Available online at www.sciencedirect.com

ScienceDirect

European Polymer Journal 43 (2007) 3471–3479

EUROPEAN POLYMER JOURNAL

www.elsevier.com/locate/europolj

Enzymatic synthesis of pH-responsive polyaniline colloids by using chitosan as steric stabilizer

Rodolfo Cruz-Silva [a], Alondra Escamilla [b], M.E. Nicho [a], Gabriela Padron [b], Antonio Ledezma-Perez [b], Eduardo Arias-Marin [b], Ivana Moggio [b], Jorge Romero-Garcia [b,*]

[a] *Centro de Investigación en Ingeniería y Ciencias Aplicadas, Universidad Autónoma del estado de Morelos, Av. Universidad 1001, Col. Chamilpa CP 62210, Cuernavaca, Mor., Mexico*
[b] *Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna # 140 CP 25140, Saltillo, Coah., Mexico*

Received 26 January 2007; received in revised form 9 May 2007; accepted 23 May 2007
Available online 2 June 2007

Abstract

Polyaniline colloidal particles were prepared by enzymatic polymerization of aniline using chitosan as steric stabilizer and toluenesulfonic or camphorsulfonic acids as doping agents. Fourier transform infrared and UV–vis spectroscopic studies indicate that enzymatic polymerization of aniline in dispersed media results in the emeraldine salt form of polyaniline. The morphology of the colloids was studied by transmission electron microscopy. Toluenesulfonic acid produced mainly oblong particles whereas rod-like shaped particles were obtained using camphorsulfonic acid. Polyaniline particles with good colloidal stability and size below 200 nm were obtained using 1.0 wt% of chitosan in the reaction media, indicating that this polymer was highly efficient as a steric stabilizer. The content of chitosan attached to the polyaniline colloids was approximately 20 wt% as indicated by elemental analysis. The colloids synthesized either with toluenesulfonic or camphorsulfonic acid showed a strong pH-dependent colloidal stability and underwent rapid flocculation in near neutral or alkaline media. This interesting behavior could be exploited in separation technology applications.

Keywords: Enzymatic polymerization; pH-sensitive; Smart flocculation; Conductive polymers

1. Introduction

Polyaniline (PANI) is one of the most promising conducting polymers because of its good chemical stability, facile synthetic route and relatively high electrical conductivity [1]. However, its application has been limited due to its infusible character and low solubility in most organic solvents. To overcome these problems, dispersion polymerization of aniline has been widely used to obtain water-dispersible colloidal particles that can be cast as films or blended with other materials to prepare composites. The synthesis of water dispersible PANI colloids is considered a “green technology” due to the reduction in the use of organic solvents during the processing of this conductive polymer. Besides

---

[*] Corresponding author. Tel.: +52 844 438 9830x1445; fax: +52 844 438 9839.
E-mail address: jromero@ciqa.mx (J. Romero-Garcia).

0014-3057/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.eurpolymj.2007.05.027

several synthetic water-soluble polymers, such as poly(vinyl alcohol) [2,3] and polyvinylpyrrolidone [4], a few biological macromolecules, such as cellulose derivatives [5,6] or proteins [7] have been used as steric stabilizers for aniline polymerization in dispersed media. One of the most interesting biological macromolecules is chitosan, an inexpensive and renewable biopolymer, which is increasingly being employed as surfactant or stabilizer [8–10]. Even though it is a naturally occurring polymer, large-scale production of chitosan is realized mainly through partial *N*-deacetylation of chitin, which is among the three most abundant biopolymers [11]. Chitosan is a soluble cationic polyelectrolyte in acidic media, and it has been previously used in PANI chemical polymerization [12]. Although to the best of our knowledge, the morphological characterization of the synthesized PANI has not been reported.

On the other hand, during the last decade, enzymatic polymerization of aniline has attracted great attention as an alternative synthetic route with lower environmental impact as compared with classical chemical oxidations because it is carried out under milder conditions and reduces the oxidation by-products to water [13–15]. One great advancement in the enzymatic polymerization of aniline was the development of the template-assisted enzymatic polymerization approach [16]. This method comprises the use of an anionic polymeric template to promote the head-to-tail coupling of aniline radicals in order to obtain a water-soluble complex of electrically conductive PANI. The template-assisted polymerization of aniline can also be performed enzymatically in a two-phase system [17], or by chemical oxidation using both synthetic [18] and biological polymers [19] yielding water-soluble polyelectrolyte complexes. However, regardless of the oxidation method employed in this approach, the separation of the PANI from the polyanion is impeded by the high degree of complexation between them.

