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Phases in temporal multiscale evolution of drug release mechanism from IPN-type chitosan based hydrogels

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Abstract. The paper proposes modeling calcein release kinetics (considered hydrophilic drug model) from interpenetrating network matrix of hydrogel type, based on combinations of two polymers, of which the chitosan is common. The release process is analyzed for different increasing time intervals, divided upon release kinetics evolution. For each time interval, a dominant release mechanism was identified and quantitative analysis were performed, probing the existence of four distinct stages during its evolution, each governed by a different kinetic model. An interesting and original aspect, analyzed through a novel approach, is that of drug release at long time scales, often overlooked. It revealed that system behaves as a complex one and its evolution can be described through a nonlinear theoretical model, that offer new insights on its order-disorder evolution.

Keywords: hydrogel, drug release, multiscale, nonlinearity

1. Introduction

One method commonly used to achieve sustained release and control of drug release process is the inclusion of drug in a matrix with three-dimensional structure (gel), hydrophobic or hydrophilic, depending on the nature of the therapeutic agent. In most cases, biologically active compounds are hydrophilic, so the matrices used are based on polysaccharides or their derivatives, proteins (collagen, gelatin) and even some synthetic polymers, provided that they are biocompatible and biodegradable poly(vinyl alcohol), poly (lactic acid), poly(glycolic acid). Since the inclusion/encapsulation of the active principle takes place in moderate condition, the distortion/degradation of sensitive drugs, during their association with the polymer matrix, is avoided.

In most cases, the polymer matrix is structured/cross-linked before being placed in contact with a concentrated solution of the drug, able to swell it (through solvent swelling technique) [1]. Drug loading is done through a diffusion process, the driving force that determines the flow of the drug within the matrix being the concentration gradient between the solution and macromolecular support [2]. A variant to obtain such systems is crosslinking the linear chains in the solution in which the drug is dissolved.

Regardless of the drug loading method in the hydrogel matrix type, its release is determined by the concentration gradient, this time between the matrix and the liquid in which the polymer-drug system is placed. Obviously, the release principle is still of diffusional nature, described by Fick's law [3].

Typical release curves, encountered most often in specialized studies, follow an exponential trend, sometimes followed by a constant plateau. But, the subsequent evolution of the process after it, especially in large scale of time, exhibits an unusual behavior, influenced by polymer matrix type: i.e. large variations for CS/GEL micro particles [4] or decrease in the amount of released drug for hydrogels [5], which are difficult to explain in the classical approach. It raises the legitimate question: how can we explain these evolutions? What kinetic models are governing these processes? What phenomena become dominant and responsible for the change in the release kinetics?

Aim of this work is to establish kinetic models most suitable to describe drug release from hydrogels based matrices at different time scales, with particular attention paid to the evolution at large time scales, of days order, when the

phenomena that appears and their time evolution are difficult to quantify in amplitude and dependence.

The present study was conducted using as polymer matrix hydrogels of semi-or full-interpenetrating type with very low porosity, obtained by chemical co-crosslinking of two pairs of polymers: chitosan-gelatin, respectively chitosan-poly(vinyl alcohol).

Chemical cross-linking agents commonly used in the synthesis of hydrogels, such as formaldehyde and glutaraldehyde, are toxic to human body and therefore attention is directed to the use of ionic crosslinker such as sodium sulfate (Na_2SO_4) and sodium tripolyphosphate (TPP). But covalent bonds induce to hydrogels better mechanical properties and, consequently, high stability in time and this is why the covalent crosslinker (glutaraldehyde) was not totally replaced in preparing the hydrogel, but substituted with the ionic one up to the limit above which the stability of the system is affected. Accordingly, the first research was aimed to find a equilibrium between the covalent (toxic in general) and the ionic (biologically acceptable) crosslinkers amounts, in order to obtain hydrogels with superior properties compared to the existing ones, especially from the point of view of biocompatibility [6, 7].

Finally, the kinetics of calcein release (water-soluble drug used as a model) from these hydrogels were analyzed and compared, by fitting, at different time scales, to the known laws for drug release in order to identify the most appropiate release mechanisms and to establish, through quantitative analysis, similarities, differences and possible correlations between the evolution of different samples.

2. Experimental results

2.1 Materials and methods

The materials used were high and medium molecular weight chitosan (HC/MC), type B gelatin (GEL), of bovine origin, poly(vinyl alcohol) (PVA) with a hydrolysis degree of 80%, glutaraldehyde (GA) (aqueous solution of 25%), sodium sulphate (Na_2SO_4), sodium tripolyphosphate (TPP), and calcein.

Hydrogels preparation is based on a partial covalent crosslinking with AG of polymers (amount of AG ensures crosslinking of 20% of the functional groups of the polymer mixture) followed by a ionic crosslinking with Na_2SO_4 or TPP. In the case of chitosan-gelatin system, both polymers participate in the both crosslinking reactions. For the chitosan-PVA system, the synthetic polymer participates only in covalent cross-linking, being free of substituents with ionic character.

Considering the presence of the amine groups of chitosan and gelatin as substituents, and that the crosslinking reaction is carried out in an acid medium, in Fig. 1 we illustrated schematically reactions that may occur between ammonium ions formed acid medium, both with covalent and with the ionic crosslinker (sulfate anion in this case), that lead ultimately to obtain interpenetrating network structure type (IPN).

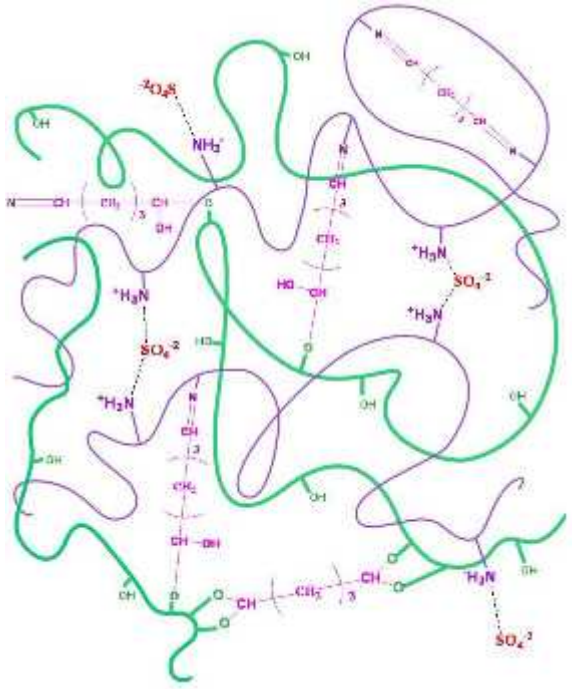


Fig. 1. Schematic presentation of the structure of the hydrogels produced by ionic cross-linking with sodium sulfate ion or covalent one with GA to form covalent imine groups or acetal cycles and semiacetalice links between GA and polymers (green chain – PVA, dark magenta - chitosan)

The procedure of obtaining the polymer films loaded with calcein is not subject of this paper, but it is described in [8]. In order to obtain drug loaded hydrogels, calcein was added to each polymer solution prior to cross-linking. The hydrogels were processed as thin films with 0,1 μm thickness.

