Thermosensitive hybrid system based on pluronic F127 and nanoclay laponite for sustained local release of lidocaine hydrochloride

Guanfeng Hou[1], Yanchun Gao[1], Yanhu Xie[1], Xiaoqing Chai[*1], Jibin Miao[*2]
1. Department of Anesthesiology, Affiliated Provincial Hospital of Anhui Medical University, Anhui Provincial Hospital, Hefei 230001, China
2. School of Chemistry & Chemical Engineering, Anhui University, Anhui Province Key Laboratory of Environment-friendly Polymer Materials, Hefei 230601, China
E-mail: lingxiaoyu1003@163.com

Abstract: A novel thermosensitive local drug release system was prepared by incorporation of biocompatible nanoclay laponite into pluronic F127 solution and characterized by rheological measurements, zeta potential measurement and in vitro drug release measurement in the presence of lidocaine hydrochloride. All the systems transited from sol to gel with increase of temperature. The lower critical solution temperature (LCST) of the composite matrix changed little with increase in the mass of incorporated nanoclay, but the modulus increased with increase in the mass of incorporated nanoclay. Thein-vitrorelease experiments revealed that the novel system provided an extended duration of drugs compared to the pluronic F127 alone. This unique feature is attributed to the interaction of nanoclay laponite with lidocaine hydrochloride and increased modulus with incoporation of nanoclay laponite. The merits of the novel system, such as good cytocompatibility, thermosensitive properties, and improved sustained local release ability, make them a promising platform for the delivery of other drugs.
Keywords: Thermalsensitive; Laponite; F127; Lidocaine; Sustained Local Release.

1. Introduction

Controlled drug delivery systems that can release drugs in a predetermined rate and to optimize the therapeutic effects have become significant in recent years [1-4]. Among these thermo-sensitive systems that can be transformed from sol to gel in situ at certain temperature play a very important role because of the conveniently controllable adjustment of temperature [5-8]. They are liquid below room temperature and gel at body temperature. Hence they are easily injected and once they fill the proper cavity they become gel as they reach body temperature. Pluronic F127 is a commercial thermo-sensitive hydrogel, which have been widely used in drug delivery systems [9-11]. It exhibits reversible thermal gelation in aqueous solution at concentrations>20% (w/v).Drug delivery via F127 gel occurs by diffusion and dissolution of the gel at the administration site. The release profiles of several drugs delivered via F127 gels have been published [12-19]. These studies have also shown that the drug diffusion coefficient in the gel decreases as F127 content increases, coinciding with an increase in gel viscosity. This has led the authors to propose that drug release rates are determined by gel viscosity [20]. Many additives like salts [21], water-soluble polymers [22], and PCEP nanoparticles [23] can change the sol-gel transition temperature, gel viscosity, drug solubilisation of F127 solutions.

In this study, we report the effects of nanoclay laponite on the release of lidocaine hydrochloride from 20% F127 gels.Laponite (LP) is a synthetic nanoclay that has a nanodisk structure (25 nm in diameter and 0.92 nm in thickness), and can be biodegraded into nontoxic products (Na^+, $Si(OH)_4$, Mg^{2+}, Li^+), very much similar to what happens with bioactive glasses (Na^+, $Si(OH)_4$, Ca^{2+}, PO_4^{3-}). The chemical structure of the LP crystals confer them a negatively charged surface and pH-dependent edge surface charges (positive at a pH lower than 9) [24]. Its unique structure, together with its good biocompatibility [25], and osteoinductivity[26], makes LP act as an ideal inorganic nanomaterial for biomedical applications [27]. Goncalves et al. studies revealed that through electrostatic interactions, DOX was able to form nanocomplexes with LP, which improved the bioactivity of the drug in cancer cells by overcoming drug resistance processes [28]. Wang *et al.* used laponite nanodisks as an efficient platform for Doxorubicin delivery to cancer cells [29]. Furthermore, LP has an ability to form strong interactions with hydrophobic molecules [30, 31], it is proposed that an amphiphilic block polymer can be used to form a protective coating on LP nanodisks, where the hydrophobic block will cover the surface of LP nanodisks working as an anchor, whereas the hydrophilic part will act as a stealth shell to maintain their stability

under physiological environment. Wang *et al.* prepared pH-sensitive drug delivery nanosystems using PEG-PLA copolymer for improving LP physiological stability [32]. F127 can also absorb to LP and improve the stability of LP system [33]. Lidocaine hydrochloride is a local anesthetic and selected as a model drug because its use is limited by the short duration of its effects.Herein, we present an elegant approach to preparing a new type of thermosensitive hybrid system based on pluronic F127 and nanoclay laponite with good cytocompatibility and stability, which are able to sustain local release of Lidocaine hydrochloride.

