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Mathematical models as tools to predict the release kinetic of fluorescein from monoglyceride colloidal liquid crystals

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Abstract: The manuscript studies the release kinetic of fluorescein from colloidal liquid crystals made up from monoglyceride and different non-ionic surfactants. The release experiments were carried out under sink conditions, and mathematical models were described as extrapolations from solutions of diffusion equation in different initial and boundary conditions imposed by pharmaceutical formulations. The diffusion equation was solved, using Laplace and Fourier transformed functions for the release kinetic from infinite reservoirs in a semi-infinite medium. Solutions represents a general square root law and can be applied for the release kinetic of fluorescein from monoglyceride colloidal liquid crystals. Akaike, Schwartz and Imbimbo criteria were used to establish the appropriate mathematical model and the hierarchy of performances of different models applied to the release experiments. The Fisher statistic test was applied to perform significance of differences among mathematical models. Differences evaluated by mathematical criteria demonstrated that small or no significant statistic differences were carried out between various applied models and colloidal formulations. Phenomenological models were preferred over to empirical and semi-empirical ones. The general square root model shows that the diffusion-controlled release of fluorescein is the mathematical models extrapolated for monoglyceride colloidal liquids, in the first part of the process.

Keywords: monoglyceride colloidal liquid crystals; release profile; mathematical models; drug delivery systems; release kinetic; square root laws.

1. Introduction

The release kinetic of drugs from pharmaceutical formulations plays a main role to study the biopharmaceutical features of payloads following their administration into the body [1]. In fact, the process of release of a drug from formulations can modulate its absorption into the various biological compartments; besides its distribution and plasma concentration, that is the rate of absorption and bioavailability in biological fluids [2]. In this attempt, technological approaches are extensively used to design pharmaceutical formulations and to obtain a controlled release of drugs. Furthermore, drug release is affected by different physiological parameters, e.g. pH of the gastric

tract, bile and pancreatic secretion, and drug dissolution from formulations [3]. Other parameters, such as physicochemical properties of biocompatible materials, their chemical structures, the thickness of polymeric or lipid shells, the interaction between drugs and plasmatic proteins, the coefficient of permeation and diffusion may be also affect the release profile of drug delivery systems [4,5].

The release profile of drugs and bio-molecules from conventional and innovative carriers, such as monoglyceride colloidal liquid crystals, could be described using mathematical approaches. In fact, liquid crystals are arranged in different supramolecular structure provided from self-assembling of surfactants [6]. Their geometrical arrangement, as well as their interaction with biological substrates, depends on thermodynamic transition from solid to liquid state of biomaterials that modulates potential applications in nanomedicine and biotechnology [7]. Monoglyceride liquid crystals can exist as thermotropic and lyotropic mesophases, and their transition from first to second supramolecular structure depends on ionic interactions between polar head groups of surfactants, hydrophobic interactions between hydrocarbon tail, surfactant concentration in water, and critical micellar concentration. All these parameters can modify the arrangement of surfactants and lipid components in liquid crystals, thus modifying their physicochemical and biopharmaceutical properties [8,9].

In this attempt, preliminary *in vitro* experiments needed to evaluate the release profile of payloads from monoglyceride colloidal liquid crystals and to predict their potential *in vivo* behavior. The prediction of physicochemical properties in colloidal formulations can be investigated using mathematical models that suitable describe the potential *in vitro/in vivo* trend [10,11].

The massive release kinetics of drugs resulting from the experimental data were further described using different mathematical models such as Noyes-Whitney [12], Higuchi [13], Peppas [14] and Weibull [15]. The mathematical models herein reported support the resulting data and demonstrated that the phenomenological models were preferred over to empirical and semi-empirical ones to estimate the release kinetic of hydrophilic drugs from monoglyceride colloidal liquid crystals.

2. Materials and Methods

2.1 Materials

Fluorescein was purchased from Sigma-Aldrich Italy (Milan, Italy). Polyoxyethylene-polyoxypropylene block copolymer 127 (Symperonic® PE/F127) was obtained from Uniquema, Croda Italiana S.p.A. (Mortara (PV) Italy). Polyethylene glycol-polypropylene glycol-polyethylene glycol block copolymer 6800 (Pluronic® PE 6800), polyethylene glycol block copolymer 10500 (Pluronic® PE 10500), Polyoxyethylene stearyl ether (Brij® 72), Polyethylene glycol oleyl ether (Brij® 92) were a kind gift of ACEF Spa (Piacenza, Italy). Glyceryl monooleate (Monomuls® 90-O18) was obtained from Cognis S.p.A. (Fino Mornasco (CO), Italy). Double-distilled pyrogen-free water was purchased from Sifra S.p.A. (Verona, Italy). Isotonic sterile saline solution (NaCl 0.9 % w/v) was a product of Fresenius Kabi Potenza S.r.l. (Verona, Italy). All other materials and solvents used in this investigation were of analytical grade (Carlo Erba, Milan, Italy).

2.2 Monoglyceride colloidal liquid crystals

Monoglyceride colloidal liquid crystals were synthesized by emulsifying hydrophobic surfactants with an aqueous solution of a poly(ethylene) glycol derivative. The mixing-dilution procedure was carried out to obtain different formulations. Glyceryl monooleate (90 mg) was dissolved in ethanol (2 ml) and the hydrophilic surfactants were dissolved in distilled water or isotonic saline solution (NaCl 0.9 % w/v) (8 ml). The aqueous phase was added drop by drop to the organic phase under continuous stirring using an Ultraturrax T25 basic homogenizer (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at a mixing speed of 18,500 rpm (3 different cycles of 5 min). Preparation was carried out at room temperature. An inclusion complex was obtained when co-surfactant was added to the ternary system ethanol-monoolein-water, thus affecting

supramolecular structure of liquid crystals. The resulting formulations were stored at room temperature for 48 h under continuous stirring (400 rpm) in order to remove any residual trace of ethanol. The residual and not assembling surfactant was then removed by using dialysis membrane with a molecular cut-off 50,000 Dalton (Spectra/Por Membranes, Spectrum Laboratories, Inc., CA, USA). The dialysis was performed for 48 h at room temperature using sterile isotonic saline solution (NaCl 0.9 % w/v) (200 ml) as receptor medium. A constant mixing rate of 400 rpm was maintained during purification and saline solution were replaced every 8 h. Four different formulations were prepared as reported in Table 1. Aqueous fluorescein solution (0.03 % w/v), used as hydrophilic drug model during experiments, was added to the aqueous phase during the preparation procedure.

Table 1. Chemical compositions of colloidal liquid crystals.

Formulations	Monomuls	Brij 72	Brij 92	Pluronic 10500	Pluronic F68	Symperonic
1	—	—	90 mg	—	50 mg	—
2	90 mg	50 mg	—	—	—	—
3	90 mg	—	—	—	—	50 mg
4	90 mg	—	—	50 mg	—	—
5	—	—	90 mg	—	—	50 mg

2.3 Release experiments

The release of fluorescein from monoglyceride colloidal liquid crystals was investigated using dialysis membrane. Regenerated cellulose membrane with molecular cut-off 25,000 Dalton (Spectra/Por Membranes, Spectrum Laboratories, Inc., CA, USA) was used for release experiments. Membrane was hydrated before analysis for 40 min using a sterile isotonic saline solution to remove any traces of sodium azide storage solvent. Membranes were then filled with 1 ml of different formulations, sealed with dialysis clips and then placed in a pyrex glass beaker containing 200 ml of sterile isotonic saline solution. Experiments were carried out at room temperature for 24 h and sink conditions were maintained during analysis. At different time points, 1 ml of the receptor solution was withdrawn, replaced with the same volume of fresh isotonic saline solution and then immediately analyzed using an UV spectrophotometer (Perkin Elmer Lambda 20, Norwalk, CT, USA). A fluorescein calibration curve was used to quantify fluorescent molecule released from monoglyceride liquid crystals, according to the following equation:

$$y = 0.88 x + 0.148 \tag{1}$$

where, x represents the fluorescein concentration ($\mu\text{g/ml}$) and y the UV/Vis absorbance (nm). The r^2 value was 0.9982. No interference was observed at fluorescein λ_{max} of 495 nm from other components of formulation.

2.4 Statistical criteria and information on selection of mathematical models

2.4.1 Akaike and Schwarz criteria

The Akaike information criterion (AIC) [16] and Schwarz criterion (SC) [17] are two different mathematical methods applied to data. They are both based on the addition of statistical errors corrected by a penalty function, which are proportional to the number of parameters (p) evaluated in the model:

$$AIC = N \ln WSS + 2p \quad (2)$$

$$SC = N \ln WSS + p \ln N \quad (3)$$

where, N represents the number of point data, and squared errors WSS is the weighted sum of squared deviations of a model with a set of p parameters, calculated according to the following equation:

$$WSS = \sum_{i=1}^n w_i (y_i^{\text{exp}} - y_i^{\text{calc}})^2 \quad (4)$$

where, W_i is the weighting factor for the respective data.