The drug release was monitored by UV–visible spectral analysis. The calcein release was performed at $37 \pm 1^{o}C$, in the dark, to protect calcein from degradation under the possible action of light radiation. Also, the release experiments were carried out into media approximating a perfect sink. These studies were performed in triplicate for each sample and the average values were used in data analysis.

2.1.1 CHITOSAN/GELATIN HYDROGELS

Hydrogels based on chitosan and gelatin (CG) have been prepared by a partial covalent crosslinking with AG followed by ionic crosslinking with Na_2SO_4 or TPP. The influence of polymers mass ratio and of ionic crosslinking agent amount was followed. The codes of synthesized matrix and their parameters are presented below (Table 1a).

2.1.2 CHITOSAN/ POLY(ALCOHOL-VINYL) HYDROGELS

These were obtained through by the same method as previous ones, except that, in all cases, the covalently cross-linked hydrogels were immersed in 4ml 1% ionic crosslinker

solutions. The codes of synthesized matrix and polymers mass ratio are illustrated in (Table 1b).

Sample	CS/GEL(w/w)	Na_2SO_4 (g)	TPP(g)
CG-S1	1,9	0,0605	-
CG-S2	9	0,064	-
CG-S3	4,5	0,079	-
CG-S4	4,5	0,0595	-
CG-T1	1,9	-	0,078
CG-T2	9	-	0,083
CG-T3	4,5	-	0,1024
CG-T4	4,5	-	0,0771

a)

Sample	CS/PAV (w/w)
CP-T1	9
CP-T2	5,7
CP-T3	4
CP-T4	3
CP-S1	9
CP-S2	5,7
CP-S3	4
CP-S4	3

b)

Table 1. Parameters used for chitosan/gelatin (a) and chitosan/ poly(alcohol-vinyl) (b) hydrogels

2.2 Experimental kinetics of calcein release from hydrogels

The experimental release kinetics are plotted in Figs. 2.

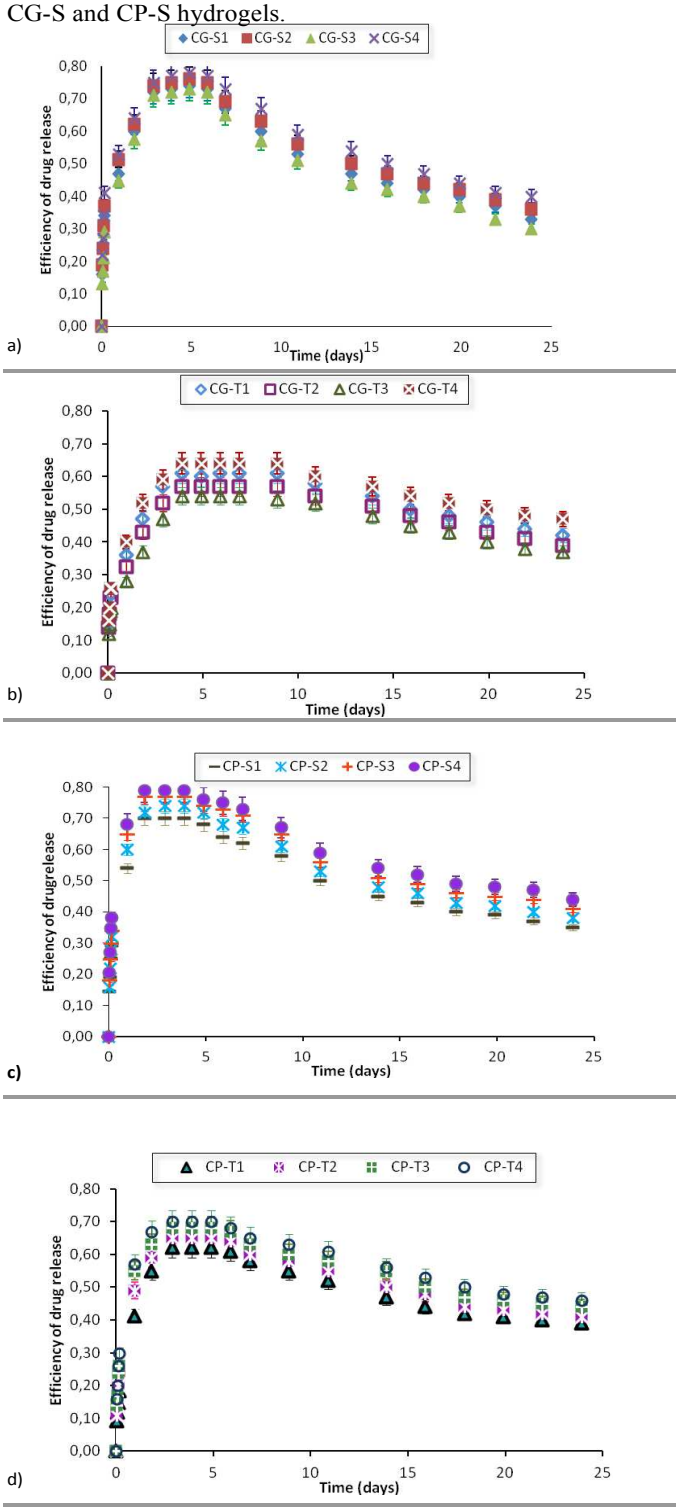
It is noted that for the CG-S hydrogels (Fig. 2a), an amount of about 80 % of the loaded drug is gradually released into the supernatant over 7 days. The parameters modified according to Table 1 does not significantly influence the amount of calcein released or release kinetics characteristics.

A similar behavior can be observed for CG-T hydrogels (Fig. 2b), but in this case there is a lower efficiency of calcein release of about 65 % and a slight influence of parameters modified according to Table 1b (molar ratio CS/GEL and TPP amount).

