Isolation of Mangiferin from Different Varieties of Mangifera Indica Dried Leaves

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Abstract

The kinetics and structural changes in the solid were studied for solid-liquid extraction from plants. Soxhlet extraction technique has been developed to extract Mangiferin from Mangifera Indica Leaves. This research aims to develop for the first time the process of mangiferin extraction from the leaves of two mango varieties, i.e. Kesar and Hapoos by using soxhlet extraction with Methanol as an extracting solvent. The study involved sampling, storage, preparation, extraction and analysis for Mangiferin was done by High Pressure Liquid Chromatography. The results found that Hapoos gave the highest yield of mangiferin as compared to Kesar.

Extraction of Mangiferin from Mangifera Indica Leaves was carried out in a series of solvents methanol, ethanol and acetone. Maximum yield of extraction of Mangiferin was estimated in Methanol on the basis of solubility and therefore was used to investigate influence of operating parameter like particle size and temperature on the recovery in a stirred batch extractor. Particle size of 0.1-0.3 mm, 70^0C temperature and methanol as a solvent were found to be the optimum parameters for the extraction of Mangiferin from the batch experimental work.

Effective intraparticle diffusivity of Mangiferin in methanol was estimated using Wilky-Chang equation and activation energy of diffusion is also estimated by using Arrhenius equation.

Keywords: Mangifera Indica Leaves, Mangiferin, Batch extraction, Soxhlet Extraction, Diffusivity (D)

1 INTRODUCTION

Mangifera indica (L.) belonging to family Anacardiaceae is one of the most important tropical plants marketed in the world. The genus Mangifera contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mangos belong to the species Mangifera indica [1]. The other edible Mangifera species generally have lower quality fruit and are commonly referred to as wild mangos. The origin of Mangifera indica is in northeast India, northestern Myanmar and Bangladesh than later spread to the rest of Asia by themselves and with the help of humans. Mangifera indica nowadays can be found in India, Sri Lanka, Bangladesh, Myanmar, Thailand, Kampuchea, Vietnam, Laos, southern China, Malaysia, Singapore, Indonesia, Brunei, the Philippines, Papua New Guinea, and the Solomon and Caroline Islands. Maximum species diversity exists in western Malaysia and about 28 species are found in this region. In India, mainly Kesar, Hapoos, Kalami, Rajapuri etc. species are available [2]. The characteristic of Mangifera indica (mango) is that the trees are deeprooted, symmetrical and height is about 25 m. The trees have simple alternate leaves that are 0.3 m to 0.5 m in length and yellow green, purple, in color when young. The mature leaves change to leathery, glossy, and deep green in color. The flower is of 0.08 m with a greenish yellow, white, pale cream, or even pinkish colour. The flower has fragrance, with 15.75 m long panicle, 0.08 m long flower buds and 36 petals. The mature terminal branches bear pyramidal flower panicles that have several hundred white flowers that are about a 1/4 inch wide when open. The fruit weighs about 0.1134 kg to 1 kg. Fruit may be round, ovate depending on the variety. Mature fruit has characteristic fragrance and a smooth, thin, tough skin. The flesh of ripe mangos is pale yellow to orange. The fruit has one seed that is flattened and sticks to the flesh [2]. Mangifera Indica is reported to possess numerous therapeutic uses viz. analgesic, anti-inflammatory, antioxidant and antidiabetic [3-5]. Phytochemical research of different parts of M. indica has demonstrated the presence of phenolic constituents, flavonoids and phytosterols [6-8]. There are several reports demonstrating the antioxidant activity of various parts of M. indica viz. leaves, bark and peels [9]. Out of these parts, mangiferin is mainly present in leaves, bark, and roots. Mangiferin is a stable C-glycoside of the xanthone group with antidiabetic, anti-inflammatory, antioxidant, antitumor, immunomodulatory & antiviral properties. It is a widely distributed flavanol glycoside containing pyranose sugar and the aglycon part is 1, 3, 6, 7-tetrahydroxy xanthone. Mangiferin is reported to be stable to acid and enzymatic hydrolysis [10].

