick R.D., Hedera P., Fink J.K., Kluin K.J., Treatment of Wilson's disease with zinc. XVII: treatment during pregnancy. Hepatology, 31(2), 364-370, 2000.

45. Najda J., Stella-Holowiecka B., Machalski M., Low-dose zinc administration as an effective Wilson's disease treatment. Biol. Trace Elem. Res., 80(3), 281-284, 2001.

46. Sturniolo G.C., Mestriner C., Irato P., Albergoni V., Longo G., D'Inca R., Zinc therapy increases duodenal concentrations of metallothionein and iron in Wilson's disease patients, Am. J. Gastroenterol., 94(2), 334-338, 1999.

47. Santon A., Irato P., Medici V., Inca R. D., Albergoni V., Sturniolo G. C., Effect and possible role of Zn treatment in LEC rats, an animal model of Wilson's disease. Biochimica et Biophysica Acta, 1637, 91-97, 2003.

48. Shimizu N., Yamaguchi Y., Aoki T., Treatment and management of Wilson's disease. Pediatr Int., 41(4), 419-422, 1999.

49. Brewer G.J., Dick R.D., Johnson V.D., Fink J.K., Kluin K.J., Daniels S., Treatment of Wilson's disease with zinc XVI: treatment during the pediatric years. J. Lab. Clin. Med., 137(3), 191-198, 2001.

50. Hungarian Pharmacopoeia VII. Edition, Medicina, Budapest, 1986.

51. European Pharmacopoeia 4th Edition, Council of Europe, Strasbourg, France, 2001.

52. Efentakis M., Vlachou M., Choulis N. H., Effects of excipients on swelling and drug release from compressed matrices. Drug. Dev. Ind. Pharm., 23, 107-112, 1997.

53. Lin S.Y., Effect of excipients on tablet properties and dissolution behavior of theophylline tableted microcapsules under different compression forces. J. Pharm. Sci., 77(Mar), 229-232, 1988.

54. Baveja S.K., Ranga Rao K.V., Effect of additives on release of centperazine from matrix tablets. Indian J. Pharm. Sci., 48(May-Jun), 69-71, 1986.

55. Dredan J., Zelko R., Bihari E., Racz I., Gondar E., Effect of polysorbates on drug release from wax matrices. Drug. Dev. Ind. Pharm., 24(6), 573-576, 1998.

56. Al-Shora H., Said S., Hammad A.L., Sustained release from inert matrices.

Part 2. Effect of polyethylene glycols on theophylline release. Int. J. Pharm., 7, 77-82, 1980.

57. Schroeder H.G., Dakkuri A., DeLuca P.P., Sustained release from inert wax matrixes. 1. Drug-wax combinations. 2. Effect of surfactants on tripelennamine hydrochloride release. J. Pharm. Sci., 67(Mar), 350-357, 1978.

58. Dakkuri A., Butler L.D., DeLuca P.P., Sustained release from inert wax matrixes. 3. Effect of povidone on tripelennamine hydrochloride release. J. Pharm. Sci., 67(Mar), 357-360, 1978.

59. Roohk B.L., Meister F.L., Hewett M.K., Rumsfeld J.A., Comparison of 16-meq and 20-meq daily potassium supplement regimens. Clin. Pharm., 7(Feb), 150-152, 1988.

60. Strom B.L., Carson J.L., Schinnar R., Sim E., Morse M.L., et-al., Upper gastrointestinal tract bleeding from oral potassium chloride: comparative risk from microencapsulated vs wax matrix formulations. Arch. Intern. Med., 147(May), 954-957, 1987.

61. Haxholdt H., Hey H., Lech Y., Matzen P., Endoscopic comparison of the effect of two formulations of potassium chloride and placebo on gastric mucosa. Curr. Ther. Res., 40(Sep), 561-566, 1986.

62. Aulton M.E., Pharmaceutics: The Science of Dosage Form Design. Churchill Livingstone, Edinburgh-London-Melbourne-New York, 1988.

