Characterization of Glow-Discharge–Treated Cellulose Acetate Membrane Surfaces for Single-Layer Enzyme Electrode Studies

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ABSTRACT: Cellulose acetate membranes (CA) were modified by means of plasma polymerization of ethylene diamine (EDA) and *n*-butylamine (*n*-BA). The motivation for this work was the application of a modified membrane for the single-layer enzyme electrode. A tubular reactor with the external radiofrequency (13.56 MHz) excitation was used. Surface modification was performed at 5, 10, and 15 W power (at 27 Pa working pressure) for 5, 10, 15 min. Modified surfaces were characterized in detail by FTIR–ATR, XPS (ESCA), contact angle, and enzyme immobilization activity. The best treatment results were obtained for EDA with 5 W and 30 min and 15 W and 10 min. These results are discussed using surface analysis data. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 1341–1352, 2001

Key words: cellulose acetate membrane; plasma polymerization; surface treatment; enzyme electrodes

INTRODUCTION

An enzyme electrode can be defined as a device that combines an electrochemical transducer with a recognition layer consisting of an enzyme. Enzyme electrodes have found wide applications in the food and biomedical industries. However, for analytical purposes their use has been limited by certain factors such as low linearity and sensitivity, long response time, interference of electrochemical compounds present in the medium, and enzyme instability.[1] These problems are caused by the performance of the recognition layer and can be overcome by the improvement of this recognition layer.

In recent years the sandwich-type recognition layer has often been used for the preparation of the sensing layer. However, the sandwich-type recognition layer still has some problems that are characteristic of a sensor. Recently, Mutlu et al.[2]

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developed a new technique, the single-type recognition layer method with polycarbonate membrane. In this method the surface of the membrane is modified to have active groups for enzyme immobilization. The single-type recognition layer is prepared by immobilization of the enzyme on the active membrane surface.

Selectivity is the other basic characteristic of an enzyme electrode. In our previous selectivity studies we determined that use of cellulose acetate (CA) membrane is more convenient than that of some other membranes (polycarbonate, polyurethane, and polyethersulphane) because it overcomes the interference of many electroactive compounds. In addition CA membranes showed selective behavior to the electroactive compound produced by enzymatic reaction (H_2O_2).[3] However, the surfaces of CA membranes are not reactive to immobilize the enzyme. This problem can be solved using amine ($-NH_2$) functional groups that provide the linkages between the enzyme and the membrane surface with the crosslinking agent. Active $-NH_2$ groups can be created on the membrane surface by several techniques such as chemical, physicochemical, or plasmochemical (or glow-discharge) techniques.[4]

Gases (e.g., argon, oxygen, air, nitrogen), nonpolymerizable polar gases (e.g., water, ammonia), and organic vapors (e.g., ethylene, fluoroethylenes, hydroxyethyl methacrylate, hexamethyldisiloxane) create nonreactive or reactive (different functional groups, e.g., carboxyl, hydroxyl, amino) layers on a material surface. Because plasma surface treatment processes, such as corona or gas plasma oxidation procedures, often produce unstable polymer surfaces that lose a considerable part of the treatment effects within a few days, the application of thin films is generally more effective.[5–7] It was found that thin films of plasma polymers are well suited for protein immobilization.[8] Therefore, we applied plasma polymerization techniques to modify the surface of polycarbonate membranes using a single-layer enzyme electrode.[2]

In this study we immobilized the enzyme on the cellulose acetate membrane surface modified by the plasma polymer containing amine groups. We aimed to optimize the parameters of the plasma polymerization process for high enzyme immobilization capacity on the surfaces. For this purpose, CA surfaces were modified for different plasma polymerization parameters and monomers, and then characterized by FTIR–ATR, XPS, contact angle measurements, and radiolabeling technique for contents of the $-NH_2$ groups and enzyme immobilization activity.

EXPERIMENTAL

Materials

Cellulose acetate (CA; 40% acetylated), acetone, ethylenediamine (EDA), glucose oxidase (GOD; E.C.1.1.3.4. from *Aspergillus niger* 292 IU/mg protein), β-D-glucose, and glutaraldehyde (50% aqueous solution) were supplied by Sigma (Poole, UK). The labeling agent ^{99m}Tc-pertechnetate was obtained from Amersham (Buckinghamshire, UK). Ethanol, *n*-butylamine (*n*-BA), phosphate buffers, $SnCl_2$–H_2O, and other standard reagents were purchased from Fisher Scientific (Springfield, NJ). All aqueous solutions were prepared in bidistilled and deionized water.

Preparation of Cellulose Acetate Membrane

Cellulose acetate membranes (with a final thickness of ~8 μm) were prepared by the solvent-casting method described elsewhere.[9,10] The composition of the casting solution was 2% CA (w/v) in acetone. A constant amount (5 mL) of the solution was cast on a petri dish. Membrane formation was achieved by evaporation of acetone at room temperature for 12 h, after which the membrane was removed from the petri dish and left to dry in air.

