Effects of Polyaniline Nanotubes on Acetylcholinesterase Activity

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Introduction: Polyaniline nanotubes (PANI - NTs) based on different polymerization paths and template formation have been reported since 2006. Polymerization depends on variables such as pH and temperature, as well as surfactants and solvents used to produce linear or branching polymer segments with different degrees of oxidation and protonation states. Zebrafish (Danio rerio) is a vertebrate model for studying biological processes, such as neurochemical alterations promoted by heavy metal toxicity, C_{60}. Here, we present a spectroscopic, morphological study in polyaniline nanotubes with acetylcholinesterase (*AChE*) activity. As a model choice, we used Zebrafish because of nanostructures' dangers and potential effects on aquatic organisms. We use spectroscopy characterization techniques such as UV-VIS-NIR and infrared spectroscopy in addition to gel permeation chromatography and scanning electron microscopy. Finally, this study combines research in different areas, such as physical chemistry, polymeric science, toxicological parameters, and nanoscience. **Materials and Method.** Black solid PANI - NTs were prepared in an aqueous solution, and their dispersion (1 mg mL^{-1} stock solutions) was prepared by sonication for 45 minutes in sterile deionized water. At their final concentrations, the suspensions exhibited a neutral pH. Animals were treated with different concentrations of nanotubes (1, 2.5, 5, 10 mg L^{-1}) suspended in water for one hour. Control animals were maintained in a tank with water for the same time as the treated animals. After treatment, fish were used for the analysis of *AChE* activity (a total of 144 animals). After PANI–NT treatment, the animals' brains were quickly dissected. Each independent experiment was performed using biological preparations from a pool of three animals for *AChE* experiments. Protein was measured via the Coomassie Blue method, which considered bovine serum albumin as the standard. *AChE* activity was expressed as mmol thiocholine (*SCh*) h^{-1} mg^{-1} protein. All experiments were performed in quadruplicate. Finally, the data for *AChE* activity was described as the means ± S.D. and were analyzed by one-way analysis of variance (*ANOVA*) following the post hoc test of Tukey, where $P < 0.05$ was considered significant. **Results and Discussions.** Synthesized products show one-dimensional structures with lengths greater than 500 nm and inner and outer diameters of 109 nm and 160 nm, respectively. With a thick wall near 40-30 nm. PANI-NTs used in this study show two values (molecular weight, polydispersity index (PI)), one at (9797, 1.03) and the other (at 4754, 1.04). From the combined analysis of electron microscopy images and gel-permeation data, we distinguished two kinds of nanotubes — the first survey related as a function of length. One nanotube has a double length that of the other, keeping the exact value of molecular weight. The second analysis shows that one kind of tube has double molecular mass as the other, keeping the same length as the tubes.

The PANI-NTs samples have benzenoid units, polarons, and delocalized free-charge carriers. The infrared spectra showed active modes at 1496 cm^{-1} of ν C-C in N=**B**=N rings, where **B** is a benzenoid unit, and 1540 cm^{-1} of ν C-C in N=**Q**=N rings, where **Q** is the quinoid unit, (Figure 2). Electronic absorption behavior shows that polaron → π* transition suffers a shift and decrement as pH increases. Absorption ratio (**AQ**/**AB** = 0.79) and vibrational active mode ($\nu_{N=B=N}/I\nu_{N=Q=N} = 0.90$) ratio confirm the doped polymer presence. All this data provides key information in terms of labeled quality indicator samples. The effect of different concentrations of PANI-NTs treatments on the *AChE* activity was investigated in zebrafish. The sample analyzed is related to the one synthesized at 1026 rpm, which is related to a better nanotube quality. As shown in Fig. 1D, there were none significant degree changes in the *AChE* activity on zebrafish brains after exposure for one hour to the tested PANI nanotubes in the concentrations (1, 2.5, 5, 10 mg L^{-1}), of 1 mg L^{-1} (53.2 ± 14.9 mmol of SCh h^{-1} mg^{-1} protein), 2.5 mg L^{-1} (57.1 ± 13.0 mmol of SCh h^{-1} mg^{-1} protein), 5 mgL^{-1} (52.9 ± 17.7 mmol of SCh h^{-1} mg^{-1} protein), and 10 mgL^{-1} (46.2 ± 14.9 mmol of SCh h^{-1} mg^{-1} protein), when compared to the control group (44.4 ± 7.6 mmol of SCh h^{-1} mg^{-1} protein; $P > 0.05$).