63. Bodmeier R., Paeratakul O., Chen H., Zhang W., Formation of sustained

release wax matrices within hard gelatin capsules in a fluidized bed. Drug Dev. Ind. Pharm., 16(9), 1505-1519, 1990.

64. Hardman J.G., Limbrid L.E., Goodman's and Gilman's The pharmacological basis of therapeutics, 9th Edition, McGraw Hill, New York, 1995.

65. Bechgaard H., Nielsen G.H., Controlled-release multiple-units and single-unit doses. A literature review. Drug. Dev. Ind. Pharm., 4, 53-67, 1978.

66. Follonier N., Doelker E., Biopharmaceutical comparison of oral multiple-unit and single-unit sustained-release dosage forms. STP Pharma Sciences, 2, 141-158, 1992.

67. Moroni A., Ghebre-Sellassie I., Application of poly(oxyethylene) homopolymers in sustained release solid formulations. Drug. Dev. Ind. Pharm., 21 (12), 1411-1428, 1995.

68. Brock C., Curry H., Comparative incidence of side effects of a wax matrix and a sustained release iron preparation. Clin. Ther., 7(4), 492-496, 1985.

69. Higuchi T., Mechanism of sustained-action medication. J. Pharm. Sci., 52, 1145-1149, 1963.

70. Carstensen J.T., Pharmaceutics of Solids and Solid Dosage Forms, John Wiley and Sons, New York, 1977, pp 63-76.

71. Evers P., Drug delivery technology: responding to medical and market needs. Pharmaceut. Technol. Eur., 8(3), 38-44, 1996.

72. Rácz I., Drug formulation. Wiley, New York, 1989, pp 367-369.

73. Blume H., Gundert-Remy U., Möller, Controlled/ Modified Release Products, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1991.

74. De Brabander C., Vervaet C., Görtz J.P., Remon J.P., Berlo J.A., Bioavailability of ibuprofen from matrix mini-tablets based on a mixture of starch and microcrystalline wax. Int. J. Pharm., 208, 81-86, 2000.

75. Zhou F., Vervaet C., Schelkens M., Lefebvre R., Remon J.P., Bioavailability of ibuprofen from matrix pellets based on the combination of waxes and starch derivatives. Int. J. Pharm., 168, 79-84, 1998.

76. Samani S.M., Montaseri H., Kazemi A., The effect of polymer blends on release profiles of diclofenac sodium from matrices. Eur. J. Pharm. Biopharm., 55, 351-355, 2003.

77. Rao Y.M., Veni J.K., Jayasagar G., Formulation and evaluation of diclofenac sodium using hydrophilic matrices, Drug Dev. Ind. Pharm., 27, 759-766, 2001.

78. Solinis M.A., De la Cruz Y., Hernandez R.M., Gascón A.R., Calvo B., Pedraz J. L., Release of ketoprofen enantiomers from HPMC K100M matrices-diffusion studies. Int. J. Pharm., 239, 61-68, 2002.

79. De Brabander C., Vervaet C, Fiermans L., Remon J.P., Matrix mini-tablets based on starch/microcristalline wax mixtures. Int. J. Pharm., 199, 195-203, 2000.

80. Colombo P., Bettini R., Santi P., Peppas N.A., Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. Pharm. Sci. Technol. Today, 3, 198-204, 2000.

81. Zhou F., Vervaet C., Remon J.P., Matrix pellets based on the combination of waxes, starches and maltodextrines. Int. J. Pharm., 133, 155-160, 1996.

82. Picker K.M., Mielck J.B., Effect of relative humidity during tableting on matrix formation of hydrocolloids: densification behaviour of cellulose ethers. Pharm. Dev. Technol., 3(1), 31-41, 1998.

83. Ries R., Moll F., Matrix formation of polyglycolic acid tablets by annealing. Eur. J. Pharm. Biopharm., 40(1), 14-18, 1994.