Besides the preparation of water-soluble PANI complexes using sulfonic or phosphonic based polyelectrolytes as templates, the preparation of polymer colloids by enzymatic synthesis has attracted a great deal of attention as well. The use of a weaker anionic polyelectrolyte, such as polyacrylic acid, during enzymatic oxidation of aniline has resulted in PANI colloids [20,21]. On the other hand, the enzymatic synthesis of PANI in micellar solutions has been reported, although the morphology of the product was not characterized [22]. Enzymatic polymerization in dispersed media has been employed to obtain polyphenol particles by using poly(vinyl methyl ether) as stabilizer in partially organic media [23]. We have shown that enzymatic polymerization of aniline using poly(vinyl alcohol) as steric stabilizer results in stable dispersions of PANI colloidal particles, with physicochemical properties similar to those synthesized by chemical oxidation [24]. Recently, we reported the “smart behavior” of polyaniline colloids prepared enzymatically by using chitosan or poly(*N*-isopropylacrylamide) as steric stabilizer [25]. In this work, we focused on the use of chitosan as steric stabilizer during the enzymatic polymerization of aniline in aqueous media to understand in a more comprehensive way the formation of these colloids. It was found that the morphology is dependent on the sulfonic acid used as doping agent. The physicochemical properties of the PANI colloids were studied by different characterization techniques whereas their morphology was analyzed by transmission electron microscopy. Water-dispersible PANI colloidal particles were obtained by a fully environmentally friendly process, which combines a biopolymer as steric stabilizer, and a biocatalytic oxidation pathway. In addition, by using an environmentally sensitive polymer as steric stabilizer, a pH-responsive colloidal stability is given to the particles.

2. Experimental section

2.1. Materials

Aniline was acquired from Baker and purified by distillation at reduced pressure over a mixture of potassium hydroxide and stannous chloride. The middle fraction was collected and stored at −28 °C in the dark prior to use. Chitosan was purchased from *Carbomer*, the deacetylation degree (87%) was calculated by FTIR spectroscopy, according to the method described by Brugnerotto et al. [26], whereas the viscosimetric molecular weight ($M_v = 9.70 \times 10^6$) was determined by the Mark–Howink equation using the procedure reported by Rinaudo et al. [27]. 4-Toluenesulfonic acid (TSA), (±)-camphor-10-sulfonic acid (β) (CSA), ammonium hydroxide (28 wt%), *N*-methyl-2-pyrrolidinone (NMP), and hydrogen peroxide (30 wt%) were acquired from Aldrich. Soybean peroxidase (RZ = 1.3, Activity 56 U/mg) was purchased from *Sigma Chemical Co*. All reagents were of analytical grade or better and used without further purification except otherwise indicated.

2.2. Polymerization reaction

In a typical reaction, 20 mL of a 0.12 mol/L solution of aniline, containing 1.2 wt% of chitosan, was adjusted to pH 3.0 with a 50 wt% aqueous solution of TSA and held in water at 60 °C under nitrogen flux and vigorous magnetic stirring for 5 min, then a water–ice bath was used to cool the reaction mixture to 1 °C. Soybean peroxidase (2.4 mg) was dissolved in 2.0 mL of deionized water and added to the reaction mixture. Once the reaction medium was homogenized, 2.0 mL of a 3.75 wt% hydrogen peroxide solution was added to the reaction using a peristaltic pump at 1.0 mL/h flow rate. Samples of 50 μL were collected and analyzed by UV–vis spectroscopy to follow the reaction. After the hydrogen peroxide addition, the reaction was left for 3 h to be completed. PANI particles were separated from the reaction mixture by redispersion-centrifugation cycles. During redispersion, water with a pH adjusted to 2.5 with TSA or CSA was used to avoid chitosan precipitation. By following this method, the enzymatically synthesized colloids could be isolated from the chitosan solution and redispersed in acidified water or dedoped by treatment in ammonia solution (0.2 N), where flocculation took place. For the characterization studies, the doped or dedoped PANI colloids were collected by centrifugation, washed with deionized water and lyophilized.

2.3. Instrumentation

UV–vis spectra were obtained on a *Shimadzu 2410* spectrophotometer. The FTIR spectra were recorded in a *Nicolet Magna 510* spectrophotometer using KBr pellets. Thermogravimetric analyses were performed in a *TA instruments Q500* thermobalance using a 20 °C/min heating rate under a 40 mL/min N_2 flow. Turbidimetric measurements were done in a *Genesys10* UV–vis scanning spectrophotomer. Elemental analyses were done in a *Perkin–Elmer CHN S/O 2400* elemental analyzer. Transmission electron images were obtained in a *JEOL 1200EXII* equipment operating at 80 kV accelerating voltage. Electrical conductivity measurements were done using a *Keithley 2400* sourcemeter. Analysis of the colloidal stability as a function of the pH was done by titrating a polyaniline dispersion with NaOH using a *Genesys10 UV–vis* spectrophotometer.