Hydrogels with PVA shows release kinetics similar with those of hydrogels with gelatin, for the same ionic cross linker, with the remark that in their case the release is faster, the amount of released calcein reaching a maximum value previous to CG hydrogels (3 days compared to 4-5 days). Moreover, these CP hydrogels show a lower stability than CG hydrogels, estimated through the time interval for which the released calcein has a constant value. In this respect, an influence of the ionic crosslinker type is noticed: 3h for CG-S, 4h for CG-T, 2h for CP-S and 3h for CP-T.

These experimental observations can be explained by assuming that the release efficiency is determined, mainly, by crosslinking density of hydrogel matrix and by considering some chracteristics of materials used:

- PVA does not participate at ionic crosslinking and it determines lower crosslinking density in CP hydrogels, reflected in faster release and higher efficiency in their case, compared to CG hydrogels;
- TPP is a stronger ionic crosslinker than Na_2SO_4 , causing a more dense hydrogel matrix, and, furthermore, longer stability and lower efficiency of CG-T and CP-T hydrogels, compared to CG-S and CP-S hydrogels.



Figs. 2. The release kinetics of calcein from hydrogels based on: chitosan and gelatin cross-linked with sodium sulphate (a) and TPP (b), chitosan and PVA cross-linked with sodium sulfate (c) and TPP (d).

CP and CG hydrogels have very similar evolutions, because, even if PVA does not participate at ionic crosslinking, the crosslinking degree if gelatin is low due its small number of amino groups, so that the main „responsible" for crosslinking density remain chitosan.

All the release kinetics, regardless the polymers and crosslinker types, shows similar system evolutions, passing through the same phases, the differeces consisting in the values of time thresholds at which „phase transitions" takes place and the time interval for each phase. The „phase transition" are observed in the release kinetics as inflections of curves shape, suggesting modifications in the release mechanism. Conducting the experiments even at large time scale (reported to the moment of reaching the maximum-approximately four time longer), the overall evolution showed a decrease in the amount of released calcein.

3. Mathematical modelling

Drug release from a polymer matrix is a complex process, that involves many interacting entities, leading to multiple phenomena, most of them superposing each other in time and space. In identifying the release mechanism, several approximations are needed, in order to simplify the system mathematics.

In our case, for a hydrogel type polymer matrix immersed in an aqueous medium, we identify the following phases in temporal evolution of drug release:

(1) In the first moments after its imersion in the release environment, the hydrogel surface wets and the burst-effect takes place, due to the release of the molecules from the matrix frontier, induced by a very high concentration gradient. This initial release is very fast and there is not enough time for water to penetrate into the matrix, so that (i) the diffusivity is constant, (ii) there is perfect sink at the interface and (iii) no swelling and erosion of the matrix takes place. In these approximations, the Fick's difussion law, in the condition of fixed boundary, can be applied and leads, for 'early' times and thin films, to an equation similar to that of Higuchi ($t^{1/2}$ time dependence of the drug release efficiency):

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{1/2} \quad (1)$$

where M_t is defined as the amount of drug released at time t, M_∞ the amount of drug released as time approaches infinity, D the diffusion coefficient and L the thickness of the film [9].

In summary, a simplified Higuchi model can be expressed as:

$$\frac{M_t}{M_\infty} = k_H t^{0,5} \quad (2)$$

where k_H is the Higuchi diffusion constant [10].

(2) After this, hydrogels retains water and begins to hydrate from the periphery towards the centre forming a viscous swollen mass. Two diffusion fronts appear: one at the interface between the dry and hydrated (swollen) polymer and second at the interface between the swollen polymer and the release environment. The diffusion coefficients are different in the two newly appeared areas (swollen and non-swollen polymer), because water penetration determine increasing network mesh, increasing dimensions of the system, increasing macromolecular mobilities and, consequently, higher diffusion coefficients, with influence on release characteristics.

In the approximations that (i) the diffusivity is constant in time, (ii) there is perfect sink at those two fronts, (iii) no erosion of the matrix takes place, (iv) swelling rates are constant in all direction and (v) no the transition from the glassy to the rubbery state of the polymer, Fick's law with moving boundary lead to a general solution in the form:

$$\frac{M_t}{M_\infty} = k_{KP}\, t^n \quad (3)$$

similar to that of Korsmeyer and Peppas, where t is the release time, M_t is the amount of drug delivered at time t, M_∞ is the total amount of drug delivered, k_{KP} is a kinetic constant, a measure of release rate and n the diffusional exponent that gives an indication of the mechanism of drug release and takes various values depending on the geometry of the release device. Thus, in our case of a thin film hydrogel, values up to 0.5 indicates a Fickian diffusion, 0.5–1.0 a anomalous (non-Fickian) transport (i.e. a mixed diffusion and chain relaxation mechanisms) and 1.0 means a Case II transport (zero order). Values of n greater than 1 reflects the so-called Super Case II-transport [11]. The smaller n values, below 0.5, are associated to drug diffusion through a partially swollen matrix and through water filled network mesh [12].

(3) After the swelling is complete, the system reaches an equilibrium state, characterized by a constant value of released drug and no concentration gradient between hydrogel matrix and release environment.

In this phase, a more general equation can be applied, that combines the contribution of Fickian (pure diffusivity phenomenon) and non-Fickian release (due to the relaxation of the polymer sections between network nodes, called and „relaxational" term), namely Peppas–Sahlin equation, expresed as the sum of two different powers of time:

$$\frac{M_t}{M_\infty} = k_D t^m + k_R t^{2m} \quad (4)$$

(1

where the first term of the right-hand side is the Fickian contribution (k_D is the diffusional constant) and the second term is the Case II relaxation contribution (k_R is the relaxation constant), and m is the purely Fickian diffusion exponent for a device of any geometrical shape, that can be determined from the plot of aspect ratio (diameter/thickness) against diffusional exponent n [13].

The k_D and k_R values were used to calculate the contribution percentage of Fickian diffusion (F_D) and relaxation (R), respectively, with the following equations:

$$F_D = \frac{1}{1 + \frac{k_R}{k_D} t^m} \quad , \quad (5a)$$

$$\frac{R}{F_D} = \frac{k_R}{k_D} t^m \quad (5b)$$

The Peppas-Sahlin equation represents actually a short time approximation of the Weibull exponential equation [14], a statistical distribution function of wide applicability, inclusively in drug release studies:

$$\frac{M_t}{M_\infty} = 1 - e^{-at^b} \quad (6)$$

where a and b are constants. The value of b is an indicator of the mechanism of drug transport through hydrogel: $b \leq 0.75$ indicate Fickian diffusion while a combined mechanism (Fickian diffusion and Case II transport) is associated with b values in the range $0.75 < b < 1$, for values of b higher than 1, the drug transport follows a complex release mechanism. This equation is criticized due to the lack of a kinetic basis for its use and the non-physical nature of its parameters.

