J Solution Chem (2008) 37: 895–917
DOI 10.1007/s10953-008-9289-8

# Solvent Effect on the Absorption and Fluorescence Emission Spectra of Some Purine Derivatives: Spectrofluorometric Quantitative Studies

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Received: 23 October 2007 / Accepted: 9 January 2008 / Published online: 22 May 2008


**Abstract** The absorption and emission spectra of six purine derivatives: adenine (I), $N(9)$-hydroxyethyladenine (II), $N(6)$-acetyladenine (III), $N(6)$-isobutyryladenine (IV), guanine (V), and $N(2)$, $N(9)$-diacetylguanine (VI) have been investigated. The effects of solvent and pH on the positions of $\lambda_{max}$ (absorption) and $\lambda_{max}$ (emission) of these compounds were determined. Correlations between the absorption wavelength ($\lambda_{max}$) of these organic compounds and the solvent parameters $(D, n, E)$ or $(K, M, N)$ show that the peak position is affected mainly by specific- and non-specific types of interactions between the solvent and solute. Solvent effects on the electronic absorption band shifts are indicative of the extent of charge reorganization of the solute molecules upon electronic excitation. The Stokes shift $(\nu_{abs} - \nu_{em})$ was correlated with the orientation polarizability $(\Delta f)$ and was found to depend mainly on the dielectric constant and the refractive index of the solvents. This shift reflects the influence of the equilibrium solvent arrangement around the excited solute molecule, which rearranges inertially due to the instantaneous charge redistribution upon radiative deactivation to the electronic ground state. A spectrofluorometric analysis technique was applied for the quantitative analysis of the components of a ternary mixture of compounds (I–III).

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**Keywords** Purine derivatives · Electronic spectra · Absorption · Fluorescence · Solvent effect · Solvent parameters · Stokes shift

## 1 Introduction

Nucleic bases have attracted the attention of scientists due to their biological importance as the chromophore of DNA [1]. In this respect, synthesis of purine derivatives targeting specific purine binding proteins could yield a variety of important compounds for use as biological probes and drug candidates [2, 3]. Consequently, alkylated [4, 5], hydroxyalkylated [6–8], acylated [9] and acetylated [10] purine derivatives were studied. The 9-(2-hydroxyethyl) purine derivatives have been evaluated for HT-1080 tumor cell invasion using the reconstituted-basement membrane Matrigel (MG), and for preventing type IV collagen degradation [8, 11]. The binding mode of some 6-substituted 9-(2-hydroxyethyl) purines to adenosine deaminase was discussed with respect to changes in the substituents at the 9-position of the purine group [7].

Experimental and theoretical efforts have been made in order to understand and correlate the electronic spectra of nucleic acid bases, which are of interest for prediction and interpretation of the ultraviolet (UV) and circular dichroism (CD) spectra of nucleic acids [12–19].

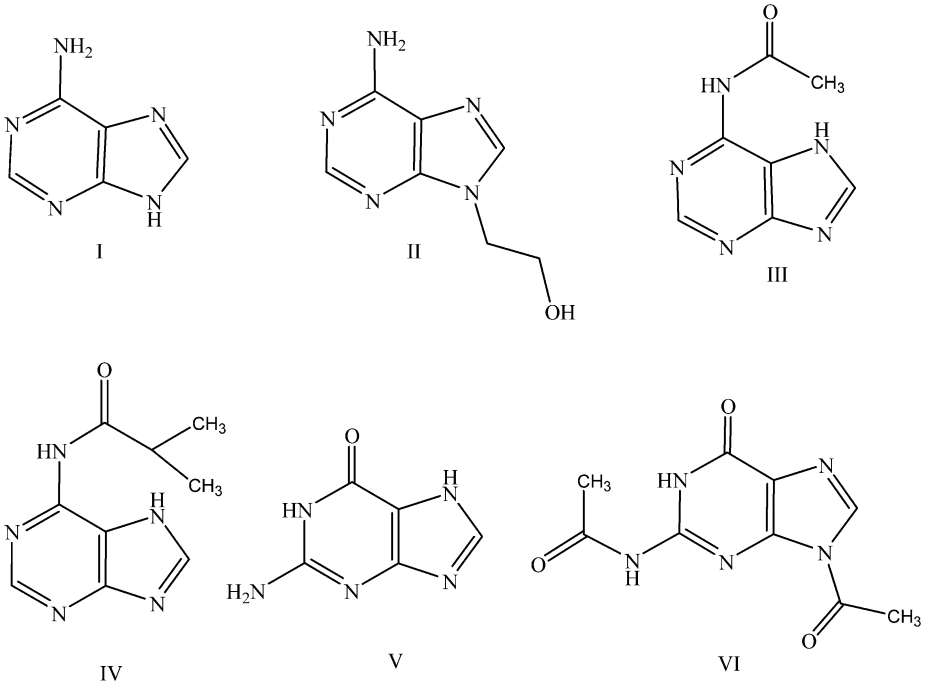
As in many organic compounds, the observed shifts of the electronic absorption and emission bands of these compounds in different media are consequences of solvent polarity that in turn depends on specific- and non-specific intermolecular interactions between the solute ions or molecules and solvent molecules [20–25]. Consequently, this effect displays remarkable regularity that allows an empirical analysis to be made that sheds light on their origin [26–32].

Examination of the ultraviolet spectra of purine and pyrimidine is very extensive [13–16]. The absorption spectrum of purine resembles that of pyrimidine in aqueous solution [13].

In our previous work, the absorption and emission spectra of some 1,6-heptadienyl pyrimidine derivatives in different solvents were elucidated, because the incorporation of modified unnatural nucleobases into an oligonucleotide chain enables the application of fluorescence techniques to nucleic acid detection and to studies of the conformation, dynamics and interactions of bio-molecules [32]. The present work is an extension to our previous work on pyrimidine [32], and is a new effort to characterize the spectral bands of some purine derivatives.

In addition, the structural and photophysical properties of nucleic bases are of great interest in order to get a better insight into UV-induced genetic damage [33]. Many technological approaches have sufficient sensitivity to measure the amounts of modified DNA constituents such as alkylated, acetylated and acylated purine [10, 34, 35], and are being used for exposure assessments such as immunohisto-chemistry [36], fluorescence and phosphorescence spectroscopy [37], gas chromatography-mass spectrometry (GC-MS) [38], and atomic absorbance spectrometry (AAS) [39].

Multi-component analysis of mixtures of organic compounds such as purines is a rather difficult task without their prior separation. The use of UV-Visible absorption and fluorescence techniques to achieve this purpose is of especially great importance. Examination of the literature revealed various spectral methods for determination of binary and ternary mixtures of compounds without requiring further separation. These methods include partial least-squares regression (PLS) [40], principal component regression (PCR) [41], multi-wavelength linear regression analysis (MLRA) [42], the H-point standard addition method




**Scheme 1** Chemical structures of adenine (**I**), *N*(9)-hydroxyethyladenine (**II**), *N*(6)-acetyladenine (**III**), *N*(6)-isobutyryladenine (**IV**), guanine (**V**) and *N*(2), *N*(9)-diacetylguanine (**VI**)

(HPSAM) [43], the double divisor-ratio spectra derivative method [44], and the mean centering of the ratio spectra [45].

In this respect, compounds (I–III), see Scheme 1, were analyzed using the spectrofluorometric derivative ratio with zero-crossing technique [46–48]. No previous determination of this mixture has been reported in the literature.

The aims of the present study are to:

(1) Characterize the spectral bands of some alkylated and acetylated aminopurine derivatives;
(2) investigate the effects of solvent polarity and pH on the absorption and emission wavelengths of the compounds (I–VI);
(3) correlate the data in order to evaluate solvent-solute interaction effects on the electronic absorption and emission spectra; and
(4) analyze compounds (I–III) spectrofluorometrically, which could become a useful tool in quantifying the presence of any modification of the adenine nucleobase.

## 2 Experimental

### 2.1 Equipment and Reagents

All chemicals and reagents used in the present work are analytical grade and were purchased from Fluka. The pHs of the solutions were measured on a GP 353 ATC pH-meter after calibration with buffer solutions at pH = 4 and 7. The electronic absorption

spectra were recorded at 25 °C with a SP-3000 OPTIMA UV-Visible spectrophotometer (Japan). The spectrofluorometric measurements were carried out at 25 °C on a Jasco FP-6200 spectrofluorometer (Japan) and supported with Jasco Spectra Manager software v. 1.05. The electronic absorption and emission spectra of these compounds (concentrations from $1 \times 10^{-5}$ to $1 \times 10^{-6}$ mol·L$^{-1}$) were investigated in various organic solvents (spectroscopic grade) having different polarities: carbon tetrachloride ($CCl_4$), chloroform ($CHCl_3$), diethylether, *N*, *N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 1,4-dioxane, acetonitrile ($CH_3CN$), ethanol (EtOH), methanol (MeOH), acetone ($Me_2CO$) and water ($H_2O$). The effect of pH change on the electronic absorption spectra was studied by adding 1 mL HCl (0.1 mol·L$^{-1}$) or NaOH (0.1 mol·L$^{-1}$) to a 3 mL methanolic solution of each compound ($10^{-5}$ to $10^{-6}$ mol·L$^{-1}$).

