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Research paper

Biological properties of printable polyaniline and polyaniline–silver colloidal dispersions stabilized by gelatin

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ABSTRACT

The oxidation of aniline with ammonium peroxydisulfate in the presence of gelatin yields spindle-like colloidal polyaniline particles having the particle size smaller than 200 nm. The similar oxidation of aniline with silver nitrate leads to hybrid composite polyaniline–silver nanoparticles with more complex morphology. The composites were characterized by transmission electron microscopy, dynamic light scattering and UV–vis spectroscopy. The cytoxicity of colloids has also been investigated. To test biointerface properties, the synthetized colloids were deposited to poly(ethylene terephthalate) foil using spiral bar coating and flexography printing technique. Prepared layers were tested for eukaryotic cell adhesion and proliferation, and antibacterial activity. The prepared surfaces do not only allow for eukaryotic cell adhesion and proliferation but also they possess significant antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*, even without silver nanoparticles. This newly prepared surface has therefore high practical potential in variety of application in regenerative medicine or biosensing.

1. Introduction

During past years we can observe growing interest in conducting or semi-conductive inks, which can be potentially processed by printing techniques like inkjet [1–3], gravure [4–9], screen printing [10–15] or by flexography [9,16,17]. Conducting polymers, such as polyaniline (PANI) [18] or polypyrrole, combined with noble metals, provide the promising way to obtain such conducting inks.

Polyaniline or polypyrrole colloids are produced by the oxidation of respective monomers in the presence of suitable water-soluble polymers [19], e.g., poly(*N*-vinylpyrrolidone) [20–22], poly(vinyl alcohol) [20,22], poly(2-acrylamido-2-methyl-1-propanesulfonic acid) [23], carboxymethylcellulose [24], hydroxypropycellulose [25], or more complex and tailor-made stabilizers [26–28]. The conductivity of printed patterns produced by such colloids may be satisfactory in many applications, such as sensors [4,12,29], but its enhancement is a challenge for the uses in printed microelectronics. The incorporation of silver seems to be the relevant strategy to achieve this goal.

Hybrid colloidal particles containing PANI and noble metals can be prepared, in principle, in two ways: (1) by the oxidation of monomers with noble-metal acids or salts [30,31] in the presence of stabilizers or (2) by chemical deposition of metals, such as silver, on conducting polymer dispersions produced earlier. In the latter approach, PANI in emeraldine form reduces silver ions to metallic silver [32]. Polyaniline is oxidized to pernigraniline at the same time. The former approach, the oxidation of aniline with silver nitrate in the solutions of gelatin, has been used for the preparation of hybrid colloids in the present report.

Gelatin has already been used for the stabilization of PANI colloids in a series of early papers published by Ptschelin in 1935–1937 [33], who used potassium dichromate as oxidant, but no follow-up studies by other authors have been reported. For that reason, we have tested gelatin as a colloidal stabilizer in present experiments and extended the studies to the preparation of hybrid PANI–silver colloidal dispersions.

Application of gelatin in food, pharmaceutical and photographic industry is well known [34]. Gelatin has often been used for the stabilization of noble-metal particles, the classical photographic emulsions being probably the best-known example. The use of gelatin in bioapplications is increasing. The gelatin generally exhibits very good film forming properties which could be next advantage regarding printing and coating application. Polyaniline and its composites has recently been successfully tested for biocompatibility [35–37] and tissue engineering [38]. The gelatin-stabilized PANI colloids with or without

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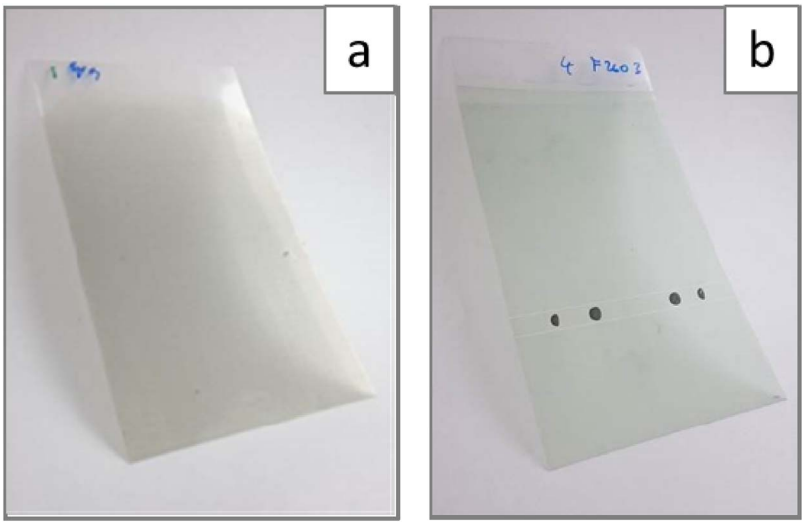

Fig. 1. Spiral-bar-coated layer of PANI–silver colloid (a) and flexography-printed PANI colloid (b).

silver thus may find uses in medicine, i.e. in devices monitoring or stimulating the processes in living tissues. For that reason, the preparation and properties of PANI–silver colloidal systems stabilized with gelatin are reported in the present paper.