(4) The fourth phase can be observed if the release is conducted at large time scales (almost four times greater than the time required to reach maximum of released drug), when the drug and/or polymer starts to degrade. Because a complete and detailed description of all the sub-phenomena at high time scales leads to very complex models and needs the knowledge of a high number of variables and parameters (which are hardly determinable), some simplifications in modelling are needed, according to the various level of details required and the different scales involved. The theoretical studies that were done on drug release from degradable polymers needed many assumptions like: „affine" deformations, no physical drug-hydrogel parts interactions, which might significantly vary with time and position, chemical reactions between drug and hydrogel parts and/or water.

In order to reduce the number of the approximations, one possible approach is the fractal one, justified by the fact that both natural and synthetic polymers are considered objects with fractal dimension and their structure and behaviour can be described by means of fractal geometry [15]. Moreover**,** the above presented laws, valid in certain approximations, are power type laws**,** specific for the fractal system evolution [16]. In this approach, we consider system complexity replaced with fractality and, therefore, the drug release process is considered to take place on curves (fractal curves) and physical quantities will be expressed through fractal functions (functions that are dependent both on coordinate field and resolution scale, continuous, but non-differentiable).

In this context, the entire system (drug loaded hydrogel in the release environment) can be considered as a medium totally lacking of interaction among component particles and its evolution can be theoretically analized in the framework of Scale Relativity Theory (SRT))[17], lead, in an arbitrary constant fractal dimension through the operator [4]:

$$\frac{\hat{\partial}}{\partial t} = \frac{\partial}{\partial t} + \hat{V}\nabla - iD(dt)^{(2/D_F)-1}\Delta + \frac{\sqrt{2}}{3}D^{3/2}(dt)^{(3/D_F)-1}\nabla^3 \quad (7)$$

where $\hat{V}$ is the complex speed field ($\hat{V} = V - iU$), V is the standard classical speed, independent of scale resolution *(dt),* whiles the imaginary part, U, is a new quantity arising from non-differentiability and resolution-dependent. Further, D is a structure coefficient, characteristic to the fractal-non-fractal transition, D_F is the fractal dimension of the drug particle trajectory, a measure of the system nonlinearity and Δ is the Laplace operator, a measure of the system dissipation [4].

Applying the fractal operator (7) to the amount of released drug M_t and accepting the principle of scale covariance in the form $(\hat{\partial} M_t / \partial t) = 0$, we obtain the generalized fractal diffusion equation in the explicit form:

$$\frac{\partial \hat{M}_t}{\partial t} = \frac{\partial M_t}{\partial t} + (\hat{V}\cdot\nabla)M_t - iD(dt)^{(2/D_F)-1}\Delta M_t + \frac{\sqrt{2}}{3}D^{3/2}(dt)^{(3/D_F)-1}\nabla^3 M_t = 0 \quad (8)$$

The equation (8) can be analyzed in two approximations of motion (dissipative and dispersive).

In the dissipative approximation of motion (equivalent with short time approximation of motion, when convective and dissipative process become dominant), results a equation similar to Weibull equation (6) [18].

In the dispersive approximation (equivalent with long time approximation, when convective and dispersive processes become dominant), the efficiency of drug release is dependent of a time function $\varepsilon(t)$ and system nonlinearity (through parameter s), in the form of a Korteweg de Vries type equation, whose explicit solution, for the unidimensional case and for a convenient choice of integration constants, has the form:

$$\frac{M_t}{M_\infty}(\varepsilon(t), s) = 2a\frac{E(s)}{K(s)} - 1 + 2a\, cn^2\left(\frac{\sqrt{a}}{s}\varepsilon(t); s\right) \quad (9)$$

where *cn* is Jacobi's elliptic function of s modulus, a is the amplitude, $K(s)$ and $E(s)$ are the complete elliptic integrals.

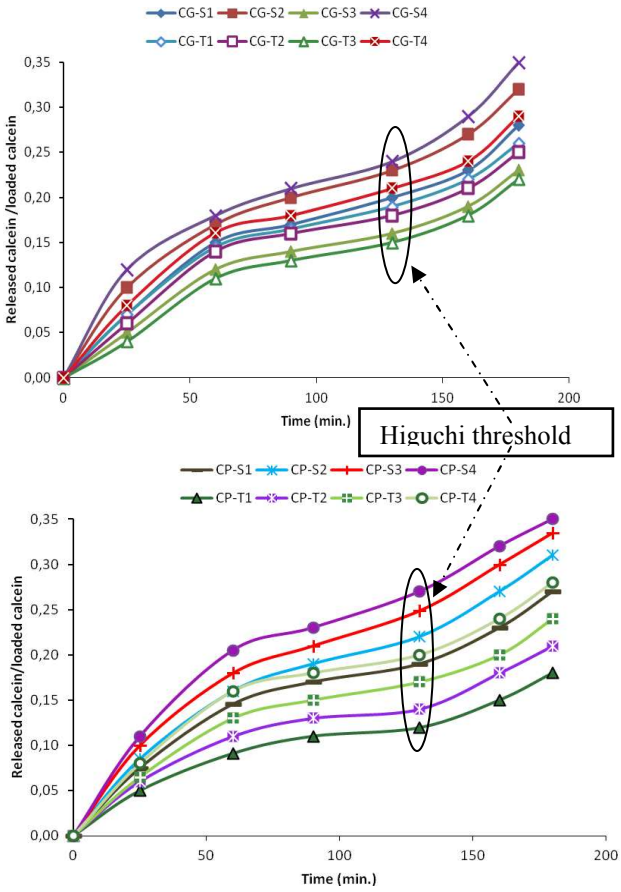
4. Results and discussion

4.1 Drug release at small time scales

4.1.1 Burst effect phase – Higuchi equation

In order to identify the time threshold up to which the burst effect takes place, the release kinetics were observed in the early times of the release, as seen in Figs. 3.

We identified the inflection points corresponding to the time threshold at approximately 130 min. for all samples, but differences in the amount of drug release can be observed, especially between hydrogels cross-linked with TPP and those with Na_2SO_4, due to different crosslinking density of the hydrogel matrix.



