



A Novel Kinetic Assay for the Examination of Solid-Liquid Extraction of Flavonoids from Plant Material

Makris D.P.

School of Environment, University of the Aegean, Mitr. Ioakim Street, Myrina, 81400, Lemnos, GREECE

Available online at: www.isca.in, www.isca.me

Received 1st October 2015, revised 20th October 2015, accepted 15th November 2015

Abstract

The medicinal plant *Artemisia inculta*, endemic to the island of Crete (southern Greece), was chosen as a matrix for the examination of solid-liquid extraction of total flavonoids, using a novel kinetic model based on a rectangular 2-parameter hyperbola function, which was established employing non-linear regression. The determination of basic kinetic parameters and the critical comparison with the well-studied and widely used second-order model, proved unequivocally that the model proposed can be implemented as an alternative means of describing solid-liquid extraction kinetics, with high reliability. The advantage over other models used in similar processes is the simplicity of the determination of the basic kinetic parameters, by utilising a "double reciprocal" linear mathematical transformation

Keywords: Flavonoids, kinetics, solid-liquid extraction.

Introduction

The solid-liquid extraction processes usually follow a kinetic curve which illustrates that the rate of mass transfer is not constant. The extraction curves usually consist of three distinct phases: constant extraction rate period, falling extraction rate period, and diffusion controlled period¹. During the first period, the easily accessible solute that surrounds the particle passes into the liquid phase with an approximately constant rate. Throughout the second period, the extraction rate decreases rapidly as a result of the decrease of the effective mass transfer area and diffusion becomes the governing phenomenon. Finally, in the last period the easily accessible solute layer is depleted and thus the extraction rate is determined exclusively by the diffusion rate of the solvent into the solid particle and of the solvent and solute from the solid particle to the bulk solvent. This mechanism characterizes a slow stage of the extraction process.

The solid-liquid extraction of polyphenolic substances has been dealt with by several examinations, which proposed various empirical mathematical models, with the aim of providing the appropriate practical tools for a reliable description of the extraction process². Conventional extraction methodologies are usually based on Fick's law derivatives models, as the parameters estimated contain physical meanings which can be used for further interpretations. However, various empirical models have also been developed from the fundamental or adapted models, as they are considered to be more suitable for non-conventional extraction processes (microwave, ultrasound-assisted etc.). Similar models have been used in several studies pertaining to adsorption³⁻⁷, which is considered the inverse phenomenon of diffusion.

To this purpose, this study was performed to examine the kinetics of batch stirred-tank flavonoid extraction from a flavonoid-rich medicinal herb, *Artemisia inculta*, using second-order and a single rectangular 2-parameter hyperbola model. The test employed to assess the total flavonoid yield is more specific compared with the widely used Folin-Ciocalteu for total polyphenol determination, thus providing more accurate data regarding the extraction kinetics. The efficiency of each model was checked by comparing parameters including the extraction rate constant, the yield at saturation, the time required for the extraction to enter the regular regime and the initial extraction rate. Kinetics was performed at increasing temperatures, to ascertain that thermal degradation of flavonoids is not implicated, a fact that could complicate the apparent kinetic values and produce misleading results.

Material and Methods

Chemicals and reagents: Rutin (quercetin 3-*O*-rutinoside) was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Aluminium chloride (AlCl₃) was from Merck (Darmstadt, Germany). Glycerol was from Fisher Scientific (New Jersey, U.S.A.).

Plant material: The material used consisted of the aerial parts of *Artemisia inculta* and it was provided by the Mediterranean Plant Conservation Center (Chania, Crete), where a voucher specimen was deposited (9493 MAIC). Following collection, the tissue was air-dried at room temperature in a dry and dark chamber for 7 days, and then ground to a fine powder (approximate mean particle diameter 1 mm) using a domestic blender (Bosch MMB 112R, Stuttgart, Germany). The ground material was placed in plastic screw-cup tubes at 4°C, until used.

