Available online at www.sciencedirect.com
ScienceDirect
Bioelectrochemistry 72 (2008) 122 – 126
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Short communication

Evaluation of the physico-chemical properties of chitosan as a potential carrier for rifampicin, using voltammetric and spectrophotometric techniques

Renê H.T. Santos, Neemias G. Santos, José P.H. Alves, Carlos A.B. Garcia, Luciane C.P. Romão, Maria Lara P.M. Arguelho *

Departamento de Química, Universidade Federal de Sergipe, 49100-000 São Cristovão – SE, Brazil

Received 29 August 2007; received in revised form 4 January 2008; accepted 11 January 2008
Available online 19 January 2008

Abstract

Rifampicin is an antibiotic which, on a carbon paste electrode, shows an oxidation response of 0.492 V (vs. Ag/AgCl) at pH 7.0, due to the electroactivity of the hydroquinone group. Interaction of rifampicin with chitosan is strongly dependent on pH, species concentrations and contact time between the latter. Compared to the carbon paste electrode, electrodes modified with chitosan showed greater sensitivity, with optimum voltammetric profile obtained at pH 8.0. Spectrophotometric measurements indicate that rifampicin is strongly absorbed by chitosan at pH less than the pKa of the pharmaceutical, such behaviour being favourable for the use of chitosan as a carrier for the controlled release of rifampicin in the intestinal tract.

Keywords: Rifampicin; Chitosan; Controlled release; Voltammetry

1. Introduction

Polymer systems for drug release have been widely used in medicine, since they enable the slow and gradual release of the active ingredient, with better targeting within the body, such as towards areas of inflammation or tumours [1,2].

Chitosan (Fig. 1), a biodegradable polysaccharide derived from chitin and found widely in nature [3], possesses properties making it particularly suitable as a carrier, including its high viscosity, charge distribution and release mechanisms. Research into the chemical properties of chitosan has demonstrated its suitability for the preparation of enzymatic biosensors for the analysis of metallic elements, proteins and lipids [4–6].

Pharmaceuticals possessing antibiotic properties have been explored as good candidates for the preparation of formulations based on polymeric controlled release systems. Development of a variety of methodologies for the preparation of bio adhesives and chitosan microcapsules inoculated with antibiotics for use in different means of administration have been described in the literature [7–10].

Produced by fermentation of *Streptomyces mediterranei,* rifampicin (Fig. 2) is a strategic medication, recommended by the World Health Organization for the treatment of endemic diseases. It shows effective action against both gram-negative and gram-positive bacteria [11], and is one of the principal chemical therapies employed in combating tuberculosis and hanseniasis [12]. However the success (or otherwise) of its therapeutic action can be influenced by physical or chemical interactions with other medications and nutrients, which may alter its bioavailability [13]. Carrier materials such as chitosan have been employed to protect the pharmaceutical agent so that it may be released under optimal absorption conditions, or to adjust the timing of release of different pharmaceutical agents administered simultaneously. In 2003, Pandey et al. undertook an *in vivo* study with rifampicin, isoniazid and pirazinamide combined with biodegradable and biocompatible glycoside nanoparticles, using mice as test organisms. Biochemical evidence

* Corresponding author. Tel.: +55 79 2105 6650; fax: +55 79 2105 6684.
E-mail address: marguelho@bol.com.br (M.L.P.M. Arguelho).

1567-5394/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2008.01.003

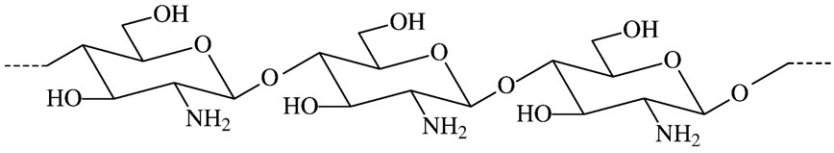


Fig. 1. Molecular structure of chitosan.

of the non-liver toxicity of this polymeric formulation was obtained [14]. In the case of chitosan acting as a carrier for rifampicin, in addition to its recognized biocompatibility [15–17] there is the additional advantage of the antilipidemic nature of chitosan, which acts as a hepatoprotector since rifampicin as well as other tuberculostatic agents show high levels of hepatotoxicity, in part due to increases in levels of cholesterol, triglycerides and fatty acids in the blood.

