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Physico-chemical properties and spectrophotometric determination of biologically active 1-alkyl-2-(2-pyridyl)pyridinium bromides

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Physico-chemical properties of compounds prepared from 2,2′-bipyridine, 1-alkyl-2-(2-pyridyl)pyridinium bromides, were investigated by DC polarography and by GC-MS. Their ionization potentials were calculated. Additionally, the formation of associates with bromothymol blue and methyl orange during the spectrophotometric determination was measured. It was determined that 1-alkyl-2-(2-pyridyl)pyridinium ions are reduced by 2 one-electron steps in a DC polarography system. The reduction potentials are not related to the ionization potential values calculated for the substances investigated. The carcinogenic potential (tg α) of the parent compound 2,2′-bipyridine and of a series of 1-alkyl derivatives was very low indicating that the compounds are not carcinogenic. The MS fragmentation patterns indicate the low stability of the 1-alkyl substituents. It was shown that 2,2′-bipyridine is either fragmented to two pyridine ions or the $-$N=CH$-$ fragments are removed. Additionally, spectrophotometric determinations of colored associates of 1-alkyl-2-(2-pyridyl)pyridinium bromides with bromothymol blue and methyl orange were investigated and the optimal condiditions for these determinations are reported.

1. Introduction

Organic ammonium salts represent an interesting group of compounds possessing a variety of biological effects, e.g. antimicrobial and herbicidal [1–4]. These result from their physico-chemical properties that also make them active as detergents, emulgators and tensides [2, 3]. Recent studies have described the relationship between the physico-chemical properties of bipyridine derivatives and their biological activity [2]. Other studies have described their ability to form biologically active metal complexes [5, 6].

The goals of this work were to of investigate some physico-chemical properties of a series of 2,2′-bipyridine alkyl derivatives, namely their polarographic reduction and potential carcinogenicity index, to investigate the degradation pattern in the GC-MS system and to calculate their ionization potentials. Additionally, we developed a spectrophotometric method of analysis for the compounds investigated.

2. Investigations, results and discussion

Polarographic reduction of the series of compounds consisting of 2,2′-bipyridine and of its 1-alkyl-2-(2-pyridyl)pyridinium bromides was performed in anhydrous conditions in dry *N*,*N*-dimethylformamide. All of the 2,2′-bipyridine alkyl derivatives were reduced by two one-electron steps. The values of the reduction potentials $E_{1/2}$ were very similar for all the alkyl derivatives investigated (Table 1). The only difference observed was in the nature of the adsorption wave present in recorded polarograms of the alkyl derivatives. The octyl derivative, ($C_{10}H_8BrN_2R$ where $R = -C_8H_{17}$), was the only compound that demonstrated an adsorption anodic-cathodic wave at -0.15 vs. SCE. All the other alkyl derivatives demonstrated an adsorption anodic wave at potentials ranging from -0.08 to -0.15 V vs. SCE. The reduction pathway of the 1-alkyl-2-(2-pyridyl)pyridinium bromides is shown in Scheme 1. It is likely that the first reduction step results in the removal of an alkyl group from a polarographed derivative and that the second step completes the reduction of the 2,2′-bipyridine moiety. This statement is justified by the fact that the first reduction wave in the reduction of the alkyl derivatives of 2,2′-bipyridine occurs at a potential of $E_{1/2}$ -0.95 to -1.00 V vs. SCE. No reduction step of the parent substance occurs at this potential.

The parent compound, 2,2′-bipyridine, was reduced by a different mechanism via three one-electron steps at potentials between -2.00 V and -2.55 V vs. SCE. It was the only compound, for which no adsorption wave was recorded. The participation of three electrons in the reduction of 2,2′-bipyridine indicates that the reduction involves intra-molecular processes that do not occur during the reduction of its alkyl derivatives.

