Spectroscopic Behavior of Acid-Base Indicators After Immobilization on Glass Supports

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This paper describes an extensive spectrophotometric study on several acid-base indicators immobilized, by means of a silylation process, on quartz powder and on controlled pore glass. The spectrophotometric properties of the immobilized indicators are at variance with those of the free indicators with respect to pH dependence and ranges. The different behavior of both types of indicators in the liquid and immobilized phases may be explained by both a modification in the electronic structure of the indicators and a distribution of the molecules on different sites. The differences among the spectrophotometric properties of the indicators, when these are immobilized on different solid supports, can be justified by taking into account the steric hindrance and the reactivity of the indicators. Particular attention was paid to the pH-sensitive bands and their position, keeping in mind that the investigated material could possibly be used as a transducer for pH fiber-optic sensors.

Index Headings: **Reflectance measurements; Fiber optic sensors; pH measurements; Acid-base indicators; Silylation.**

INTRODUCTION

In recent years the use of fiber-optic sensors for the measurement of chemical parameters has been extensively studied in view of several advantages which they offer, such as the possibility of high miniaturization, electrical insulation, and continuous monitoring in hostile environments. Due to these characteristics, fiber-optic sensors are very useful both in biomedical and in industrial applications and also in environmental control.

Particular attention has been paid to the evaluation of H^+ concentration, since a knowledge of pH is extremely important in many industrial processes and also, as far as biomedical applications are concerned, for the human body. Several optical-fiber pH sensors have been developed which make use of fluorescent or absorption substances as transducer.[1] The use of an indicator in solution, connected with the optical fibers, is not practical because of the problems due both to the use of a highly selective membrane, which can give rise to a long response time, and to the structure of the probe, which is often too fragile. Hence, immobilization of the chromophore on solid supports is more suitable.

Some studies on immobilization of the chromophore using an adsorption technique have been reported.[2,3] Although very simple, this technique suffers from the progressive leakage of the chromophore, and does not appear to be very practical. Linking of the chromophore on suitable supports by means of a covalent bond seems to be more efficient and is already widely applied, especially for fluorescent substances, since it guarantees the almost total absence of chromophore leakage. The appropriate choice of the chromophore is another important aspect. Indeed, fluorescent materials, characterized by high sensitivity, may suffer from interferences by other substances, so that the accuracy of the pH measurement is decreased. Moreover, the low-excitation wavelength, generally in the near-UV region, often prevents the use of light-emitting diodes (LEDs) as sources, requiring more expensive or cumbersome devices (laser, halogen lamp, etc.). Instead, substances working on absorption, even if they are less sensitive, are less affected by interferences and, since the working wavelength is usually in the visible region, they can be used with LEDs as sources, allowing the realization of a cheaper and easily transportable optoelectronic system. It is important to stress the fact that changes in the optical properties can occur after immobilization. For this reason, an accurate and thorough spectrophotometric analysis is necessary in order to investigate the properties of the immobilized chromophore. Studies on different materials used for the development of optical-fiber chemical sensors have been reported, in particular regarding indicators for pH[4,5,6,7] and oxygen[8,9] determination and complexing agents for metallic ions.[10,11]

The present work consists of a spectrophotometric study, in the visible range, of acid-base indicators covalently bound on glass supports by means of a silylation process. Their spectroscopic properties are compared with those of the free indicators in solution. Since the aim of our work is to realize a fiber-optic pH sensor, particular attention has been paid to the occurrence of photodecomposition, which can affect the accuracy of the sensor, and to the working wavelengths of the immobilized dyes, which affect the ability to use LEDs as sources in the optoelectronic instrument.

EXPERIMENTAL

Materials. N,N-dimethylaniline (DMA), *n*-propylamine, sodium dithionite, sodium nitrite, the reagent for the buffer solutions, and all the tested indicators were obtained from Farmitalia Carlo Erba (Milan); *p*-nitrobenzoyl chloride and quartz powder were obtained from Merck; γ-aminopropyltriethoxysilane (γ-APTSi) and Controlled-Pore Glass (CPG) (120–200 mesh, pore size 700 Å) were obtained from Fluka (Switzerland). Spectrophotometric measurements were made with a Perkin-Elmer spectrophotometer (Model 552). Diffuse reflectance spectra of the immobilized indicators at different pH values were recorded in the range 380 to 800 nm by using an external integrating sphere, coated with $BaSO_4$ and connected to the spectrophotometer via optical fibers. Measurements were performed by placing a 5-mL beaker, containing a sufficient amount of the quartz or

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CPG to ensure a complete absorption of the light, over the hole of the integrating sphere. The beaker was filled with the suitable buffer and covered with a black cloth to avoid interference from ambient light. With regard to quartz powder, the buffer was changed by making use of a syringe having a needle with a 0.4-mm internal diameter. This procedure could not be applied for CPG, owing to the fineness of its format; in this case the change of buffer was performed with consecutive washing and centrifugation.

Immobilization on Quartz Supports. Surface derivation using silanes is a common process for imparting a particular chemical character or reactivity to solid substrates having hydroxyl groups on their surfaces. Silane coupling agents are characterized by one end which reacts with the glass surfaces via one or more reactive groups and the other end which gives the glass a new functionality. For this work, the silylating agent used is γ-APTSi, which allows the introduction of amino groups on the surface; such a chemically modified surface can be further derivated in order to synthetize surfaces bearing a variety of pH indicators.[12,13] At first, three slightly different silylation procedures were studied in order to maximize the bound amine and, consequently, the final bound pH indicator. In all cases, quartz powder was degreased overnight in boiling heptane, then rinsed with acetone and oven dried.

In the first method, the silylation was achieved by dissolving the γ-APTSi in a 95% ethanol/5% water solution to yield a 2% final concentration; the solution was allowed to stand for five minutes for hydrolysis, and then the carrier was soaked for five minutes and subsequently rinsed twice with ethanol and cured for 24 h in a desiccator.

In the second method, the silylation was performed by covering the carrier with γ-APTSi in a sealed tube at 90°C for 24 h. The product was subsequently washed with benzene, ethanol, and water.