3. Results and discussion

3.1. Enzymatic polymerization in dispersed media

After the addition of the first drop of hydrogen peroxide, the reaction media developed a dark green color within 30 s indicating a very fast aniline oxidation without induction period. Fig. 1 displays the UV–vis spectra of the resulting PANI colloidal particles synthesized using CSA or TSA as doping agents dispersed in acid aqueous medium. The UV–vis spectra of both samples exhibit the typical spectral features of the emeraldine PANI salt [4–6], with a 360 nm band assigned to the benzenoid π–π^* electronic transitions, and a shoulder at 430 nm and a broad band close to 755 nm assigned to the polaron bands of PANI. The reaction mixture required the green color for the whole period of the reaction, indicating that emeraldine salt is produced without pernigraniline formation. On the contrary, chemically synthesized PANI dispersions present a blue color during the oxidation step, due to the formation of pernigraniline salt [3]. Unlike the chemical oxidation mechanism, peroxidase catalyzes the rapid and direct oxidation of aniline, and the radicals produced undergo coupling to form the PANI in its emeraldine salt form. As will be discussed further, these particles could not be dispersed in

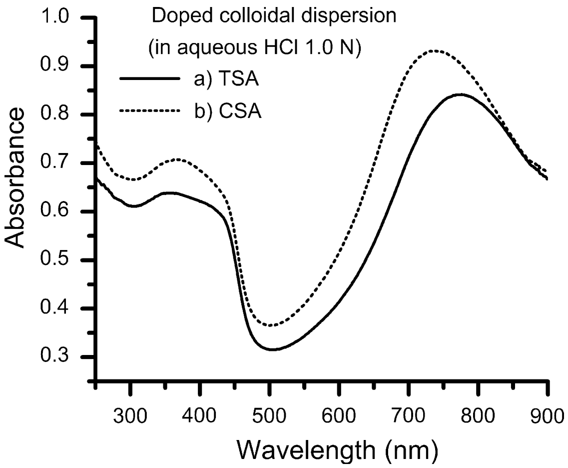

Fig. 1. UV–vis spectra of the colloidal dispersions of doped enzymatically synthesized PANI colloids, which were synthesized in the presence of chitosan using toluenesulfonic acid or camphorsulfonic acid as doping agent.

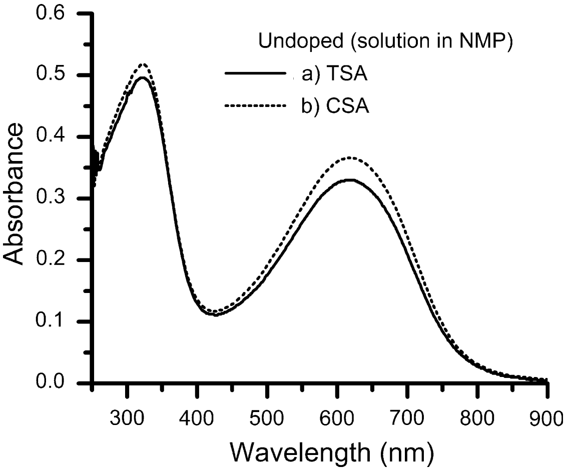

Fig. 2. UV–vis spectra of the dedoped enzymatically synthesized PANI using chitosan as steric stabilizer and toluenesulfonic acid or camphorsulfonic acid as doping agent. The solutions were prepared by dissolving the dedoped enzymatically synthesized PANI colloids in *N*-methyl-2-pyrrolidinone.

alkaline medium, thus their electronic absorption spectra in its dedoped form were obtained by dissolving the dedoped particles in NMP. Fig. 2 shows the spectra of the dedoped PANI synthesized in the presence of TSA or CSA. The spectra of these solutions are characteristics of the emeraldine base form of the PANI. The band at 323 nm is due to the benzenoid π–π* signal whereas the band at 620 nm is due to the exciton emission from the quinoid moieties [2]. The ratio among these bands depends on the oxidation degree, and indicates that enzymatically synthesized PANI has a similar degree of oxidation to that of chemically synthesized PANI [3].

3.2. PANI colloids characterization

In Fig. 3, the FTIR spectra of the PANI samples prepared with chitosan as steric stabilizer are compared with that of PANI enzymatically synthesized in the absence of chitosan as a control sample (Fig. 3a), and with that of chitosan (Fig. 3e). The spectrum of the sample synthesized using TSA as doping agent and chitosan as steric stabilizer after dedoping (Fig. 3b) shows the characteristics bands of PANI, such as those at 1582 and 1495 cm^{-1} due to C–C stretching of the quinoid and the benzenoid rings, respectively, the weak C—N= stretching absorption at 1376 cm^{-1}, and the 1302 cm^{-1} due to C–N– stretching [22,28]. The band at 828 cm^{-1} corresponds to the C–H out of plane bending of the adjacent hydrogens in the 1,4-disubstituted aromatic ring [29], supporting the formation of a linear PANI structure. The spectra of PANI colloids doped with TSA or CSA, (Fig. 3c and d, respectively) show an increase in the absorption of the 1140 cm^{-1} band with respect to the dedoped form, that can be assigned to a vibrational mode of the protonated imine–quinone units [30], which are indicative of the protonation process of polyaniline. This band overlaps the 1160 cm^{-1} signal due to the aromatic C–H in-plane bending, the latter clearly visible in the dedoped form (Fig. 3b). The presence of TSA and CSA in the doped PANI colloids is confirmed by the peaks that appear at 1010 and 1030 cm^{-1} due to S=O symetric and O=S=O asymmetric stretching [22,31]. Although the signals of the sulfonic moieties are weaker in the CSA doped PANI, the carbonyl stretching at 1732 cm^{-1} confirms the presence of the doping agent in the colloids. On the other hand, the adsorption of chitosan on the PANI colloids surface is evidenced by the appearance of two weak bands due to chitosan at 1100 and 1030 cm^{-1} (indicated by the arrows) that are observed in the dedoped PANI sample (Fig. 3b). In the doped samples, these bands are overlapped by those corresponding to the S=O stretching and the vibrational mode of the protonated imine–quinone groups.