The model equation having the lowest AIC and/or SC were selected for the description of time course plots [18].

2.4.2 Imbimbo criterion

The Imbimbo criterion is based on the mean area between the limits of a 90 % confidence interval for calculated values according to model, $\hat{y}_i = y_i^{\text{calc}}$ [19] using the following equation:

$$I_p = \frac{t_{(N-p,0.05)}}{\overline{y_i^{\text{calc}}}} \sqrt{SS_p \left(\frac{1}{(N-p)} - \frac{1}{N} \right)} \quad (5)$$

where, $\overline{y_i^{\text{calc}}}$ is the mean of estimated concentrations versus time, $t_{(N-p,0.05)}$ is 0.05 quintile for Student distribution with $N-p$ degree of freedom, and SS_p is the above-mentioned WSS in the case of models with p parameters.

In fact the index is approximately the ratio between area of the confidence limits and area under a theoretical curve. The model equation with the minimum I_p value generates the narrowest confidence interval for the estimated released amounts of drug from different formulations.

2.4.3 Fisher (F) test criterion

We can compare simple model having q parameters with complex model having supplementary k parameters with $p = q + k$ using the F ratio (Fisher 1971) according to the following equation:

$$F = \frac{WSS_q - WSS_p}{WSS_p} \frac{df_p}{df_q - df_p} \quad (6)$$

where, WSS_q is the sum of standard errors for the selected reference mathematical model; while WSS_p corresponds to the more complex model. The number of freedom degrees represents the difference between the number of experimental data, n , and the number of parameters:

$$df_p = n - p \quad \text{and} \quad df_q = n - q \quad (7)$$

The analysis makes statistical significance when the two models are nested, i.e. the model with lower number of parameters can be considered as degenerated from the model with more parameters, by keeping constant a number of parameters. In fact, by reporting the ratio in the following equation:

$$F = \frac{(WSS_q - WSS_p) / WSS_p}{(df_q - df_p) / df_p} \quad (8)$$

It has been shown that the mathematical test could be compared to the relative decreasing for the sum of errors and the relative increasing of the number for freedom degrees.

2.5 Model construction of diffusion by using Fick's first law

The flux J of molecule through virtual interfaces in solution was described using Fick's first law:

$$\frac{1}{A} \frac{dm}{dt} = J = -D \frac{\partial c}{\partial x} \quad (9)$$

where, m is the transferred mass, A area of the interface, D diffusion coefficient and c concentration.

By extrapolating the fluorescein delivery at virtual interfaces from solutions to interfaces of monoglyceride colloidal liquid crystals in release medium, the Fick's equation can be used to generate all mathematical models and analyze data of the diffusion-controlled release processes.

The fluorescein delivery by the limit of the stationary layer of thickness (δ) appears in the receptor solution at the border with monoglyceride liquid crystals, which is not affected by the convection currents in the fluid. This limit layer is similar to the fixed electrical double layer present at the interfaces of particles. The concentration gradient is usually considered linear in this limit layer. Furthermore, the concentration of molecules in the immediate neighborhood of pharmaceutical formulation is equal to its maximum value c_s (noted also frequently by S), which depends on its solubility. Parameters herein reported represent the concentration of compound at the stationary layer (c_δ) and its solubility at the maximum concentration in the medium (S). Consequently the following approximation can be made:

$$\frac{\partial c}{\partial x} \approx \frac{c_\delta - S}{\delta} \quad (10),$$

and Fick's equation is transformed into:

$$\frac{1}{A} \frac{dm}{dt} = -D \frac{(c_\delta - S)}{\delta} \quad \text{or} \quad \frac{dm}{dt} = -\frac{AD}{\delta} (c_\delta - S) \quad (11),$$

thus finally getting the Nernst-Brunner equation. This equation cannot evaluate experimentally neither the thickness, δ , of the limit layer nor the diffusion coefficient, D , neighbor to interface. The area of interface, A , is also no easy to be calculated and it is not constant over time. For this reason,

we can suppose in the following studies that the time interval of release for the expression $\frac{AD}{\delta}$ could be considered constant. This theoretical assumption allows simplifying the Fickian first law using the following equation:

$$\frac{dc}{dt} = k(c_s - c_\delta) \quad (12)$$

In fact, Noyes and Whitney, which has been-established experimentally more than a century ago, can be easily solved when the initial conditions is expressed as $c_{\delta}(t = 0) = 0$ thus obtaining the following solution:

$$-\ln\left(1 - \frac{c_{\delta}}{c_s}\right) = kt \tag{13}$$

By accepting the assumption that beyond the limit layer of particle narrow size distribution, the homogenization is rapid and the drug concentration is the same of C_{δ} in the bulk of the dissolution

media $c(x,t) = c_{\delta}(t)$ for $x \geq \delta$, the representation of $-\ln\left(1 - \frac{c_{\delta}}{c_s}\right)$ as a function of time leads to an approximately straight line and we consider this result as the evidence that the process follows the Noyes-Whitney law.

Noyes-Whitney law represents a model having a single parameter k . Sometimes solvents penetrate or swell through the pharmaceutical formulations. In such cases, the representation is a straight line, $\alpha + kt$, without passing through the origin. In this case, the model has two parameters, α and k , and it is, in fact, no longer then a solution where Noyes-Whitney law can be applied using the same initial conditions. If α is considered as a time lag, it is possible to simplify the Noyes-Whitney law.

When the release occurred into medium having a constant volume V and release was reported

as the ratio of $\frac{c_{\delta}}{c_s}$, the new fraction $\frac{V \cdot c_{\delta}}{V \cdot c_s}$ can be written in the following form $\frac{m(t)}{m_{\infty}}$,

where, m_{∞} represents the maximum amount of compounds, which can be released at the detection limit of solubility as reported in the following equation:

$$-\ln\left(1 - \frac{m(t)}{m_{\infty}}\right) = kt \tag{14}$$

Basically, V is increased thus obtaining $c_{\delta}(t) \approx 0$ in dissolution tests. This value could represent the sink conditions in a mathematical model. In such cases, we have a complete release of drugs at infinite time, while a few amount of drug is released form pharmaceutical formulations at time 0. Consequently, it is possible to calculate the amount of drug released at different times as a percentage of m_{∞} , although it is difficult enough to define exactly m_{∞} .

In order to obtain a more flexible model, it is possible to replace t with t^{β} and include this parameter in the Equation 14, thus obtaining in the following equation:

$$-\ln\left(1 - \frac{m(t)}{m_{\infty}}\right) = kt^{\beta} \quad \text{and} \quad 1 - \frac{m(t)}{m_{\infty}} = e^{-kt^{\beta}} \tag{15}$$

A more general model is obtained empirically (new model) since there is no theoretical justification for considering a power of t . This model is firstly applied for describing dissolution of drugs from pharmaceutical formulations by using Weibull law [15].

Equation 15 can be rewritten in the following form:

$$\frac{m(t)}{m_{\infty}} = 1 - e^{-\alpha t^{\beta}} \quad (16)$$

This equation allows analyzing α as the scale factor and β as the shape factor in the Weibull distribution.

The following transformation is then used to describe the following equation:

$$\frac{m(t)}{m_{\infty}} \times 100 = R \quad (17)$$

A linear dependence can be obtained by transforming the previous equation into a linear form. A second order logarithm transformation is applied and the following mathematical equations are then obtained:

$$e^{-\alpha t^{\beta}} = 1 - R/100 \quad (18)$$

$$\alpha t^{\beta} = -\ln(1 - R/100) \quad (19)$$

$$\ln(-\ln(1 - R/100)) = \ln \alpha + \beta \ln(t) \quad (20)$$

Consequently, the previous equations can be reported in the following form $\ln(-\ln(1 - R/100)) - \ln(t)$, thus showing a straight trend line, which can be assumed as Weibull empirical dependence between R and t . This function was frequently applied to analyze the dissolution and release of drugs from pharmaceutical formulations in different experimental conditions [20–24].

In different simulations [25] of power laws, the Weibull function and the fitting of experimental data, concerning of the release of diltiazem and diclofenac [10], demonstrated that the exponent β , in case of polymeric matrices is an indicator of the mechanism of transport for the drug through the polymer matrix. A value of $\beta \leq 0.75$ was associated with Fick diffusion in either fractal or Euclidian spaces; while a combined mechanism (Fick diffusion and swelling controlled transport) was associated with β values in the range $0.75 < \beta < 1$.

For values of β higher than 1, it was demonstrated that the drug transport follows a complex release mechanism.

2.6 Construction of diffusion models by using Fick's second law

Fick's second law predicts how the diffusion process can modify the concentration of drugs during the time. The dependence between the drug concentration and the time is described by the following equation:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (21)$$

where, c represents the concentration of molecule at point x , t is time; D is the diffusion coefficient.

