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Purification of a conducting polymer, polyaniline, for biomedical applications

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ABSTRACT

A conducting polymer, polyaniline, was prepared in globular and nanotubular morphologies. The protonated forms were converted to the corresponding bases and both types of samples were tested for cytotoxicity. The polyanilines were then suspended in *N*-methylpyrrolidone or in concentrated sulphuric acid, and the soluble parts were precipitated into methanol acidified with sulphuric acid. Such a dissolution/precipitation cycle was tested as a purification procedure for polyaniline, which would remove the potential low-molecular-weight components. The original morphology of polyaniline was destroyed in soluble part (18–24 wt.%) but maintained in the fraction insoluble in *N*-methylpyrrolidone. The fraction soluble in sulphuric acid was higher (56–64 wt.%). The original morphology converted to fragments after reprecipitation, and the samples became amorphous. The conductivity was reduced on average by two orders of magnitude. FTIR spectroscopy was used to assess the molecular structure, hydrogen bonding, and their changes. The cytotoxicity of polyaniline salt determined on mouse embryonic fibroblast cell line NIH/3T3 was reduced after reprecipitation from *N*-methylpyrrolidone when compared to the initial polymer and showed the absence of cytotoxicity at the extract concentration of 5 and 10% in the case of globular and nanotubular polymer, respectively. A corresponding positive effect was not observed for polyaniline reprecipitated from sulphuric acid.

1. Introduction

Polyaniline (PANI) [1] is one of the most frequently studied conducting polymers. In addition to its electrical, optical, or responsive properties, scientists have also been attracted by the variety of nanostructures this polymer produces [2,3]. In addition to standard globular PANI, which is obtained by the oxidation of aniline in strongly acidic aqueous media, nanotubes are produced in solutions of moderate acidity [4–7], and nanofibres typically under a diluted regime [3,8,9].

Medicine and biology are promising areas, in which conducting polymers, such as polyaniline or polypyrrole, could be used for monitoring vital function in living organisms, as well as for their stimulation [10]. The mixed electronic and proton conductivity of conducting polymers makes them unique materials that are expected to mediate the interfacial transfer between ionic and electronic charge transport [11–15] at the interface of biological objects and electrodes. The interaction with living tissue is of interest especially with respect to cardiac [16–18] and neural tissues [19–21] or brain and other cells [22]. Many potential applications of conducting polymers, however, are not based on conductivity. Among them, the antimicrobial compositions [23–25], cell-proliferation supports [26,27], and the use in photothermal tumour destruction [28], may serve as examples.

The biocompatibility of PANI has recently been tested [22,29,30] and, for some applications, improvement is still needed. The deprotonation–reprotonation cycles performed on PANI led to reduction in cytotoxicity. Polyaniline, being completely insoluble and stable in aqueous media, can hardly be toxic. Thus it seems that prevailing hazards with respect to cytotoxicity on biological objects are associated with low-molecular-weight components. These are of two types: (1) reaction by-products and aniline-based oligomers

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[31,32], and (2) the acids that constitute the salts with PANI. For that reason, the purification of PANI with respect to the former group is of importance.

The reprecipitation of polymers is a routine method used in polymer science for the removal of residual monomers and any other low-molecular-weight compounds. In this procedure, a polymer is dissolved in a suitable solvent and added drop-wise to a large excess of non-solvent. The polymer precipitates but low-molecular-weight compounds stay dissolved and can be separated. So far, for PANI, this method has not been tested due to the small selection of solvents, which is limited to *N*-methylpyrrolidone and concentrated sulphuric acid and, even in these solvents, the solubility is not complete. The present study tests these reprecipitation procedures as the means for the purification of PANI for applications in medicine or biosciences.

An additional question of interest is connected with the morphology and conductivity changes caused by the reprecipitation. Globular and nanotubular PANI have therefore been selected for this study, and their fate in this procedure is reported.

2. Experimental

2.1. Polyaniline preparation

Standard globular PANI (PANI-S) was prepared by the oxidation of 0.2 M aniline hydrochloride (Lachner, Czech Republic) with 0.25 M ammonium peroxydisulphate (APS; Lachner, Czech Republic) in water [1] at room temperature. The nanotubular PANI (PANI-NT) was similarly synthesized by the oxidation of 0.2 M aniline (Fluka, Switzerland) with 0.25 M APS in 0.4 M acetic acid [4]. Polyaniline powders were separated by filtration, rinsed with acetone, and dried under ambient conditions. They were subsequently deprotonated to the PANI bases by suspension in excess of 1 M ammonium hydroxide and dried as above.