### 2.2 Synthesis of the Organic Compounds

Adenine (I), guanine (V) and *N*(2), *N*(9)-diacetylguanine (VI) were purchased from Fluka, whereas compounds (II), (III), and (IV) were prepared according to the literature, purified through column chromatography, and their structures confirmed via FT-IR, NMR, and elemental analysis [9, 49–51].

## 3 Calculation Methods and Results

The solvent effect and the specific interaction between solute and solvent molecules (hydrogen bonding) are major factors in explaining their spectral behavior. For the regression analysis, the following equation was used:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + \cdots. \tag{1}$$

The $a_1, a_2$ and $a_3$ constants are different regression coefficients, and the constant $a_0$ is the regression intercept. The observed peak location $\lambda_{max}$ is treated as the dependent variable $Y$, while the independent variables $(X_1, X_2, X_3, \ldots)$ are those for the solvent interaction mechanisms $(D, n, E)$ or $(E, K, M, N)$, see Tables 1 and 2. The quantity $D$ is the dielectric constant of the solvent, $n$ is the refractive index and $E$ is an empirical solvent polarity parameter sensitive to both solvent-solute hydrogen bonding and dipolar interactions. The quantity $K$ depends on $D$, and is a measure of the polarity of the

**Table 1** Solvent dielectric constant $D$, refractive index $n$, empirical polarity $E$, and parameters used in spectral correlation equations [52]

| Solvent | $D$ | $n$ | $E$ | $K$ | $M$ | $N$ |
|---|---|---|---|---|---|---|
| $CCl_4$ | 2 | 1.426 | 32.5 | 0.2 | 0.22 | 0.01 |
| Diethylether | 4.2 | 1.353 | 34.6 | 0.34 | 0.18 | 0.3 |
| 1,4-Dioxane | 2.2 | 1.422 | 36 | 0.22 | 0.2 | 0.03 |
| Chloroform | 4.7 | 1.443 | 39.1 | 0.36 | 0.21 | 0.29 |
| Water | 78.5 | 1.33 | 63.1 | 0.49 | 0.17 | 0.76 |
| Dimethylformamide | 36.7 | 1.427 | 43.8 | 0.48 | 0.2 | 0.67 |
| Dimethyl sulfoxide | 48.9 | 1.478 | 45 | 0.49 | 0.22 | 0.66 |
| Acetonitrile | 37.5 | 1.344 | 46 | 0.48 | 0.18 | 0.71 |
| Ethanol | 24.3 | 1.361 | 51.9 | 0.47 | 0.18 | 0.67 |
| Methanol | 32.6 | 1.329 | 55.5 | 0.48 | 0.17 | 0.71 |

**Table 2** $\lambda_{max}$ of absorption (nm) for adenine (I), $N(9)$-hydroxyethyladenine (II), $N(6)$-acetyladenine (III), $N(6)$-isobutyryladenine (IV), guanine (V) and $N(2)$, $N(9)$-diacetylguanine (VI) in different solvents

| | Compound | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| Solvents | | | | | | |
| $CCl_4$ | | 259 | 260, 284, 294 | | | |
| Ether | 216, 259 | 217, 260 | 279, 294 | | | 280 |
| 1,4-Dioxane | 261 | 261 | 257, 276, 283, 294 | 248, 257, 277, 284, 294 | | 257, 287, 298, 312 |
| Chloroform | 233, 262 | 230, 262 | 275, 284, 294, | 249, 256, 277, 284, 294 | | 252, 297, 307, 322 |
| DMF | 270 | 271 | 269, 276, 283, 293 | 268, 278, 284, 296 | 278 | 270, 288, 325 |
| DMSO | 266 | 265 | 259, 277, 284, 295 | 263, 278, 285, 296 | 263 | 264, 290, 324 |
| Acetonitrile | 209, 259 | 204, 260 | 256, 275, 282, 292 | 256, 275, 283, 293 | | 254, 288, 306, 320 |
| Ethanol | 207, 262 | 209, 262 | 255, 274, 282, 292 | 257, 275, 282, 293 | 248 | 261, 283 |
| Methanol | 210, 262 | 212, 262 | 255, 274, 282, 291 | 256, 275, 282, 292 | 248 | 258, 282 |
| Water | 209, 262 | 210, 262.5 | 256, 275, 281, 292 | 255, 275, 281, 292 | 248 | 261, 283 |

**Table 3** Multiple correlation coefficients for compounds (I–IV and VI) at $\lambda_{max}$ (262, 262, 282, 292 and 282 nm, respectively) using the $D, n$, and $E$ solvent parameters

| Parameter | Compound | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | VI |
| $D$ | 0.308 | 0.391 | 0.621 | 0.172 | 0.089 |
| $n$ | 0.574 | 0.357 | 0.919 | 0.894 | 0.708 |
| $E$ | 0.085 | 0.213 | 0.84 | 0.635 | 0.263 |
| $D, n$ | 0.729 | 0.626 | 0.959 | 0.908 | 0.711 |
| $D, E$ | 0.43 | 0.451 | 0.851 | 0.932 | 0.347 |
| $n, e$ | 0.731 | 0.611 | 0.954 | 0.895 | 0.719 |
| $D, n, E$ | 0.741 | 0.634 | 0.96 | 0.925 | 0.722 |

solvent, $K = (D - 1)/(2D + 1)$. The quantity $M$ depends on the solvent refractive index ($n$) and is a measure of solute permanent dipole-solvent induced dipole interactions, $M = (n^2 - 1)/(2n^2 + 1)$, whereas $N$ is a measure of permanent dipole–permanent dipole interactions, $N = (D - 1)/(D + 2) - (n^2 - 1)/(n^2 + 2)$ [25, 32, 52].

A multiple regression analysis was performed in each case, and the fits were obtained as a function of one, two or three of these parameters. The results are listed in Tables 3 and 4.

The coefficients $K_1$, $K_2$, and $\nu$(vapor) were calculated using the multiple regression technique based on the following equation:

$$\nu(\text{solution}) = \nu(\text{vapor}) + K_1(2D - 2)/(2D + 1) + K_2(2n^2 - 2)/(2n^2 + 1) \qquad (2)$$

**Table 4** Multiple correlation coefficients for compounds (I–III) at $\lambda_{max}$ (262, 262 and 282 nm, respectively) using the $E$, $K$, $M$, and $N$ solvent parameters

| Parameter | Compound | | |
|---|---|---|---|
| | I | II | III |
| $E$ | 0.085 | 0.231 | 0.84 |
| $K$ | 0.379 | 0.16 | 0.962 |
| $M$ | 0.5 | 0.5 | 0.522 |
| $N$ | 0.317 | 0.455 | 0.618 |
| $E, K$ | 0.468 | 0.453 | 0.966 |
| $E, M$ | 0.671 | 0.577 | 0.867 |
| $E, N$ | 0.417 | 0.547 | 0.852 |
| $K, M$ | 0.72 | 0.672 | 0.962 |
| $K, N$ | 0.516 | 0.662 | 0.963 |
| $M, N$ | 0.733 | 0.569 | 0.865 |
| $E, K, M$ | 0.727 | 0.674 | 0.969 |
| $E, K, N$ | 0.519 | 0.668 | 0.967 |
| $E, M, N$ | 0.736 | 0.587 | 0.925 |
| $K, M, N$ | 0.747 | 0.672 | 0.978 |

**Table 5** Values of $K_1$, $K_2$, $\nu$(vapor) and correlation analysis data for the compounds (I–IV) and (VI) at different $\nu/\mathrm{cm}^{-1}$)

| Compound | $\nu$(vap)/cm$^{-1}$ | $K_1$ | $K_2$ | $MCC$ | $R^2(\nu, D)$ | $R^2(\nu, n)$ |
|---|---|---|---|---|---|---|
| I | 42929.16 | −9401.865 | −1493.562 | 0.794 | 0.14 | 0.33 |
| II | 42386.681 | −7944.968 | −1544.233 | 0.759 | 0.255 | 0.125 |
| III | 36442.63 | −3018.337 | 105.932 | 0.935 | 0.287 | 0.847 |
| IV | 36040.609 | −273.025 | −4595.716 | 0.935 | 0.031 | 0.801 |
| VI | 414341.2 | −444991 | 84760.612 | 0.793 | 0.419 | 0.053 |

**Table 6** $\lambda_{max}$(abs) values of compounds (I–VI) in different solvent media

| Compound | MeOH | MeOH + HCl | MeOH + NaOH |
|---|---|---|---|
| | $\lambda_{max}$ (abs) | $\lambda_{max}$ (abs) | $\lambda_{max}$ (abs) |
| I | 211, 262 | 208, 258, 265 | 211, 272, 281 |
| II | 210, 262 | 213, 261 | 211, 262 |
| III | 255, 273, 282, 291 | 254, 275, 282 | 283, 292 |
| IV | 274, 282, 292 | 276, 283 | 282 |
| V | | 252, 277 | 278 |
| VI | 259, 282 | 261 | 274 |

where, $\nu$(vapor) is the frequency of the spectral maximum in the absence of solvents, and $D$ and $n$ have already been defined. The multiple correlation coefficient and the square of the correlation coefficient were calculated for each $R^2(\nu, n^2)$ and $R^2(\nu, D)$ combination, and the results are summarized in Table 5.