An infinite number of solutions can be obtained by using Fick's second law in the following form. In particular, three limit cases are reported when this equation is used for pharmaceutical applications: i) release from a membrane of thickness $2l$, having an initial concentration (c_1), toward

an environment where the concentration of drug remains constant, c_0 . The concentration in the membrane will be:

$$c(x, t) = c_0 + (c_1 - c_0) \frac{4}{\pi} \sum \frac{1}{2k+1} \sin \frac{2k+1}{2l} \pi x e^{-\frac{(2k+1)^2}{4l^2} \pi^2 D t} \quad (22)$$

where, $c(x, t)$ represents the drug concentration in the point x at time t ;

ii) release kinetic by infinite reservoir with constant concentration (c_1) across a membrane of thickness l in a medium at a constant concentration (c_0). The concentration in the membrane will be calculated according to the following equation:

$$c(x, t) = c_0 + (c_1 - c_0) \left(\frac{x}{l} - \frac{2}{\pi} \sum \frac{(-1)^n}{n} \sin \frac{n\pi x}{l} e^{-\frac{n^2 \pi^2 D t}{l^2}} \right) \quad (23);$$

iii) release kinetic by infinite reservoir with a constant concentration (c_s) in a semi-infinite medium. Concentration in the medium will be calculated using the following equation:

$$c(x, t) = c_s \left(1 - \operatorname{erf} \frac{x}{\sqrt{4 D t}} \right) \quad (24)$$

where, c_s is the concentration of drugs at their maximum solubility, while $\operatorname{erf}(z)$ represents the error function:

$$\operatorname{erf}(z) = \int_0^z e^{-\frac{u^2}{2}} du \quad (25)$$

The different solutions of Fick's second law depend on the initial and boundary conditions. In fact it is difficult to quantify, by using an experimental model, the concentration of drugs for different points in liquid medium at the same time. It is possible to theoretical estimate their distribution in semisolid media and transfer course by using an experimental Franz diffusion cell model.

For a drug concentration slightly affected by time, it is generally estimated the amount of drug at the steady state. However, for solid pharmaceutical formulations, it is generally created an independent convection flow in order to homogenize the concentration of drugs in the release medium and to approximately obtain the sink conditions.

2.7 Higuchi square root law

Fifty years ago Higuchi applied Fick's first law to describe the release of drugs in a limit layer at the surface of a pharmaceutical matrix (e.g. ointment, tablet) toward an external solvent, which acts as a perfect sink under pseudo steady-state conditions. Since the assumptions of the model are approved only in the first part of the release process, the application of this law is recommended only for the first 60% of the release curve [26]. In the evaluation of release profiles from ointments and insoluble matrices, the Higuchi law is expressed as a square root function as following reported:

$$m = \sqrt{D C_s (2 C_0 - C_s)} t = \alpha t^{1/2} \quad (26)$$

where, D is the diffusion coefficient, C_0 is the initial drug concentration in the matrix and C_s the solubility of the drug.

2.8 Other square root laws

A similar square root law to Higuchi equation was further used to describe the release kinetic of drugs from pharmaceutical formulations, which can be considered as an infinite reservoir at the

interface with large or semi-infinite solution [27–29]. The concentration of drugs inside solution can be expressed using the following equation:

$$c(y, t) = c_s \left(1 - \operatorname{erf} \left(\frac{y}{\sqrt{4Dt}} \right) \right) \quad (27)$$

where, y is the distance from the interface and $\operatorname{erf}(z)$ is the error function calculated as the area under the curve $\frac{2}{\sqrt{\pi}} e^{-x^2}$, with the limit between 0 and z :

$$\operatorname{erf}(z) = \int_0^z \frac{2}{\sqrt{\pi}} e^{-x^2} dx \quad (28)$$

The integration of flux of the interface in the range between 0 and t further provided the following square root equation:

$$m(t) = \frac{2}{\sqrt{\pi}} A c_s \sqrt{Dt} \quad (29)$$

where, A is area of interface between reservoir and diffusion medium.

For mathematical point of view, the diffusion equation is the same describing the heat transfer, the impulse in fluid movement, and the probability in quantum mechanics, as following reported:

$$\square_a u(x_1, x_2, \dots, x_n; t) = 0 \quad (30)$$

$$\square_a = a\Delta - \frac{\partial}{\partial t} \quad (31)$$

A fundamental result is the theoretical solution obtained when one unit of mass is delivered in one unit of time, similar to bolus intravenous administration:

$$a \frac{\partial^2 u(x; t)}{\partial x^2} - \frac{\partial u(x; t)}{\partial t} = \delta(x) \times \delta(t) = \delta(x; t) \quad (32)$$

If we apply Fourier transform to $u(x, t)$ for x parameter and then the Laplace transformation as function of t , we can obtain the following equation:

$$-\alpha^2 a L(F(u)) - p L(F(u)) = 1(\alpha) \times 1(p) = 1 \quad (33)$$

Based on the Equation 33, the double transformed of $u(x, t)$ can be reported as:

$$L(F(u)) = -\frac{a}{p + a\alpha^2} \quad (34)$$

By applying the inverse Laplace function, Equation 34 is transformed into:

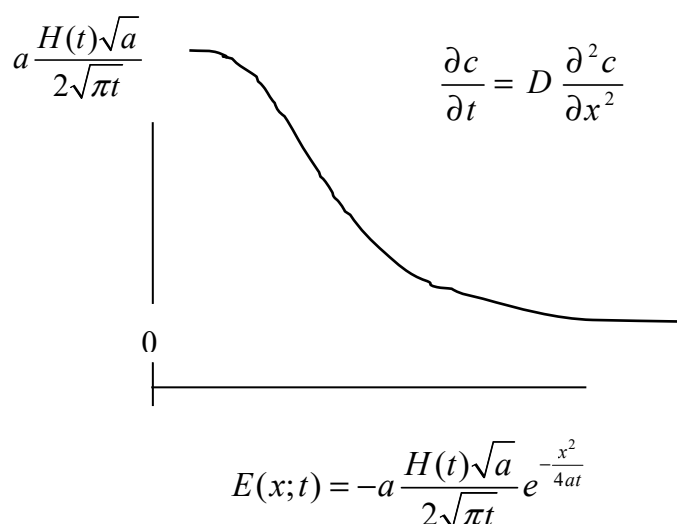
$$F[u(x; t)] = -aH(t)e^{-a\alpha^2 t} \quad (35)$$

where, $H(t)$ is the Heaviside function.

By further applying the inverse of Fourier transformed function, Equation 35 is transformed into:

$$E(x;t) = -a \frac{H(t)\sqrt{a}}{2\sqrt{\pi t}} e^{-\frac{x^2}{4at}} \quad (36)$$

Based on the Equation 36, the square root of time laws could arise in a more general frame. In case of monoglyceride colloidal liquid crystals, if the release of fluorescein is rapid, the process could be controlled by diffusion across membrane containing the colloid formulations. The inner interface of membrane can be considered as source acting short time at initial phase of process. The further evolution of concentration in the membrane could be described by fundamental solution.



2.5.3 The Power-Law (Peppas) Model

The release profile of formulations in a specific drug range concentration was analyzed using a power law equation proposed by Peppas, which derived from considering both the effects of diffusion and the erosion on drug release kinetics from colloidal systems [14]:

$$\frac{m(t)}{m_{\infty}} = \alpha t^{\beta} \quad (37)$$

The law is semi-empirical and represents a generalization of Higuchi's law. The value of β , obtained by fitting experimental data with $m(t)/m_{\infty} \leq 60\%$ from polymeric controlled delivery systems is indicative of the nature of release mechanism. In fact the model could be considered a degeneration of the Weibull model for low values of αt^{β} :

$$\frac{m(t)}{m_{\infty}} = 1 - e^{-\alpha t^{\beta}} \approx 1 - (1 - \alpha t^{\beta}) \approx \alpha t^{\beta} \quad (38)$$

In the general frame presented above, the delivery of drug through the interface, depended on two dimensional diffusion as reporting in the following equation:

$$E(x, y; t) = -\frac{\theta(t)}{4\pi} e^{-\frac{r^2}{4at}}, \quad r = \sqrt{x^2 + y^2} \quad \leftarrow \begin{array}{|c|} \hline \uparrow \\ \hline \leftarrow \quad \rightarrow \\ \hline \downarrow \\ \hline \end{array} \quad \begin{array}{|c|} \hline \uparrow \\ \hline \leftarrow \quad \rightarrow \\ \hline \downarrow \\ \hline \end{array}$$

$$M(t) = kt \quad (39),$$

and in three dimensional diffusion as reporting in the following equation:

$$E(x, y, z; t) = -\frac{\theta(t)}{8\pi\sqrt{\pi at}} e^{-\frac{R^2}{4at}}, \quad R = \sqrt{x^2 + y^2 + z^2} \quad M(t) = kt\sqrt{t} \quad (40)$$

In particular, for diffusion process, a symbol is reported as D .