The parameter of potential carcinogenicity tg α was determined in the presence of α-lipoic acid (LA) by a method described previously [7–11]. LA has an ability to form polarographically recognized complexes with carcinogens. However, 2,2′-bipyridine was the only compound from

Table 1: Compounds investigated with data regarding their polarographic reducibility, potential carcinogenicity and ionization potential

Formula	R	MW (g/mol)	$E_{1/2}$ (V) SCE	tg α	Ionization potential (kcal)	Retention time of 1-bromoalkans formed during GC (min)
$C_{10}H_8N_2$	–	156.19	−2.00 −2.25 −2.55	0.0046	13.41	16.05
$C_{10}H_8BrN_2R$	C_8H_{17}	349.32	−0.13 −1.00 −2.51	–	13.00	8.88
$C_{10}H_8BrN_2R$	$C_{10}H_{21}$	377.38	−0.15 −1.00 −2.55	–	12.81	13.67
$C_{10}H_8BrN_2R$	$C_{11}H_{23}$	391.40	−0.09 −0.95 −2.45	–	12.65	16.08
$C_{10}H_8BrN_2R$	$C_{12}H_{25}$	405.42	−0.10 −0.95 −2.45	–	12.50	17.75
$C_{10}H_8BrN_2R$	$C_{13}H_{27}$	419.14	−0.08 −0.95 −2.46	–	12.34	18.08
$C_{10}H_8BrN_2R$	$C_{14}H_{29}$	433.49	−0.09 −0.95 −2.42	–	12.24	21.47
$C_{10}H_8BrN_2R$	$C_{15}H_{31}$	447.16	−0.09 −0.95 −2.42	–	12.17	23.45

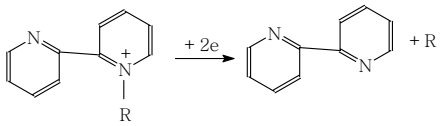
this series interacting with LA, and even in this case the new polarographic wave of the complex of 2,2′-bipyridine with LA was poorly defined. The potential carcinogenicity value of 2,2′-bipyridine determined was 0.0046 (Table 1), thus indicating no carcinogenic potential.

No alkyl derivative formed a new polarographic wave in the presence of LA indicating no potential carcinogenicity of these compounds.

From the ionization potential data (Table 1), it is obvious that this parameter is not related to the reducibility of the compounds investigated. While the ionization potential values decrease in a linear manner, the reduction potential values $E_{1/2}$ are very similar for all the alkyl derivatives under investigation. The increase of the number of carbon atoms in the alkyl group decreases the ionization potential of the substances. This is in good agreement with the induction effects of alkyls and is not surprising. The unsubstituted, parent compound – 2,2′-bipyridine – had the highest calculated ionization potential.

The behaviour of 2,2′-bipyridine and its alkyl derivatives was also investigated using GC-MS. The GC-MS analysis was performed using the standard conditions described in the Experimental section. The retention time of 2,2′-bipyridine was 16.05 min (Fig. 1). During the GC analysis, all the alkyl derivatives were decomposed forming the 1-bromoalkanes. The retention times of these 1-bromoalkanes were in the range from 8.88 min for 1-bromooctane to 23.45 min for 1-bromopentadecane. The retention time of the 1-bromoalkanes was linearly dependent on the number of carbon atoms in the molecule. Because of the decomposition of the alkyl compounds investigated, no molecular peak was registered during the MS measurements.

Scheme 1



The MS analysis revealed that 2,2′-bipyridyl was degraded in the manner outlined in Scheme 2. The main peaks formed were those with m/z value of 156 (the molecular peak), 129, 102, 78 and 51. Clearly, the fragmentation of 2,2′-bipyridine proceeded via the removal of fragments of 27 (m/z value) during MS analysis either from 2,2′-bipyridine or from the pyridine ions thus constituting two degradation pathways. The decrease of m/z value by 27 indicates the removal of =N−CH= fragments.

