دراسة تكوين معقد بين حامض الفوليك والفلورين باستخدام مطيافي الأشعة تحت الحمراء والأشعة فوق البنفسجية

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الخلاصة

أظهرت دراسة تكون معقد بين حامض الفوليك والفلورين انموذجا للمركبات الهيدروكربونية متعددة الحلقات الاروماتية من خلال ظهور حزمة أشعة تحت الحمراء جديدة في 2401 سم$^{-1}$ تعود لجزء NH_2–C=N في حلقة البتيرين لحامض الفوليك ونشوء قمتي امتصاص أشعة فوق البنفسجية في(217، 278 نم) للتمازج في نظام واهب – مستقبل π-π . النتائج تؤكد أن تكون المعقد غير بالضرورة توزيع الشحنة مع المحيط ومن ثم ليس فقط عملية التحول الحيوي للحامض بل وسمية المركبات الهيدروكربونية متعددة الحلقات الاروماتية.

Folic Acid With Fluorene: A Complexation Study by UV and FTIR Spectroscopies

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Abstract

The complexation between folic acid and a typical polyaromatic hydrocarbon, fluorene, was investigated using FTIR and UV spectra. Appearance of a new IR band at 2401cm^{-1} demonstrates that NH_2–C=N moiety on pterin ring in folic acid is protonated when fluorene is introduced. The emergence of two charge transfer bands at 217 nm and 278 nm in UV difference spectra shows the presence of π-π complexation between folic acid and fluorene. These experiments confirm that fluorene could combine with the pterin ring of folic acid through π-π 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 fluorene with folic acid necessarily change 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.

Introduction

Folic acid is composed of p- amino benzoic acid, glutamic acid, and pterin ring (see scheme -1-). Folic acid, which plays 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.[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 was demonstrated.[3-6]

The formation of π-π complexes between fluorene and some model humic _-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 2362cm^{-1} demonstrates that NH_2–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, fluorene, as a representative compound of them by using FTIR and UV spectroscopies.

Experiment

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

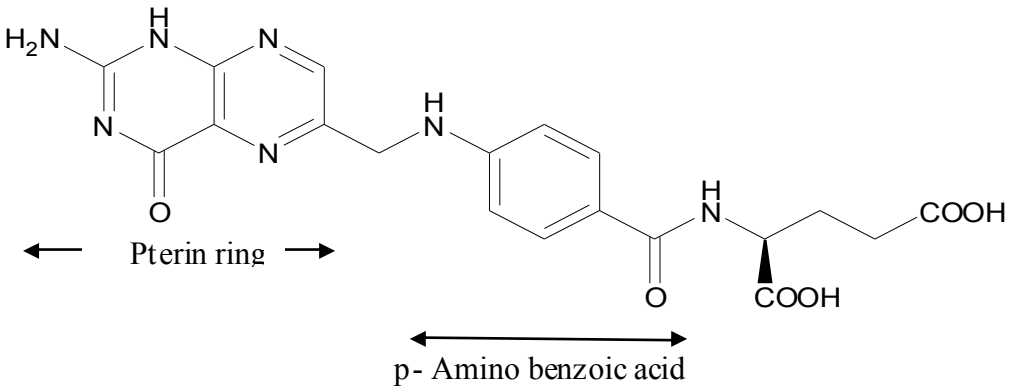
0.01 g fluorene was dissolved in 100 ml methanol, and the supernatant liquid was taken out as fluorene saturated solution. 2.50×10^{-4} mol l^{-1} folic acid stock solution was prepared by dissolving 0.0221 g folic acid in 1:1 methanol/water (v:v) or water in 200 ml volumetric flask. Also HCl, NaOH were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

Experiment methods [8]

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

UV absorption spectroscopy: UV absorption spectra have often been used to study the structure of the complex. UV absorption spectra were recorded as follows: eight of $5.0\times10-5$ mol 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 50µl fluorene saturated solutions were respectively added into each 10ml of the solutions from group A, which were tagged as group B. Fluorene solution of the same concentration without folic acid were used as spectra control. The UV absorption spectra of the test solutions were recorded using a UV–Vis spectrophotometer (UV-1650PC, Shimadzu, Japan) against a solvent blank (for group A) or fluorene blank (for group B).



