Synthesis and Characterization of Polyvinyl Alcohol-Polyaniline-Glutaraldehyde Composite Disc: a Probable Matrix for Protein Immobilization

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Neste trabalho são apresentados os resultados da síntese e caracterização de um compósito de álcool polivinílico-glutaraldeído-polianilina (PVAG-PANIG). O compósito foi analisado por espectros de infravermelho e absorção UV-visível e por análise termogravimétrica. As análises de microscopia eletrônica de varredura e da superfície dos poros demonstraram que o PVAG-PANIG é um material macroporoso. O material também apresentou condutividade elétrica em baixa temperatura. O compósito preservou características do PVA (hidrofilicidade, possibilidade de ser moldado à mão) e da PANI (propriedades eletrocrômicas). As propriedades encontradas neste material sugerem que possa ser utilizado como suporte em técnicas de imobilização de proteínas.

Palavras-chave: *PVA, PANI, imobilização de proteínas.*

This work presents the results from the synthesis and characterization of a composite of polyvinyl alcohol-glutaraldehyde-polyaniline-glutaraldehyde discs (PVAG-PANIG). The composite was analyzed through infrared spectra, UV-visible absorption and themogravimetric analysis. Scanning electron microscopy and surface porosity analysis showed that PVAG-PANIG is a macroporous material. Electrical conductivity was detected at low temperatures. The composite preserved some properties from the PVA (hydrophilicity, hand molding) and PANI (electrochromical properties). The properties found in this material suggest that it could be used as a support for protein-immobilization applications.

Keywords: *PVA, PANI, protein immobilization*.

Introduction

Synthetic organic polymers are materials whose chemical diversity and versatility make them the most investigated compounds for biotechnological applications. Many properties of these polymers are exploited in industry. In the field of biochemical engineering for example, several synthetic polymers have been used to solve problems such as prosthesis implants, development of electrochemical cells, and support for enzyme immobilization in construction of biosensors or bioreactors.

Polyaniline (PANI) is a widely studied polymer and has received much attention from researchers in the last 30 years. There are many properties that make polyaniline a versatile material, such as its environmental stability, [1] electrochemical behavior,[2, 3] simple acid-base doping/de-doping chemistry,[4, 5] thermal stability, [6, 7] optical properties[8] and low cost of its synthesis. [9] Moreover, the property of electrical conductivity and the capacity to immobilize biomolecules have made polyaniline a component in biosensors.[10, 11, 12, 13, 14]

Nevertheless, polyaniline presents some disadvantages. PANI has been described by some authors to be intractable because of its high thermal stability and insolubility. [1, 15, 16] Moreover, the polymer resulting from the chemical synthesis process is a fine powder which is difficult to separate from the solvent because of its dispersibility property.[1]

To overcome these problems, preparation of PANI-based composites or blends is considered a very promising alternative in that both the functional properties of PANI and the mechanical properties of the matrix can be simultaneously preserved.[16]

One of the polymers frequently used as support in composite structures is polyvinyl alcohol (PVA). This organic polymeric material has great flexibility, hydrophilicity, transparency, crystalinity, biodegradability and biocompatibility properties, and can be shaped during its synthesis. Because of these properties PVA was used as a polymer stabilizer,[17, 18] as a remover of agricultural pesticides,[19] as a model of mass transfer,[20] as a chromatographic matrix gel[21] and as a matrix for protein immobilization.[22, 23]

In our research group amylase, xantine oxidase,[24] peroxidase,[25] glucoamylase[26] and some antigens of tropical diseases were covalently immobilized on PANI and PVA.[23, 27, 28] Here we present a synthesis and characterization of PVA-glutaraldehyde discs coated with polyaniline, named PVAG-PANIG. This material is based on the synthesis of the PVA-glutaraldehyde network followed by the chemical synthesis of polyaniline on the surface of the network. As a consequence, we obtained a composite which has a mixture of hydrophilic/hydrophobic groups, has electrochromic properties and the advantages of a few number of steps for synthesis as well as lower costs. Finally, PVAG-PANIG composite can be removed from the bulk of chemical reactions manually allowing them to have more precise control.