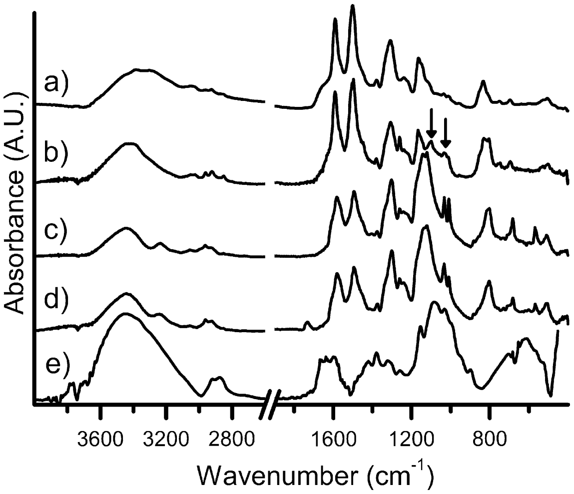

Fig. 3. FTIR spectra of the enzymatically synthesized PANI samples compared with that of the steric stabilizer: (a) dedoped PANI prepared as control in the absence of chitosan and using TSA; (b) dedoped PANI colloids prepared using TSA; (c) PANI colloids doped with TSA; (d) PANI colloids doped with CSA; (e) chitosan (steric stabilizer).

Fig. 4 shows the thermogravimetric scans of the PANI sample prepared as control in the absence of chitosan (Fig. 4a), the dedoped PANI sample

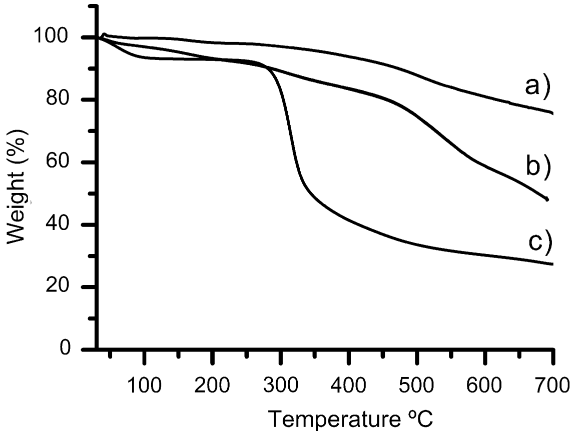

Fig. 4. Thermogravimetric analysis of the PANI particles: (a) PANI synthesized as control in the absence of chitosan; (b) dedoped PANI colloids prepared using TSA; and (c) chitosan.

synthesized in the presence of chitosan by using TSA as steric stabilizer (Fig. 4b), and the scan of chitosan (Fig. 4c). In a previous work, we have shown that the PANI enzymatically synthesized in aqueous media has good thermal stability, slightly lower to that of chemically synthesized PANI [32]. The thermal stability of the PANI synthesized using chitosan as steric stabilizer (Fig. 4b) is lower than the one synthesized as control (Fig. 4a). A possible explanation is because chitosan has a considerably lower thermal stability (Fig. 4c), thus, it is expected that the adsorption of chitosan on the PANI colloids lowers their thermal stability. In addition, the high specific surface of the colloids may contribute to this thermal behavior due to faster evolution of the volatile degradation products. In addition, the lower polymer crystallinity may reduce the thermal stability of the PANI, since the as-synthesized polymer in aqueous medium is semicrystalline [32].

The amount of adsorbed chitosan in the colloids was studied by comparing the elemental analysis of the samples with the theoretical elemental composition of model chitosan and PANI (see Table 1). Carbon content in chitosan is 45 wt% whereas in PANI it is 79 wt%. The synthesized PANI particles have intermediate values, as expected due to adsorbed chitosan on the PANI. The carbon content from elemental analysis indicates that the PANI fraction in the dedoped colloidal particles is about 75 wt% for those synthesized using TSA, and 80 wt% for those synthesized using CSA. The lower adsorption of the hydrophilic chitosan is ascribed to the slightly more hydrophobic character of the PANI doped with CSA, compared to that doped with TSA.

