



5-Fluorouracil Release from Chitosan-Based Matrix. Experimental and Theoretical Aspects

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Abstract: A series of four drug release formulations based on 5-fluorouracil encapsulated into a chitosan-based matrix were prepared by *in situ* hydrogelation with 3,7-dimethyl-2,6-octadienal. The formulations were investigated from structural and morphological aspects by FTIR spectroscopy, polarized light microscopy and scanning electron microscopy. It was established that 5-fluorouracil was anchored into the matrix as crystals, whose dimension varied as a function of the crosslinking density. The *in vitro* drug release simulated into a media mimicking the physiological environment revealed a progressive release of the 5-fluorouracil, in close interdependence with the crosslinking density. In the context of Pharmacokinetics behavioral analysis, a new mathematical procedure for describing drug release dynamics in polymer-drug complex system is proposed. Assuming that the dynamics of polymer-drug system's structural units take place on continuous and nondifferentiable curves (multifractal curves), we show that in a one-dimensional hydrodynamic formalism of multifractal variables the drug release mechanism (Fickian diffusion, non-Fickian diffusion, etc) are given through synchronous dynamics at a differentiable and non-differentiable scale resolutions. Finally, the model is confirmed by the empirical data.

Keywords: drug release, dynamics, polymer, non-differentiable scale, multifractal curves

1. Introduction

Chitosan based formulations are of increasing interest in the drug delivery field, due to its intrinsic properties such as biocompatibility and biodegradability, which recommend it for *in vivo* applications [1-3]. In order to further improve the chitosan ability to anchor large amounts of drugs and to release them in a controlled manner, many attempts were pursued consisting mainly in chitosan crosslinking with various agents. In this line of thoughts, an eco-friendly method was developed by crosslinking chitosan with eco-friendly monoaldehydes [4-10]. The method proved to be a successful one, providing hydrogels whose properties can be simple controlled by the nature of the aldehyde. Thus, by using the natural aldehyde: 3,7-dimethyl-2,6-octadienal, also known under the commercial name: citral, hydrogels, with excellent biocompatibility and biodegradability which were further developed as matrix for drug delivery systems, were obtained.

On the other hand, the homogeneity assumption in its various forms (homogenous kinetic space, law of mass etc.) has become almost dogmatic in Pharmacokinetics (PK). The functionality of such a hypothesis allowed the development of a class of differentiable models in the description of dynamics

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of biological systems (i.e. “compartmental” analysis) and mainly, of drug release dynamics in such systems. However, biological systems are nowadays understood as inherently non-differential (fractal). Specifically, the microenvironments where any drug molecules with membrane interface, metabolic enzymes or pharmacological receptors are unanimously recognized as unstirred, space-restricted, heterogeneous and geometrically fractal. It is thus necessary to define a new class of models, this time non-differentiable, in describing biological system dynamics and particularly drug release dynamics in such systems.

Usually, such an approach, known as Fractal Pharmacokinetics, implies the use of fractional calculus, expanding on the notion of dimension etc. As such, it is possible in the context of “compartmental analysis” [11], to describe diffusion in dense objects [12], dynamics in polymeric networks [13, 14], diffusion in porous and fractal media [4], kinetics in viscoelastic media [15] etc. More recently, “compartmental analysis” through PK allowed the modeling of processes such as drug dissolution [16], absorption [17], distribution [18], whole disposition [19], kinetics with bio-molecular reactions [20] etc.

In this paper, in the context of “compartmental analysis”, a new method for describing drug release dynamics in complex systems (evidently discarding to fractional derivative and other standard “procedures” used in PK), considering that drug release dynamics can be described through continuous but non-differentiable curves (multifractal curves) is proposed. Then, instead of “working” with a single variable described by a strict, non-differentiable function, it is possible to “operate” only with approximations of these mathematical functions, obtained by averaging them on different scale resolutions. As a consequence, any variable purposed to describe drug release processes will still perform as the limit of a family of mathematical functions, this being non-differentiable for null scale resolutions and differentiable otherwise. Finally, the theoretical model is confirmed by the empirical date related to the 5-fluorouracil release from chitosan-based matrix.

