International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.11 pp 171-182, 2015

Modelling and simulation of displacement in liquid chromatography using characteristics of industrial columns for the insecticide Malathion

Jorge Eliecer Buitrago Salazar, Olga Lucia Ramos Sandoval, Dario Amaya Hurtado

Universidad Militar Nueva Granada, Facultad de Ingeniería en Mecatrónica, Bogotá, Colombia; Grupo de Aplicaciones Virtuales – GAV.

Abstract: Pesticides like Malathion are used in agriculture due their broad insecticidal power, however, these properties depend of purity level that have the compound. Therefore, for optimum performance in crops, it is required that the compound has a high purity. In the case of pesticides, the purification process most commonly used is the preparative chromatography. This is a separation technique, which take advantage the property of chemical affinity, to isolate the different components of a sample through the retention time in the column. Therefore, it is necessary obtaining a model, which can determine the displacement of Malathion. With the model can set a configuration in which the resolution of the separation is increased. In this work is show the results of mathematical modeling and simulation of a column of liquid chromatography (LC), using material balances in the purification of Malathion. It is make a comparison between a standard column and two columns used in the industry, the industrial columns have good separation for a length of 0.45 to 0.6 of Tao. The diffusivity in the internal particle is the principal problem in the actual columns, this parameter is improved through the optimization of the radio.
Keywords – Liquid chromatography, Malathion, Column.

Introduction

Chromatography is a process, in which the separation of various components of a mixture by their properties is performed. Thus, is used as a specific separation method[1]. To achieve this separation the system count mainly with two phases, the mobile phase which contains the sample and the stationary phase that could be alumina, silica or ion exchange resins. Through this phase the dissolved sample flows. Chemical interactions between the sample and the layers determine the degree of migration and separation of the components contained. Different types of chromatography are characterized by the state of the phases, for liquid chromatography, the mobile phase is in liquid state and the stationary in phase solid state[2-4].

This technique of process is widely used in industry for the separation and purification of reactants and products, between the main mixtures to be purified are some natural compounds, enzymes, proteins and contaminants compounds in crops such as pesticides and herbicides[5-7].

The use of chromatography and the lack of specific experiments for each compound, raise the cost of industrial implementation. For which is necessary the development of models for this technique. The model of

liquid chromatography, is based on the ideal model raised by Wicke in 1939 and a year later by Wilson[8,9]. These models were developed for a single compound using the Langmuir isotherm, to describe the adsorption of molecules on a solid surface. Due to the complexity in developing these models, few people have taken the task of developing them. However, with the increased use of this technique, have begun to offer specialized models. In 1995 Tingyue Gu proposed a model based on the balance of matter and energy for a liquid chromatography column, which is based in use dimensionless numbers of mass transfer and energy such as Biot, the Peclet, among others[10].

Malathion is an organophosphate compound, widely used in agriculture because of its wide insecticidal power. It is characterized as an inhibitor of acetylcholinesterase (AChE) in mammals and insects, therefore, it is mainly used in crops which are often attacked by plagues of flies (*Sciaridae Phoridae, Cecydomidae sp.)* such as, avocados, artichokes, mushrooms, among others. When ingested in humans can cause cardiovascular collapse in some muscles, muscle cramps, tachycardia, hypertension, etc. [11]

Most organophosphorus compounds used as insecticides, are classified as moderately toxic by the Environmental Protection Agency of the United States (US-EPA), however, the mixture of chemicals due to incorrect synthesis or a deficient purification, they can even cause, that the resulting mixture has a greater toxicity.[12]

Therefore, it is given the need to find methods, with which, it can decrease the amount of impurities that are present in the pesticides used in agriculture. In addition, the reduction of the cost of equipment used for that purpose, choosing the appropriate column through the inner characteristics of each column and the elution pattern of the compound through the same.

Materials and methods

Model Development

According to the work done by Tingyue Gu in 1995[10]. The balance of matter for liquid chromatography column (**Error! Reference source not found.**), was raised, in the model, the following assumptions were taken into account

- Isothermal process throughout the column.
- The pores of the stationary phase are spherical and have auniform diameter.
- Radial concentration gradients are negligible.
- There is a local balance between pore surface and the surrounding fluid.