Thus physico-chemical studies of the interactions between pharmaceuticals and chitosan are essential for the development of new and more efficient drugs to combat diseases which can cause widespread harm in tropical countries. According to the 2005 epidemiological census, there are on average 100,000 new cases of tuberculosis annually in Brazil which, together with Peru, accounts for 50% of the reported cases in the Americas [18]. Despite the existence of free tuberculosis treatment, in 30% of cases the treatment is abandoned, due in part to the collateral effects of administration of antibiotics for prolonged periods of, on average, between 18 and 24 months [19,20].

In this work the degree of interaction between chitosan and rifampicin is investigated using voltammetric and spectrophotometric techniques, envisaging possible applications in new formulations based on optimization of the therapeutic dose in order to improve the quality of life of patients.

2. Experimental

2.1. Materials and methods

Rifampicin, obtained from Glaxo Welcome, was used at a concentration of 5.6×10^{-5} mol/L in distilled water for the preparation of solutions for voltammetric studies. For spectrophotometric studies 4.6 μmol L^{-1} solutions in B-R/pH 8.0 buffer were employed. Pure chitosan, 96% deacetylized, was obtained from Primex Ingredients A. S. (Norway).

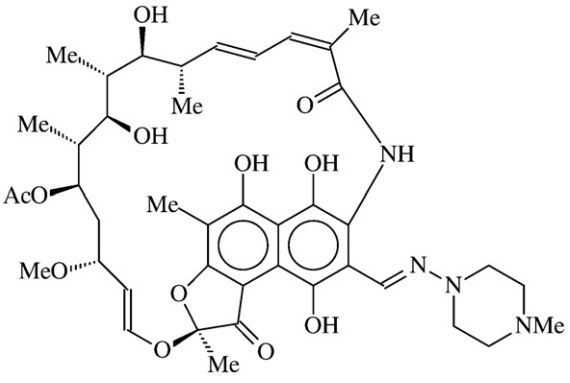


Fig. 2. Molecular structure of rifampicin.

For the spectrophotometric measurements the buffered solutions containing chitosan and rifampicine were kept in the dark at a temperature of 25 °C.

For the pH studies solutions having pH between 2 and 12 were used, prepared by varying the percentages of an acid solution, containing 0.04 mol/L of acetic acid, 0.04 mol/L of boric acid, and 0.04 mol/L of phosporic acid, and a basic solution containing 0.2 mol/L of NaOH (all chemicals supplied by Merck), according to the procedure described by Britton–Robinson [21] (B-R).

The electrochemical measurements were made using an Ecochemie Model Autolab 30 potentiostat/galvanostat, connected to a 133 MHz microcomputor and controlled using GPES v. 4.8 software. The system used consisted of a Metrohm VA Stand 633 polarography cell (V=10 mL), containing a glassy carbon electrode and a reference electrode (Ag/AgCl, KCl 3.0 mol/L). The carbon paste microelectrode (geometric area 0.1 cm^2) was prepared using a 3:1 ratio of carbon and mineral oil.

Microelectrodes modified with chitosan (MEC) were obtained by homogenizing chitosan with carbon paste at ratios between 1:7 and 1:1. The compressed pastes were filled into a pipette tip and electrical connection was made using a platinum wire (Fig. 3). Renewal of the exposed surface was undertaken after each experiment.

Coulometric analyses of 0.01 μmol/L solutions of rifampicin at pH 8.0 were undertaken on a 1.0 cm^2 geometric area carbon paste electrode at a potential of E=0.650 V (vs. Ag/AgCl) for a period of 10 min.

Spectrophotometric experiments were undertaken on a FEMTO 800 XI spectrophotometer. Measurements were made at 1 h intervals, at a wavelength bandwidth of 330–800 nm.