3.3. Morphology

The transmission electronic microscopy images of the PANI colloids are displayed in Fig. 5. Spherical and slightly oblong particles shapes were obtained when TSA was used as doping agent, with a size ranging between 50 and 130 nm diameter (Fig. 5a), whereas needle-like morphologies of 25 nm diameter and approximately 200 nm length were produced when CSA was used as doping agent (Fig. 5b). The change in the particles shape is in part related to the stabilizer efficiency [5], in this case, between chitosan and the PANI doped either with TSA or CSA. Aniline–camphorsulfonic salts are however known to self-assemble in solution, which can also affect the morphology of the particles synthesized with this doping agent [33]. In addition, polymerization of aniline at pH above 2 can lead to polyaniline nanotubes, due to a template effect of PANI oligomers [34]. To investigate the reason for the different morphology observed for PANI colloids prepared with the two doping agents, a wide-angle X-ray diffraction (Fig. 6) study of the

Table 1
Elemental composition of the enzymatically synthesized PANI colloidal particles

Sample	%C	%H	%N	%S
Chitosan[a]	45.5	7.9	8.3	0.0
Emeraldine base	79.6	6.2	14.3	0.0
Emeraldine salt doped with TSA[b]	65.4	5.0	7.9	9.0
Emeraldine salt doped with CSA[b]	63.0	5.9	7.0	8.0
Dedoped PANI colloidal particles synthesized using CSA	73.0	4.8	13.4	1.0
Dedoped PANI colloidal particles synthesized using TSA	71.1	4.7	12.5	1.4
PANI colloidal particles doped with CSA	63.5	5.1	8.7	6.1
PANI colloidal particles doped with TSA	61.9	4.9	8.7	6.9

The first four rows indicate theoretical compositions.

[a] Assuming an 87% of deacetylation degree.

[b] Assuming a 50% of doping level.

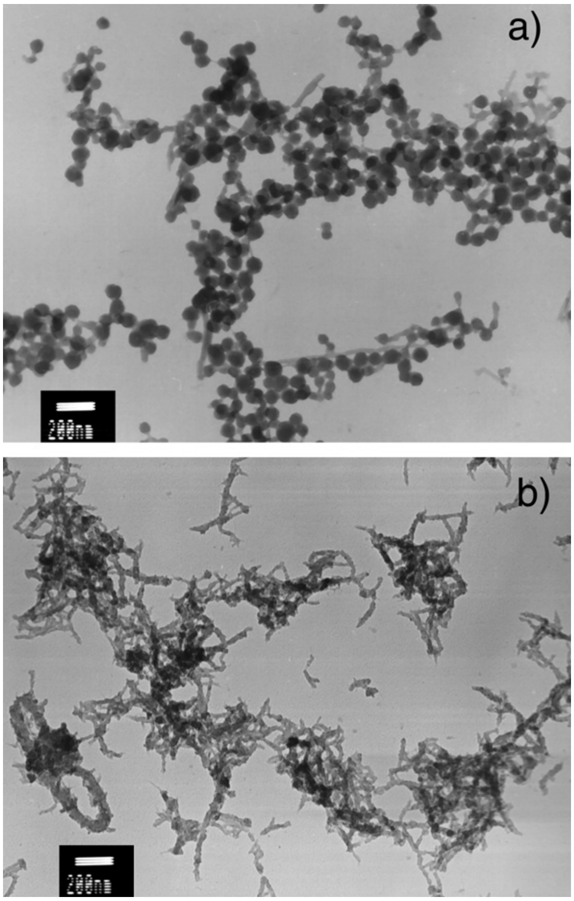

Fig. 5. TEM images of PANI colloidal particles enzymatically synthesized using chitosan as steric stabilizer, and (a) TSA or (b) CSA as doping agent.

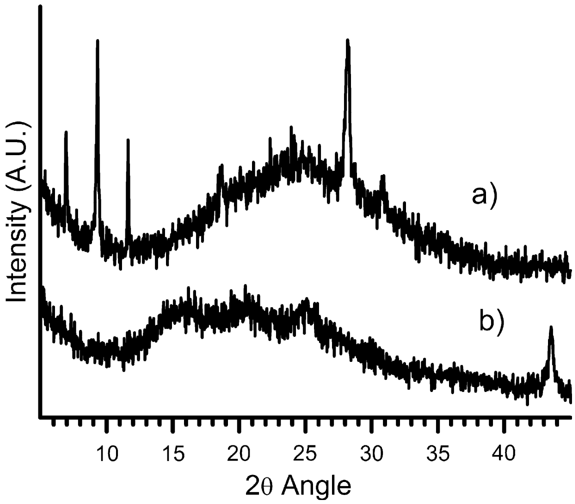

Fig. 6. Wide angle X-ray diffraction of the enzymatically synthesized PANI particles using chitosan as steric stabilizer, and (a) TSA or (b) CSA as doping agent.