2.2. Reprecipitation

Polyaniline bases (10 g) were suspended in 250 mL of *N*-methylpyrrolidone (NMP) or concentrated sulphuric acid (96 wt.%). The suspensions were occasionally shaken. After 30 days, the insoluble part was separated by filtration. The insoluble part was well rinsed with methanol containing sulphuric acid, and dried in an ambient atmosphere. The solutions containing the soluble part of the PANI base were added drop-wise into 1.5 L of methanol containing 15 mL of concentrated sulphuric acid. The precipitate, PANI sulphate, was collected on a filter and dried in air at room temperature.

2.3. Characterization

Infrared spectra in the range 400–4000 cm^{-1} were recorded using a fully computerized Thermo Nicolet NEXUS 870 FTIR Spectrometer with a DTGS TEC detector. Samples were dispersed in potassium bromide and compressed into pellets. Raman spectra, excited with a HeNe 633 nm laser, were collected on a Renishaw inVia Reflex Raman spectroscope. The conductivity was measured by a four-point van der Pauw method using a current source SMU Keithley 237 and a Multimeter Keithley 2010 voltmeter with a 2000 SCAN 10-channel scanner card on pellets compressed at 540 MPa with a manual hydraulic press.

Wide-angle X-ray scattering (WAXS) experiments were performed using a pin-hole camera (Molecular Metrology System) attached to a microfocused X-ray beam generator (Osmic MicroMax 002) operating at 45 kV and 0.66 mA (30 W). The camera was equipped with a removable and interchangeable Imaging Plate $23 \times 25\ cm^2$ (Fujifilm). Experimental setup covered the momentum transfer range $q = (4\pi/\lambda) \sin\theta$ of 0.25–3.5 $Å^{-1}$, where $\lambda = 1.54$ Å is the wavelength and 2θ is the scattering angle. Calibrations of the centre and sample-to-detector distance were made by using silicon powder. Samples were measured in the transmission mode.

2.4. Cytotoxicity test

Prior to *in-vitro* cytotoxicity testing, the samples were sterilized by a dry heat at 121 °C for 20 min, homogenized in a mortar, and subsequently extracted, according to ISO 10993-12, at 0.2 g of the PANI per 1 mL of culture medium. The extraction was performed in chemically inert closed containers using aseptic techniques at 37 ± 1 °C under stirring for 24 ± 1 h. The parent extracts (100%) were then diluted in a culture medium to obtain a series of dilutions with concentrations of 50, 25, 10, 5 and 1%. All extracts were used within 24 h. The ability of cells to respond to cytotoxic substances was verified by application of sodium dodecyl sulphate solution (SDS; Sigma, Czech Republic).

Cytotoxicity testing was conducted in accordance with EN ISO 10993-5 using mouse embryonic fibroblast cell line NIH/3T3 (American Type Culture Collection, HB-8065), cultivated according to the protocol recommended by the supplier. Cells were pre-cultivated for 24 h and the culture medium was subsequently replaced with PANI extracts. As a reference experiment providing 100% cell proliferation, the pure extraction medium was used. To assess cytotoxic effect, the MTT assay (Invitrogen Corporation, USA) was performed after one-day cell cultivation in extracts at 37 ± 0.1 °C. All the tests were performed in quadruplicates. The absorbance was measured at 570 nm by Infinite M200 PRO (Tecan, Switzerland). Dixon's *Q* test was used to remove outlying values and means were calculated. The cell viability, expressed as percentage of cells present in the corresponding PANI extract relatively to cells cultivated in pure extraction medium (100% viability) was determined. The morphology of the cells was assessed after their cultivation in extracts for 24 h. The cells from each culture plate were observed by an inverted Olympus phase contrast microscope (Olympus IX81, Japan) at 40× magnification.

Table 1
The conductivity of globular and nanotubular polyanilines and of their respective components soluble and insoluble in *N*-methylpyrrolidone or concentrated sulphuric acid.

Type	Solvent	Soluble fraction [wt.%]	Conductivity, soluble part [$S\ cm^{-1}$]	Conductivity insoluble part [$S\ cm^{-1}$]	Crystallinity[a] [%]
Globular	Original PANI base	–	1.3		15
	NMP	18.6	0.028	0.035	18
	Sulphuric acid	64.0	0.073	0.14	0
Nanotubular	Original PANI base	–	0.029		35
	NMP	24.4	4.2×10^{-5}	1.3×10^{-4}	35
	Sulphuric acid	56.0	8.8×10^{-4}	5.0×10^{-4}	0

[a] Degree of crystallinity of original or insoluble parts. All soluble parts were amorphous.