The electronic absorption spectra of compounds (I–VI) in different media are summarized in Table 6, and the emission spectral data of these compounds are summarized in Table 7.

**Table 7** $\lambda_{max}$(abs), $\lambda_{max}$ (em, and $\Delta\nu/cm^{-1}$ for compounds (I–VI) and $\Delta f$ values in different solvents

| Solvent | Compound | λ(abs) | λ/em | $\Delta\nu$ | $\Delta f$[a] |
|---|---|---|---|---|---|
| $CCl_4$ | I | | 340 | | |
| | II | 259 | 430 | 15354.2 | |
| | III | 260 | 326 | 7786.69 | |
| | IV | 260 | 357 | 10450 | |
| | V | 266 | 348 | 8858.35 | |
| | VI | 259 | 363 | 11061.82 | |
| Diethylether | I | 259 | 317 | 7064.29 | |
| | II | 260 | 313 | 6512.65 | 0.162 |
| | III | | 326 | | |
| | IV | | 311, 357 | | |
| | V | | 311, 346 | | |
| | VI | | 361 | | |
| 1,4-Dioxane | I | 261 | 307 | 5740.86 | |
| | II | 261 | 307 | 5740.88 | 0.02 |
| | III | 257 | 313 | 6961.62 | |
| | IV | 257 | 311 | 6756.16 | |
| | V | | 305 | | |
| | VI | 257 | 310 | 6652.44 | |
| $CHCl_3$ | I | 262 | 326 | 7493.09 | |
| | II | 262 | 324 | 7303.74 | 0.148 |
| | III | | 329 | | |
| | IV | | 363 | 11514.29 | |
| | | 256 | | | |
| | V | | 327, 355 | | |
| | VI | 252 | 366 | 12360.13 | |
| DMF | I | 270 | 325 | 6267.8 | |
| | II | 271 | 332 | 6779.88 | 0.274 |
| | III | 269 | 344 | 8104.95 | |
| | IV | 268 | 326 | 6638.58 | |
| | V | | | 5483.41 | |
| | VI | 270 | 341 | 7711.52 | |
| DMSO | I | 266 | 322 | 6538.08 | |
| | II | 265 | 321 | 6583.2 | 0.263 |
| | III | 259 | 342 | 9370.27 | |
| | IV | 263 | 326 | 7347.96 | |
| | V | 263 | 324 | 7158.61 | |
| | VI | 264 | 354 | 9630 | |
| $CH_3CN$ | I | 259 | 311 | 6455.69 | |
| | II | 260 | 377 | 11936.33 | 0.305 |
| | III | 256 | 407 | 14492.47 | |
| | IV | 256 | 322 | 8006.6 | |
| | V | | 344 | | |
| | VI | 254 | 311, 387 | 13530.28 | |

**Table 7** (*Continued*)

| Solvent | Compound | λ(abs) | λ/em | Δν | Δf[a] |
|---|---|---|---|---|---|
| Ethanol | I | 262 | 321 | 7015.29 | |
| | II | 262 | 321 | 7015.29 | 0.289 |
| | III | 255 | 325 | 8446.45 | |
| | IV | 257 | 325 | 8141.27 | |
| | V | 248 | 364 | 12850.05 | |
| | VI | 261 | 323, 367 | 11066.22 | |
| Methanol | I | 262 | 314 | 6320.8 | |
| | II | 262 | 317 | 6622.19 | 0.309 |
| | III | 255 | 327 | 8634.64 | |
| | IV | 256 | 325 | 8293.26 | |
| | V | 248 | 342 | 11082.81 | |
| | VI | 258 | 378 | 12304.66 | |
| Water | I | 262 | 312 | 6116.65 | |
| | II | 262.5 | 321 | 6942.60 | 0.32 |
| | III | 256 | 325 | 8293.27 | |
| | IV | 255 | 327 | 8634.64 | |
| | V | 248 | 344 | 11252.81 | |
| | VI | 260 | 374, 435 | 11723.57 | |

[a] $\Delta f = [(D-1)/(2D+1) - (n^2-1)/(2n^2+1)]$

The Stokes shift $\Delta\nu = (\nu_{abs} - \nu_{em})$ of the same electronic transition was correlated with the orientation polarizability ($\Delta f$) of the solvents [53–57] by

$$\Delta f = [(D-1)/(2D+1) - (n^2-1)/(2n^2+1)] \tag{3}$$

and

$$\Delta\nu = \left\{\frac{2(\mu e - \mu g)^2}{hca^3}\right\}\Delta f + K \tag{4}$$

where $\nu_{ab}$ and $\nu_{em}$ are the peak absorption and emission frequencies in $cm^{-1}$, $\mu e$ and $\mu g$ are the dipole moments of each compound in their excited and ground states, $D$ and $n$ have already been defined, $h$ is Planck's constant, $c$ is the velocity of light, $a$ is the Onsager cavity radius for each molecule, and $K$ is a constant.

## 4 Discussion

The electronic absorption and fluorescence emission spectra of these compounds (see Tables 2 and 7, Figs. 1–10) were recorded in solvents of different physico-chemical properties, mainly different dielectric constants and the refractive indexes, and in different media.

Generally, the effect of different solvents on the absorption bands of these substances consists of displacements, and does not involve a fundamental change in the general form of the spectrum. The absorption and emission spectra of these compounds are related to the organic solvent parameters.

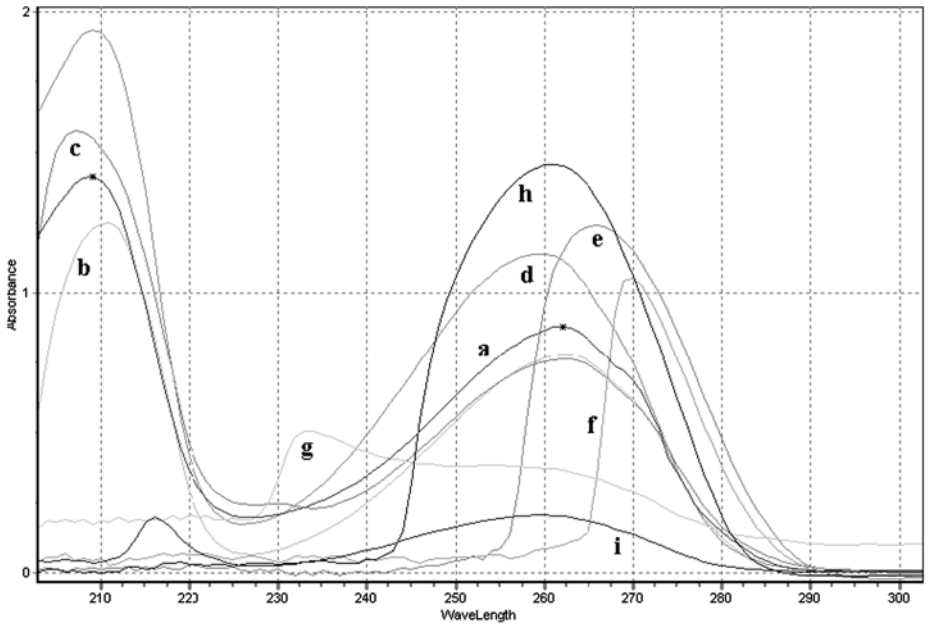



**Fig. 1** Electronic absorption spectra of adenine in different solvents: (**a**) water, (**b**) methanol, (**c**) ethanol, (**d**) acetonitrile, (**e**) dimethyl sulfoxide, (**f**) dimethylformamide, (**g**) chloroform, (**h**) 1,4-dioxane, and (**i**) diethylether

## 4.1 Spectrophotometric Study

### *4.1.1 Electronic Absorption Spectra*

Molecules containing both the amine (-$NH_2$) or hydroxyl (-OH) groups, and the imine (-N=C) or carbonyl (>C=O) groups, show an important process known as excited state intramolecular proton transfer, provided that the two groups are connected by intramolecular hydrogen bonding in the ground state [58]. These types of systems have been widely used as light photodetectors, materials in continuous lasers, as well as having many applications in biology, and many studies has been conducted in order to understand the dynamic and photophysics of this process [59, 60].

The adenine derivatives (III) and (IV) and the guanine derivative (VI) show extra absorption bands when compared to the parent compounds adenine (I) and guanine (V), due to the presence of the carbonyl chromophore at N(6) in compounds (III) and (IV), and at N(2) and N(9) in compounds (VI). These are of the $\pi - \pi^*$ (>C=O), one charge transfer (involving $e^-$ transfer from an adjacent N to the >C=O group), and $n - \pi^*$ (>C=O) types. The hydroxyethyl group at the N(9) position of (II) does not cause any observable increase in the number of absorption bands in comparison to adenine.