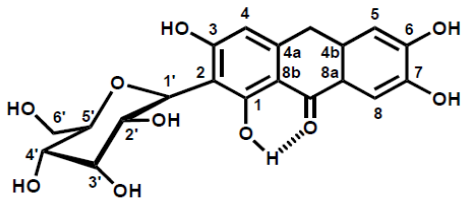

Figure 1 Chemical structure of Mangiferin [12]

The objectives of the present study were to isolate and characterize mangiferin from different varieties viz. Kesar and Hapoos of Mangifera Indica dried leaves by using Soxhlet Extraction technique and to find optimum parameters such as temperature, particle size for the Extraction of Mangiferin from Mangifera Indica Leaves.

2 Materials and Methods

Leaves of Mangifera indica were purchased from the local market of Nashik, State-Maharashtra, Country-India. Leaves were washed with tap water and shade dried (at a room temperature for 4-5 days) and grinded to get powder. The powder of the leaves was stored at the room temperature. All organic solvents n-Hexane, Methanol, Ethanol and acetone were AR grade and solvents used for chromatographic separations were of HPLC grade purchased from S.D. Fine Chemicals, India.

2.1 Soxhlet Extraction

The objective of the soxhlet extraction is to compare the yield of Mangiferin obtained from different varieties (Kesar and Hapoos) of Mangifera Indica by performing Soxhlet extraction by using solvent Methanol, 625 gm of screened solid powder material (approx. 200 gm per batch) was taken in soxhlet apparatus with 1500 ml solvent such as methanol per batch, and extraction was carried out for 48 hours. After completion of extraction, methanolic extract (free from leaves powder) collected at the end. The extract was distilled off and dried extract was obtained and analyzed by reverse phase HPLC to determine concentrations of the extracted Mangiferin. The process of soxhlet extraction for Methanolic extraction is described as follows:

Mangifera Indica dried leaves is subjected to exhaustive Soxhlet extraction with methanol as a solvent to isolate Mangiferin. The point of completion of extraction is determined by reaction with iodine vapors. A small spot is applied on a TLC plate by using capillary tube and placed it in iodine chamber, if colourless spot is observed that indicates completion of extraction.

Fractionation of Methanolic Extract with Ethyl Acetate:

Methanolic extract consist of many other constituents other than mangiferin, so for purification of methanolic extract, it is treated with ethyl acetate. The methanolic extract is concentrated and concentrated extract is repeatedly treated with ethyl acetate to separate ethyl acetate soluble and ethyl acetate insoluble extract. Mangiferin is present in ethyl acetate soluble extract because it is soluble in ethyl acetate. Then ethyl acetate soluble extract was distilled off to separate ethyl acetate and dried extract is analyzed by reverse phase HPLC to determine concentrations of the extracted Mangiferin.

2.2 Batch Extraction

The objective of the batch experiments is to measure global kinetics of extraction and to analyze the influences of the operating parameters such as effect of particle size, temperature and the influence of the solvent polarity on the percentage extraction of mangiferin. As methanol is highly toxic in nature but mangiferin is highly soluble in methanol than ethanol and acetone, therefore we are using methanol for the extraction of mangiferin. For the experimentation 10 gm of leaves powder of Hapoos was taken in a four baffled 300 ml stirred borosilicate glass vessel 8 cm ID, and 6.5 cm height along with 210 ml solvent. A four pitched bladed turbine type agitator (2 cm diameter) was used to stir the mass at constant rpm and the revolution was monitored through speedometer. Figure 2 shows the schematic diagram of batch extraction.

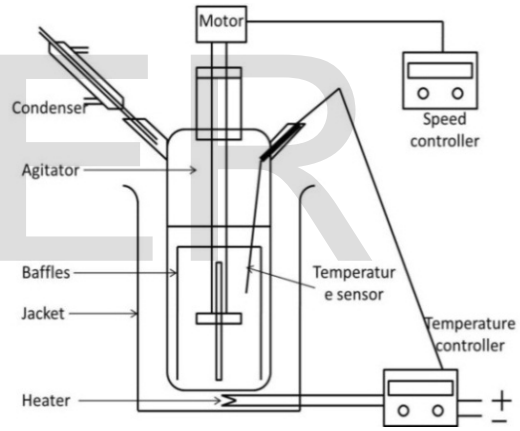

Figure 2 Schematic view of Batch Extraction

2.3 High-performance liquid chromatography (HPLC) analysis

All unknown samples of Mangiferin were analysed by HPLC system make of Analytical Technologies Limited with model no. HPLC 3000 and software is HPLC workstation. The system consist of P3000-M series with controller, a Rheodyne 7725i injector with 5µL sample loop, dual λ absorbance detector (UV detector) at λ=254 nm for Mangiferin with 0.01 aufs sensitivity. Mangiferin is analyzed in a symmetry C-18, (4.6×250 mm, 5µm) column equipped with automatic temperature controller. Column temperature was maintained at 25°C.The isocratic mobile phase is methanol: 2% glacial acetic acid=40:60 v/v with flow rate of 1.0 ml/min was selected for identification of Mangiferin.