Figs. 3. Early time release – Burst effect phase

In order to determine the system parameters for the first phase of release (Higuchi constant – k_H, diffusion coefficient D), the release data up to Higuchi threshold are fitted with the coresponding Higuchi model equation. The values obtained (Table 2a, b) shows, as expected, that the diffusion coefficients calculated are higher for hydrogels crosslinked with Na_2SO_4, because the diffusion is favoured by their larger network mesh, that allows a higher average free path of drug molecules in the hydrogel superficial layer. Comparing the values of Higuchi constant, an indicator of release rate, it is observed that it has the lowest value for CG-T3 (for CG hydrogels) and CP-T1 (for CP hydrogels), samples with denser matrix determined by the highest amount of TPP used (CG-T3) and by the highest amount of chitosan used, the main participant at crosslinking.

For all samples, the correlation coefficients are higher than 0,99.

Sample	CS/GEL (w/w)	Na2SO4 (g)	k_H	D(cm2/s) x 10^{11}
CG-S1	1,9	0,060	0,72	1,71
CG-S2	9	0,064	0,86	2,44
CG-S3	4,5	0,079	0,60	1,19
CG-S4	4,5	0,060	0,98	3,16
Sample	CS/GEL (w/w)	TPP (g)	kH	D(cm2/s) x 10^{11}
CG-T1	1,9	0,078	0,66	1,43
CG-T2	9	0,083	0,64	1,36
CG-T3	4,5	0,102	0,54	0,96
CG-T4	4,5	0,077	0,72	1,72

Tables 2a. Higuchi parameters for CG hydrogels

Sample	CS/PAV (w/w)	k_H	D(cm2/s) x 10^{11}
CP-S1	9	0,68	1,50
CP-S2	5,7	0,77	1,94
CP-S3	4	0,87	2,47
CP-S4	3	0,96	3,01
Sample	CS/PAV (w/w)	kH	D(cm2/s) x 10^{11}
CP-T1	9	0,42	0,60
CP-T2	5,7	0,50	0,83
CP-T3	4	0,62	1,26
CP-T4	3	0,72	1

Tables 2b. Higuchi parameters for CP hydrogels

4.1.2 Swelling phase – Korsmeyer and Peppas equation

The Korsmeyer Peppas threshold (t_{KP}) is considered to be the moment at which the system reaches the equilibrium state with approximately constant calcein concentration (no gradient concentration between the hydrogel and the release environment). These moments are visualized in the release kinetics as the beginning of constant concentration plateau (Figs. 4) and their values can be seen in Table 3a, b (average values for each sample group). t_{KP} can be equivalent with complete swelling time and, as expected, the hydrogels follows similar swelling behaviors as when immersed in water: CG hydrogels, with higher crosslinking density, swells slower than others and reach last the equilibrium state.

Sample	CS/GEL (w/w)	Na2SO4 (g)	t_{KP} (days)	k_{KP}	n
CG-S1	1,9	0,060	3	0,50	0,32
CG-S2	9	0,064		0,53	0,29
CG-S3	4,5	0,079		0,47	0,37
CG-S4	4,5	0,060		0,56	0,26

Sample	CS/GEL (w/w)	TPP (g)	t_{KP} (days)	k_{KP}	n
CG-T1	1,9	0,078	4	0,40	0,30
CG-T2	9	0,083		0,37	0,30
CG-T3	4,5	0,102		0,33	0,33
CG-T4	4,5	0,077		0,43	0,29

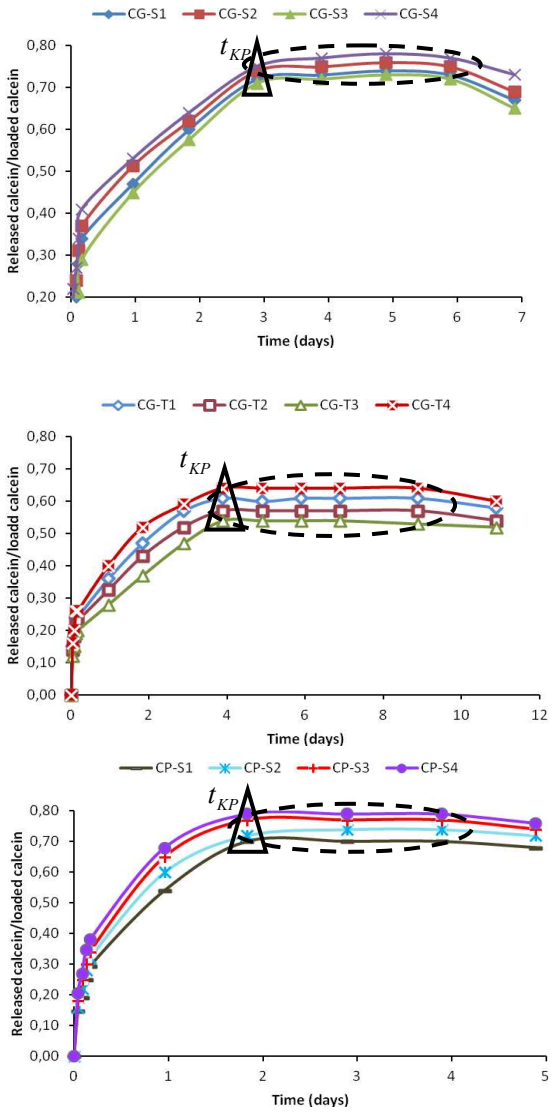
Sample	CS/PAV (w/w)	tKP (days)	kKP	n
CP-S1	9	2	0,55	0,40
CP-S2	5,7		0,60	0,38
CP-S3	4		0,63	0,36
CP-S4	3		0,65	0,33

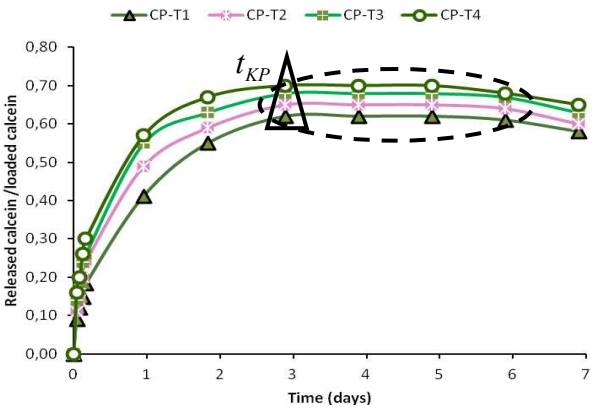
Sample	CS/PAV (w/w)	tKP (days)	kKP	n
CP-T1	9	3	0,40	0,44
CP-T2	5,7		0,46	0,38
CP-T3	4		0,50	0,35
CP-T4	3		0,53	0,33

Tables 3a. Korsmeyer Peppas parameters for CG hydrogels

Table 3b. Korsmeyer Peppas parameters for CP hydrogels

Also, the Korsmeyer Peppas parameters obtained by fitting are presented in Tables 3a,b.