Experimental

SUPPORT SYNTHESIS

PVAG-PANIG discs were synthesized according to Araújo *et al*.[22] and Fernandes *et al*.[5] Briefly: PVA (Vetec Química Fina, Brazil; 200 mg) was dissolved in distilled water (10 mL) by heating at 65 ºC and 25% (v/v) of glutaraldehyde (Vetec Química Fina, Brazil; 1.5 mL) was added. This mixture was vigorously stirred for 50 min after which aliquots (20 µL) were introduced into flat wells of microplates (Mumc, Denmark) containing 3.0 mol L^{-1} HCl (120 µL). The microplates containing the network of PVA cross-linked with glutaraldehyde (PVAG) were incubated for 24 h at 25 ºC. Afterwards, the discs (1.0 g) were treated with 0.61 mol L^{-1} ammonium persulphate (Carlo Erba, Italy) prepared in 2.0 mol L^{-1} HCl for 30 min. Then the treated discs (1.0 g) were incubated with 0.44 mol L^{-1} aniline (Merck, Germany) for 60 min to allow the PANI to be formed. The PVAG-PANI discs (1.0 g) were thoroughly washed with 2.0 mol L^{-1} HCl, incubated with 2.5% (v/v) glutaraldehyde at 60 ºC and finally washed five times in succession with 0.1 mol L^{-1} sodium phosphate buffer, pH 7.6.[16] These discs (PVAG-PANIG) were kept at 4 ºC in a 0.1 mol L^{-1} sodium phosphate buffer pH 7.6.

PVAG-PANIG CHARACTERIZATION

Spectrophotometry

PVA, PVAG and PVAG-PANIG discs were analyzed by infrared spectra (FT-IR; KBr method) in the spectral

range 400-4000 cm^{-1} (Hartman & Braun MB series - Michelson). Visible-ultraviolet spectrophotometry analyses of the PVAG and PVAG-PANIG were carried out in the range of 250-900 nm with 2 nm intervals (Beckmann DU-70, Beckmann Instruments GmbH, Germany).

Thermal gravimetry (TGA)

Discs of PVAG-PANIG (15 mg) previously stored in phosphate buffer 0.1 mol L^{-1} pH 7.0 were washed in distilled water, dried using filter paper (Whatman n. 1) and then transferred to a thermo-analyzer (Shimadzu DTG 60H). To avoid discs deformation no dehydration step was used in this procedure. The samples were heated from 23 ºC to 800 ºC using the following heating rates: 5 ºC min^{-1} in the 23 ºC-250 ºC range; 2 ºC min^{-1} in 251 ºC-700 ºC and 5 ºC min^{-1} in the 701- 800 ºC.

Surface Area Analysis

The surface area measurements of the PVAG-PANIG discs were carried out by using an accelerated surface area and porosimetry analyzer (ASAP 2010, Micromeritics Corporate Headquarters, USA). The initial mass of the sample was 0.072 g with P/P_0 equal to 0.200 and the pore measurement capacity of 1.7 at 35 nm.

Scanning electron microscopy

The discs were first dehydrated in acetone solutions from 30% (v/v) to 100% (v/v) and dried at the critical point (Balzers, model CPD 030, England). Samples were fixed on stubs followed by metallization with gold in an argon atmosphere. Finally, they were analyzed by scanning electron microscopy (JEOL JSM-840A, Japan).

Electrical conductivity

PVAG-PANIG discs were submitted to electrical conductivity variation using a Keithley 602 electrometer at 40 V and the temperature ranging from -146 ºC to 18.7 ºC (126.6 K to 291.67 K).

Results and Discussion

About 240 discs of 15 mg (PVAG-PANIG) were synthesized using the procedure described here (11.5 mL of the mixture and three microplates) and typical discs are shown in Figure 1, namely, PVAG disc (yellow, transparent) and PVAG-PANIG disc (dark green).

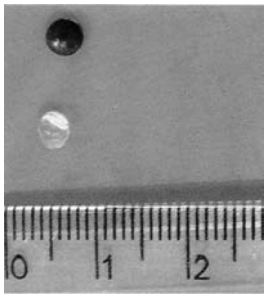

Figure 1. Discs of PVAG (yellow) and PVAG-PANIG (dark green).