PANI colloidal samples was carried out. While the PANI colloids synthesized using TSA showed a single broad peak, indicative of amorphous PANI, the colloids synthesized using CSA showed three broad peaks at 16°, 21°, and 25°. The latter two have been ascribed to the periodicity parallel and perpendicular to the polymer chain in PANI nanotubes synthesized using CSA/aniline mixtures in aqueous media [33]. These results support the self-assembly mechanism of aniline–CSA in the aforementioned sample to produce the elongated colloids. Electrical conductivity measured on compressed pellets prepared with the colloidal PANI samples was in the range of 10^{-3} to 10^{-4} S/cm, which are values in the same order of those found for chemically synthesized PANI dispersions using poly(vinyl alcohol) as steric stabilizer [35]. The lower conductivity of the dispersed polymerized PANI compared to enzymatically synthesized in aqueous media [32] is due to the insulating character of the adsorbed layer of the stabilizer.

3.4. Colloidal stability and further discussion

Although the colloidal stability of the PANI dispersions in chitosan was very good and they can be stored without flocculation for months, once isolated from the chitosan solution, the PANI particles prepared using TSA showed stability in acidic water (pH 2.5) for about 2 weeks, followed by a slight aggregation, whereas PANI particles prepared using CSA were stable only for 3–4 days in the same medium. The stability of the particles has a strong dependence with the pH of the medium as shown in Fig. 7. When the pH of the dispersion was increased from pH 3.0 to pH 6.0, a continuous increase in the absorption occurs, which is attributed to the partial agglomeration of colloids, resulting in higher light scattering. Flocculation of the isolated PANI particles rapidly occurred when the pH of the medium reaches a critical point at 6.9, slightly above the pK_a of the chitosan (approximately 6.3 [36]). This behavior suggests that PANI particles are sterically stabilized by the chitosan, which is soluble only in acidic medium. It is worthy to point out that during synthesis, although interaction through hydrogen bonds between chitosan and PANI is likely to occur, both polymers (emeraldine salt form of PANI and cationic chitosan) are positively charged, leading to electrostatic repulsion. Nonetheless, the adsorption of chitosan on the

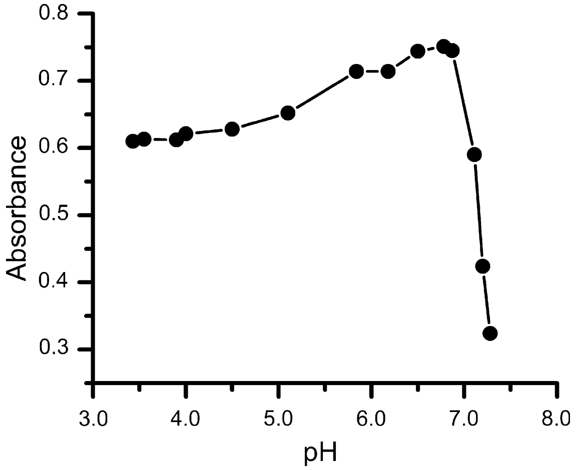

Fig. 7. Colloidal stability of the isolated PANI dispersion synthesized using TSA as doping agent at different pH. The absorbance at 500 nm as a function of the pH was measured as indication of the turbidity.

colloids was enough to stabilize the particles using only 1.0 wt% of this polymer in the reaction media. Besides steric stabilization due to the chitosan, both PANI and chitosan are positively charged in acidic conditions, and electrostatic repulsion may contribute to avoid aggregation of the colloids. However, above its pK_a, chitosan undergoes a coil-to-globule transition [37], loosing simultaneously solubility and cationic charge. We suppose that the particles studied in this work are stabilized mainly by steric effects since dedoped PANI particles stabilized with poly(vinyl alcohol), a non-ionic polymer, exhibit good colloidal stability in acidic or alkaline media [4–6,24]. Dispersion of the flocculated particles was completely reversible as long as the flocculated particles were not dried. It is noteworthy to point out that although PANI colloids have been prepared by chemical oxidation using a wide range of water-soluble polymers [2–7], the synthesis of PANI colloids showing a stability dependent on pH has just recently been reported [25]. The high surface area, pH sensitivity, and abundance of amino functional groups of these PANI colloids makes them interesting for applications such as enzyme immobilization [38], since this colloids could be dispersed at pH as high as 6.5 and easily recovered under neutral or slightly alkaline conditions. Another potential application could be the recovery of proteins [39] with isoelectric points below 6.5, which under these conditions should attach to the positively charged surface of the chitosan stabilized PANI colloids.