Figs. 4. Korsmeyer Peppas threshold (t_{KP}) and equilibrium plateau (surrounded areas)

Referring to *n* values, simple Fickian diffusion mechanisms (*n=0.5*), does not occur for none of our samples; *n* values lies below 0.5, demonstrating that the mechanism of drug release is the complex Fickian diffusion, consisting of drug diffusion through the swollen hydrogel and/or water filled pores.

Besides insights in the release mechanism, the Korsmeyer Peppas equation offers information on release rate, through k_{KP}; it follows the same evolution as in burst effect phase: for CG-S hydrogels, the slower release manifests for CG-T3, with the most dense network due to higher amount of TPP, and the faster for CG-S4, for which the ionic cross linker is weaker and in smaller amount. In CP-T hydrogels case, for which the amount of ionic cross linker was the same for all samples, the faster release exhibits for the one cross-linked with TPP and highest chitosan/gelatin molar ratio (CP-T1) and the slowest for the one cross-linked with Na_2SO_4 and lowest chitosan/PVA molar ratio (CP-S4).

For all samples, the correlation coefficients are higher than 0,99.

4.1.3 Equilibrium phase

The equilibrium phases are that of constant concentration and their length (surrounded areas from Figs. 4) proved to be dependent of sample type, more precisely of the bonds strength that are stronger for CG-T hydrogels and weaker for CP-S hydrogels. Based on the observations of the release kinetics in the equilibrium area, we can establish a hierarchy of the stability degree detailed in Table 4. Also, a time threshold for this phase can be estimated.

Sample	Starting moment (days)	Time of equilibrium phase (days)	Equilibrium threshold (days)
CG-T	4	5	9
CP-T	3	3	6
CG-S	3	3	6
CP-S	2	2	4

Table 4. Equilibrium phase timing

In addition to the predicted evolution of CG-T and CP-S hydrogels, one observation can be done for CG-S and CP-T hydrogels: they are comparable as stability, acting as polymer properties (gelatin/PVA) are compensated by those of ionic cross linker (TPP/ Na_2SO_4)

The equilibrium phase will be analyzed through two equations: Peppas-Sahlin and Weibull, both known to describe the „entire" release kinetics, considered up to the constant plateau.

4.1.3.1 PEPPAS-SAHLIN EQUATION

The Peppas Sahlin parameters resulted by fitting the experimental data to this equation are presented in Table 5a,b. The value of m for our films was 0.4 as determined from the plot of aspect ratio (diameter/thickness) against diffusional exponent *n* [19].

Negative value of k_R were obtained, fact that indicates an insignificant effect of relaxation compared to the Fickian diffusion of drug release, continuing the Fickian release from swelling phase. Moreover, it is possible to express the preponderance of the Fickian mechanism compared to the relaxation one, as the values of k_D are larger than k_R, and less dependence on the relaxation of the polymer chains, although it is necessary that both phenomena take place [20].

Sample	CS/GEL (w/w)	Na_2SO_4 (g)	k_D	k_R
CG-S1	1,9	0,060	0,67	-0,15
CG-S2	9	0,064	0,74	-0,18
CG-S3	4,5	0,079	0,59	-0,10
CG-S4	4,5	0,060	0,80	-0,21
Sample	CS/GEL (w/w)	TPP (g)	k_D	k_R
CG-T1	1,9	0,078	0,53	-0,11
CG-T2	9	0,083	0,49	-0,10
CG-T3	4,5	0,102	0,42	-0,08
CG-T4	4,5	0,077	0,58	-0,13

Table 5a. Peppas Sahlin parameters for CG hydrogels

Sample	CS/PAV (w/w)	k_D	k_R
CP-S1	9	0,68	-0,15
CP-S2	5,7	0,76	-0,18
CP-S3	4	0,84	-0,22
CP-S4	3	0,92	-0,26
Sample	CS/PAV (w/w)	k_D	k_R
CP-T1	9	0,49	-0,08
CP-T2	5,7	0,60	-0,13
CP-T3	4	0,68	-0,17
CP-T4	3	0,75	-0,20

Table 5b. Peppas Sahlin parameters for CP hydrogels

From these parameters the contribution of the Fickian diffusion to the overall release (Eq. 5a) was calculated. The results for the representative samples of each group (CG-S4, CG-T3, CP-S4, CP-T1) versus time are plotted in Fig. 5.

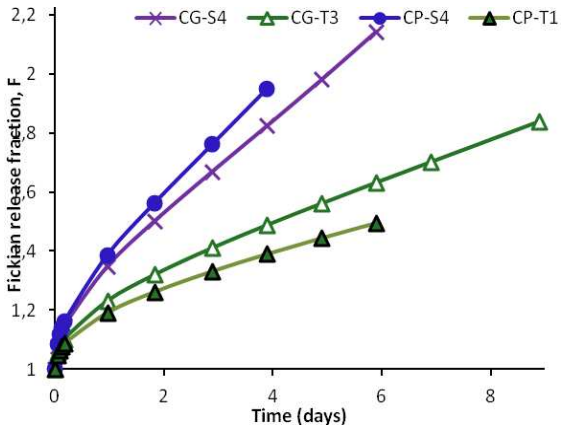


Fig. 5. The contribution of Fickian diffusion to overall release

It is obvious that Fickian diffusion is more dominant for hydrogels cross-linked with Na_2SO_4, for which the diffusion is faster due to a polymer network with high porosity.

For all samples, the correlation coefficients are higher than 0,99.

4.1.3.2 Weibull equation

The same analyze was performed also for Weibull equation and we obtained for parameter b values smaller than 0.75, indicating also a Fickian diffusion, consistent with the conclusion from the analysis based on Peppas Sahlin equation.

Since both equations, Peppas-Sahlin and Weibull, led to the same conclusion and the correlation factors are very close (0,99 for Peppas Sahlin equation and 0.98 for Weibull equation), it raises the question what equation is recommended to further analysis. We consider that the selection must be taken based on what information are needed: Weibull offers simple information on release mechanisms, while Peppas Sahlin can quantify the diffusion and relaxation contribution to the overall release.