In the third method, the quartz was soaking in a 10% γ-APTSi solution in toluene; after refluxing for 22 h, the quartz was washed with toluene, methanol, and acetone and then oven dried at 80°C for 1 h.

The amidization procedure was the same in all three methods. A solution of *p*-nitrobenzoyl chloride in chloroform containing triethylamine was added to the silylated quartz. Initial amine, acid chloride, and triethylamine were present, in an approximately 1:3:4 mole ratio. This mixture was refluxed for 20 h; after cooling, the quartz powder was washed with chloroform. Reduction of the nitro group was conducted in boiling dithionite for 1 h; an approximately 3-fold molar excess of dithionite (as compared to the original amine) was used, resulting in a 3–5% dithionite solution. After the reaction, the product was washed with water. The diazotization process was made by placing the quartz powder in a 2% sodium nitrite solution in 2 M HCl, cooled with an ice bath (0–3°C). The mixture was stirred for 30 min, washed with water, and immediately added to the coupling solutions.

Coupling with DMA. Diazotized quartz powder was placed immediately in N,N-dimethylaniline, allowed to stand for 30 min, and then washed with benzene, ethanol, and water. The quartz was slightly orange in the basic and neutral solutions, and red in the acid solution.

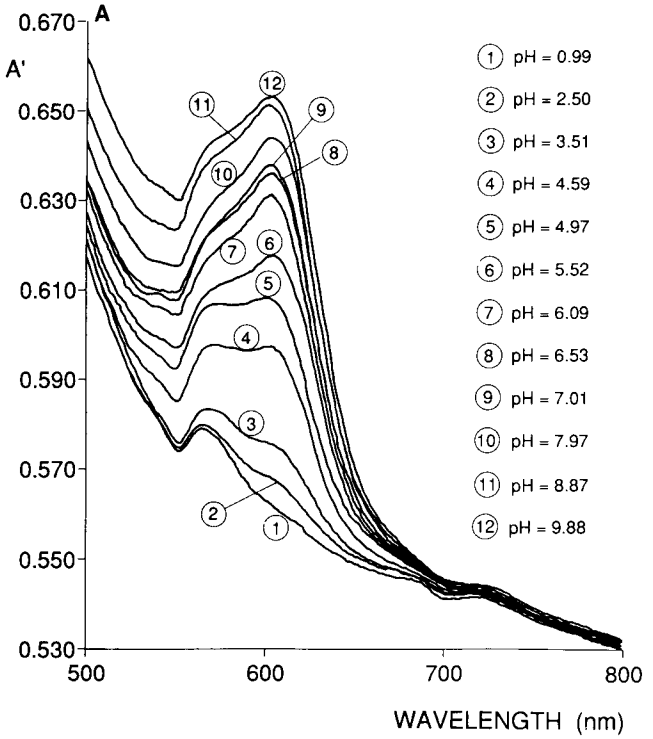


FIG. 1A. Reflectance spectra of BPB immobilized on quartz powder for different pH values following the third immobilization method.

Coupling with Sulfonphthalein Indicators. The diazotized quartz powder was added immediately to an aqueous solution containing 0.05 g of the indicator. The reaction was carried out at 4°C for 24 h. The quartz was washed several times with water until no detection of

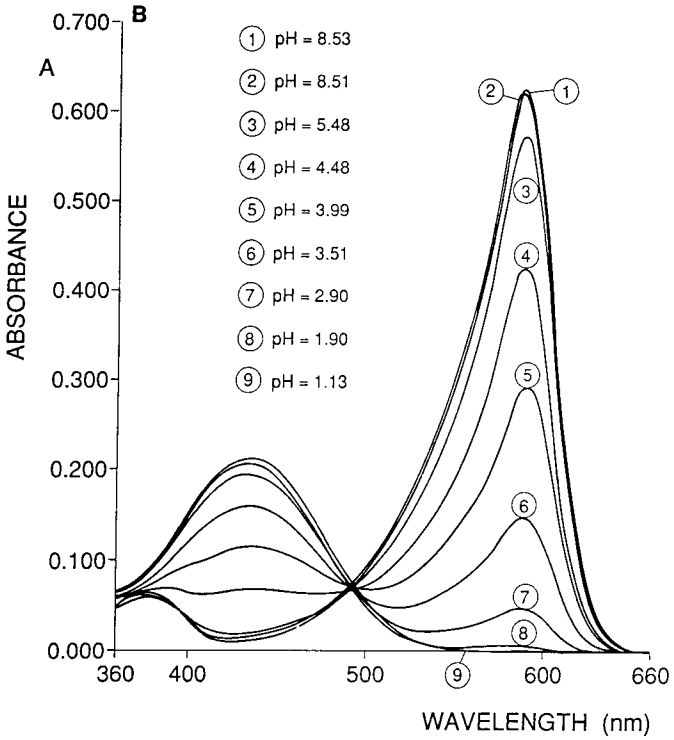


FIG. 1B. Absorption spectra of BPB in solution ($C = 10^{-5}$ M) for different pH values.

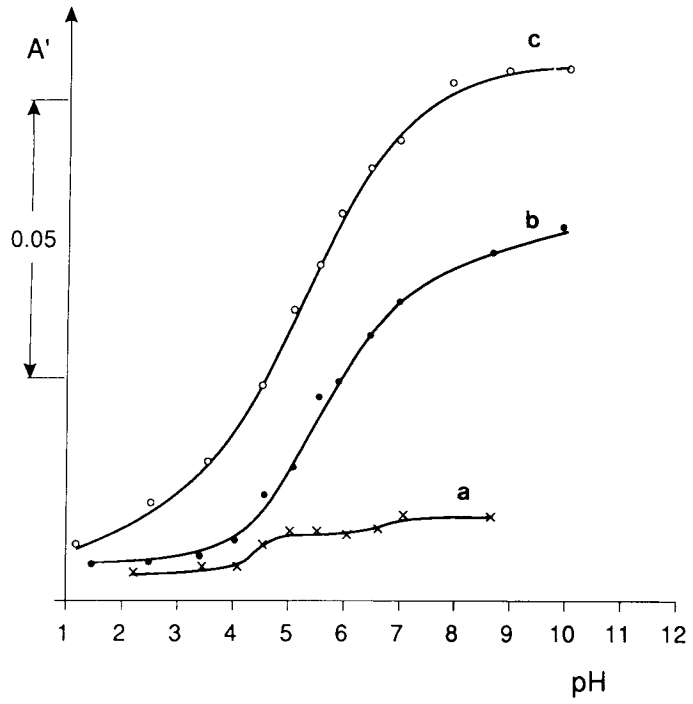


FIG. 2. A' values measured at the absorption peak vs. pH for BPB for the three methods followed: (*a*) first method; (*b*) second method; (*c*) third method.

indicator was observed in the spectrophotometer. The carriers were maintained in water until used.