3. Results and discussion

Fig. 4 illustrates the differential pulse voltammogram on the carbon paste electrode for rifampicin at pHs 2.0, 6.0 and 8.0. It

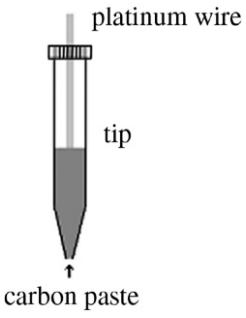


Fig. 3. Structure of the carbon paste microelectrode.

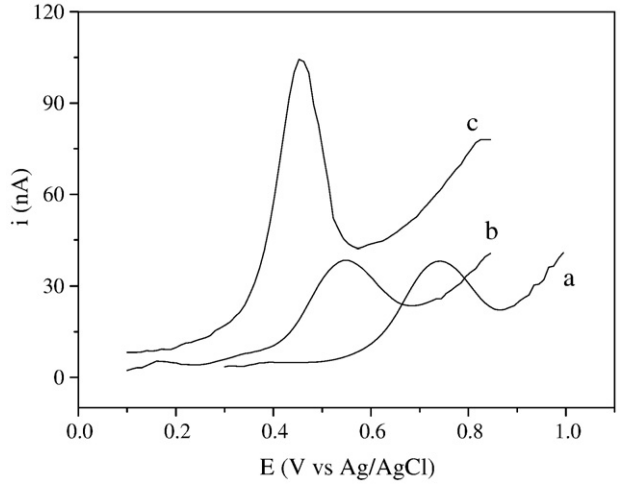
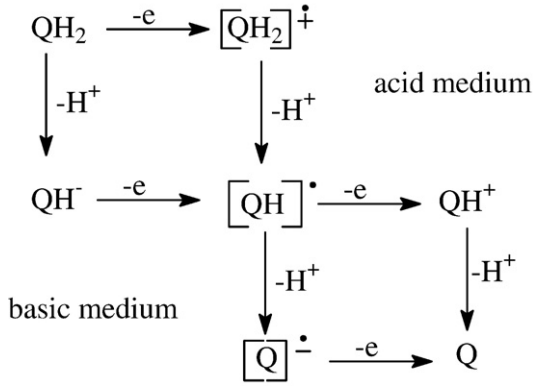


Fig. 4. Differential pulse voltammograms for rifampicin (2.0 μmol/L) at pHs: a) 2.0, b) 6.0 and c) 8.0 (v=0.03 V/s and ΔE=100 mV) using a carbon paste electrode.

was observed that the electrode response was strongly dependent on hydrogen ion concentration. When the acidity of the medium was reduced there was a shift of the oxidation potential towards less positive values, accompanied by an increase in the peak current. Optimum response was obtained at pH 8.0, when the peak current reached a maximum, while at higher pHs there was a progressive separation of the oxidation processes into two stages, which substantially reduced signal intensity.

As seen in Fig. 4, in aqueous medium the pharmaceutical shows a well defined oxidation response at 0.458 V (vs. Ag/AgCl), with the half height peak width ($W_{1/2}$) corresponding to 105 mV. Complementary microelectrolysis results, using the 1.0 cm^2 area electrode, indicated the transfer of two electrons per molecule of rifampicin.

Electrochemical oxidation of rifampicin in aqueous medium, involving transfer of two electrons and two protons, shows current and potential behaviour which can be attributed to the typical protonation/oxidation equilibrium of hydroquinones [22–24] (Scheme 1), where the sequence of electron transfer and chemical reaction steps comprising the process occurring at



Scheme 1. Stages of the electrochemical oxidation of hydroquinone derivatives.

the electrode depend on the proton availability of the medium and on the pKa of the hydroquinone derivative (QH_2).

The operating parameters of the differential pulse techniques were optimized in order to obtain the best voltammetric profile (Table 1).

Factors such as electrical conductivity and paste compaction influence microelectrode response [25]. We used different proportions of paste and modifying agent, with the best result obtained for a 1:7 ratio of chitosan to paste, when a 23.4% increase in the oxidation current of rifampicin was observed.