The experimental amount of released drug is proportional to $\sqrt{t}$ or t or $t\sqrt{t}$; these data can be interpreted as one-dimensional, two-dimensional or three-dimensional diffusion processes.

2.9 Graphical representation of data

The SigmaPlot software was used for the graphical representation of results. These data represent the average of three different experiments $\pm$ standard deviation.

3. Results and Discussion

Colloidal formulations loading payloads show different kinetic release profiles, which depend on the physicochemical properties of drugs. Polymeric suspensions and colloidal carriers provide a release kinetic and profiles similar to various pharmaceutical formulations, and a zero or first order kinetic is basically obtained during the experiments. For mathematical criteria, the controlled release of drugs from supramolecular-, micro- and nano- carriers depends on some mechanisms, which modulate the release of payloads and affect the choice of model. In this attempt, three criteria can explain the mechanism of release of drugs from monoglyceride colloidal liquid crystals, and particularly: i) diffusion-controlled, ii) swelling-controlled, and iii) chemical-controlled release [30].

Different mathematical models are extensively proposed to analyze the release and dissolution kinetics of drugs from pharmaceutical formulations [25,31].

The release kinetic profiles of hydrophilic molecule (fluorescein) from monoglyceride colloidal liquid crystals were carried out using the Fickian's law, which studies the flux of drugs through a polymeric and/or lipid shells as a function of time and different physicochemical parameters, such as the drug distribution between the internal compartment of the formulations and the external medium, the coefficients of diffusion and repartition, the thickness of the limit layer, and the surface adsorption of the drug. These parameters also modulate the release of the drugs from a bulk or colloidal matrix in the aqueous compartment [3,26].

Among the different mathematical models, the Higuchi process could be considered a theoretical model for a specific portion of drug release when the entire range is not applied [32].

Biopharmaceutical analysis showed that the fitting of results and predictions, obtained by applying the Higuchi law, are somewhat different from those obtained by applying directly Fickian's law, in the case of colloidal carriers. These differences result by analyzing the mechanism of drug diffusion through the colloidal matrix, as well as the aqueous solution, and they are also strictly affected by the interaction between the external surface of colloidal formulations and the internal compartment of colloidal carriers [33]. Our research group previously demonstrated that a square root equation similar to Higuchi's could be obtained by transforming initial and boundary conditions in solving the Fick's second law of diffusion [28]. Recent data showed that this model could be extensively used to investigate the release kinetics of dextran microspheres [34], poloxamer gels [35] and cylinder matrix systems [36].

Various scientists [3,37,38] demonstrated that mathematical models could be classified in two different categories: i) empirical models, which are mainly used for fitting experimental data with a given power or exponential function, and ii) phenomenological models which take physicochemical phenomena, e.g. mass diffusion transfer or processes of chemical reaction, into account.

The drug release kinetic is also affected by the composition of carriers and technological parameters, such as excipients, biomaterials, drug loading, geometry, size and shape. Furthermore, for conventional formulations, the amount of drug measured in the dissolution medium depends both on the payloads released in the receptor medium and the bulk of drug, which is still un-dissociated from formulations [3]. In particular, the amount of drugs contained in the un-dissociated formulations could be also used to describe their release kinetic.

The extrapolation of theoretical criteria, herein reported for conventional formulations, allowed describing the release kinetic profiles of monoglyceride colloidal liquid crystals by considering the distribution of drug in two different compartments; particularly, for a small inner fraction ($\leq 10\%$) and a sequestered fraction ($\geq 40\%$). A small time lag could be also observed for monoglyceride colloidal liquid crystals besides an equilibration time, which occurred between the supramolecular carrier and the receptor of the release apparatus.

3.1 Description and analysis of obtained release kinetics

The release kinetic of fluorescein, which is used as a hydrophilic drug candidate, from different monoglyceride colloidal liquid crystals was investigated as a function of liquid crystal compositions (Table 1). As shown in Figure 1, surfactants forming colloidal liquid crystals affect the release kinetic of formulations, and any time lag was observed for different formulations.

The release kinetic of various formulations arise the steady state and saturation of the medium within the first six hours in the range of incubation from 0 to 24 h. This value was considered as the amount of fluorescein, which is released from the monoglyceride colloidal liquid crystals during the incubation time. Consequently, the mathematical model of the release kinetic profile was normalized to values obtained after 6 h of incubation, and the amount of fluorescein released at 6 h

was considered as m_{∞} , or 100% (Figure 1).

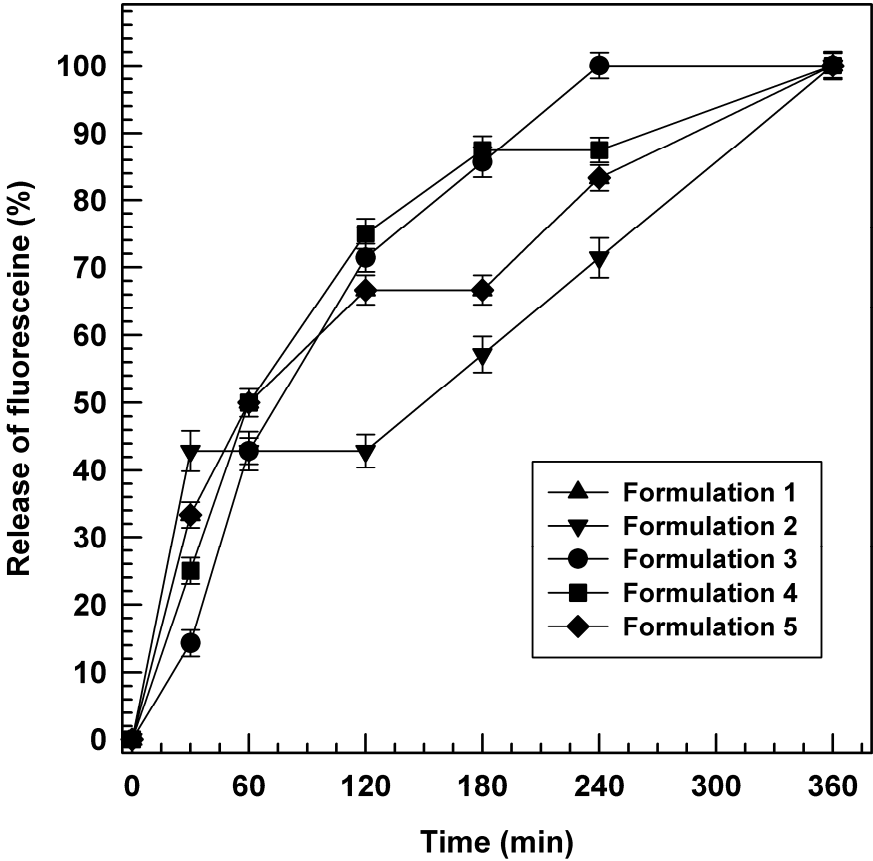


Figure 1. Release profile of fluorescein by various monoglyceride colloidal liquid crystal formulations. Symbols: (▲) formulation 1; (▼) formulation 2; (●) formulation 3; (■) formulation 4; (◆) formulation 5. Data are the average of three different experiments $\pm$ standard deviation. Error bar if not shown is within the symbol.

A standard procedure for testing different cluster models was designed. The theoretical models already reported (linear, Higuchi, Noyes-Whitney, Peppas and Weibull) were selected and the analyses were carried out with or without considering the lag time of different data. Partial time and/or entire time intervals were evaluated by fitting the experimental data. In particular, a hierarchy of the fitting success was established by applying Akaike, Schwartz and Imbimbo information criteria. The statistical significance of the differences between parent and degenerated models was tested using the *F*-test. The mechanistic component of these phenomenological models was selected as most reliable factor for the analysis that was fitted for the partial or full range time of the experiments. The comparison of the best models resulting from the different formulations was carried out. The closely related formulations were merged into a single model, although the active surface factors could lead to critical phenomena and significant changes of the structure of formulations.

Significant differences were observed in the fitting performances deriving from the direct linear regression model and the Peppas model after their transformation and linearization. In particular, the release profiles of formulation 1 showed that the sum of the squared errors remained lower ($WSS = 128$) when the Peppas model was applied with respect to the WSS (140) obtained in the case of direct linear regression (Figures 2a and b, respectively).