Immobilization on CPG. We also studied CPG as a carrier; it is characterized by a large surface area suitable for bonding. The reactions are the same as above, and the method used for silylating is the third one, since it appears to be the most efficient. If the values of the wetting surface of silane per gram (W_s) and the surface area of the carrier per gram (σ) are known, calculation of the necessary amount of silane to obtain uniform coverage can be obtained, from the simple formula:[14]

$$P_s = \frac{P_c \cdot \sigma}{W_s} \qquad (1)$$

where P_s and P_c are the amount of the silane and of the carrier in grams, respectively. The CPG used is characterized by $\sigma = 27.6\ m^2\ g^{-1}$, while for γ-APTSi, W_s is 353 $m^2\ g^{-1}$.

Free Indicator Dye from N,N-Dimethylaniline (FIDMA). The free indicator obtained from N,N-dimethylaniline (FIDMA) was prepared in order to compare the behavior of bound DMA, which becomes an indicator only after the immobilization. *n*-Propylamine (3.25 g) was placed in 150 mL of water containing 7.95 g of Na_2CO_3 and 9.27 g of *p*-nitrobenzoyl chloride.[15] After heating at 50–60°C for 90 min, the white precipitate of benzamide was filtered and washed. Reduction of the nitro group of benzamide was achieved with $NaBH_4$, with the use of Pd/C 5% as catalyst. After stirring in an ice bath for 60 min,[16] the mixture was extracted with chloroform in the presence of Na_2CO_3. The organic extracts were evaporated to several milliliters of a yellow oil. The diazonium salt was formed by dissolving the reduction product in ice water containing 0.69 g of $NaNO_2$ and 2 mL of HCl 5 M; 1.08 mL of DMA and 0.5 mL of acetic acid were

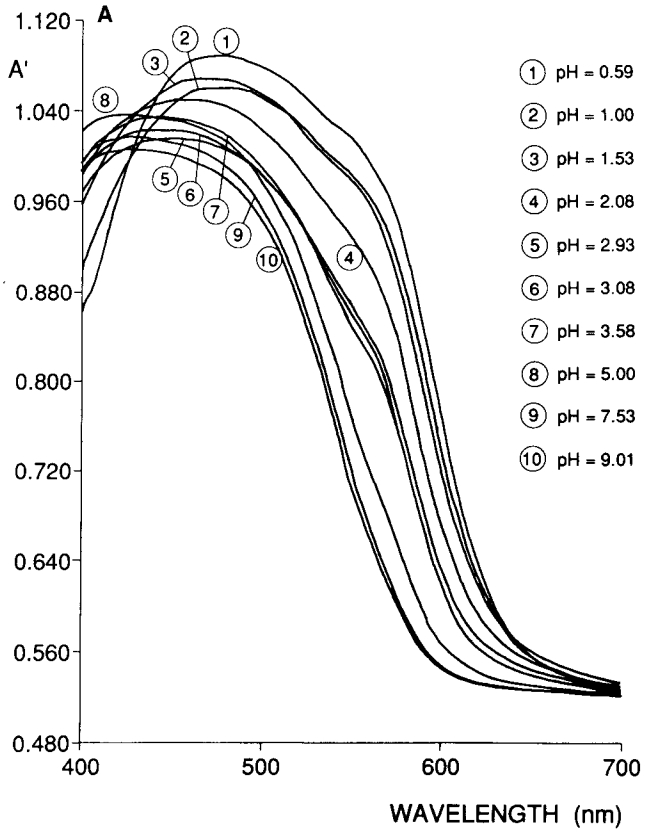


FIG. 3A. Spectra of DMA immobilized on quartz powder for different pH values according to the third immobilization method.

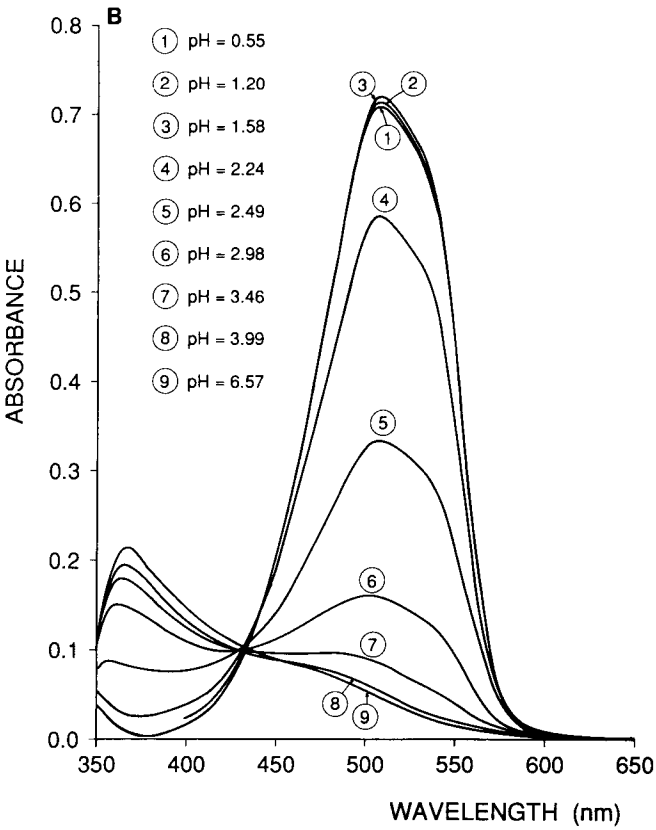


FIG. 3B. Absorption spectra of FIDMA in solution ($C = 10^{-5}$ M) for different pH values.