3 Results and Discussion

3.1 Soxhlet Extraction

As mango leaves are abundantly available, non-edible as well as they are more economical than other herbs, therefore mango leaves were selected for extraction purpose. This is the first time attempt to compare yield of mangiferin for two different varieties of Mangifera Indica viz. Kesar and Hapoos. Table 1 shows that % yield and % extraction of mangiferin is much higher in Hapoos as compared to Kesar. As we have highest yield in Hapoos, it is more beneficial specie for the extraction of Mangiferin. Thus, leaves show better diffusivity towards solvent methanol at temperature 60^{0}C.

TABLE 1
COMPARATIVE YIELD OF MANGIFERIN IN KESAR AND HAPOOS

Varieties of Mangifera Indica (Mango)	Mangiferin	
	% Extraction	% Yield
Keasar	34.95	1.75
Hapoos	48.66	2.43

3.2 Batch reactor

3.2.1 Selection of Solvent

The solvent selection for the extraction of Mangiferin depending on the physical properties of Mangiferin e.g. solubility. Solvents of varying polarity (Methanol, Ethanol and Acetone) were used for the mass transfer kinetics and strength of solute matrix interaction were chosen to extract mangiferin from Mangifera Indica leaves at 60^{o}C temperature. In batch extraction dry leaves of 10 gm taken along with each solvent and stirred at 60 ^{0}C for 3 hrs. Figure 4 shows the concentration of extracted Mangiferin from batch extraction.

Mangiferin showed maximum extraction in methanol and it decreased gradually in ethanol to acetone. Around 59% Mangiferin extracted in three hours using methanol as solvent at 60^{o}C. This is due to varying solubility values of Mangiferin in the chosen solvents. The size of solvent molecule also plays a crucial role in the extraction. The smaller size of methanol helps it to penetrate better into the cellulose matrix of leaves.

TABLE 2
DATA FOR SELECTION OF SOLVENT FOR EXTRACTION OF MANGIFERIN FROM MANGIFERA INDICA LEAVES

Solvent	% Extraction of Mangiferin
Methanol	59
Ethanol	30.68
Acetone	10.93

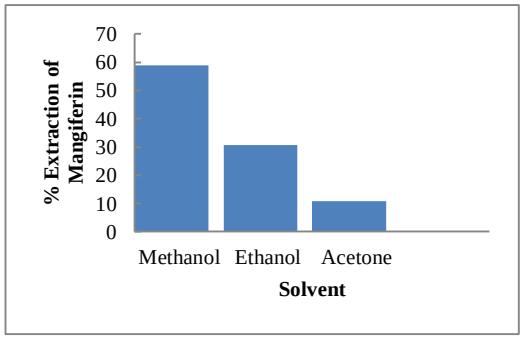

Figure 4 Selection of Solvent for extraction of mangiferin at temperature 60^{0}C

3.2.2 Effect of Particle Size

The batch extraction with methanol as solvent at 700 rpm speed were performed at 60 ^{0}C temperature by using different particle size (0.1-0.3, 0.3-0.4, 0.4-0.5, and 0.5-0.6 mm). As particle size is reduced, the rates of extraction for Mangiferin increased as shown in Figure 5. For the smaller particles, pore diffusional resistance path decreases, and surface area increases. Both of these help in increased recovery of Mangiferin. It is a classical result since the pore diffusion path increases with the particle size [11].