Three compounds of the series were chosen for a spectrophotometric study of the formation of associates of 1-alkyl-2-(2′-pyridyl)pyridinium bromides with indicators. The associate formation was investigated for 2,2′-bipyridine as the parent compound and for three substances with 1-alkyl substituents containing eight, eleven and fifteen carbons (the substances containing the shortest, the intermediate and the longest 1-alkyl substituent). The conditions for the formation of ionic associates with color indicators were determined for bromothymol blue (BTB) representing sulfonphthalein substances and for methyl orange (MO) representing azo substances. Chloroform was used to extract the aqueous phases (1 : 1 volumes). By varying the pH of the aqueous phase, it was shown that optimal association of 1-alkyl-2-(2-pyridyl)pyridinium ions with BTB and MO occurrs when the pH of the aqueous phase is 10 (Fig. 2 and 3). The recorded λ_{max} of these associates is 424 nm.

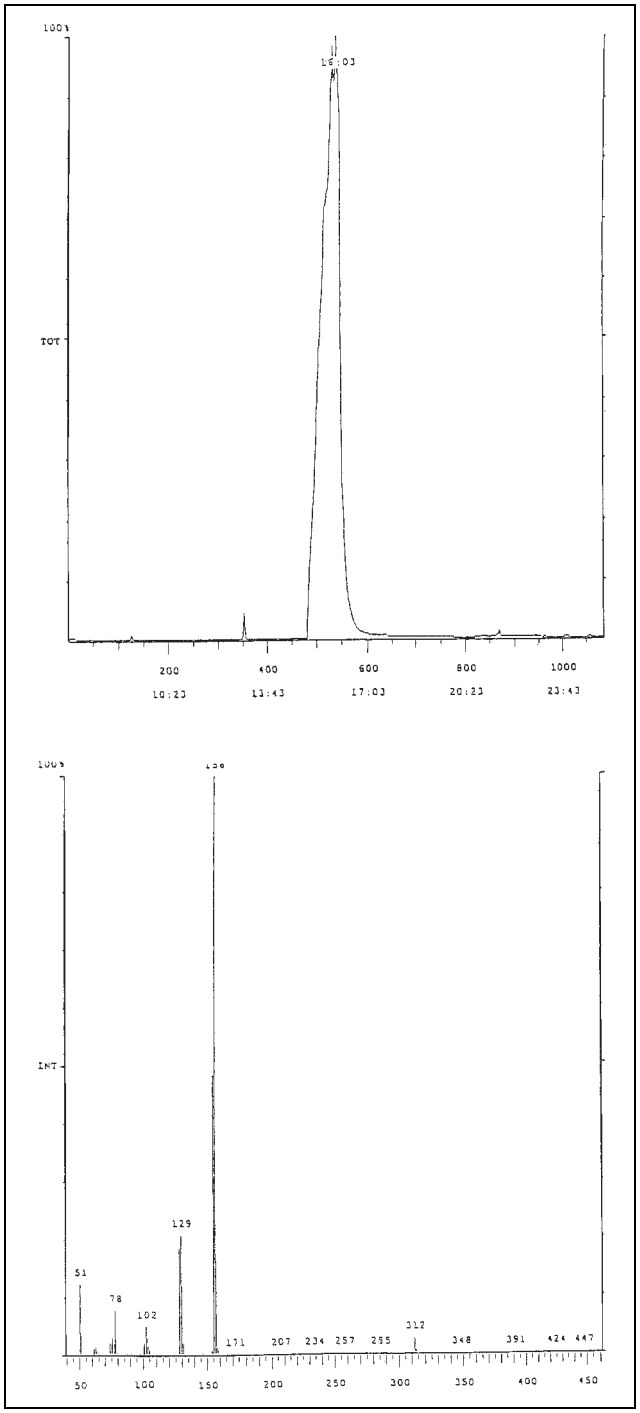


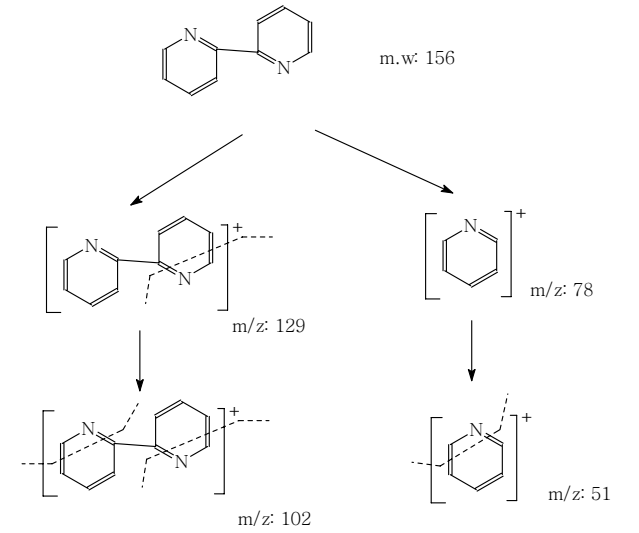
Fig. 1: Full-scan GC-MS spectrum of 2,2′-bipyridine

Table 2: Linearity parameters of the associate absorbance and concentration of three 1-alkyl-2-(2-pyridyl)pyridinium bromides: $y = a + bx$

Indicator	Compd.	a	b	r
BTB	I	0.035	14397	0.9907
	II	−0.031	13968	0.9928