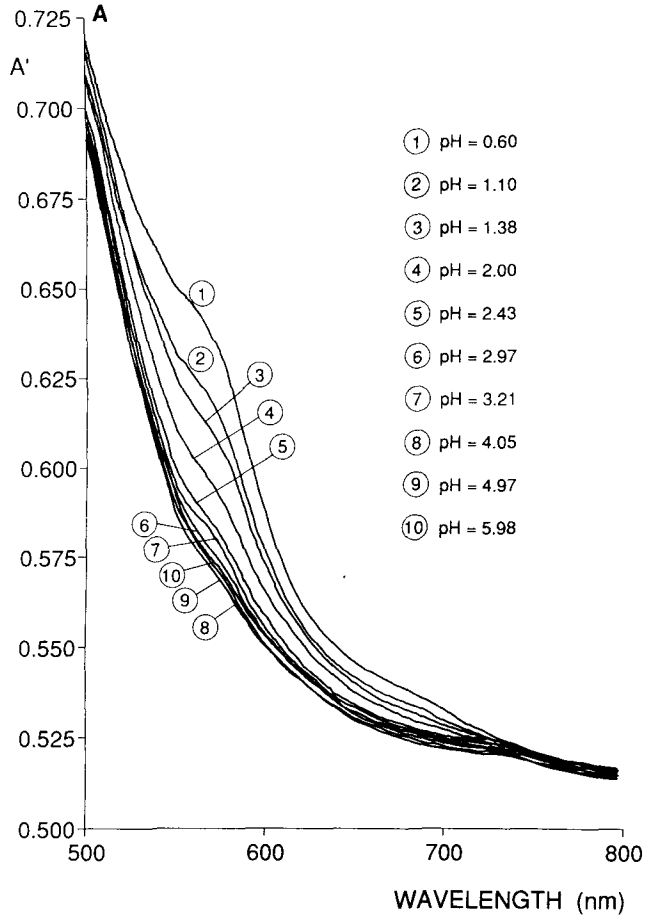


FIG. 4A. Reflectance spectra of XB immobilized on quartz powder in the acidic range following the third immobilization method.

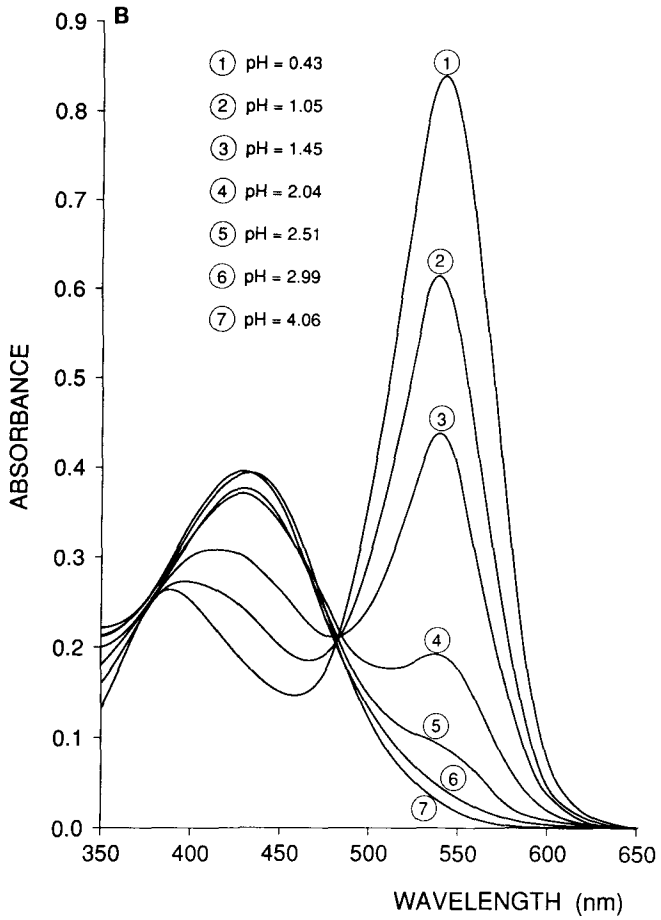


FIG. 4B. Absorption spectra of XB in solution ($C = 10^{-5}$ M) in the acidic range.

then added. After 15 min, the pH of the solution was adjusted to 9. The precipitate was filtered and washed with water. The product was recrystallized from ethanol/water (1:1).

RESULTS AND DISCUSSION

Many indicators were tested, and a first series of measurements was performed in solution so as to exclude the use of indicators characterized by strong photodissociation. In fact, it was impossible to obtain stable absorption spectra in solution for methyl green, brilliant green, malachite green, crystal violet, or quinaldine red; accordingly, these indicators were no longer investigated.

The three different methods of silylation described above were first tested on quartz powder, which has a very low cost in comparison to CPG. Figure 1A shows the pH dependence of the reflectance spectra of bromophenol blue (BPB) immobilized on quartz powder according to the third method. For easier comparison, the reflectance measurements are expressed in an analogous way to those for absorbance ($A' = \log 1/R$, where R is the diffuse reflectance). The spectra for BPB immobilized on quartz powder following the first and second method are not reported, because these methods give results that are quite similar with respect to the shape of the curves; the only difference is their lesser sensitivity. Spectra below 500 nm are not reported, because they are practically insensitive to pH changes. In these spectra and in all the spectra recorded on quartz powder, two small bands centered, respectively, at 550 and 730 nm are present. The presence of these bands, which are independent of pH, is probably due to metal ion impurities in the glass beaker. It is interesting to compare the spectra in Fig. 1A with the spectra in solution for BPB for the different pH values (Fig. 1B). The relevant points are: (1) a red-shift of about 15 nm for the absorption peak of the basic form of the immobilized indicators; (2) the disappearance or a shift below 400 nm of the band of the acidic form originally at 435 nm; (3) an increase in the pH range, i.e., the range over which a nearly linear relationship can be observed between pH and absorbance. In Fig. 2 the relationship between the absorbance at $\lambda = 605$ nm and pH is reported for the three methods. The best sensitivity of the material treated by the third method of silylation, in comparison with the other two, is apparent. On the basis of these results, the first method (the least sensitive one) was no longer followed for the other indicators. Similar results were obtained for bromocresol purple (BCP) and phenol red (PR).