The synthesis of PVAG-PANIG was monitored by infrared spectra in the three stages: PVA, PVAG and PVAG-PANIG (Figure 2) and the pattern of the bands from polyvinyl alcohol, glutaraldehyde and polyaniline were observed. It is possible to observe peaks at the region of the 3400 cm^{-1} in all the stages, related to the stretching of -OH from the PVA.[29, 30] Figure 2C shows a band at 2942 cm^{-1}, related to the presence of $-CH_2$ groups.[30] Another band from PVA was found at 1429 cm^{-1} related to the -OH deformation (Figures 2A-C). Additionally, the spectrum of PVA (Figure 2A) indicates an elevated isotacticity degree in the sample used in this work (1140 cm^{-1}). Carbonyl bands from glutaraldehyde were observed at the 1700 cm^{-1} region in Figures 2B and 2C[31, 32, 33] and at 2840 cm^{-1}. Mansur *et al.*[32] explained the presence of the carbonyl bands in PVA-glutaraldehyde preparations as an incomplete reaction of the glutaraldehyde with –OH groups from PVA during the crosslinking network formation. This can be an advantage for covalent immobilization processes, when a glutaraldehyde arm is necessary to link support and immobilize enzymes.

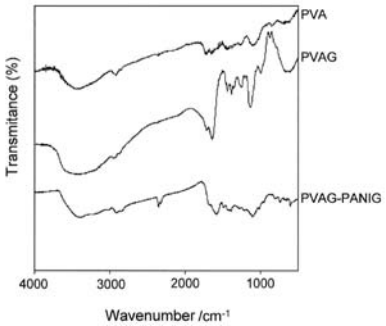

Figure 2. Infrared spectra of the PVA, PVAG and PVAG-PANIG. Samples were prepared using KBr pellet technique.

The bands from polyaniline in Figure 2C revealed a predominance of 1577 cm^{-1} under 1500 cm^{-1}. This finding, according to Melo *et al*.,[31] indicates that the polyaniline content in the PVAG-PANIG disc appears in the reduced form, named leucoemeraldine. These authors explain that this feature is based on the hypothesis of redox interactions occurring between glutaraldehyde and polyaniline, yielding the aldehyde groups from the first compound to carboxyl radicals and the quinoid structures from polyaniline to benzenoid groups (leucoemeraldine).

In situ visible-ultraviolet spectra (Table 1) showed peaks from PVA and PVAG-PANIG. In 250-300 nm there is a region correspondin g to the chemical groups from polyaniline. Tang *et al*.,[33] studying optical properties from polyaniline and some derivatives, related a peak at 208 nm and suggest it as a π–π^* electron transition within the benzenoid segments. According to these authors, a peak at 300 nm is originated from the charged cationic species known as polarons. Other peak at 320 nm in PVAG-PANIG spectrum confirms this finding.[18]

One band identified in PVAG spectra was at 280-300 nm, generally found when glutaraldehyde is present.[34] It was not possible to observe peaks at 280 nm or 330 nm, related to carbonyl functionality of PVA.[17] We believe this is a consequence of the crosslinking action of glutaraldehyde.

Table 1. In situ visible-ultraviolet spectrophotometry of the PVAG and PVAG-PANIG. Samples were prepared using one solid disc of PVAG or PVAG-PANIG.

	Bands/Peaks	**Reference**
PVAG	280-300 nm	[34]
PVAG-PANIG	250-300 nm	[33, 34]
	320 nm	[18]

Thermal gravimetry analysis of the PVAG-PANIG is presented in Figure 3. Weight loss was observed in the ranges 50-100 ºC, 200-300 ºC and 400-450 ºC. The first one (64.04% of weight loss) was attributed to water evaporation from the composite. The hydrophilic behavior of PVA, due to its high hydroxyl content, makes it a polymer able to interact with water molecules. Since PVAG-PANIG samples used in this procedure were washed and stored in a phosphate buffer environment we believe this high water content is also due to the presence of the buffer in the pores of the discs. Phosphate buffer was chosen, in this case, to preserve the support surface and to avoid glutaraldehyde oxidation that occurs in the oxygen atmosphere.