Surface Modification of Cellulose Acetate Membrane by a Plasma Polymerization Process

The CA membrane surface was subjected to a low-pressure plasma–radiofrequency discharge, operating in the two different monomers (EDA and *n*-BA), to substitute NH_2 groups on the CA surface for enzyme immobilization. Details of the plasma polymerization were described in our previous publication.[11] The glow-discharge apparatus is schematically shown in Figure 1. The reactor was a Pyrex glass tube (inner diameter, 5.6 cm; length, 35 cm). Two copper electrodes (7 × 17 cm) were externally placed on the reactor. One electrode was connected to a radiofrequency of 13.58 MHz (Model T-RF-1200; Tasarim Ltd., Turkey) through a matching network unit (Model T-RF-1100; Tasarim), whereas the other electrode was grounded. The monomer tank was connected to the reactor through a flowmeter (F-1200, Size 2; Gilmont, Barrington, IL, USA) and a needle

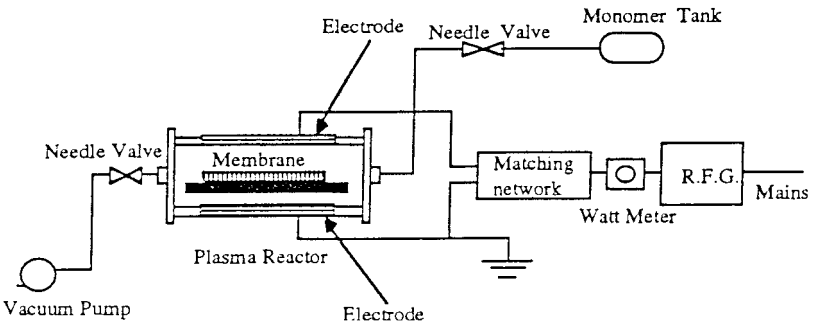

Figure 1 Schematic representation of plasma polymerization apparatus.

valve (Model 1335 CA, Brooks, Hatfield, PA, USA).

EDA and *n*-BA were used as active gases (monomers) to create amine- and/or aminolike active groups on the CA membranes surfaces, to prepare them for the activation by glutaraldehyde and then for the covalent binding of the enzyme. In a typical glow-discharge process two membranes attached to each other (outer surfaces facing plasma) were placed in the middle of the reactor. Nitrogen and oxygen gases in the reactor were swept out with an argon stream prior to the glow discharge. The reactor was evacuated to 10^{-3}–10^{-4} mbar. One of the monomers (e.g., EDA) was allowed to flow through the reactor at a constant flow rate of 20 mL/min, establishing a working gas pressure at 27 Pa. The surfaces of the CA membranes were exposed to EDA–plasma for different time periods (5, 10, 30 min) at different glow-discharge powers (5, 10, 15 W). After the plasma modification the CA membranes were kept for 1 month in the open air before characterization.

Surface Characterization of the Treated Membranes

The changes in the surface structure and the characteristics of glow-discharge–modified CA membranes were determined by FTIR spectroscopy and by X-ray–induced photoelectron spectroscopy (XPS) techniques. The surfaces were also characterized by contact angle measurements to determine the changes in hydrophobicity and to relate them to the nitrogen concentration in the surface. The FTIR and XPS results were correlated to those obtained by immobilizing radiolabeled enzymes on the glow-discharge–modified surfaces.

Contact Angle Measurement

Advancing and receding air/water contact angles were measured at room temperature by an image analyzing system, using the air bubble technique.[12] The advancing angle was obtained by placing a needle in bidistilled and deionized water and creating a bubble on the sample surface (1–1.5 μL) and carefully expanding the bubble volume until the part of the bubble adjacent to the sample surface stopped changing. Similarly, the receding angle was obtained when the air was sucked out of the bubble. On each separately prepared sample surface at least five measurements were taken on different parts of the central sample area and average values were calculated.

FTIR Spectroscopy

Infrared measurements were carried out using a Nicolet Impact 400 FTIR spectrophotometer (Nicolet Instruments, Madison, WI) in an H_2O-purged environment. An ambient-temperature deuterated triglycine sulfate (DTGS) detector was used for the wavelength range from 400 to 4000 cm^{-1}. A Happ–Genzel apodization function was used. Spectral resolution was set at 2 cm^{-1}. Approximately 300 scans were coadded to achieve the signal-to-noise ratio shown.

Because our first attempts to perform the sample measurements in the classical IR light transmission arrangement appeared to be insufficiently sensitive to the surface changes, the baseline horizontal attenuated total reflection (ATR) accessory with ZnSe crystal (n = 2.4 at 1000 cm^{-1}) was used for the measurements of infrared spectra of the samples. In these experiments, the effective path length was approximately a few micrometers (angle of incidence, 60°; seven reflections). The ATR correction was made to eliminate the dependence of the effective path length on the wavelength. The measurements of the vibration spectra of each membrane were repeated three times using three different samples of the same membrane. No changes of spectral features were observed between the results. All studied samples

were arranged in the same way (as identically as possible) to get the possibility of direct comparison.

X-ray–Induced Photoelectron Spectroscopy (XPS)

An X-ray–induced photoelectron spectrometer (ADES 400; VG Scientific, UK) was used to obtain information about the composition and chemical bonding of elements in the analyzed region of the layer. AlKα radiation (1487 eV) was used for XPS photoelectron spectra excitation. A hemispherical electron energy analyzer was adjusted at either 20 or 100 eV pass energy. Spectra were recorded in the regions of C 1s, N 1s, and O 1s at the normal emission angle. Areas of the peaks were determined following an electron inelastic background subtraction (Shirley method). Photoelectron spectra were charge-corrected with respect to the hydrocarbon component of the C 1s line, which peaked at 285.0 eV.[13] All samples were analyzed in an as-received state without any further treatment.

Atomic concentrations were determined semiquantitatively within a model of a solid homogenous within a depth at least equal to the probing depth of the method used,[14] accounting for the photoelectron cross sections, asymmetry parameters,[15] the inelastic mean free paths,[16] and the experimentally determined transmission function of the hemispherical energy analyzer.[17] Deconvolution of the C 1s, O 1s, and N 1s lines was performed using a standard curve synthesis procedure of the SPECTRA program (VG Scientific).