2. Materials and methods

2.1 Materials

3,7-dimethyl-2,6-octadienal (95%), chitosan (243 kDa, DA: 87%), 5 fluorouracil and phosphate buffer solution with a $pH = 7.4$, have been purchased from Aldrich and used as received.

2.2 Formulation preparation

The formulations were prepared by in situ hydrogelation of chitosan with 3,7-dimethyl-2,6-octadienal in the presence of 5-fluorouracil, following an reported protocol [21]. Shortly, a 2% solution of 3,7-dimethyl-2,6-octadienal mixed with 5-fluorouracil was slowly dropped into a 3% solution of chitosan dissolved in aqueous acetic acid (1%), produced by Aldrich. The amount of drug and chitosan were kept constant, while the quantity of 3,7-dimethyl-2,6-octadienal was varied to reach different molar ratios of the amine/aldehyde groups, from 1/1 to 4/1, and thus to obtain hydrogels with different crosslinking density [7]. The hydrogelation time increased as the amount of aldehyde decreased. Thus, it instantly occurred for a 1/1 molar ratio of amine/ aldehyde functional groups and proceeded slowly, during 24 h for a 4/1 molar ratio. Finally, the obtained hydrogels were lyophilized and submitted to analysis. The formulations were coded **U1**, **U2**, **U3**, **U4**, the number corresponding to the molar ratio of amino/aldehyde groups.

2.3 Methods

The gelation time was determined when visually the reaction mixture was transformed from viscous to rubbery state. The xerogels have been obtained by lyophilization from the corresponding hydrogels, using a Labconco FreeZone Freeze Dry System, (FreeZoner2.5 Liter Freeze Dry Systems) equipment for 24 h at $-50^{\circ}C$ and 0.04 mbar. The formulations were characterized by FTIR spectroscopy, on a FT-IR Bruker Vertex 70 Spectrophotometer. The xerogels morphology was investigated with a field emission Scanning Electron Microscope SEM EDAX – Quanta 200 at accelerated electron energy of 20 keV.

The release kinetics from the developed drug delivery systems has been monitored by registering the absorbance at 265 nm from the supernatant in which the release was done, after which the concentration was calculated using the Beer-Lambert law. The UV-vis spectra of the supernatant were registered on a Horiba Spectrophotometer, and the absorbance was fitted on a prior drawn calibration curve. The calibration curve for the 5-fluorouracil was traced using the absorption maximum from its spectrum, at 265 nm.

3. Results and discussions

The presence of the 5-fluorouracil into formulations was evidenced by polarized light microscopy (Figure 1), which revealed the clear segregation of the drug into the hydrogels with high crosslinking density (U1, U2), while for the hydrogel formulations with lower crosslinking density (U4) a birefringent, granular texture was observed, characteristic for submicrometric dimensions of the crystals, which fall under the detection limit of the equipment [22,23].

