

Spectroscopic Study of a Typical Polyaromatic Hydrocarbon (Naphthalene) and a Biological π Acceptor (Folic acid) complex

BY

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Abstract

The complexation between folic acid and a typical polyaromatic hydrocarbon, naphthalene, was investigated using FTIR and UV spectra. Appearance of a new IR band at 2376 cm^{-1} demonstrates that $\text{NH}_2\text{-C}=\text{N}$ moiety on pterin ring in folic acid is protonated when naphthalene is introduced. The emergence of two charge transfer bands at 235 nm and 296 nm in UV difference spectra show the presence of $\pi\text{-}\pi$ complexation between folic acid and naphthalene. These experiments confirm that naphthalene could combine with the pterin ring of folic acid through $\pi\text{-}\pi$ donor-acceptor interaction and induce the protonation process in folic acid upon strengthening electron accepting ability of pterin ring. The results suggest that complexation between naphthalene and folic acid necessarily changes their charge distribution and the surroundings. It is inferred that not only biotransformation process of folic acid, but also the toxicity of polyaromatic hydrocarbons could be changed.

Keywords: Naphthalene, folic acid, UV, FTIR, spectroscopic

الخلاصة:

أظهرت دراسة تكون معقد بين حامض الفوليك والنفتالين كنموذج للمركبات الهيدروكربونية متعددة الحلقات العطرية من خلال ظهور حزمة أشعة تحت الحمراء جديدة في 2376 سم⁻¹ تعود لجزء $\text{NH}_2\text{-C}=\text{N}$ في حلقة البتيرين لحامض الفوليك ونشوء قمتي امتصاص أشعة فوق البنفسجية في (235، 296) نانومتر للتمازج في نظام واهب - مستقبل النتائج تؤكد إن تكون المعقد قد غير بالضرورة توزيع الشحنة مع المحيط وبالتالي ليس فأن عملية التحول الحيوي للحامض وسمية المركبات الهيدروكربونية متعددة الحلقات العطرية قد تغيرت سوية.

1. Introduction

Folic acid is composed of p- amino benzoic acid, glutamic acid, and pterin ring (see scheme -1-). Folic acid, which plays a key role 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.[1,2] Polyaromatic hydrocarbons are a class of electron rich aromatic pollutants with three or more fused benzene rings that are widespread in natural or artificial forms. Some of them are known to be mutagenic and/or carcinogenic. Transformation and toxicity of Polyaromatic hydrocarbons which were strongly depended on the surroundings has demonstrated.[3-6]

The formation of π - π complexes between phenanthrene and some model humic π - acceptor subunits such as o-phenanthroline, pyridine had been investigated and then explained the dependence of the environmental transformation of polyaromatic hydrocarbons on the surroundings.[7] Y.Y. He et al [8] reported that toxicity and transformation process of polyaromatic hydrocarbons is strongly depended on the interaction between polyaromatic hydrocarbons and the coexisting compounds. Complexation between folic acid and a typical polyaromatic hydrocarbon, anthracene, was investigated using FTIR and UV spectra. Appearance of a new IR band at 2362 cm^{-1} demonstrates that $\text{NH}_2\text{-C=N}$ moiety on pterin ring in folic acid is protonated when anthracene is introduced.

The shift of the characteristic IR band of the pterin ring and the emergence of two charge transfer bands at 254 nm and 246 nm in UV difference spectra show the presence of π - π complexation between folic acid and anthracene. These experiments confirm that anthracene could combine with the pterin ring of folic acid through π - π donor-accepter interaction and induce the protonation process in folic acid upon strengthening electron accepting ability of pterin ring.

The aim of this work is to study the complexation between folic acid and another typical polyaromatic hydrocarbon, naphthalene, as a representative compound of them by using FTIR and UV spectroscopies.

Experimental Details

1- Material:

Naphthalene, methanol, folic acid, hydrochloric acid, and sodium hydroxide were from BDH Chemicals Ltd Poole, England.

