

MODELLING OF TRANSFER KINETICS OF TRICHLORPHON PESTICIDE ACROSS CUPROPHANE DIALYSIS MEMBRANE IN PRESENCE AND ABSENCE OF ANTIDOTE POWDERS

GHEORGHE CORLAN, GABRIELA PAHOMI, ROXANA SANDULOVICI, IRINA PRASACU, MIHAELA IONESCU, VALENTINA ANUTA*

¹ "Carol Davila" University of Medicine and Pharmacy, 37 Dionisie Lupu St., Bucharest, Romania

*corresponding author: vali_anuta@yahoo.com

Abstract

The objectives of the paper were to analyse and to model the data concerning diffusion through a membrane with or without adsorptive powders of a hydrophilic organophosphorous pesticide, Trichlorphon (TCP), using *in vitro* experimental models and to estimate the antidote efficacy of an adsorptive powder.

Data concerned the *in vitro* transfer across a dialysis membrane using a static Franz diffusion cell battery and a flow through cell system. A mixture of cellulose acetate powder and bentonite powder was applied on the membrane in order to reduce the transfer of toxics across membrane.

As mathematical models, linear biphasic regression was tested, first order kinetics and a square root law derived from diffusion equation. Fitting of experimental data with theoretical models was performed using least square methods. For selecting one or two phase linear regression models it was used the F-test.

The release kinetics was biphasic: a first model for 50 – 60 % of the transfer of available fraction of toxic, followed by a more or less saturation phase. Adsorptive powder applied as antidote candidate significantly reduced the amount of transferred Triclorfon across membrane – at least for some hours, suggesting a possible similar effect at the level of intact skin. Presence of powders changed the mathematical transfer model from a model dominated by a first order kinetic to a square root model.

Rezumat

Obiectivele acestei lucrări au fost de a analiza și modela datele cu privire la transferul unui pesticid organofosforic hidrofil – Triclorfon prin membrane, folosind modele experimentale *in vitro*. S-a estimat de asemenea eficacitatea antidotică a unei pulberi adsorbante.

Datele, publicate anterior de autori au privit transferul *in vitro* printr-o membrană de dializă folosind un sistem "flow-through". A fost evaluat efectul unui amestec de acetat de celuloză pulbere și bentonită pulbere aplicat pe membrană pentru a reduce penetrarea toxicului.

Modelele matematice folosite la testare au fost regresia liniară bifazică și o lege a radicalului derivată din ecuația difuziei. Potrivirea datelor experimentale cu modele

teoretice a fost făcută folosind metoda celor mai mici pătrate. Pentru selectarea a unui sau a două modele de regresie lineară fazică, a fost folosit testul F.

Cinetica de eliberare a fost bifazică: un model inițial pentru 50-60% din transferul fracției disponibile a toxicului, urmat de o etapă mai mult sau mai puțin de saturare. Pulberea testată a redus semnificativ cantitatea de toxic transferată prin membrane – cel puțin pentru câteva ore, sugerând un posibil efect similar la nivelul pielii intacte. Prezența pulberilor a schimbat modelele matematice de transfer de la un model predominant de cinetică de ordin 1, la un model predominant de cedare proporțională cu radicalul timpului.

Keywords: transfer across membrane; organophosphorous pesticides; adsorptive powders, square root laws, biphasic linear regression

Introduction

Mathematical modelling and statistical analysis of transfer and release of active substances provide tools to analyse experimental data and the critical factors which determine ultimately the pharmacokinetics of active substances. It is therefore expected that a lot of papers have been devoted to developing models for the drug transfer phenomena [1-7]

The archetypical mechanistic model of drug release is the Higuchi square root of time law [8,9]. In fact equation of mass transfer phenomena, heat transmission, fluid mechanics, quantum mechanics are based on same mathematical equation, solved in different initial and boundary conditions [10 - 13], and solutions are easy transferable from one domain to other.