Batch extraction procedure: The optimised method previously described was used⁸. Amount of 1 g of the pulverised material was added in 100 mL of 90% (w/v) aqueous glycerol in a plastic container and extracted under stirring at 600 rpm, with a teflon-coated magnetic stirrer, for 160 min. Heating was accomplished by an oil-bath placed on a hot plate (Yellow Line MST Basic C, Richmond, VA, U.S.A.), set at 50, 70 and 80±1.5°C. Temperature was controlled by a Yellow Line TC1 thermostat. Sampling was carried out at predetermined intervals (5 - 160 min) and all samples were centrifuged in a table centrifugator (Hermle, Wehingen, Germany) at 10,000 rpm for 10 min and filtered through 0.45-µm syringe filters prior to determinations.

Determination of total flavonoid yield (Y_{TFn}): A previously published protocol was used⁹, with modifications. A volume of 0.25 mL sample was combined with 0.75 mL AlCl₃ reagent [0.16% (w/v) AlCl₃ in 5% (v/v) acetic acid in methanol] and allowed to react for 30 min, at ambient temperature. The absorbance was recorded at 415 nm (A₄₁₅) and the total flavonoid concentration (C_{TFn}) was estimated from a calibration curve, constructed with rutin (quercetin 3-O-rutinoside) as standard. Yield in total flavonoids (Y_{TFn}), expressed as mg rutin equivalents (RtE) per g of dry weight, was determined using the following equation:

$$Y_{TFn} (mg RtE g^{-1} dw) = \frac{C_{TFn} \times V}{m} \quad (1)$$

Where *V* is the volume of the extraction medium (L) and *m* the dry weight of the plant material (g).

Statistical analyses and kinetics: All treatments were performed in duplicate. Determinations were carried out in triplicate and values were averaged. Kinetics was established by non-linear regression between Y_{TFn} and *t*. Non-linear and linear regressions were performed with Sigma Plot™ 12.0, at least at a 95% significance level.

Results and Discussion

Second-order model: The model fitted to the extraction kinetics using non-linear regression between extraction yield in TFn (Y_{TFn}) values and *t* (figure-1, upper plot), was a modified hyperbola described by the equation:

$$y = \frac{ax}{1+bx} \quad (2)$$

For all temperatures tested, fitting was statistically significant (table-1), suggesting that extraction yield as a function of *t* can be adequately predicted by the equation-2. This equation actually describes a second-order extraction kinetics, as previously reported^{10,11}, when the boundary conditions *t* = 0 to *t* and Y_{TFn(0)} = 0 to Y_{TFn(t)} are considered:

$$Y_{TFn(t)} = \frac{Y_{TFn(s)}^2 kt}{1 + Y_{TFn(s)} kt} \quad (3)$$

Table-1
Statistical parameters calculated after non-linear regression between Y_{TFn} and *t* values

T (°C)	R ²	<i>p</i>
Second-order		
50	0.9857	< 0.0001
70	0.9964	< 0.0001
80	0.9938	< 0.0001
Double reciprocal		
50	0.9857	< 0.0001
70	0.9964	< 0.0001
80	0.9938	< 0.0001

Y_{TFn(s)} and *k* are the TFn yield at saturation and the extraction rate constant, respectively. Transformation of equation-3 yields the following linear expression:

$$\frac{t}{Y_{TFn(t)}} = \frac{1}{kY_{TFn(s)}^2} + \frac{t}{Y_{TFn(s)}} \quad (4)$$

When *t* approaches 0, the initial extraction rate, *h*, given as Y_{TFn(t)/t}, may be expressed as:

$$h = kY_{TFn(s)}^2 \quad (5)$$

If *t*/Y_{TFn(t)} is plotted against *t*, there would be a straight line with a slope = 1/Y_{TFn(s)} and intercept = 1/*h* (figure-2, upper plot). Therefore, for every extraction performed, Y_{TFn(s)}, *k* and *h* could be determined graphically. In all cases examined, the correlations between *t*/Y_{TFn(t)} and *t* were very high and statistically significant (table-2).