Scheme-1-: Structures of folic acid*

Results and Discussion

Exact information about the functional groups involved in the interaction process would be given by the comparison of FTIR of folic acid with that of the product after interaction with fluorene. As shown in Figure -1-, the bands for pure folic acid between 3600 - 3400cm^{-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

* The Merck Index ,2000 by Merck & Co Inc., Whitehouse station, NJ, USA

band at 1607cm^{-1} relates to the bending mode of NH vibration. The bands between 1511 – 1482 cm^{-1} are attributed to characteristic absorption band of the phenyl and pterin ring.[9]

When folic acid coexists with fluorene as shown in Figure- 1-, a new absorption band appears at 2375cm^{-1}, and it is ascribed to N^{+}–H stretching vibration band of C= N^{+}H on pterin ring.[10]

The appearance of the new band demonstrates that the N atom on pterin ring is protonated when fluorene 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 1482cm^{-1} suggests that phenyl or pterin ring is also involved in the interaction of folic acid with fluorene [11].

Research performed has demonstrated that a planar molecular with rich electrons, such as polyaromatic hydrocarbons, can interact with opposing system to produce complexes named as π-π electron donor–accepter system. [12].

Pterin ring, a planar N-heterocyclic (see scheme -1-) , would parallel the planar π system of fluorene to produce a complex by π-π electron donor–accepter interaction through suggested face to face geometry [8]. In consequence, the electron clouds would deflect from fluorene 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 with fluorene is studied by using the UV spectra and UV difference spectra as displayed in Figure -2- UV spectra of folic acid shows a strong absorb pterin ion band around 283 nm during pH 3.0–8.0 , and it is assigned to the π-π* transition of pterin ring.[13]

The dominant form of pterin ring of folic acid is as 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 [14], which are respectively ascribed to the dissociation of the protonated groups of O=C–N and NH_2–C=N.

This is consistent with the fact that its λ_{max} of the UV band is remaining unchanged during pH 3.0–8.0. Absorption band of fluorene at 289 nm is assigned to π-π * transition of conjugated π system and it is not interfered by pH.

When folic acid and fluorene coexist, the UV spectra against fluorene blank shows the appearance of two charge transfer bands at 217 and 278 nm during pH 3.0–8.0.These two bands are the evidence of the π-π complexation between folic acid and fluorene, 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 NH_2–C=N on pterin ring is much smaller than the inflection. This fact proves that the complexation between folic acid and fluorene indeed changes the charge distribution of the folic acid, especially the pterin ring. Consequently, the proton accepting ability of pterin ring increases.

References

1.Lippincott,s Illustrated Reviews (2005): Biochemistry, Champe ,P. C.; Harvey, R. A. and Ferrier, D. R. (Editors), Lippincott Willams & wilkins, USA. P. 372,