It was suggested that transfer across artificial membranes is less influenced by the nature of the membrane and much more by the nature of the toxic [14].

The present paper modeled the transfer and accumulation of a largely used pesticides as well as the efficacy of an adsorptive powders as non-specific antidote for retention of toxic and desorption. These type of results could be useful in predicting the risks of later undesired effects *in vivo* [15].

Materials and Methods

All reagents were purchased from Sigma. Membrane transfer concerning organophosphorous compound dimethyl 2,2,2 - trichloro - 1 - hydroxyethylphosphonate (Trichlorfon –TCP, Neguvon - NEG) presented the $\log P_{o/w}$ of 0.52 [16]. Data were published previously [16] and concerned the transfer across a membrane used for the dialysis filters in a flow-through diffusion cell.

Results and Discussion

First order kinetics model for evaluation of TCP transfer through membranes. TCP with an octanol/water distribution $\log P_{o/w}$ of 0.52 is slightly more lipophilic than hydrophilic. The amount transferred in the first three hours was significantly large (60 - 80 %), as can be seen in figure 1 and 2.

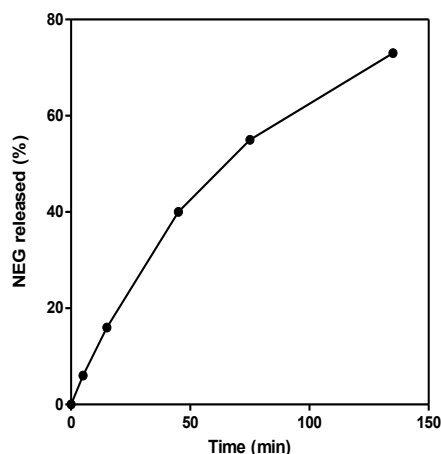


Figure 1
Amount of TCP transferred across
Cuprophane membrane

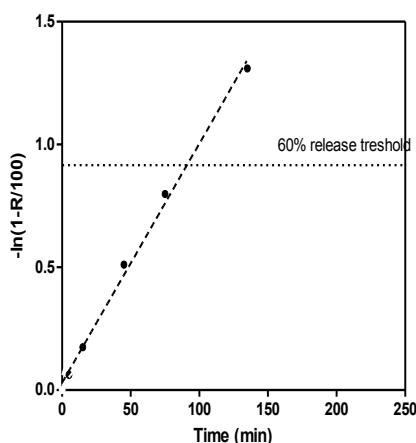


Figure 2
First order kinetic model of TCP
transfer

It is reasonable to think that the transfer between compartments is the result of a series of processes including the transfer to the interface TCP solution – membrane, diffusion across the membrane, accumulation in the membrane, the transfer at the interface membrane – receptor fluid, diffusion in the receptor fluid etc. The kinetics of the entire process is given by the rate - determining (slowest) step. If that step obeys the kinetics of a first order model, the mass transferred in one time unit – m is proportional with the mass of toxic in the upper compartment:

$$\frac{dm}{dt} = -km$$

This equation has the solution $m(t) = m_0(1 - e^{-kt})$, where m_0 is the initial quantity in the donor compartment. Rearranging this equation we obtain

$$\left(1 - \frac{m}{m_0}\right) = (1 - R/100) = e^{-kt}, \text{ where } R \text{ is the percentage of released amount of}$$