84. Fassihi A.R., Continous matrix formation for controlled drug release: compression of isotropic polymeric system. Int. J. Pharm., 34,169-172, 1986.

85. Dredan J., Zelko R., Bihari E., Racz I., Effect of physicochemical properties of polyethylene glycol derivatives on drug release from wax matrices. Acta Pharm. Hung., 68(4), 210-213, 1998.

86. Adikwu M.U., Chukwu A., Chiori C.O., Sealant effect of dika fat in the microcapsules of a highly water-soluble drug: chloroquine phosphate. Acta Pharm. Jugosl., 41(1), 61-65, 1991.

87. Emori H., Ishizaka T., Koishi M., Effects of acrylic acid polymer and its arrangement on drug release from a wax matrix. J. Pharm. Sci., 73, 910-915, 1984.

88. Giannola L.I., De Caro V., Rizzo M.C., White beeswax microspheres with valporic acid. Drug Dev. Ind. Pharm., 21(7), 793-807, 1995.

89. Dredan J., Antal I., Rácz I., Evaluation of mathematical models describing

drug release from lipophilic matrices. Int. J. Pharm., 145, 61-64, 1996.

90. Su X.Y., Al-Kassas R., Li Wan Po A., Statistical Modelling of Ibuprofen Release from Spherical Lipophilic Matrices. Eur. J. Pharm. Biopharm., 40(2), 73-76, 1994.

91. Langenbucher F., Parametric representation of dissolution-rate curves by the RRSBW distribution. Pharm. Ind., 38(5), 472-477, 1976.

92. Peppas N.A., Analysis of fickian and nonfickian drug release from polymers. Pharm. Acta. Helv., 60, 110-111, 1985.

93. Ravelli V., Rossi R., Prolonged release formulation technology. Pharm. Manufact. Int., 171-172, 1995.

94. Huang H-P., Mehta S.C., Radebaugh G.W., Fawzi M.B., Mechanism of drug release from an acrylic polymer-wax matrix tablet. J. Pharm. Sci., 83(6), 795-797, 1994.

95. Otsuka M., Matsuda Y., Effect of cogrinding time on the release of pentoxifylline from waxy matrix tablets. J. Pharm. Sci., 83, 956-961, 1994.

96. Macheras P., Dokoumetzidis A., On the Heterogenity of Drug Dissolution and Release. Pharm. Res., 17(2), 108-112, 2000.

97. Rácz I., Zelkó R., Bihari E., Bucsek M., Effect of Eudragit type polymers on the drug release from magnesium oxide granules produced by laboratory fluidization. Drug Dev. Ind. Pharm., 21(18), 2085-2096, 1995.

98. Rácz I., Zelkó R., Bihari E., Mathematical Modelling of Magnesium Oxide Release from Granules Produced by Laboratory Fluidization. Drug Dev. Ind.

Pharm., 22(3), 225-230, 1996.

99. Dredán J., Zelkó R., Antal I., Bihari E., Rácz I., Effect of chemical properties on drug release from hydrophobic matrices.
Int. J. Pharm., 160 (2), 257-260, 1998.

100. Dredán J., Zelkó R., Antal I., Bihari E., Rácz I., Effect of the Particle Size and the Coating Level on the Diffuse Reflectance of Wax Matrices. J. Pharm. Pharmacol., 50, 139-142, 1998.

101. Antal I., Zelkó R., Roczey N., Plachy J., Rácz I., Dissolution and diffuse reflectance characteristics of coated theophylline particles.
Int. J. Pharm., 155(1), 83-89, 1997.

102. Cooke N.E., Chen C., A contribution to a mathematical theory for polymer-based controlled release devices. Int. J. Pharm., 115, 17-27, 1995.

103. Zelkó R., Antal I., Bihari E., Dredán J., Benko B., Rácz I., Substrate-binder interactions in theophylline granules.
Die Pharmazie, 53(3), 205-206, 1998.

