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Research Article

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Thermodynamic parameters for phenanthrene interaction with a biological π-acceptor (folic acid) by spectroscopic measurements

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ABSTRACT

The complexization between folic acid and typical polyaromatic hydrocarbon (phenanthrene) was studied by using FTIR and UV spectra. The negative values of Gibbs free energies for these interactions at different temperatures showed that the process is spontaneous, in the direction of equilibrium and changed with the increasing in temperature. The enthalpy of interaction had appositive value that means the process is endothermic and the system requires input of energy. The experiments confirmed that combination was through π-π donor-accepting interaction and inducing the protonation process in folic acid upon strengthening electron accepting ability of the acid so the changing of their charge distribution and the surroundings became necessarily. In another words, this inferred that not only biotransformation process of folic acid, but also the toxicity of polyaromatic hydrocarbons could be understood.

Keywords: Folic acid, phenanthrene, polyaromatic hydrocarbons, spectroscopic studies, thermodynamic parameters.

INTRODUCTION

Folic acid, (N- [[2-Amino-1,4-dihdro-4-oxo-6-pteridinyl) methyl1] amino] benzoyl] –L- glutamic acid0, hematopoietic vitamin presents, free or combined with one or more additional molecules of L(+) glutamic acid, in liver, kidney, mushrooms, spinach, green leaves, and grasses [1].Folic acid, which plays as a key in one-carbon metabolism, is essential for biosynthesis of several compounds. The pterin ring changed by reductase enzyme to tetrahydrofolate which receives one carbon fragments from donors (monocarbonic units) such as serine, glycine, and histidine then transfers them to intermediates in the synthesis of amino acids, purines, thymine, and pyrimidine found in DNA[2,3]

Polyaromatic hydrocarbons are a group of organic compounds consisting of two or more fused benzene rings. They are priority pollutants in many industrial areas and occur in living organisms at significant concentrations near these sites [4].

The natural levels of ultraviolet radiation in sunlight enhance the toxicity of polyaromatic hydrocarbons in aquatic animals [5] and aquatic plants [6] which results from oxidation to more toxic from [7], photosensitization reaction [8], or combination of both of them [9]. Polyaromatic hydrocarbons toxicity and transformation have been demonstrated [10, 11].

The aim of this paper is to study the complexization between folic acid with a typical polyaromatic hydrocarbon (phenanthrene) by using FTIR and UV spectroscopies and thermodynamic parameters then comparing the obtained results with the recent studies.

EXPERIMENTAL SECTION

Materials: (All materials were from BDH chemicals Ltd Poole, England)

Solution:
Folic acid stock solution of ($7*10^{-5}$M) concentration was prepared in 100ml methanol: water (1:1) as a solvent. Phenanthrene stock solution of ($6*10^{-5}$M) was prepared in methanol as a solvent. Other solutions of folic acid and phenanthrene were prepared by dilution from its stock solution at pH (7.4).

Spectrophotometric Measurements
Absorption spectra were taken with the UV-Vis spectrophotometer 1650 PC, Shimadzu, Japan. The absorbance of folic acid and phenanthrene were measured in a wave length (200-400 nm). The rate constant for the interaction of phenanthrene and folic acid in a series of solutions (1:1) complex were prepared by mixing ($4 * 10^{-5}$) folic acid stock solution with phenanthrene solution in a range of ($1* 10^{-5}$ to $6 * 10^{-5}$) concentrations and the absorbance was recorded with time at constant temperature (25, 37, 40)°C in a time interval (0 – 25) min.

FTIR spectroscopy [12] : Microscopic FTIR-spectra of the complex formed between folic acid and phenanthrene as well as the spectra of folic acid and phenanthrene were recorded at FTIR spectrophotometer (IR Prestige – 21. Shimadzu, Japan) after packed with potassium bromide at room temperature. Solid folic acid and phenanthrene were used directly to obtain microscopic FTIR spectra, while the samples of complex were prepared by mixing the appropriate quantity of folic acid with phenanthrene.

RESULTS AND DISCUSSION

The absorption law, also known as the Beer-Lambert law or just Beer's law, tells us quantitatively how the amount of attenuation depends on the concentration of the absorbing molecules and the path over which absorption occurs. In the simplest case, in which the molecules of one absorbing solute act independently of each other, and in which the influence of a colorless solvent is constant with concentration each molecule of solute absorbs the same fraction of the light transmitted by a given thickness of solution decreases exponentially with increasing concentration of solute, or the absorbance increases linearly with concentration [13].