The spectrophotometric behavior of DMA after immobilization on quartz powder (Fig. 3A) is compared with the behavior in solution of the free indicator (FIDMA), suitably prepared (Fig. 3B). Here, the relevant points are: (1) a broadening and a blue shift of 20–30 nm of the absorption band of the acidic form; (2) the dis-

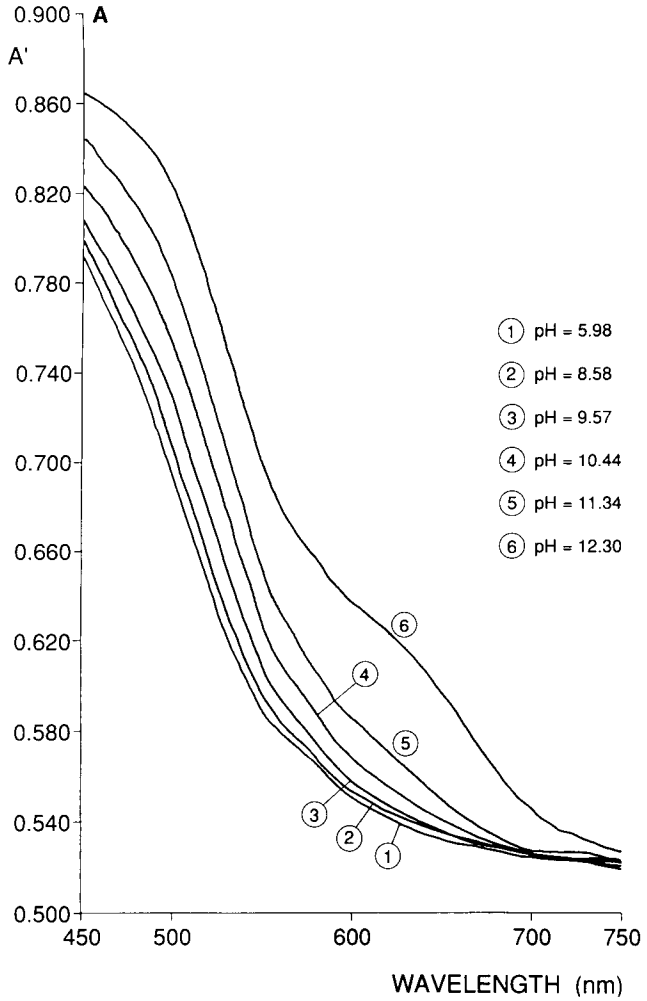


FIG. 5A. Reflectance spectra of XB immobilized on quartz powder in the basic range following the third immobilization method.

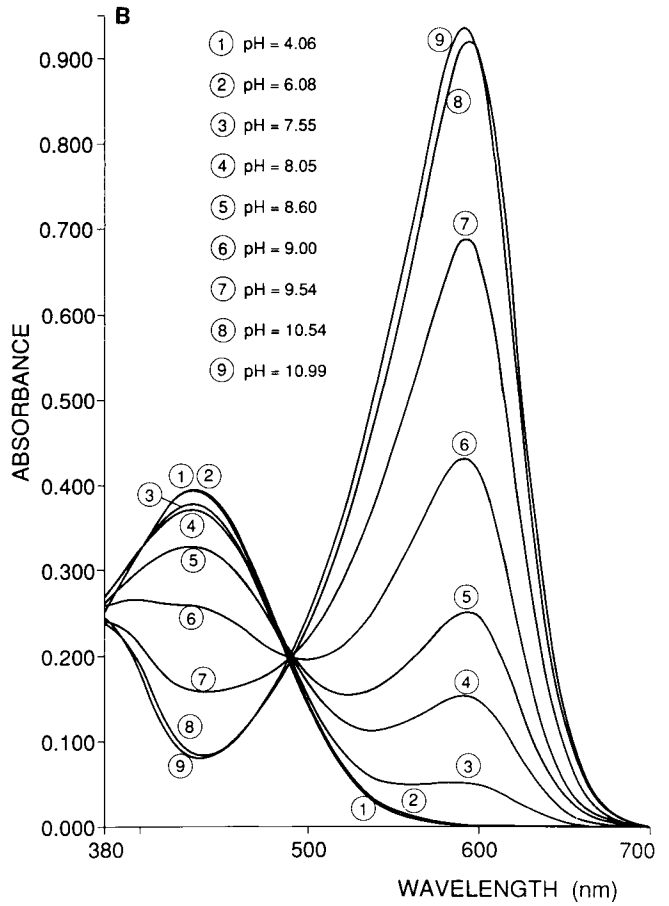


FIG. 5B. Absorption spectra of XB in solution ($C = 10^{-5}$ M) in the basic range.

appearance in the visible range of the characteristic band of the basic form, centered at 370 nm in solution.

As *p*-xylenol blue (XB) is a diprotic acid, it is characterized by two dissociation constants and, accordingly, there are two intervals in which the indicators are sensitive to a pH change. Both the intervals were investigated. The spectra for the indicators immobilized on quartz powder and in solution are shown, both for the range below pH = 7 (Fig. 4A, 4B) and for the range above pH = 7 (Fig. 5A, 5B). Remarkable changes are apparent, not only in the shape of the curves, in which a shoulder (and no longer a maximum) is present, but also in the working range, particularly for the interval above pH = 7.

The results for these five indicators are summarized in Table I. It is not a simple matter to account for the different spectrophotometric behavior of the dyes before and after immobilization. The shift in the absorption

TABLE I. pH ranges and related wavelengths for the indicators being investigated in solution and after immobilization on quartz powder. The values for the first immobilization method are not reported.