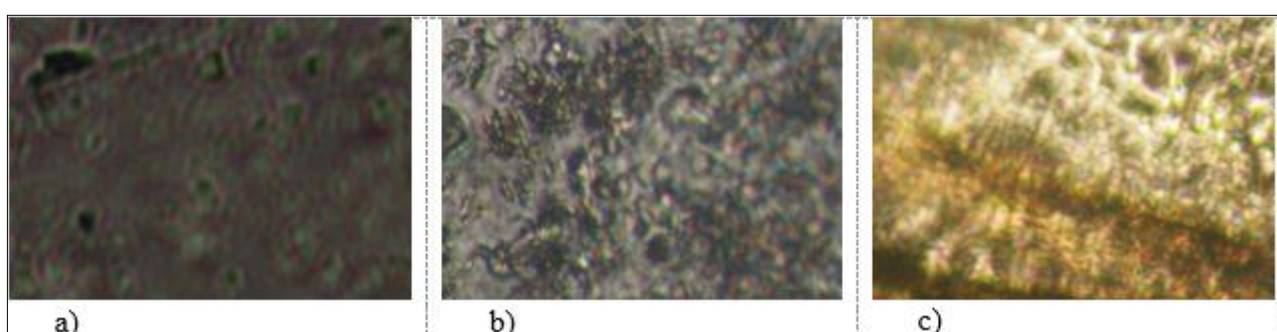


Figure 1. Representative POM images of the formulations a) U1; b) U2; c) U4

Further, the formulation morphology was assessed by scanning electron microscopy. As can be seen in Figure 2, they have a porous morphology, with evident drug crystals encapsulated into the pore walls (Figure 2). The diameter of the drug crystals decreased as the crosslinking degree was diminished, in line with the polarized light microscopy observation, as also observed for other chitosan based formulations [24].

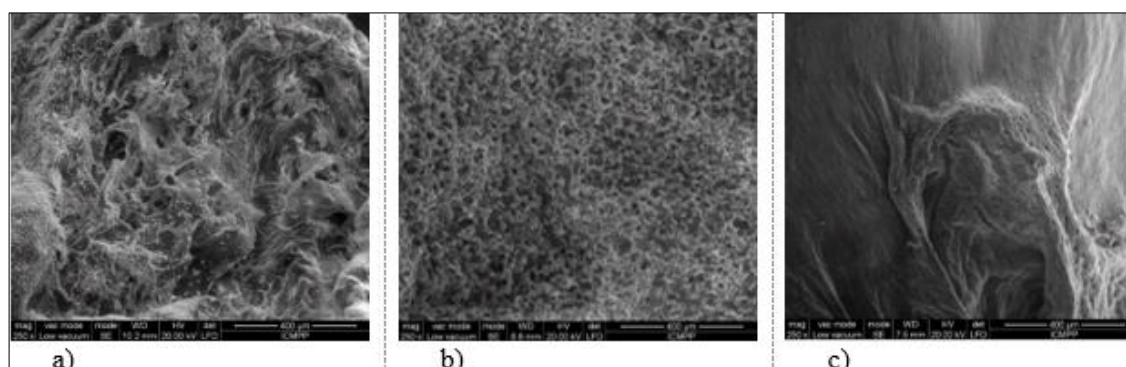


Figure 2. Representative SEM images of the formulations: a) U1; b) U3; c) U4

The in vitro drug release of the 5-fluorouracil showed a different trend, depending by the crosslinking density. Thus, the formulation U1 with the highest crosslinking density exhibited the fastest release rate, reaching almost 100% drug released in less than 24 h. The release rate slow down as the crosslinking degree decreased along with the total percent of the drug released, reaching in the case of the formulation U4 around 75 % drug release in less than 24 h (Figure 3). This correlated very well with the size of the drug into formulations; as the drug crystals size decreased, the release rate diminished, in agreement with the stronger anchoring of the drug into the chitosan based matrix [25].

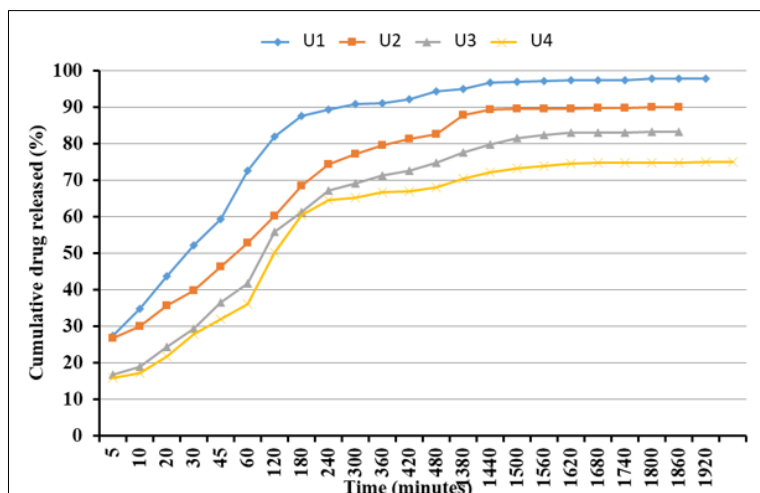


Figure 3. The 5-fluorouracil release from the formulations (blue: U1; orange: U2; grey: U3, yellow: U4)