	III	0.000	8655	0.9863
MO	I	−0.093	31168	0.9967
	II	−0.076	28810	0.9976
	III	−0.012	23117	0.9946

x = concentration of 1-alkyl-2-(2-pyridyl)pyridinium bromide in mol/l and y = absorbance (n = 6). Compounds: I – 1-octyl-2-(2-pyridyl)pyridinium bromide (C_8H_{17}); II – 1-undecyl-2-(2-pyridyl)pyridinium bromide ($C_{11}H_{23}$); III – 1-pentadecyl-2-(2-pyridyl)pyridinium bromide ($C_{15}H_{31}$)

Scheme 2



It was determined that at alkaline pH, the indicator and 1-alkyl-2-(2-pyridyl) pyridinium ions combine in a 1 : 1 ratio. Excess indicator (BTB or MO) stays in the aqueous phase (Fig. 4 and 5) and does as not show any absorbance at this wavelength. At acidic pH the parent compound 2,2′-bipyridine forms an associate with BTB only and not with MO. Within the concentration range 1.0–8.0×10^{-5} mol/l there was a linear dependence of concentration and absorbance for the substances investigated [A = f(c)] (Table 2).

In conclusion, 2,2′-bipyridine and its 1-alkyl-2-(2-pyridyl)-pyridinium bromide derivatives are reduced by DC polarography. Three one-electron steps are needed for the reduction of 2,2′-bipyridine and two one-electron steps are required for the reduction of the alkyl derivatives. The determined carcinogenic potential is negligible both for 2,2′-bipyridine and its alkyl derivatives. The ioniza-