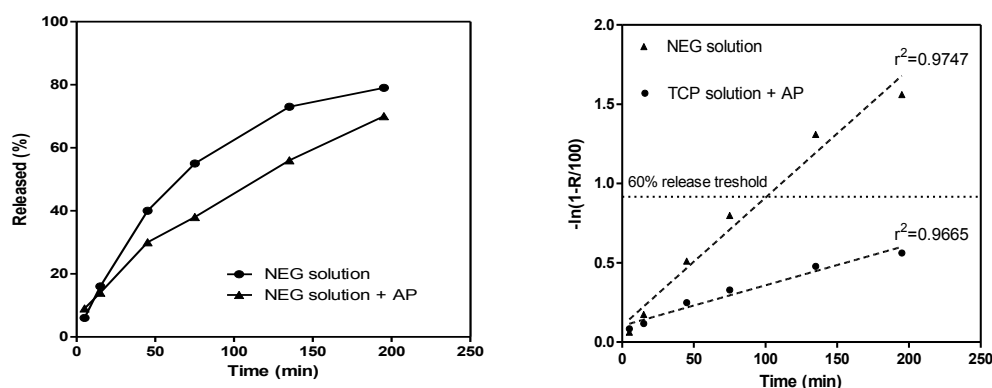
toxic at time t . After logarithmic transformation it can be obtained a straight line - $\ln(1 - R/100) = -kt$.

It is not possible to prove that this is the real picture of evolution, but the model is reliable since, as it can be seen in fig. 2, a good linear dependence $-\ln(1 - R/100) : kt$ for first two hours was obtained. The time interval for the linear correlation concerns the transfer of less than 60 % of the toxic. This result is similar to what is obtained in the case of diffusion controlled models of release of active substances from solid and semisolid pharmaceutical formulations [17].

The curve didn't present a lag-time. In the case of skin transfer it appears in all cases a long lag-time with the exception of the skin without the *stratum corneum* [16]. This means a much lower resistance to transfer across artificial membranes than in the case of integer skin.

Comparison of first order kinetics model and square root model for transfer of TCP across dialysis membrane in presence of an adsorptive powder.

The influence of adsorptive powder on the transfer of the toxic is presented in figure 3. It can be seen that applying the powder, the amount of the transferred toxic in the 0 - 3 h time interval was reduced. In absence of the adsorptive powder, the first order release kinetics characterized only the transfer in the first hour, in the case of powder the model was followed in the entire three hours interval. Consequently it can be considered that the addition of adsorptive powders changed the transfer model.



a – percent of toxic transferred

b – first order kinetic model

Figure 3

Transfer kinetics of TCP across cuprophane membrane in flow-through cell
(AP – absorptive powder)

Since in the absence of adsorptive powders first order transfer kinetics proved a good fit of experimental data, it was tried the same model to transfer in the presence of adsorptive powder. As can be seen in figure 3 for the transfer of the first 60 % amount of TCP (corresponding to $\ln(1-R/100)=1.3$) it was obtained a good fitting in both cases.

But in the prediction for results at the end of the measuring interval it seems to appear a bias. This can be interpreted as a sign that diffusion controls the release kinetic only in the first hour. If we examine in detail the data concerning transfer in presence of adsorptive powder the evolution appears to be better described by biphasic linear regression $\ln(1-R/100)$ - time, as can be seen in figure 4.

