Journal of Molecular Catalysis B: Enzymatic 74 (2012) 1–5

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

α-Amylase immobilization on the silica nanoparticles for cleaning performance towards starch soils in laundry detergents

Majid Soleimani [a,*], Azam Khani [a], Kolsoom Najafzadeh [b]

[a] Department of Chemistry, Imam Khomeini International University (IKIU), Qazvin, Iran
[b] Department of Instrumental Laboratory, Mahde taban, Qazvin, Iran

ARTICLE INFO

Article history:
Received 7 March 2011
Received in revised form 26 June 2011
Accepted 20 July 2011
Available online 25 August 2011

Keywords:
Detergent
Silica nanoparticle
α-Amylase
Immobilization
Cleaning performance

ABSTRACT

In this study, α-amylase was immobilized on silica nanoparticles and immobilized α-amylase was used in formulation of detergent powder for enhancing removal of starch soils. Detergent products contain very components which may affect the free enzyme activity and stability. Also various factors such as temperature, pH and humidity reduced enzyme activity and cleaning efficiency. Therefore the effect of enzyme immobilization on the removal of starch based soil was investigated on cotton fabrics as the model soil. The effect of temperature and humidity on stability of free and immobilized enzyme was compared. It was found that the immobilized enzyme increased cleaning efficiency toward starch soil removal on cotton fabrics, whereas free enzyme imposed a small effect on the enzymatic activity towards the same soil substrates. In addition, stability immobilized enzyme against temperature and humidity was much more than free enzyme.

1. Introduction

Enzymes are biological catalysts that have been used for centuries in the food industry for fermentation and in detergents for enhancing removal of some soils. In the last year, enzymes have been an important factor in the development and improvement of detergent products. The major types of enzymes used in the cleaning industries are protease, amylase, lipases and celluloses, which break down protein, starch, grease and dust, respectively. Not only enzymes increase performance in cleaning but also, have provided environmental benefits by reducing energy and water consumption and lower washing temperatures. Most of the commercially available detergents contain a complicated complex of enzymes, surfactants, builders, polymers and bleach [1]. Enzyme is sensitive to some detergent ingredients, both during the shelf life of the product and in the washing cycle. Among them, enzyme–surfactants compatibility is the notable. It has been shown that surfactant can either enhance [2,3] or inhibit [4,5] enzyme hydrolysis by decreasing adsorption of enzyme onto the substrate. When detergent product absorbs humidity, bleaches like percarbonate or perborate release small amounts of hydrogen peroxide which oxidizes the enzymes [6].

The immobilization of enzymes on solid supports that are either organic or inorganic is an area of intense research due to a very effective way to increase enzyme stability and operational lifetime [7–10]. There have been many reports about immobilization of various enzymes on inorganic supports, e.g. immobilization on silica [11], clay [12,13], collagen [14] and zirconia [15]. Furthermore, the immobilization of α-amylase on alumina for the hydrolysis of starch to low molecular weight carbohydrates [16], functional glass beads for covalent attachment [17], immobilization of enzymes on magnetic nanoparticles [18] and amino functionalized magnetic supports for covalent immobilization of Candida cylindrical lipase [19] have been reported.

Among inorganic materials, silica nanostructures for the immobilization of enzymes have been explored extensively because they are in the range of nm which are similar to sizes of enzyme molecules, high surface areas, ordered structures, high stability to chemical and mechanical forces, and resistance to enzyme attack. Moreover, enzyme immobilization on these carriers can simply be achieved by physical adsorption which has the least effect on enzyme structures compared to other methods such as covalent bonding [20,21,10,22–24].

In the present study, stability and cleaning efficiency of α-amylase in detergent products was increased. For this purpose, α-amylase was immobilized on silica nanoparticles. Immobilized amylase was used in formulations of detergent powder. Then, immobilized amylase stability (against humidity and temperature) and cleaning efficiency was compared with detergent powder containing the free enzyme by standard test methods.

* Corresponding author. Tel.: +98 9125258506; fax: +98 2813780040.
E-mail address: m-soleimani@hotmail.com (M. Soleimani).