The electronic absorption spectra of compounds (I), (II), (III), (IV) and (VI) in different solvents show a weak absorption band (band A) in the range 256 to 271 nm due to a $\pi - \pi^*$ electronic transitions, and this band is red shifted when proceeding from the nonpolar solvent $CCl_4$ ($\lambda_{max} = 259$ nm) to the polar solvent DMF ($\lambda_{max} = 271$ nm), see Table 2 and Figs. 1–3. This shift is mainly due to solute-solvent interactions that cause stabilization of the $\pi^*$ orbital more than the $\pi$ orbital in polar solvents. The first $\pi - \pi^*$ band of some

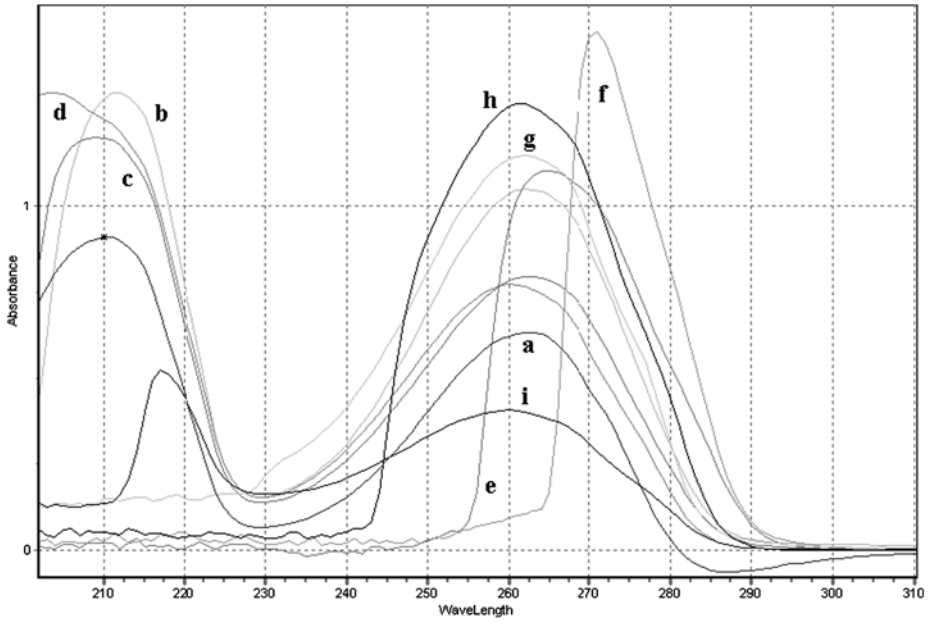



**Fig. 2** Electronic absorption spectra of $N(9)$-hydroxyethyladenine in different solvents: (**a**) water, (**b**) methanol, (**c**) ethanol, (**d**) acetonitrile, (**e**) dimethyl sulfoxide, (**f**) dimethylformamide, (**g**) chloroform, (**h**) 1,4-dioxane and (**i**) diethylether

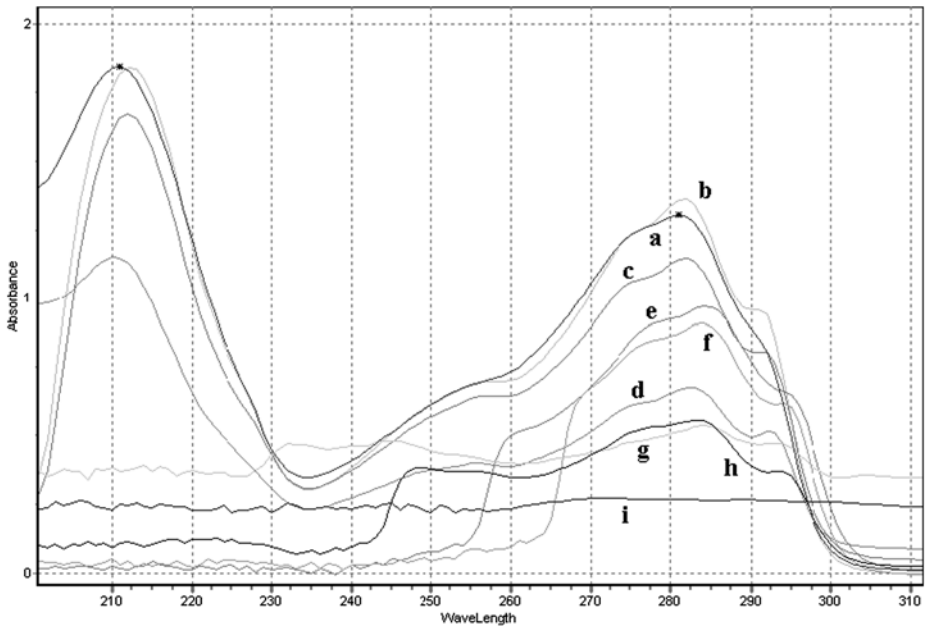



**Fig. 3** Electronic absorption spectra of $N(6)$-acetyladenine in different solvents: (**a**) water, (**b**) methanol, (**c**) ethanol, (**d**) acetonitrile, (**e**) dimethyl sulfoxide, (**f**) dimethylformamide, (**g**) chloroform, (**h**) 1,4-dioxane, and (**i**) carbontetrachloride

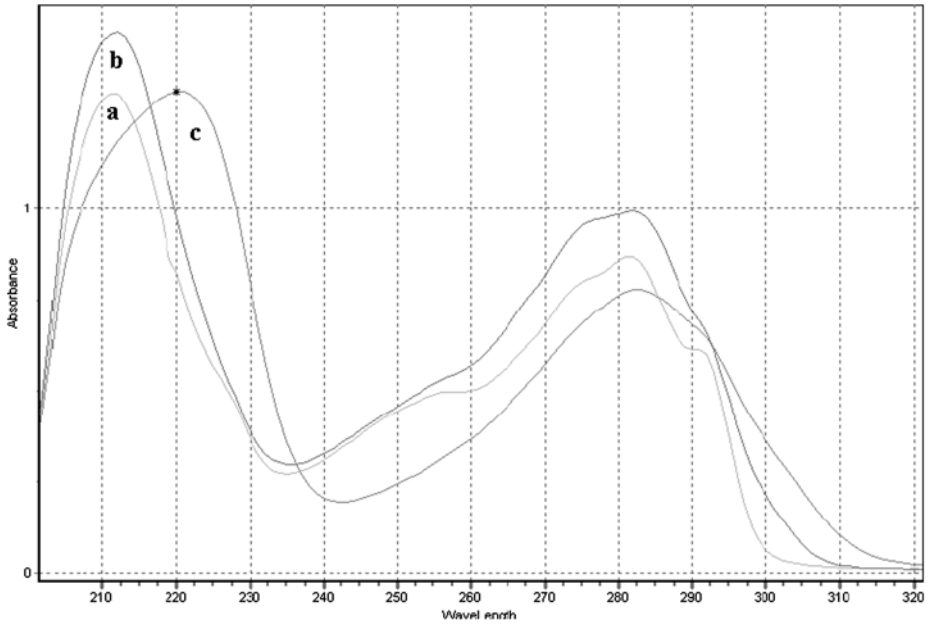



**Fig. 4** Electronic absorption spectra of *N*(6)-acetyladenine at different pHs: (**a**) pH = 6.4 (methanol), (**b**) pH = 1.5 (3:1 methanol-water mixture) and (**c**) pH = 9.6 (3:1 methanol-water mixture)

substituted pyrimidines and purines was treated as being an analogue of the 260 nm band of benzene [61]. Quantum mechanical calculations place one or more $n - \pi^*$ states in this energy region as the lowest $\pi - \pi^*$ state for compound (I).

The second band (band B) in these compounds falls in the range of 270 to 279 nm, and is due to a $\pi - \pi^*$ transition as revealed by the bathochromic shift in the spectrum of compound (IV) when proceeding from the solvents 1,4-dioxane and $CHCl_3$ ($\lambda_{max} = 277$ nm) to DMF and DMSO ($\lambda_{max} = 278$ nm). This shift can be explained by the hydrogen-donor ability of the compounds and the hydrogen-acceptor nature of DMF and DMSO. The band B shows a small blue shift in its $\lambda_{max}$ value in ethanol (see Table 2) compared to those found in DMF and DMSO. This shift can be explained based on the amphiprotic nature of ethanol, i.e., the -OH moiety behaves as a proton donor or acceptor toward the NH, N-C=O groups via H-bonding, thereby compensating each other as indicated by the small blue shift observed in alcohol that is the net effect of increased solvent polarity.

The broad nature of some bands is probably due to mixing of the ground state of the hydrogen-bonded complex with different electronic states, resulting from charge transfer from the electron donor atom.

The spectra of compounds (III), (IV) and (VI) show a new band (band C) in the range 281 to 285 nm that is attributed to a charge transfer or to $\pi - \pi^*$ transition that shows a red shift when proceeding from protic solvents (water, $\lambda_{max} = 281$ nm; methanol, $\lambda_{max} = 282$ nm) to aprotic solvents (DMF, $\lambda_{max} = 283$ nm; DMSO, $\lambda_{max} = 284$ nm).

It is well known that compounds containing lone electron pairs undergo a blue shift (to higher energy) in their $n - \pi^*$ electronic transition when solvation occurs. The reason for this blue shift is a lowering of the energy of the lone-pair orbital caused by the molecule's strong interaction with protic solvents, such as water or alcohols, through hydrogen-bond formation.