As for the carbon paste microelectrode (CPME), investigations of pH were also performed for carbon microelectrodes modified with chitosan (MEC), when it was seen that, compared to the CPME, the presence of the polymer caused an increase of the sensitivity to rifampicin of the microelectrode, in acid medium (Fig. 5). This is due to the presence of the amine group within the chitosan structure, which is protonated in an acid medium, hence attracting the nucleophilic regions of rifampicin and favouring its oxidation. Maximum sensitivity for the CPME was achieved at pH 8.0.

Another parameter important for the performance of the chitosan modified electrode is the extent of deacetylation of chitosan. According to the literature [26–28], chitosan is obtained via a process of alkaline hydrolysis. Acetyl groups are removed from chitin, thus exposing the amine groups responsible for the cationic nature of chitosan. Higher deacetylation of chitosan results in higher availability of amine groups able to absorb organic or inorganic compounds. This feature accounts for the versatility of chitosan as a polymeric matrix. A chitosan microelectrode modified with epichlorhydrin, which links to chitosan via the amine group, was used to evaluate the importance of these amine groups in the interaction with rifampicin. Comparing the behaviour of rifampicin on chitosan microelectrodes (MEC) with that on the microelectrode containing chitosan modified with epichlorhydrin, (MECEP), it was seen that when a portion of the chitosan amine groups were rendered unavailable for linking, the interaction of rifampicin with chitosan diminished, with a consequent reduction in the anodic current (Fig. 6). Other examples of the interaction of pharmaceuticals with the chitosan amine group may be found in the literature [29–31], an important parameter being the degree of chitosan deacetylation, which here was 96%.

Spectrophotometric experiments, in which the optical properties of rifampicin were employed to follow the progress of the reaction, were undertaken in order to investigate whether the interaction between rifampicin and chitosan could also occur in solution.

The molecular absorption spectrum of rifampicin showed the presence of an absorption band at 472.4 nm, due to the presence

Table 1
Optimized parameters for detection of rifampicin on the carbon paste microelectrode

Voltammetric parameters		Optimized conditions
Pulse height, (ΔE_p)/mV	(10–200)	100
Scan rate, v/(mV/s)	(5–100)	30
pH	(2–12)	8.0

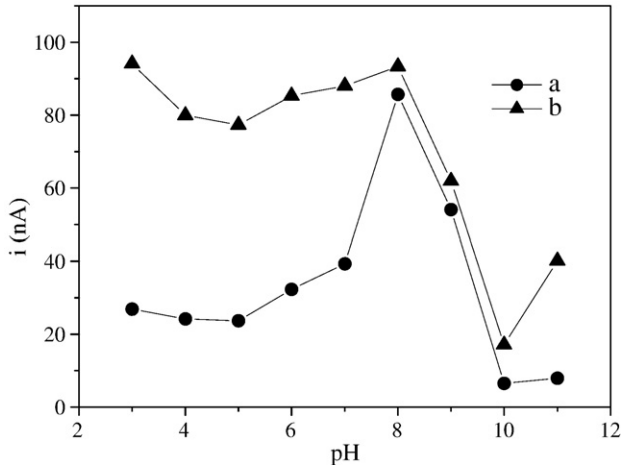


Fig. 5. Behaviour of the anodic current of rifampicin (2.0 μmol/L) as a function of pH for a) the carbon paste microelectrode (CPME), and b) the microelectrode modified with chitosan (MEC). Conditions: v=0.03 V/s and ΔE=100 mV.

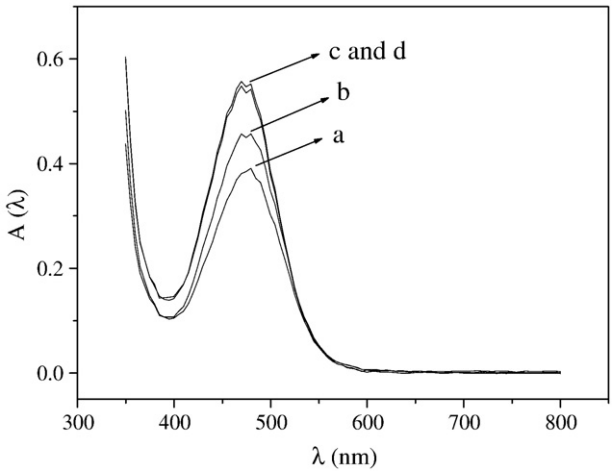


Fig. 7. Absorption spectra for 2.0 μmol/L rifampicin solutions at pHs: a) 3.0, b) 6.0, c) 8.0 and d) 10.0.