Enzyme Immobilization

Besides the analytical investigation of the glow-discharge–modified CA membrane surfaces, we also used an enzyme-immobilization procedure to see the final result of the modification and to show the convenience of using new surfaces for enzyme electrode preparation (Fig. 2). For that particular purpose, the model enzyme glucose oxidase (GOD) was selected and labeled with ^{99m}Tc-pertechnetate by the chloramine-T method.[18] The plasma-treated CA membrane surfaces (5 cm^2) were activated by incubation with 10 cm^3 of 2% (v/v) glutaraldehyde solution at 20°C for 2 h. The surface-activated membranes were then washed with distilled water to remove any unbounded glutaraldehyde present on the surfaces. The ^{99m}Tc–GOD was immobilized on the active membrane surface in phosphate-buffered solution (pH 6) containing 2 mg/ml of ^{99m}Tc–GOD at 30°C for 3 h. At the end of the immobilization period, the membrane was removed from the solution and the unbounded enzyme was washed out by the buffer and by water. Immobilized radiolabeled enzyme content per square centimeter was measured by gamma scintillation counter (Wallac 1480 Wizard 3; Wallace Inc., Akron, OH, USA) and the amount of enzyme on a unit surface of the membrane was calculated. The details of a typical enzyme immobilization procedure were given elsewhere.[2]

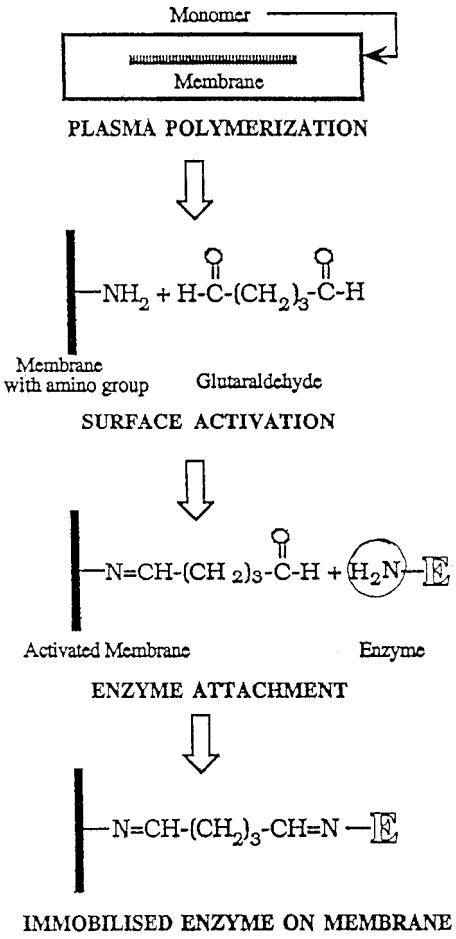

Figure 2 Scheme of membrane modification.

RESULTS AND DISCUSSION

Effect of the Glow-Discharge Treatment

FTIR

FTIR spectra were measured for pristine CA membranes and for treated samples prepared un-

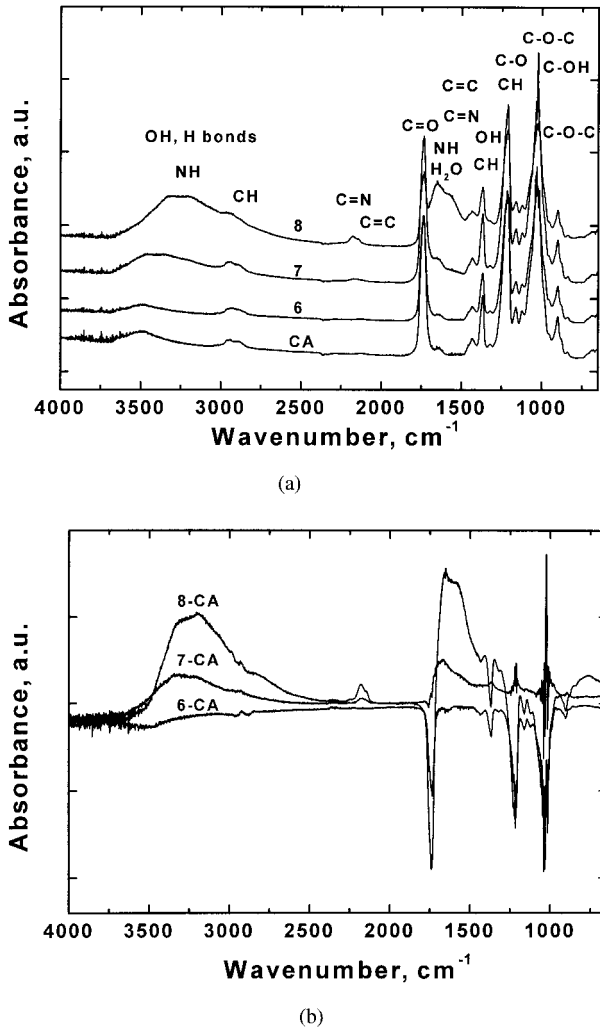

Figure 3 FTIR spectra of pristine CA membrane and of membranes plasma-treated by EDA at 15 W (see Table I, samples 6, 7, and 8). (a) ATR absorption spectra; (b) differential spectra.

der different combinations of both the power and the treatment time used in the plasma modification of the CA surfaces. Typical absorption spectra are presented in Figure 3(a). Here the spectra of pristine CA membrane and of a series of samples of the membranes treated at 15 W for different time periods (samples 6, 7, and 8; see Table I) are plotted. For clarity, the spectra are mutually shifted along the absorbance axis, although their scales match. Above the spectra, some characteristic regions of the vibration of the bonds are shown. One can see that, after the modification process, new features in the spectra appear and become stronger for longer process periods, especially those connecting with NH_2 groups that are present. These are of special interest because they prove that the modification was successful. On the other hand, the appearance of the bands typical for double and triple bonds between carbon atoms and between carbon and nitrogen atoms shows that the modification is not a simple process.