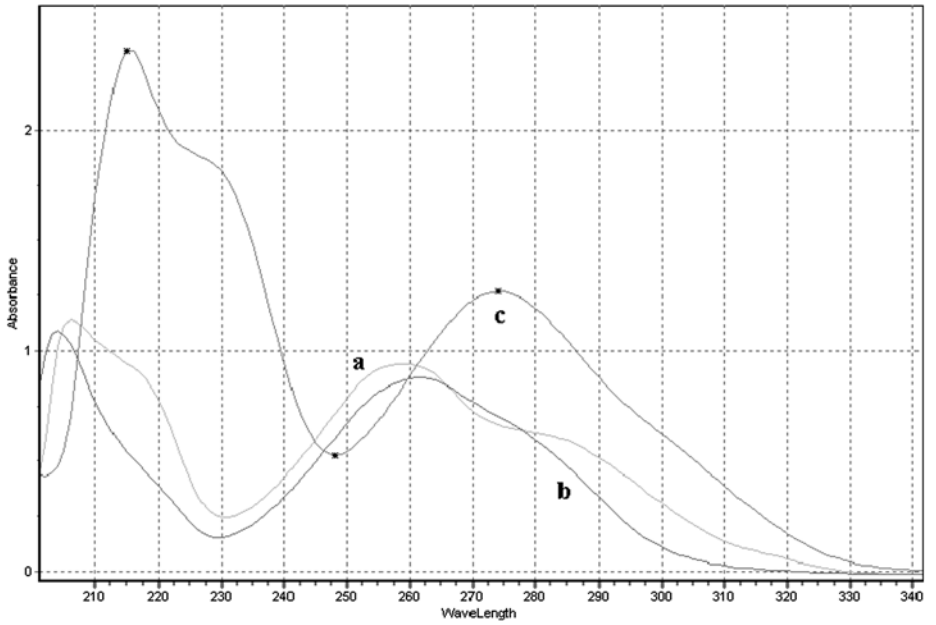



**Fig. 5** Electronic absorption spectra of: $N(2), N(9)$-diacetylguanine at different pHs: (**a**) pH = 6.4 (methanol), (**b**) pH = 1.5 (3:1 methanol-water mixture) and (**c**) pH = 9.6 (3:1 methanol-water mixture)

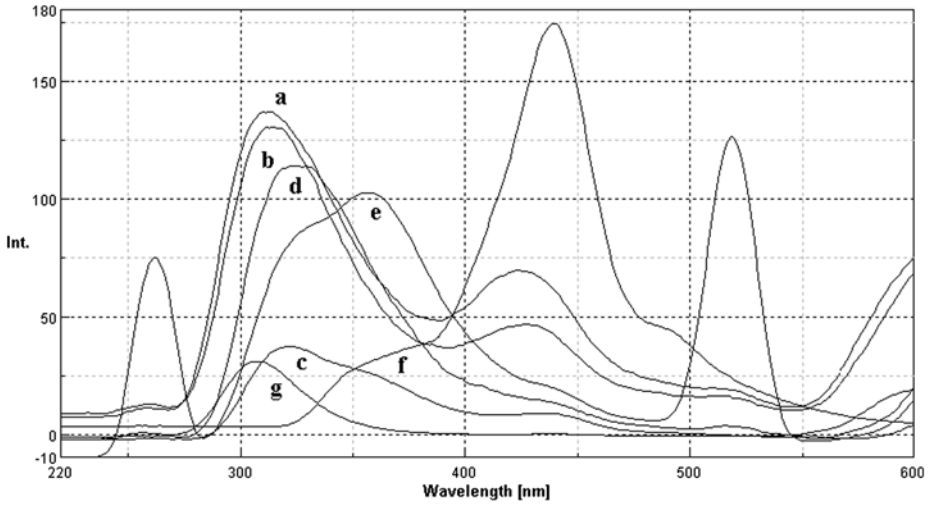



**Fig. 6** Excitation-emission spectra of adenine in different solvents: (**a**) water, (**b**) methanol, (**c**) dimethyl sulfoxide, (**d**) dimethylformamide, (**e**) chloroform, (**f**) acetone, and (**g**) 1,4-dioxane

The presence of lone electron pairs on the "N" and "O" atoms in compounds (III), (IV) and (VI) resulted in spectral bands (band D) that appear as a shoulder near 290 nm for a $n - \pi^*$ transition, due to substituents and solvent effect (mainly hydrogen bonding with the solvents). This assignment is supported by the spectral behavior of these compounds in

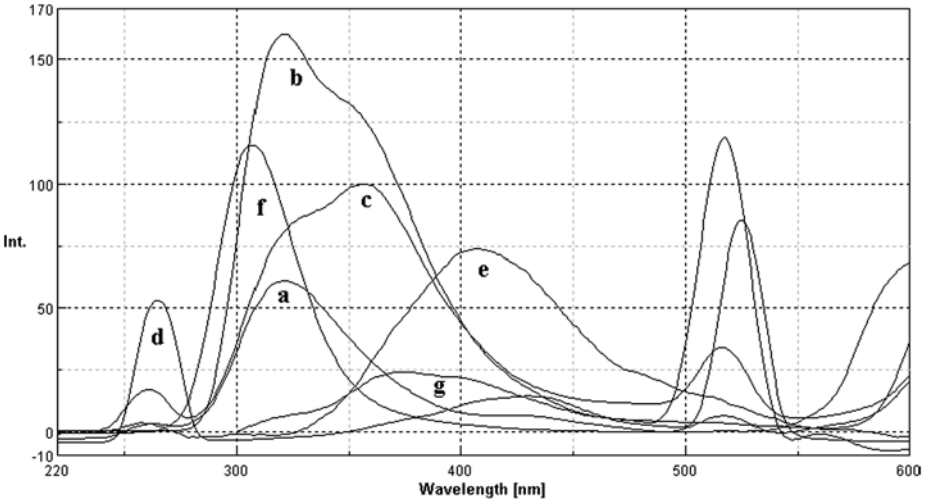



**Fig. 7** Excitation-emission spectra of $N(9)$-hydroxyethyladenine in different solvents: (**a**) ethanol, (**b**) dimethyl sulfoxide, (**c**) chloroform (**d**) carbontetrachloride, (**e**) acetone, (**f**) 1,4-dioxane, and (**g**) acetonitrile

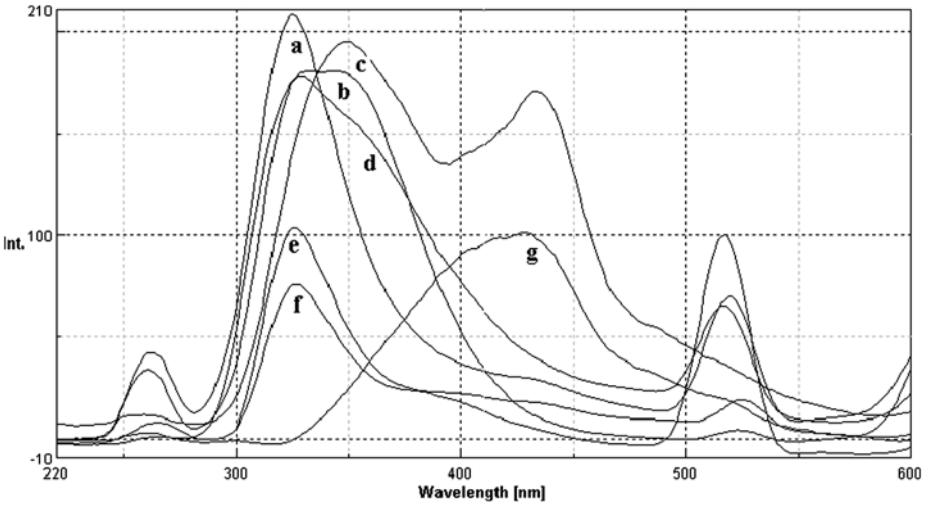



**Fig. 8** Excitation-emission spectra of $N(6)$-acetyladenine in different solvents: (**a**) ethanol, (**b**) dimethyl sulfoxide, (**c**) dimethylformamide, (**d**) chloroform, (**e**) diethylether, (**f**) carbontetrachloride, and (**g**) acetone

acidic solutions of varying pH, where the $n - \pi^*$ band disappears in acidic medium due to protonation, see Figs. 4 and 5. Thus, excitation of $n$-electrons is expected to be hindered. This $n - \pi^*$ transition shows a shift to shorter wavelengths with increasing polarity: $CCl_4$ ($\lambda_{max} = 294$ nm), $CHCl_3$ ($\lambda_{max} = 294$ nm), ethanol ($\lambda_{max} = 292$ nm), methanol ($\lambda_{max} = 291$ nm), and then water ($\lambda_{max} = 292$ nm), see Table 2 and Fig. 3. This shift is caused by the ground state being stabilization more than the excited state due to superior solvation of the polar ground state by the polar solvent. This has been attributed to factors such as specific interactions, e.g., hydrogen bonding in hydroxylic solvents, and more generally to solvent