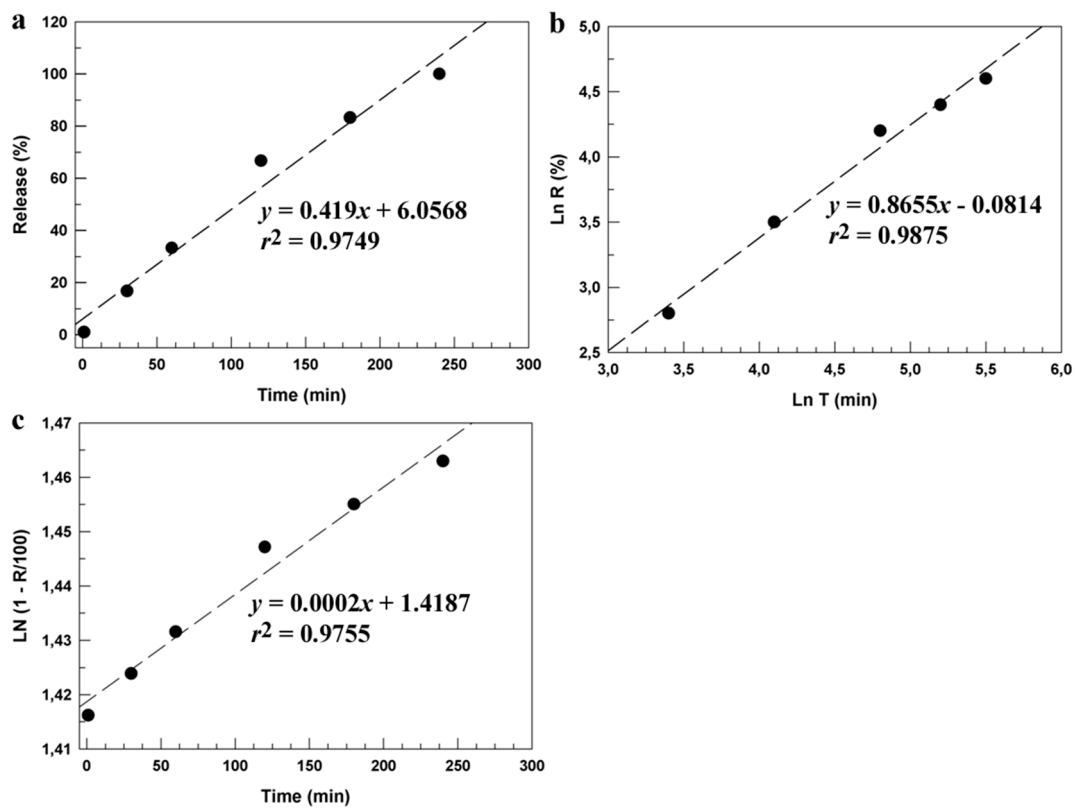


Figure 2. Evaluation of the release profile of fluorescein from monoglyceride colloidal liquid crystal formulation 1 using the following mathematical models: (a) Peppas; (b) Linear regression; (c) Noyes-Whitney. Data represented the average of three different measurements.

Furthermore, the r^2 value obtained by using the Peppas model for data analysis was 0.988; while that obtained using the linear regression model was 0.975. Furthermore, some point values need to be discarded because they are not included in the linear trend of the equation. Data analysis, obtained by applying the Fisher test and considering the linear model, as a degeneration of power law, demonstrated that the increase of release kinetic was rather a random effect. In fact, Peppas law basically described the model release profiles of drugs from polymer based colloidal systems [39], which could be similar to our formulations.

Noyes-Whitney linear model demonstrated that all the mathematical models have different mechanisms of release in the first and in the last 2 hours. In fact after the first 2 hours of incubation, a decrease of release rate was carried out (Figure 2c).

No further significant difference was observed when the Higuchi and Noyes-Whitney equations were applied for the analysis of data. The r^2 values were similar for the Higuchi and Noyes-Whitney models; while the predicted data obtained by applying the Noyes-Whitney law could be overlaid with those of Peppas analysis (Figure 2). The Higuchi model did not provide the better or worse model for the analysis, but provided some advantages approximating a maximum number of experimental points.

Since in case of small values of αt^β , the Weibul model degenerates to Higuchi or Peppas models,

$$\frac{m(t)}{m_\infty} = 1 - e^{-\alpha t^\beta} \approx 1 - (1 - \alpha t^\beta) \approx \alpha t^\beta$$

, it was possible to validate the application of F test for evaluating the significance of the increasing fluorescein release fitting with the performances obtained using the Weibul model. These results are not enough with this theoretical model, and demonstrated that the Higuchi and Peppas models can be used to extrapolate these data with respect to the Weibul model. Furthermore, the Higuchi model showed some advantages for fitting a

large number of experimental points, thus increasing the prediction power and the statistical significance of the analysis.

The mathematical analysis of formulation 2 showed that the release profile of fluorescein using Higuchi, Peppas and Weibull models could be suitable evaluated by starting from 2 hours and ending the analysis after 8 hours (Figure 3).

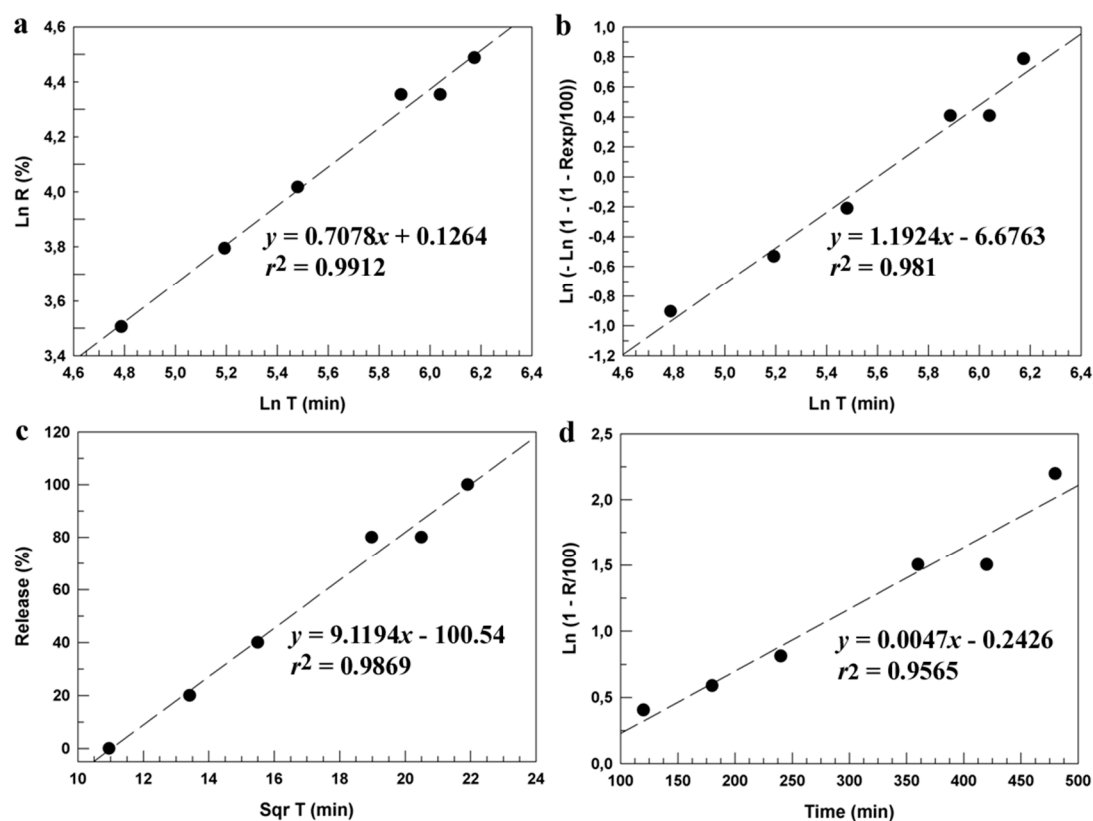


Figure 3. Evaluation of the release profile of fluorescein from monoglyceride colloidal liquid crystal formulation 2 using the following mathematical models: (a) Peppas; (b) Weibull; (c) Higuchi; (d) Noyes-Whitney. Data represented the average of three different measurements.

Results obtained using statistical criteria did not allow having the best model describing the release kinetic between those herein reported.

Significant differences of parameters in the selected mathematical model could be obtained when surfactant compositions were modified in supramolecular carriers [32]. The Akaike and Schwarz criteria showed that the Weibull equation represents the best equation to analyze these data. This hypothesis is in agreement with data previously reported [10,23]. The Imbimbo criterion showed that the Higuchi model provides detailed information about the release kinetic of fluorescein. Bhaskar and co-workers also obtained similar data for the nitrendipine released from solid lipid nanoparticles and nanostructured lipid carriers [40].

No significant difference was obtained by using the *F*-test analysis with the models previously reported. In particular, the *F*-test demonstrated that the Peppas model seemed to be more significant than the other models (Figure 3a). In fact, the r^2 value was 0.991 and all points of analysis fitted with the linear trend of the applied equation. The correlation coefficients r^2 obtained by using Weibull, Higuchi and Peppas methods showed a 0.956, 0.993 and 0.991 values, respectively. These data fitted with the linear trend describing graphically the analysis, and the trend demonstrated significance with theoretical model (Figure 3).