4. Conclusions

PANI colloids were successfully prepared by enzymatic polymerization in dispersed media using chitosan as steric stabilizer. The effectiveness of chitosan as stabilizer is good and stable dispersions were obtained using only 1.0 wt% of chitosan in the reaction medium. FTIR and UV–vis spectroscopy analysis indicate that the synthesis yields PANI structurally similar to the chemically synthesized one, whereas elemental and thermal analyses indicate that chitosan was adsorbed on the PANI colloidal particles. Enzymatic polymerization of aniline is fast and occurs without pernigraniline formation during the oxidation stage. Oblong particles with diameter ranging from 50 to 130 nm were obtained using TSA as doping agent whereas needle-like morphologies about 200 nm long and 25 nm wide were obtained when CSA was used instead of TSA. The stability of the dispersed particles in aqueous medium was strongly dependent of the pH, with good stability in acidic condition whereas rapid destabilization occurred at near neutral or alkaline conditions. The environmental sensitivity of these colloids could be further exploited for applications such as separation technology, enzyme immobilization, and smart materials.

Acknowledgments

This work was partially funded by CONACYT through the SEP-2004-CO1-46046 and J50313 projects. The authors acknowledge Jorge Sepúlveda (IFC-UNAM) for TEM images, Paulina Roman for technical assistance, and Blanca Huerta and Enrique Vielma (CIQA) for thermal and elemental analyses.

References

[1] MacDiarmid AG. “Synthetic metals”: a novel role for organic polymers. Curr Appl Phys 2001;1(4–5):269–79.

[2] Stejskal J, Kratochvil P, Helmstedt M. Polyaniline Dispersions. 5. Poly(vinyl alcohol) and poly(*N*-vinylpyrrolidone) as steric stabilizers. Langmuir 1996;12(14):3389–92.

[3] Chakraborty M, Mukherjee DC, Mandal BM. Dispersion polymerization of aniline in different media: a UV-visible spectroscopic and kinetic study. Langmuir 2000;16(6): 2482–8.

[4] Ghosh P, Siddhanta SK, Chakrabarti A. Characterization of poly(vinyl pyrrolidone) modified polyaniline prepared in stable aqueous medium. Euro Polym J 1999;35(4):699–710.

[5] Stejskal J, Spirkova M, Riede A, Helmstedt M, Mokreva M, Prokes J. Polyaniline dispersions 8. The control of particle morphology. Polymer 1999;40(10):2487–92.

[6] Chattopadhyay D, Banerjee S, Chakravorty D, Mandal BM. Ethyl(hydroxyethyl)cellulose stabilized polyaniline dispersions and destabilized nanoparticles therefrom. Langmuir 1998;14(7):1544–7.

[7] Eisazadeh H, Gilmore KJ, Hodgson AJ, Spinks G, Wallace GG. Electrochemical production of conducting polymer colloids. Colloids Surf A: Physicochem Eng Aspects 1995;103:281–8.

[8] Ngimhuang J, Furukawa J, Satoh T, Furuike T, Sakairi N. Synthesis of a novel polymeric surfactant by reductive *N*-alkylation of chitosan with 3-O-dodecyl-glucose. Polymer 2004;45(3):837–41.

[9] Marie E, Landfester K, Antonietti M. Synthesis of chitosan-stabilized polymer dispersions, capsules, and chitosan grafting products via miniemulsion. Biomacromolecules 2002;3(3):475–81.

[10] Huang H, Yang X. Synthesis of chitosan-stabilized gold nanoparticles in the absence/presence of tripolyphosphate. Biomacromolecules 2004;5(6):2340–6.

[11] Aberg CM, Chen T, Payne GF. Renewable resources and enzymatic processes to create functional polymers: adapting materials and reactions from food processing. J Polym Envir 2002;10(3):77–84.

[12] Yang S, Tirmizi SA, Burns A, Barney AA, Risen WM. Chitaline materials: soluble chitosan-polyaniline copolymers and their conductive doped forms. Synth Met 1989;32(3): 191–200.

[13] Liu W, Kumar J, Tripathy S, Senecal KJ, Samuelson LA. Enzymatically synthesized conducting polyaniline. J Am Chem Soc 1999;121(1):71–8.

[14] Liu W, Cholli AL, Nagarajan R, Kumar J, Tripathy SK, Bruno FF, et al. The role of template in the enzymatic synthesis of conducting polyaniline. J Am Chem Soc 1999;121(49):11345–55.

[15] Trakhtenberg S, Hangun-Balkir Y, Warner JC, Bruno FF, Kumar J, Nagarajan R, et al. Photo-cross-linked immobilization of polyelectrolytes for enzymatic construction of conductive nanocomposites. J Am Chem Soc 2005; 127(25):9100–4.

[16] Samuelson LA, Anagnostopoulos A, Alva KS, Kumar J, Tripathy SK. Biologically derived conducting and water soluble polyaniline. Macromolecules 1998;31(13):4376–8.

[17] Rumbau V, Marcilla R, Ochoteco E, Pomposo JA, Mecerreyes D. Ionic liquid immobilized enzyme for biocatalytic synthesis of conducting polyaniline. Macromolecules 2006;39(25):8547–9.