TABLE 3
DATA FOR EFFECT OF PARTICLE SIZE ON EXTRACTION OF MANGIFERIN WITH METHANOL AS SOLVENT FROM MANGIFERA INDICA LEAVES

Particle Size in mm	% Extraction of Mangiferin
0.1-0.3	72.8
0.3-0.4	46
0.4-0.5	38.7
0.5-0.6	20.4

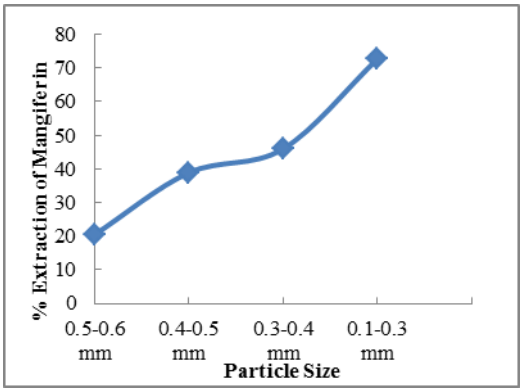

Figure 5: Effect of Particle Size on Extraction of Mangiferin with Methanol as a Solvent 700 rpm speed at temperature 60^0 C.

3.2.3 Effect of Temperature

Generally, higher extraction temperature is better for extraction because of the increased solubility. The effect of temperature on Mangiferin extraction was studied at 40, 50, 60 and 70°C by maintaining the speed of agitation at 700 rpm and 0.1-0.3 mm particle size. Heat treatment is routinely performed to accelerate the mechanism of diffusional process when extracting from the plant. However, it must be verified that the products are not degraded at higher temperatures [11].

Figure 6 shows that with increase in temperature, the percentage recovery of Mangiferin is increased. This is due to the expected thermodynamic effect of the temperature on the solubilization of Mangiferin inside the solid matrix. With increase in temperature, viscosity of solvent decreases and solubility of Mangiferin increases. The kinetic energy as well as diffusivity of solvent increased with temperature, and thus solvent penetrated better inside the cellular matrix leading thereby faster release of the mangiferin molecules took place. At 70°C, we get 49.2% extraction of Mangiferin, this is probably due to the higher temperature that causes intermolecular interaction within the solvent to increases, giving rise to higher molecular motion and causing higher local temperature in the solid matrix [11]. Diffusion coefficient can be written as a function of the temperature and the viscosity i.e. D= f (K (T/η)), the coefficient of diffusion D will increase with the temperature [11].The data for the effect of temperature and % extraction of Mangiferin is shown in Table 4.

TABLE 4
DATA FOR EFFECT OF TEMPERATURE ON EXTRACTION OF MANGIFERIN FROM MANGIFERA INDICA LEAVES

Temperature	% Extraction of Mangiferin
40°C	21.28
50°C	25.44
60°C	34.22
70°C	49.2

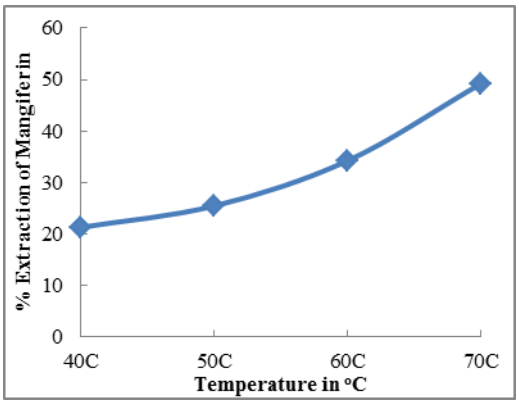

Figure 6: Effect of Temperature on Extraction of Mangiferin with Methanol as Solvent, 0.1-0.3 mm particle size 700 rpm speed, at different temperature

4 Determination of Activation Energy

Activation energy can be calculated from the diffusion coefficients at different temperatures by using Arrhenius law equation, [11]

Arrhenius law equation as shown below,

$$D = D_0 \times e^{-E/RT}$$

where, D= Diffusivity Coefficient (m²/sec),

D_0=Constant,

E=Activation Energy (kJ/mol),

R=Gas constant

By plotting ln D Vs 1/T the slope gives activation energy and Intercept gives constant value of Do.

$$\ln D = -E/RT + \ln D_0$$

The diffusivity at different temperatures is calculated by using Wilke-Chang equation at 700 rpm speed, 5% loading, and 0.106-0.42 mm size as mentioned in table 5.

