Controlled Release of Model Drug Delivery Species from Polymer Matrix to Simulated Body Fluids

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2. Způsoby, principy, faktory a kinetiky uvolňování léčiv z těchto systémů.
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Praktická část:
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1. UHRICH, K.; E., CANNIZZARO, S. M.; AND ROBERT, S. L. Polymeric Systems for Controlled Drug Release, Chem. Rev. 1999, no.99, Pp.3181–3198.
2. LAKSHMI, S., N.; CATO T., L. Polymers as Biomaterials for Tissue Engineering and Controlled Drug Delivery, Adv. Biochem Engin/Biotechnol, 2006, no. 102, Pp. 47–90.
3. GRASSI, M.; GRASSI, G. Mathematical Modelling and Controlled Drug Delivery: Matrix Systems, Current Drug Delivery, 2005, no. 2, Pp. 97–116
4. COSTA, P.; MANUEL, J.; LOBO, S. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences, 2001. Sv. 13, c. 2, s. 123–133. ISSN 0928–0987.

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ABSTRAKT

Tato bakalářská práce je zaměřena na uvolňovací studie krystalové violeti z PVC matrice. V teoretické části jsou popsány vhodné polymery pro přípravu matric, typy matric, modely popisující kinetiku uvolňování a způsoby vyhodnocování uvolněné či neuvolněné látky z polymeru. Praktická část shrnuje přípravu, charakterizaci vlastností připravených filmů a samotné uvolňovací studie.

Klíčová slova: přírodní a syntetické polymery, řízené uvolňování, matricové tablety, farmakokinetické modely, uvolňování léčiva, rozpouštění

ABSTRACT

This bachelor thesis focuses on release studies of crystal violet from PVC matrices. Theoretical part describes polymers which are suitable for matrix preparations, matrix types, release kinetic models and methods which evaluate released or unreleased substances from polymer. Practical part deals with preparation of samples, their properties characterization and the release studies.

Keywords: natural and synthetic polymers, controlled release, matrix tablets, pharmacokinetic models, drug release, dissolution procedures

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INTRODUCTION

Nowadays, polymers offer a high quantity of applications in a lot of fields. They are used for manufacturing products which are used in everyday life. For example bottles, packets, toys .etc.

In the pharmaceutical and medical field they mostly have utilization as suitable biodegradable or non-biodegradable drug carriers. Their utilization leads to slow release of active substance, increasing the solubility and better opportunities for targeting and controlled release in present pharmacotherapy [1].

The "controlled release" term appears since seventies of the past century. Dosage forms with controlled release are frequently used because of theirs several advantages in comparison with classical dosage forms, such as decreased frequency of drug dosage, reduction of the fluctuation in plasmatic level of the used drug substance, decreased occurrence of the side effects and with this related improved compliance of the patient.

Controlled release can be further divided to:

- Prolonged release.
- Delayed and pulse release.

Prolonged release secures the right level of drug in blood plasma for desired time, which means for longer time than occurs after one application. Delayed release is advantageous when desired absorption of drug is in the intestinal tract or in its specific part (colon) [2].

I. THEORETICAL PART

1 POLYMERS AS DRUG CARRIERS

Polymers are used in drug delivery when the control of oral absorption or the delivery of genes to the specific cells interior is desired. Macromolecules are nowadays presented as extraordinary components of a variety of drug delivery systems. Polymeric systems are suitable for pharmaceutical and medicinal application for many reasons. They can:

- Prolong the drug action by entrapping the drug within matrices.
- Shift the drug distribution in the direction of tumors.
- Shunt the therapeutic genes or oligonucleotides into cells.
- Enable the drug absorption at optimum GIT absorptive sites.
- Make the drug available only when there is defined change in the temperature or pH or while activated by an enzyme.

Polymers are also preferred because of their unique cooperative properties that are not found in low-molecular-weight compounds. A simple example can be the ability of polymers to restrict the diffusion of low-molecular-weight compounds into a matrix. Also, the manipulation of water solubility by increasing their chain length through cross-linking or by hydrophobising or hydrophilizing them with copolymers and other groups, proceeds a lot of materials with a wide spectrum of possible applications. The resulting materials are capable for a several drug-enhancing functions such as:

- Prolong the drug availability if pharmaceuticals are formulated as hydrogels or microparticles.
- Favorably alter biodistribution if they are packed into dense nanoparticles.
- Enable hydrophobic drug administration if it is formed into micelles.
- Transport a drug to its usually inaccessible site of action if they are formulated as gene medicines.
- Make drugs available in response to stimuli.

It is really important to choose the right polymer. For example a drug delivery device or system have some critical key properties as the need to release the drug at the desired rate, and in order to obtain clinical success, the delivery vehicle polymer components must swell (or not), degrade (or not), dissolve (or not) or be retrieved (if necessary) after the drug is depleted [3].

Polymers can be basically divided to two groups:

- Synthetic polymers
- Natural polymers

The choice of natural polymers is more suitable for biomedical or pharmaceutical applications due to their excellent biocompatibility, good ability to simulate native cellular environments, unique mechanical properties and biodegradability by enzymatic or hydrolytic mechanisms. On the other hand, synthetic polymers have enormous advantages over natural ones from the material side. Their synthetic flexibility allows development of polymers with a wide spectrum of properties with excellent reproducibility. Variation of their structure leads to the feasible fine-controlling of the degradation rate [4,5].

1.1 Synthetic polymers

1.1.1 Polyethylene (PE), Polypropylene (PP)

PE is used in drug delivery as polymer matrix. Drug zero-order temporal control is achieved by drug diffusion from PE matrices. Also prolonged pseudo-first order release of drug acetoaminophen in gastrointestinal tract (GIT) is positive effect of PE matrix.

PP is applied in ophthalmic drug delivery.

1.1.2 Polyvinylchloride (PVC), Polyvinylalcohol (PVA), Polyethylene-vinylacetate (pEVAc)

PVC is used as polymer matrix and membrane devices that form volatile agents into air and non-volatile agents into aqueous solutions.

PVA stabilizes surface in microsphere formulation and it is useful in production of bioadhesive hydrogels.

pEVAc is used in magnetically controlled temporal release, ultrasound-stimulated release, subcutaneous implants for cancer pain relief and as chemotherapeutic agents.

1.1.3 Polyacrylamides

Polyacrylamid is plasma expander useful as polymer-drug conjugate for distribution control. It has enzyme cleavable side-chains employed to target release in the colon. It is also a component of photosensitive delivery systems.

1.1.4 Polyacrylates, Polyethyleneglycol (PEG)

Polyacrylates have been utilized in controlled drug release as polymer-drug conjugates and also in topical applications.

PEG is used as diffusion-limited tablet formulation, cross-linked hydrogels and polymer-drug conjugates [6].

1.1.5 Aliphatic polyesters

The commonly used monomers for aliphatic polyesters are lactide, glycolide and caprolactone. This group of polymers contains Polyglycolic acid, polylactic acid, polycaprolactone, polylactide-*co*-glycolide, polydioxanone, polyclyconate and polypropylene fumarate.

They have highly crystalline (polyclygolic acid) or semicrystalline structure (other polymers in this group). Their melting temepratures are between 50-200 °C. Except polyglycolic acid they have good solubility in common organic solvents.

The aliphatic polyesters undergo bulk degradation, where material is lost from the entire polymer volume at the same time due to the bulk water penetration. The rate of degradation of these polymers is determined by the extent of water accessibility to the matrix, which depends on several factors as the hydrophobicity, hydrophilicity, crystallinity of the polymer and the dimension of the sample.

In drug delivery applications, the drug release patterns show a marked initial burst release which is followed by a slow continuous release.

1.1.6 Polyorthoesters

Polyorthoesters are hydrophobic polymers with hydrolytically sensitive backbone. They were developed by Alza Corporation.

The high hydrophobicity of the matrix reduces the water penetration into the bulk so these polymers undergo surface erosion and the rate of degradation can be controlled by using diols having different degrees of chain flexibility as well as by the incorporation of acidic and basic excipients.

There are four types: Polyorthoester I, Polyorthoester II, Polyorthoester III and Polyorthoester IV.

1.1.7 Polyanhydrides

Polyanhydrides are surface-eroding and the most hydrolytically unstable polymers. They have a highly hydrophobic backbone and at the same time highly hydrolytically sensitive anhydride bond. This hydrophobicity impedes water penetration into the matrix. The degradation and erosion of the polymer are essentially confined to the surface as evidenced from the linear mass lost kinetics of polymer during degradation. These matrices are directed by zero-order drug release.

Aliphatic polyanhydrides are highly crystalline and they have very high degradation rates.

Aromatic polyanhydrides have very high hydrolytic stability and very high melting points.

For example polysebacic anhydride, polycarboxy-phenoxy propane-sebacic acid and polyanhydride-*co*-imides belong to this group.

1.1.8 Polyalkylcyanoacrylates

They form a unique class of biodegradable polymers where the carbon-carbon bonds in the polymer are cleaved by hydrolysis.

These polymers exhibit suitable properties as high rates of degradation ranging and what makes them good candidates for drug delivery and drug targeting.

1.1.9 Polyphosphoesters

Polyphosphoesters are a class of inorganic polymers with a unique backbone consisiting of phosphorus atoms attached to a carbon or oxygene atom. They degrade under the physiological conditions due to the hydrolytic and enzymatic cleavage of the phosphate bonds in the backbone [7].

1.2 Natural polymers

1.2.1 Gelatin

Gelatin belongs to natural polymers which are derived from collagen and it is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments. Gelatin contains a large number of glycine, proline and 4-hydroxyproline residues. It is a denaturated protein and can be obtained by acid and alkaline processing of collagen. The mostly used types of gelatin are basic gelatin with isoelectric point of 9.0 and acidic gelatin with isoelectric point at 5.0. Theoretically it

is possible to form polyion complexes of gelatin with any type of charged biomolecules, although the strength of the interaction is determined by the type of used molecules. For acidic drugs or molecules, basic gelatin with an isoelectric point 9.0 is better as a matrix, while gelatin with an isolelectric point 5.0 is preferable for alkaline molecules.

1.2.2 Chitosan

Chitosan is a cationic polysacharide obtained from chitin. Chitosan is biodegradable and bioadhesive material. The adhesive properties of chitosan in a swollen state persist well during repeated contacts of chitosan and the substrate. For the purposes of drug delivery, chitosan exhibits a pH-sensitive behavior as a weak poly-base due to the large quantities of amino groups in its chain. Chitosan dissolves easily at low pH while it is insoluble at higher pH ranges. The mechanism of pH-sensitive swelling involves the protonation of amine groups of chitosan under low pH conditions.

1.2.3 Starch

With its inherent biodegradability, overwhelming abundance and renewability starch can be classified as the most promising natural polymer. Starch is composed of α-amylose and amylopectin.