2. Materials and methods

2.1 Materials

Lidocaine hydrochloride (LC), Pluronic F127 (12,600Da, 70%w/w PEO), cellulose acetate dialysis tubes (Cutoff M_W at-7000) and Dulbecco's phosphate buffer saline (PBS, without Ca^{2+} and Mg^{2+}) solution were purchased from Sigma-Aldrich, USA. The de-ionized water used was treated with Millipore-Q water purification system. Laponite RDS (LP) was friendly offered from Rockwood Additives Limited, UK.

2.2 Sample preparation

All the samples were prepared on a weight basis using the cold method [34]. Concentrations of Pluronic polymer F127, LP and LC are expressed by weight percentage (% w/w). For the preparation of F127/LC sample, Pluronic polymer was dissolved in de-ionized (DI) water and stirred at 4 °C. The solution was then put into the refrigerator at 4 °C for 1~2 days until it became clear. Then LC was added to the solution, stirred at 4 °C until it was dissolved completely. In order to obtain a F127/LP/LC samples, we first dissolved LP in DI water while stirring for 24 h. In order to further exfoliate the LP, the solution was subsequently sonicated for 1 h. The LP solution was placed into an ice bath and the F127 polymer was added. In order to ensure that the polymer was completely dissolved, the entire solution was then refrigerated at 4 °C. LC was added at last.

2.3 Characterizations

Rheological measurements were conducted on a TA AR-G2 rheometer using the system of coaxial cylinders (stator inner radius of 15.00 mm, rotor inner radius of 14.00 mm, cylinder immersed height of 42 mm). The concentric cylinder geometry was loaded with the polymer solutions at 4 °C, in order to ensure that they were in the liquid state. Temperature sweep measurements were performed from 4 °C to 45 °C at a constant heating rate of 1 °C/min. The storage modulus *G*' and loss modulus *G*" were measured as a function of temperature at an angular frequency of 1 rad/s within a linear range of viscoelasticity. The frequency dependence of the complex modulus was determined between 0.01 and 100 rad/s. A thin layer of low-viscosity silicone oil was used to cover the free surface of the solution to prevent evaporation of solvent.

The hydrodynamic diameter and the surface charge of LP, LP/LC and LP/LC/F127 water solution were measured at room temperature using a Zetasizer Nano ZS (Malvern Instruments, UK).The hydrodynamic diameters were determined with a detection angle of 173°. Zeta potential measurements were performed with a detection angle of 17° and calculated using the Smoluchowsky model for aqueous suspensions. Before measurement, LP, LP/LC and LP/LC/F127 solutions prepared in 2.2 were diluted with 6 times water and sonicated (BRANSON 2510,100 W) for 15 min.

Ultraviolet-visible (UV-vis) spectroscopy was performed using a Lambda 25 UV-vis spectrometer (PerkinElmer25). Before measurement, LP/LC and LP/LC/F127 solutions prepared in 2.2 were dialyzed against UP water, for 12 h (the dialysis membrane had a molecular weight cutoffof 14 000 Da, Spectrum Laboratories, Inc.), to obtain the final products named as LP/LC and LP/LC/F127nanohybrids, respectively. Free LC, and LC-loaded nanohybrids were dispersed in water before measurements. LP/F127 solution was also tested as controls.

2.4 In vitro drug release

In vitro release response of LC was carried out in the USP eight stage dissolution rate test apparatus with the dialysis bag technique [35, 36]. Buffer solutions of pH 7.4 were used as dissolution medium. Amounts of LC, F127/LC, and LP/F127/LC, dispersed in 5 ml release medium in cellulose dialysis tubes (cutoff molecular mass of 7000) were immersed in 500 ml release medium. The temperature was maintained at 37 ±0.5 °C. The rotation frequency was kept at 100 rpm. Aliquots (5 ml) were withdrawn at predetermined periods and were immediately replaced by the same volume of fresh medium. The aliquots, after suitable dilution, were analyzed spectrophotometrically at 262 nm [37]. These studies were performed in triplicate for each sample, and the average values were reported.