The model (**Error! Reference source not found.**) can be derived from the equations of continuity of the book Transport Phenomena by Bird et al.[13]Also, it can be get through the material balances in dynamic state, for the particle (Eq. 1) and the fluid passing through the column (Eq. 2).

$$-D_b\frac{\partial^2 C_b}{\partial Z^2}+v\frac{\partial C_b}{\partial Z}+\frac{\partial C_b}{\partial t}+\frac{3k_i(1-\epsilon_b)}{\epsilon_b R_p}\left(C_b-C_{p_{R=R_p}}\right)=0 \qquad \text{Eq. 1}$$

$$(1-\epsilon_p)\frac{\delta C_p^*}{\delta t}+\epsilon_p\frac{\delta C_p}{\delta t}-\epsilon_p D_p\left[\frac{1}{R^2}\frac{\delta}{\delta R}\left(R^2\frac{\delta C_p}{\delta R}\right)\right]=0 \qquad \text{Eq. 2}$$

Boundary conditions (BC):

$$Z=0 \qquad \frac{\delta C_b}{\delta Z}=\frac{v}{D_b}*(C_b-C_f(t)) \qquad \text{Eq. 3}$$

$$Z=L \qquad \frac{\delta C_b}{\delta Z}=0 \qquad \text{Eq. 4}$$

$$R=0 \qquad \frac{\delta C_p}{\delta R}=0 \qquad \text{Eq. 5}$$

$$R=R_p \qquad \frac{\delta C_p}{\delta R}=\frac{K_i}{\epsilon_p D_p}\left(C_b-C_{p_{R=R_p}}\right) \qquad \text{Eq. 6}$$

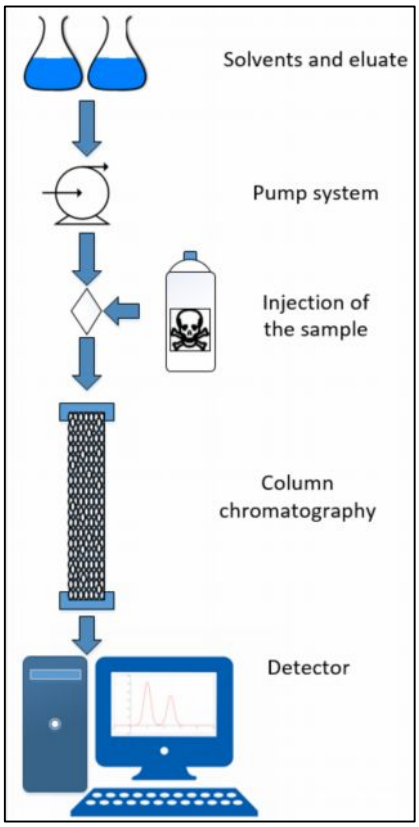

Figure1: Separation technique by liquid chromatography.

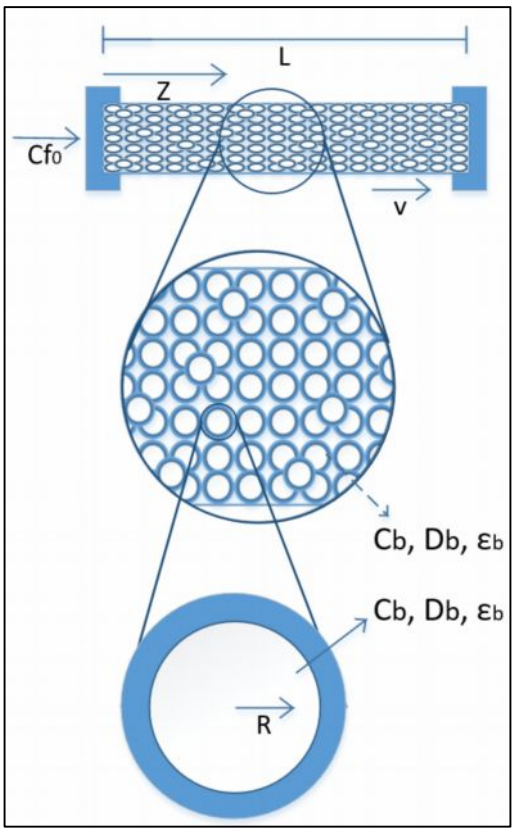

Figure 2: Chromatography column.

For these equations, the length is given only for the chromatographic column, it is not taken into account the length of storage of samples. The time zero was defined as the time in that the sample finished of enter the column. For analysis, the sample comes in the form of a single pulse, to simplify the model, the following dimensionless parameters were defined:

$$c_b = \frac{C_b}{C_0} \quad \text{Eq. 7}$$

$$c_p = \frac{C_p}{C_0} \quad \text{Eq. 8}$$

$$\tau = \frac{vt}{L} \quad \text{Eq. 9}$$

$$r = \frac{R}{R_p} \quad \text{Eq. 10}$$

$$z = \frac{Z}{L} \quad \text{Eq. 11}$$

$$Pe_L = \frac{vL}{D_b} \quad \text{Eq. 12}$$

$$Bi = \frac{KR_p}{\epsilon_p D_p} \quad \text{Eq. 13}$$

$$\eta = \frac{\epsilon_p D_p L}{R_p^2 v} \quad \text{Eq. 14}$$

$$\xi = \frac{3Bi\eta(1-\epsilon_b)}{\epsilon_b} \qquad \text{Eq. 15}$$

Three dimensionless parameters were used, the Peclet number (Pe_L) which takes account the axial dispersion, the Biot number (Bi) for mass transfer and a dimensionless factor for Malathion (η) used in liquid chromatography.