4.2 Drug release at large time scales

In this case, the experimental results shows that the amount of drug existed in the release environment decreases. This decrease can be attributed to interactions appearing between calcein and hydrogel fragments resulting from degradation that bonds each other, so the amount of free calcein measured decreases as the degradation is more advanced and the number of detached fragments that bind calcein increases.

According to equation (9), in this situation the efficiency of drug release is dependent of a time function *ε(t)* and system

nonlinearity, through parameter s (we point out that nonlinearity can be interpreted as disorder in system evolution). This dependence is illustrated in Fig. 6.

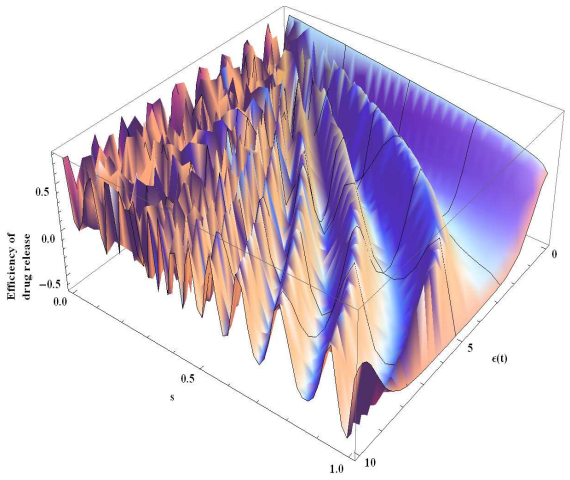


Fig. 6. 3D dependence of drug release efficiency on a time function ($\varepsilon(t)$) and system nonlinearity (s)

For each sample, the normal plane section that fits best the entire release kinetics was identified and, thus, the system nonlinearity and complexity can be estimated. The intersection between the normal section and the ($\varepsilon(t)$, s) plane of the tridimensional plot represents a straight line whose equation can be written:

$$s = s_0 + a\varepsilon(t) \quad (9)$$

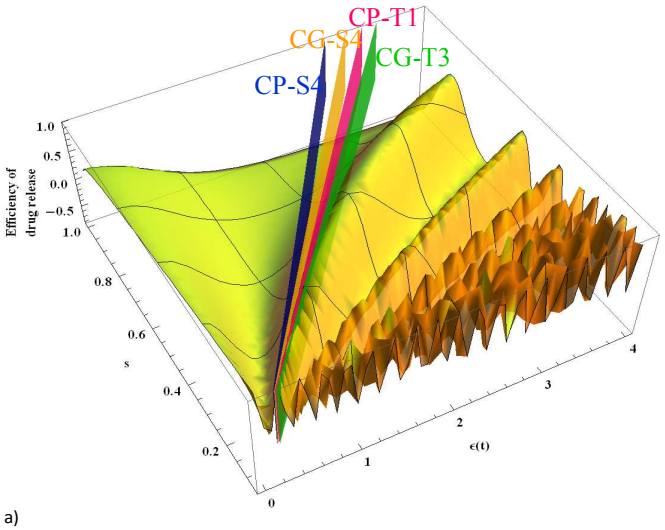
where s_0 represents initial system nonlinearity and a the line slope, with the physical meaning of the rate of evolution to unstable and disordered states, with high degree of nonlinearity. As a consequence of the similarity of release kinetics for the samples within a group, the associated parameters were very close, so in what follows will consider only the representative samples CG-S4, CG-T3, CP-S4, CP-T1. For these, the intersection line parameters are given in Table 6.

Sample	CG-T3	CP-T1	CG-S4	CP-S4
s_0	0.002	0.001	0.001	0.003
a	0.29	0.31	0.33	0.36

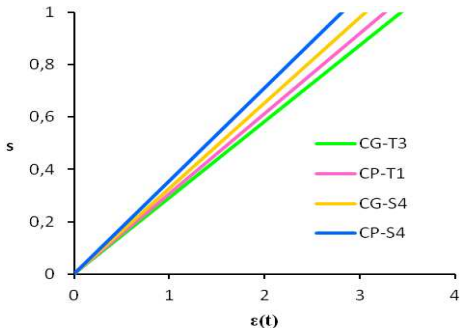
Table 6. The parameters of the intersection line between section plane and 3D plot

First observation is referring to s_0 values, that are very small, indicating thus stable, organized systems, in the initial state of the release. The a values confirm the stability hierarchy from Table 4, the most stable samples (CG-T) exhibiting the slowest evolution to unstable states.

The corresponding section planes and intersection lines with 3D plot (Fig. 6), that fits best with experimental data, are illustrated in Figs. 7.



a)

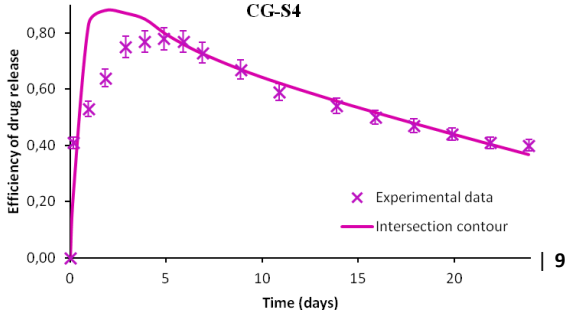


b)

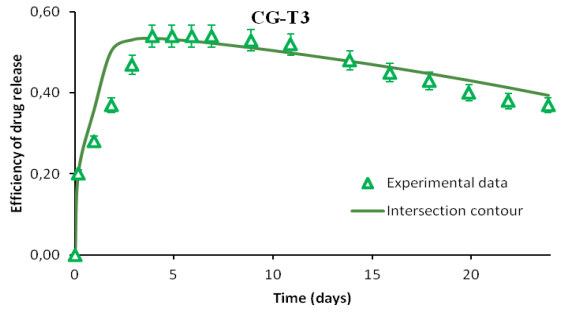
Figs. 7. The section planes (a) and intersection lines (b) for CG-S4, CG-T3, CP-S4, CP-T1

Fig. 7b reflects better that, at a certain moment in systems evolution (a given $\varepsilon(t)$) the nonlinearity degrees in these samples increases in the order CG-T3, CP-T1, CG-S4, CP-S4, a reverse order to that of stability (Table 4), as expected.

