# MATHEMATICAL MODELING OF FOLIC ACID FACILITATED PERTRACTION

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**Abstract:** Using the statistical analysis and factorial experiments of second order, two mathematical correlations for the folic acid facilitated pertraction using Amberlite LA-2 as carrier have been established. These equations describe the dependences between the solute initial mass flow, respectively the permeability factor, and the main parameters influencing the separation efficiency (pH-values of the feed and stripping phases, carrier concentration). For both cases, the considered variables control the separation process in a 96.1 - 96.8% extent, the carrier concentration exhibiting the most important influence.

**Keywords**: *folic acid, vitamin $B_9$, Amberlite LA-2, liquid membranes, pertraction, carrier, mathematical modeling, regression equation, determination coefficients.*

## INTRODUCTION

The folic acid, also called pteroylglutamic acid or vitamin $B_9$, is one of the important members of vitamins B group. It is a growth factor, essential for making genetic material (DNA and RNA), red blood cells, for building muscle tissues, especially during periods of infancy, adolescence and pregnancy. It also intervenes in many metabolic functions, like as the choline and amino acids biosynthesis, has a direct effect on lessening depression, helps regulate sleeps and appetite and prevents some birth defects [1]. Folic acid deficiency can cause poor growth, tongue inflammation, gingivitis, loss of appetite, irritability, mental sluggishness etc.

Folic acid could be obtained by extraction at acidic pH from vegetable (spinach leaves, cereals, lemons) or animals (liver) organs [2]. But, this method needs high amount of raw materials and laborious stages for separation and purifications.

Its chemical synthesis is applied at industrial scale and consists on condensation of p-amino-(L)-glutamic acid with 2,3-dibromopropionaldehide and 2,4,5-triamino-6-hydroxipyridine/HCl in basic media [1, 3]. The overall yield on folic acid does not exceed 40 - 50%, the acid purity being of 80%.

The condensation reactions can be catalyzed also by enzymes. Thus, the folic acid can be obtained from precursors as glutamic acid, p-aminobenzoic acid or 2-amino-4-hydroxipyridine-6-carboxyaldehide using enzymes extracted from *Escherichia coli*, *Lactobacillus arabinosus* or *Mycobacterium avium*, with or without needing supplementary stages of chemical synthesis type [1]. Owing to their economic limitations, these methods are used only at laboratory scale.

The folic acid production by biosynthesis has been recently developed, the obtained product possessing a superior biodisponibility compared to that obtained by chemical pathways [4]. This method is based on the high metabolic potential of mutant *Bacillus subtilis* for biosynthesize the folic acid on glucose substrate. The obtained conversion of glucose to folic acid was of 0.16 mole folic acid/mole glucose, for several times greater than that reached when wild strains of *Bacillus subtilis* have been used.

The separation and purification of folic acid from fermentation broths implies the biomass filtration and acid sorption on anionic exchangers, after the preliminary purification of filtrate by thermal or chemical treatments. These stages are not difficult, but its elution from anionits with basic solutions (sodium hydroxide) must be gently carried out, owing to the low stability of folic acid in the strong alkaline pH-domain, this representing an important limitation of separation technology.

The separation of biosynthetic products from fermentation broths by extraction constitutes an efficient method for many technologies. For the compounds which dissociate in aqueous solutions, the performances of extraction process can be enhanced by reactive extraction with an extractant added into the organic phase [5-7]. The reactive extraction using extractants of organophosphoric or height molecular amines derivatives types have been successfully applied to the separation of some carboxylic acids, namely as: acetic acid, lactic acid, citric acid, succinic acid, malic acid, ascorbic acid or beta-lactamic antibiotics [5-12].

Due to the insolubility of folic acid in organic solvents [1], its separation by physical extraction is impossible. But, as it was demonstrated in the previous papers, its extraction became possible by adding into the solvent of an extractant which reacted with folic acid, leading to the formation of a hydrophobic compound, as in the case of above mentioned carboxylic acids extraction. In this purpose, our previous studies have been focused on the reactive extraction of folic acid with Amberlite LA-2 (lauryl-trialkyl-methylamine), the efficiency of reactive extraction reaching over 95% [13].