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doi:10.1016/j.molcatb.2011.07.011

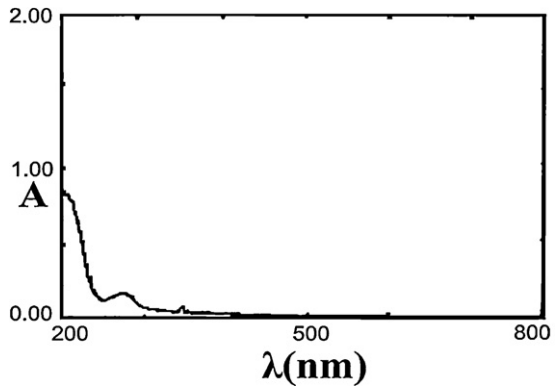

Fig. 1. UV spectrum of original α-amylase solution (1 mg/ml).

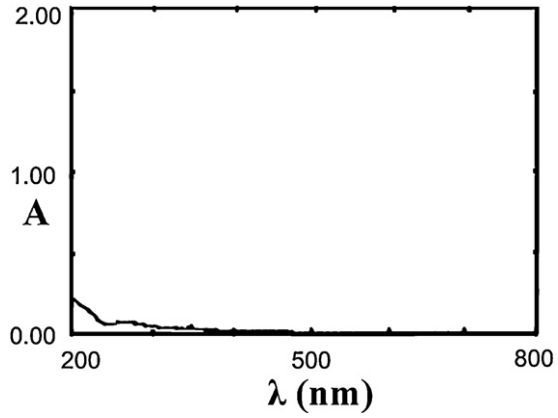

Fig. 2. UV spectrum of residual α-amylase in the supernatant after adsorption.

2. Experimental

2.1. Apparatus and reagents

Fumed nanosilica (Aerosil A200, spherical shape with surface area 200 m^2/g) was purchased from Evonik Degussa Co. (China). The α-amylase (termamylaze) was obtained from Novozymes Co. (Tehran, Iran). Starch soil on cotton fabrics (EMPA161) was used as the model soil. Deionized water which was prepared with an ion exchange system was applied to prepare enzyme solutions. Tergotometer Apparatus (7243 ES, USA) and Spectraflash SF 450 (USA) were used to perform cleaning or laundering performance. The concentration of α-amylase solution was determined by a UV–vis spectrophotometer (Shimadzu UV 160 A) at an absorption wavelength of 210 nm. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Tensor 27 Spectrometer employing a potassium bromide (KBr) pellet method.

2.2. Immobilization of α-amylase on silica nano particles

In immobilization process, solid silica nanoparticles were added to α-amylase solution (1 mg/ml) under constant stirring. At different time intervals, 5 ml of the suspensions were withdrawn and immediately centrifuged to obtain clear supernatant, which was used to determine the residual α-amylase concentration by a UV Spectrometer at a wavelength of 210 nm (Figs. 1 and 2). The amount of immobilized α-amylase onto silica NPs was calculated from the original α-amylase concentration and the amount of α-amylase in the supernatant after adsorption. After the adsorption had reached the equilibrium, the suspension was filtered and rinsed with deionized water to remove entrapped α-amylase molecules.

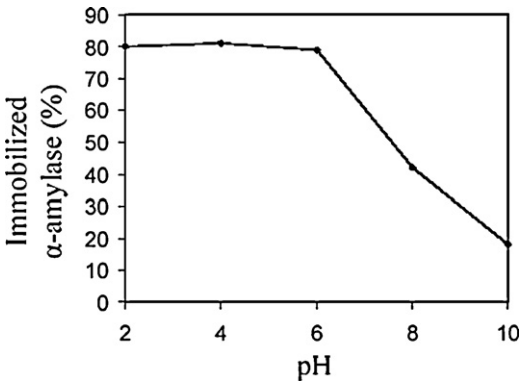

Fig. 3. Effect of pH on α-amylase immobilization.

The immobilized α-amylase on nanoparticles were dried and used for subsequent laundering performance to quantificate enzymatic activity toward the soil substrate. Effect of the pH, time and amylase/silica ratio on immobilization process was investigated. Conformational changes in the protein secondary structure on immobilized amylase were investigated by using FTIR analysis.