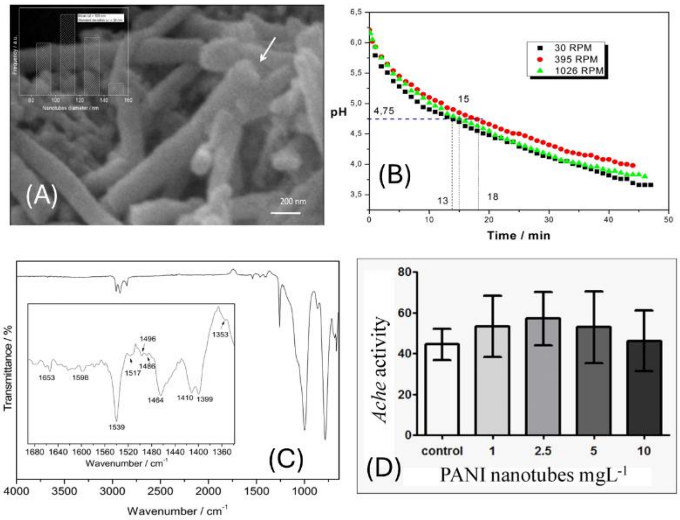

Figure 1. *(A) Electron microscopy of PANI-NTs. Insert the histogram frequency, where (d) mean outer diameter and (σ) is the standard deviation. White arrow shows the round closed-end tips tube.* ***(B)*** Diagram pH vs. time of PANI nanotubes at different agitation speed levels. ***(C)*** Infrared spectra of the PANI nanotubes on the range of 4000-640 cm^{-1}. The sample analyzed corresponds to the one synthesized at 1026 rpm. Insert on it the spectrum in the range of 1690-1340 cm^{-1}. ***(D)*** *Zebrafish* AChE *activity after PANI nanotube treatments. The activity was evaluated after treatment with different nanotube concentrations. Bars represent the mean ± S.D. of at least eight different experiments. The data were analyzed by a one-way ANOVA test followed by a Tukey's multiple range test in which $P \leq 0.05$ was considered significant (F).*

We chose low nanotube concentration due to the possible interference of color with the biological test. Polymeric tubes were synthesized in water to distinguish the effects of the interactions between tube solvents and tube tubes. Water tube solubility is an essential issue for this kind of essay. We found that *AChE* activity was significantly enhanced when the zebrafish were exposed to 2.5 mg L^{-1} of PANI nanotubes dissolved in water. *AChE* brain activities after one hour of exposition at low concentrations ≤ 10 mg L^{-1} were about 30 % higher than the control. A derivative UV-VIs *ex-situ* spectra of PANI-NTs sample after the treatment and the control sample showed a slight shift wavelength at 258 nm and 283 nm bands, which are associated with benzene ring distortions, which means that uptake, elimination, and compartmental distribution dynamics could be affected by the weak basicity of aniline, nitrogen charge moieties, and nanotube lipophilicity. **Conclusion**. In summary, Despite the *AChE* results after one hour of exposition of PANI nanotubes, 30 % higher activity than the control group is observed. The spectroscopic findings show that polymerization produces tail-to-tail (benzidine) segments polymer, and two kinds of doped tubes were identified. **Acknowledgements.** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors thank the financial support of CNPq and FAPERGS and VL thanks for the guidance and support given to C. Bonan and G. Cognato at the time.

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