It is used for drug delivery applications, including cancer therapy and nasal administration of insulin.

1.2.4 Alginates

Alginates are found as structural components of marine brown algae and as capsular polysaccharides in some soil bacteria.

Alginate-based materials are pH-sensitive. Molecules released from alginate-based materials in low pH solutions are reduced and they can give an advantage in the development of a delivery system. Alginate shrinks at low pH and the encapsulated drugs are not released. This pH dependent behavior of alginate is exploited to adjust release profiles and in the development of ‘smart’ systems. At a higher pH, alginate undergoes a rapid dissolution which may results in a burst release of protein drugs and subsequently their denaturation by proteolytic enzymes. Therefore, many modifications in the physicochemical properties are needed for the prolonged controlled release of drugs [8,9].

2 KINETICAL MODELS OF DRUG RELEASE

Mathematical modeling of controlled drug delivery provides a scientific knowledge base concerning the mass transport mechanisms which are involved in the control of drug release. Modeling is used for simulations of the device and design parameters effects on the resulting drug release kinetics. Type of drug, kind of excipients and the composition of the device strongly influence the choice of the appropriate mathematical model. Also route of drug administration is an important criterium that needs to be considered. Optimization of existing and development of new pharmaceutical products is significantly facilitated by mathematical modeling [10].

There are number of kinetically models, which describe the overall release of drug from dosage forms. Methods describing the drug release are divided into three categories:

- Statistical methods (exploratory data analysis method, repeated measures design, MANOVA: multivariate analysis of variance)
- Model dependent methods (for example zero order, first order, Higuchi, Weibull model, etc.)
- Model independent methods [11].

2.1 Fundamentals of drug release kinetics

2.1.1 Noyes-Whitney equation

The first experiment with dissolution of solid substances was performed by A. A. Noyes and W. R. Whitney in 1897. They published fundamental equation for kinetic of drug release.

$$\frac{dM}{dt} = kS\ (C_s - C_t) \qquad (1)$$

Where k is constant, M is mass transferred with respect to time t ,by dissolution from the solid particle of instantaneous surface S, under the effect of prevailing concentration driving force $(C_s - C_t)$. C_t is the concentration at the time t and C_s equilibrium solubility of the solute at the experimental temperature. The dM/dt is the rate of dissolution and it describes the dissolved amount per unit area per unit time and can be expressed for solid in g/cm^2s^1 .

2.1.2 Nernst and Brunner equation

This equation is based on the diffusion layer concept and the second Fick´s law and it describes a relationship between constant in the equation (1) and the diffusion coefficient of the solute.

$$k = \frac{DS}{h\gamma} \tag{2}$$

where D is diffusion coefficient, S is the area of dissolving surface or area of the diffusion layer, γ is the solution volume and h is the diffusion layer thickness [12,13].

2.2 Statistical methods

2.2.1 Exploratory data analysis methods

These analysis´ mediate an improved understanding of the dissolution data of controlled release formulation and its main task is to compare dissolution profile data in numerical and also in graphical manner. These methods are not still validated by FDA (U.S. Food and Drug Administration), but their use is recommended.

2.2.2 Multivariate approach (MANOVA)

Manova is based on repeated measurements, where the repeated factor is time and dependency of percent dissolved part is variable. The most known and most used statistic is Wilks´ lambda. The compound symmetry assumption requires that the variances and covariances of the different repeated measures are homogenous.

2.3 Model dependent methods

Different mathematical functions describe the dissolution profiles.

2.3.1 Zero order model

Slow drug release from drug dosage forms that are not dissolving can be represented by this equation:

$$Q_t = Q_0 + K_0 t \tag{3}$$

where Q_t is drug amount dissolved in time t, Q_0 the initial amount of drug (mostly $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time.

This relationship is used for some transdermal drugs, matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

2.3.2 First order model

First order model is used for description of drug absorption and drug elimination. Drug release is then expressed by equation:

$$\frac{dC}{dt} = -Kc \tag{4}$$

where K is first order rate constant in time units-^{1}.

Equation (4) can be also expressed as:

$$logC = \log C_0 - \frac{Kt}{2{,}303} \tag{5}$$

where C_0 is initial drug concentration, K the first order rate constant and t is the time.
This equation describes dissolution of drugs from porous matrix with water-soluble drug inside [14].

2.3.3 Higuchi model

Higuchi model was initially designed for description of drug release from matrix tablets in planar systems. Later it was modified for different geometries and matrix structures including porous systems. It is the most famous and the most often used mathematical equation.

$$\frac{M_t}{A} = \sqrt{D(2\,c_0 - c_s)c_s t} \qquad \text{for } c_0 > c_s \tag{6}$$

where M_t is the cumulative absolute amount of released drug at time t, A is the surface area of the controlled release device exposed to release medium, D is drug diffusivity in the polymer carrier, c_0 initial drug concentration and c_s drug solubility in polymer.

This equation has an advantage of its simplicity, but there are assumptions that need to be considered and kept in mind when applying Higuchi model to controlled drug delivery systems:

- The initial drug concentration needs to be higher than drug solubility.
- Mathematical analysis is based on one-dimensional diffusion so that the edge effects are supposed to be negligible.
- Swelling or polymer carrier dissolution are also negligible.

- The drug diffusivity is constant.
- Perfect sink conditions are maintained [15].

The simplified Higuchi model is expressed by following equation:

$$f_t = Q = K_H \times t^{1/2} \quad (7)$$

where, K_H is the Higuchi dissolution constant. The data obtained were plotted as cumulative percentage drug release versus square root of time [16].

2.3.4 Hixson- Crowell model

Hixson and Crowell have found out that the cylindrical surface of a dissolving substance changes with the time in practice. For example, if spherical particles dissolve in well- agitated bulk fluids the radius of the spheres decreases. Their experiments lead to the " cube-root law" or also known as Hixson-Crowell equation:

$$\sqrt[3]{M_t} = \sqrt[3]{M_0} - kt \quad (8)$$

where M_0 is mass of the particle at time $t=0$, M_t mass of the particle at time t and k is positive constant. Plotting of the cube-root of the remaining particles as a function of time gives a linear dependence. And this equation is based on three following assumptions:

- The dissolved substance concentration does not fundamentally change with the time and perfect sink conditions needs to be provided.
- The dissolving particles have spherical geometry and geometry does not change with the time.
- The particles remain intact and do not disintegrate into smaller fragments during dissolution [17].

2.3.5 Korsmeyer- Peppas model

Korsmeyer-Peppas model is used for purposes of describing mechanism composed of drug diffusion (Fickian transport) and Case II transport (non-Fickian) controlled by polymer chain relaxation. It can predict the release mechanism during the first 10 hours and it is described by equation:

$$\frac{M_t}{M_\infty} = K.t^n \quad (9)$$

where Mt/M_∞ is the drug fraction released at time t, K is the rate constant and n is the diffusion exponent that indicates release mechanism. Values of n between 0.5 and 1 refer to anomalous transport kinetics. The n about 0.5 is for pure diffusion. Values of n below 0.5 indicate drug diffusion through the swollen matrix and water filled pores. This model can be applied for release profile analysis in polymeric systems [18].

2.3.6 Baker- Lonsdale model

This model was developed from Higuchi equation (6) and it is used for description of controlled drug release from a spherical matrix and its representation is following equation:

$$f_t = \frac{3}{2}\left[1 - (1 - \frac{M_t}{M_\infty})^{2/3}\right] - \frac{M_t}{M_\infty} = kt \qquad (10)$$

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

This equation is applied for linearization of release data from formulations of microcapsules or microspheres [19].

2.3.7 Weibull model

Weibull model is important because it successfully describes and compares dissolution profile data and it is given by equation:

$$- \frac{M_t}{M_\infty} = 1 - exp(-at^b) \qquad (11)$$

where M_t is amount of drug release at time $_t$, M_∞ amount of drug released at infinite time again and a,b are constants with physical meaning. The exponent b originates from the fact that a depletion zone is created gradually near the boundaries of the release device, and that is why the drug concentration in the device is not uniform [20].

2.3.8 Hopfenberg model

The release of drugs from surface-eroding devices with several geometries is analyzed by Hopfenberg model. This model describes drug release from slabs, spheres and infinite cylinders displaying heterogeneous erosion:

$$\frac{M_t}{M_\infty} = 1 - \left[1 - \frac{k_0 t}{C_0 a_0}\right]^n \qquad (12)$$

where Mt is the amount of drug dissolved in time t, M_∞ is the total amount of drug dissolved when the pharmaceutical dosage form is exhausted, M_t/M_∞ is the fraction of drug dissolved, k_0 is the erosion rate constant, C_0 is the initial concentration of drug in the matrix and a_0 is the initial radius for a sphere or cylinder or the half-thickness for a slab. The value of n is 1, 2 and 3 for a slab, cylinder and sphere, respectively [21].

2.3.9 Gompertz model

Gompertz model describes *in-vitro* dissolution profiles. It is a simple exponential model and it is given by equation:

$$X_{(t)} = X_{max} exp\left[-\alpha e^{\beta \log t}\right] \tag{13}$$

where $X_{(t)}$ = percent dissolved at time t divided by 100, X_{max} = maximum dissolution, α determines the undissolved proportion at time $t=1$ and described as location or scale parameter, β= dissolution rate per unit of time described as shape parameter.

This model has its application in comparison of the drug release profiles where drugs have good solubility and intermediate release rate.

2.3.10 Regression model

For optimizing the formulations from *in-vitro* studies are used several types of regression models.

- Linear or first order regression model- describes linear systems and the empirical model relating response variable to the independent variables is expressed by equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \tag{14}$$

  Where Y is response, X_1 and X_2 are the two variables, β_0 signifies the intercept of the plane, β_1 and β_2 are regression coeficients.
- Quadratic model or second order regression model
- Non-linear model- This method was designed using software to detect factors contributing to differences in the dissolution process of drug delivered in dosage form.

2.4 Model independent methods

2.4.1 Model independent method with utilization of similarity factor

This method is used for comparing dissolution profiles. It has two factors, difference factor (it calculates the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves) and similarity factor (it is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent dissolution between the two curves). Model independent methods are suitable for dissolution profile when three to four or more dissolution time points are available [22].

3 MATRIX TABLETS

Matrix tablets are simple dosage forms. Tablet works as one whole system and its property depends on carrier´s character and character of drug substance. There are four types of matrix tablets:

- Polymeric in-soluble tablets
- Lipophilic tablets
- Hydrophilic gel tablets
- Coated matrix tablet

There are some disadvantages of matrix tablets. Release rate is decreasing with time. This fact is caused by the tablet surface reduction (hydrophilic and lipophilic matrix) and for the polymer in-soluble matrix problem is distance extension in diffusion of drug solution [23].