Pristine CA Membrane. The membranes were made of partly (40%) acetylated cellulose and were stored in the open air before testing. Their IR spectra [curve CA in Fig. 3(a)] also contain, besides the known[19,20] peaks of cellulose, the three absorption peaks described in the literature[20] as typical of CA: at 1750 cm^{-1} [vibration ν(C═O)], at 1375 cm^{-1} [δ(C—CH_3)], and at 1240 cm^{-1} [ν(C—O)]. The two latter maxima, as well as the further pronounced maximum around 1030 cm^{-1} are multicomponential. The last one contains, beside others, the antisymmetric vibration ν_a(C—O—C). Its symmetric counterpart, ν_s(C—O—C), is seen as a smaller maximum near 900 cm^{-1}. The presence of OH groups is visible as a broad maximum around 3500 cm^{-1} and as a small one at 1640 cm^{-1}. The broad peak reflects not only the OH groups of CA but probably also the presence of some amount of water in the material.

Membranes Treated by EDA. The changes of the spectra caused by the treatment are seen in Figure 3(a) and still better in Figure 3(b), in which the difference spectra of the treated samples are plotted, that is, their measured spectra from which the spectrum of CA membrane was subtracted. In both figures the height of the bands belonging to amine groups [$\nu_a(NH_2)$ at 3330 cm^{-1}, $\nu_s(NH_2)$ at 3200 cm^{-1}, and $\delta(NH_2)$ at 1680 cm^{-1}] allows one to follow the results of the modification as a function of both the period of the modification and the power used. As a semiquantitative measure of the amount of the NH_2 groups in the samples, the heights of the former two typical peaks are given in Table I. One can see that there is a good agreement with the corresponding data from XPS given in the same table. By longer and/or more intense modification, the contents of NH_2 groups are greater.

On the other hand, the strong band belonging to vibration ν(C═O) decreases with the progress of modification. This is in agreement with the results of XPS, that during the plasma modification the oxygen content at the surface decreases. This may be partly caused by the shielding of the substrate surface, which results from the growing

Table I Plasma Surface Modification Parameters of the CA Samples and Their Surface Characterization

	Plasma Treatment			FTIR: Height of Absorption Peaks (a.u.)		XPS					Immobilized Enzyme Activity (IU/cm^2)
Sample No.	Monomers	Power (W)	Time (min)	ν_a (NH_2) 3330 cm^{-1}	ν_s (NH_2) 3200 cm^{-1}	O (at %)	C (at %)	N (at %)	N/C	N in NH_2 (at %)	
1	EDAe	5	5	0.03	0.02	29.5	51.5	18.9	0.37	4.3	0.16
2	EDAe	5	10	0.18	0.16	19.0	43.5	37.5	0.86	11.9	0.28
3	EDAe	5	30	0.28	0.30	9.2	59.5	31.3	0.52	14.5	0.52
4	EDAe	10	5	0.13	0.14	13.6	53.8	32.6	0.61	13.4	0.32
5	EDAe	10	10	0.10	0.11	12.4	58.2	29.5	0.51	11.9	0.18
6	EDAe	15	5	0.005	0.024	30.4	60.7	9.0	0.15	—	0.25
7	EDAe	15	10	0.21	0.19	16.8	61.2	22.0	0.36	7.8	0.38
8	EDAe	15	30	0.52	0.57	8.8	57.5	33.7	0.59	16.8	0.16
9	*n*-BA	5	5			34.7	65.3	0	0	—	0.04
10	*n*-BA	5	10			35.7	60.4	3.9	0.065	—	0.18
11	*n*-BA	5	30			36.9	63.1	0	0	—	0.29
12	*n*-BA	10	5			15.0	73.2	11.8	0.16	3.7	0.03
13	*n*-BA	10	10			—	—	—	—	—	0.08
14	*n*-BA	15	5			—	—	—	—	—	0.02
15	*n*-BA	15	10			—	—	—	—	—	0.09
16	*n*-BA	15	30			—	—	—	—	—	0.04
17	Untreated CA	0	0	0	0	38.8	61.2	0	0	0	0

layer of aminelike plasma polymer. However, taking into account the penetration depth of the evanescent wave in the ATR measurement, it may be at least partly ascribed to a plasma-activated chemical reaction between the carbonyl groups and the NH_2 groups of EDA, in which oxygen is released in the form of reaction water.

From the spectra, one can also notice some other features of the process. A simultaneous decrease of the intensity of the absorption bands containing the δ(C—CH_3) and the ν(C—O) vibrations suggests that the plasma-modification process is rather energetic and the surface acetyl groups are not only modified but also, more probably, often completely destroyed. This finding is also supported by the observation that the two peaks containing the ν(C—O—C) vibrations also decrease. This indicates that even ring oxygen in CA is degraded by various species (the ions and the radicals) from the plasma. Further supporting arguments come from the spectral region around 2150 cm^{-1}. Here, with a progressing modification the bands corresponding to the vibration of triple bonds between two carbon atoms, of nitrile groups, and of structures of the type —N═C═N— appear and become stronger. The same is valid for vibrations of double bonds C═N and C═C at about 1570 cm^{-1}.