Applying these new parameters in equations 1 and 2, it is obtained for the fluid that pass through the column the Eq. 16 and for the particle the Eq. 17.

$$-\frac{1}{Pe_L}\frac{\delta^2 c_b}{\delta z^2}+\frac{\delta c_b}{\delta z}+\frac{\delta c_b}{\delta \tau}+\xi*(c_b-c_{p,r=1})=0 \qquad \text{Eq. 16}$$

$$\frac{\delta}{\delta \tau}[(1-\epsilon_p)c_p^{\cdot}+\epsilon_p c_p]-\eta\left[\frac{1}{r^2}\frac{\delta}{\delta r}\left(r^2\frac{\delta c_p}{\delta r}\right)\right]=0 \qquad \text{Eq.17}$$

Boundary conditions (BC):

$$z=0 \qquad \frac{\delta C_b}{\delta z}=Pe_L\left[c_b-\frac{c_f(\tau)}{c_0}\right] \qquad \text{Eq. 18}$$

$$z=L \qquad \frac{\delta C_b}{\delta z}=0 \qquad \text{Eq. 19}$$

$$R=0 \qquad \frac{\delta C_p}{\delta r}=0 \qquad \text{Eq. 20}$$

$$r=1 \qquad \frac{\delta C_p}{\delta r}=Bi(c_b-c_{p,r=1}) \qquad \text{Eq.21}$$

The parameter c_0 is used to normalize the concentration, in this case is taken as the highest value concentration in the column.

Calculation of parameters

Table1: Malathion properties.

Property	Value	Units
T_{Fb}	513,15	K
MW	330,358	$\frac{g}{mol}$
μ	1,00E-03	$Pa\,s$
Vm	0,2785	$\frac{m^3}{kgmol}$
ρ	1,186	$\frac{g}{cm^3}$

For the calculation of the parameters involved in the solutions of the equations, input values of the molecule and characteristics of the column were required, for programming the initial problem, the initial data for the molecule (Table1) and the column (Table 2) were used.

Table 2: Initial values for the calculation of parameters in the column 1.

Parameter	Value	Units
MW	10000	g/mol
Q	1,0000	mL/min
L	10,000	cm
d_c	1,0000	cm
R_p	0,0113	cm
ϵ_b	0,4000	
τ_{tor}	4,0000	
d_p	300,00	Å
ϵ_p	0,5000	

With the initial values can be calculated the parameters required for the solution of the equations 16 and 17. The parameters and equations used are shown in Table 3.

Table 3: Equations used to calculate the parameters.

Parameter	Equation	Equation number	Units
V_{in}	$V_{in} = \pi * R_c^2 * L$	22	cm^3
v	$v = \frac{Q}{\frac{\pi}{4} d_c^2 \epsilon_b}$	23	$\frac{cm}{s}$
d_m	$d_m = 1.44(MW)^{\frac{1}{3}}$	24	Å
D_m [14]	$D_m = \frac{9.96x10^{-16}T}{\mu * V_m^{\frac{1}{3}}}$	25	$\frac{cm^2}{s}$
D_p [15]	$D_p = \frac{D_m\left[1 - 2.104 * \left(\frac{d_m}{d_p}\right) + 2.09\left(\frac{d_m}{d_p}\right)^3 - 0.95\left(\frac{d_m}{d_p}\right.\right.}{\tau_{tor}}$	26	$\frac{cm^2}{s}$
K [16]	$K = 0.687 v^{\frac{1}{3}} \left(\frac{\epsilon_b R_p}{D_m}\right)^{\frac{2}{3}}$	27	$\frac{cm}{s}$

To calculate the molecular diffusivity, was required the viscosity, the atomic volume and the temperature at which the component was inside the system. Therefore, to be applied to a pesticide, it is required to have these parameters.