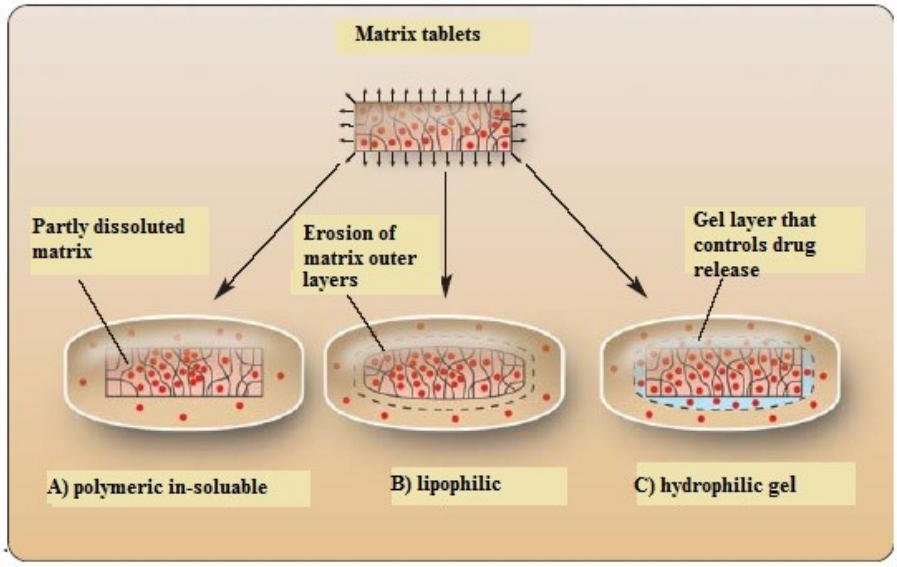

Figure 1 Types of polymeric matrices [24].

3.1 Polymeric in-soluble matrix

The bases are in-soluble polymers. The most used belongs to amonioalkylmethacrylate copolymers (Eudragit RS and Eudragit RL) and ethylcelulose. They are made by simple technologies as direct moulding of powders or moulding of granulation products (granulated powders). Polymeric carrier creates very high-strength porous structure that is called skeleton. The skeleton provides slow release of the active dispersed substance in the gastrointestinal tract (GIT), and thus its absorption and prolonged effect. The shape of the tab-

let does not change while its exposition in GIT and away from organism goes like dissoluted polymeric skeleton. A solution of active substance eludes from matrix by channels filled with water and the permeability of channels depends on porosity of matrix.

3.2 Lipophilic matrix

Basic carriers of lipophilic matrix tablets are fatty acids, fatty alcohols and their esters. Examples are hexadecane-1-ol, glycermonostearate, carnauba wax,…etc.

Drug substances are incorporated to lipophilic matrix by technologies of spray cooling, thermoplastic granulation or direct moulding.

Drug released is occurred on the basis of erosion that is gradual reduction of tablet due to hydrolysis, dissolution of fat and wax due to the enzymes and pH changes in the GIT. Surface erosion of the lipophilic matrix and the rate of drug release depend on the concentration and properties of the carrier and other excipients that are added.

3.3 Hydrophilic matrix

Their basic components are swelling hydrophilic polymers as cellulose derivative (for example methylcellulose, hydroxyethylcellulose,..etc.) or natural substances as gelatin, alginic acid, natural gums. Process of drug release is based on different principles than previous matrix types. Hydrophilic matrix is upon contact with an aqueous medium moistened and release an initial dose of drug dissolved from its surface. Polymer of the matrix surface begins its hydration; solvent increases the mobility of polymer chains and their loosening leads to formation of a swelling gel. A gel barrier on the surface of the matrix is really important for the controlled release of contained drug. Protective gel layer allows water to penetrate into the tablet continuously without being disintegrated. Polymer chains are loosened and escapes into the medium. Original gel layer is gradually dissolved and needs to be replaced by new one that must be strong enough to decrease the diffusion and extend drug release. Consistency of the surface gel layer determines viscosity, polymer concentration, and its chemical structure.

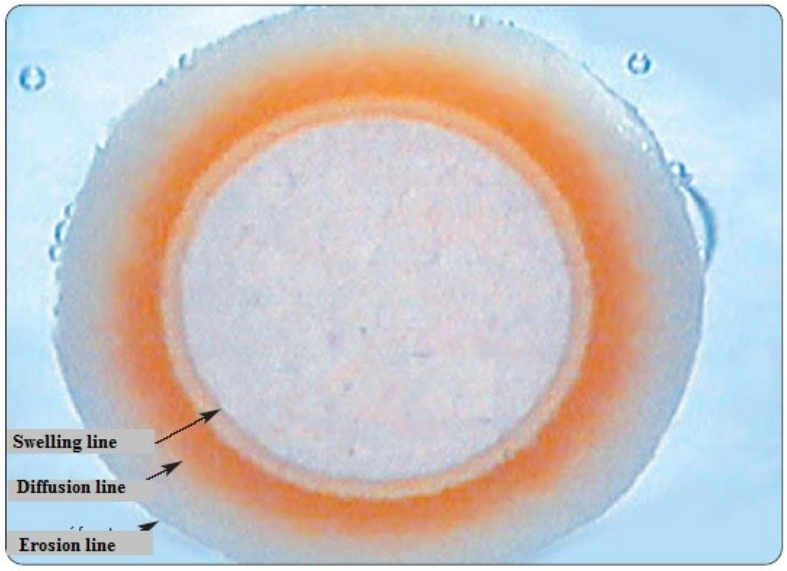

Figure 2 Hydrophilic matrix in the water [25].

3.4 Coated matrix tablets

Coating has mostly correctional or protecting function. These tablets are divided in two groups- permeable and semi-permeable.

Matrixes with permeable coatings are based on controlled dissolution and controlled diffusion. Drug diffusion is implemented by pore diffusion when hydrophilic components are dissolved. Semi-permeable coatings allow penetration of digestive fluids to the matrix nucleus but drug stays in matrix and does not elude out of the matrix. Because of this process there is originated high osmotic pressure and drug can eludes to medium with lower osmotic pressure, for example to the GIT. This process is called controlled osmosis and its advantage is constant release rate.

Release in the intestinal tract is mediated by acid resistant coatings. These coatings protect the matrix from the dissolution in stomach [26,27].

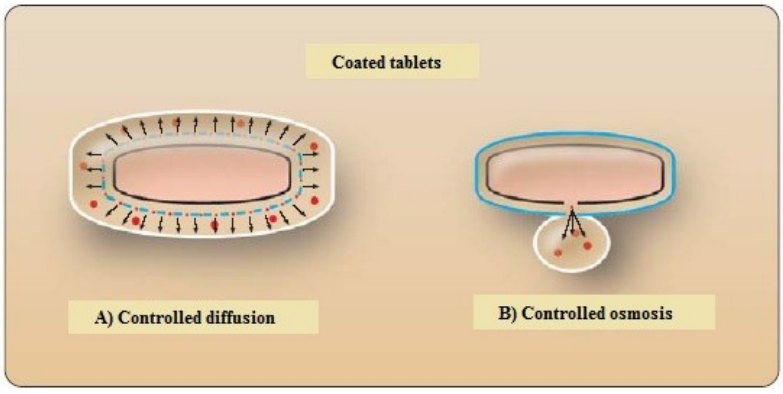

Figure 3 Types of drug release from coated matrix [28].

4 PRINCIPLES OF DRUG RELEASE FROM POLYMER MATRIX

Releasing of drugs from dosage forms is the beginning of pharmacokinetical process that follows after drug´s administration. Releasing is typical for solid dosage forms, because in other forms, as semi-solid and liquid, releasing is coalesced with absorption [29].

Drug release from polymer matrix is based on combination of molecular diffusion, where released drug goes through the gel layer, and erosion of gel layer, that is characteristic for hardly-soluble and practically non-soluble substances [30].

4.1 Mechanisms of diffusion and osmosis

4.1.1 Diffusion

Drug diffusion occurs due to the molecular diffusion or pore-diffusion. Degree of crystallinity and crystal size, degree of swelling, porous structure and tortuosity of polymers affect drug release rate in pore-diffusion. Molecular diffusion is divided into two types- active and passive transport. Passive transport does not require an external energy source and drug travels from a region with high concentration to region with low concentration. Active transport, on the other hand, needs an external source of energy, because the drug is carried against the concentration gradient. The external energy can be given by enzymes or biochemical carriers. Passive molecular diffusion is described by the first Fick´s law, which says that an amount of material flowing through the unit-cross section of barrier in unit time that is also known as flux is proportional to the concentration gradient.

$$J = \frac{dM}{S.dt} \tag{15}$$

where is J = flux in g/cm^{2}s, S = cross section of barrier in cm^2, dM/dt = rate of diffusion in g/s (M = mass in g, t = time in sec).

The flux is proportional to the concentration gradient dC/dx:

$$J = -\frac{dC}{dx} \tag{16}$$

where is D is diffusion coefficient of penetrant in cm^2/s , C is concentration in g/cm^3 or g/mL and x is distance in centimeters of movement perpendicular to the surface of the barrier.

The diffusion coefficient D is physical chemical property of the drug molecule. It is not constant and it changes with concentration, temperature, pressure, solvent properties and chemical nature of the diffusant [31].

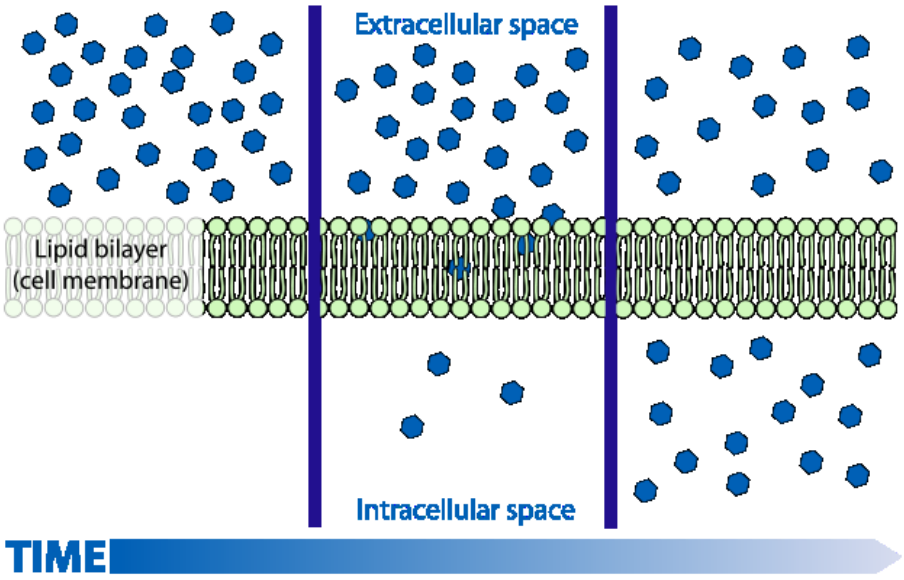

Figure 4 Mechanism of diffusion [32].