Membranes Treated by n-*BA*. Changes of the form of the spectra of these samples were of the same type as that by EDA, only they were much smaller. In fact, again in agreement with the XPS results, certain changes were observed only in the spectrum of the most intensively treated sample.

XPS

Carbon, oxygen, and nitrogen atomic concentrations in the near-surface region (6–8 nm) of the reference and the plasma-treated CA membranes are summarized in Table I. There is a close agreement between the carbon and oxygen concentrations measured for the reference membrane and the expected values.[13]

As the two monomers used for the plasma-membrane modification contain no oxygen species, an oxygen 1s photoelectron intensity decay may be considered as a qualitative measure of the thickness of the thin film deposited from the plasma.

In Table I (EDA), the oxygen content at measured surfaces gradually decreased with EDA plasma process time. This result—a decrease or disappearance of the C 1s signal from the carbonyl functional group (mentioned below) and the occurrence of the N 1s spectral line intensity—indicates a thin-film deposition at the modified surfaces. This seems not to be the case, however, for *n*-BA membranes plasma modified at a power of 5 W. Here, a plasma-etching process likely dominates over the deposition. Considerable decreases of the oxygen concentration and the C 1s spectral line intensity of the carbonyl functional group are observed for sample 12, which indicates that the deposition process from *n*-BA plasma starts at a glow-discharge power of 10 W. The idea of the deposition and etching is further supported by the particular values of nitrogen concentrations. The high values close to or above 30 at % of nitrogen are found for the membranes treated by EDA for 10 and 30 min, except for sample 4. Because only amine functional groups are expected to be effective in the successive enzyme immobilization, the N 1s was fitted using two Gaussian functions and, tentatively, a signal originated from nitrogen atoms in the amine functional group was separated and is displayed in Table I. The highest amine functional group concentration was found for sample 8, treated by EDA at 15 W and 30 min. Comparing both monomers using the samples treated by *n*-BA plasma revealed lower nitrogen content, mainly as a result of the lower nitrogen content in the *n*-BA monomer.

Typical high-resolution C 1s, O 1s, and N 1s spectra of the reference CA membrane and the EDA plasma-treated membranes are shown in Figure 4(a)–(c). The C 1s spectrum of the CA reference surface formed a two-peak structure: a dominating broad structure with a flat maximum at 286–267 eV and a less-pronounced peak at 289 eV. The former peak could consist of four different contributions[13]; however, an additional contribution originating from a surface carbon contamination had to be added to obtain a satisfactory fit. The peak at 289 eV corresponds to carbon in the carbonyl functional group.[13] Similar features are preserved for the C 1s line of sample 1, indicating that 5 W/5 min EDA plasma treatment does not create an overlayer of sufficient thickness. As the probing depth of the method used is estimated to be 6–8 nm,[21–23] the estimated average thickness of the overlayer may be below 1 nm. The C 1s spectra of the EDA plasma-treated samples 3 and 8 hide the CA original structure; they reveal a weak or no signal in the region of 289 eV. This indicates, together with the nitrogen and oxygen contents, that overlayers grown during EDA