The Imbimbo comparison of Higuchi and Weibull models of the release kinetic profile of formulation 2 did not show any significant difference (Table 2). The absence of significant differences between the various models showed that the Higuchi model could be preferentially used

for the analysis of data, because it is easy to use and provides a suitable analysis of phenomenological data. Conversely, the Higuchi model was only applied for the first part of experimental set of data.

Table 2. Comparison of validation models for colloidal liquid crystal release kinetic profile.

Mathematical Model	Akaike	Schwarz	Imbimbo	F criterium
Higuchi	29.7	31.3	0.057	0.242
Weibul	25.8	25.4	0.059	—

The release profile of formulation 3 showed that fluorescein was rapidly released from monoglyceride colloidal liquid crystals after 4 h of incubation and a plateau was obtained from 4 h up to 24 h (Figure 1). A biphasic profile of the release kinetic was obtained for formulation 3, and the release kinetic represented two different models, i.e. the first part (0-4 h), which could be represented by a linear profile, and the second part (4-24 h), which showed a saturated phase. The sums of standard errors and the correlation coefficients showed that the mathematical correlation was suitable for the analysis of data; while any significant difference occurred in the linear part of the release kinetic of fluorescein for Peppas and Higuchi models (Figure 4). The F-test analysis showed any statistical significant increase of the curve by changing the mathematical model from Peppas to Weibul (Figure 4 a-c). The direct linear fitting dependence demonstrated a poor correlation between the resulting data (Figure 4d).

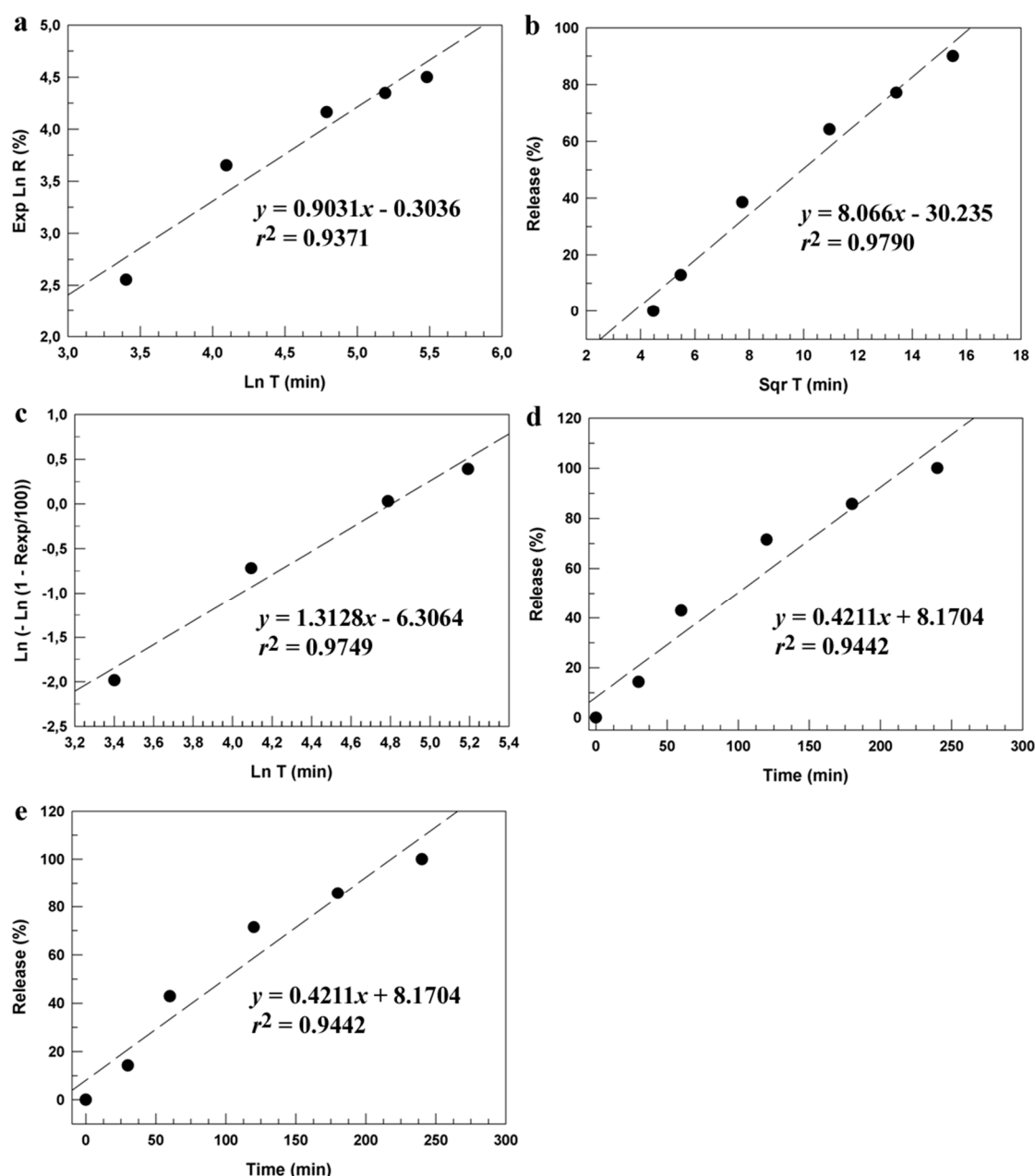


Figure 4. Evaluation of the release profile of fluorescein by monoglyceride colloidal liquid crystal formulation 3 using the following mathematical models: (a) Peppas; (b) Higuchi; (c) Weibull; (d) linear regression; (e) Noyes-Whitney. Data represented the average of three different measurements $\pm$ standard deviation.

All models fitted data in the first 6 h, except Peppas and Weibull models, could be applied on the full range of analysis. Any suitable result was obtained when the Noyes-Whitney model was applied for the analysis of formulation 3 (Figure 4e).

The mathematical analysis of the release profile of formulation 4 showed that the Noyes-Whitney model was not suitable to predict the release of fluorescein from monoglyceride colloidal liquid crystals in physiological solution and aqueous media. For this reason, the release kinetic of formulation 4 was evaluated by using the Higuchi model. In particular, Higuchi analysis (Figure 5a) showed that this mathematical model can be used to analyze the drug release in the first 3 h (Figure 1). In order to extend the experimental model up to 5 h of incubation, the Peppas and Weibull empirical models were applied (Figures 5b and c). Both models increased the statistical significance of the analysis for the release kinetic of formulations, thus demonstrating that Peppas

semi-empirical and Weibull empirical models (Figures 5b and c) allowed obtaining similar results. These results further demonstrated that the Peppas model could be used to predict the release kinetic of fluorescein from monoglyceride colloidal liquid crystals.

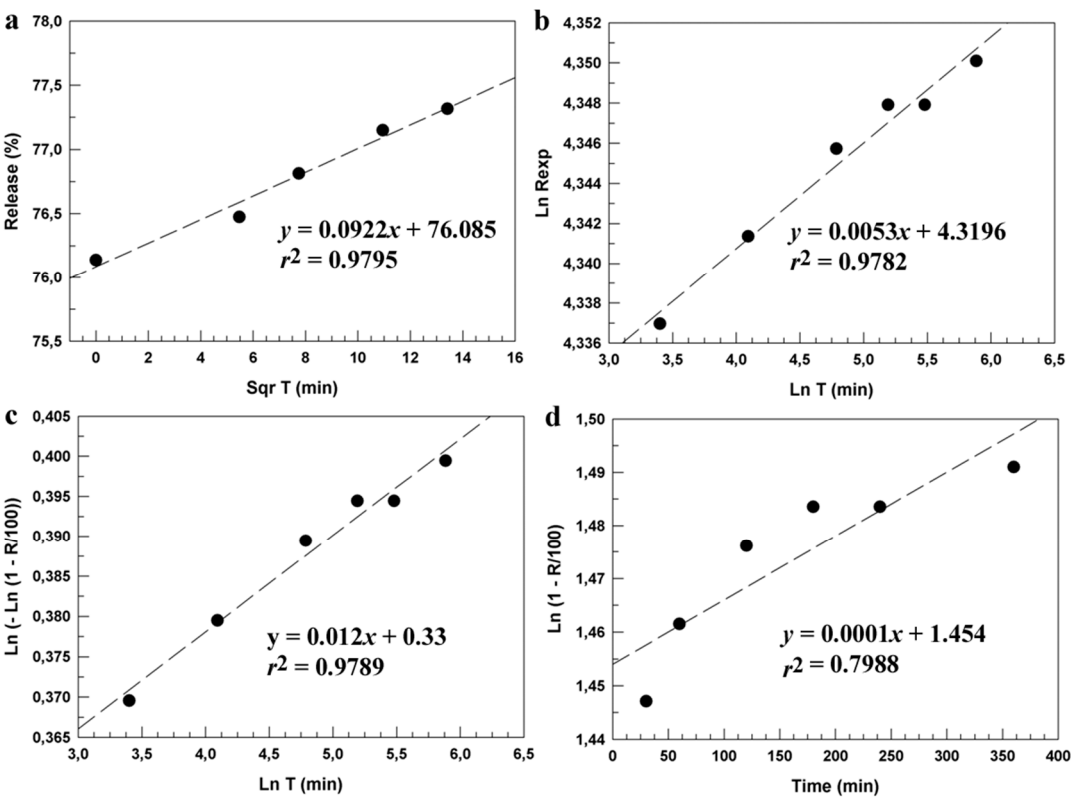


Figure 5. Evaluation of the release profile of fluorescein from monoglyceride colloidal liquid crystal formulation 4 using the following mathematical models: (a) Higuchi; (b) Peppas; (c) Weibull; (d) Noyes-Whitney. Data represented the average of three different measurements $\pm$ standard deviation.