2. Birdsall,B.; Feeney, J. S Tendeler,J.B.; Hammond,S.J. and Roberts, G.C.K.(1989), Biochemistry 28:2297–2305.

3. Noordman,W.H.; Bruining,J.W.; Wietzes,. D.and Janssen, B. (2000), J. Contam. Hydrol. 44: 119–140.
4. Gensemer, R.W. ; Dixon,D.G.; B.M. Greenberg (1998), Ecotoxicol. Environ. Safety 39:57–64.
5. Chi,F.H. and Amy, G.L. (2004), Chemosphere 55: 515–524.
6. Akkanen, J. ; Penttinen, S.; Haitzer, M. and Kukkonen ,J.V.K. (2001), Chemosphere 45: 453–462.
7. Wijnja,H. ; Pignatello,J.J.and Malekani ,K. (2004), J. Environ. Qual. 33 :265–275.
8. He,Y.Y. ; Wang,X.C. ; Jin b,P.K. and Zhao,Bo Xiaoyuan Fan (2009), Spectrochimica Acta Part A 72 :876–879.
9. Zhang,J. ; Rana,S. ; Srivastava,R.S. and Misra, R.D.K. (2008), Acta Biomater 4:40–48.
10. Bellamy L.J. (1980), The Infrared Spectra of Complex Molecules, Chapman and Hall, London.
11. Szakács,Z. and Noszál B. (2006), Electrophoresis 27:3399-3409.
12. Mulliken ,R.S. (1952), J. Am. Chem. Soc. 74: 811–824.
13. Thomas,A.H.; Suلrez,G. ; Cabrerizo,F.M. ; Martino,R. and Capparelli ,A.L. (2000), J. Photochem. Photobiol. A 135:147–154.
14. Albert,A. (1953) Biochem. J. 54 646–654, He,Y.Y. ;Wang,X.C. ; Jin b, P.K. and Zhao, Bo Xiaoyuan Fan (2009), Spectrochimica Acta Part A 72: 876–879.