The course of extraction, from fast to slow, occurs when approximately 80% of the extraction efficiency has been reached¹². This period is called “regular regime” and characterises the second, slow phase of the extraction, where small increases in the extraction rate are recorded within a significant period of time. The time required for the extraction to achieve the regular regime, *t_R*, can be determined as follows¹³:

$$t_R = \frac{1}{kY_{TFn(s)}} \quad (6)$$

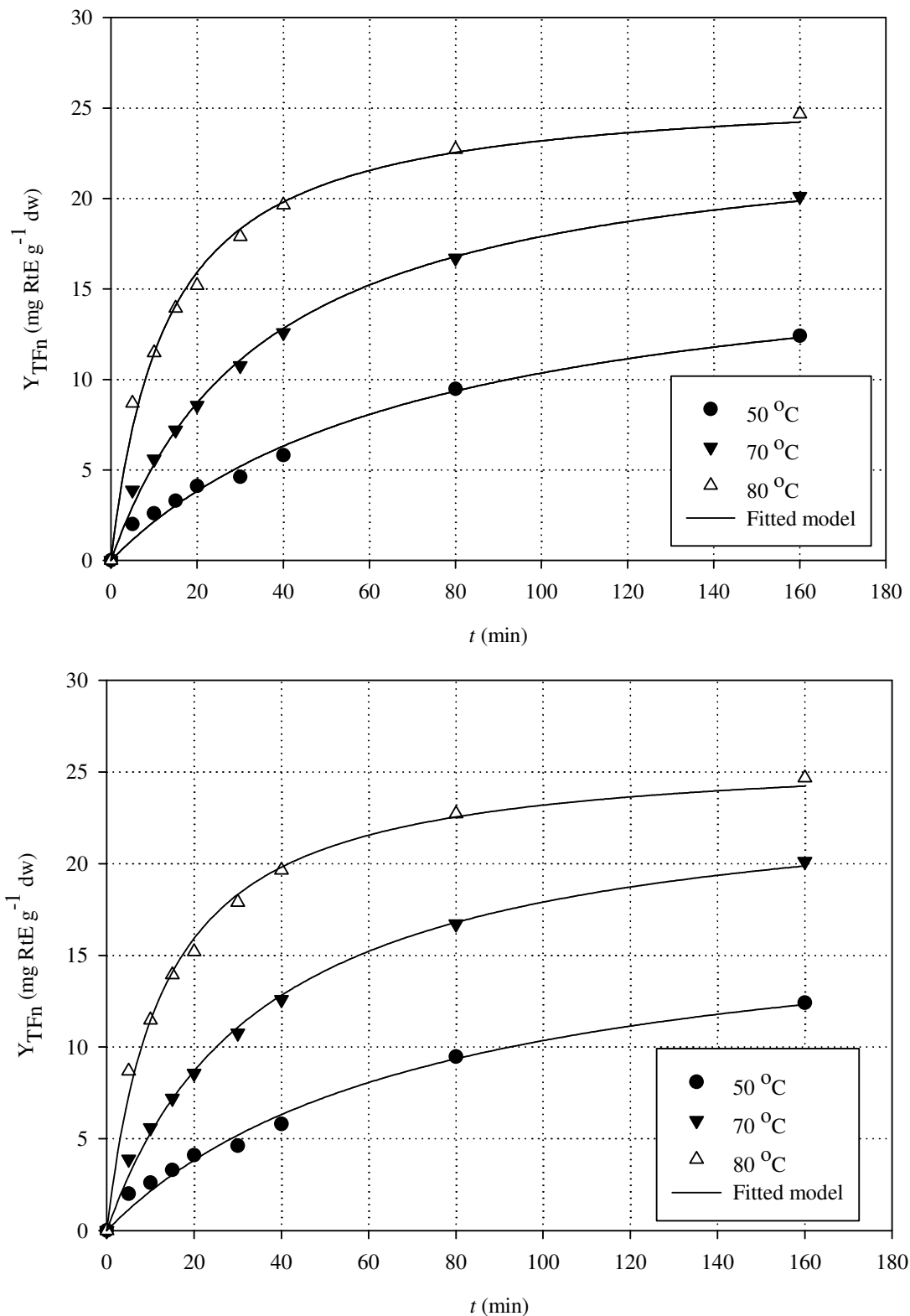


Figure-1

Non-linear regression between Y_{TFn} and t , during extraction of TFn from *A. inculta*, using 90% (w/v) glycerol. Extractions were carried out under continuous stirring at 600 rpm, with $R_{L/S} = 100 \text{ mL g}^{-1}$. Upper and lower plot display model fitting according to the equation-2 and 7, respectively