3.1. Theoretical model

Let it be considered the one-dimensional multifractal hydrodynamic-type equations [26-30], in the form:

$$\partial_t V_D + V_D \partial_x V_D = -\partial_x \left[-2\lambda(dt) [f(\alpha)]^{-2} \frac{\partial_x \partial_x \sqrt{\rho}}{\sqrt{\rho}} \right] \quad (1)$$

$$\partial_t \rho + \partial_x (\rho V_D) = 0 \quad (2)$$

In the above – written relations, x is the fractal spatial coordinate, t is the non – fractal time having the role of an affine parameter of the motion curves, V_D is the differential velocity independent on the scale resolution dt , $f(\alpha)$ is the singularity spectrum of order α and $\sqrt{\rho}$ is the states function amplitude.

These equations for the initial and boundary conditions:

$$V_D(x, t = 0) = V_0, \quad \rho(x, t = 0) = \frac{1}{\sqrt{\pi}\alpha} \exp \left[-\left(\frac{x}{\alpha}\right)^2 \right] \quad (3)$$

$$V_D(x = V_0 t) = V_0, \quad \rho(x = -\infty, t) = \rho(x = +\infty, t) = 0 \quad (4)$$

with V_0 the initial velocity and α the parameter of Gaussian distribution of positions, using the mathematical procedures from [30-36], admit the solution:

$$V_D(x, t, \sigma, \alpha) = \frac{V_0 \alpha^2 + \left(\frac{\sigma}{\alpha}\right)^2 xt}{\alpha^2 + \left(\frac{\sigma}{\alpha}\right)^2 t^2} \quad (5)$$

$$\rho(x, t, \sigma, \alpha) = \frac{\pi^{-1/2}}{\left(\alpha^2 + \left(\frac{\sigma}{\alpha}\right)^2 t^2\right)^{1/2}} \exp \left[-\frac{(x - V_0 t)^2}{\alpha^2 + \left(\frac{\sigma}{\alpha}\right)^2 t^2} \right] \quad (6)$$

with

$$\sigma = \lambda(dt) [f(\alpha)]^{-1} \quad (7)$$



the multifractal degree. From here, the non-differentiable velocity V_F takes the form:

$$V_F(x, t, \sigma, \alpha) = \sigma \frac{(x - V_0 t)}{\alpha^2 + \left(\frac{\sigma}{\alpha}\right)^2 t^2} \tag{8}$$

Introducing the non-dimensional variables:

$$\xi = \frac{x}{V_0 \tau_0}, \quad \eta = \frac{t}{\tau_0} \tag{9}$$

and non-dimensional parameters

$$\mu = \frac{\sigma \tau_0}{\alpha^2}, \quad \phi = \frac{\alpha}{V_0 \tau_0} \tag{10}$$

with τ_0 the specific time, (5), (6) and (8) become:

$$V \equiv V_D(\xi, \eta, \mu) = \frac{V_D(x, t, \sigma, \alpha)}{V_0} = \frac{1 + \mu^2 \xi \eta}{1 + \mu^2 \eta^2} \tag{11}$$

$$\rho(\xi, \eta, \mu, \phi) = \pi^{\frac{1}{2}} \alpha \rho(x, t, \sigma, \alpha) = (1 + \mu^2 \eta^2)^{-\frac{1}{2}} \exp \left[-\frac{(\xi - \eta)^2}{\phi^2 (1 + \mu^2 \eta^2)} \right] \tag{12}$$

$$U \equiv V_F(\xi, \eta, \mu) = \frac{V_F(x, t, \sigma, \alpha)}{V_0} = \mu \frac{(\xi - \eta)}{1 + \mu^2 \eta^2} \tag{13}$$