Different results were obtained for formulation 5. In this case, the Higuchi model showed that a specific fitting occurred when the analysis was carried out up to 20 h of incubation (Figure 6). Theoretical and experimental analysis are strictly correlated by applying the Higuchi model. Any significant differences of the release kinetic of various formulations were obtained analyzing data with Peppas and Weibull (Figure 6b and c), as well Higuchi (Figure 6a) and Noyes-Whitney (data not reported) models.

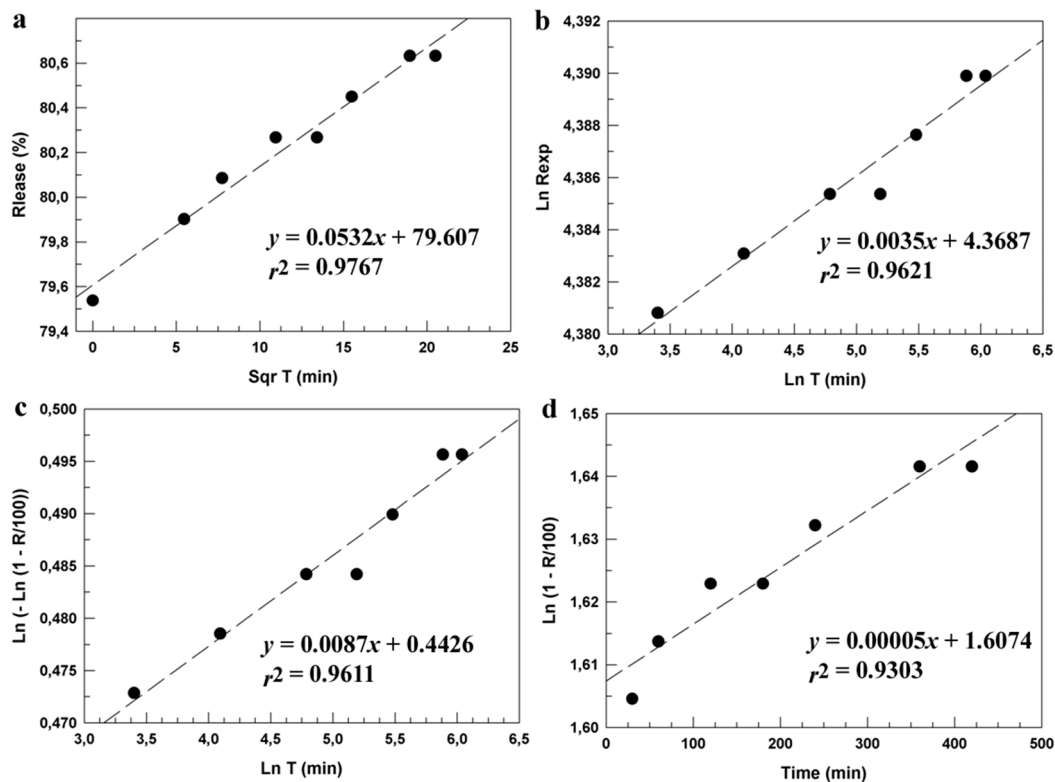


Figure 6. Evaluation of the release profile of fluorescein by monoglyceride colloidal liquid crystal formulation 5 using the following mathematical models: (a) Higuchi; (b) Peppas; (c) Weibull, (d) Noyes-Whitney. Data represented the average of three different measurements $\pm$ standard deviation. Error bar if not reported was included in the symbol.

Results showed that by applying the square root model data theoretical and experimental data fitted enough for five different formulations; and was allowed to improve the statistical significance of experimental data compared to linear regression. The linear regression of different formulations (1-5) further demonstrated that data have the same trend and similar r^2 values (Supplementary Figure 1). The t -test significance of different data was calculated by comparing equations previously reported (Supplementary Information) [41].

4. Conclusions

Our findings demonstrated that the graphical analysis of the release profile from various monoglyceride colloidal liquid crystal formulations were characterized by a continuous release of fluorescein up to 6 h of incubation with except for the formulation 3 that showed a rapid release phase during the first hour followed by a gradual and continuous release up to 6 h of incubation. In particular, the trend of release did not show a linear behavior since saturation phase appeared in all cases, probably due to a complete drain of available fraction of fluorescein from colloidal liquid crystals.

Results obtained by using mathematical models could be used to predict the release profile from supramolecular carriers. These mathematical models could also be applied to validate the obtained results.

Our findings showed that a square root model represented most efficacious mathematical model to describe the release profile of hydrophilic compounds from monoglyceride colloidal liquid crystals independently on chemical compositions and/or structure of supramolecular carriers. The type of surfactant and its amount did not modify the release profile of the fluorescein. The criteria applied for the analysis highlighted a linearity of different transformed curves as a function of the time, and experimental data well fitted with predicted values of fluorescein release. The general

information obtained from square root model could be further improved by modeling using Weibull and Peppas models. In fact, these models allowed obtaining more detailed information concerning the release profile of hydrophilic compounds from colloidal liquid crystals.

Both two models represented a valid alternative to square root model; however, it showed the advantage to be easily applied and directly obtained using the diffusion equation. Furthermore, the composition of monoglyceride colloidal liquid crystals did not drastically affect the release profile of fluorescein, regression line release as function of square root of time being statistically the same for all four of five formulations. **Supplementary Materials:** The following are available online, Figure S1: *T*-test applied to compare the linear regression for square root equation for formulations 1, 3, 4 and 5; Equation S1: The extreme values of slopes of different formulations.

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References

1. Larsen, C.; Larsen, S.W.; Jensen, H.; Yaghmur, A.; Ostergaard J. Role of in vitro release models in formulation development and quality control of parenteral depots. *Expert Opin. Drug Deliv.* **2009**, *6*, 1283–1295. DOI: 10.1517/17425240903307431
2. Karalis, V.; Macheras, P.; Van Peer, A.; Shah, V.P. Bioavailability and bioequivalence: focus on physiological factors and variability. *Pharm. Res.* **2008**, *25*, 1956–1962. DOI: 10.1007/s11095-008-9645-9.
3. Dokoumetzidis, A.; Macheras, P. A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system. *Int. J. Pharm.* **2006**, *321*, 1–11. DOI: 10.1016/j.ijpharm.2006.07.011
4. Abdul, S.; Poddar, S.S. A flexible technology for modified release of drugs: multi layered tablets. *J. Control. Release* **2004**, *97*, 393–405. DOI: 10.1016/j.jconrel.2004.03.034
5. Siepmann, J.; Siepmann, F. Mathematical modeling of drug delivery. *Int. J. Pharm.* **2008**, *364*, 328–348. DOI: 10.1016/j.ijpharm.2008.09.004
6. Rizvi, T.A. Liquid crystalline biopolymers: A new arena for liquid crystal Research. *J. Mol. Liquids* **2003**, *106*, 43–53. DOI: 10.1016/S0167-7322(03)00019-9
7. Woltman, S.J.; Jay, G.D.; Crawford, G.P. Liquid-crystal materials find a new order in biomedical applications. *Nat. Mater.* **2007**, *6*, 929–938. DOI: 10.1038/nmat2010
8. Fong, W.K.; Hanley, T.; Boyd, B.J. Stimuli responsive liquid crystals provide 'on-demand' drug delivery in vitro and in vivo. *J. Control. Release* **2009**, *135*, 218–226. DOI: 10.1016/j.jconrel.2009.01.009