The second loss between 200-300 ºC (72.51-75.32 % of weight loss) was attributed to the chemical alterations of the PVA structure, according to Helen *et al*.,[35] and to Holland and Hay.[36] These authors compared the TG pattern with the infrared bands in the TA-FTIR spectroscopy analysis. According to them the thermal degradation of PVA between 260 and 290 ºC showed keto-enol tautomerization (1705 cm^{-1}) and the formation of small amounts of alkyne units (2050 cm^{-1}). In this range (200-300 ºC) there is a lost reported to HCl evaporation[7] used in PANI-doping.

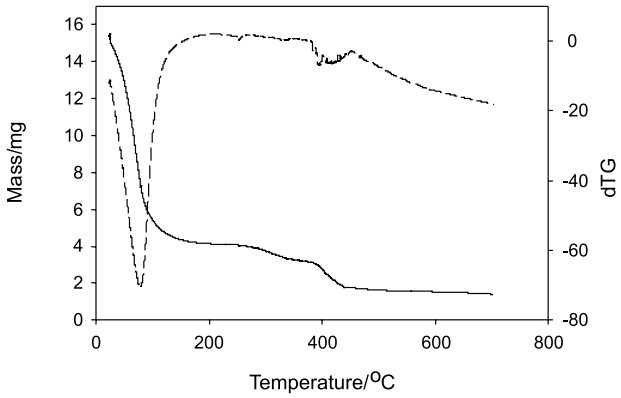

Figure 3. Thermal gravimetry analysis of the PVAG-PANIG. Straight line represents weight loss (mg) and dash line shows the resulting derivative transformation for thermogravimetry.

The weight loss above 400 ºC is related to the thermal decomposition of the carbon backbone of PVA and PANI, according to Gangopadhyay *et al*.,[18] Helen *et al*.,[35] and Wei and Hsueh.[7] Finally, at the end of the thermal treatment 10% of residues did not presented any weight loss. This is according to some PANI TGA analyses, and show that the presence of the buffer did not alter the inorganic composition of the PVAG-PANIG discs.[18, 37]

Figure 4 presents the results from the porosity analysis of the PVAG-PANIG. Under the analysis condition (0-40 nm), PVAG-PANIG discs presented a small number of pore sizes from 5 to 35 nm in diameter (mesopores

range). The major adsorbed volume was attributed to the number of pores with a diameter greater than 35 nm. Pore volume capacity varied from 0.9 x 10^{-4} cm^3 g^{-1} to 3.58 x 10^{-4} cm^3 g^{-1}.

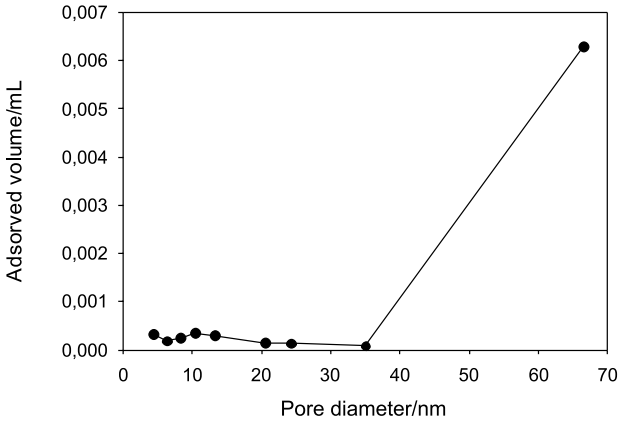

Figure 4. Pore distribution for PVAG-PANIG.

PVAG-PANIG adsorption analysis (Figure 5) displayed a behavior comparable to the isotherms of type IV (pore size greater than 50 nm in diameter) proposed by Brunauer in 1938.[38, 39] On the other hand, the pore area measurement of the PVA has been reported to be type I (microporous materials) according to Zhang *et al*.[29] We hypothesize that polymerization of aniline during support preparation had covered PVA's micropores.