2. Experimental

2.1. Preparation of polyaniline and polyaniline–silver dispersions

Colloidal PANI or PANI–silver dispersions were prepared by the oxidation of aniline (0.2 M, Fluka, Switzerland) with ammonium peroxydisulfate (0.25 M, Lach-ner, Czech Republic) or silver nitrate (0.5 M, Lach-Ner, Czech Republic) in the presence of gelatin (2, 4, and 8 wt.%, porcine type, Fluka, Switzerland) in 1 M methanesulfonic acid (MSA) solution at 40 °C. The methanesulfonic acid has been used to constitute the acidic medium required for the polymerization of aniline; elevated temperature prevented the gelation of gelatin. The reaction between aniline and silver nitrate was accelerated by 0.002 M of *p*-phenylenediamine (Sigma-Aldrich) [39,40].

2.2. Characterization of dispersions

The particle size was determined on colloidal dispersions diluted with water by the dynamic light scattering apparatus Malvern AutoSizer Lo-C at 40 °C. At least 5 measurements had been performed and the results were averaged. The dispersity index, a relative width of the particle-size distribution was obtained by assuming the logarithmic-normal distribution of particle sizes. Colloids morphology was investigated by transmission electron microscope JEOL JEM 2000 FX (Jeol, Japan). The ultraviolet–visible (UV–vis) spectra of PANI and PANI–Ag colloids were recorded in 1 M ammonium hydroxide with a Perkin Elmer Lambda 20 spectrometer (United Kingdom).

2.3. Cytotoxicity test

Prior to *in-vitro* cytotoxicity testing, the sterilization of dispersions was conducted. The dispersions were exposed to dry heat at 80 °C for 1 h for 3 times, always after 24 h. For the test of cytotoxicity primary mouse embryonic fibroblasts (MEF; a kind gift of Dr. Jiří Pacherník) were used. The ATCC-formulated Dulbecco's Modified Eagle's Medium (DMEM, BioSera, France) was used as the culture medium. Calf serum in concentration 20% (BioSera, France), 100 U mL^{-1} of penicillin/streptomycin (BioSera, France) and 7 µL L^{-1} of 2-mercaptoethanol (Serva, Germany) were added to the DMEM. The tested samples were diluted to concentrations of 0.1–10% in the culture medium. The test of cytotoxicity was performed according to the EN ISO 10993-5, with modification. Cells were pre-cultivated for 24 h in the concentration 1×10^5 cells per mL. After pre-cultivation time, the culture medium was aspirated and the cells were treated with individual samples in different concentrations. Cells cultivated in a pure culture medium were assigned as a reference. For the determination of the cytotoxicity, metabolic assay MTT (Invitrogen Corporation, USA) was used. MTT assay was performed after 24 h of cultivation of cells treated with colloidal PANI. MTT assay was carried out in 4 repetitions. The absorption was taken at the wavelenght 570 nm on the Infinite M200 Pro NanoQuant (Tecan, Switzerland). Dixon's Q test was used to remove outlying values, and mean values were calculated. Moreover, cell morphology was observed after 24 h of cell cultivation with the tested samples. For the better visibility, cells were stained with fluorescent dyes. Hoechst 33258 (Invitrogen, USA) was used for the nucleus staining and actin filamentous were stained using ActinRed™ 555 (Thermo Fisher Scientific, USA). Firstly, cells were fixed and permeabilized. For the fixation, cells were covered by 4% formaldehyde solution (Penta, Czech Republic) for 15 min. Subsequently, cells were washed by phosphate buffer saline (PBS, Invitrogen, USA). This step was followed with the permeabilization. Triton X-100 (Sigma-Aldrich) in concentration 0.5% was applied to the cells for 5 min. In the next step, cells were washed by PBS for three times. Finally, PBS was added to the cells and they were stained using two drops per 1 mL of ActinRed™ 555 and 5 µg mL^{-1} of Hoechst 33258. The cells were left to incubate for 30 min in the dark and then observed under Olympus inverted fluorescent microscope (Olympus IX 81, Japan).

2.4. Coating and printing of polyaniline and polyaniline–silver dispersions

Colloidal PANI or PANI–silver dispersions were used as an ink formulation for coating of poly(ethylene terephthalate) (PET) substrate using spiral bar coating technique (Fig. 1a). Given technique was used as a standard deposition technique (TQC AB3120, Netherlands) because uniformity of deposited wet films for all colloidal inks. The 30 µm spiral bar was used and deposition speed was set to 10 mm s^{-1}. The 175 µm PET substrate Melinex ST504 was used in all experiments including flexography printing. Flexography printing represents one of the most common mass production techniques which could suitable for large area printing of developed cytotoxic layers (Fig. 1b). Anilox ceramic cylinder was engraved at a 60° with line screen 260 cells per inch, where the cell volume was 8.8 cm^3 m^{-2}. As a printing form, the EPDM rubber cylinder with hardness 65° Shore was used. Dispersions were printed three times by "wet to dry" technique, where the drying between printings was 20 s at lab temperature. All layers were left in hot-