The development of the reactive extraction of folic acid was made by studying its extraction and transport through liquid membranes, technique also called *pertraction* or *permeation through liquid membranes*. This separation method consists in the transfer of a solute between two aqueous phases of different pH which are separated by a solvent layer of various sizes.
Commonly, liquid membranes can be obtained either by emulsification (*liquid membrane extraction*) when its stability is poor, by including the solvent in a hydrophobic porous polymer matrix (*supported liquid membrane extraction*), or by using special equipments (*free liquid membrane*) [8, 9].
The pertraction efficiency could be significantly enhanced by adding of a carrier in liquid membrane, such as organophosphoric compounds, long chain amines or crown-ethers, the separation process being called *facilitated pertraction*.
Among the factors which control the efficiency of permeation through liquid membranes (pH-gradient between the aqueous phases, carrier concentration, mixing intensity, physical and chemical characteristics of the system components), the pH-difference between the feed and stripping phase exhibits the main influences. For transport systems through liquid membranes, the pH-values of the two aqueous phases will control the yields of the extraction and re-extraction processes, on the one hand, and the rate of the solute transfer through the solvent layer, on the other hand.
The previous experiments on the mechanism and influencing factors of the facilitated pertraction of folic acid with Amberlite LA-2 as carrier have been continued by mathematical modeling, using the statistical analysis, respectively the factorial experiment of second order. The proposed models take into account the influences of pH-values of the feed and stripping phases and of the carrier concentration on the vitamin mass flows and permeability factor.

## EXPERIMENTAL

The experiments have been carried out using a patented pertraction equipment that allows obtaining and easily maintaining the free liquid membrane [14]. The pertraction cell consists on a U - shaped glass pipe having an inner diameter of 45 mm and a total volume of 400 mL, the volume of each compartment being equal.
The aqueous solutions are independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having a rotation speed of 500 rpm. In order to reach high diffusional rates through the solvent layer, the organic phase has been mixed with a stirrer of the same design, at a constant rotation speed of 500 rpm. The area of mass transfer surface, both for extraction and for re-extraction, was of $1.59.10^{-3}$ $m^2$. The interfaces between the phases remained flat, and hence the interfacial area constant, for the used mixing intensity.
The experiments were carried out in a continuous system, at the steady state conditions for aqueous solutions, these solutions being separately fed with a volumetric flow of 1.9 L/h.
The liquid membrane phase consists of a solution of 40, 60, 80 g/L Amberlite LA-2 as carrier dissolved in 1,2-dichloromethane.
The feed phase contains a solution of $5.3.10^{-2}$ g/L folic acid. The stripping phases consist on solutions of sodium hydroxide of different pH values varied between 8 and 12.

The feed solution pH adjustment has been made with a solution of 5% sulfuric acid, depending on the desired pH value, namely 3, 4, 5. The stripping solution pH has been corrected with a 5% sodium hydroxide solution at the following values: 8, 9 and 10. The pH values were determined using a digital pH meter C831 type Consort. The pH values of the two aqueous phases have been registered throughout each experiment and any pH change was recorded.
The evolution of pertraction was followed by means of the folic acid mass flows and permeability factor through liquid membrane. For calculating these parameters, the folic acid concentration in the initial solution was measured by high performance liquid chromatography technique (HPLC) with a Shim-pack CLC-ODS column (6 mm diameter and 150 mm length) and UV detector at 210 nm. The mobile phase was a mixture of 100 mM phosphate buffer (pH = 6) and 0.8 mM n-octylsulphonate sodium salt/acetonitrile in a volume ratio of 9/1. For the calculation of the folic acid concentration in the solvent layer the mass balance has been used. Samples removal was carried out from the aqueous phases' evacuation outlet.