4.1.2 Osmosis

The effect of osmosis occurs when membrane is not permeable to the osmolytes. Water equates concentrations of the impermeable solutes on both sides of the membrane and this is given by water flow through the semipermeable membrane. Difference in the chemical potentials of water on both sides and concentration gradient determine the water flow through membrane. These chemical potentials depend on both chemical concentration of osmolytes and the thermodynamic compatibility of water with osmolytes. For the small osmolyte molecules (for example salts), its osmotic pressure is determined by osmolyte concentrations according to van´t Hoff´s law. When the osmolytes are polymers, osmotic pressure is influenced by polymer concentration and polymer/water compatibility. In an adequately hydrated membrane water molecules are in contact with each other and, as a consequence, there is a correlation of neighboring molecules´ motions. Osmotic flow can be reduced by partial permeability of membrane to the osmolytes. As water flows into a device that contains osmolytes, it dilutes osmolytes and lowers the osmotic pressure.

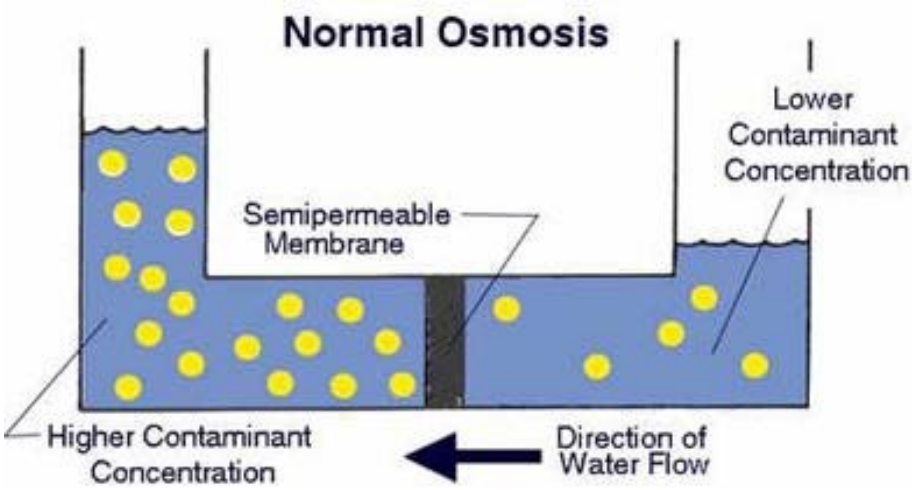

Figure 5 Mechanism of osmosis [33].

4.2 Erosion and degradation

Covalent bonds of polymer can be attacked by components which are released by the medium (especially water) causing polymer erosion. There are two types of erosion- bulk erosion and surface erosion.

When water invades the polymer, than bulk erosion (homogeneous erosion) is occurred. Water establishes its presence throughout the matrix and chain scission is initiated everywhere. Hydrolysis may be really slow especially if the polymer chains are long. Drug release from bulk eroding polymers has three stages. The first one is drug release from the device surface or from pores connected to the surface. The second corresponds to the little degradation of the polymer and trapping the remaining drug. The third is rapid release of trapped drug when the polymer disintegrates.

Surface erosion (heterogeneous erosion) unlike bulk erosion can occur by slow water invasion or rapid hydrolysis. Only surface is affected by erosion, not the inner parts of matrix. Device dimensions decreases with time and also erosion rate decreases with time due to reduction in exposed surface area [34,35].

4.3 Affecting the permeability of gel layer

There are a lot of physical and chemical factors that could affect the permeability of gel layer. Important influence has a type of polymer, its concentration, molecular weight, speed of polymer hydratation, polymer particle size, etc.

4.4 Increasing the solubility of drug substance

Chemical structure, solubility, polymorphism and addition of pharmaceutical accessories-additives have influence on the incorporated drug. Addition of cyklodextrines (CD) leads to better solubility of drug, because molecules of CD have ability to trap other molecules inside their chiral cavities.

4.5 Interaction of components from polymer matrix

Dissolution profile can be influenced by interaction with another component of polymer matrix or interaction of polymer carriers. This can be exploited on drug substances with ionic activity, because interactive products originate by reaction of drug substance with ionic polymers, ion exchangers or surfactants. Change of dissolution profile is caused by change of solubility and growth of molecular weight.

4.6 Change of pH in microenvironment of polymer matrix

Solubility of drug substances highly depends on pH. Since the parts of GIT have different pH, buffer solutions are used to ensure the constant release. Incorporated buffers formulate microregions with pH that ensure uniform release of drug substance without dependence on pH of ambient medium. Effectivity of buffers increases with growth of his power that is given by its dissociation constant and decreases with growth of its solubility in dissolution medium.

Weak acids and salts of weak acids or strong bases are dissoluted better in alkaline medium. Alkaline buffers are used for their better dissolution in acid pH, for example in stomach (pH 1.0- 3.5). As alkaline buffers are used sodium hydrogenphosphate, sodium dihydrogenphosphate, sodium carbonate, calcium carbonate, sodium hydrogencarbonate, magnesium oxide or magnesium hydroxide.

Weak bases and salts of weak bases or strong acids are opposite to weak acids and their salts and better medium for their dissolution is acidic. For their better dissolution in small intestine (pH 6.8- 7.4), acidic buffers as fumaric acid, citric acid, succinic acid, adipic acid, ascorbic acid, glutaric acid and sorbic acid are used [36].

4.7 Critical factors in drug release from polymer matrix

There is a lot of factors influencing drug release like molecular weight, drug solubility, temperature, ionic strength, dose …etc.

4.7.1 Molecular weight

Molecular weight (MW) of polymer determines the strength of polymeric gels and gel strength influences erosion of the polymer matrix. The higher the MW is, the erosion of matrix is more difficult. Also MW of used drug has an influence on the release. Drugs with high MW have lower tendency to diffuse than drugs with low MW.

High MW of polymer and drug causes slower release.

4.7.2 Drug solubility

There are two types of drugs-water soluble and water insoluble. Water soluble drugs are in general released by mechanism of diffusion that has quicker release rates, while water insoluble drugs have tendency to be released by erosion.

4.7.3 Particle size

Particle size affects the entry of water to the polymer matrix.

4.7.4 Viscosity

Polymers have better resistance to erosion when the polymer matrix forms really viscous gel when water enters it. The reason is that the more viscous gel have better ability to capture water and polymer swelling is rapid [37].

5 METHODS FOR EVALUATION ANALYSIS

Nowadays, there are lot analyses methods for whatever evaluation, but in drug delivery following analytical methods, that detect concentration of dissolved or not dissolved drug from polymer, are the most used.

5.1 Ultraviolet and Ultraviolet-visible spectrometry

The UV-VIS spectrometry belongs to the spectral methods and is based on the interaction between the mass and electromagnetic radiation.

Particle (atom, ion, molecule) changes its state to the excited state when energy quantum is absorbed. When the radiation goes through the environment that absorbs, attenuation of the initial radiation can be observed. The absorbent spectra are dependent on this attenuation and wavelength or wavenumber of used radiation. Particles can absorb only that much energy quantum that needs for the transition to an excited state, but remain in the excited state very short time and then return to elementary state.

Principle of the absorption in UV-VIS is that the light coming from the source after setting the system of mirrors, lenses and gaps, goes through the measured absorbing environment and impacts on the detector. Usually there is monochromator in front of the sample or behind the sample. The more absorbing (colored) substance is in the measured sample, the greater is the attenuation of the initial radiation.

- For the visible zone the source is wolfram lamp and for the UV zone it is a deuterium discharge lamp.
- Monochromator determines light in limited (narrow) range of wavelengths.
- Energy of impacted light on detector causes electrical, chemical or thermic effect. The output of detector (analog or digital) is called signal and this signal is in relation with studied parameter (concentration, amount of the analyte…etc.).

Absorbance is defined by this equation:

$$A = -log\frac{I}{I_0} \tag{17}$$

where I is intensity of attenuated radiation and I_0 intensity of radiation that went through blank sample.

Relation between the absorbance of measured solution and concentration of colored substance is described by Lambert-Beer law:

$$A_\lambda = \varepsilon_\lambda . c . l \qquad (18)$$

Where l is length of absorbent layer, c is concentration of colored substance in solution and ε_λ is the molar absorption coefficient expressed in $mol^{-1}.dm^3.cm^{-1}$ [38].

5.2 Chromatography methods

Chromatography is a separation method in which components of substance are separated, and represent qualitative and quantitative analysis of the sample.

Sample is introduced between two mutually immiscible phases. Stationary phase is immobile and mobile phase is fluctuant. Sample is situated to the start of stationary phase and with movement of mobile phase over stationary is sample carried by this system. Components of the sample can be captured by stationary phase and that is why their movement is delayed. Components that have less delay will reach the end of stationary phase as first.

Types of chromatography:

- Liquid chromatography (LC)
- Gas chromatography (GC)
- Paper chromatography (PC)
- Thin layer chromatography (TLC)
- Partition chromatography
- Adsorption chromatography
- Ion exchange chromatography (IC)
- Gel chromatography
- Affinity chromatography

5.3 Atomic emission spectrometry (AES)

The AES principle is monitoring of emission of electromagnetic radiation from free atoms of substances in gaseous state. There are exploited analysis lines (the most intensive selected spectral patterns of the substance). If sample concentration decreases, the least intensive lines from spectral pattern disappear.

5.4 Atomic absorption spectrometry (AAS)

The basic principle of AAS method is absorption of appropriate electromagnetic radiation by free atoms in gaseous state. Radiation that will be absorbed needs to fulfill following requirement:

$$E_1 - E_0 = \frac{hc}{\lambda} \tag{19}$$

Where E_0 is energy of datum level, E_1 is energy of excitation level, h is Planck constant, c is light velocity and λ is wavelength.

The AAS is very sensitive method which can detect ng in 1 mL of substance and with electrotermic atomization it can be even pg in 1 mL. This method is used for analysis of drinking water, in medicine, food industry, for detection of heavy metals in matrices, etc [39].

5.5 Infrared spectroscopy

In infrared spectroscopy, IR radiation passes through the sample and some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Two unique molecular structures cannot produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

IR spectroscopy is useful for:

- Identification of unknown materials.
- Determination of quality or consistency of the sample.
- Determination of the amount of components in the mixture [40].

II. EXPERIMENTAL PART

6 AIMS OF WORK

This bachelor thesis is focused on:

- Preparation of PVC films with active additives.
- Characterization of mechanical, antibacterial, optical properties of the films.
- Studying of active additives release from polymer matrix.

7 USED MATERIALS

For this experiment was prepared blend of polyvinylchloride (PVC) and crystal violet (CV). This blend was dissolved in cyclohexanone (CYH).