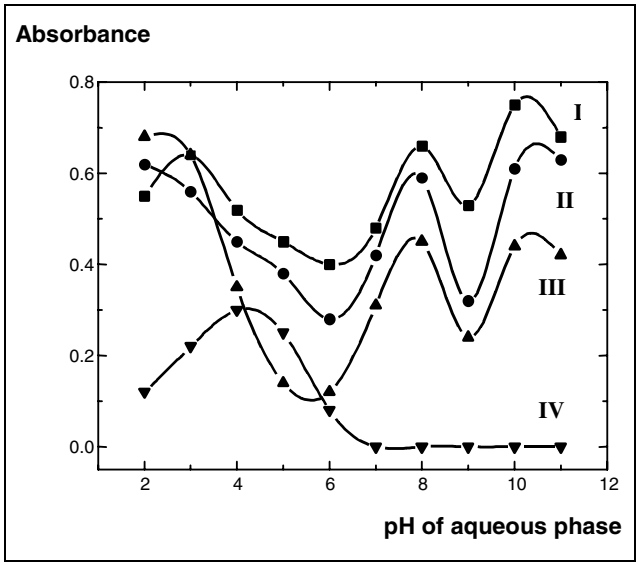


Fig. 2: Absorbance of associates of 1-alkyl-2-(2-pyridyl)pyridinium ions or 2,2′-bipyridine with BTB in the relation to pH of aqueous phase. I – 1-octyl-2-(2-pyridyl)pyridinium bromide (C_8H_{17}); II – 1-undecyl-2-(2-pyridyl)pyridinium bromide ($C_{11}H_{23}$); III – 1-pentadecyl-2-(2-pyridyl)pyridinium bromide ($C_{15}H_{31}$); IV – 2,2′-bipyridine

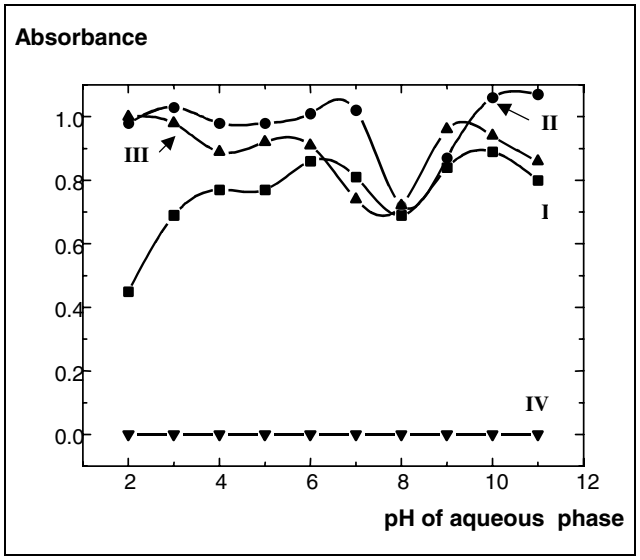


Fig. 3: Absorbance of associates of 1-alkyl-2-(2-pyridyl)pyridinium ions or 2,2′-bipyridine with MO in the relation to pH of aqueous phase. I – 1-octyl-2-(2-pyridyl)pyridinium bromide (C_8H_{17}); II – 1-undecyl-2-(2-pyridyl)pyridinium bromide ($C_{11}H_{23}$); III – 1-pentadecyl-2-(2-pyridyl)pyridinium bromide ($C_{15}H_{31}$); IV – 2,2′-bipyridine

tion potential values are not related to the reducibility of the compounds investigated. Additionally, MS fragmentation of alkyl derivatives proceeds initially through removal of an alkyl part of the molecule and then fragmentation proceeds as elucidated on the basis of the MS spectra recorded for 2,2′-bipyridine. It may also be concluded the basis of the polarographic and MS data and the ionization potential values that the alkyl part of these substances is responsible for changes to some physical properties of the compounds but that the parts of the molecules responsible for biological and chemical behavior are located in the 2,2′-bipyridine moieties. The 1-alkyl derivatives can form associates with colored indicators (BTB and MO). This can be used for spectrophotometric determination of their concentration, as the intensity of the color of the associates formed is linearly dependant on their concentration.