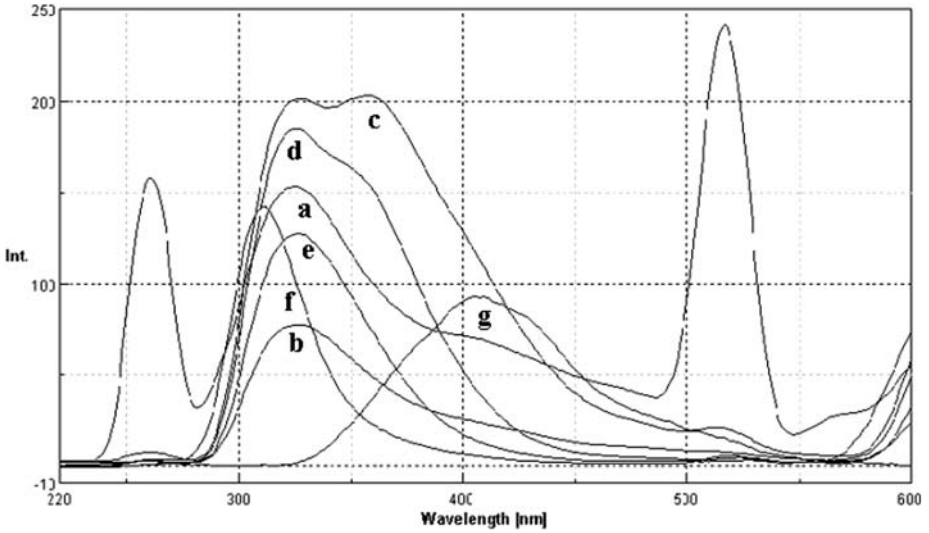



**Fig. 9** Excitation-emission spectra of $N(6)$-isobutyryladenine in different solvents: (**a**) water, (**b**) methanol, (**c**) ethanol, (**d**) dimethyl sulfoxide, (**e**) dimethylformamide, (**f**) 1,4-dioxane, and (**g**) acetone

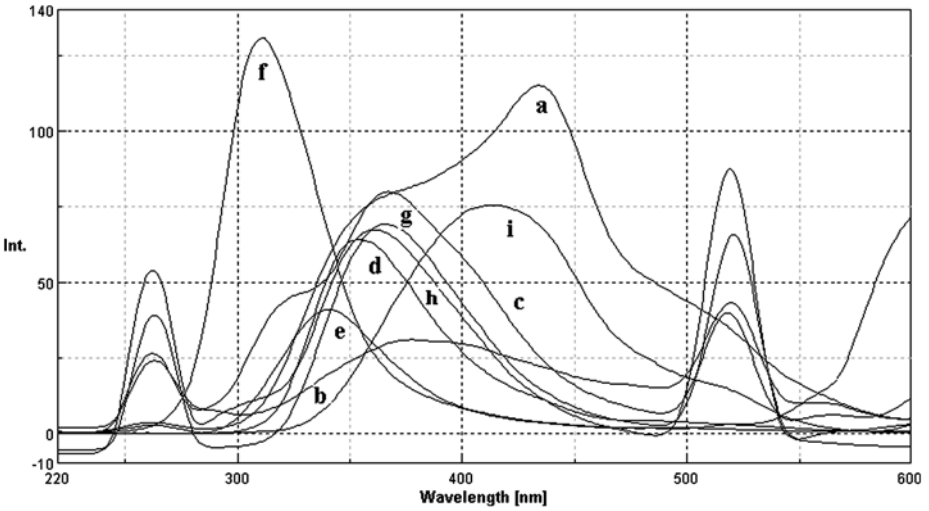



**Fig. 10** Excitation-emission spectra of $N(2), N(9)$-diacetylguanine in different solvents: (**a**) water, (**b**) methanol, (**c**) ethanol, (**d**) dimethyl sulfoxide, (**e**) dimethylformamide, (**f**) 1,4-dioxane, (**g**) chloroform, (**h**) diethylether, and (**i**) acetone

dielectric effects such as dipole-dipole and dipole-induced-dipole interactions, as well as packing and orientation strain in the Franck-Condon excited state.

However, the absence of $n - \pi^*$ bands in the spectra of the other compounds can presumably be ascribed to their being submerged into the $\pi - \pi^*$ or intramolecular charge-transfer bands.

### *4.1.2 Solvatochromism Absorption Correlation Studies*

The spectral shifts in solvents of different polarities are consequences of differences in solvation of species between the ground state and excited state. These shifts in the spectral position reflect the influence of solvent polarity that depends, according to Reichardt [22], on the solvation power of the solvent, which in turns depends on the action of specific and non-specific intermolecular interactions between solute and solvent molecules. Multi-parameter treatments of solvent effects were studied as candidates for a complete description of the solute spectral changes.

From the correlation of $\lambda_{max}$ (band A) for compounds (I) and (II), $\lambda_{max}$ (band C) for compounds (III) and (VI), and $\lambda_{max}$ (band D) for compound (IV) with the solvent parameters, an idea was reached about the type of interaction between the solute and solvent. The solvatochromic shift reveals the effect of the dielectric constant and refractive index of the solvent as well as solute-solvent H-bond interactions.

The correlations of parameter $E$ for compounds (I) and (II) at $\lambda_{max}$ of band A are 0.085 and 0.213, respectively (see Table 3). This indicates that there is no remarkable solute-solvent hydrogen bonding or dipolar interactions, in comparison to compounds (III) (band C) and (IV) (band D) with an acetyl or isobutyryl group present at the N(6) position where the values for the correlations of $E$ are 0.84 and 0.635, respectively (see Table 3). In addition, the simultaneous influence of the solvent's dielectric constant $D$ and refractive index $n$ on the position of $\lambda_{max}$ is evident by the high multiple-correlation coefficient ($MCC$ greater than 0.5) when using a two-parameter $(D, n)$ equation; the $MCC$ is equal to 0.729, 0.626, 0.959, 0.908 and 0.711 for compounds (I), (II), (III), (IV) and (VI), respectively (see Table 3). Moreover, for compounds (III) and (IV), improvement of the fits occurred when using three parameters ($D, n$ and $E$) since higher correlation coefficients were obtained in that case ($MCC = 0.96$ and 0.925). This indicates that H-bonds play a role in the observed spectral shift. In addition, an influence of the refractive index on the spectral shift of $\lambda_{max}$ is clearly observed for these compounds as in case of compound (VI) where a higher $MCC$ (0.708) was obtained for $\lambda_{max}$ of (Band C). However, the $MCC$ value obtained when using other parameters is 0.089 for the dielectric constant $D$ and 0.263 for electronic parameter $E$, indicating there were no significant influences of these parameters on the spectra shift of compound (VI) due to solvent change (see Table 3).

Correlations among the $\lambda_{max}$ values of compounds (I), (II), and (III) with either $E$ (solvent polarity parameter), $K$ (function of the dielectric constant), $M$ (function of the refractive index) or $N$ (a measure of permanent dipole-permanent-dipole interaction) parameters, see Table 4, shows similar dependences of $\lambda_{max}$ of these compounds on the $M$ parameter ($MCC > 0.5$). A larger influence was observed for the other parameters on the wavelength of compound (III) as indicated by the high correlation with the $E$ ($MCC = 0.84$), $K$ ($MCC = 0.962$), $M$ ($MCC = 0.522$), or $N$ ($MCC = 0.618$) parameters compared to the lower correlation values for compounds (I) and (II) ($MCC < 0.5$).

Compounds (I) and (II) show higher correlations using two-parameter equations with $(M, N)$ than with either the $(E, M)$ or $(E, N)$ parameter pairs. Using a three-parameter equation, compound (III) shows high correlations for the $(E, K, M)$, $(E, N, K)$, $(E, M, N)$, and $(M, N, K)$ parameter triplets ($MCC > 0.9$).

The values of $K_1$, $K_2$, $\nu$(vapor), $R^2(\nu, D)$, $R^2(\nu, n)$ and $MCC$ for compounds (I–III) were computed and listed in Table 5. The data indicate that both the dielectric constant and refractive index of the solvents affect the electronic absorption spectra of compounds, but to varying degrees. The negative values of $K_1$ and $K_2$ indicate the occurrence of strong solute-solvent interactions that cause a decrease in energy for the electronic transition from LUMO to HOMO, in comparison with the vapor phase.

#### *4.1.3 pH Effect on the Absorption Spectra*

The effect of pH change on the electronic absorption spectra, see Figs. 4 and 5, was studied for compounds (I–VI) in different media (see Table 6).

The appearance of new bands for compound (I) at $\lambda_{max} = 258$ nm in acidic (pH = 1.5) and at $\lambda_{max} = 281$ nm in basic medium (pH = 9.6) confirms the formation of new cationic and anionic species, respectively. For compound (II) there is minor shift in $\lambda_{max}$ while going from acidic (pH = 1.5) to basic medium (pH = 9.6), see Table 6.

The shape of the absorption curve for compound (III) shows a difference in the three media, see Fig. 4, where the bands at $\lambda_{max} = 254$ nm and 275 nm become shoulders in acidic media (pH = 1.5) but disappear in basic media (pH = 9.6), which implies the formation of cationic-anionic species or keto-enol tautomerism. The disappearance of the peak at $\lambda_{max} =$ 290 nm for compounds (III), (IV) and (VI), see Figs. 4 and 5, in acidic media (pH = 1.5) confirms the assignments of this band to a $n - \pi^*$ transition.

### 4.2 Fluorescence

#### *4.2.1 Fluorescence Measurements*

The shape of the excitation spectrum is shown to coincide reasonably well with the absorption spectrum. The fluorescence excitation spectra were measured on samples with maximum absorbance less than 0.1 in order to avoid inner filter distortion. The excitation wavelength was fixed at 260 nm, and the emission spectra were recorded in different solvents.