7.1 Characteristics of materials

7.1.1 Polyvinylchloride (PVC)

For the experiment was used medical grade PVC compound RB3 which is delivered by Modenplast Medical. Its characteristic properties from material list are listed in table below.

Table 1 Properties of PVC compound RB3

Density (ISO/R1183)	g/cm^3	1.23
Hardness (ISO868)	Shore A/15´´	75
Apparent density (ASTM D 1895)	g/cm^3	0,67
Thermal stability (UNI 5637)	Min at 190 ° Black/ Yellow point	20´/ 80´
Chemical properties (European Pharmacopoeia compliance)		YES
Biocompatibility (ISO 10993, USP, Class VI)		YES
Suitable applications		Tubing

7.1.2 Crystal violet (CV)

Crystal violet is used as an indication substance in the analytical chemistry. It is delivered by PENTA Company. Physical and chemical properties from the material list are displayed in the table below.

Table 2 Properties of CV

Shape, Color, Odor	Powder, Dark green with metallic shine, without odor
Molecular weight	407.98
Melting Temperature	189 - 205 °C
Flammability (solid, gaseous state)	Non flammable
Outburst temperature	-
Density for 20 °C (solid state)	-
pH range	2.5 – 3.5 (10g/L, 20°C)
Solubility and miscibility with water (20 °C)	10 g/L

7.1.3 Cyclohexanone (CYH)

CYH is a solvent which is used in analytical chemistry and for laboratory synthesis. It is delivered by PENTA Company and its physical and chemical properties from material list are listed in the following table.

Table 3 Properties of CYH

State, Color, Odor	Liquid state, Transparent, Prickly odor
Molecular Weight	98.15
Boiling Temperature	81 °C
Flammability	Flammable
Outburst temperature	44 °C
Density for 20 °C	0.947 g/cm^3
pH range	~ 7 (70g/L, 20 °C)
Solubility and miscibility with water (20 °C)	90 g/L

7.2 Used devices

- UV-VIS spectrophotometer Cary 300. It is a UV- VIS spectrometer with wave-length range between 200 – 800 nm delivered by Agilent Technologies.
- External sipper with flow cell.
- FTIR spectrometer with adapter ID5 ATR delivered by Thermo Scientific compa-ny.

- Testometric M350- 5CP supplied by LABOR machine, Ltd.
- Scanning electron microscope Vega II LMU (Tescan, Czech Republic)

7.3 Used tools and laboratory glass

For this experiment were used several types of tools and laboratory glass, such as:
Beakers 50 mL, measuring cylinders, measuring flasks 25 mL and 250 mL, Erlenmeyer flasks, Petri dish, plastic Paster´s pipettes, micropipettes, tweezers, analytical balance.

8 PREPARATION OF THE SAMPLES

Preparation of the samples had three steps: Mixing of blends, pouring of blends to laboratory glass (Petri dish) and blend evaporation.

The PVC/CV and PVC films were prepared by a solvent casting technique. First was prepared solution of CV and CYH. The 0.2522 g of CV was added to 250 ml of CYH to get solution with concentration of 1g/L. Then CV solution was left to dissolute for 16 h on the shaking table (speed 150/RPM). The PVC granules of weight 20.005 g were dissolved in 300 ml of CYH on the shaking table (speed 150/RPM) for 16h. Once, the PVC solution was prepared, the amount of 250 ml of CV solution was added and this blend was left to dissolute for 8h more. After this 8 h, it was necessary to use an ultrasonic bath for 15 minutes, because CV was not dissoluted enough and some small crystals appeared in the blend.

The PVC control sample was prepared through the previous procedure without the CV addiction and ultrasonic bath.

The samples were poured to Petri dishes. To achieve a film thickness of 500 μm, dishes had specific diameters.

9 EXPERIMENTAL METHODS

Laboratory conditions of experiments:

Temperature in the laboratory: 25°C

Humidity in the laboratory: 35%

9.1 FTIR analysis

FTIR analysis was used to compare PVC pellets and PVC, PVC/CV films. The aim of this analysis was to study if modification with CV has not change the structure and chemical properties of materials.

A measurement was proceeding by FTIR spectrometer with adapter ID5 ATR and attenuated total reflection method was used. Each sample was scanned 64 times. For measurement was used GeSe crystal.

9.2 SEM analysis

The micrographs of the prepared materials were taken by the scanning electron microscope Vega II LMU (Tescan, Czech Republic), after coating with a thin layer of gold/palladium by the sputter coater SC 7640 (Quorum Technologies Ltd, UK).

9.3 Tensile test

Tensile test was performed on testing advice Testometric M350- 5CP. For tensile test were prepared five samples of PVC/CV film and five samples of PVC control film. Sample for test were cut out from film with parameters of width 5 mm and work length 36 mm. In this test was investigated tensile resistance of prepared films. Samples were inserted to Testometric measurement device and left there till their rupture break. Speed on Testometric was set to 500 mm/min.

9.4 Antibacterial tests

9.4.1 Inhibition growth test

Antimicrobial properties were determined by an agar diffusion test. Round samples (8 mm in diameter) were placed on Petri dishes with inoculated agar with the solution of microorganisms (concentration of 1.0. 10^7 CFU.mL^{-1}). The samples were tested against Gram neg-

ative Escherichia coli (EC) 4517, Gram positive Staphylococcus aureus (SA) 4516, and fungi Candida albicans (CA) CCM 8215 and Aspergillus braziliens (AB) 8222. After the incubation period (72 hours at 23 °C for fungi and 24 hours at 37 °C for bacteria), the samples were visually analyzed by measuring of inhibition zones in four directions. Three samples were tested and the average values were then calculated. The test was done duplicated.

9.5 Releasing of CV from PVC matrix

For studying of release was prepared samples (PVC/CV) with diameter 12.7 mm and solutions were chosen: distilled water, physiological saline solution and buffer solution about pH 7.4. Measurement of release of active additives was proceeding by UV-VIS spectrophotometer Cary 300 with sipper and flow cell. Samples were added into 50mL of the solution and then samples were mixing. Frequency of mixing was 60 revolutions per minute. Sampling was carried during interval of each 3 minutes interval in the first two hours of measurement, then each 30 minutes until each 2 hours.

Concentration of released CV to the solution was calculated from the UV-VIS calibration curves which were measured for wavelength 580 nm. In this wavelength CV absorbs the highest amount of radiation.

10 RESULTS AND DISCUSSION

10.1 FTIR analysis

In the figure 6 FTIR spectra comparing PVC material and PVC pellets are demonstrated. The infrared spectrum of polyvinylchloride contains the bands typical of aliphatic CH groups, except that the band due to CH_2 deformation vibration is shifted by about 30 cm^{-1} to lower wavenumbers, to near 1430 cm^{-1}. In addition to the aliphatic CH bands, the spectra of PVC contain contributions due to the the C-Cl vibrations. A broad, strong band is observed in the region 710-590 cm^{-1} due to the C-Cl stretching vibration. Since there are a very large number of additives possible, great care needs to be taken in the analysis of PVC samples. A Band near 1720 cm^{-1} is often observed in the infrared spectra of commercial samples of PVC. This band may be assigned to a carbonyl group present in the plasticizer employed and hence is assigned to the C=O stretching vibration.

The FTIR of CV displays all characteristic peaks of CV, namely 1587 cm^{-1} due to C=C stretching in aromatic nuclei, 1365 cm^{-1} due to C-H deformation in methyl, 1174 cm^{-1} due to C-H stretching in aromatic ring, and 1128 cm^{-1} due to C-N vibration

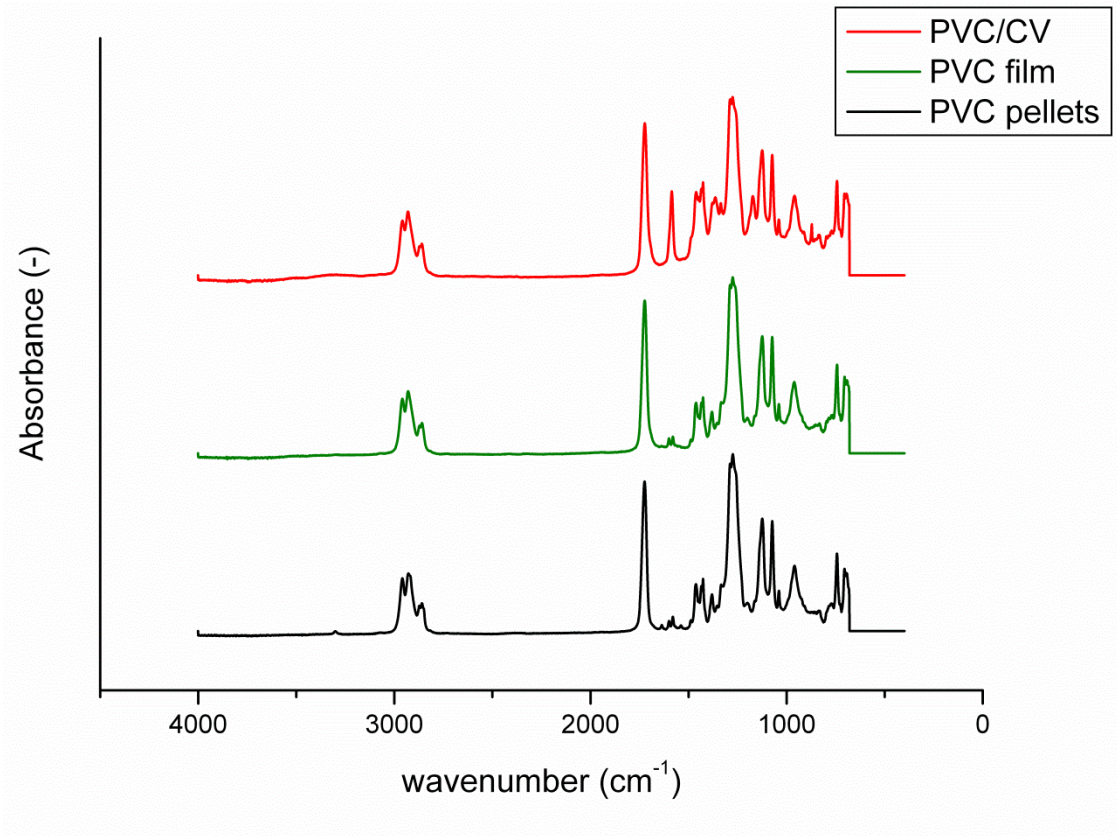

Figure 6 FTIR spectra of PVC pellets, PVC film and PVC with CV

Spectra show no change in structure between PVC pellets and PVC film, thus structures of materials was not influenced by preparation of these materials. However, chemical properties of polymer matrix were not influenced by the crystal violet mixed in PVC.