Solving the system of equations

From Equation 16, the concentration profile according to column length and duration of the chromatography was obtained. Due to the complexity of the equations, the model was developed through a matrix, in which the boundary conditions were placed, making an analysis of the system. For example for the time zero, the concentration is show in Equation 28, because the sample is just inject, this is at the length zero of the column, while the rest of the column is empty.

$$\tau = 0 : \begin{cases} z = 0 & \rightarrow c_b = 1 \\ z \neq 0 & \rightarrow c_b = 0 \end{cases} \quad \text{Eq. 28}$$

For development of derivatives, the central method of derived for partial differential equations was used (Equations 29 and 30) [17]. The subscripts of the equations indicate the basis of the derivative, while the parameters h and k are the step sizes that advances in length (z), radio (r) and time (τ) respectively.

$$U_{zz} = \frac{U_{z+1,\tau} - 2U_{z,\tau} + U_{z-1,\tau}}{h^2} \quad \text{Eq. 29}$$

$$U_{z\tau} = \frac{U_{z+1,j+1} - U_{z-1,\tau+1} - U_{z+1,\tau-1} + U_{i-1,j-1}}{2hk} \quad \text{Eq. 30}$$

The same process was performed for the partial derivatives of equation 17, wherein the concentration of component depend on the particle radius and the time. Due to the standardization of parameters (equations 7-15), the boundary values of z and r variables were zero, for the time when the sample just entered to the column and 1 in the time the sample arrives the limit of the variable.

Table 4: Matrix used to solve the equations 16 and 17 for "z" and "r" respectively.

$z\ (i)$ / τ ó $r\ (j)$	0	1	...	19	20
0	$X_{i;j} = X_{0;0}$	$X_{i*h;j} = X_{0,0526;0}$	...	$X_{i*h;j} = X_{0,9474;0}$	$X_{i*h;j} = X_{1;0}$
1	$X_{i;j*k} = X_{0;0,2}$	$X_{i*h;j*k} = X_{0,0526;0,2}$	...	$X_{i*h;j*k} = X_{0,9474;0,2}$	$X_{i*h;j*k} = X_{1;0,2}$
...	...	...	...	...	...
5	$X_{i;j*k} = X_{0;0,8}$	$X_{i*h;j*k} = X_{0,0526;0,8}$	...	$X_{i*h;j*k} = X_{0,9474;0,8}$	$X_{i*h;j*k} = X_{1;0,8}$
6	$X_{i;j*k} = X_{0;1}$	$X_{i*h;j*k} = X_{0,0526;1}$	...	$X_{i*h;j*k} = X_{0,9474;1}$	$X_{i*h;j*k} = X_{1;1}$

Considering, that for the variables "z" and r 20 points were defined, with step size h, and for the variable τ 6 points were defined, it has an array of equations (Table 4), in which the system solution is when the equation is zero at every.

Multivariate iterative method for solving equations was used, because the equations 17 and 18 are equalized to 0, this parameter as a constraint for the solution of equations, in which random values are assigned to the variables used until all equations in the matrix are zero. These equations were solved with the help of Microsoft Excel® Solver function 2013.

To calculate the error in the equalization of the equations to zero, the mean, median and standard deviation was calculated. This procedure for each of the columns studied was realized, to verify the performance of Malathion versus time.

Results and discussion

Model Development

In most of equipment, the area where is located the column, is surrounded by a furnace, which keeps the temperature constant during the separation process, we must also emphasize that pesticides are mostly compounds highly volatile, whereby during chromatography, the internal temperature should not exceed the boiling point of the substance. This ensures, that the heat lost due to interaction with the environment will be low, with this you can reach optimal control of the indoor temperature without consuming much energy in the oven.Nowadays, one of the main advantages of existing columns is that the resolution of the chromatogram is increased, because they have a greater length and a smaller diameter, this promotes can be neglected the radial gradients of concentration at any point in the column.

The filling or the stationary phase of the column, is one of the more controlled parameters during production, because, for enhanced the high quality, it must form pores more uniforms, whereby the system can quickly reach equilibrium. Whereupon the assumptions applied in the model does not generate significant differences with the real behavior.

The properties of Malathion used in the different columns for the simulation are shown in Table1, however, three different configurations for columns (Table 5) were tested. The specifications of column 2 and column 3 are used industrially.

Table 5: Initials values for the columns.

Parameter	Value Column 1	Value Column 2	Value Column 3
T (K)	513,15	413,15	393,15
MW (g/mol)	330,3580	330,3580	330,3580
Q ($\frac{mL}{\min}$)	1,0000	1,0000	1,0000
L (cm)	10,0000	2000,0000	4000,0000
d_c (cm)	1,0000	0,0010	0,0010
R_p (cm)	0,0113	0,0113	0,0001
ϵ_b	0,4000	0,4000	0,6000
τ_{tor}	4,0000	4,0000	5,0000
d_p (Å)	300,0000	200,0000	200,0000
ϵ_p	0,5000	0,4500	0,4500

The principal parameter affected was the length of columns, which directly affects the calculation of the Peclet number, because is the relationship between fluid advection and diffusion rate, a big number indicates that the system is mainly governed by fluid displacement. The porosity of the particle (ϵ_p) and stationary phase (ϵ_b) are predetermined for each column and depend on the material which is manufactured the column. For the column 2 and 3 the material used is polydimethylsiloxane (PDMS), which is effective for non-polar compounds. Tortuosity (τ_{tor}) is the degree of rotation that has the column, between longer is the column, a higher degree of tortuosity is required to store the column without affecting the volume occupied by it. For the three columns the same flow rate (Q) was used, because at higher flow rates, the separation is inefficient by the drag of the components to be separated.