## RESULTS AND DISCUSSION

The previous studies on separation of folic acid by facilitated pertraction underlined the major influences of the pH-gradient between the feed and stripping phase, the carrier concentration in the solvent layer, and the mixing intensity of the aqueous phases on the separation efficiency [15]. But, the magnitude of these influences has to be correlated with the reactive extraction in solvent layer.
For reaching the maximum initial and final mass flows of the folic acid, the following process conditions must be respected: the pH-value of the feed phase near to 5, the pH-value of stripping phase over 10, the carrier concentrations over 80 g/L at intense mixing of both aqueous phases. On the other hand, for transporting the total amount of folic acid through the liquid membrane, high pH-gradient between the aqueous phases, low concentration of carrier in liquid membrane and high stirrers' rotation speed are required [15].
For mathematical modeling of separation of folic acid by facilitated pertraction with Amberlite LA-2, two factorial experiments of second order have been used. The mathematical models quantify the influences of pH values of aqueous phases and Amberlite LA-2 concentration in organic layer on the initial mass flow, N, and permeability factor, P, and are established by statistical analysis. Thus, the real values of the process variables were chosen arbitrarily, their limits and coding being given in Table 1.

***Table 1.*** *The limits and coding of process variables for modeling the folic acid facilitated pertraction*

| Variable | Code | Variable level | | | Step |
|---|---|---|---|---|---|
| | | -1 | 0 | +1 | |
| pH value of feed phase | $x_1$ | 3 | 4 | 5 | 1 |
| pH value of stripping phase | $x_2$ | 8 | 9 | 10 | 1 |
| Carrier concentration, g/L | $x_3$ | 40 | 60 | 80 | 20 |

In order to settle the correlations between the vitamin initial mass flow, N, or permeability factor, P, and the above mentioned parameters, the following model of polynomial equation type has been proposed:

$$Y = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_{12} \cdot x_1 \cdot x_2 + b_{13} \cdot x_1 \cdot x_3 + b_{23} \cdot x_2 \cdot x_3 + \\ + b_{11} \cdot x_1^2 + b_{22} \cdot x_2^2 + b_{33} \cdot x_3^2 \quad (1)$$

where $b_0$,..., $b_{33}$ are the regression coefficients.
For both cases, the plan of the factorial experiment of second order is given in Table 2.

***Table 2.*** *The experimental matrix*

| No. exp. | $x_1$ | $x_2$ | $x_3$ |
|---|---|---|---|
| 1. | -1 | -1 | -1 |
| 2. | 1 | -1 | -1 |
| 3. | -1 | 1 | -1 |
| 4. | 1 | 1 | -1 |
| 5. | -1 | -1 | 1 |
| 6. | 1 | -1 | 1 |
| 7. | -1 | 1 | 1 |
| 8. | 1 | 1 | 1 |
| 9. | -1 | 0 | 0 |
| 10. | 0 | 0 | 0 |
| 11. | 0 | -1 | 0 |
| 12. | 0 | 1 | 0 |
| 13. | 0 | 0 | -1 |
| 14. | 0 | 0 | 1 |
| 15. | 0 | 0 | 0 |
| 16. | 0 | 0 | 0 |
| 17. | 0 | 0 | 0 |

By means of the obtained data, the regression coefficients have been calculated using the following relations [16]:

$$b_0 = \overline{Y}_{15-17}, \quad b_j = \frac{\sum_{i=1}^{15} x_{ji}.Y_i}{\sum_{i=1}^{15} {x_{ji}}^2}, \quad b_{jk} = \frac{\sum_{i=1}^{15} x_{ji} x_{ki} Y_i}{\sum_{i=1}^{15} {x_{ji}}^2 {x_{ki}}^2}, \quad b_{jj} = \frac{\sum_{i=1}^{15} x_{ji}^{/} Y_i}{\sum_{i=1}^{15} \left(x_{ji}^{/}\right)^2}$$

$$x_{ji}^{/} = x_{ji}^2 - \frac{1}{15} \cdot \sum_{i=1}^{15} x_{ji}^2 \quad (2)$$

i = 1...15 number of experiments   j = 1...3 number of variables.
The values of regression coefficients obtained for the initial mass flow and permeability factor correlations are listed in Table 3.
For checking the normal results from the program center (experiments no. 15 - 17), the Q test was used [16]. Thus, the calculated Q value is:

$Q = \frac{|a_1 - a_2|}{A} = 0.66$ for initial mass flow, respectively $Q = 0.60$ for permeability factor

where: $a_1$ - the uncertain value ($9.6.10^{-3}$ mole/m$^2$.h, respectively 0.79);
$a_2$ - the closest to the uncertain value ($9.4.10^{-3}$ mole/m$^2$.h, respectively 0.76);
A - the amplitude (difference between the most distant values: $0.3.10^{-3}$ mole/m$^2$.h, respectively 0.05).

For a certain threshold of 0.05, Q = 0.77 was found in literature [16]. Since both the calculated values, 0.66 for the initial mass flow and 0.60 for the permeability factor, are lower than the tabulated one, it could be concluded that the uncertain values of $9.6.10^{-3}$ mole/m$^2$.h, respectively 0.79 are also normal values. Consequently, all of the three obtained values for the folic acid initial mass flow and permeability factor (experiments no. 15 - 17) have to be taken in calculation.

***Table 3.*** *The values of regression coefficients for folic acid facilitated pertraction*

| Regression coefficient | Regression coefficients values | |
|---|---|---|
| | Final mass flow | Permeability factor |
| $b_0$ | 0.943 | 0.763 |
| $b_1$ | 0.821 | -0.101 |
| $b_2$ | 0.774 | 0.063 |
| $b_3$ | 1.124 | -0.118 |
| $b_{12}$ | 0.054 | 0.008 |
| $b_{13}$ | -0.031 | -0.004 |
| $b_{23}$ | 0.161 | 0.034 |
| $b_{11}$ | 0.581 | -0.022 |
| $b_{22}$ | 0.548 | 0.014 |
| $b_{33}$ | 0.796 | -0.026 |

Hence, the regression equations may be written as:

- for the final mass flow:

$$Nx10^2 = 0.943 + 0.821 \cdot x_1 + 0.774 \cdot x_2 + 1.124 \cdot x_3 + 0.054 \cdot x_1 \cdot x_2 - 0.031 \cdot x_1 \cdot x_3 + 0.161 \cdot x_2 \cdot x_3 + 0.581 \cdot x_1^2 + 0.548 \cdot x_2^2 + 0.796 \cdot x_3^2 \quad (3)$$

- for the permeability factor:

$$P = 0.763 - 0.101 \cdot x_1 + 0.063 \cdot x_2 - 0.118 \cdot x_3 + 0.008 \cdot x_1 \cdot x_2 - 0.004 \cdot x_1 \cdot x_3 + 0.034 \cdot x_2 \cdot x_3 - 0.022 \cdot x_1^2 + 0.014 \cdot x_2^2 - 0.026 \cdot x_3^2 \quad (4)$$

The experimental and calculated values of the folic acid initial mass flow and permeability factor were tabulated in Table 4.

The limits between which the values of parameter Y, initial mass flow or permeability factor, calculated with the regression equation oscillate around the experimental values are determined with the relation [16]:

$$Y_{calc_i} = Y_{\exp_i} \pm t \cdot S_{Yx}, \% \quad (5)$$

The standard deviation $S_{Yx}^2$ was calculated using the following relationship [16]:

$$S_{Yx}^2 = \frac{\sum_{i=1}^{8}\left(Y_{\exp_i} - Y_{calc_i}\right)^2}{n-(k+1)} \tag{6}$$

where n is the number of experiments and k the number of variables taken into account. According with the above equations, the standard deviations for the two modeled parameters are as follows:

- for the final mass flow

$$S_{Nx}^2 = 1.125 \cdot 10^{-5}$$

- for the permeability factor

$$S_{Px}^2 = 5.5 \cdot 10^{-4}$$

***Table 4.*** *The experimental and calculated values for folic acid initial mass flow and permeability factor*

| No. exp. | Initial mass flow, $Nx10^2$, mole/m$^2$.h | | Permeability factor, P | |
|---|---|---|---|---|
| | Experimental | Calculated | Experimental | Calculated |
| 1. | 0.32 | 0.33 | 0.92 | 0.92 |
| 2. | 1.29 | 1.26 | 0.71 | 0.71 |
| 3. | 1.01 | 1.05 | 0.99 | 0.97 |
| 4. | 3.25 | 3.26 | 0.88 | 0.87 |
| 5. | 1.68 | 1.64 | 0.66 | 0.64 |
| 6. | 3.78 | 3.79 | 0.49 | 0.51 |
| 7. | 4.07 | 4.08 | 0.86 | 0.83 |
| 8. | 5.76 | 5.77 | 0.61 | 0.61 |
| 9. | 0.71 | 0.70 | 0.79 | 0.81 |
| 10. | 2.33 | 2.34 | 0.62 | 0.61 |
| 11. | 0.70 | 0.72 | 0.66 | 0.68 |
| 12. | 2.29 | 2.26 | 0.76 | 0.78 |
| 13 | 0.62 | 0.61 | 0.83 | 0.82 |
| 14 | 2.54 | 2.51 | 0.63 | 0.64 |
| 15 | 0.93 | | 0.74 | |
| 16 | 0.94 | 0.943 | 0.76 | 0.763 |
| 17 | 0.96 | | 0.79 | |

The t values are to be found in the tables for Student distribution [16], for a confidence threshold of 0.05 and 15 experiments, namely t = 2.131. Therefore, the calculated folic acid initial mass flow and permeability factor oscillate around their experimental values into the following limits:

- for the initial mass flow:

$$N_{calc\,i} = N_{\exp_i} \pm 7.15 \cdot 10^{-3}\text{, mole/m}^2\text{.h} \tag{7}$$

- for the permeability factor:

$$P_{calc\,i} = P_{\exp_i} \pm 4.99 \cdot 10^{-2} \tag{8}$$

The individual influence of the factors under consideration on the parameter Y describing the folic acid pertraction is estimated by means of the value of the correlation coefficient, $r_{Yx}$ [16]:

$$r_{Yx_i} = \frac{\sum_{i=1}^{8}\left[x_i \cdot \left(Y_i - \overline{Y}\right)\right]}{\sqrt{\sum_{i=1}^{8} x_i^2 \cdot \sum_{i=1}^{8}\left(Y_i - \overline{Y}\right)^2}} \tag{9}$$

that indicating the nature of dependence between the process variables and the extraction yield. The determination coefficient, which is the square of correlation coefficient, represents the fraction of folic acid initial mass flow or permeability factor that can be explained by variable $x_i$ variation. For the two cases, the calculated values of determination coefficients are:

- for the initial mass flow:
  $r_{Nx_1}^2 = 0.250$ $r_{Nx_2}^2 = 0.262$ $r_{Nx_3}^2 = 0.449$
- for the permeability factor:
  $r_{Px_1}^2 = 0.289$ $r_{Px_2}^2 = 0.166$ $r_{Px_3}^2 = 0.513$

These values suggest that the considered parameters influence the initial mass flow of folic acid in 96.1% extent and the permeability factor in 96.8%, Amberlite LA-2 concentration in organic layer being the most important factor. The differences to the 100% can be attributed to the effect of other factors that were not taken into account, namely: the mixing intensity into the liquid membrane, the volumetric flow etc.

## CONCLUSIONS

By means of the statistical analysis and using factorial experiments of second order, the folic acid separation by facilitated pertraction with Amberlite LA-2 has been modeled. Thus, two mathematical correlations between the vitamin initial mass flow and permeability factor and the main parameters influencing the extraction (pH-value of feed phase, pH-value of stripping phase, carrier concentration in liquid membrane) have been established. For the studied extraction system, the considered variables control the pertraction process in a 96.1 - 96.8% extent, Amberlite LA-2 concentration in organic layer being the most important factor exhibiting the most important influence.

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