10.2 SEM

A SEM micrograph of the prepared PVC film with CV is shown in figure 7. Cross-section of polymer matrix is observed by BSE detector and enables to distinguish the composition of particles by material contrast showing phases containing elements with a higher atomic number brighter. For SEM were selected PVC/CV, which they were released different time. Figure 7a represent sample that was not released. Figure 7b represent sample that was released by 3 days. Structures of both samples are very similar. In the first case, surface of matrix is wrinkled with hollows and there can be seen a lot of small crystal of CV. In the second surface of polymer matrix is conformable, but amount of CV crystals is lower than in the first case.

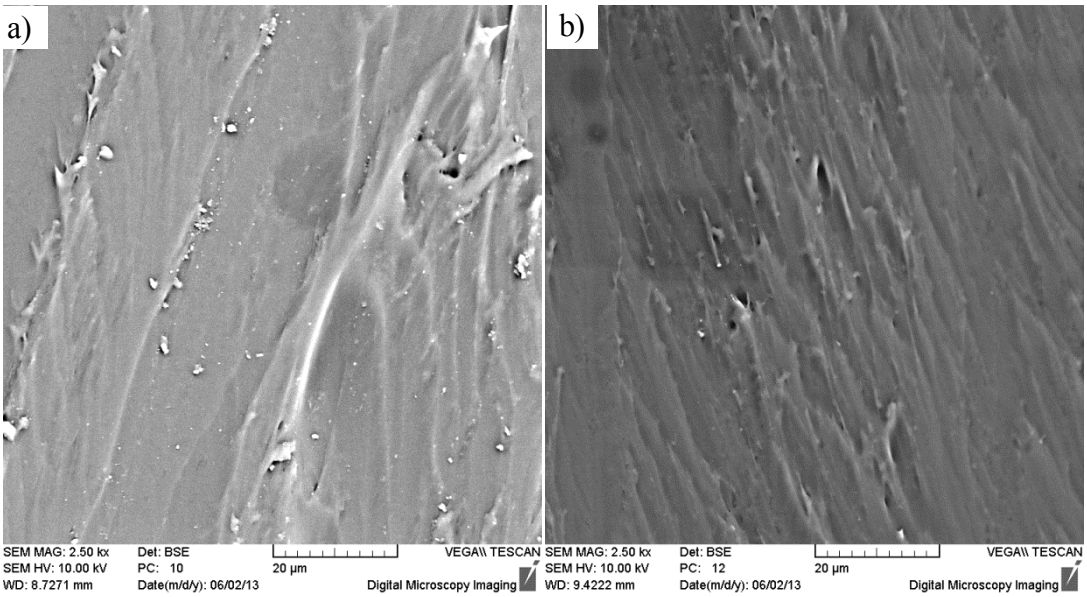

Figure 7 BSE microphotographs of PVC cv with release time a) 0 day, b) 3 days

10.3 Tensile test

The evaluation of mechanical tensile test can be seen in Table 4 and Table 5. Mechanical properties of PVC film and PVC/CV film are very similar. Young's modulus of pure PVC is 4.95 MPa and of PVC/CV is 4.81 MPa. However, PVC with CV shows higher deformation properties than PVC sample. Strain at break for pure PVC film is average value 493.117 %, but for PVC with CV film strain at break shows average value 616.542 %

while tensile stress for PVC pure is 11.156 MPa and 13.281 MPa for PVC/CV. The standard deviations (SD) are also displayed in following tables.

Table 4 Results of tensile test for PVC film

Sample no.	Young´s modulus (MPa)	Strain at break (%)	Break force (N)	Tensile stress at break (MPa)
1	5.202	551.006	36.255	9.006
2	4.686	505.944	27.050	8.825
3	5.254	465.708	42.900	10.154
4	4.039	464.206	28.005	13.306
5	5.569	478.722	26.440	14.488
Min	4.039	464.206	26.440	8.825
Average	4.950	493.117	32.130	11.156
Max	5.569	551.006	42.900	14.488
SD	0.600	36.435	9.332	2.961

Table 5 Results of tensile test for PVC with CV

Sample no.	Young´s modulus (MPa)	Strain at break (%)	Break force (N)	Tensile stress at break (MPa)
1	4.689	658.678	59.870	15.965
2	5.000	668.283	51.570	17.631
3	5.399	579.908	37.140	10.876
4	4.733	565.408	35.280	9.800
5	4.231	610.431	48.420	12.135
Min	4.231	565.408	35.280	9.800
Average	4.810	616.542	46.456	13.281
Max	5.399	668.283	59.870	17.631
SD	0.430	45.953	10.267	3.367

10.4 Antibacterial tests

Antibacterial activity of PVC and PVC with additives against S. aureus E. coli and C. albicans is displayed in following table. Pure PVC material has no antibacterial properties, but PVC with crystal violet shows antibacterial activity against all tested bacteria.

Table 6 Antibacterial activity of the PVC and PVC/CV films

SAMPLE	SA	EC	CA
PVC	-	-	-
PVC with CV	14.8 ± 0.9	10.3 ± 1.0	16 ± 3

- No inhibition zone

Following pictures demonstrate inhibition zone colonies of bacteria around samples of antibacterial material (PVC/CV) tested by agar diffusion test.

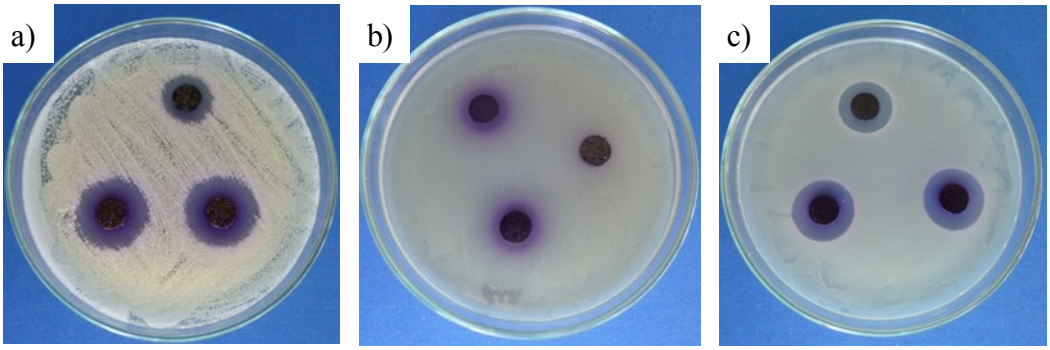

Figure 8 Diffusion agar test against a) C. albicans, b) E. coli c) S. aureus

10.5 Releasing of CV from PVC

The samples, from the macroscopic aspect, have not changed their properties that mean there was not observed swelling, either dissolution of the matrix. And this effect was proved with analytical weighing and also with simple hand touch. Neither the color change of the sample was observed with human eye. Whereas in the solution was presence of CV evident, because liquid above the sample was colored.

With simple measurement of the absorbance and then conversion to the concentration in dissolution media was obtained an integral plot of the CV release from the sample. This integral plot for water is displayed in the figure 9, for physiological saline solution in the figure 10 and for buffer solution in the figure 11. The first order kinetic model, which corresponds with Noyes-Whitney fundamental model, was chosen with consideration of matrix relative passivity in the used solutions and one-dimensional geometry of the process (big flat sample of small thickness).