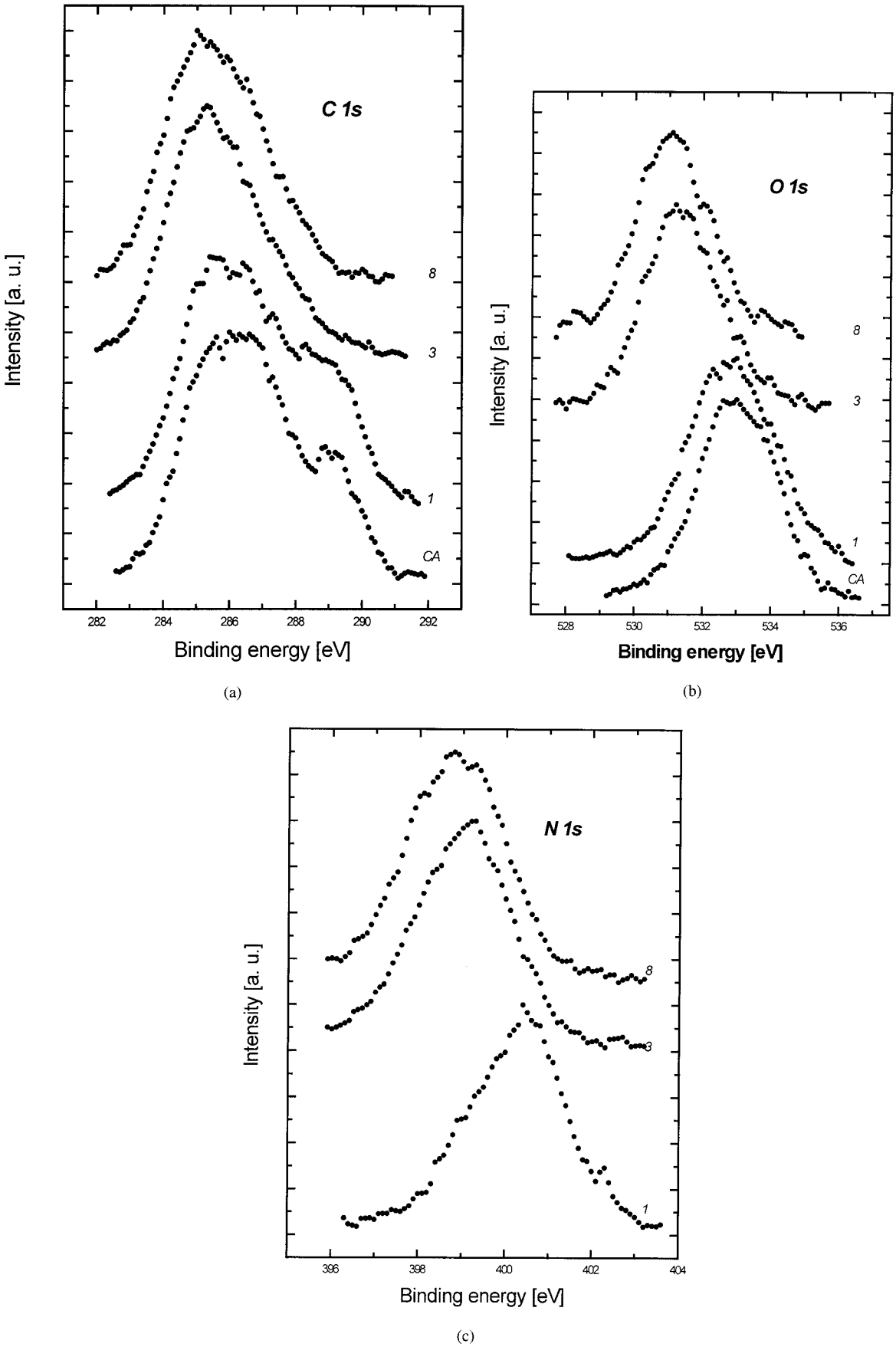

Figure 4 High-resolution photoelectron spectra of CA membranes plasma-treated in EDA. (a) C 1s for samples 1, 3, and 8 and pristine CA; (b) O 1s for samples 1, 3, and 8 and pristine CA; (c) N 1s for samples 1, 3, and 8 and pristine CA.

plasma modification (i.e., plasma polymer deposition) are thick enough compared to the probing depth.

It is important to note that the C 1s spectral line shapes of the *n*-BA plasma-treated CA membranes (not shown) resemble those of the EDA plasma-treated membranes: samples 9, 10, and 11 possess the C 1s line shape typical for the untreated foil.[14] Sample 12, with a relatively high nitrogen content, possesses the C 1s line shape practically the same as that for the EDA plasma-treated samples 3 or 8.

The O 1s peaks [see Fig. 4(b)] of the pristine CA membrane and sample 1 form a broad, almost symmetric line peaked at about 533 eV, typical for oxygen in the CA.[13] This finding is consistent with the behavior of the corresponding C 1s peaks. The O 1s peaks of samples 3 and 8 are shifted toward low binding energy by about 2 eV. The origin of these two spectral line intensities can be attributed to the exposure of the sample surfaces to air during their transfer to the XPS chamber and/or with the chemical reaction of broken original oxygen bonds of CA during the plasma process.

The N 1s peak [see Fig. 4(c)] of sample 1 is asymmetric, with a maximum at about 400.5 eV, and consists of at least two individual lines. The N 1s peaks of samples 3 and 8 are located at lower binding energy at about 399 eV. The lower binding energy part of the N 1s envelopes at about 399 eV originates from nitrogen atoms in the amine functional group.[14,24] A residual signal at higher binding energy can be ascribed to C—N bonding in different configurations.[25]

The changes of the elemental composition (Table I) of the modified surface layers of CA membranes, dependent on the time period of the modification, are shown in Figure 5(a) (5 W) and Figure 5(b) (15 W).

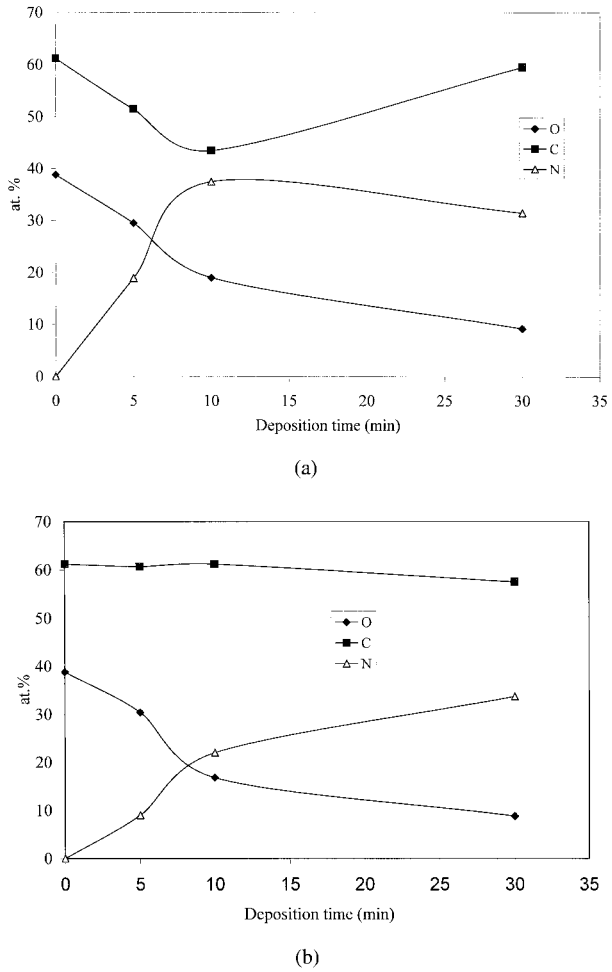

Figure 5 Elemental composition versus plasma-treatment time. (Experimental error in elemental composition determination is 2%. Curves obtained by connecting measured points show tendencies only.) (a) EDA, power 5 W; (b) EDA, power 15 W.