With the initial values for the three columns, was calculated the parameters necessary for solving the derivatives (Table 6), it should be noted that the number of mass transfer Biot indicates the ease of molecular diffusion inside the column. Due the column has a smaller diameter, the intra-particle diffusion will be small, so that the concentration within the particle will have many variations.This dimensionless number depends directly on the mass transfer coefficient, it is intended that this value be as high as possible on any system to facilitate the exchange of molecules between the two phases, this factor depends on the Reynolds number, which characterizes movement of a fluid, in liquid chromatography is intended that the fluid passing through the column is in laminar regime, as a result the molecular transfer between the phases is facilitated.

Table 6: Calculated parameters for the columns.

Parameter	Equation	Column 1	Column 2	Column 3	Units
V_{in}	22	7,8540	0,0016	0,0031	cm^3
v	23	0,0531	53051,6477	35367,7651	$\frac{cm}{s}$
d_m	24	9,9546	9,9546	9,9546	Å
D_m	25	$7{,}81x10^{-10}$	$6{,}29x10^{-10}$	$5{,}98x10^{-10}$	$\frac{cm^2}{s}$
D_p	26	$1{,}82x10^{-10}$	$1{,}41x10^{-10}$	$1{,}34x10^{-10}$	$\frac{cm^2}{s}$
K	27	0,0011	0,0007	0,0097	$\frac{cm}{s}$
Pe_L	12	222,2222	44444,4444	$5{,}926x10^{6}$	
η	14	$1{,}35x10^{-4}$	$1{,}89x10^{-8}$	$4{,}31x10^{-4}$	
Bi	13	994,9387	123440,1339	18025,2633	
ξ	15	83,8212	0,0104	19,42117032	

The internal volume of the column is a very important factor in terms of operating cost, a higher values, it requires a larger quantity of mobile phase, leading to the existence of more waste when the equipment is operating. Currently, in the columns this value is reduced each time more, this is achieved by making the column diameter and the internal pore diameter smaller.

Diffusivity is a factor that indicate how a solute is displace in a predetermined solvent, in the model were defined the effective diffusivity (D_p) and the molecular diffusivity (D_m) for Malathion. These values depends mainly on the temperature and fluid viscosity. Column 1 shows the highest values of diffusivity, because the initial values are based on the parameters of an ideal column, however the difference with the columns used industrially is not significant.

The dimensionless factor for Malathion (η), indicates the relationship between the diffusivity that has the component in relation to the radius of the particle and the interstitial speed on the system. The smaller is this factor, the system will be governed by the displacement fluid, whereby the interaction between the particle and fluid is poor, reason why the system will not reach the equilibrium. Generally, this factor takes values between $1x10^{-2}$ and $1x10^{-6}$, outside these values, the system is considered as non-equilibrium system. The column 2 has this value outside the normal range, for which the system shows many oscillation and the peaks to observe will not have a good definition.

Solving the model for Malathion

The step size for each one of the variables is changeable, if this value is high, the system loses accuracy, and while to low step sizes the error at each step builds up, so that the system will behave oscillatory. To solve the system of equations, the steps were 0.0526 and 0.2000 for h and k respectively. The step size h, was defined for the change in length "z" and radio$r$, because they are the main variables on which was based the studio, while the variable k, was assigned to the variable of timeτ.

To verify the system solution through the proposed method, the average, median, and standard deviation was determined (Table 7) for each column. These values were calculated using the data obtained from each point of the matrix, in total 240 equations.

Table 7: Error calculate in the solution of the system of equations.

Parameter	Column 1	Column 2	Column 3
Average	0,080	-0,045	-0,154
Median	3,880E-05	1,001E-06	1,546E-05
Standard deviation	1,366	1,828	1,703

The average for the three columns was very close to the desired value, which is confirmed by the standard deviation, this parameter show the dispersion of data, for which in this case is low, it indicate the most part of data was near of zero, whereby the 240 equations for the solution method, tend to reach its desired value.