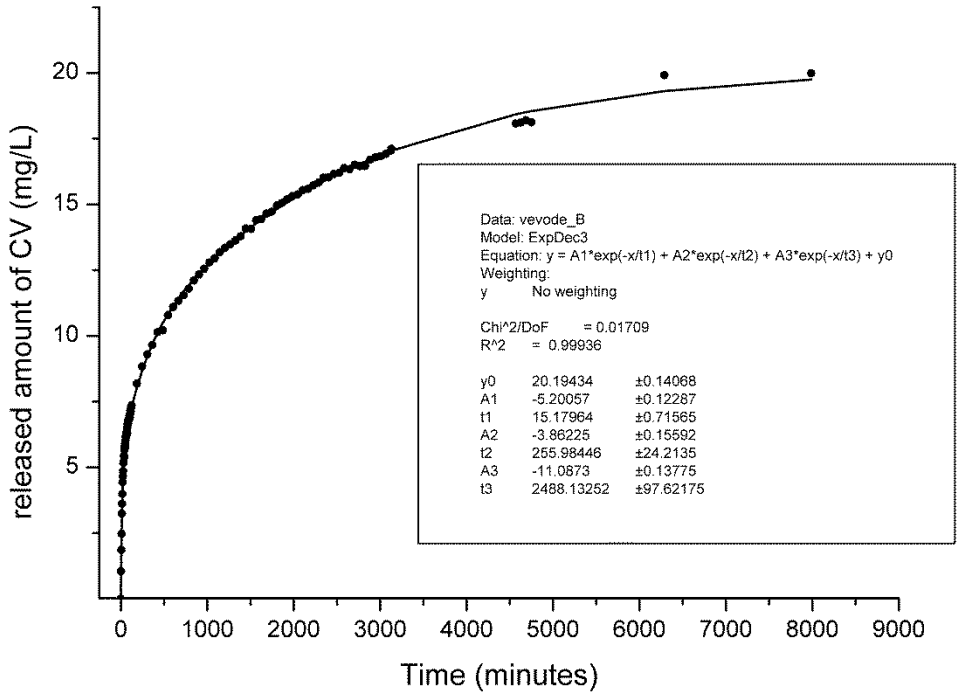

Figure 9 Release in the water

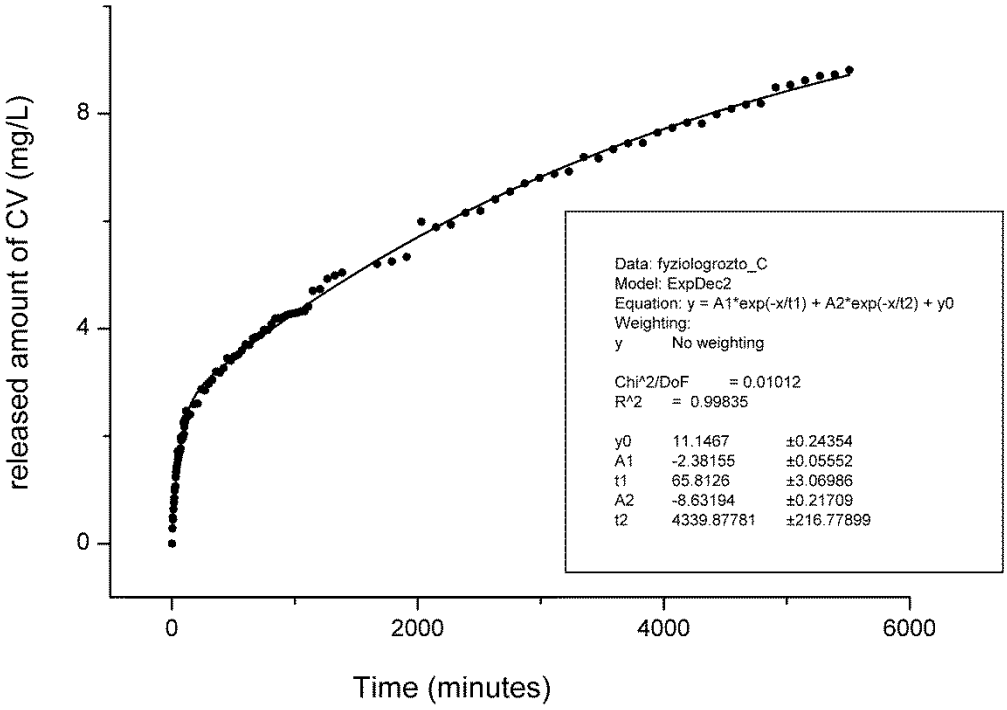

Figure 10 Release in the physiological solution

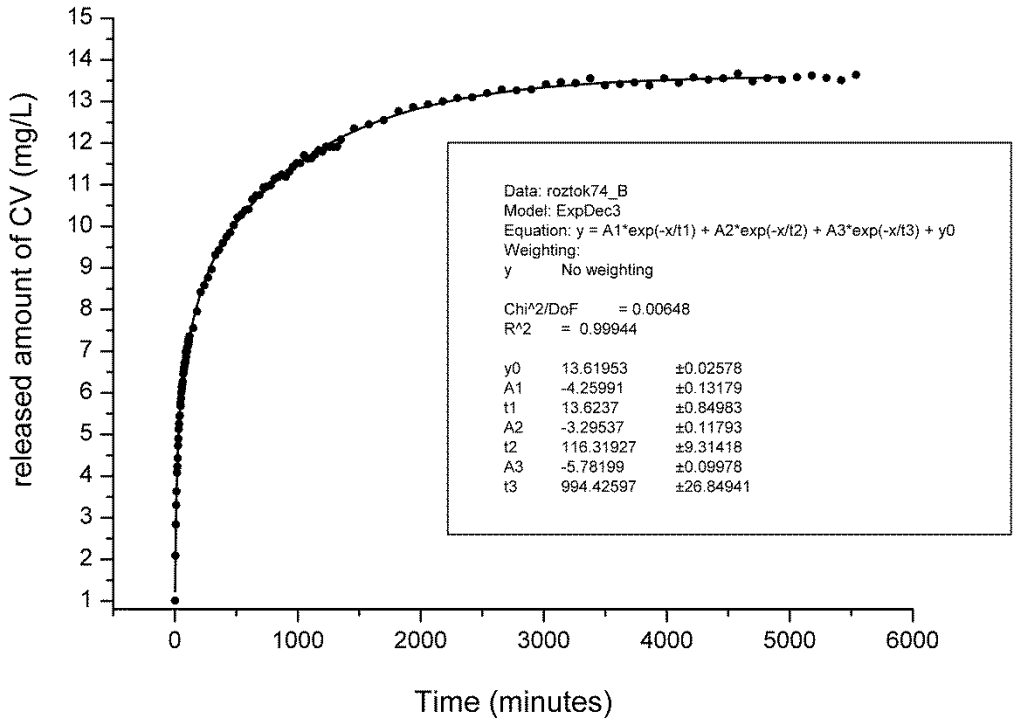

Figure 11 Release in the buffer solution of pH about 7.4

Experimental lines were interspersed by series of these three processes (water, buffer) and by two of them with physiological solution. The general equation is in the following form:

$$y = A_1.\exp\left(-\frac{x}{t_1}\right) + A_2.\exp\left(-\frac{x}{t_2}\right) + A_3.\exp\left(-\frac{x}{t_3}\right) + y_0 \qquad (18)$$

which describes the profile and the release kinetics. And parameter y_0 is the maximal amount above the sample, A_1, A_2, A_3 are constants describing physical parameters of the matrix and t_1, t_2 and t_3 are half-life of the process. The results are summarized in the table 7.

Table 7 Dissolution constants which describe kinetic of released CV

	y_0	A_1	t_1	A_2	t_2	A_3	t_3
Water	20.19	-5.20	15.18	-3.86	256.00	11.09	2488.13
physiological solution	11.15	-2.38	65.81	-8.63	4339.88	-	-
buffer	13.62	-4.26	13.62	-3.30	116.32	-5.78	994.43

The first exponential component (with the shortest half-life) probably represents releasing from of CV from the matrix surface, because this process is the shortest one and could be limited by CV solubility in the water. This solubility is the concentration value, which cannot be exceeded in the matrix surface. The second release phase is slower, because CV from the matrix surface is already depleted and CV from the subsurface layers needs to cross an energetic barrier before releasing. The third phase is characterized by solution saturation onset, when the gradient between the matrix surface and the solution volume is decreased and the extractable CV is also depleted at the same time. CV which is placed in the deeper layers is not released to the solution in the relevant time horizon.

These three phases were observed for sample released in the water and the buffer solution. The sample in the distilled water has higher saturation value (y_0) than sample in the buffer solution, because the buffer solution has higher ionic strength than distilled water and ionic strength highly influences the saturation value. The sample in the physiological saline solution shows slower first and second phase. The third phase was not observed for the physiological solution, because the sample was plotted only by two exponential lines. In this case, solubility of CV is highly influenced by presence of the chloride anion, which is commonly shared between CV and physiological solution. CV would need to leave polymer matrix as cation Cl^+ and anion Cl^- always in the rate 1:1, due to electronegativity. This condition is satisfied in the cases of the first two samples (water, buffer), whereas in the third case this pair would be release to medium with high concentration of chloride anions. And this anionic release would be possible only till the limitation by CV solubility product, is reached. CV solubility product is $K_s = 6.10^{-4}$ $mol^2.dm^{-6}$ and it has been calculated from CV solubility 10g/L. Solution of the following equation:

$$\left(x + 0{,}154\frac{mol}{L}\right).x = K_s \tag{19}$$

where x is critical CV concentration, gives only one positive base, namely x=0.0038 mol/L, which corresponds to the CV concentration 1.55 g/L. This value is about six and half times lower than limitation for the sample in distilled water.

The first release phase in physiological solution is about five times slower in comparison with half-times of the other processes. This first original consideration ignores active coefficient, either CV capability to exist in the solution in undissociated forms or ion-associates. Detailed identification of described processes would require analytical analysis, which exceeds the framework of the bachelor thesis in its instrumental requirements and in its time range.

CONCLUSION

The films from PVC with addiction of 1% CV were prepared by solvent casting technique. With mechanical test was proved, that CV addiction does not influence mechanical properties in a negative, either positive way. It can be considered as a positive result, because used PVC medical compound RB3 has its mechanical properties adapted to its portfolio of the future applications. There was also tested antibacterial activity of the prepared samples by zone diffusion test on agar plates. In all three cases, against S.aureus, E.coli and C.albicans, was observed and proved antibacterial effect. Colonies inhibition zones originated around the samples. It is obvious, that material is releasing active substance by the mechanism of diffusion. Although, CV is effective against gram+, gram- and yeast cells, there is some antipathy for its utilization because of the carcinogenic effect suspicion. The question remains, if there will be search for compromise between these two effects or if it will be better to search another active substance. In the case of new search, studies of the bachelor thesis will be also useful, namely in input of the active substance into polymer matrix.

Release kinetics was observed in three different media with physiological relevance and it was described with first order model process for three respectively two phases. It is characteristic, that CV has slow release rate and small CV concentrations in the solution were achieved after long period of time. The release process is even more decelerated by omnipresent chloride anions in the biological fluids. In this aspect, system can be considered as mild in its effects and beneficial due to possible side effects of the CV.

Thick coating appears as the most suitable application due to perspective aspect. This thick coating would be applied on the volume product (for example from the same PVC resin) and would be prepared by solvent casting technique described in this thesis. Thick coating application is suitable because CV release rate from the surface is small. Alternative option is application of thick coating on the extruded profile by coextrusion, however this method has a negative aspect and it is thermal degradation of the active substance in polymer melt. Unresolved question of this application remains adhesion of thick coating to the surface and this question will be necessary to experimentally verify, although there is a strong assumption that PVC will have good adhesion.

BIBLIOGRAPHY

[1] VAVŘÍKOVÁ, E. and J. VINŠOVÁ. Chitosan a jeho farmaceutické aplikace. Chemické listy [online]. 2009, Vol. 103, Issue 1, p. 56-65 [cit. 2013-06-03]. Available at: http://chemicke-listy.cz/docs/full/2009_01_56-65.pdf

[2] RABIŠKOVÁ. Miloslava. Perorální formy s řízeným uvolňováním léčiv [online]. Praktické lékárenství. Olomouc: Solen. 2008. vol. 4. issue 4. p. 186-190 [cit. 2013-05-18]. ISSN 1803-5329. Available at: http://solen.cz/pdfs/lek/2008/04/09.pdf

[3] UCHEGBU. I.F. and A.G. SCHÄTZLEIN. Polymers in drug delivery. Boca Raton. FL: CRC/Taylor & Francis. 2006. p. 35-108. ISBN 0-8493-2533-1.

[4] NAIR. L.S. and C.T. LAURENCIN. Polymers as Biomaterials for Tissue Engeneering and Controlled Drug Delivery. *Advanced Biochem Engin/ Biotechnol.* 2005. Vol.102. Issue October. p. 47-90. DOI: 10.1007/b137240.

[5] UHRICH. K.E.. S.M. CANNIZZARO. R.S. LANGER and K.M. SHAKESHEFF. Polymeric systems for controlled drug release. *Chemical reviews.* 1999. Vol. 99. Issue 11. p. 3181-3198.

[6] UHRICH. K.E.. S.M. CANNIZZARO. R.S. LANGER and K.M. SHAKESHEFF. Polymeric systems for controlled drug release. Chemical reviews. 1999. Vol. 99. Issue 11. p. 3181-3198.

[7] NAIR. L.S. and C.T. LAURENCIN. Polymers as Biomaterials for Tissue Engeneering and Controlled Drug Delivery. *Advanced Biochem Engin/ Biotechnol.* 2005. Vol.102. Issue October. p. 47-90. DOI: 10.1007/b137240.