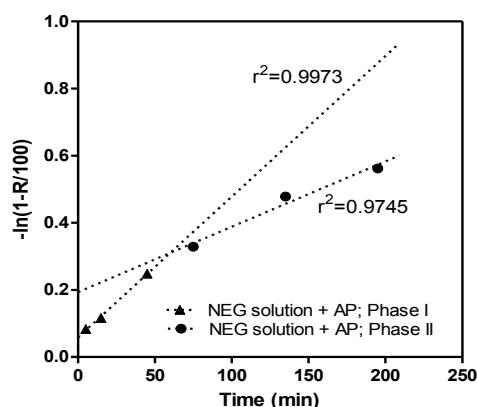


Figure 4a

Biphasic linear first order kinetics

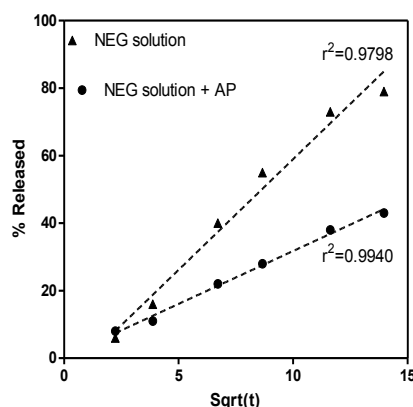


Figure 4b

Square root model

This was the reason to try, for describing the transfer in presence of adsorptive powders, at least for the first 60 % of released amount, a square root model $R = k\sqrt{t}$. As can be seen in figure 4 the model fits experimental data well enough not only for points lower than 60 %, but also for further points. The square root model works better in the case of transfer in presence of adsorptive powders. The shift from one model to other could be thought as connected with the decrease of the amount of toxic available for transfer following its adsorption on powders.

If square root model was introduced in describing the release kinetics from solid and semisolid pharmaceutical formulation fifty years ago by Higuchi, such an extension of the law to transfer across membranes is rather an unexpected result.

Mathematical deduction of a square root model for transfer across membranes.

Representation of the released amount as function of square root of time revealed a very good correlation. In fact, we can think to a rapid adsorption followed by a release from powder and further transfer across membrane. If release from the powder is less rapid than the transfer, the entire process has the release kinetic. Let us consider further that the release is, in the first phase a diffusion from an infinite reservoir in an infinite large volume. If saturation of the membrane is very rapid, concentration at the membrane/solution interface can be considered constant, c_o .

Consequently we have to find the solution of the diffusion equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial y^2}$$

with initially and boundary conditions $c(t, 0) = c_o$ and $\lim_{y \rightarrow \infty} c(t, y) = 0$

Applying the Laplace transform the equation becomes an ordinary differential equation which can be easily solved [12] and, after application of inverse transform, it results the solution [18,19]

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

where $\operatorname{erf}(z)$ is the “error function”, being defined as the area under the curve $\frac{2}{\sqrt{\pi}} e^{-x^2}$ between 0 and z :

If we compute the flux of drug across the interface $y=0$, it results for the quantity of released active substance

$$Q(t) = m(t) = \frac{2}{\sqrt{\pi}} A c_o \sqrt{Dt}$$

where A is the area of membrane, i.e. a linear dependence on the square root of time, which seems to be the case in our experiment.

From figure 4b it can be seen also that transfer from solution in absence of powders cannot be considered to follow the same rule.

A natural question concerns the selection between the two models. If we put the graphs together it can be seen that it is difficult to make a choice.

Therefore it appears the problem that data were transformed in different ways into the two models. A more precise evaluation has to start from the sum of squared differences between experimental and theoretical point in the initial, non-transformed data.

$$MSS = \sum_1^n (Exp - Rth)^2 / n$$

The calculus of Mean Square Error (MSS) for the two models indicated that the square root model is more adequate than the first order kinetics model in fitting the experimental data.

Conclusions

The transfer kinetics in absence of the absorptive powder followed a first order kinetics, i.e. the amount transferred was proportional with the concentration in the upper compartment.

Addition of powders changed the model. First order kinetics described also well enough the evolution of concentration of toxic in receptor compartment, but the evolution was rather biphasic: a linear regression of $\ln(1 - R/100)$ as function of time in the first hour and another one with a lower slope in the next two hours.

An even better fitting was obtained with the square root model.

Considering a rapid release of toxic from the upper compartment in the membrane, and later from the membrane in an infinite large volume, it was obtained that the solution of the diffusion equation with initially and boundary conditions

$$c(t, 0) = c_0 \text{ and } \lim_{y \rightarrow \infty} c(t, y) = 0$$

is $c(t, y) = c_0(1 - \operatorname{erf} \frac{y}{\sqrt{4Dt}})$ and, for the transferred amount across

membrane $Q(t) = m(t) = \frac{2}{\sqrt{\pi}} AS\sqrt{Dt}$, i.e a linear dependence of the transfer on square root of time. The model worked for describing the transfer of 60 – 70 % of the toxic.