The solution of system of equations was determined, Equation 16 and 17 for each column. With these solutions, the displacement of the maximum peak of concentration was determined, of the pesticide Malathion through the column (Figure3). For column 1, the change in maximum peak concentrations over time was evident, to low values of tao τ, the peak did not change significatively, this is because the diffusion of Malathion inside the particle does not performed uniformly (Figure4), thereby causing concentration peaks between 0,6 and 0,9 of radius. For high values of tao τ, the peaks were more diffuses and had a lower resolution, some of them enters in the oscillatory zone where was the accumulated error, whereby it becomes difficult to read an adequate value.

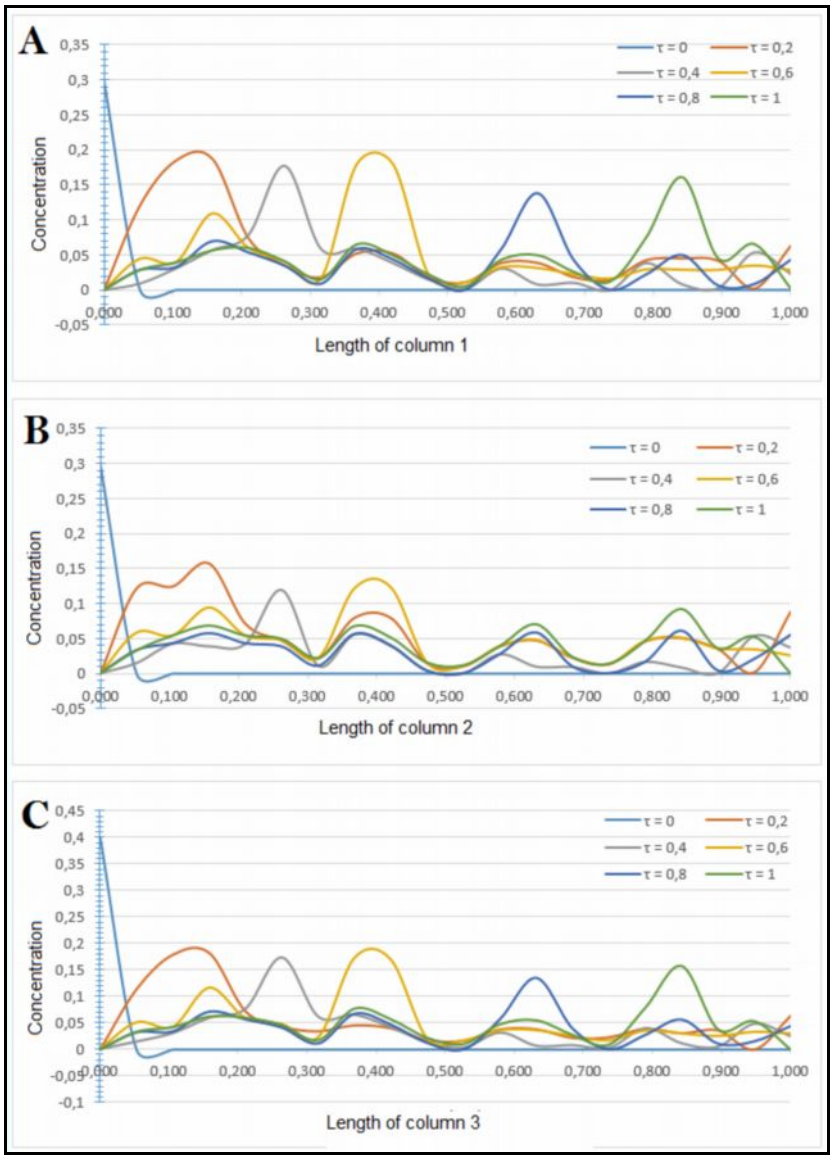

Figure3: Concentration profile of Malathion through the column at different times. A. Column 1, B. Column 2, C. Column3.

For column 1 (Figure3 , Part A), the ideal length for a tao of 1 is close to the value of 0,85 for the variable "z", in which, the observed peak resolution of Malathion was enough. This promote the separation of this compound, reducing the costs required in the purification of this pesticide used in many crops. For columns 2 and 3, the ideal value of the variable "z" is close to 0,45, for a tao of 0.6, but the resolution of these peaks is less than that obtained in column 1. It cannot use the highest peak for the other taos, due the instability of the system. At these points the separating capacity of the column low, reason why the maximum efficiency of the process down.

Column 2, showed the greatest dispersal in the data, this was because the parameters calculated for this column were out of the limits(Table 6). This error, also was due to the low diffusion of the pesticide at inner the particle (Figure4, Part B), regardless of value of tao, the concentration of Malathion is low for any internal radius. Reason why did not given an adequate mass transfer in the system. While the intra-particle diffusion in column 3 was dispersed to different Taos. This helps that exist a greater adsorption and desorption in each step at the column. Directly affecting the retention of the pesticide and have a better separation in the purification of this compound.