2- Solution preparation

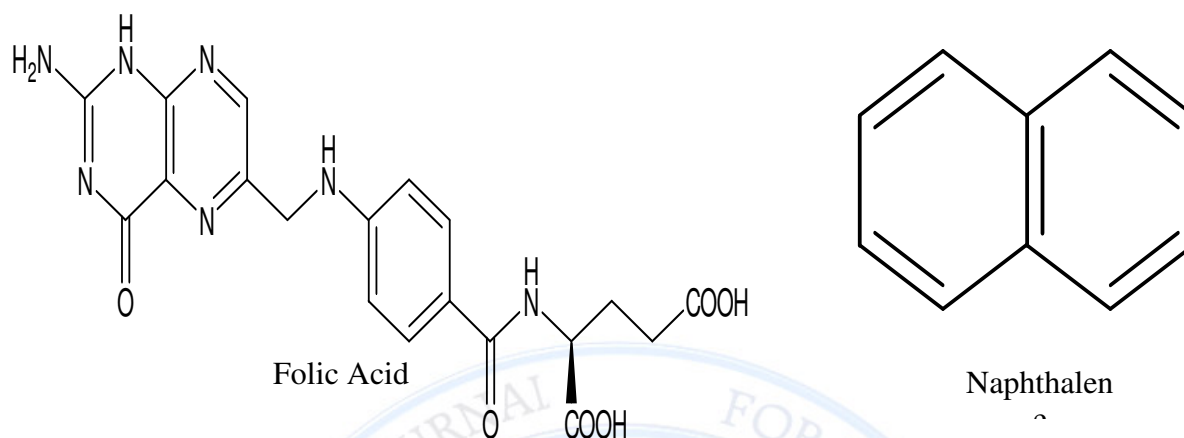
0.01 g naphthalene was dissolved in 100 ml methanol, and the supernatant liquid was taken out as naphthalene saturated solution. 2.50×10^{-4} mole l^{-1} folic acid stock solution was prepared by dissolving 0.0221 g folic acid in 1:1 methanol/water (v:v) or water in 200 ml volumetric flask. Also HCl, NaOH were used for pH adjustment.

Experiment methods

FTIR spectroscopy: Microscopic FTIR-spectra of the complex formed between folic acid and naphthalene as well as the spectra of folic acid and naphthalene were recorded using FTIR spectrophotometer (IR Prestige - 21, Shimadzu, Japan) after packed with potassium bromide at room temperature. Solid folic acid and naphthalene were used directly to obtain microscopic FTIR spectra, while the samples of complex were prepared by mixing the appropriate quantity of folic acid with naphthalene. [8]

UV absorption spectroscopy: UV absorption spectra were recorded using UV-Vis spectrophotometer (UV-1650PC, Shimadzu, Japan) as follows: Eight of 5.0×10^{-5} mole l^{-1} folic acid working solutions in 1:1 methanol/water (v:v) were respectively adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 with HCl and NaOH, which were tagged as group A.

Then 50 μ l naphthalene saturated solutions were respectively added into each 10ml of the solutions from group A, which were tagged as group B. Naphthalene solution of the same concentration without folic acid were used as spectra control. The UV absorption spectra of the test solutions were recorded using against a solvent blank (for group A) or naphthalene blank (for group B). [8]



Scheme-1-: Structures of folic acid and naphthalene

2. Results and discussion

Exact information about the functional groups involved in the interaction process would be given by comparison of FTIR of folic acid with that of the product after interaction with naphthalene. As shown in Figure -1-, the bands for pure folic acid between $3600 - 3400\text{cm}^{-1}$ are due to the hydroxyl (OH) stretching bands of glutamic acid moiety and NH group of pterin ring. The stretching vibration peak of C=O appears at 1696cm^{-1} , while the band at 1607cm^{-1} relates to the bending mode of NH vibration. The bands between $1511 - 1482\text{cm}^{-1}$ are attributed to characteristic absorption band of the phenyl and pterin ring.[9]

When folic acid coexists with naphthalene as shown in Figure- 1-, a new absorption band appears at 2376cm^{-1} , and it is ascribed to $\text{N}^+\text{-H}$ stretching vibration band of $\text{C}=\text{N}^+\text{H}$ on pterin ring.[10]

The appearance of the new band demonstrates that the N atom on pterin ring is protonated when naphthalene coexists. Because there is no other source of the proton, the change of the IR bands would be caused by the proton transfer from carboxyl at glutamic acid moiety to N atoms at pterin ring. It is well known that the lone-pair electrons on N atoms at pterin ring are not conjugated with ring π system, so they are capable to combine with proton to produce

positive salt. The shift of IR bands at 1511 and 1482 cm^{-1} suggests that phenyl or pterin ring is also involved in the interaction of folic acid with naphthalene.

Research performed has demonstrated that a planar molecule with rich electrons, such as polyaromatic hydrocarbons, can interact with opposing system to produce complexes named as π - π electron donor-accepter system.[11]

Pterin ring, a planar N-heterocyclic, resembles the planar π system of naphthalene to produce a complex by π - π electron donor-accepter interaction. In consequence, the electron clouds would deflect from naphthalene to pterin ring, and then the proton-accepting ability of N atoms could become stronger so that the proton transformation from the carboxyl of glutamic acid to N atoms happens. The interaction between folic acid and naphthalene is studied using the UV spectra and UV difference spectra as displayed in Figure -2- UV spectra of folic acid shows a strong pterin ion band around 283 nm from pH 3.0-8.0, and it is assigned to the π - π^* transition of pterin ring.[12] The dominant form of pterin ring of folic acid is shown in Scheme -1- during pH 3.0-8.0, and the other acid or basic form could be ignored because the pKa values of the pterin ring are 8.1 and 2.4 [13], which are respectively ascribed to the dissociation of the protonated groups of O=C-N and NH₂-C=N (atom numbering is shown in Scheme 1).