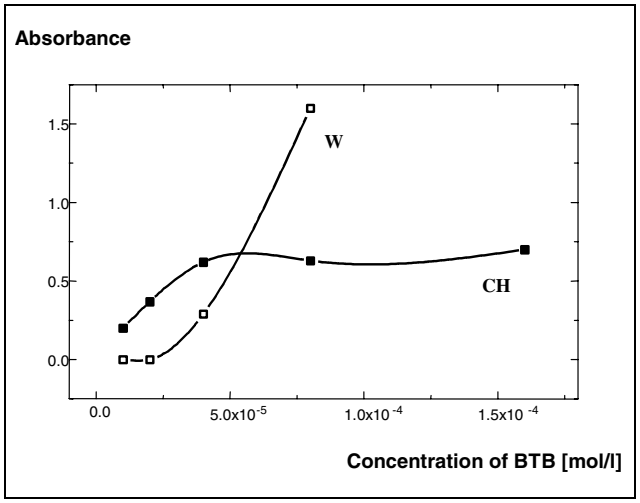


Fig. 4: Effect of BTB concentration on the formation of associates at pH of aqueous phase 10. CH – associate in chloroform phase, W – BTB in aqueous phase

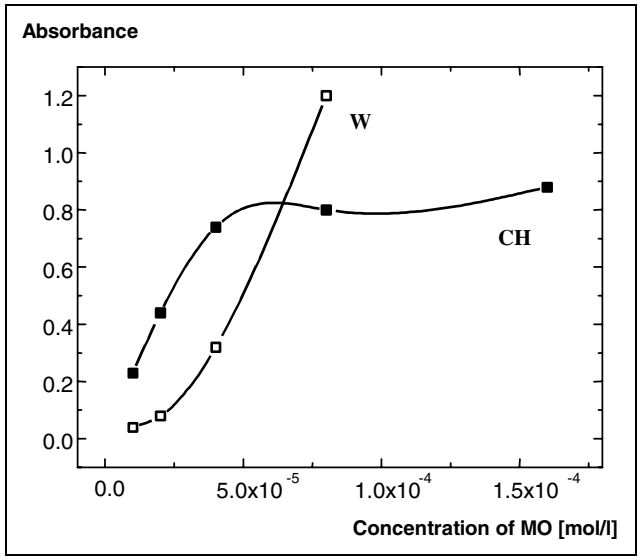


Fig. 5: Effect of MO concentration on the formation of associates at pH of aqueous phase 10. CH – associate in chloroform phase, W – BTB in aqueous phase

3. Experimental

3.1. Materials

The compounds studied, 1-alkyl-2-(2′-pyridyl)pyridinium bromides, were prepared from 2,2′-bipyridine and 1-bromoalkanes according to the published procedure [7]. *N,N*-dimethylformamide (DMF) was from Fluka (Fluka, Buchs, Switzerland). The DMF was additionally purified prior to all electrochemical measurements by double vacuum distillation in a dry nitrogen atmosphere [8]. Water content of the DMF did not exceed 0.01 wt.%. Tetrabutylammonium perchlorate (TBAP) was used as the supporting electrolyte at a concentration of 0.15 mol/l, and was purchased from Fluka. α-Lipoic acid (D,L-6,8-thioctic acid) was purchased from Koch Light Laboratories, Colnbrook, United Kingdom. Bromothymol blue used as a 0.1% solution in 0.01 mol/l sodium hydroxide was from Fluka. Methyl orange was purchased from Merck and was used in the form of a 0.1% aqueous solution. For the spectrophotometric study, the Britton Robinson buffer was used. All other chemicals, including boric, phosphoric and acetic acids and also sodium hydroxide, were of the highest quality available.

3.2. DC polarographic analysis

All polarographic measurements were performed using the three-electrode setting on a PA 2 polarographic analyzer equipped with an XY 4106 two-line recorder from Laboratorni pristroje Prague, Czech Republic. Polarographic experiments were performed in a polarographic cell adapted for work in an anhydrous system. As the indicating electrode, a dropping mercury electrode (DME) was used with a drop time of 3 s and a flow rate of 2.27 $mg \cdot s^{-1}$ at a mercury column height h_{Hg} of 81 cm. As the reference electrode, a saturated calomel electrode (SCE) modified for anhydrous conditions was used. The auxiliary electrode was a platinum electrode.
All polarographic measurements were carried out at room temperature in a stream of dry nitrogen in order to exclude atmospheric oxygen and humidity from the polarographic cell as described previously [9]. The concentrations of polarographed compounds in the polarographic cell were 5×10^{-4} mol/l.