2.3. Measurement of the cleaning performance on starch soils

The effect of enzyme immobilization onto silica nanoparticles on soil removal was investigated by laundering performance (ASTM: D4265-98). To perform laundering performance towards starch soils on cotton fabrics, formulation of detergent powder containing 15–20% surfactant, 25% sodium tripolyphosphate, 35% sodium sulfate, 10% sodium silicate and 0.1% α-amylase (free or immobilized enzyme) was prepared. Then washing test method was carried out under standard conditions: temperature, 45 °C; concentration of mentioned detergent powder, 4 g/l in distilled water (water hardness = 300 mg/l) and washing cycle 15 min with 100 rpm.

Evaluation of the cleaning performance of enzymes (free or immobilized enzyme) was performed by measuring the intensity of reflectance light ($R\%$) at λ = 460 nm on the washed or unwashed cotton fabrics containing starch soils. $\Delta R\%$ was calculated as follows:

$$\Delta R\% = R_{\text{washed}} - R_{\text{unwashed}} \quad (1)$$

The $\Delta R\%$ defined digestion power of detergent powder and was calculated from averaging 4 replicate measurements for each formulation.

3. Results and discussion

3.1. The effective parameters on amylase immobilization

To investigate the effect of pH on immobilization of α-amylase on silica nanoparticle, 3.75 mg of SiO_2 NPs was added to 5 ml of 1 mg/ml enzyme solution and the pH of the solution was adjusted by HCl and NaOH in the range of 2–10. Fig. 3 shows that maximum adsorption was obtained when the pH is lower than 6.0. The adsorption amount decreased when the pH increased from 6.0 to 10.0. This can be relative to the fact that the positively charged surface of SiO_2 NPs was desirable for the adsorption of α-amylase. When the pH value is higher than 6.0, the net charge of the SiO_2 NPs was negative. Therefore, immobilization of α-amylase on SiO_2 NPs was done in native pH (5.5).

To investigate the effect of amylase/silica ratio in immobilization process, different ratios of SiO_2 NPs were added to 5 ml of 1 mg/ml enzyme solution in the native pH. Fig. 4 shows that maximum of immobilized amylase percentage is 79% when

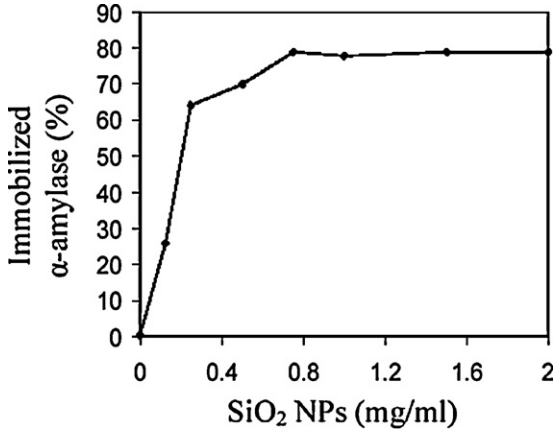

Fig. 4. Effect of amylase/silica ratio on α-amylase immobilization.

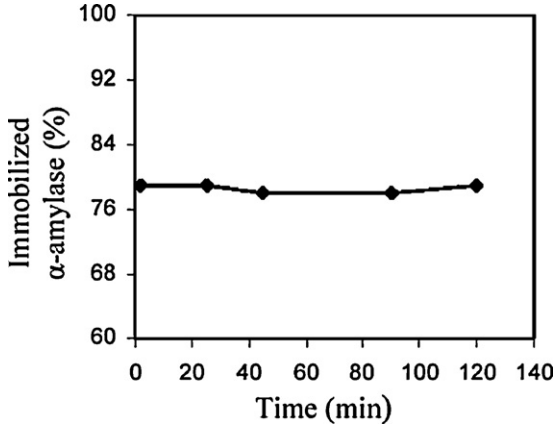

Fig. 5. Effect of time on α-amylase immobilization.

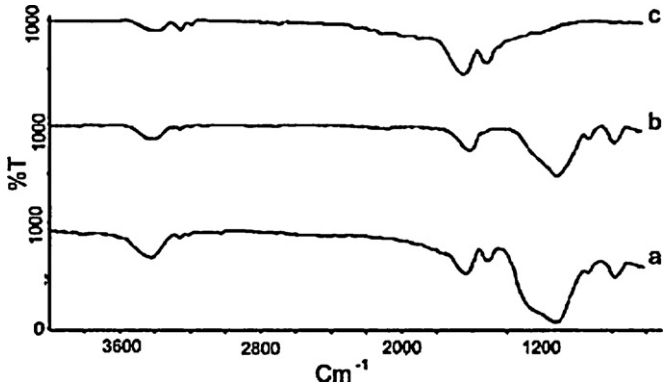

Fig. 6. The FTIR spectra of α-amylase-immobilized on silica nanoparticles (a), silica nanoparticles (b), and free α-amylase (c).

amylase/silica ratio is 1/0.75. Therefore the amount of immobilized α-amylase on solid silica nanoparticles is 1056 mg/g silica.