This is consistent with the fact that λ_{max} of the UV band is remained unchanged at pH 3.0-8.0. Absorption band of naphthalene at 219 nm is assigned to π - π^* transition of conjugated π system and it is not interfered by pH. When folic acid and naphthalene coexist, the UV spectra against naphthalene reference shows the appearance of two charge transfer bands at 235 and 296 nm at pH 3.0-8.0. These two bands are the evidence of the π - π complexation between folic acid and naphthalene, and they are ascribed to the disturbance of the complexation on π - π^* transition of conjugated π system. The intensities of charge transfer bands should be proportional to the interaction strength, and the interaction strength should be positive correlation with the protonation degree of π accepter, so the intensities of charge transfer bands would be relative to the protonation degree of N-atoms on the pterin moiety. The pKa value 2.4 of the protonated group NH₂-C=N on pterin ring is much smaller than the inflection. This fact proves that the complexation between folic acid and naphthalene indeed changes the charge distribution of the folic acid, especially the pterin ring. Consequently, the proton accepting ability of pterin ring increases.



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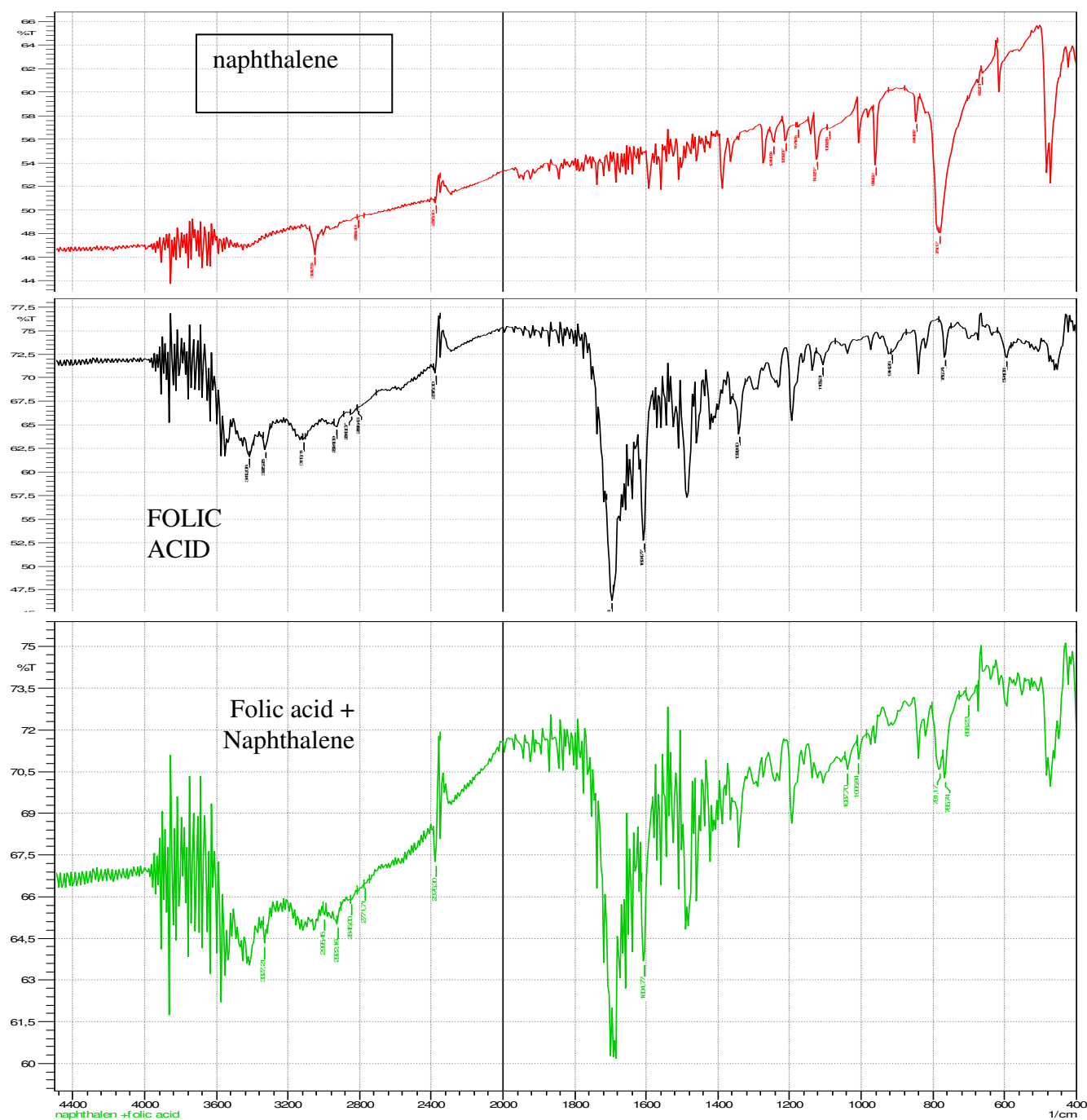