3. Results and discussion

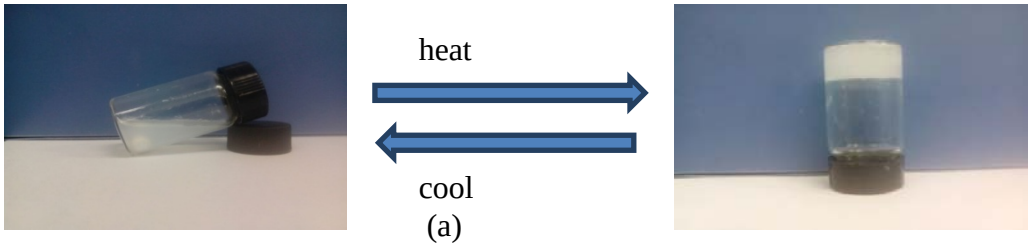

Fig.1 Directly observation of obtained drug delivery system. After the tube was taken out of the water bath, the photo was taken immediately. (a) 4 °C; (b) 37 °C

Pluronic F127 is a polyoxyethylene-polyoxypropylene-polyoxyethylene triblock polymer. At low temperature both the PEO and the PPO blocks of the Pluronic F127 are hydrophilic and water soluble forming a transparent solution. The triblock copolymers exist as unimers solvated by water. With increasing temperature the PPO blocks dehydrate, and F127 form spherical micelles which have a core consists mainly of PPO and the hydrated PEO swollen outer shell molecules. Above certain concentration (≥10 wt%) there are micellar clusters due to the coalescence of spherical micelles. Finally, a liquid–crystalline phase of disk-like micelles forms [37]. The sol transform to a gel with increasing of temperature. At present study the concentration of F127 is 20% (w/v). With addition of lidocaine hydrochloride and laponite, the complex system can also transit from a sol to a gel as shown in figure .1.At 4 °C, the sol could flow because of its low viscosity, favor for injected. When the temperature increased to 37 °C, gel formed and it could not flow again. It can act as a depot for the sustained release of drugs.

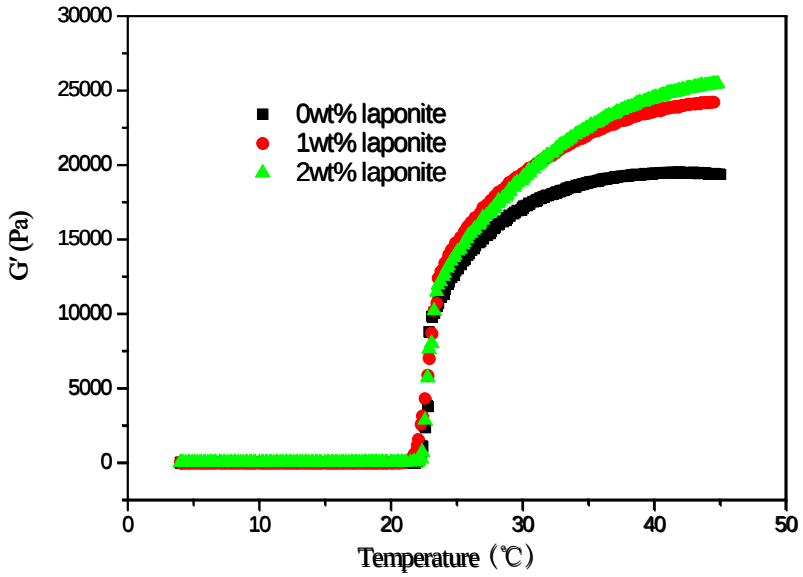

Fig.2 Storage modulus *G′* for complex with different concentrations of LP as a function of temperature

The temperature dependence of rheological properties of the complex was studied at a range of 4~45 °C. In Fig.2, we plot storage modulus G′ for complex with different concentrations of LP as a function of temperature. The storage modulus is low at low temperature but they increase dramatically with increasing temperature as a result of the gel forming process. The sol-gel transition temperature of the complex change little with increase of LP concentration. But the storage modulus increases with increase in the mass of incorporated LP. Since LP is hydrophilic, when the LP concentration exceeds 2% they are able to form a gel at temperatures due to charge-charge interactions. So the presence of the LP in the complex provides resistance to the shear, hence effectively increasing the modulus of the solution [38].