Now taking out the quadratic term in η between (11) and (13), it results that for $\xi = const.$ the ratio $\frac{V}{U}$ is homographic dependent of ξ by the form:

$$\frac{U}{V} = \frac{\mu(\xi - \eta)}{1 + \mu^2 \xi \eta} \tag{14}$$

From here, the condition (dynamical simultaneity):

$$d\left(\frac{U}{V}\right) = 0 \Leftrightarrow V = const U \tag{15}$$

(i.e. the extension of the first principle of Newton to any scale resolution, or equivalently, “synchronizations” of drug release dynamics at differentiable scale with drug release dynamics at non-differentiable scale), implies correlations between phase and amplitude of the shape function, by the form:

$$\ln \rho = \rho_0 \exp[const(s - s_0)] \tag{16}$$

where ρ_0 and s_0 are integration constants. Thus, it is stated that various “mechanisms” involved in the drug release process can be mimed through period doubling, quasi-periodicity, intermitences etc. (for details see [37]).

Because through the restriction (15) given, for example, by $V = -U$, the multifractal type conservation laws (1) and (2) take the form of the multifractal type “diffusion” equation:

$$\partial_t \rho = \lambda (dt)^{\left[\frac{2}{f(\alpha)}\right]^{-1}} \partial_l \partial^l \rho = \sigma \partial_l \partial^l \rho \tag{17}$$

it results that these “mechanisms” “manifest”/are “perceived” as diffusions at various scale resolutions in a multifractal space (fickian-type diffusion, non-fickian-type diffusion etc.) To expand on this

hypothesis, we approach on investigating the following scenario: the one-dimensional drug diffusion of multifractal type from a controlled-release polymeric system with the form of a plane shut, of thickness δ . If drug release of multifractal type occurs under perfect sink condition, the following initial and boundary conditions can be assumed:

$$\begin{aligned} t = 0, & \quad -\frac{\alpha}{2} < x < \frac{\alpha}{2}, & \quad \rho = \rho_0 \\ t > 0, & \quad x = \pm \frac{\alpha}{2}, & \quad \rho = \rho_1 \end{aligned} \tag{18}$$

where ρ_0 is the initial drug states density of the multifractal type in the “device” of multifractal type and ρ_1 is the drug states density at the “polymer-fluid” interface of multifractal type. This solution equation under these conditions can take the following form (for details in the classical case see [37]). In Figure 4 there are represented the

$$f = \frac{\rho_t}{\rho_\infty} = 2 \left(\frac{\sigma t}{\delta^2} \right)^{\frac{1}{2}} = \left\{ \pi^{-1/2} + \sum_{n=1}^{\infty} (-1)^n \operatorname{erfc} \left[\frac{n\delta}{2(\sigma t)^{\frac{1}{2}}} \right] \right\} \tag{19}$$

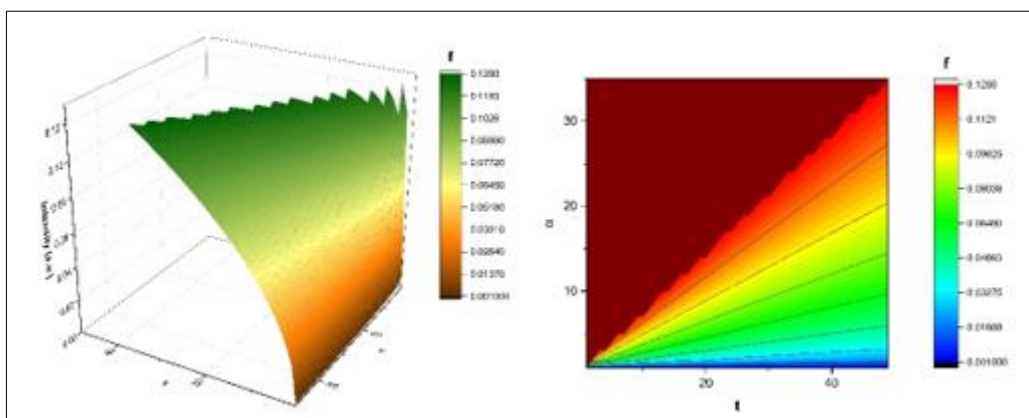


Figure 4. 3D (left-side) and contour plot (right-side) representations of our multifractal function use for drug release mechanism analysis