	Free dye $C = 10^{-5}$ M $H_2O/EtOH$	Immobilization on quartz powder	
		2nd method	3rd method
N,N-Dimethylaniline	2.0–3.0 pH units[a] λ = 510 nm[a]	1.0–3.0 pH units λ = 480 nm	0.5–3.0 pH units λ = 490 nm
Phenol red (PR)	6.5–8.0 pH units λ = 570 nm	8.0–11.0 pH units λ = 575 nm	7.0–10.0 pH units λ = 575 nm
Bromophenol blue (BPB)	3.0–5.0 pH units λ = 590 nm	4.5–7.5 pH units λ = 605 nm	3.5–6.0 pH units λ = 605 nm
Bromocresol purple (BCP)	5.0–7.5 pH units λ = 595 nm	7.0–10.0 pH units λ = 600 nm	6.5–10.5 pH units λ = 600 nm
p-Xylenol blue (XB)	0.5–2.5 pH units λ = 540 nm 7.5–10.0 pH units λ = 595 nm		0.5–3.0 pH units λ = 570 nm, sh 9.5–12.0 pH units λ = 620 nm, sh

[a] Values obtained for the indicator (FIDMA) suitably prepared from N,N-dimethylaniline.

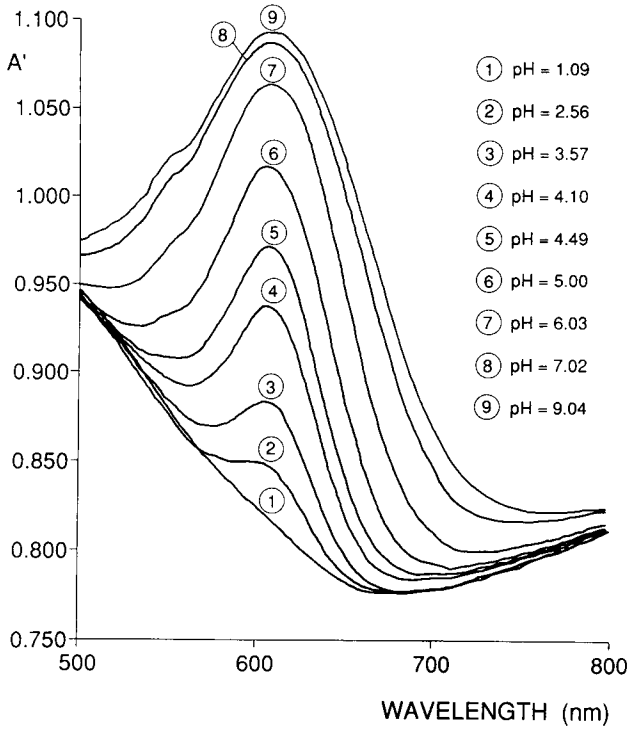
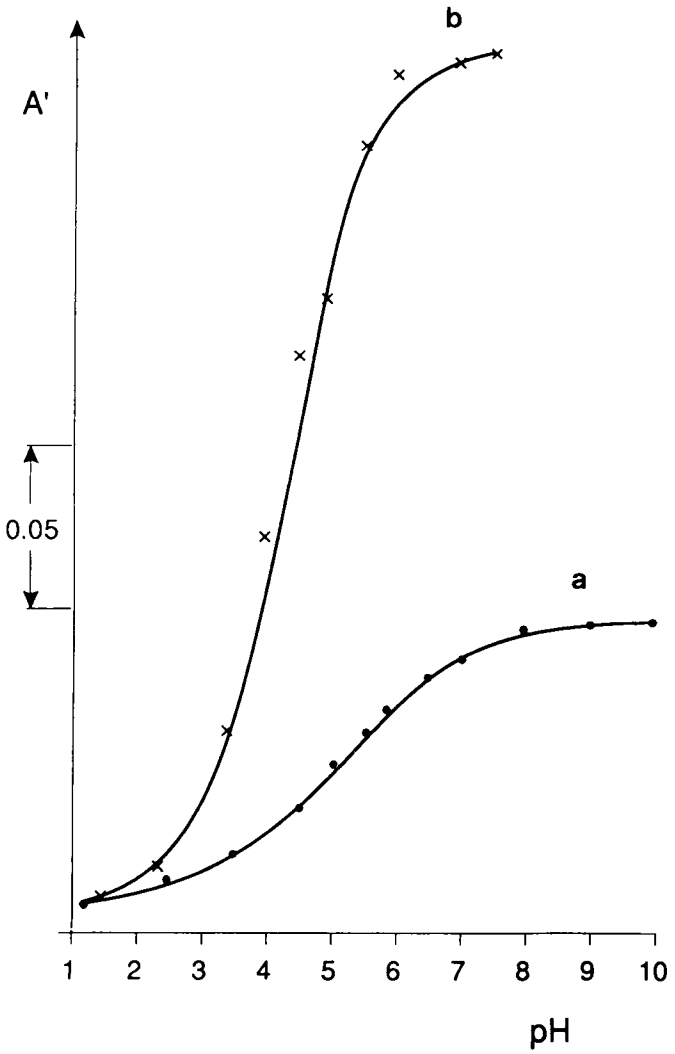


FIG. 6. Reflectance spectra of BPB immobilized on CPG for different pH values.



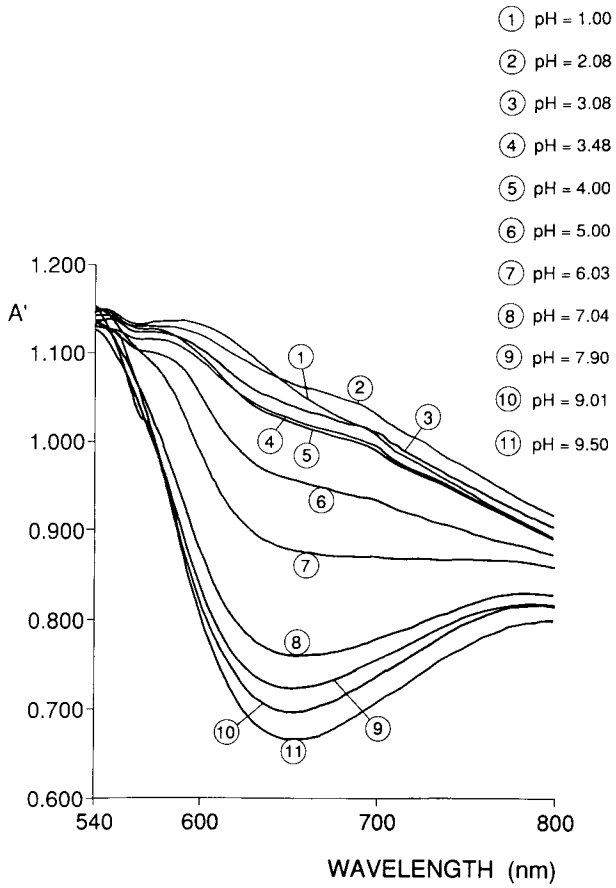


FIG. 8. Reflectance spectra of DMA immobilized on CPG for different pH values.