of chromophore groupings on the molecule (−Ar, C=O and C=C). The absorption spectrum shows a hyperchromic effect with increasing pH, which is related to the formation of the deprotonated form of rifampicin, whose maximum absorption occurs at the same wavelength as the protonated species (Fig. 7). Between pH 2.0 and 8.0, the absorbance increases gradually, then remains constant at higher pH. These results indicate that the pKa$_1$ and pKa$_2$ values obtained for rifampicin are in agreement with those described in the literature [32] (pKa=1.7 and 7.9, respectively). They are in line with the results achieved using the voltammetric method (illustrated in Fig. 5).

The rifampicin calibration curve obtained spectrophotometrically is described by the linear equation: $A=-0.026+1.2\times10^{-4}$C ($r$=0.9970, n=8). A value for εb of 12,469.1 mol cm/L was obtained, close to the value of the molar absorptivity of rifampicin (ε) described in the literature [33] (15278.9 mol/L, b=1.0 cm).

As can be seen in Fig. 8, the intensity of the spectrophotometric signal for rifampicin decreased exponentially with the contact time between rifampicin and chitosan in solution. The decrease was ~33% after 4 h under agitation at 500 rpm, resulting in a decrease in the concentration of free rifampicin equivalent to 2.0 μmol/L for a mass ratio between rifampicin and chitosan of 1:740 w/w, this quantity of chitosan corresponding to the mass employed in preparation of the modified electrode. After 1 h of experiment the reduction of the rifampicin concentration can be described for the equation $A(t)=0.453-4.475t$ (r=0.9768, n=11). The reaction followed second order kinetics with a constant of 1.11 L/mol s. These results, whether obtained by spectrophotometry or by voltammetry, show that even at high concentrations and over extended periods, chitosan in an alkaline medium shows low affinity for rifampicin. Regulation of the affinity between the pharmaceutical and the biopolymer is pH dependent, which might offer a convenient mechanism for controlled release of the pharmaceutical, particularly when considering the intestinal tract, where the pH is slightly alkaline [34,35].

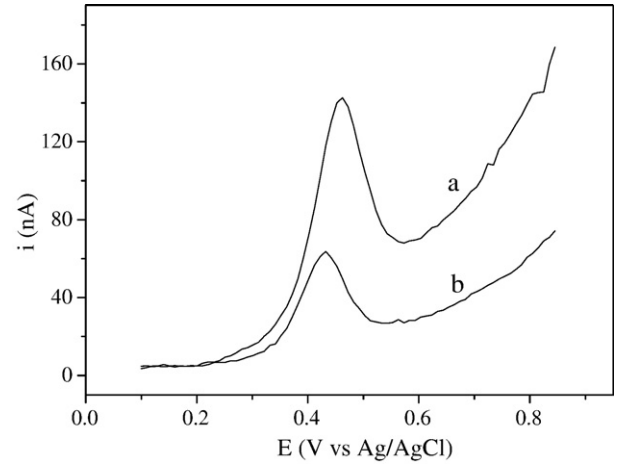


Fig. 6. Rifampicin (a) on a chitosan paste electrode, and (b) on a chitosan paste electrode modified with epichlorhydrin. Conditions: rifampicin 2.0 μmol/L, pH 8.0, v=30 mV/s, ΔE=100 mV.

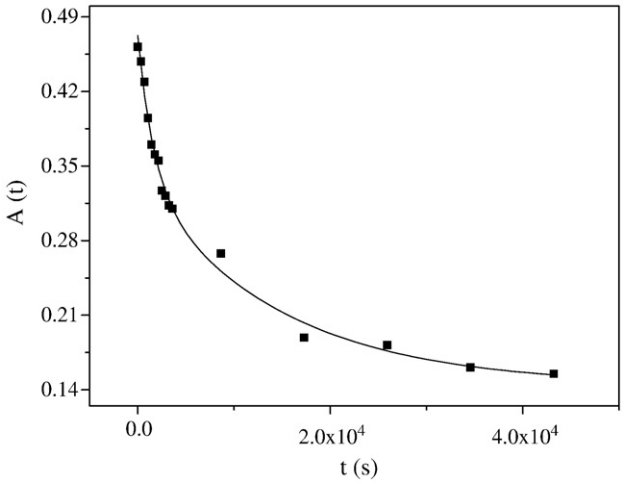


Fig. 8. Absorbance at 472.4 nm of a 4.5 μmol/L solution of rifampicin in B-R/pH 8.0 buffer in the presence of chitosan.