For therapeutic delivery, the size of a nanoplatform plays an important role on its biodistribution. It has been reported that nanoparticles with a size around 100 nm are more able to permeate the immature malignant neovasculature and accumulate in the tumor site through the enhanced permeation and retention (EPR) effect while their uptake by the reticuloendothelial system (RES) occurs in a slower rate [39-41]. Table 1 shows the

hydrodynamic diameter (size) of LP, LP/LC, LP/LC/F127 analyzed by dynamic light scattering (DLS) and the corresponding ζ-potentials. The Z-average size of LP was32±4 nm, indicating it is well dispersed state in water as individual nanodisks [26].The addition of LC resulted in the successful formation of nanocomplexes of LP/LC (120 ±5nm), probably through their strong electrostatic interactions[25].The incorporation of F127 appeared to further increase the hydrodynamic size of LP/LC, indicating the successful coating of the nanocomplexes, which was further confirmed by the less negative ζ-potential of LP/LC/F127(−0.09±0.02 mV) when compared to that of LP/LC (−0.62±0.1mV). Pluronic F127 having both PEO and PPO segments is a triblock polymer. The more hydrophoic PPO segments have a higher affinity for the LP surface, while the hydrophilic PEO segments dangling into solution [33].

Table 1 Characterization of LP,LP/LC,LP/LC/F127 nanohybrids in water

Sample identity	Size (nm)	Zeta potential (mv)
LP	32±4	-6.7±0.7
LP+LC	120±5	-0.62±0.1
LP+LC+F127	150±7	-0.09±0.02

The LP/LC and LP/LC/F127 nanohybids were further characterized by UV-vis spectroscopy, with the spectra shown in Fi.3. From their UV-vis spectra , we can see that, for the free LC, LP/LC and LP/LC/F127 nanohybrids, there is an absorption peak at around 262 nm, which is absent in LP/F127 spectra, indicating the successful loading of LC in the nanohybrids [37].

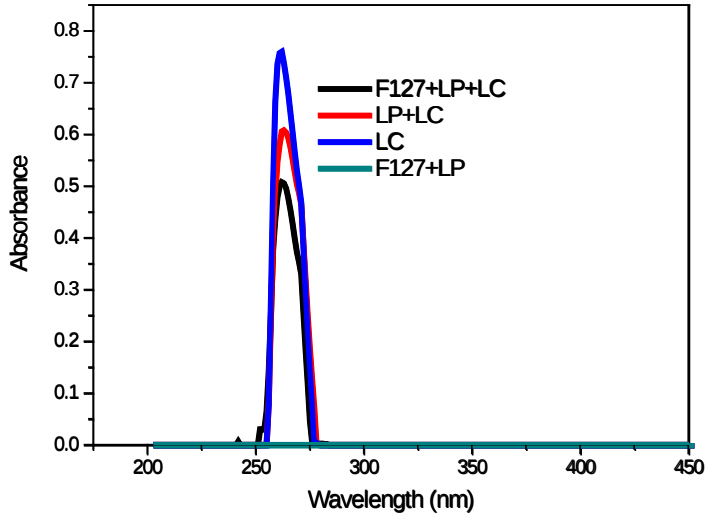

Fig.3 UV−vis spectra of LC, LP/LC, LP/LC/F127and LP/F127

At phosphate buffer pH=7.4, the release of pure LC was ~77% in 2.5 h and 81% up to 25 h (Fig. 4). In the case of LC/F127, and LC/LP/F127, 44% and 27% LC were released within 5h. The maximum amount of LC released was 54% and 34.5% up to 25 h. At present temperature and concentration, F127 can form gel. The increase in solution viscosity reduces the lidocaine release rate because drug diffusion through the gel matrix is prolonged. With addition of LP, LC can absorb to LP through strong electrostatic interaction. At the same time, LP can also increase the viscosity of the solution. So the release rate of lidocaine was slowest for LC/LP/F127 solution. Therefore, compounding of LC/F127 with LP had a desirable effect on the delivery of LC. The delivery mechanism of this system is proposed as followed in Fig. 5.