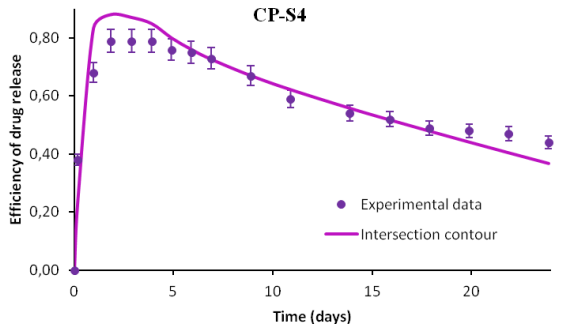
The intersection contours and experimental release profiles fits with correlation factors higher than 0.9 (Figs. 8).



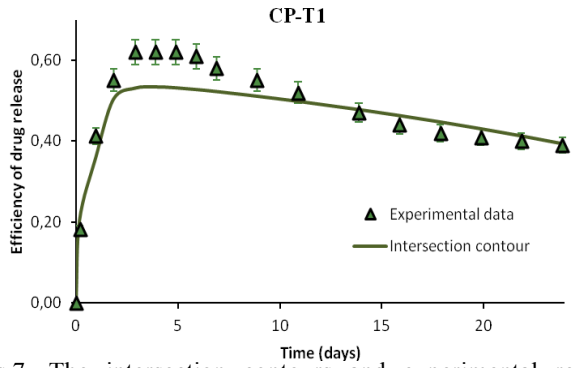
a)



b)



c)



d)

Figs.7. The intersection contours and experimental release profiles for samples CG-S4 (a), CG-T3 (b), CP-S4 (c), CP-T1 (d)

The above results reconfirm the validity of the fractal approach for the analysis of drug release at large time scales, offering insights on systems evolution to unstable, disordered states.

Conclusions

1. Interpenetrated networks based on chitosan, double cross-linked, are capable to release hydrophilic drugs through a multi-scale mechanism, characterized by four distinct phases, each characterized by a different kinetic model.

2. The first phase, of burst effect, can be best described by the Higuchi equation. The corresponding threshold is not significantly influenced by the polymers or cross linker type, being conditioned mainly by the concentration gradient.

3. The second one is dominated by the swelling of polymer matrix and the most appropriate model for it is that of Korsmeyer and Peppas. Its temporal length is proportional with the crosslinking density of polymer matrix, and, implicitly, depends on polymers or cross linker type.

4. The analysis of Korsmeyer Peppas parameters shows that the mechanism of drug release is the complex Fickian diffusion, consisting of drug diffusion through the swollen hydrogel and/or water filled pores and that the release rate depends, also, on polymers or cross linker type.

5. The equilibrium phase is the third one and is characterized by constant concentration and its time length is dependent of sample type, more precisely of the crosslinking density. The equilibrium phase can be analyzed through two equations: Peppas-Sahlin and Weibull. The quantitative analysis of these equations parameters shows the preponderance of the Fickian mechanism compared to the polymer matrix relaxation

6. Since both equations, Peppas-Sahlin and Weibull, led to the same conclusion, the decision of which equation can be used in further analysis must be taken based on what information are needed: Weibull equation offers simple information on release mechanisms, while Peppas Sahlin equation can quantify the diffusion and relaxation contribution to the overall release.

7. In situations where drug release is monitored at long time scales, the results show unusual evolution (in our case, the amount of released drug decreases). The phenomena that determine this (drug and polymer matrix degradation, physical and chemical interaction between them) are difficult to estimate, so that the nonlinear mathematical approach is appropriate. It shows that the efficiency of drug release is dependent of time and system nonlinearity. Moreover, the nonlinearity of all samples in the initial state has small values, indicating thus stable, organized systems; further, the rate of evolution to unstable and disordered states, with high degree of nonlinearity, is influenced by polymer matrix stability: the most stable samples exhibiting the slowest evolution to disorder.

References

[1] N. A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, *Eur. J. Pharm. Biopharm.*, 2000, **50**, 27.
[2] G. Tataru, M. Popa, J. Desbrieres, *J. Bioact. Compat. Polym.*, 2000, **24**, 525.

[3] J. Siepmann, R. Siegel, M. Rathbone, *Fundamentals ans Applications of Controlled Release Drug Delivery*, Springer, 2012.
[4] E. S. Băcăiţă, C. Bejinariu, B. Zoltan et. al, *J. Appl. Math.*, 2012, doi:10.1155/2012/653720.
[5] C. Vasile, G. E. Zaikov, *Environmentally Degradable Materials Based on Multicomponent Polymeric Systems*, Brill, 2009.
[6] C.A.Peptu, G.Buhus, M.Popa, A. Perichaud, D. Costin, *J. Bioact. Compat. Polym.,* 2010, **25**, 98.
[7] N. Jătariu, M.Popa, S. Curteanu, C.A. Peptu, *J. Biomed. Mater. Res. A*, 2011, **98A**, 342.
[8] B. C. Ciobanu, A. N. Cadinoiu, M. Popa, J. Desbrières, C. A. Peptu, *Cell. Chem. Technol.* (in press).
[9] L. L. Lao, N. A. Peppas, et. al, *Int. J. Pharm*., 2011, **418**, 28.
[10] T. Higuchi, *J. Pharm. Sci.*, 1963, **52**, 1145.
[11] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, *Int. J. Pharm.*, 1983, **15**, 25.
[12] E.B. Souto, *Eur. J. Med. Chem.*, 2013, **60**, 249.
[13] N.A. Peppas, J.J. Sahlin, *Int. J. Pharm.*, 1989, **57**, 169.
[14] V. Papadopoulou, K. Kosmidis, et. al, *Int. J. Pharm.*, 2006, **309**, 44.
[15] G.V. Kozlov, G.E. Zaikov, *Fractals and Local Order in Polymeric Materials*, Nova Science Publishers Inc. New York, 2001.
[16] V.U. Novikov, G.V. Kozlov, *Russ. Chem. Rev.*, 2000, **69**, 323.
[17] L. Nottale, *Scale Relativity and Fractal Space-Time – A New Approach to Unifying Relativity and Quantum Mechanics*, Imperial College Press, London, 2011.
[18] S. Băcăiţă, C. Urîtu, M. Popa, A. Uliniuc, C. Peptu, M. Agop, *Smart Mater. Res.*, 2012, doi:10.1155/2012/264609.
[19] N. A. Peppas, J.J. Sahlin, *Int. J. Pharm.*, 1989, **57**, 169.
[20] P. R. Oliveira, L. S. Bernardi,O. L. Strusi, et. al, *Int. J. Pharm.*, 2011, **405**, 90–96.