#### *4.2.2 Solvent Effect on Fluorescence Spectra*

The shape of the emission spectrum in all of the different solvents studied is independent of the excitation wavelength. The solvent effect on the spectral positions of the fluorescence bands of compounds (I–VI) is summarized in Table 7 and in Figs. 6–10. The spectral shift is correlated with the polarity parameters (dielectric constant and refractive index) of the solvent, see Fig. 11. The Stokes shift is one of the quantitative parameters that is useful for understanding the origin of the variation of the spectral shift in organic solvents, and can be used to obtain information about the type of solute-solvent interactions. The large Stokes shift (see Table 7) indicates that an increase in the dipole moment occurs upon excitation. This behavior has been attributed to a charge redistribution of the excited state with respect to the ground state.

The Stokes shift $(\Delta\nu) = (\nu_{ab} - \nu_{em})$ was plotted versus the solvent orientation polarizability $(\Delta f)$ for compounds (I–III) in the various solvents, see Fig. 11. For compound (I) the plot shows an almost linear dependence of $\Delta\nu$ on $\Delta f$ with $R^2 = 0.84$, which validates the Lippert-Mataga equation [53–57] while excluding the solvents DMSO, acetonitrile, and $CHCl_3$. This linearity confirms that the slope depends on the change in dipole moment that occurs upon excitation. A similar plot for compound (II) also shows a linear dependence with $R^2 = 0.88$ when acetonitrile and $CHCl_3$ are excluded. A value of $R^2 = 0.9$ was obtained in the case of compound (III) in different solvents excluding acetonitrile, 1,4-dioxane and DMSO. This anomaly with 1,4-dioxane and acetonitrile has been documented before in the literature [62], and is due to conformation polarization and solute dipole-solvent quadrupole interactions in the case of dioxane, and to self-association in the case of acetonitrile. The red shift found in the emission spectra in water (and other protic solvents) as shown for compound (VI) could be due to hydrogen bonding of water to specific sites of the purine rings, because the electron density greatly increases in the rings in their excited state through

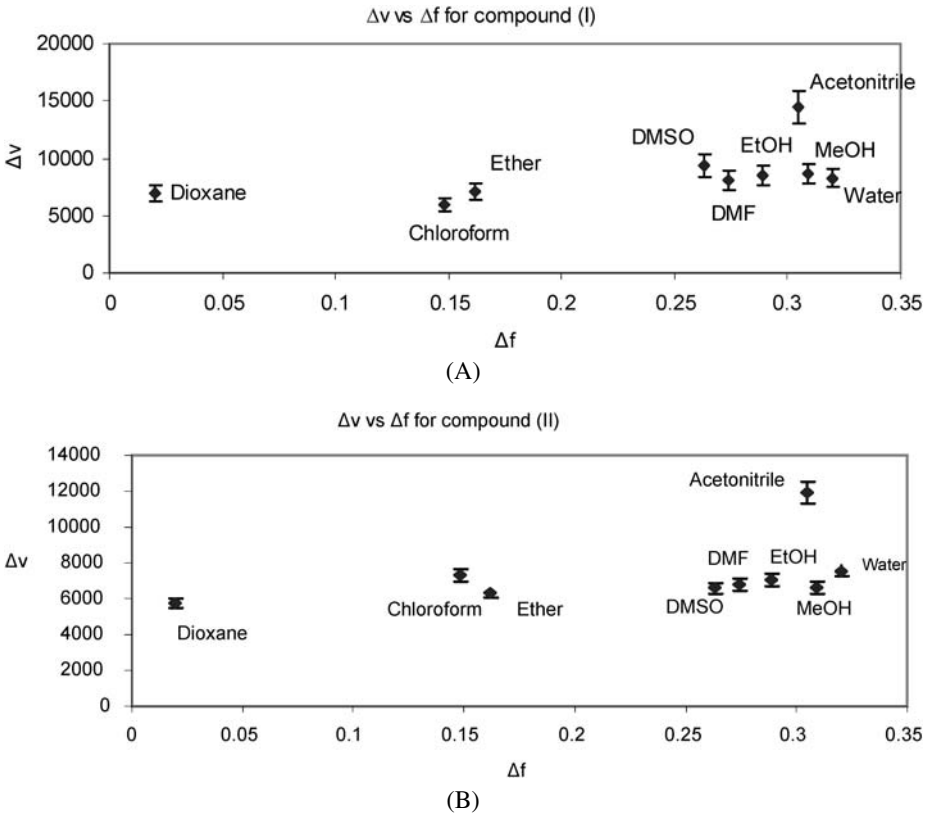



**Fig. 11** Plots of the Stokes shift ($\Delta\nu$) versus the solvent polarity parameter ($\Delta f$): (**A**), compound (I), and (**B**), compound (II), in solvents with different polarities

charge transfer from the amino group. Indeed, the above facts suggest that solvent-solute complexation occurs in the first excited singlet state.

The fluorescence spectra of adenine and $N(9)$-hydroxyethyladenine, see Figs. 6 and 7, show an emission at $\lambda_{max} = 340$ nm in $CCl_4$ and at $\lambda_{max} = 326$ nm in $CHCl_3$ due to a $n - \pi^*$ transition that undergos a blue shift with increasing polarity and hydrogen-bonding capacity of the solvents ($\lambda_{max} = 314$ and 312 nm in methanol and water, respectively). Compounds (III) and (IV) show an emission band, see Table 7, at $\lambda_{max} = 326$ nm and $\lambda_{max} = 353$ nm, respectively, which is blue shifted when going to more polar solvents ($\lambda_{max} = 325$ nm in ethanol), thus indicating a $n - \pi^*$ transition. Compound (VI) shows a red shift, see Table 7, when switching from DMF ($\lambda_{max} = 341$ nm) and DMSO ($\lambda_{max} = 354$ nm) to water ($\lambda_{max} = 374$ nm) and methanol ($\lambda_{max} = 378$ nm) due to a $\pi - \pi^*$ transition. The emission of compound (V) at $\lambda_{max} = 348$ nm in $CCl_4$ shows a blue shift when going to more polar solvents ($\lambda_{max} = 344$ nm in water) thus indicating a $n - \pi^*$ transition [19, 63].

### 4.3 Spectrofluorometric Determination of the Components of a Ternary Mixture of Adenine, $N(9)$-hydroxyethyladenine and $N(6)$-acetyladenine

Salinas et al. [46] introduced a method for resolving binary mixtures based on the use of the first derivative of the ratio spectrum. The absorption spectrum of the mixture is divided by

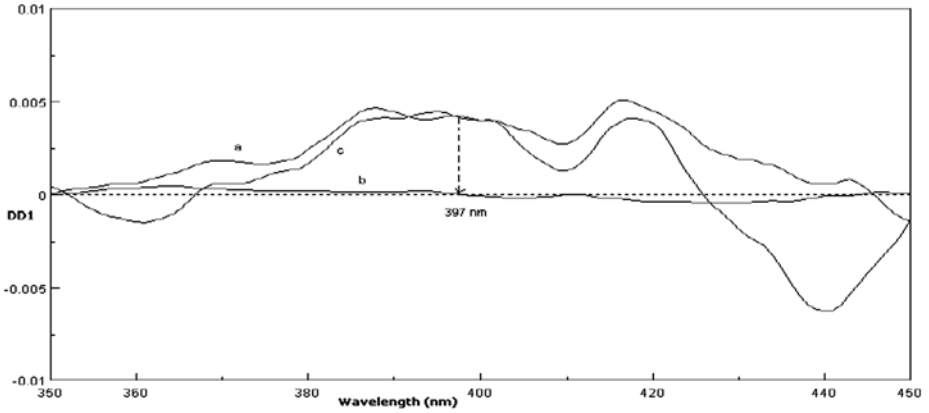



**Fig. 12** First derivative ratio spectra of: (**a**) $1.1 \times 10^{-7}$ mol·L$^{-1}$ (I), (**b**) $1.1 \times 10^{-7}$ mol·L$^{-1}$ (III), (**c**) mixture of $1.1 \times 10^{-7}$ mol·L$^{-1}$ of compounds (I), (II) and (III) using (II) as the divisor

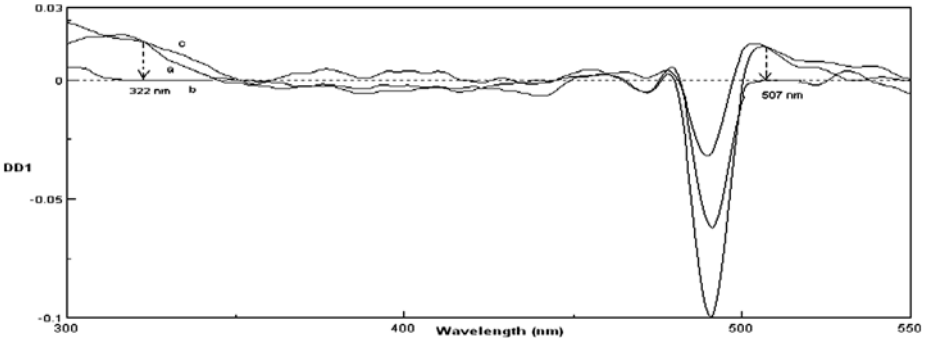



**Fig. 13** First derivative ratio spectra of: (**a**) $2 \times 10^{-6}$ mol·L$^{-1}$ (III), (**b**) $2 \times 10^{-6}$ mol·L$^{-1}$ (II) and (**c**) $2 \times 10^{-6}$ mol·L$^{-1}$ mixture of compounds (I), (III) and (II) using (I) as the divisor

the absorption spectrum of a standard solution of one of the three components, and the first derivative of the ratio spectrum is obtained. The concentrations of the active components are then determined from the calibration curves obtained from measuring the amplitude at points corresponding to the minimum or maximum wavelengths. This method has been extended to determine ternary mixtures spectrophotometrically, by the use of Salinas's method and the zero-crossing technique [47, 48].