Fig. 5 shows immobilization of α-amylase on silica nanoparticles on different time intervals. From Fig. 3 it can be seen that the adsorption of α-amylase onto solid silica nanoparticles involves only one stage and quickly reaches to equilibrium.

Fig. 6 shows the FTIR spectrums: (a) α-amylase-immobilized on silica nanoparticles, (b) silica nanoparticles and (c) free α-amylase. In FTIR spectrum of silica nanoparticles, the peaks of 3430 cm^{-1}, 1630 cm^{-1}, 1100 cm^{-1}, 800 cm^{-1}, and 957 cm^{-1} are the non-symmetric stretching vibration of O–H groups bound with the surface of silica materials, the flexural vibration of H–O–H

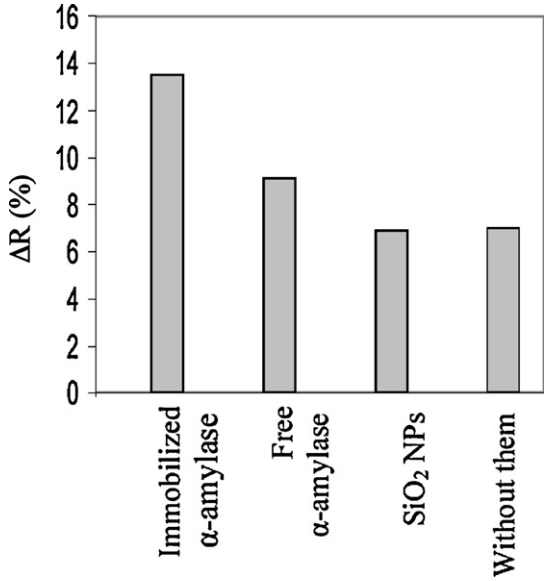

Fig. 7. Digestion power of four detergent powder formulations containing immobilized enzyme on SiO_2 NPs, free enzyme, pure SiO_2 NPs and without them.

groups adsorbed onto the surface of silica materials, the non-symmetric and symmetric stretching vibration of Si–O–Si groups and the stretching vibration of Si–OH groups, respectively. As it is well known, the amide linkages between amino acid residues in polypeptides and protein give the well-known fingerprints from the IR spectrum [25]. The position of the amide I and II bands in the FTIR spectra of proteins is a sensitive indicator of conformational changes in the protein secondary structure [26] and has been used in the study to investigate the immobilized enzymes molecules. The amide I peak at about 1650 cm^{-1} can be observed at α-amylase and α-amylase-immobilized silica. The peak at 1530 cm^{-1}, corresponding to the amide II band [27], can also be clearly seen in α-amylase-immobilized silica. These spectral characteristics indicate that the secondary structure of the protein is maintained in the immobilized α-amylase molecules.

3.2. Effect of immobilization on activity and the cleaning performance of α-amylase

To investigate the effect of immobilization process on α-amylase activity or cleaning efficiency, four detergent powder formulations containing free enzyme, immobilized enzyme on SiO_2 NPs, pure SiO_2 NPs and without any of them were prepared. Each of the formulations was washed with mentioned washing test method at standard conditions. When immobilized enzyme was used in detergent powder formulation, the best digestion power or cleaning efficiency was obtained (Fig. 7). It was found that detergent ingredients mostly affected free enzyme comparison with immobilized enzyme.

During the washing cycle, the pH of detergent solutions is about 9–10. In the alkaline solution, enzyme and silica NPs have a weak interaction together. Therefore enzyme desorbs from the carrier surface and adsorbs on to the soil surface.