4. Conclusion

A spectrochemical/electrochemical methodology has been developed to investigate the physico-chemistry of the interaction between rifampicin and chitosan. The fact that rifampicin is absorbed by chitosan in an acid medium, and released in an alkaline medium, is vital information needed for optimization of the absorption of rifampicin during transit through the intestine, and controlling its behavior in body fluids. pH adjustment can reduce the potential for interactions between medicines and alimentary materials during transport through the digestive system, which in the case of complexation reactions with nutrients could render the pharmaceutical unavailable for the intended therapeutic action.

Acknowledgement

To Prof. Cestari (UFS) for the chitosan samples.

References

[1] R. Jayakumar, N. New, S. Tokura, H. Tamura, Sulfated chitin and chitosan as novel biomaterials, Int. J. Biol. Macromol. 40 (2007) 175–181.
[2] M. George, T.E. Abraham, Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan, J. Control. Release 114 (2006) 1–14.
[3] M. Terbojevich, R.A.A. Muzzarelli, Chitosan, in: G. Phillips, P. Williams (Eds.), Handbook of Hydrocolloids, Woodhead, Cambridge, UK, 2000, pp. 367–378.
[4] X.B. Lu, Q. Zhang, L. Zhang, J.H. Li, Direct electron transfer of horseradish peroxidase and its biosensor based on chitosan and room temperature ionic liquid, Electrochem. Commun. 8 (2006) 874–878.
[5] I.R.W.Z. Oliveira, I.C. Vieira, Immobilization on procedures for the development of a biosensor for determination of hydroquinone using chitosan and gilo (solunum gilo), Enzyme Microb. Techn. 38 (2006) 449–456.
[6] J. Tkac, J.W. Whittaker, T. Ruzgas, The use of single walled carbon nanotubes dispersed in a chitosan matrix for preparation of a galactose biosensor, Biosens. Bioelectron. 22 (2007) 1820–1824.
[7] G.D. Colo, Y. Zambito, S. Burgalassi, I. Nardini, M.F. Saettone, Effect of chitosan and of *N*-carboxymethylchitosan on intraocular penetration of topically applied ofloxacin, Int. J. Pharm. 203 (2004) 37–44.
[8] S. Torrado, P. Prada, P.M. Torre, S. Torrado, Chitosan-poly(acrylic) acid polyionic complex: in vivo study to demonstrate prolonged gastric retention, Biomaterials 25 (2004) 917–923.
[9] A.K. Anal, W.F. Stevens, Chitosan-alginate multilayer beads for controlled release of ampicillin, Int. J. Pharm. 290 (2005) 45–54.
[10] F-L. Mi, Y-B. Wu, S-S. Shyu, A-C. Chao, J-Y. Lai, C-C. Su, Asymmetric chitosan membranes prepared by dry/wet phase separation: a new type of wound dressing for controlled antibacterial release, J. Memb. Sci. 212 (2003) 237–254.
[11] A.M.F. Pinho, Chemoprophylaxis for tuberculosis and survival of HIV infected patients in Brazil, AIDS 15 (2001) 2129–2135.
[12] World Health Organization, Treatment of Tuberculosis. Guidelines for National Programmes 3rd Ed.Geneva, 2003.
[13] C. Zent, P. Smith, Study of the effect of concomitant food on the bioavailability of rifampicin, isoniazid and pyrazinamide, Tuber. Lung Dis. 76 (1995) 109–113.
[14] R. Pandey, A. Zahoor, S. Sharma, G.K. Khuller, Nanoparticle encapsulated anti-tubercular drugs as a potential oral drug delivery system against tuberculosis, Tuberculosis (Edinb) 83 (2003) 373–378.