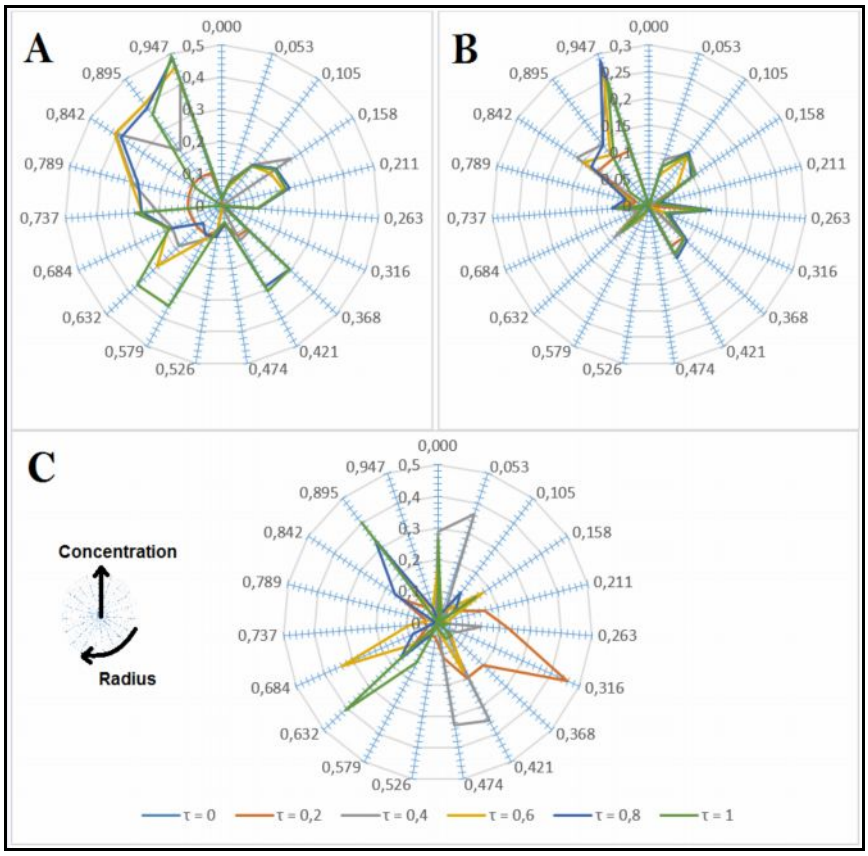

Figure4: Concentration profile of Malathion through the particle radius at different times. A. Column 1, B. Column 2, C. Column3.

Conclusions

The model was developed, on the basis of the material balances for the system of a column of liquid chromatography. Due to the complexity of the model were found the ideal step size according the importance of the variable analyzed. That step size decreases the accumulated error through each point of the matrix.

The solution of the system of equations, depends on the initial values to be taken, however, the pore diameter and the molecular diffusivity affect the dimensionless parameters of mass transfer, which dictate the behavior of the pesticide in the column. It should be noted that these parameters also depend on the molecule, in addition, the parameters for the flow in the column have to reach the minimum and maximum established.

For the design of a column, it is seeks that the parameters of mass transfer are very similar to the values used for column 1, which is an ideal column, reason why provides the best results. The column 2 is not suitable for the separation of Malathion, however, the column three, has a good separation with a length of 0.45 and tao of 0.6.

Acknowledgements

The authors would like to offer their special gratitude to the Research Vice-chancellorship of Nueva Granada Military University for financing the research project IMP_ING 1777 titled: "Análisis de residuos de plaguicidas en frutas tropicales en Colombia para la predicción de posibles efectos en la salud humana", 2015.

Nomenclature

Bi	Biot Number
c	Dimensionless Malathion concentration
C	Malathion concentration $\left(\frac{mol}{L}\right)$
d	Diameter (cm)
$\mathbf{d_c}$	Inner diameter of a column (cm)
$\mathbf{D_m}$	Molecular diffusivity for Malathion $\left(\frac{cm^2}{s}\right)$
$\mathbf{D_p}$	Effective diffusivity for Malathion $\left(\frac{cm^2}{s}\right)$
ϵ_b	Bed void volume fraction
ϵ_p	Particle porosity
ξ	Dimensionless mass transfer constant
h	Step size for z and r
K	Mass transfer coefficient for Malathion
k	Step size for τ
L	Length of study area (cm)
MW	Molecular Weight $\left(\frac{g}{mol}\right)$
η	Dimensionless constant for Malathion
Pe	Peclet number
Q	Mobile phase volumetric flow rate $\left(\frac{mL}{min}\right)$
τ	Dimensionless time
t	Time (s)
r	Dimensionless particle radio
R	Radial coordinate (cm)
v	Interstitial velocity $\left(\frac{cm}{s}\right)$
V_m	Atomic volume $\left(\frac{m^3}{kgmol}\right)$
V	Volume (cm^3)
Z	Column length (cm)
z	Dimensionless column length
Subscripts	
0	Initial point
b	Mobil phase
c	Column
in	Inner part
L	Axial column coordinate
p	Particle phase