An accurate expression can be obtained for small values of t since the second term of (20) disappears and then it becomes:

$$\frac{\rho_t}{\rho_\infty} = 2 \left(\frac{\sigma t}{\delta^2} \right)^{\frac{1}{2}} = \operatorname{const}(t)^{\frac{1}{2}} \tag{20}$$

In such a context, $\frac{\rho_t}{\rho_\infty}$ can be assimilated to the fraction of dissolved drug i.e. $\frac{M_t}{M_\infty} \equiv \frac{\rho_t}{\rho_\infty}$, where M_t is the amount of drug dissolved in time t and M_∞ is the total amount of time dissolved when the pharmaceutical dosage form is exhausted [29, 37]. A verification of our model is presented in Figure 5, for the drug release of 5-fluorouracil release from chitosan-based matrix. The empirical data was fitted with the mathematical function. The figure shows that the model is well equipped to predict the drug-release dynamics [38].

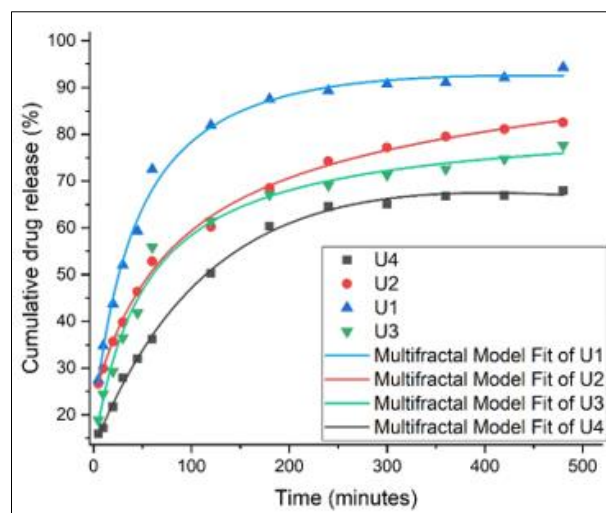


Figure 5. Experimental showcase of the 5-fluorouracil release from the formulations fitted by the multifractal theoretical model

4. Conclusions

A series of drug delivery systems were prepared by encapsulation of the 5-fluorouracil into a hydrogel formed by crosslinking chitosan with 3,7-dimethyl-2,6-octadienal. The hydrogel proved excellent ability to anchor the drug, assuring its prolonged release during 24 h. The release rate has been tuned by varying the crosslinking density, reaching 96 % for a high crosslinking density and a 75 % rate for a lower one. A theoretical model in a multi fractal paradigm was developed for understanding the drug release dynamics, considering that these behaviors are described by continuous but non-differentiable curves. In such a context the irrotational type dynamic of the polymer drug structural units implies the functionality of a multifractal type hydrodynamic formalism. For the unidimensional case of this multifractal type hydrodynamic formalism, we can see that ratio between the differentiable velocity and the non-differentiable one for a certain distance depends in a homographic manner on time. The conditions for the simultaneous dynamics imply the synchronization of the drug release mechanisms at the two scale resolutions, expressed through diffusion functions of multifractal type (the diffusion process depends on the scale resolutions). The model is confirmed by experimental data .

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References

1. PRAUSNITZ M.R., LANGER R., Transdermal drug delivery. *Nat. Biotechnol.* Vol. 26, 2008, p. 1261-1268.
2. PATEL A., CHOLKAR K., AGRAHARI V., MITRA A.K., Ocular drug delivery systems: An overview. *World J. Pharmacol.* Vol. 2, 2013, p. 47-64.
3. JACOB J., HAPONIUK J.T., THOMAS S., GOPI S., Biopolymer based nanomaterials in drug delivery systems: A review. *Mater Today Chem.* Vol. 9, 2018, p. 43-55.
4. MARIN L., AILINCAI D., MARES M., PASLARU E., CRISTEA M., NICA V., SIMIONESCU B.C., Iminochitosan biopolymeric films. Obtaining, self-assembling, surface and antimicrobial properties, *Carbohydr. Polym.*, Vol. 117, 2015, p. 762-770.
5. AILINCAI D., MARIN L., MORARIU S., MARES M., BOSTANARU A.C., PINTEALA M., SIMIONESCU B.C., BARBOIU M., Dual crosslinked iminoboronate-chitosan hydrogels with strong antifungal activity against *Candida* planktonic yeasts and biofilms, *Carbohydr. Polym.*, Vol.152, 2016, p. 306-316.