As an experimental model phenanthrene (π-donor) with o- phenanthroline or pyridine (humic π – acceptor) that forms a complex give a primary explanation of the dependence o the environmental transformation of polyaromatic hydrocarbons on the surrounding [14].

Recently, Y. He et al. reported that these processes of polyaromatic hydrocarbons are strongly depended on the interaction between polyaromatic hydrocarbons and the co-existing compounds [12].

In our present study, folic acid or phenanthrene solution of six different concentration ranging from ($1*10^{-5}$ M to $6*10^{-5}$ M) at pH 7.4 were prepared and the spectrum was recorded . For the studying of the interaction between folic acid and phenanthrene, UV spectra showed that folic acid has a strong absorb around (283 nm) for π-π^* transition of pterin ring at pH 7.4 [15] while absorption band of phenanthrene (250 nm) assigned to π-π^* transition of this conjugated system.

As a result a straight line obtained showing that (folic acid or phenanthrene) solutions obey beer's law, and the slope equal to the molar absorptivity "ε" at (λ_{max}=283 nm) for folic acid and (λ_{max}=250 nm) for phenanthrene. The behavior of folic acid and phenanthrene solutions separately in their solvent at human physiological pH 7.4, were studied first to make sure that there substances were stable in their solvent.

The mixture of folic acid and phenanthrene showed a shift in λ_{max} and a change in the absorbance due to a complex formation between them and the existent of the equilibrium (Table (1)). The new band (244 nm) for folic acid – phenanthrene complex which is in the same range of our previous studies for naphthalene – folic acid complex (235,296) nm, fluorine- folic acid complex (217, 278) nm, (210,302) nm for folic acid –acenaphthylene complex, and (247,330) nm for folic acid- acenaphthene complex [16].