3.3. Determination of the index of potential carcinogenicity

The potential carcinogenic activities of 2,2′-bipyridine and of 1-alkyl-2-(2-pyridyl)pyridinium bromides were determined on the basis of their ability to form complexes with α-lipoic acid [9–12]. Briefly, investigation of the polarographic behaviour of α-lipoic acid in strictly anhydrous DMF showed that at concentrations of up to 10^{-2} mol/l the substance exhibits no polarographic wave of its own but affects the polarographic reduction of a variety of carcinogens. During the polarographic reduction of such compounds in an anhydrous system in the presence of α-lipoic acid, the value of the diffusion current I_d [μA] of the first polarographic wave increases or a new wave with a more positive $E_{1/2}$ value is formed. The increase in current reflected in the height of the new polarographic wave is linear and it increases with increasing concentrations of α-lipoic acid. The linear increase follows the equation

$$y = kx + q\,,$$

where y is the diffusion current, x is the concentration of α-lipoic acid, and k and q are constants. The tangent of the angle α between the straight line and the x coordinate is used as a basis for evaluation of the potential carcinogenic activity of the compounds under study. (The diffusion current I_d values in μA are plotted against the concentrations of α-lipoic acid shows a linear relationship.) The tangent values of this angle (tg α) obtained for some polycyclic aromatic hydrocarbons with known carcinogenic activity are known [9]. No such effect of the concentration of α-lipoic acid was found for inactive or non-carcenogenic polyaromates and their tg α values are close to zero [9]. The concentration ratio of 2,2′-bipyridine: α-lipoic acid ranged from 7.5×10^{-5} mol/l to 4×10^{-4} mol/l.

3.4. Calculation of ionization potentials

The molecules were prepared with the molecular modeling package Quantum CAChe, marketed and distributed by FQS, Krakow, Poland (http://www.fqspl.com.pl), running on a PC. Optimizations were performed with the semi-empirical molecular orbital package MOPAC2000 using the PM3 Hamiltonian [13]. The Eigenvector Following (EF) method was used for optimization and the keyword Precise was specified in each case. The ionization potentials of the quaternary amines were calculated in the absence of counterions.

3.5. GC-MS analysis

The compounds were analyzed by a Finnigan-Mat GCQ-MS (quadrupole ion trap mass analyzer) using an AT-WAX fused silica column (30 m length × i.d. 0.25 mm, 0.25 μm film thickness) from Altech, U.S.A. The temperature was programmed to start at 70 °C, held for 5 min and then increased to 145 °C at a rate of 5 °C per min and held at 145 °C for 5 min. Helium was used as a carrier gas (70 kPa head pressure) with a flow rate of 1 ml/min. The conditions for mass detection were as follows: 70 eV, positive EIMS in Full Scan mode for m/z values 50–450. The temperatures of ion source and transfer line were 180 °C and 275 °C, respectively. Compounds were introduced to the column in the spilt-less mode at an injection temperature of 230 °C. The detection limit of 2-2′-bipyridine was approximately 15 pg on column.

3.6. Spectrophotometric determination

The measurements of formation of associates of the investigated compounds with BTB and MO were performed using a Hewlett Packard 8453 spectrophotometer equipped with a 0.999 cm silicon cuvet. The experiments were performed as follows: 0.1 ml of BTB or MO (0.004 ml/l) was mixed with 0.1–0.6 ml of the bipyridyl derivatives (0.00033 mol/l) and 4.3–4.8 ml of a buffer solution. The final volume of the mixture (a water phase) was 5.0 ml. To this, 5.0 ml of chloroform were added and the mixture was shaken for 20 min. The aqueous and chloroform phases were separated in a separation funnel after 30 min. Determinations were performed versus a blank solution at least in triplicates.

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