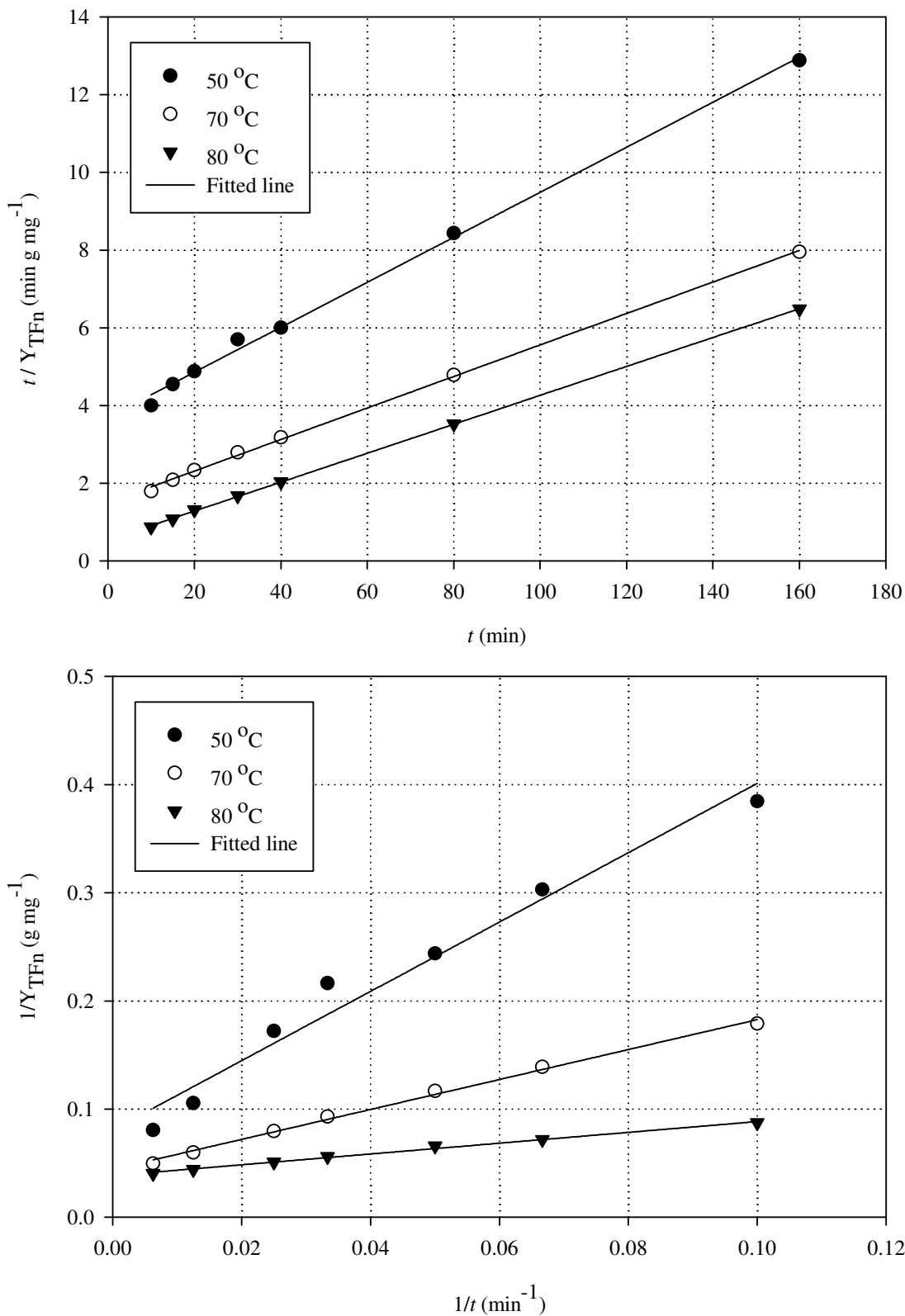


Figure-2

Second-order (upper plot) and double reciprocal (lower plot) kinetic model of TFn extraction from *A. inculta* using 90% (w/v) glycerol. Extractions were carried out under continuous stirring at 600 rpm, with $R_{LS} = 100 \text{ mL g}^{-1}$

Table-2

Statistical parameters calculated after linear regression between t/Y_{TFn} and t values (second-order model) or $1/Y_{TFn}$ and $1/t$ values (double reciprocal)