4. Conclusions

In summary, we develop a novel thermosensitive local drug release system which was prepared by incorporation of biocompatible nanoclay laponite into pluronic F127 solution. LP can form nanohydrbid with LC through strong electrostatic interaction and increase the viscosity of the solution. Pluronic F127 can form gel with increase of temperature and also increase the viscosity of the solution. So the drug delivery can be controlled. The combination of LP and F127 may be useful for the controlled delivery of other drugs.

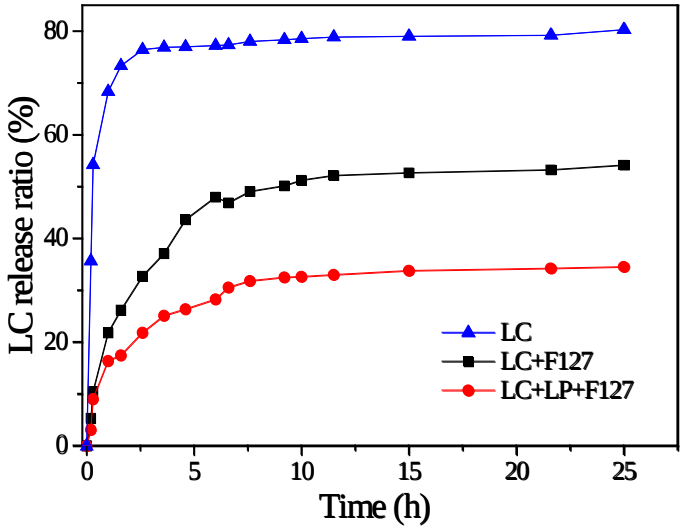

Fig.4 Release profiles of LC in intestinal fluid (pH 7.4) at37 ±0.5 °C

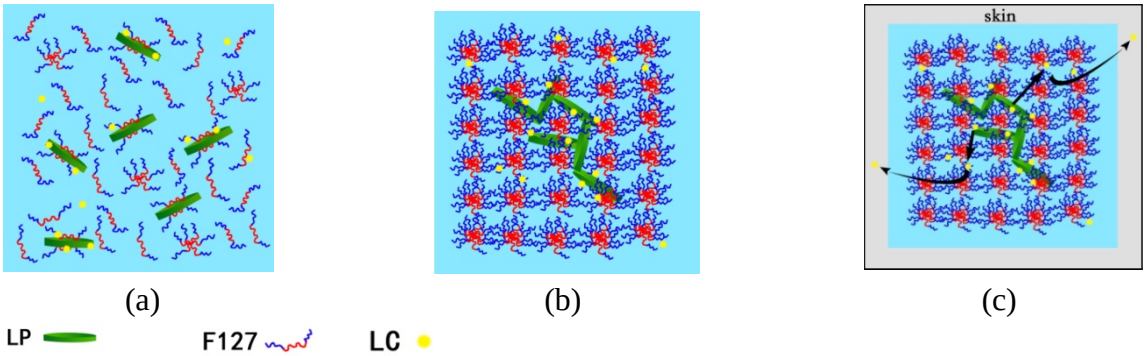

Fig.5 Possible sol-gel transition and LC release mechanism of LP/LC/F127 systems. (a) 4 °C; (b) 37 °C; (c) 37 °C, LC desorbs from LP and diffuses from F127 gel to skin

References

[1] D. Dipankar, G. Paulomi, D. Santanu, P. Asit, P. Sagar, Applied materials＆interfaces 7 (2015) 4791
[2] P. Numata,S. Yamazaki,N. Naga, Biomacromolecules13 (2012) 1383
[3] J. Wei, F. Chen,J.W. Shin,H. Hong,C. Dai,J. Su, C. Liu, Biomaterials 30 (2009) 1080
[4] T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney,Nat. Biotechnol 19 (2001) 1029
[5] R. Langer, D. A. Tirrell,Nature 428(2004)487
[6] B. Jeong, Y. H. Bae, D. S. Lee, S. W. Kim, Nature 388 (1997) 860
[7] J. Kost, R. Langer, Adv. Drug Deliv.Rev.46 (2001) 125
[8] D. Schmaljohann, Adv.Drug Deliv. Rev. 58 (2006) 1655
[9] S. M. Moghimi, A. C. Hunter, Trends Biotechnol. 18 (2000)4120
[10] Y. Liu, W. L. Lu, J. C. Wang, X. Zhang , H. Zhang, X. Q. Wang, T. Y. Zhou, Q. Zhang, J. Control. Release 117 (2007) 387
[11] G. Dumortier, J. L. Grossiord, F. Agnely, J. C. Chaumeil, Pharm. Res. 23 (2006) 2709
[12] M. L. Veyries, G. Couarraze, S. Geiger, F. Agnely, L. Massias, B. Kunzli, F. Faurisson, B. Rouveix, Int. J. Pharm.192 (1999) 183
[13] A. Paavola, P. Tarkila, M. Xu, T. Wahlstrom, J. Yliruusi, P. Rosenberg, Pharm.Res. 15(1998) 482
[14] A. Paavola, I. Kilpelaine, J. Yliruusi, P. Rosenberg, Int. J. Pharm. 199 (2000) 85
[15] M. Scherlund, M. Malmsten, A. Brodin, Int. J. Pharm. 173 (1998) 103
[16] M. Scherlund, M. Malmsten, P. Holmqvist, A. Brodin, Int. J. Pharm. 194 (2000), 103
[17] S. Y. Kin, H´ a, J. C., Y. M. Lee, J. Control. Release 65 (2000) 345