[8] VAVŘÍKOVÁ, E. and J. VINŠOVÁ. Chitosan a jeho farmaceutické aplikace. Chemické listy [online]. 2009, Vol. 103, Issue 1, p. 56-65 [cit. 2013-06-03]. Available at: http://chemicke-listy.cz/docs/full/2009_01_56-65.pdf

[9] MALAFAYA. P.B.. G.A. SILVA and R.L. REIS. Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Advanced Drug Delivery Reviews* [online]. 2007. Vol. 59. Issue 4-5. p. 207-233 [cit. 2013-05-28]. DOI http://dx.doi.org./10.1016/j.addr.2007.03.012 Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0169409X07000257/1-s2.0-S0169409X07000257-main.pdf?_tid=0c9fe7be-c7c0-11e2-9546-00000aab0f6c&acdnat=1369764023_3b286944b07cd96836e94719e972c304

[10] Mathematical modeling of controlled drug delivery. *Advanced drug delivery reviews* [online]. 2001. vol. 48. issue 2-3. p. 137-138 [cit. 2013-05-22]. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0169409X01001119/1-s2.0-S0169409X01001119-main.pdf?_tid=02443faa-c2ec-11e2-9d0b-00000aacb35e&acdnat=1369233148_afcfcfe1ed02d2bb20c11f94ca36150a

[11] DASH. S.. P.N. MURTHY. L. NATH and P. CHOWDHURY. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica- Drug Research.* 2010. Vol. 6. Issue 3. p. 217-223. ISSN 0001-6837

[12] DOKOUMETZIDIS. A. and P. MACHERAS. A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System. *International Journal of Pharmaceutics* [online]. 2006. Vol. 321. Issue 1-2. p. 1-11 [cit. 2013-05-23]. ISSN 0378-5173. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0378517306005813/1-s2.0-S0378517306005813-main.pdf?_tid=fc5735b8-c3cf-11e2-a570-00000aab0f6b&acdnat=1369331063_9cfe5326aedf77c65f01b389005e2333

[13] DASH. S.. P.N. MURTHY. L. NATH and P. CHOWDHURY. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica- Drug Research.* 2010. Vol. 6. Issue 3. p. 217-223. ISSN 0001- 6837

[14] DASH. S.. P.N. MURTHY. L. NATH and P. CHOWDHURY. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica- Drug Research.* 2010. Vol. 6. Issue 3. p. 217-223. ISSN 0001- 6837

[15] SIEPMANN. J. and N.A. PEPPAS. Modeling of drug release from delivery sys tems based on hydroxypropyl methylcellulose (HPMC). *Advanced drug delivery reviews.* 2012. Vol. 64. Supplement. p. 163-174. DOI: 10.1016/j.addr.2012.09.028

[16] DASH. S.. P.N. MURTHY. L. NATH and P. CHOWDHURY. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica- Drug Research. 2010. Vol. 6. Issue 3. p. 217-223. ISSN 0001- 6837

[17] SIEPMANN. J. a F. SIEPMANN. Mathematical modeling of drug dissolution. *International Journal of Pharmaceutics* [online]. 2012. In press [cit. 2013-05-26]. DOI: http://dx.doi.org/10.1016/j.ijpharm.2013.04.044. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0378517313003451/1-s2.0-S0378517313003451-

main.pdf?_tid=7ab2383a-c647-11e2-b211-00000aab0f27&acdnat=1369602287_7ce5d92eb78adf4c690a9edc85392995

[18] JOSE. S. et al Predictive modeling of insulin release profile from cross-linked chitosan microspheres. *European Journal of Medicinal Chemistry* [online]. 2013. Vol. 60. February 2013. p. 249-253 [cit. 2013-05-26]. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0223523412007337/1-s2.0-S0223523412007337-main.pdf?_tid=b85d2b8a-c648-11e2-aa23-00000aab0f02&acdnat=1369602820_330d224fdee2defc25f4d36c4aafef4a

[19] COSTA. P. and J.M. SOUSA LOBO. Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences* [online]. 2001. Vol. 13. Issue 2. p. 123-133 [cit. 2013-05-27]. DOI: http://dx.doi.org./10.1016/S0928-0987(01)00095-1. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0928098701000951/1-s2.0-S0928098701000951-main.pdf?_tid=b544508a-c64e-11e2-a7e8-00000aacb362&acdnat=1369605392_b50c641c80ff401cf725cbaf1623de64

[20] KOSMIDIS. K. and P. MACHERAS. Monte Carlo simulations of drug release from matrices with periodic layers of high and low diffusivity. *International Journal of Pharmaceutics* [online]. 2008. Vol. 354. Issue 1-2. p. 111-116 [cit. 2013-05-27]. DOI: http://dx.doi.org /10.1016/j.ijpharm.2007.10.036. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0378517307008642/1-s2.0-S0378517307008642-main.pdf?_tid=dfeed3ae-c654-11e2-81a8-00000aab0f02&acdnat=1369608041_8d5062782b30c8469d01d2a902d7538e

[21] COSTA. P. and J.M. SOUSA LOBO. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences [online]. 2001. Vol. 13. Issue 2. p. 123-133 [cit. 2013-05-27]. DOI: http://dx.doi.org./10.1016/S0928-0987(01)00095-1. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0928098701000951/1-s2.0-S0928098701000951-main.pdf?_tid=b544508a-c64e-11e2-a7e8-00000aacb362&acdnat=1369605392_b50c641c80ff401cf725cbaf1623de64

[22] DASH. S.. P.N. MURTHY. L. NATH and P. CHOWDHURY. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica- Drug Research*. 2010. Vol. 6. Issue 3. p. 217-223. ISSN 0001- 6837

[23] RABIŠKOVÁ. M. Perorální matricové tablety s řízeným uvolňováním léčiva. *Remedia*. Praha. 2007. Issue 2. ISSN 0862-8947.

[24] RABIŠKOVÁ. M. Typy matricových tablet [online]. *Remedia*. Praha. 2007[cit. 2013-06-03]. Available at:

http://www.remedia.cz/Images/Articles/Main/vtextu20070428013205.jpg

[25] RABIŠKOVÁ. M. Liberace léčiva z hydrofilní matrice [online]. *Remedia*. Praha. 2007 [cit. 2013-06-03] Available at:

http://www.remedia.cz/Images/Articles/Main/vtextu20070428013223.jpg

[26] RABIŠKOVÁ. M. Perorální formy s řízeným uvolňováním léčiv [online]. *Praktické lékárenství*. Olomouc: Solen. 2008. vol. 4. issue 4. p. 186-190 [cit. 2013-05-18]. ISSN 1803-5329. Available at: http://solen.cz/pdfs/lek/2008/04/09.pdf

[27] RABIŠKOVÁ. M. Perorální matricové tablety s řízeným uvolňováním léčiva. *Remedia*. Praha. 2007. Issue 2. ISSN 0862-8947.

[28] RABIŠKOVÁ. M. Typy obalů u matricových tablet [online]. Remedia. Praha. 2007 [cit. 2013-06-03]. Available at:

http://www.remedia.cz/Images/Articles/Main/vtextu20070428013236.jpg

[29] CHALABALA. Milan et al. Technologie léků. 2nd edition. Praha: Galén. 2001. 402 p. ISBN 80-7262-128-9.

[30] DVOŘÁČKOVÁ. K.. Principy uvolňování léčiv z perorálních matricových tablet obsahujících hypermelosu [online]. *Chemické listy*. 2009. Vol. 103. Issue 1. p. 66-72 [cit. 2013-05-18]. ISSN 1213-7103. Available at: http://chemicke-listy.cz/docs/full/2009_01_66-72.pdf

[31] MAHATO. R. I. Dosage form and drug delivery. In: *APhA´s complete review for pharmacy* [online]. 2013. p. 37-63 [cit. 2013-05-22]. Available at: http://www.uthsc.edu/pharmacy/pharmsci/faculty/rmahato/Publications_PDF%20files/Mahato_NAPLEX_05.PDF

[32] Diffusion. [online]. *www.biologyguide.net* [cit. 2013-06-03] Available at:

http://www.biologyguide.net/biol1/3b_exchange.htm

[33] Normal osmosis [online]. *www.nicewater.com* [cit. 2013-06-03] Available at:

http://www.nicewater.com/edu_technologies.html

[34] SIEPMANN. J.. R.A.. SIEGEL and M.J. RATHBORNE. Overview of controlled release mechanism. *Fundamentals and Applications of Controlled Release Drug Delivery* [online]. New York: Springer US. 2012. p. 35-40 [cit. 2013-05-20]. ISBN 978-1-4614-0881-9. DOI: 10.1007/978-1-4614-0881-9. Available at: http://link.springer.com/book/10.1007/978-1-4614-0881-9/page/1

[35] SIEPMANN. J. and GÖPFERICH. Mathematical modeling of bioerodible. polymeric drug delivery systems. *Advanced Drug Delivery Reviews* [online]. 2001. vol. 48. issue 2-3. p. 229-247 [cit. 2013-05-22]. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0169409X01001168/1-s2.0-S0169409X01001168-main.pdf?_tid=56803d3c-c2ea-11e2-9368-00000aab0f02&acdnat=1369232430_183862a4cce2aa9e558831ec6cbb0453

[36] DVOŘÁČKOVÁ. K.. Principy uvolňování léčiv z perorálních matricových tablet obsahujících hypermelosu [online]. *Chemické listy*. 2009. vol. 103. issue 1. p. 66-72 [cit. 2013-05-18]. ISSN 1213-7103. Available at: http://chemicke-listy.cz/docs/full/2009_01_66-72.pdf

[37] MADERUELO. C.. A. ZARZUELO and J.M. LANAO. Critical factors in the release of drugs from sustained release hydrophilic matrices [online]. *Journal of controlled release.* Elsevier. 2011. vol. 154. issue 1. p. 2-19 [cit. 2013-05-20]. ISSN 0168-3659. DOI: 10.1016/j.jconrel.2011.04.002. Available at: http://ac.els-cdn.com.proxy.k.utb.cz/S0168365911002124/1-s2.0-S0168365911002124-main.pdf?_tid=d0c32556-c13b-11e2-815e-00000aab0f6c&acdnat=1369047522_5ed386ea0534d917ae44d661290df665

[38] http://www.sci.muni.cz/~analchem/files/pdf/bartusek_skripta.pdf

[39] KLOUDA. P.. Moderní analytické metody. Ostrava. Nakladatelství Pavel Klouda. 1996. ISBN 80-902155-5-5

[40] http://mmrc.caltech.edu/FTIR/FTIRintro.pdf

LIST OF ABBREVIATIONS

GIT	Gastrointestinal tract
PE	Polyethylene
PP	Polypropylene
PVC	Polyvinylchloride
PVA	Polyvinylalcohol
pEVAc	Polyethylene-vinylacetate
PEG	Polyethyleneglycol
FDA	United States Food and Drug Administration
CD	Cyklodextrines
MW	Molecular weight
UV-VIS	Ultraviolet-visible
UV	Ultraviolet
AES	Atomic emission spectrometry
AAS	Atomic absorption spectrometry
IR	Infrared
CV	Crystal violet
CYH	Cyklohexanone
SA	Staphylococcus aureus
CA	Candida albicans
AB	Aspergillus braziliens
FTIR	Fourier-transformation infrared spectroscopy
SD	Standard deviation
SEM	Scanning electron microscope
	Third abbreviation meaning.

LIST OF FIGURES

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