Figure (1) : IR spectra of naphthalene and folic acid before and after interaction.

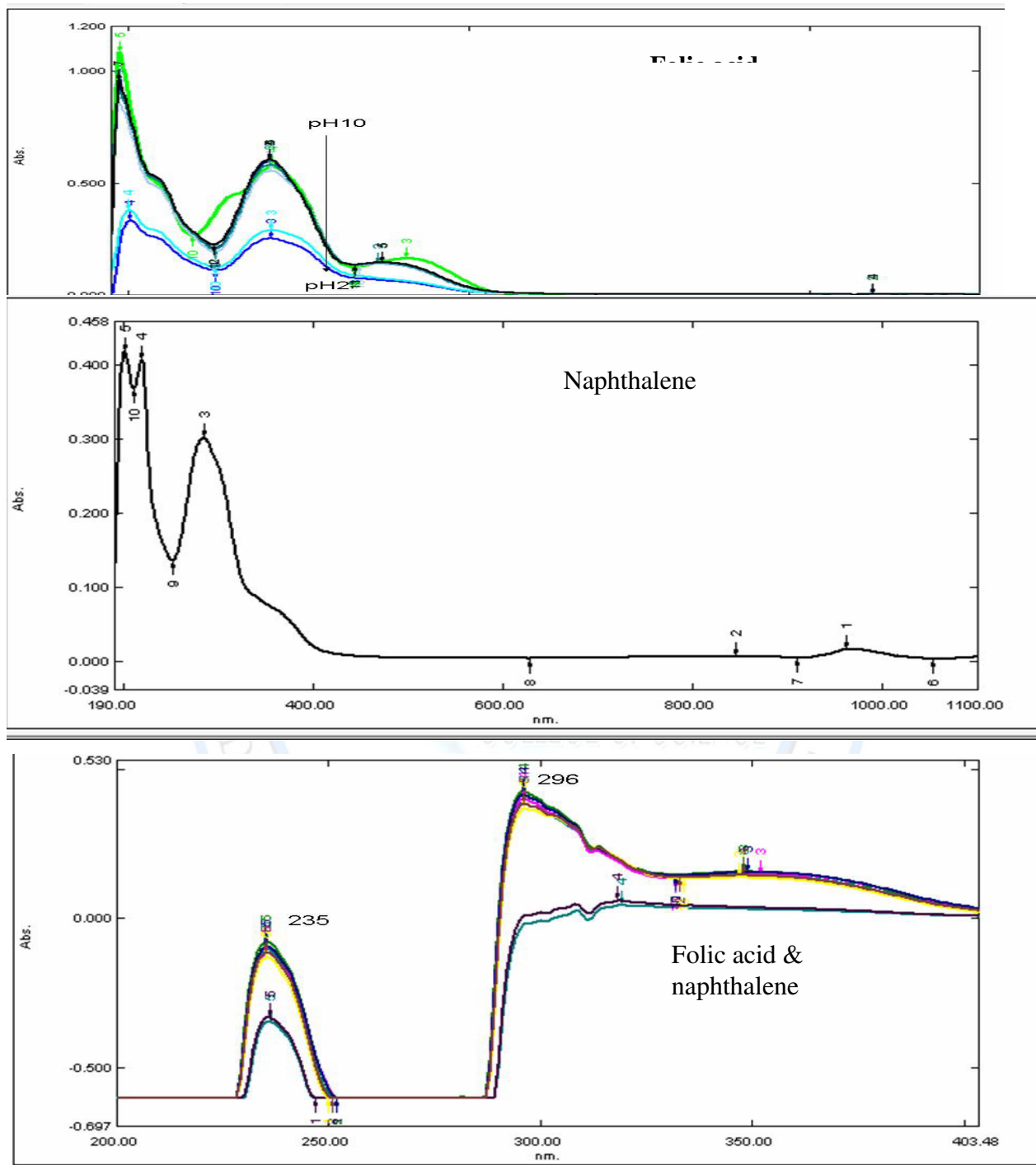


Figure (2) : UV absorption spectra of folic acid and naphthalene, before and after interaction against naphthalene blank respectively.