T (°C)	R ²	p
Second-order		
50	0.9971	< 0.0001
70	0.9991	< 0.0001
80	0.9998	< 0.0001
Double reciprocal		
50	0.9716	< 0.0001
70	0.9960	< 0.0001
80	0.9944	< 0.0001

Double reciprocal: In this case fitting of the data obtained was performed using single rectangular 2-parameter hyperbola, described by the following equation:

$$y = \frac{ax}{b+x} \quad (7)$$

As for the second-order model, fitting was highly significant, giving identical statistical parameters (table-1). Based on the previous considerations for the second-order model, equation-7 would correspond to a kinetic model, as given below:

$$Y_{TFn(t)} = \frac{Y_{TFn(s)}t}{t_{0.5} + t} \quad (8)$$

The term b of the equation-7 can be assigned to a parameter termed as $t_{0.5}$, as it should be called from now and on, because it has dimensions of time and when $t = t_{0.5}$, then $Y_{TFn(t)} = \frac{Y_{TFn(s)}}{2}$. Therefore, $t_{0.5}$ represents the time where 50% of the $Y_{TFn(s)}$ has been attained.

The linearized expression of equation-8 would be:

$$\frac{1}{Y_{TFn(t)}} = \frac{t_{0.5}}{Y_{TFn(s)}} \frac{1}{t} + \frac{1}{Y_{TFn(s)}} \quad (9)$$

Equation-9 dictates that a plot of $1/Y_{TFn}$ as a function of $1/t$ would yield a straight line (figure-2, lower plot), with the intercept on the y-axis being equal with $1/Y_{TFn(s)}$ and the intercept on the x-axis with $-1/t_{0.5}$. Thus both $t_{0.5}$ and $Y_{TFn(s)}$ can be estimated graphically. The initial rate of the extraction, h , and the second-order extraction rate, k , could then be determined as follows:

$$h = \frac{Y_{TFn(s)}}{t_{0.5}} \quad (10)$$

$$k = \frac{1}{Y_{TFn(s)}t_{0.5}} \quad (11)$$

Table-3 Displays the kinetic parameters, as determined with the two models. It can be seen that in all cases, the two models provided almost identical values. This outcome was unequivocal proof that the alternative approach proposed can describe the extraction kinetics adequately and with high reliability, as judged by comparison with the second-order model.

Table-3
Kinetic parameters of the extraction process, calculated after implementation of the two kinetic models

T (°C)	Kinetic parameters			
	$k (\times 10^{-3}) (\text{g mg}^{-1} \text{min}^{-1})$	$H (\text{mg g}^{-1} \text{min}^{-1})$	$t_R (\text{min})$	$Y_{TFn(s)} (\text{mg RtE g}^{-1})$
Second-order				
50	0.750	0.243	74.07	18.00
70	1.140	0.678	35.97	24.39
80	2.967	2.035	12.87	26.19
Double reciprocal				
50	0.749	0.243	74.08	18.02
70	1.144	0.678	35.92	24.34
80	2.970	2.036	12.86	26.18

Conclusion

The kinetics of the solid-liquid extraction of total flavonoids from *A. inculta* was determined using second-order model, but also a novel approach based on a rectangular 2-parameter hyperbola function. The kinetic parameters determined with the two models were identical, a fact that proved the validity of the alternative model proposed. The simple mathematical transformation into a linear expression allows for the kinetics to be estimated by experimental data obtained within relatively short assay periods. It is to be said, however, that the model proposed is empirical and its general applicability is to be demonstrated by further studies. Thus implementation of this model requires case experimentation, the appropriate fitting of data provided.

Nomenclature

- C_{TFn} : total flavonoid concentration (mg RtE L⁻¹)
 h : initial extraction rate (mg g⁻¹ min⁻¹)
 k : second-order extraction rate constant (g mg⁻¹ min⁻¹)
 $R_{L/S}$: liquid-to-solid ratio (mL g⁻¹)
 t : time (min)
 T : temperature (°C or K)
 Y_{TFn} : yield in total flavonoids (mg RtE g⁻¹)
 $Y_{TFn(s)}$: yield in total flavonoids at saturation (mg RtE g⁻¹)

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