The Diffusivity of continuous phase is calculated by using Wilky-Chang equation as shown below,

$$D_{AB} = [(117.3 \times 10^{-18}) \times (M_B \times \Phi)^{0.5} \times T] / [\mu \times V_A^{0.6}]$$

Where, M_B= Molecular weight of Solvent

Φ = Association factor for Solvent

(For Methanol φ = 1.9)

T= Temperature of extraction in K

μ= Viscosity of continuous phase in Kg/m.sec

V_A= Molar volume of solute

TABLE 5
DATA FOR DIFFUSIVITY D_{AB} AT DIFFERENT TEMPERATURES

Temperature in °K	Diffusivity D_{AB} or D (m²/sec)
313	1.3299E-9
323	1.7921E-9
333	2.3934E-9
343	3.1685E-9

TABLE 6
DATA FOR LN D AND 1/T

Diffusivity D (m²/sec.)	ln D	T (°K)	1/T
1.3299E-9	-20.44	313	3.1949E-3
1.7921E-9	-20.14	323	3.0959E-3
2.3934E-9	-19.85	333	3.0030E-3
3.1685E-9	-19.57	343	2.9155E-3

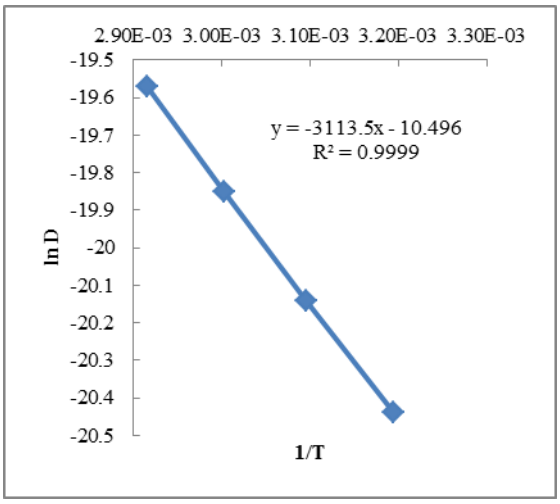

Figure7: Activation Energy Calculation for Mangiferin extraction in MeOH at 700 rpm with 0.1-0.3 mm particle size at different temperature.

Figure 7 shows the estimation of Activation energy for Mangiferin extraction from Mangifera Indica Leaves by using methanol as solvent. The lower value of activation energy is depending on initial percentage of solute present in the matrix and nature of cellulose matrix. If the cellulose matrix is soft, then release of solute with solvent will be fast. The activation energy for Mangiferin in methanol is 25.887 KJ/mole. This value of activation energy shows how much energy is required to diffuse the molecule from cellulose matrix. This lower value of activation energies represents better extraction of Mangiferin from cellulose matrix of Mangifera Indica results in higher diffusion coefficients, also showed the higher solubility in the selected solvent & penetrates easily from cellulose matrix.

5 Calculation of Mass Transfer Coefficient for Solid-Liquid Batch Extraction

The mass transfer coefficient for the Diffusion (Extraction) of Mangiferin from Mangifera Indica Leaves is calculated by using formula,

$K_C = \{1.46 \times D_{AB} \times [((D_i \hat{}2\ N\ \varrho)/\mu) \hat{}0.65 \times Sc \hat{}(\ 0.33)]\}/dv$

where, K_C = Mass Transfer coefficient in m/sec

D_{AB}= Diffusivity of continuous phase in m^2/sec

dv= Diameter of vessel in m

D_i= Diameter of impeller in m

N= Revolution in per unit time

ϱ = Density of Continuous phase in Kg/m^3

μ = Viscosity of continuous phase in Kg/m.sec

Sc= Schmidt number (Dimensionless)

The Schmidt number (Sc) equation is given by,

$Sc = \mu/(\varrho \times D_{AB})$

The Diffusivity of continuous phase is calculated by using Wilky-Chang equation as shown in below,

$$D_{AB} = [(117.3 \times 10^{-18}) \times (M_B \times \Phi)^{0.5} \times T] / [\mu \times V_A^{0.6}]$$

where, M_B= Molecular weight of Solvent

Φ = Association factor for Solvent (For Methanol $\varphi = 1.9$)

μ= Viscosity of continuous phase in Kg/m.sec

V_A= Molar volume of solute

The Mass transfer coefficient for the diffusion of Mangiferin from Mangifera Indica Leaves is varied from 1.0227E-4 to 2.4367E-4 m/sec at temperature 40 ^{0}C to 70 ^{0}C as shown in Table 7.