104. Weyer L.C., Near infrared spectroscopy of organic substances. Appl. Spectrosc. Rev., 21, 1-43, 1985.

105. Morisseau K.M., Rhodes C.T., Pharmaceutical uses of near-infrared spectroscopy. Drug Dev. Ind. Pharm., 21, 1071-1090, 1995.

106. Rácz I., Dredán J., Antal I., Gondár E., Comparative evaluation of microcapsules prepared by fluidization atomization and melt-coating process. Drug Dev. Ind. Pharm., 23, 1-5, 1997.

107. Rhodes C.T., Morisseau K., Tablet Evaluation Using Near-Infrared Spectroscopy. In: James Swarbrick, James C. Boylan (editors): Encyclopedia of Pharmaceutical Technology, Second Edition, Marcel Dekker, 2002, p. 2689-2700.

108. Pharmaceutical Excipients 2004 (Gelatine monograph), (Editor: Raymond C Rowe, Paul J Sheskey, Siân C Owen) Pharmaceutical Press and American Pharmacists Association, London, UK

PUBLICATIONS AND LECTURES

Publications

1. **J. Nagy**, M. Vajna, L. Dévényi, R. Zelkó: Comparison of zinc sulphate release and morphology of matrices prepared for the individual therapy of Wilson's disease.
Die Pharmazie, 59(4), 327-328, (2004). IF=0,696

2. R. Zelkó, Á. Orbán, **J. Nagy**, G. Csóka, I. Rácz: Coating polymer-plasticizer interaction in relation to the enthalpy relaxation of polymer.
Journal of Thermal Analysis and Calorimetry. 68(2), 531-537 (2002).
IF=0,598

3. **Nagy J.**, Vincze Z., Folhoffer A., Horváth A., Csák T., Zelkó R.: A Wilson-kór patomechanizmusa és gyógyszeres kezelése.
Acta Pharmaceutica Hungarica, 73, 237-241 (2003).

4. **J. Nagy**, A. Folhoffer, A. Horvath, T. Csak, G. Taba, K. Szentmihályi, F. Szalay, R. Zelko: Kinetic study of zinc sulphate release from lipophilic matrices prepared for the therapy of Wilson's disease.
Die Pharmazie, közlésre elfogadva. (Ref: 4122) IF=0,696

Lectures

1. **Nagy Judit**, Dr Zelkó Romána, Dr Vincze Zoltán: Wilson-kór individuális terápiáját biztosító bevont tabletta formulálási vizsgálata.
Gyógyszer az ezredfordulón IV. Továbbképzo Konferencia, Sopron, 2001. október 24-27.

2. **Nagy Judit**: Wilson-kór individuális terápiájának biztosítása cink-szulfát mátrix tablettával.
XXXVII. Rozsnyai Mátyás Emlékverseny, Kaposvár, 2002. május 23-25.

3. **Nagy Judit**: Wilson-kór individuális terápiájának biztosítása cink-szulfát mátrix tablettával. *Ph. D. Tudományos Napok 2002, Semmelweis Egyetem Doktori Iskola, Budapest, 2002. június 7-8.*

4. **Nagy Judit**, Vajna Márton, Zelkó Romána: Cink-szulfát mátrix rendszerek eloállítása és vizsgálata Wilson-kór individuális terápiájához.
XIV. Országos Gyógyszertechnológiai Konferencia. Hévíz, 2002. november 8-10.

5. **Nagy Judit**: Formulation and evaluation of zinc-sulphate matrices for the therapy of Wilson's disease.
Ph. D. Tudományos Napok 2003, Semmelweis Egyetem Doktori Iskola, Budapest, 2003. április 10-11.

6. **Nagy Judit**, Vajna Márton, Zelkó Romána, Vincze Zoltán: Cink-szulfát mátrix rendszerek formulálása és vizsgálata Wilson-kór individuális terápiájára.
Congressus Pharmaceuticus Hungaricus XII., Budapest, 2003. május 8-10.

OFFPRINTS OF THE PUBLICATIONS