In addition, the same method has been applied for the analysis of the fluorescence spectra of ternary drug mixtures [64]. In the present paper, we apply this technique to the spectrofluorometric determination of a mixture of compounds (I), (II) and (III), and was carried out to analyze any component in the presence of the others (see Figs. 12–16). The aim is to develop a method to monitor any modification that could happen to the basic structure of nucleobases due to alkylating or acetylating agents. The emission spectra of the three components strongly overlap, so no direct determination could be carried out, see Fig. 14. Moreover, the application of the 1$^{st}$ and 2$^{nd}$ derivative techniques could not solve their overlap, see Figs. 15 and 16, due to the lack of any zero-crossing point. For this reason it was useful to develop a spectrofluorometric derivative ratio method for the determination of their concentrations, see Figs. 12 and 13.

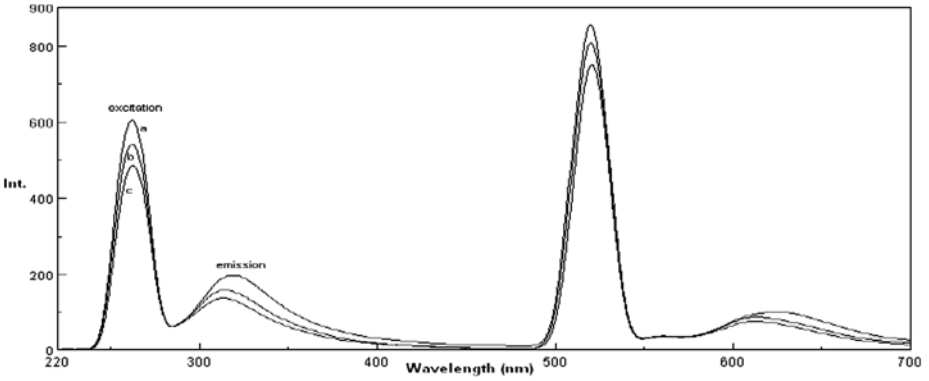



**Fig. 14** Excitation and emission spectra of: $10^{-5}$ mol·L$^{-1}$ of (**a**) (III), (**b**) (I), and (**c**) (II)

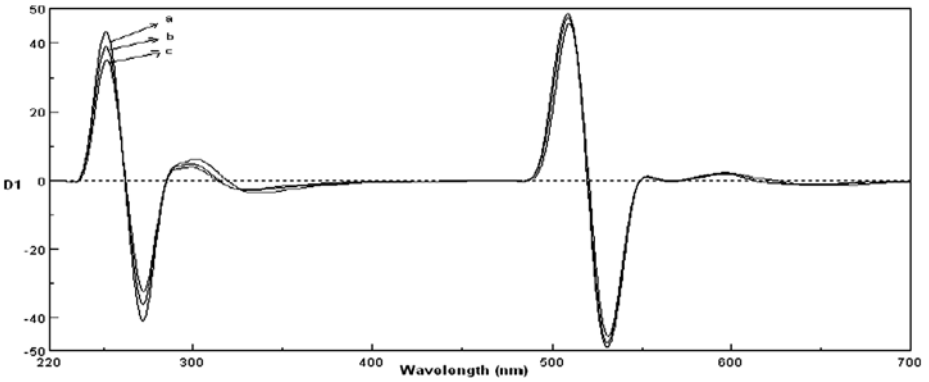



**Fig. 15** First derivative spectra of $10^{-5}$ mol·L$^{-1}$ of: (**a**) (III), (**b**) (I), and (**c**) (II)

The excitation spectrum of each component (I–III) was measured and the excitation wavelengths were found to be 273, 285, and 295 nm. The emission of each of the three components were studied at the three excitation wavelengths. The optimum excitation wavelength was found to be the one that yielded the highest emission for all three components, 274 nm. Emission spectra for the three components were measured and stored. To determine each of the three components, the other two components were tried as a divisor, and selection of the suitable divisor was based on finding zero-crossing points upon calculation of the first and second derivative.

Therefore, for the determination of compounds (I) and (III), the stored emission spectra of standard solutions of (I), (II), (III), and solutions of their mixtures in acetonitrile were divided (amplitude by amplitude at appropriate wavelengths) by the emission spectrum of a standard solution of $1.1 \times 10^{-7}$ mol·L$^{-1}$ of (I). Then, the first derivatives of the obtained ratio spectra were calculated with $\Delta\lambda = 1$ nm (see Fig. 13). This figure showed that the first derivative of the ratio spectrum of the mixture consists only of those of compounds (II) and (III), where the corresponding values for compound (I) were equal to zero. From this figure, the amounts of compounds II and III can be determined in this mixture by measuring the amplitude at 507 nm (zero-crossing point of compound III) and 322 nm (zero-crossing point of compound II).

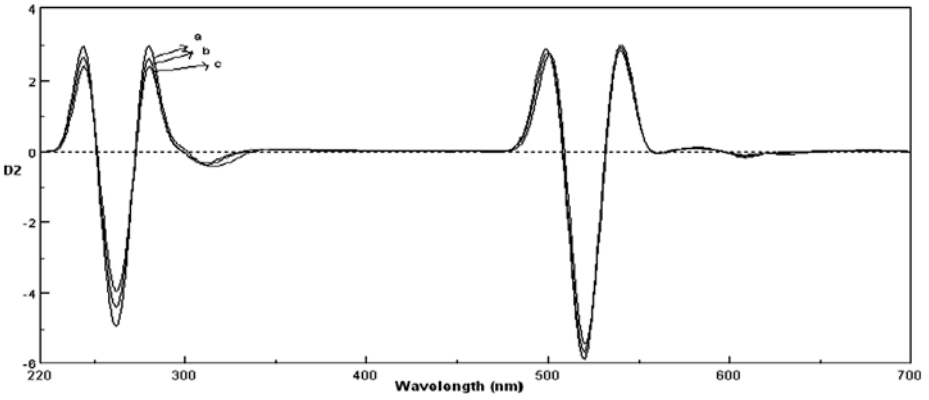



**Fig. 16** Second derivative spectra of $10^{-5}$ mol·L$^{-1}$ of: (**a**) (III), (**b**) (I), and (**c**) (II)

On the other hand, for the quantization of compound (I), an analogous procedure was followed, where the emission spectra of the three standard solutions and the mixture solutions were divided (amplitude by amplitude) by the emission spectrum of a $1.1 \times 10^{-7}$ mol·L$^{-1}$ standard solution of compound (II), and the first derivative of the developed ratio spectra were obtained with $\Delta\lambda = 1$ nm (see Fig. 12). This figure showed that the first derivative of the ratio spectrum of the mixture consists only of those of compounds (III) and (I), where the corresponding values of compound (II) were equal to zero. Thus, compound (I) can be assayed by measuring the amplitude at 397 nm that corresponds to the zero-crossing point of compound (III).

## 5 Conclusion

The electronic absorption and emission spectra of a series of purine derivatives (I–VI) were determined in solvents having different physico-chemical properties.

The results presented here help us develop a qualitative understanding of medium effects (solvent and pH) on the spectral properties of some purine molecules. We conclude that the effects of solvent properties on the UV-VIS and fluorescence spectra of nucleic bases and their analogues cannot be ignored. Moreover, the presence of different functional groups at various sites on the pyrimidine and imidazole rings affects the shift in the spectra of each solute molecule in different solvents. In each case, variations in correlations among the values of $\lambda_{max}$ of the solute and physical properties of the solvent are controlled by the interaction between the functional group of the solute (electron donor, electron acceptor or charge transfer ability) and the solvent molecule. These are due to specific and non-specific types of interactions.

The multi-parameter Eqs. 1 and 2 for analyzing absorption spectra gave different multiple correlation coefficient values. This indicates that the empirical expressions used are successful for evaluating solvent effects on the electronic spectra of compounds (I–VI).

A considerable shift in the emission spectra of these compounds was observed in the organic solvents. The spectral shift was correlated, see Eq. 4, with the polarity parameters ($D$ and $n$) and this is indicative of an increase in the dipole moment upon excitation. However, we cannot neglect the involvement of the H-bond in the emission spectral shift where its contribution is different for each solute. The data suggest that non-specific interactions are the key factors in shifting the fluorescence maxima for these molecules.

**Acknowledgement** The authors thanks Dr. Fawzi El-Yazbi for his contribution to the spectrofluorometric analysis.

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