[15] S. Santhosh, T.K. Sini, R. Anandan, P.T. Mathew, Effect of chitosan supplementation on antitubercular drugs-induced hepatotoxicity in rats, Toxicology 219 (2006) 53–59.
[16] Y. Chan, J.P. Zimmer, M. Stroh, J.S. Steckel, R.K. Jain, M.G. Bawendi, Incorporation of luminescent nanocrystals into monodisperse core-shell silica microspheres, Adv. Mater. 16 (2004) 2092–2096.
[17] D. Gerion, F. Pinaud, S.C. Williams, W.J. Parak, D. Zanchet, S. Weiss, A.P. Alivisatos, Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots, J. Phys. Chem., B 105 (2001) 8861–8871.
[18] World Health Organization. Tuberculosis. Accessed at http://www.who.int/tb/en/ on 27 July 2007.
[19] R. Frothingham, J.E. Stout, C.D. Hamilton, Current issues in global tuberculosis control, Int. J. Infect. Dis. 9 (2005) 297–311.
[20] A.M. Ditto, R.E. Story, Drug rash with eosinophilia and systemic symptoms (DRESS) secondary to anti-tuberculosis therapy with features of isoniazid toxicity, J. Allergy Clin. Immunol. 113 (2004) S309.
[21] H.T.S. Britton, Hydrogen Ions, 4th ed. Chapman Hall, London, 1952.
[22] D.H. Evans, in: A.J. Bard, H. Lund (Eds.), In encyclopedia of electrochemistry of the elements, vol. 12, Marcel Dekker, Inc., New York, 1978, p. 198.
[23] M.J. Preigh, M.T. Stauffer, F-T. Lin, S.G. Weber, Anodic oxidation mechanism of a spiropyran, J. Chem. Soc., Faraday Trans. 92 (1996) 3993–3996.
[24] R. Adams, Electrochemistry at Solid Electrodes, Marcel Dekker, Inc. New York, 1969, pp. 351–356; 363–369.
[25] P.T. Kissinger, W.R. Heineman, Laboratory Techniques in Electroanalytical Chemistry, 2[a] ed., Marcel Dekker, New York, 1996, p. 986.
[26] R.A.A. Muzzarelli, Structural and functional versatility of chitins, in: S. Dimitriu (Ed.), Structural Diversity and Functional Versatility of Polysaccharides, Marcel Dekker Inc., New York, 1998.
[27] E. Guibal, Interactions of metal ions with chitosan-based sorbents: a review, Sep. Purif. Tech. 38 (2004) 43–74.
[28] R.A.A. Muzzarelli, Natural Chelating Polymers, Pergamon, Oxford, UK, 1973, p. 254.
[29] P. Tengamnuay, A. Sahamethapat, A. Sailasuta, A.K. Mitra, Chitosans as nasal absorption enhancers of peptides: comparison between free amine chitosans and soluble salts, Int. J. Pharm. 197 (2000) 53–67.
[30] P.R. Rege, D.J. Shukla, L.H. Block, Chitinosans as tableting excipients for modified release delivery systems, Int. J. Pharm. 181 (1999) 49–60.
[31] M.L. Lorenzo-Lamosa, C. Remuñan-Lopez, J.L. Vila-Jato, M.J. Alonso, Design of microencapsulated chitosan microspheres for colonic drug delivery, J. Control. Release 52 (1998) 109.
[32] Drug Information for Health Care Providers USPDI, V. I, The United States Pharmacopoeia Convention Inc., 1984, p. 259.
[33] Merck Index, An Encyclopedia of Chemicals Drugs and Biologicals, 11th Ed Merck & Col, 1989.
[34] H.P. Rang, M.M. Dale, J.M. Ritter, in: S.A. Koogan (Ed.), Farmacologia 4th Ed., pp. 51–57.
[35] B.A. Hendriksen, M.V.S. Felix, M.B. Bolger, The composite solubility versus pH profile and its role in intestinal absorption prediction, AAPS Pharm. Sci. 5 (1) (2003) E4.