[18] Li W, McCarthy PA, Liu D, Huang J, Yang SC, Wang HL. Toward understanding and optimizing the template-guided synthesis of chiral polyaniline nanocomposites. Macromolecules 2002;35(27):9975–82.

[19] Yuan GL, Kuramoto N. Water-processable chiral polyaniline derivatives doped and intertwined with dextran sulfate: synthesis and chiroptical properties. Macromolecules 2002;35(26):9773–9.

[20] Thiyagarajan M, Samuelson LA, Kumar J, Cholli AL. Helical conformational specificity of enzymatically synthesized water-soluble conducting polyaniline nanocomposites. J Am Chem Soc 2003;125(38):11502–3.

[21] Cholli AL, Thiyagarajan M, Kumar J, Parmar VS. Biocatalytic approaches for synthesis of conducting polyaniline nanoparticles. Pure Appl Chem 2005;77(1):339–44.

[22] Liu W, Kumar J, Tripathy SK, Samuelson LA. Enzymatic synthesis of conducting polyaniline in micelle solutions. Langmuir 2002;18(25):9696–704.

[23] Uyama H, Kurioka HN, Kobayashi S. Preparation of polyphenol particles by peroxidase-catalyzed dispersion polymerization. Colloids Surf A: Physiochem Eng Aspects 1999;153(1–3):189–94.

[24] Cruz-Silva R, Ruiz-Flores C, Arizmendi L, Romero-Garcia J, Arias-Marin E, Moggio I, et al. Enzymatic synthesis of colloidal polyaniline particles. Polymer 2006;47(5): 1563–1568.

[25] Cruz-Silva R, Arizmendi L, Del-Angel M, Romero J. pH- and thermosensitive polyaniline colloidal particles prepared by enzymatic polymerization. Langmuir 2007;23(1):8–12.

[26] Brugnerotto J, Lizardi J, Goycoolea FM, Argüelles-Monal W, Desbrières J, Rinaudo M. An infrared investigation in relation with chitin and chitosan characterization. Polymer 2001;42(8):3569–80.

[27] Rinaudo M, Milas M, LeDung P. Characterization of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. Int J Biol Macromol 1993; 15(5):281–5.

[28] Zheng W, Angelopoulos M, Epstein AJ, MacDiarmid AG. Experimental evidence for hydrogen bonding in polyaniline: mechanism of aggregate formation and dependency on oxidation state. Macromolecules 1997;30(10):2953–5.

[29] Trchova M, Sedenkova I, Konyushenko EN, Stejskal J, Holler P, Ciric-Marjanovic G. Evolution of polyaniline nanotubes: the oxidation of aniline in water. J Phys Chem B 2006;110(19):9461–8.

[30] Ping Z, Nauer GE, Neugebauer H, Theiner J, Neckel A. Protonation and electrochemical redox doping processes of polyaniline in aqueous solutions : investigations using in situ FTIR–ATR spectroscopy and a new doping system. J Chem Soc, Faraday Trans 1997;93(1):121–9.

[31] Lu X, Ng HY, Xu J, He C. Electrical conductivity of polyaniline–dodecylbenzene sulphonic acid complex: thermal degradation and its mechanism. Synth. Met. 2002; 128(2):167–78.

[32] Cruz-Silva R, Romero-García J, Angulo-Sánchez JL, Ledezma-Pérez A, Arias-Marín E, Moggio I, et al. Template-free enzymatic synthesis of electrically conducting polyaniline using soybean peroxidase. Euro Polym J 2005; 41(5):1129–35.

[33] Zhang L, Wang M. Synthesis and characterization of self-assembled polyaniline nanotubes doped with D-10-camphorsulfonic acid. Nanotechnology 2002;13(6):750–5.

[34] Stejskal J, Sapurina I, Trchová M, Konyushenko EN, Holler P. The genesis of polyaniline nanotubes. Polymer 2006; 47(25):8253–62.

[35] Stejskal J, Kratochvil P, Armes SP, Lascelles SF, Riede A, Helmstedt M, et al. Polyaniline dispersions. 6. Stabilization by colloidal silica particles. Macromolecules 1996;29(21): 6814–9.

[36] Sorlier P, Denuziere A, Viton C, Domard A. Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan. Biomacromolecules 2001; 2(3):765–72.

[37] Bodnar M, Hartmann JF, Borbely J. Preparation and characterization of chitosan-based nanoparticles. Biomacromolecules 2005;6(5):2521–7.

[38] Vazquez-Duhalt R, Tinoco R, D'Antonio P, Topoleski LDT, Payne GF. Enzyme conjugation to the polysaccharide chitosan: smart biocatalysts and biocatalytic hydrogels. Bioconjugate Chem 2001;12(2):301–6.

[39] Casal E, Corzo N, Moreno FJ, Olano A. Selective recovery of glycosylated caseinmacropeptide with chitosan. J Agric Food Chem 2005;53(4):1201–4.