Annex 1

Laplace transform method in solving equation of diffusion in a semiinfinite medium

Let us consider a substance or a drug formulation at the bottom of a height vessel. Let us look the evolution of concentration of active substance as time and space function. Let us consider also that dissolution – diffusion balance is so that at the interface with solution the concentration is constant – the saturation concentration C_s . Let us consider also that height of vessel is enough to assure that in time interval in which we are interested the front of substance does not reach the upper surface.

In the above conditions, we can write the following initial and boundary conditions

Let's solve the diffusion equation in these conditions

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}$$

We apply the Laplace transform to concentration as function of time

$$L(c(x, t)) = \int_0^{\infty} c(x, t) e^{-pt} dt = C(x, p)$$

Where we noted the image function by C

Starting from the model definitions initial and boundary conditions are

$$x = \infty \quad \lim_{x \rightarrow \infty} c(x, t) = 0; \quad t = 0 \quad c(x, 0) = 0; \quad x = 0 \quad c(0, t) = c_s$$

Starting from definition of Laplace transform, it obtains easy

$$L\left(\frac{\partial c}{\partial t}\right) = pC(x, p) - c(x, 0) = pC(x, p) \quad \text{and} \quad L\left(\frac{\partial^2 c}{\partial x^2}\right) = \frac{\partial^2 C}{\partial x^2}.$$

Applying the transform in both members it obtains

$$pC(x, p) = D \frac{\partial^2 C}{\partial x^2}$$

Considering $C(x, p)$ as function of x, we obtained the ordinary differential equation $DC'' - pC = 0$

Looking for solutions of the form e^{rx} (Euler method) it obtains the characteristic

$$\text{equation } x^2 - \frac{p}{D} = 0 \text{ with two roots } \sqrt{\frac{p}{D}} \text{ si } -\sqrt{\frac{p}{D}}.$$

Consequently the general solution is

$$C(x, p) = \alpha e^{-\sqrt{\frac{k}{D}}x} + \beta e^{\sqrt{\frac{k}{D}}x}$$

where α and β are constants which can be identified using from the initial and boundary conditions

Since $c(0, t) = c_s$ it obtains

$$C(0, p) = \int_0^{\infty} c(0, t) e^{-pt} dt = \int_0^{\infty} c_s e^{-pt} dt = \frac{c_s}{-p} e^{-pt} \Big|_0^{\infty} = \frac{c_s}{p}$$

$$\text{But, on other hand } C(0, p) = \alpha e^{-\sqrt{\frac{k}{D}}0} + \beta e^{\sqrt{\frac{k}{D}}0} = \alpha + \beta$$

Futher

$$\lim_{x \rightarrow \infty} c(x, t) = 0 \Rightarrow \lim_{x \rightarrow \infty} \int_0^{\infty} c(x, t) e^{-pt} dt = \int_0^{\infty} \lim_{x \rightarrow \infty} c(x, t) e^{-pt} dt = \int_0^{\infty} 0 e^{-pt} dt = 0$$

But $\lim_{x \rightarrow \infty} \int_0^{\infty} c(x, t) e^{-pt} dt = \lim_{x \rightarrow \infty} C(x, p) = \lim_{x \rightarrow \infty} \left(\alpha e^{-\sqrt{\frac{k}{D}} x} + \beta e^{\sqrt{\frac{k}{D}} x} \right)$ and last limit is zero only in the case $\beta = 0$

Since previously was established $\alpha + \beta = \frac{c_s}{p}$ it obtains $\alpha = \frac{c_s}{p}$ and

$$C(x, p) = \frac{c_s}{p} e^{-\sqrt{\frac{p}{D}} x}$$

From tables of original functions corresponding to different image functions (found in all engineering handbooks)

$$c(x, p) = c_s \left(1 - \operatorname{erf} \frac{x}{\sqrt{4Dt}} \right) \text{ unde } \operatorname{erf} \text{ is "errors function"} \quad \operatorname{erf} x = \frac{2}{\pi} \int_0^x e^{-u^2} du$$

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