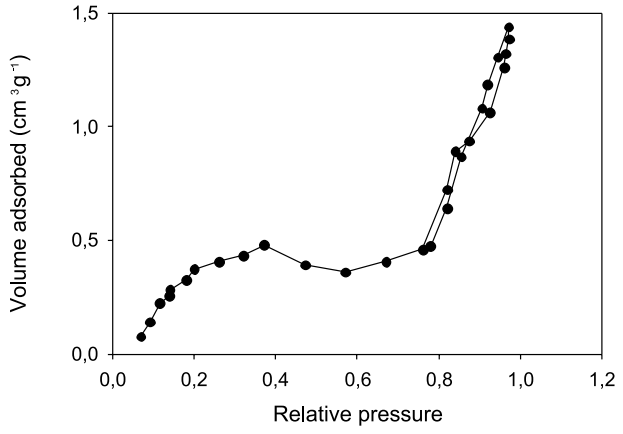

Figure 5. PVAG-PANIG adsorption isotherm.

The absence of micropores in the PVAG-PANIG can also be confirmed by the scanning electron microscopy (Figure 6) that shows the presence of depressions and protuberances 500 nm apart from each other. According to Gregg and Sing[37] this is a characteristic of the macroporous structure (pore size greater than 50 nm). This feature can be an advantage when an enzyme which recognizes voluminous substrate/product is immobilized at the surface of the disc of PVAG-PANIG. A macroporous structure permits the transit of a substrate from the bulk of the reaction to the microenvironment of an immobilized enzyme, with less mass transfer limitations.

Figure 7 presents data related to the influence of temperature on the electrical resistance of the PVAG-PANIG. No change in the electrical conductivity/resistance was observed in the 120-240 K range (-153 to -73 ºC). After that, a dramatic decrease occurred yielding a resistance equal to about 106 Ω. Similar conductivity variation influenced by the temperature has been reported for PVA and PANI.[14, 35, 40] This phenomenon observed for PANI is explained by the HCl evaporation responsible for the conductivity properties of the polymer.[6, 7, 14] Moreover, the use of glutaraldehyde as a chemical arm may cause an increase in the benzoid content in the PANI structure,[31] promoting a decrease in the conductivity by decreasing the quinoid-benzoid pair at the polymer. Finally, the incubation of the PVAG-PANIG discs in alkaline buffer solution could enhance the counter ion dissociation and hence, reduce the conductivity of the composite produced in this work.

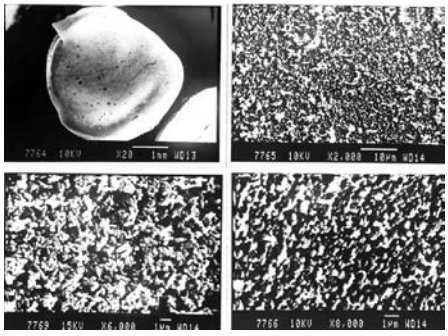

Figure 6. Scanning electronic microscopy of PVAG-PANIG.

Conclusions

PVAG-PANIG composite discs were synthesized via a glutaraldehyde network. Properties from PVA and PANI could be identified by infrared and UV-Vis spectra, and by

TG analysis. At room temperature, PVAG-PANIG appears to be a hydrophilic and non-conductive macroporous composite. The SEM surface studies indicated that PANI recovered the PVA matrix. The properties of the composite produced in this work, namely hydrophilicity, the presence of reactive groups and macroporosity are very interesting, and suggest that this material is a promising support for protein immobilization.

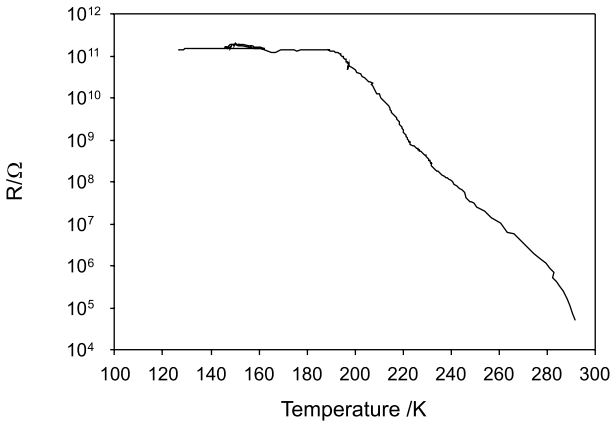

Figure 7. The influence of the temperature on the electrical resistance of the PVAG-PANIG.

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