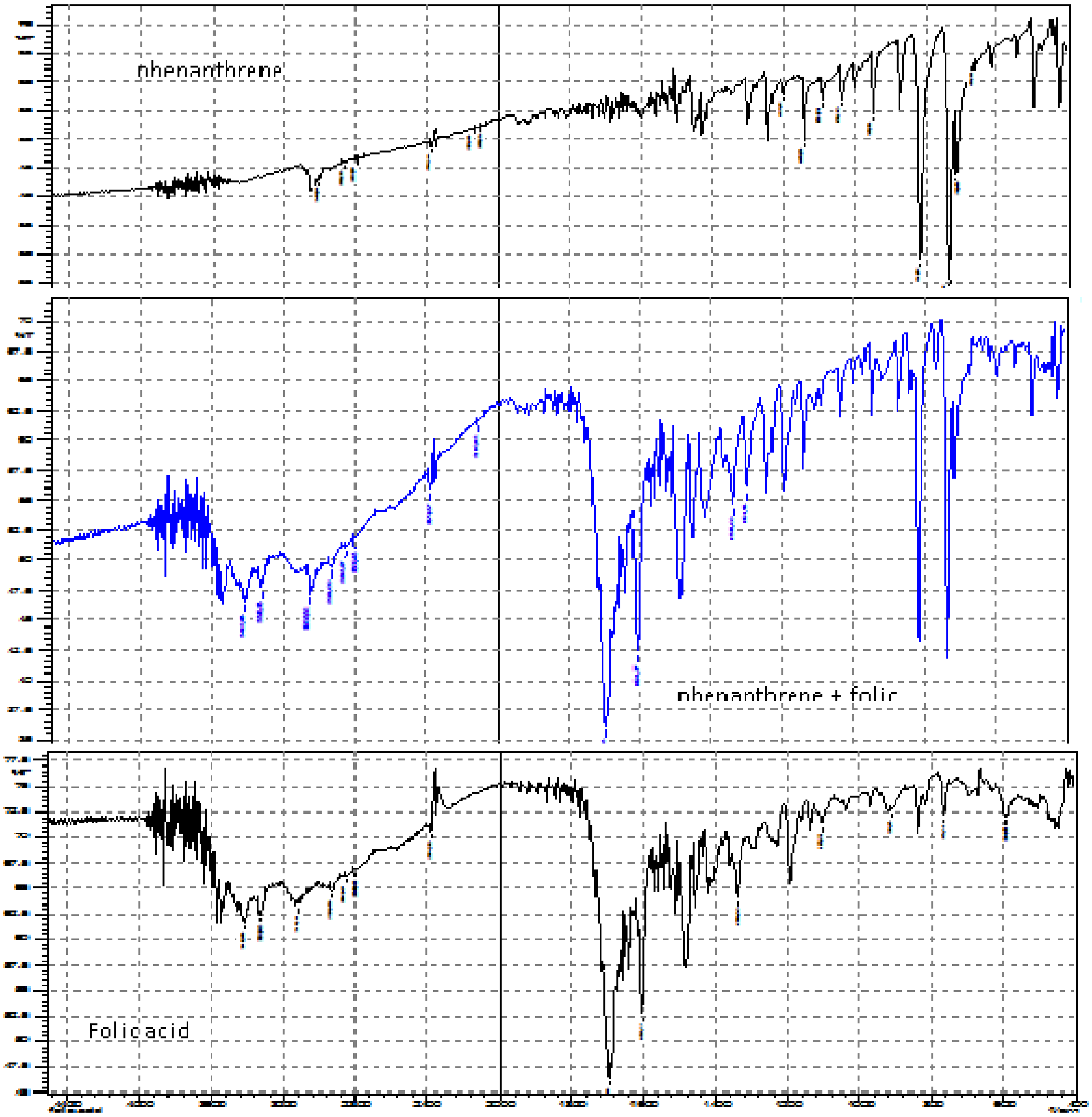

Figure -1- : FTIR spectra of folic acid and phenanthrene before and after interaction.

The equilibrium constant can be calculated by using the continuous variation method [17.18.19]. With experimenting, the formula of the complex between folic acid and the phenanthrene (1:1), it was possible to determine the equilibrium constant of these complexes. The concentration of the complex formed at equilibrium was calculated [20]. The equilibrium constant for the interaction of phenanthrene with folic acid was then calculated. At three different temperature (25, 37, 40) °C, the result in table (2) showed that the equilibrium constant for the complex changed as the temperature changed, and reached the maximum value near the normal temperature of the human body (37°C).

Table (1): Values of the molar absorptivity at pH 7.4 and λ_{max}

Solution	λ_{max} (nm)	Molar absorptivity (ε) ($cm^{-1}.L.mol^{-1}$)
Folic acid	283	23930
Phenanthrene	250	41380
Phenanthrene-folic acid complex	244	16703

The thermodynamic parameters and their relation to equilibrium of studied biochemical systems were calculated [21, 22]. The enthalpy of a reaction can be determined [23] by measuring the equilibrium constant for a system at different temperature then from the slope of the resulting liner van't Hoff plot of $\ln k_{eq}$ versus (1/T). The entropy change for the system can then be calculated (ΔG°=ΔH°-TΔs).

Table (2): Thermodynamic parameters for complex

Temp. (K)	k_{eq} (L.mol^{-1})	ΔG° (KJ.mol^{-1})	ΔH° (KJ.mol^{-1})	ΔS° (KJ.mole^{-1} K^{-1})
298	1.83x10^5	-30.0304	+50.3495	0.2697
310	5.07x10^5	-33.8605	+50.3495	0.2716
313	4.31x10^5	-33.7669	+50.3495	0.2687

So firstly the evidence of this complexization can be noticed by the appearance of new band due to disturb of the complexization on π-π^* transition of conjugated π system so the accepting ability of proton increases. Secondly from the resulted thermodynamic values where the Gibbs free energy is deciding the direction of processes and positions of equilibrium in biochemical systems. The negative values of Gibbs free energies for these interactions at different temperatures meant that process is spontaneous, in the direction of equilibrium and changed with the increasing in temperature. The enthalpy of interaction had a positive value that means that the process is endothermic and the system requires input of energy. The positive enthalpy and entropy change also refer to the type of interaction between the two molecules.

FTIR spectroscopy is an appropriate technique to understand the organic compound reaction effects on the functional groups absorptions for both the reactants and the products. New absorption band appears at 2372 cm^{-1} for folic acid – phenanthrene suggested the protonation of the N atom on the pterin ring and producing a positive salt may be resulted while the pure folic acid is characterized with (3600-3000) hydroxyl stretching and NH- stretching vibrations, 1697 cm^{-1} for the C=O bond stretching vibration of carboxyl group, and 1636 cm^{-1} belongs to C=O bond stretching vibration of- CONH$_2$ group beside 1604 cm^{-1} NH- bending vibration [24].

The shift of IR band around 1500 cm^{-1} confirms the interaction between the folic acid –phenanthrene to produce a complex of π-π electron donor- acceptor. The above IR bands confirm our previous works which showed at 2376 cm^{-1} for naphthalene – folic acid complex, 2401 cm^{-1} for fluorine – folic acid complex, 2381 cm-1 for folic acid – acenaphthylene and 2372 cm^{-1} with acenaphthene [16].

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