To investigate the effects of residual silica NPs on the fabric after the washing, incrustation test or ash content test (ISO 4312) was carried out. Two detergent powder formulations containing free and immobilized enzyme were prepared. According to the mentioned test method condition (ISO 4312), washing test was performed on cotton fabrics by two powder formulations. Washed and unwashed cotton fabrics incinerated in furnace (Heraeus D63450, Germany) at 800 °C. The residual deposition of solid inorganic

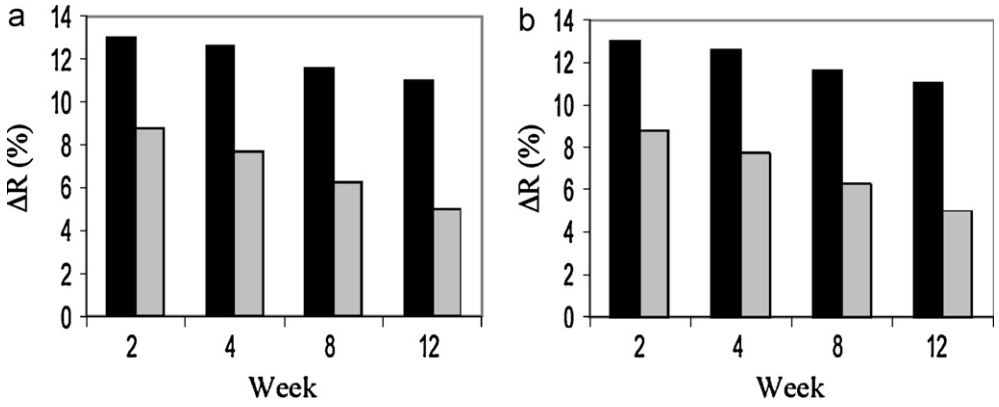

Fig. 8. Effect of temperature on digestion power of free and immobilized (■) enzyme in 40 °C (a) and 50 °C (b).

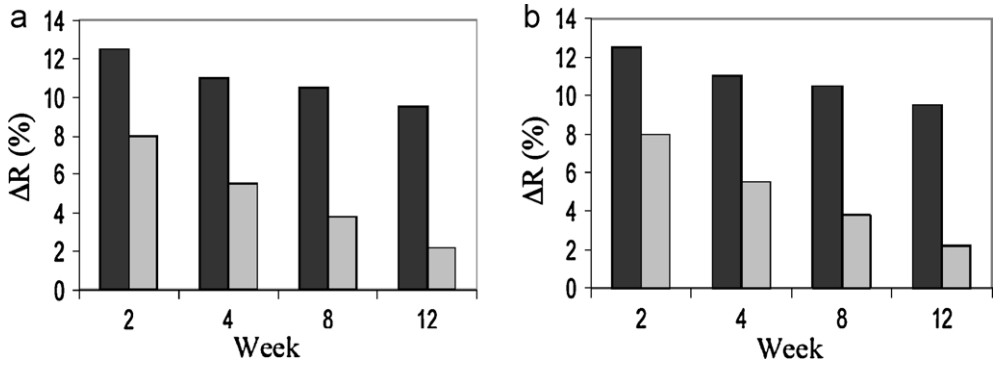

Fig. 9. Effect of humidity on digestion power of free and immobilized (■) enzyme in 50% (a) and 70% humidity (b).

matter on cotton fabrics was calculated from the percentage ash on the washed and unwashed cotton fabrics. The result shows that there is no difference between incrustation values of two formulations.

3.3. Effect of temperature and humidity on activity or the cleaning performance of free and immobilized α-amylase

To investigate the effect of temperature on α-amylase activity, two detergent powder formulations containing free and immobilized enzyme were prepared and then placed at 40 and 50 °C in the oven at different time periods. The mentioned washing test method was carried out (Fig. 8). As Fig. 8a and b illustrates, digestion ability or cleaning efficiency of immobilized enzyme is more than free enzyme on starch soils of cotton fabric at 40 °C and 50 °C.

To investigate the effect of humidity on α-amylase activity, above method was performed under different humidity (50 and 70%) in an incubator (ELYEA KCL-2000W) at different time periods (T = 44 °C), then the mentioned washing test method was carried out. As Fig. 9a and b shows, digestion ability or cleaning efficiency of immobilized enzyme is more than free enzyme.