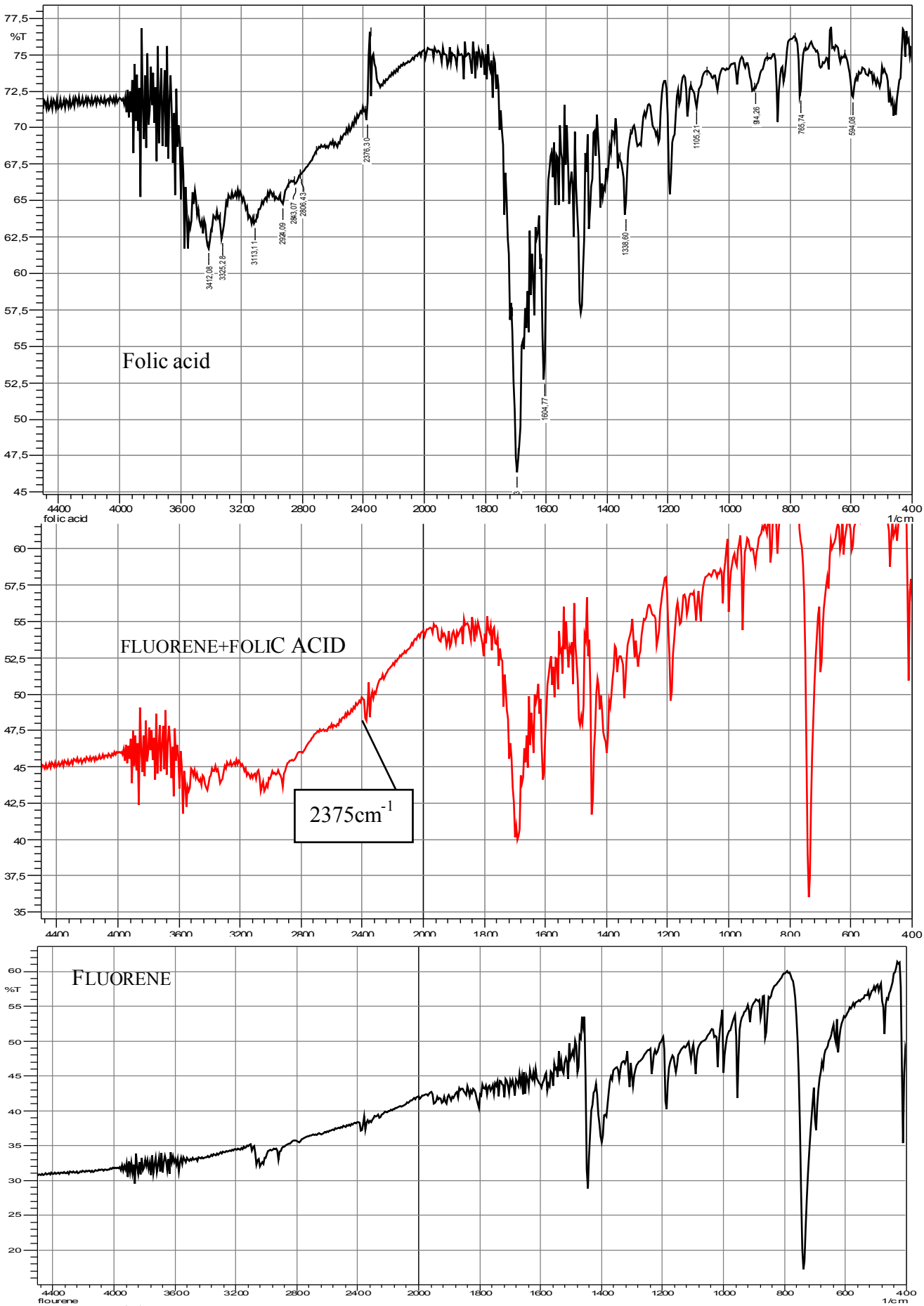
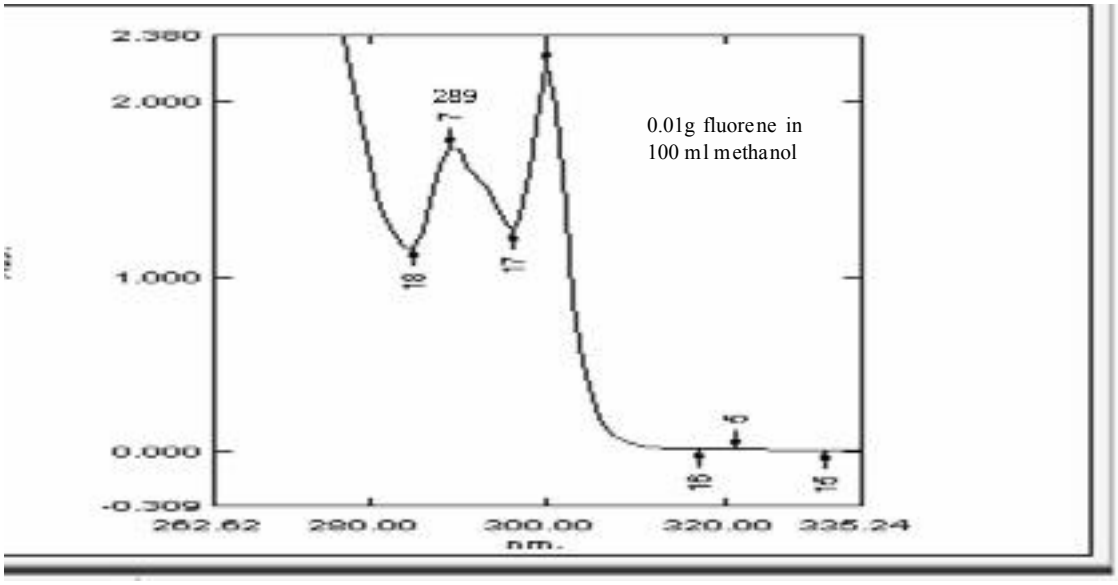
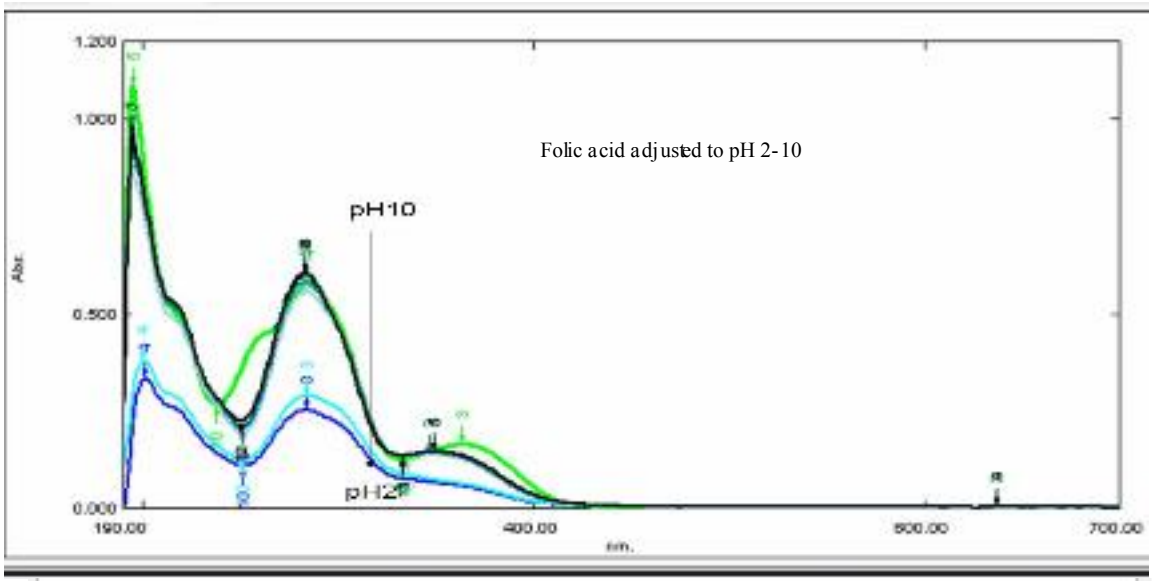