References

1. D. A. Ahumada, L. W. Aparicio, J. C. Fuentes, J. A. Guerrero, and B. I. Checa, “Comparación de dos aproximaciones para la estimación de la incertidumbre en análisis de residuos de plaguicidas mediante cromatografía de gases,” *Rev. Colomb. Quím.*, vol. 41, no. 3, pp. 377–394, 2012.

2. D. S. Hage, J. A. Anguizola, R. Li, R. Matsuda, E. Papastavros, E. Pfaunmiller, M. Sobansky, and X. Zheng, "Chapter 1 - Affinity Chromatography," in *Liquid Chromatography*, S. F. R. H. F. P. S. Lloyd, Ed. Amsterdam: Elsevier, 2013, pp. 1 – 23.
3. G. Guiochon and S. Golshan-Shirazi, "A retrospective on the solution of the ideal model of chromatography," *J. Chromatogr. A*, vol. 658, no. 2, pp. 173 – 177, 1994.
4. K. Pourghazi and M. Amoli-Diva, "Magnetic nanoparticles solid phase extraction based on the formation of supramolecular mixed hemimicelle aggregates for the determination of naproxen in biological fluids using high-performance liquid chromatography-UV," *Micro Nano Lett. IET*, vol. 9, no. 9, pp. 577–581, Sep. 2014.
5. J. Giacometti and D. Josić, "Chapter 7 - Protein and Peptide Separations," in *Liquid Chromatography*, S. F. R. H. F. P. S. Lloyd, Ed. Amsterdam: Elsevier, 2013, pp. 149 – 184.
6. F. Hernández and M. Ibáñez, "Chapter 12 - Multiresidue Methods for Pesticides and Related Contaminants in Food," in *Liquid Chromatography*, S. F. R. H. F. P. S. Lloyd, Ed. Amsterdam: Elsevier, 2013, pp. 319 – 336.
7. R. Boden, S. Ogden, and K. Hjort, "Microdispenser With Continuous Flow and Selectable Target Volume for Microfluidic High-Pressure Applications," *Microelectromechanical Syst. J. Of*, vol. 23, no. 2, pp. 452–458, Apr. 2014.
8. E. Wicke, "Empirische und theoretische Untersuchungen der Sorptionsgeschwindigkeit von Gasen an porösen Stoffen II," *Kolloid-Z.*, vol. 86, no. 3, pp. 295–313, 1939.
9. J. N. Wilson, "A Theory of Chromatography," *J. Am. Chem. Soc.*, vol. 62, no. 6, pp. 1583–1591, 1940.
10. G. Tingyue, *Mathematical Modeling and Scale-Up of Liquid Chromatography*, Second. Switzerland: Springer International Publishing.
11. R. Mosquera and G. A. Peñuela, "Biodegradación del malatión utilizando microorganismos nativos de suelos agrícolas," *Rev. Colomb. Cienc. Pecu. Colomb. J. Anim. Sci. Vet. Med.*, vol. 22, no. 2, pp. 189–198, 2009.
12. D. G. F. A. Md, L. C. M. G. Md, and D. C. F. A. Md, "Intoxicación por organofosforados," *Rev. Med*, vol. 18, no. 1, pp. 84–92, 2010.
13. R. B. Bird, W. E. Stewart, and E. N. Lightfoot, *Fenomenos de transporte/ Transport Phenomena*. Editorial Limusa S.A. De C.V., 2007.
14. A. Polson, "The Some Aspects of Diffusion in Solution and a Definition of a Colloidal Particle.," *J. Phys. Colloid Chem.*, vol. 54, no. 5, pp. 649–652, 1950.
15. A. M. Striegel, W. W. Yau, J. J. Kirkland, and D. D. Bly, "The Column," in *Modern Size-Exclusion Liquid Chromatography*, John Wiley & Sons, Inc., 2009, pp. 130–144.
16. E. J. Wilson and C. J. Geankoplis, "Liquid Mass Transfer at Very Low Reynolds Numbers in Packed Beds," *Ind. Eng. Chem. Fundam.*, vol. 5, no. 1, pp. 9–14, 1966.
17. F. C. Tito, *Métodos Numéricos Para estudiantes de ingeniería*, Tercera. Universidad Nacional de Colombia, 2011.

*****