Contact Angle Measurement

Contact angle measurements based on advancing and receding angle values are a quite simple and reliable technique for investigating surface energy changes. They directly give information related to wettability. According to Liston et al.[26] the increase of nitrogen content in the surface increases its wettability. In our case the wettability can also be enhanced by the presence of oxygen in COOH and OH groups. Similarly to the method of Liston et al.[26] we plotted our contact angle results versus N/C ratio measured by XPS (Table I). These plots, related to EDA-modified CA membranes, are shown in Figure 6(a) (5 W) and Figure 6(b) (15 W), showing maxima at 0.4 and 0.4–0.5, respectively. The occurrence of the maxima may be explained as follows. Generally, with the increased time of plasma modification the average thickness of the plasma polymer film increases. This means that the original CA surface, which contains a number of CO and COOH polar groups, is gradually covered by continuous amine-like plasma polymer. The function of the original surface is shielded and, apparently, the contact angle increases. However, when at convenient plasma conditions the N content increases, the decrease of the contact angle takes place because of the increased presence of amino groups. Therefore, the plots of contact angle versus N/C show certain maxima. The error in determination of the contact angle

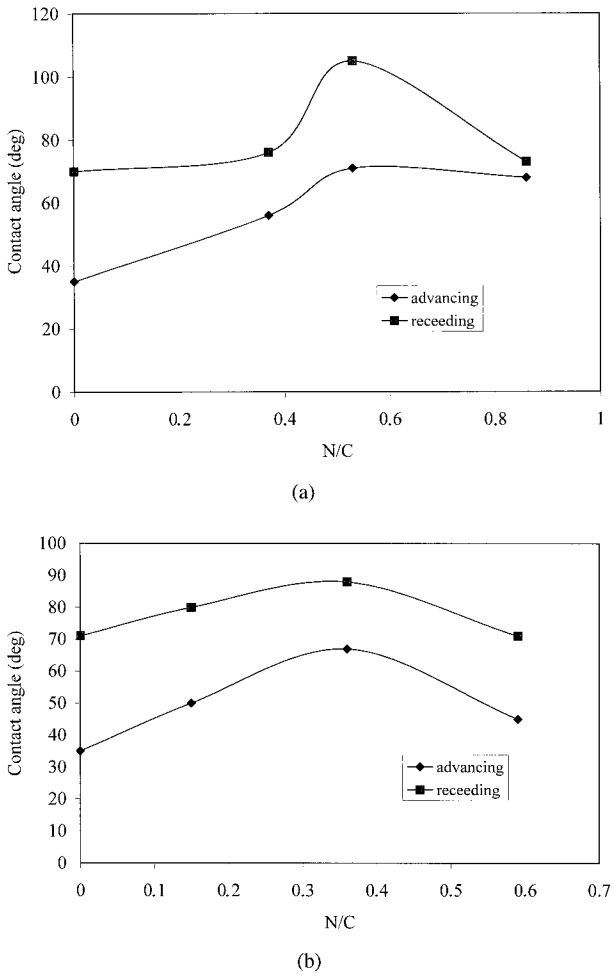

Figure 6 Contact angle versus ratio N/C from Table I. (Experimental error in N/C determination is 5% and in contact angle 4%, respectively. Curves obtained by connecting measured points show tendencies only.) (a) EDA, power 5 W; (b) EDA, power 15 W.

measurement may be assessed as about 4% and, in the case of N/C, about 5%.

Enzyme Immobilization Capacity

The plasma-treated membranes were studied by the above-described enzyme immobilization method using radiolabeled glucose oxidase (^{99m}Tc–GOD). Table I shows the amount of immobilized ^{99m}Tc–GOD dependent on the plasma-treatment parameters and the type of the monomer. Again, one can see that the EDA monomer creates surfaces with active groups more effectively than does the *n*-BA monomer. This situation can be explained by the C/N ratio of the monomers. In EDA the C/N ratio is 1, although this value increases up to 4 in *n*-BA. Therefore, under glow-discharge conditions the probability of the formation of amino groups on the membrane surface or the concentration of possible N ions or radicals in the reaction volume is relatively higher when EDA is used.

The highest amount of immobilized GOD was observed on the surface treated with EDA monomer at 5 W and 30 min. In this mild regime, the immobilization capacity rises with the rising amount of NH_2 groups present at the surface (and determined by the other analytical methods). On the other hand, the enzyme immobilization capacity of the plasma-treated surfaces is decreased by increased power and longer treatment period. These observations suggest that the concentration of NH_2 groups present at the surface is not the only decisive parameter for the successful treatment of the membranes. It should be understood that the highest amino group presence on the surface does not mean the highest enzyme immobilization capacity of the membrane and the best performance of the electrode. Because of the conformational orientation of the enzyme molecules and the stearic hindrance, the large amount of amino groups on the surface cannot be used properly, or the dense packing and binding of enzymes could not show sufficient activity. In such cases the criterion of the immobilized enzyme systems is not the total amount but the total apparent activity of the enzymes.

For comparison we plotted the results of enzyme immobilization capacity measurements against N/C in Figure 7(a) and (b). The highest immobilization effect occurs at N/C = 0.4–0.5, in the same region where the contact angle also achieves its maximum. Both methods show that the best treatment results were achieved at 5 W and 30 min and at 15 W and 10 min. Increasing the power from 5 to 15 W then a shorter time of modification is required to obtain optimal concentration of NH_2.