TABLE 7
DATA FOR DIFFUSIVITY D_{AB} AND MASS TRANSFER COEFFICIENT KC AT DIFFERENT TEMPERATURE

Temperature (^{0}C)	Diffusivity D_{AB} (m^2/sec)	Mass Transfer Coefficient Kc (m/sec)
40	1.3299E-9	1.0227E-4
50	1.7921E-9	1.3782E-4
60	2.3934E-9	1.8403E-4
70	3.1685E-9	2.4367E-4

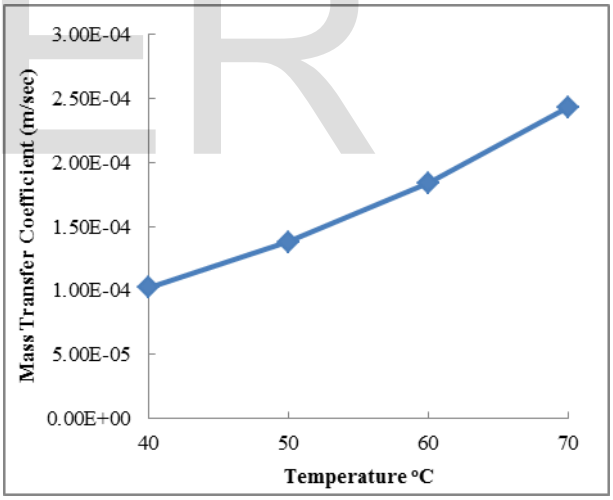

Figure 8: Variation of Diffusivity D_{AB} and Mass Transfer coefficient Kc for different temperatures

6 Calculation For the time required for the internal diffusion

The time required for the internal diffusion of Mangiferin from Mangifera Indica Leaves is calculated by [13,14],

$t = L \hat{}2/D$

where, t = Diffusion time (hr)

L= Particle length in m

D= Diffusivity or Diffusivity coefficient m/sec.

TABLE 8
DATA FOR DIFFUSIVITY D AND DIFFUSION TIME

Temperature (^{0}C)	Diffusivity (D)m^2/sec	Diffusion time (hr)
40	1.3299E-9	30.08
50	1.7921E-9	26.46
60	2.3934E-9	16.72
70	3.1685E-9	12.62

From above results, it is clear that diffusivity varies from 1.3299× 10^{-9} to 3.1685×10^{-9} m^2/s in methanol with respect to increase in the temperature from 40^{o}C to 70^{o}C. It shows that as temperature increases diffusivity increases and there is decrease in diffusion time with there is no degradation in compounds. The activation energy for diffusion of Mangiferin in methanol is 25.887 KJ/mole as calculated by using Arrhenius equation and the mass transfer coefficient for the diffusion of mangiferin from Mangifera Indica Leaves is varied from 1.0227E-4 to 2.4367E-4 m/sec at temperature 40 ^{0}C to 70 ^{0}C.

R. Wongkittipong also found activation energy by using Arrhenius law equation for extraction of andrographolide compound [11].

7 Conclusions

Herb as Mangifera Indica having great importance in daily life of human as well as animals. The Mangiferin showed effect for analgesic, anti-ulcer, anti-inflammatory and anti-tumor activity. In this study, extraction of mangiferin from Mangifera Indica Leaves as a cheaper source was carried out in a soxhlet and batch extractor. Soxhlet experiments were performed in order to obtain the comparative study of % extraction of mangiferin. It is observed that % extraction of mangiferin is highest in Hapoos than in Kesar. So, Hapoos variety is more beneficial for extraction of different phytoconstituents.

The batch extraction experiments were performed in order to study the influences of the operating parameters such as particle size and temperature. In this investigation, it has been found that the sizes of the particles have very little effect on extraction. Methanol has higher solvation power, and it shows greatest release on extraction of mangiferin than ethanol and acetone. Particle size of 0.1-0.3 mm and 70^{0}C temperature and methanol as a solvent, this combination were found to be optimal composition to obtain higher percentage extraction of mangiferin from the experimental work. The Diffusivity is higher when temperature (T) increases, hence the extraction is high at higher temperature (approximately at boiling point of methanol). The diffusivity varies from 1.3299× 10^{-9} to 3.1685×10^{-9} m^2/s in methanol solution depending on the temperature. The activation energy for diffusion of Mangiferin in methanol is 25.887 KJ/mole is calculated by using Arrhenius equation.

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