- 687 9. Lee, K.W.; Nguyen, T.H.; Hanley, T.; Boyd, B.J. Nanostructure of liquid crystalline matrix
688 determines in vitro sustained release and in vivo oral absorption kinetics for hydrophilic model
689 drugs. *Int. J. Pharm.* **2009**, *365*, 190–199. DOI: 10.1016/j.ijpharm.2008.08.022
- 690 10. Papadopoulou, V.; Kosmidis, K.; Vlachou, M.; Macheras, P. On the use of the Weibull function
691 for the discernment of drug release mechanisms. *Int. J. Pharm.* **2006**, *309*, 44–50. DOI:
692 10.1016/j.ijpharm.2005.10.044
- 693 11. Dokoumetzidis, A.; Valsami, G.; Macheras P. Modelling and simulation in drug absorption
694 processes. *Xenobiotica* **2007**, *37*, 1052–1065. DOI: 10.1080/00498250701502114
- 695 12. Noyes, A.A.; Whitney, W.R. The rate of solution of solid substances in their own solutions. *J. Am.*
696 *Chem. Soc.* **1987**, *19*, 930–934. DOI: 10.1021/ja02086a003
- 697 13. Higuchi, T. Rate of release of medicaments from ointment bases containing drugs in suspension.
698 *J. Pharm. Sci.* **1961**, *50*, 874–875. DOI: 10.1002/jps.2600501018
- 699 14. Siepmann, J.; Peppas, N.A. Modeling of drug release from delivery systems based on
700 hydroxypropyl methylcellulose (HPMC). *Adv. Drug Delivery Rev.* **2001**, *48*, 139–157. DOI:
701 10.1016/S0169-409X(01)00112-0
- 702 15. Langerbucher, F. Linearization of dissolution rate curves by the Weibull distribution. *J. Pharm.*
703 *Pharmacol.* **1972**, *24*, 979–981. DOI: 10.1111/j.2042-7158.1972.tb08930.x
- 704 16. Yamaoka, K.; Nakagawa T. Application of Akaike's information criterion (AIC) in the evaluation
705 of linear pharmacokinetic equations. *J. Pharmacokin. Biopharm.* **1978**, *6*, 165–175. DOI:
706 10.1007/BF01117450
- 707 17. Schwarz, G. Estimating the dimension of a model. *Ann. Stat.* **1978**, *6*, 461–464.
- 708 18. Sandulovici, R.; Prasacu, I.; Mircioiu, C.; Voicu, V.; Medvedovici, A.; Anuța V. Mathematical and
709 phenomenological criteria in selection of pharmacokinetic model for M1 metabolite of
710 pentoxifylline. *Farmacia* **2009**, *57*, 235–246.
- 711 19. Imbibo, B.P.; Imbibo, E.; Daniotti, S.; Verotta, D., Bassotti, G. A new criterion for selection of
712 pharmacokinetic multiexponential equations. *J. Pharm. Sci.* **1988**, *77*, 784–789. DOI:
713 10.1002/jps.2600770914
- 714 20. Van Vooren, L.; Krikilion, G.; Rosier, J.; De Spiegeleer, B. A novel bending point criterion for
715 dissolution profile interpretation. *Drug. Dev. Ind. Pharm.* **2001**, *27*, 885–892. DOI:
716 10.1081/DDC-100107254
- 717 21. Adams, E.; Coosmans, D.; Smeyers-Verbeke, J.; Massart, D.L. Non-linear mixed effects models
718 for the evaluation of dissolution profiles. *Int. J. Pharm.* **2002**, *240*, 37–53. DOI:
719 10.1016/S0378-5173(02)00127-8
- 720 22. Costa, F.O.; Sousa, J.J.S.; Pais, A.A.C.C.; Formosinho, S.J. *J. Control. Release* **2003**, *89*, 199–212. DOI:
721 10.1016/S0168-3659(03)00033-6
- 722 23. Koester, L.S.; Ortega, G.G.; Mayorga, P.; Bassani, V.L. Mathematical evaluation of in vitro release
723 profiles of hydroxypropylmethylcellulose matrix tablets containing carbamazepine associated to
724 beta-cyclodextrin. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 177–179. DOI: 10.1016/j.ejpb.2004.03.017

- 725 24. Varma, M.V.; Kaushal, A.M.; Garg S. Influence of micro-environmental pH on the gel layer
726 behavior and release of a basic drug from various hydrophilic matrices. *J. Control. Release* **2005**, *103*,
727 499–510. DOI: 10.1016/j.jconrel.2004.12.015
- 728 25. Kosmidis, K.; Argyrakis, P.; Macheras, P. A reappraisal of drug release laws using Monte Carlo
729 simulations: the prevalence of the Weibull function. *Pharm. Res.* **2003**, *20*, 988–995. DOI:
730 0724-8741/03/0700-0988/0
- 731 26. Macheras, P.; Iliadis, A. Modeling in biopharmaceutics, pharmacokinetics and
732 pharmacodynamics: homogeneous and heterogeneous approaches. (Springer, New York, **2006**), pp.
733 57-88
- 734 27. Mircioiu, C.; Mircioiu, I.; Voicu, V.; Miron, D. Dissolution-bioequivalence non-correlations. *Basic*
735 *Clin. Pharmacol. Toxicol.* **2005**, *96*, 262–264. DOI: 10.1111/j.1742-7843.2005.pto960324.x
- 736 28. Mircioiu C. Release of drug from an infinite reservoir. An alternative method to derive the square
737 root (Higuchi) law, 6th, **2013**. Int. conference “Perspectives in percutaneous penetration”, Leiden.
- 738 29. Dokoumetzidis, A.; Kosmidis, K.; Macheras, P. *Int. J. Pharm.* **2011**, *418*, 100–103. DOI:
739 10.1016/j.ijpharm.2010.11.046
- 740 30. Narasimhan B. Mathematical models describing polymer dissolution: consequences for drug
741 delivery. *Adv. Drug Delivery Rev.* **2001**, *48*, 195–210. DOI: 10.1016/S0169-409X(01)00117-X
- 742 31. Macheras, P.; Dokoumetzides, A. On the heterogeneity of drug dissolution and release. *Pharm.*
743 *Res.* **2000**, *17*, 108–112.
- 744 32. Siepmann, J.; Göpferich A. Mathematical modeling of bioerodible, polymeric drug delivery
745 systems. *Adv. Drug Delivery Rev.* **2001**, *48*, 229–247. DOI: 10.1016/S0169-409X(01)00116-8
- 746 33. Peppas, N.A.; Wu, J.C.; von Meerwall E.D. Mathematical Modeling and Experimental
747 Characterization of Polymer Dissolution. *Macromolecules* **1994**, *27*, 5626–5638. DOI:
748 10.1021/ma00098a017
- 749 34. Chen, F.M.; Wu, Z.F.; Sun, H.H.; Wu, H.; Xin, S.N.; Wang, Q.T; Dong, G.Y.; Ma, Z.W.; Huang S.;
750 Zhang, Y.J.; Jin, Y. Release of bioactive BMP from dextran-derived microspheres: a novel delivery
751 concept. *Int. J. Pharm.* **2006**, *307*, 23–32. DOI: 10.1016/j.ijpharm.2005.09.024
- 752 35. Ricci, E.J.; Lunardi, L.O.; Nanclares D.M.; Marchetti, J.M. Sustained release of lidocaine from
753 Poloxamer 407 gels. *Int. J. Pharm.* **2005**, *288*, 235–244. DOI: 10.1016/j.ijpharm.2004.09.028
- 754 36. Martínez, L.; Villalobos, R.; Sánchez, M.; Cruz, J.; Ganem, A.; Melgoza, L.M. Monte Carlo
755 simulations for the study of drug release from cylindrical matrix systems with an inert nucleus. *Int. J.*
756 *Pharm.* **2009**, *369*, 38–46. DOI: 10.1016/j.ijpharm.2008.10.023
- 757 37. Lánský, P; Lánská, V.; Weiss, M. A stochastic differential equation model for drug dissolution
758 and its parameters. *J. Control. Release* **2004**, *100*, 267–274. DOI: 10.1016/j.jconrel.2004.08.021
- 759 38. Dokoumetzidis, A.; Macheras, P. Fractional kinetics in drug absorption and disposition
760 processes. *J. Pharmacokinet. Pharmacod.* **2009**, *36*, 165–178. DOI: 10.1007/s10928-009-9116-x
- 761 39. Ward, J.H.; Peppas, N.A. Preparation of controlled release systems by free-radical UV
762 polymerizations in the presence of a drug. *J. Control. Release* **2001**, *71*, 183–192. DOI:
763 10.1016/S0168-3659(01)00213-9

- 764 40. Bhaskar, K.; Krishna-Mohan, C.; Lingam, M.; Prabhakar-Reddy, V.; Venkateswarlu, V.;
765 Madhusudan-Rao. Y. *Drug Dev. Ind. Pharm.* **2008**, *34*, 719–725. DOI: 10.1080/03639040701842485
- 766 41. Mircioiu, C.; Sandulovici, R.C. *Aplicatii Numerice de Biostatistica si Statistica in Farmacie*, 2nd
767 Edn. (Editura Universitaria “Carola Davila, Bucurest, **2010**), pp. 88-124.