Fig. (1): IR spectra of fluorene and folic acid before and after interaction with fluorene.





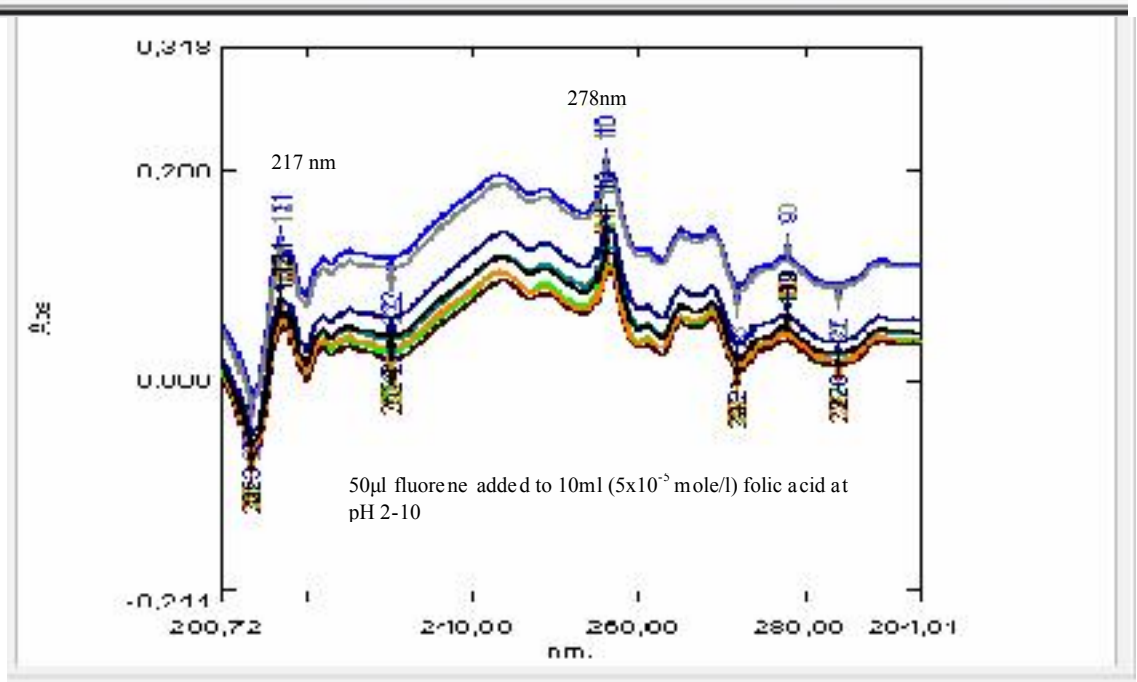


Fig.(2): UV absorption spectra of fluorene, folic acid, and their mixture against fluorene blank respectively