peak can be justified by considering that the electronic structure of the indicator is modified by the introduction of the diazo group, which surely alters the π and π^* orbitals of the free indicator. On the other hand, the broadening of the pH range can be understood if we realize that the basic (or acidic) dye molecules are probably distributed on the quartz powder over slightly different sites, whereas they are equivalent in solution, so that, in the former case, a distribution of the dye dissociation constant may occur.

On the contrary, the disappearance of one band of the indicator, compared to the behavior in solution, is more intriguing. Some hypotheses can be made also by keeping in mind that the band which disappears or shifts is centered at shorter wavelengths and is characterized by smaller absorbance changes as a function of pH.

The hypotheses are: (1) a filtering effect due to the glass, which evens up the intensity of the recorded light; (2) a light-scattering effect, which is more apparent for the lowest wavelengths and could prevail over the changes in absorption as a function of pH; and (3) a strong blue-shift of the acidic form absorption, due to modification of the electronic structure. At present we are not in a position to give support to any one of the above hypotheses.

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FIG. 7. A' values measured at the absorption peak vs. pH for BPB immobilized on quartz powder (●) and CPG (×), respectively, following the third immobilization method.

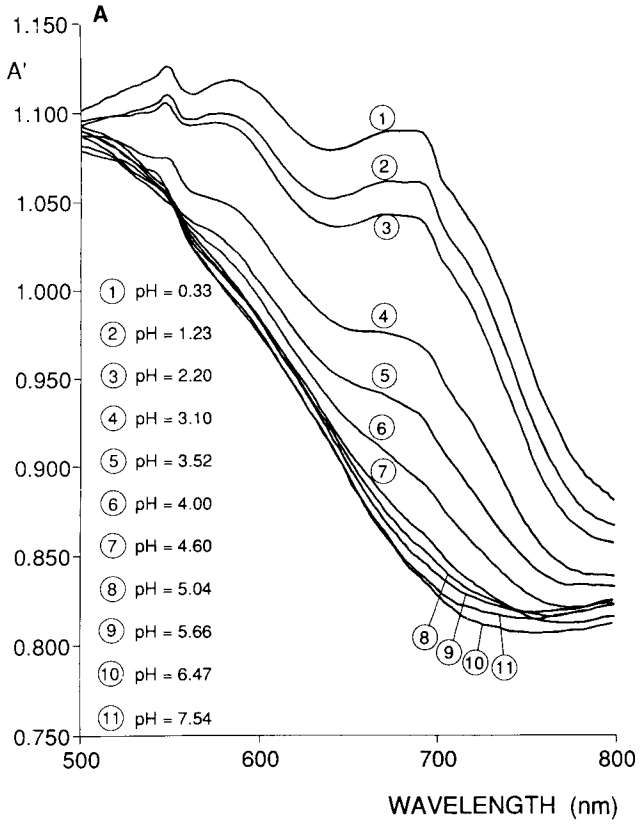


FIG. 9A. Reflectance spectra of XB immobilized on CPG for different pH values in the acidic range.

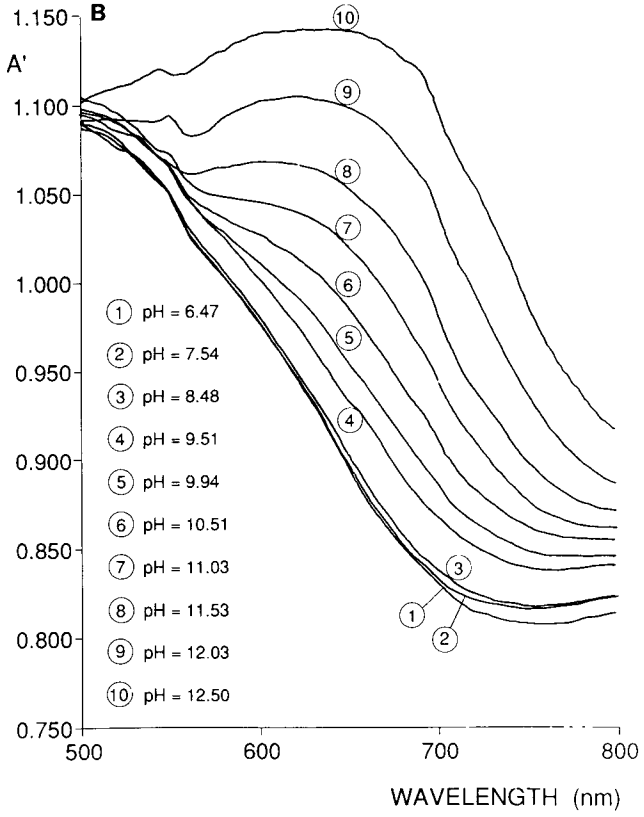


FIG. 9B. Reflectance spectra of XB immobilized on CPG for different pH values in the basic range.

On the basis of the above results, immobilization of the indicators on CPG was performed according to the third method, which is the most sensitive. As expected, the sensitivity of the treated support increases, in comparison with that of the quartz powder, due to the larger surface available on CPG for formation of the covalent bond. Figure 6 shows the reflectance spectra of BPB immobilized on CPG for different pH values. In Fig. 7 the relationship between absorbance at $\lambda = 605$ nm and pH is shown, together with the same relationship obtained for the immobilization on quartz powder following the same method, already reported in Fig. 2. Similar results were obtained for BCP. For both BPB and BCP, the main difference in the immobilization on CPG and on quartz powder is an increase in sensitivity, while the spectrum shape remains practically unchanged.