Process Outcomes

During (and after) the plasma treatment of surfaces, several processes can occur:

1. plasma ablation (etching) of the original surface
2. deposition of plasma polymer films (growth of crosslinked macromolecules)
3. competitive processes of both deposition and ablation
4. postdeposition reactions of frozen-in free radicals with the air in the open atmosphere.

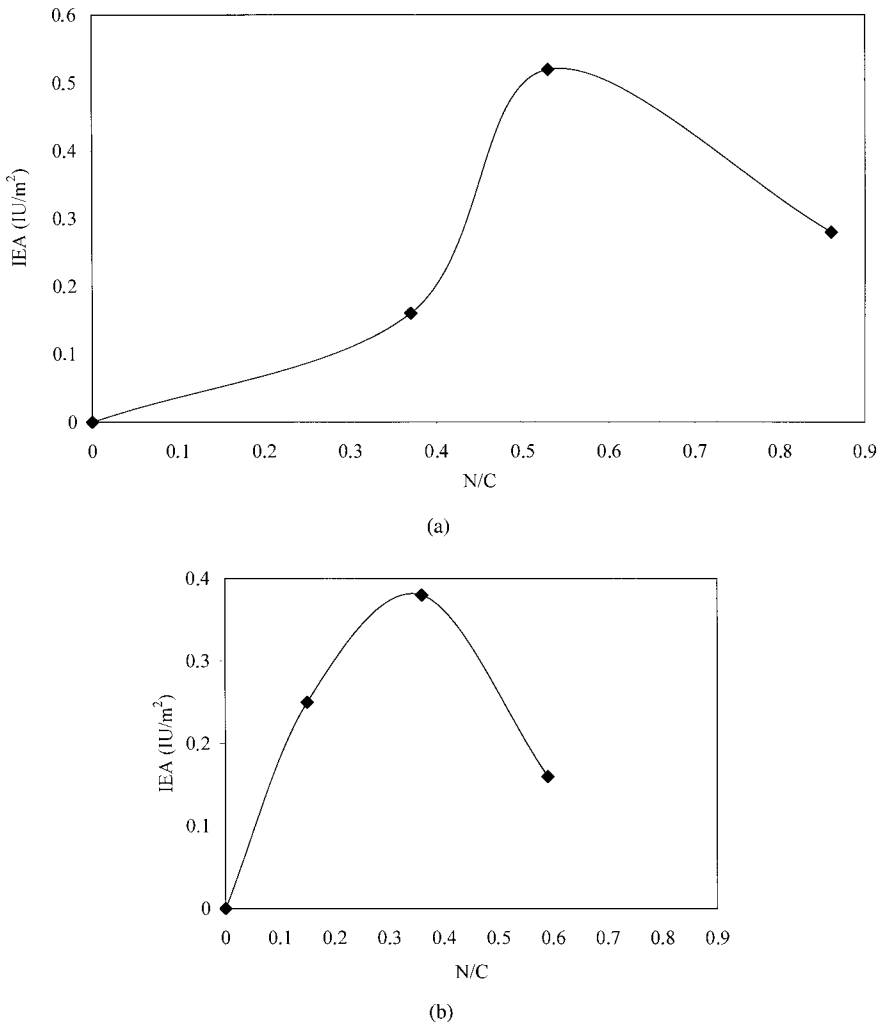

Figure 7 Immobilized enzyme activity IEA versus ratio N/C. (Experimental error in N/C determination is 5% and in IEA 4%, respectively. Curves obtained by connecting measured points show tendencies only.) (a) EDA, power 5 W; (b) EDA, power 15 W.

Probably all these processes played some role in the present experiments and probably mainly in the same order as given. It seems that in the mild regime of the modification (treatment) by EDA (samples 1 and 2) almost all the parts of the EDA molecules are first fixed on the surface (by a chemical reaction and/or by the formation of a plasma polymer film). The thickness of this film is low (up to the order of 1 nm, as assessed in previous discussions under Membranes Treated by EDA and XPS) and, therefore, this plasma polymer film is more or less discontinuous. At longer times and/or at higher powers a continuous plasma polymer film with increasing thickness up to several 10 nm is deposited [see Table I and Fig. 5(a) and (b)]. However, with the increasing power, the N/C ratio in the resulting film decreases.

The immobilization treatment of the modified surfaces is carried out using water solutions. Usually, long plasma modification periods and high power produce very disordered effects that, together with the rising amount of double and triple bonds, probably represent a competing mechanism to the growing concentration of NH_2 groups.

CONCLUSIONS

Both XPS and FTIR results reveal increasing nitrogen content in the treated CA surface layer with increasing duration and intensity of the modification. In the case of *n*-BA, some threshold power is necessary to start the surface chemical

changes. The success of the treatment was also confirmed by contact angle and enzyme immobilization measurements.

Despite different information depths in the two analytical methods (here, some 6 to 8 nm for XPS and several micrometers for FTIR–ATR), there is agreement of results on the presence of different types of bonds in the treated surface layer. Moreover, both methods have shown that nitrogen is only partly present in the desired form in the amine groups, and that a considerable part of it makes double or triple bonds.

Enzyme immobilization capacity measurements have shown that the contents of NH_2 groups on the modified surface is not the only decisive parameter. Because of the complexity of the plasma-treatment process, the resulting immobilization capacity of the surface also depends on the parameters of the treatment procedure. A correlation was found between the immobilization capacity and the wettability of the surface, although it is still under investigation.

The reason that EDA modification at 15 W and 30 min, which according to XPS shows the highest presence of NH_2, does not show the highest immobilization capacity cannot be fully explained at present.

Studies concerning the preparation of a high-linearity, single-layer enzyme electrode are still under investigation.

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