6. IFTIME M.M., MORARIU S., MARIN L., Salicyl- imine-chitosan hydrogels: Supramolecular architecturing as a crosslinking method toward multifunctional hydrogels, *Carbohydr. Polym.*, Vol. 165, 2017, p. 39-50.
7. MARIN L., AILINCAI D., MORARIU S., TARTAU-MITITELU L., Development of biocompatible glycodynameric hydrogels joining two natural motifs by dynamic constitutional chemistry, *Carbohydr. Polym.* Vol. 170, 2017, p. 60-71.
8. OLARU A.M., MARIN L., MORARIU S., PRICOPE G., PINTEALA M., TARTAU-MITITELU L., Biocompatible based hydrogels for potential application in local tumour therapy, *Carbohydr. Polym.*, vol. 179, 2018, p. 59-70.
9. BEJAN A., AILINCAI D., SIMIONESCU B.C., MARIN L., Chitosan hydrogelation with a phenothiazine based aldehyde-toward highly luminescent biomaterials, *Polym. Chem.*, vol. 9, 2018, p. 2359-2369.
10. IFTIME M.M., MARIN L., Chiral betulin- imino-chitosan hydrogels by dynamic covalent sonochemistry, *Ultrason. Sonochem.*, Vol. 45, 2018, p. 238-247.
11. PEREIRA, L.M., *Computational and Mathematical Methods in Medicine*, Vol. 11, no.2, 2010, p. 161-184.
12. LEMEHAUTE, A., CREPY, G., *Solid State Ionics*, Vol. 9-10, 1983, p.17-30.
13. BARKAI, E., KLAFTER, J., *Physical Review Letters*, Vol. 81, no.5, 1998, p.1134-1134.
14. HONCIUC, M., PAUN, V.P., *Rev. Chim.*, **54**(1), 2003, 74-76.
15. LAZAR, B., STERIAN, A., PUSCA, S., et al., Conference: International Conference on Computational Science and Its Applications (ICCSA 2006), *Computational Science and Its Applications - ICCSA 2006, PT 1 Book Series: LECTURE NOTES IN COMPUTER SCIENCE*, Vol. 3980, 2006, p. 779-784.
16. STANA, R.; BOTEZ, I. CASIAN; PAUN, V. P., et al., *Journal of Computational and Theoretical Nanoscience*, Vol. 9, no. 1, 2012, p. 55-66.
17. IORDACHE, D., PUSCA, S., TOMA, G., et al., Conference: International Conference on Computational Science and Its Applications (ICCSA 2006), *Computational Science and Its Applications - ICCSA 2006, Pt 1 Book Series: LECTURE NOTES IN COMPUTER SCIENCE*, Vol. 3980, 2006, p. 804-813.
18. KOSMIDIS, K., ARGYRAKIS, P., MACHERAS, P., (2003). *The Journal of Chemical Physics*, Vol.119, no.12, 2003, p.6373-6377.
19. HIGAKI, K., YAMASHITA, S., AMIDON, G.L., *Journal of Pharmacokinetics and Pharmacodynamics*, Vol. 28, no. 2, 2001, p.109-128.
20. KARALIS, V., TSANTILI-KAKOULIDOU, A., MACHERAS, P., *European Journal of Pharmaceutical Sciences*, Vol. 20, no. 1, 2003, p.115-123.
21. AILINCAI D., MITITELU TARTAU L., MARIN L., Drug delivery systems based on biocompatible imino-chitosan hydrogels for local anticancer therapy, *Drug Deliv.*, 25, 2018, 1080-1090.
22. ZABULICA A., BALAN M., BELEI D., SAVA M., SIMIONESCU B. C., MARIN L., Novel luminescent phenothiazine-based Schiff bases with tuned morphology. Synthesis, structure, photophysical and thermotropic characterization, *Dyes and Pigments*, 96, 2013, 686-698.
23. MARIN L., POPESCU M.C., ZABULICA A., UJI-I H., FRON E., Chitosan as a matrix for biopolymer dispersed liquid crystal systems, *Carbohydr. Polym.*, 95, 2013, 16-24.
24. IFTIME M.M., AILIESEI G.L., UNGUREANU E., MARIN L., Designing chitosan based eco-friendly multifunctional soil conditioner systems with urea-controlled release and water retention, *Carbohydr. Polym.* 223, 2019, 115040.
25. CRACIUN A.M., MITITELU TARTAU L., PINTEALA M., MARIN L., Nitrosalicyl-imine-chitosan hydrogels based drug delivery systems for long term sustained release in local therapy, *J. Colloid Interface Sci.*, Vol. 536, 2019, p. 196-207.