4. Conclusions

The present investigation describes the amylase immobilization on silica nanoparticles. The immobilized amylase was used in the formulation of detergent powder. Then its cleaning efficiency and stability were compared with the free enzyme by laundering performance of standard starch soils on cotton fabrics. This study shows that cleaning efficiency and also digestion power of immobilized enzyme are much more than the free enzyme. Immobilization process decreased enzyme sensitivity to some detergent ingredients. On the other hand, enzyme immobilization on SiO_2 NPs increased its stability during storage and shelf life of the product.

References

[1] Y.A. Galante, C. Formantici, Curr. Org. Chem. 7 (2003) 1399–1422.
[2] T. Eriksson, J. Brjesson, F. Tjerneld, Enzyme Microb. Technol. 31 (2002) 353–364.
[3] H. Oshima, M. Sakata, Y. Harano, Biotechnol. Bioeng. 28 (1986) 1727–1734.
[4] E. Jurado, V. Bravo, G. Luzon, M. Fernandez-Serrano, M. Garcia-Roman, D. Altmajer-Vaz, et al., J. Surfactants Deterg. 10 (2007) 61–70.
[5] M.R. Stoner, D.A. Dale, P.J. Gualfetti, T. Becker, M.C. Manning, J.F. Carpenter, et al., Enzyme Microb. Technol. 34. (2004) 114–125.
[6] N. Eriksen, Detergents in Industrial Enzymology, 2nd ed., Macmillan, New York, 1996, pp. 189–200.
[7] C.H. Jang, B.D. Stevens, P.R. Carlier, M.A. Calter, W.A. Ducker, J. Am. Chem. Soc. 124 (2002) 12114–12115.
[8] A.F. Abdel-Fattah, M.Y. Osman, M.A. Abdel-Naby, J. Chem. Eng. 68 (1997) 189–196.
[9] C.M.F. Soares, M.H.A. Santana, G.M. Zanin, H.F. De Castro, Biotechnol. Progr. 19 (2003) 803–807.
[10] Y. Han, S.S. Lee, J.Y. Ying, Chem. Mater. 18 (2006) 643–649.
[11] L. Cao, U.T. Bornscheuer, R.D. Schmid, J. Mol. Catal. B: Enzym. 6 (1999) 279–285.
[12] G. Sanjay, S. Sugunan, Catal. Commun. 6 (2005) 525–530.
[13] G. Sanjay, S. Sugunan, Catal. Commun. 6 (2005) 81–86.
[14] C.A. Groom, J.M. Meising, B.N. White, Appl. Microbiol. 28 (1988) 8–13.
[15] R. Reshmi, G. Sanjay, S. Sugunan, Catal. Commun. 8 (2007) 393–399.
[16] R. Reshmi, G. Sanjay, S. Sugunan, Catal. Commun. 7 (2006) 460–465.
[17] M. Vezir Kahraman, G. Bayramoglu, N. Kayaman-Apohan, Food Chem. 104 (2007) 1385–1392.
[18] A.K. Johnson, A.M. Zawadzka, L.A. Deobald, R.L. Crawford, A.J. Paszczynski, J. Nanopart. Res. 10 (2008) 1009–1025.
[19] L. Xianqiao, G. Yueping, S. Rui, L. Huizhou, J. Chromatogr. B 822 (2005) 91–97.

[20] A.S.M. Chong, X.S. Zhao, Appl. Surf. Sci. 237 (2004) 398.
[21] H. Ma, J. He, D.G. Evans, X. Duan, J. Mol. Catal. B: Enzym. 30 (2004) 209–217.
[22] C.H. Lei, Y.S. Shin, J. Liu, E.J. Ackerman, J. Am. Chem. Soc. 124 (2002) 11242–11243.
[23] R.B. Bhatia, C. Jeffrey Brinker, Chem. Mater. 12 (2000) 2434–2441.
[24] M. Miyazaki, J. Kaneno, R. Kohama, M. Uehara, K. Kanno, M. Fujii, H. Shimizu, H. Maeda, Chem. Eng. J. 101 (2004) 277–284.
[25] A. Dong, P. Huang, Biochem. J. 31 (1992) 182–189.
[26] J.F. Rabolt, F.C. Burns, N.E. Schlotter, J.D. Swalen, J. Chem. Phys. 78 (1983) 946–952.
[27] A. Gole, G. Thakar, M. Sastry, Colloids Surf. B 28 (2003) 209–214.