The same fact cannot be affirmed for the other dyes tested, which, although characterized by an increase of sensitivity, also show a different shape for the spectrum.

There is a drastic change for DMA immobilized on CPG (Fig. 8). The shape of the spectra is totally different from the shape of the spectra recorded in solution or after the immobilization on quartz. This change could be due to the fact that the maximum peak is shifted below 400 nm, and only the lateral side of the band appears in the investigated range; however, the filtering effect of the glass hinders the detection of such absorption.

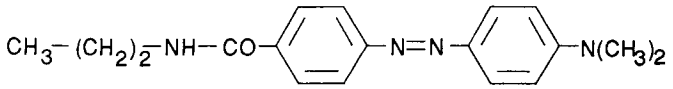
An appreciable change is also observable for XB on CPG, where the shape of the spectra is different from that in solution or after immobilization on quartz powder. In the pH range below 7 (Fig. 9A), a new band appears at 680 nm, in addition to the band at 580 nm, which is analogous (although slightly shifted) to the band recorded for XB immobilized on quartz powder (see Fig. 4A). In the pH range above 7 (Fig. 9B), only one broad band appears, centered at 620 nm, probably due to the overlapping of two bands similar to those observed in the range under pH = 7.

Very similar behavior was observed for thymol blue (TB) and *m*-cresol purple (*m*CP). As far as phenol red is concerned, only one broad band, shifted at 680 nm, appears, and the shape of the spectra is analogous to the one observed for XB in the range above pH = 7.

In all these spectra, a small absorption band appears at 550 nm, possibly still due to metal impurities of the beaker. All the results obtained with CPG as supports are reported in Table II, together with the results obtained in solution.

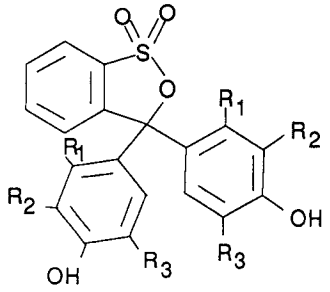
In order to explain the different behavior of the indicators when immobilized on quartz powder or on CPG, particular attention must be devoted to their structure before the immobilization. These indicators differ among themselves in the substituents on the aromatic rings (Fig. 10). In particular, two aspects must be taken into account: the different activation of the aromatic rings with respect to the electrophilic attack, and the hindrance of the substituents.

For BCP and BPB there is a disactivation and a steric factor due to the presence of one or two bromines, respectively, on a single aromatic ring. This leads to a reduced reactivity, which gives rise to a substrate which



Free indicator from N,N-dimethylaniline (DMA)

FIDMA



$R_1 = R_2 = R_3 = H$	Phenol red (PR)
$R_1 = H$, $R_2 = R_3 = Br$	Bromophenol blue (BPB)
$R_1 = H$, $R_2 = CH_3$, $R_3 = Br$	Bromocresol purple (BCP)
$R_1 = H$, $R_2 = R_3 = CH_3$	p-Xylenol blue (XB)
$R_1 = CH_3$, $R_2 = H$, $R_3 = CH(CH_3)_2$	Thymol blue (TB)
$R_1 = CH_3$, $R_2 = R_3 = H$	m-Cresol purple (mCP)

FIG. 10. Chemical structures of the investigated indicators.

is not crowded, without mutual interaction between the indicator molecules, and to a rigid structure due to steric hindrance. The simultaneous occurrence of these aspects can yield very few (as well as deep) potential wells for the substrate, with a consequent limited distribution of chromophores. Instead, for the other indicators, steric hindrance is not present (or, at least, is very reduced), and there is activation for the aromatic rings with respect to electrophilic reaction. Owing to the small size of the substituents, a crowded structure is possible with a distribution of quasi-energetic conformational states, which produces a broadening of the absorption spectrum.

CONCLUSIONS

The main purpose of our work was to characterize a solid system, working on absorption, to be utilized for a pH sensor. The use of CPG with covalently bound indicators seems to be promising, even if some aspects must still be explained. In particular, the electronic structure of the bound indicators and the real configurations of the molecules on the surface of the carriers must be investigated more thoroughly. Another interesting aspect, which we are going to investigate, is the influence of CPG porosity on the structural and spectroscopic characteristic of immobilized indicators. Further studies are under way, in order to understand the superficial structure of the materials in connection with the support and with the preparation methods.

TABLE II. pH ranges and related wavelengths for the indicators being investigated in solution and after immobilization on CPG according to the third immobilization method.

	Free dye H_2O/EtOH, $C = 10^{-5}$ M	CPG 3rd method
N,N-Dimethylaniline	2.0–3.0 pH units[a] λ = 510 nm[a]	2.0–7.5 pH units λ = 650 nm, sh
Phenol red (PR)	6.5–8.0 pH units λ = 570 nm	5.0–9.0 pH units λ = 680 nm
Bromophenol blue (BPB)	3.0–5.5 pH units λ = 590 nm	3.0–6.0 pH units λ = 605 nm
Bromocresol purple (BCP)	5.0–7.5 pH units λ = 595 nm	5.0–8.0 pH units λ = 600 nm
p-Xylenol blue (XB)	0.5–2.5 pH units λ = 540 nm 7.5–10.0 pH units λ = 595 nm	1.0–5.0 pH units λ = 680 nm 9.0–12.5 pH units λ = 620 nm
Thymol blue (TB)	1.0–3.0 pH units λ = 545 nm 8.0–9.5 pH units λ = 595 nm	2.0–6.5 pH units λ = 690 nm 10.5–12.5 pH units λ = 630 nm
m-Cresol purple (*m*CP)	0.5–2.5 pH units λ = 525 nm 7.0–9.5 pH units λ = 580 nm	2.0–6.0 pH units λ = 670 nm 9.5–12.0 pH units λ = 630 nm

[a] Values obtained for the indicator (FIDMA) suitably prepared from N,N-dimethylaniline.

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