26. IRIMICIUC S.A., NICA P.E., AGOP M., FOCSA, C. Target properties - Plasma dynamics relationship in laser ablation of metals: Common trends for fs, ps and ns irradiation regimes Appl. Surf. Sci., Vol. 506, 2020, 144926.
27. IRIMICIUC S., BULAI G., AGOP M., GURLUI S., Influence of laser-produced plasma parameters on the deposition process: In situ space- and time-resolved optical emission spectroscopy and fractal modeling approach, Appl. Phys. A Mater. Vol. 124, 2018. 615.
28. IRIMICIUC S.A. BULAI G., GURLUI S., AGOP M., On the separation of particle flow during pulse laser deposition of heterogeneous materials-A multi-fractal approach, Powder Tech. Vol. 339, 2018, p. 273-280.
29. COBZEANU B.M., IRIMICIUC S., VAIDEANU D., GRIGOROVICI A., POPA O., Possible Dynamics of Polymer Chains by Means of a Ricatti's Procedure-an Exploitation for Drug Release at Large Time Intervals, *Mater. Plast.*, **54**(3) 2017, 531-534
30. MERCHES I., AGOP M., *Differentiability and fractality in dynamics of physical systems*, World Scientific, New Jersey, 2016.
31. MANDELBROT, B.B., *The Fractal Geometry of Nature*, W. H. Freeman and Co., San Francisco, 1982.
32. BORDESCU, D., PAUN, M.A., PAUN, V.A., PAUN, V.P., University Politehnica of Bucharest Scientific Bulletin, Series A-Applied Mathematics and Physics Vol. 80, no. 4, 2018, p. 309-320.
33. NICHITA, M.V., PAUN, M.A., PAUN, V.A., PAUN, V.P., University Politehnica of Bucharest Scientific Bulletin-Series A-Applied Mathematics and Physics Vol. 81, no. 1, 2019, p. 273-284.
34. NOTTALE, L., *Scale Relativity and Fractal Space-Time: A New Approach to Unifying Relativity and Quantum Mechanics*, Imperial College Press, London, 2011.
35. MERCHES, I., AGOP, M., *Differentiability and fractality in dynamics of physical systems*, World Scientific, New Jersey, 2016.
36. AGOP, M., PAUN, V.P., *On the new perspectives of fractal theory. Fundaments and applications*, Romanian Academy Publishing House, Bucharest, 2017.
37. AILINCAI, D., DOROBANȚU, A.M., DIMA, B., IRIMICIUC, S.A., LUPAȘCU, C., AGOP, M., OLGUTA, O., Journal of Immunology Research, 2020, "in press".
38. CRANK, J., *Diffusion in a plane shut in: The Mathematics of Diffusion*, 2nd Edition, Oxford Press, Oxford, 1965.

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