[18] J. M. Barichello, M. Morichita, K. Takayama, I. Nagai, Int. J. Pharm. 184(1999) 189
[19] J. M. Barichello, M. Morichita, K. Takayama, Y. Chiba, S. Tokiwa, T. Nagai, Int. J.Pharm. 183 (1999) 125
[20] N. K. Pandit, D. Wang, Int. J. Pharm. 167 (1998) 183
[21] E. J. Ricci, L. O. Lunardi, D. M. A. Nanclares, J. M. Marchetti, Int. J.Pharm. 288 (2005) 235
[22] C. P. Oliveiraa, M. E. N. P. Ribeiro, N. M. P. S. Ricardo, T. V. de P. Souza, C. L. Mouraa, C.Chaibundit, S.G. Yeates, K.Nixonc, D. Attwood, Int. J. Pharm. 421 (2011) 252
[23] M. Gou, X. Li, M. Dai, C. Gong, X. Wang, Y. Xie,H. Deng, L. Chen, X. Zhao, Z. Qian, Y. Wei. Int. J.Pharm.359 (2008) 228
[24] D. W. Thompson, J. T. Butterworth, J. Colloid Interface Sci. 151 (1992) 236
[25] Y. Li, J. Santos, D. Maciel, H. Tomás, J. Rodrigues, J. Controlled Release 152(Suppl 1) (2011) e55
[26] A. K. Gaharwar, S. M. Mihaila, A. Swami, A. Patel, S. Sant, R.L.Reis, A. P. Marques, M. E. Gomes, A. Khademhosseini, Adv. Mater. 25 (2013)3329
[27] J. I. Dawson, R. O. Oreffo, Adv. Mater. 25 (2013) 4069
[28] M. Goncalves, P. Figueira, D. Maciel, J. Rodrigues, X. Qu, C. Liu, H. Tomás, Y. Li, Acta Biomater. 10 (2014) 300
[29]S. Wang, Y. Wu, R. Guo, Y. Huang, S. Wen, M. Shen, J. Wang,X. Shi, Langmuir 29 (2013) 5030
[30] H. Jung, H. M. Kim, Y. Bin Choy, S. J. Hwang, J. H. Choy, Int. J. Pharm. 349 (2008) 283
[31] T. Takahashi, Y. Yamada, K. Kataoka, Y. Nagasaki,J. Controlled Release107 (2005)408
[32] G. Wang, D. Maciel, Y. Wu, J. Rodrigues, X. Shi,Y. Yuan, C. Liu, H. Tomás, Y. Li, Applied materials& interface,6 (2014) 16687
[33] A. Nelson, T. Cosgrove, Langmuir 21 (2005) 9176
[34] N. Pandit, T. Trygstad, S. Croy, M. Bohorquez , C. Koch, J Colloid Interface Sci 222 (2000)213
[35] G. V. Joshi, B. D. Kevadiya, H. A. Patel, H. C. Bajaj, R. V. Jasra, Int. J.Pharm. 374 (2009) 53
[36] G. V. Joshi, H. A. Patel, B. D. Kevadiya, H. C. Bajaj, Appl. Clay Sci. 45 (2009) 248
[37] B. D. Kevadiya, G. V. Joshi, H. M. Mody, H. C. Bajaj, Applied Clay Science 52 (2011) 364
[38] C. C. Perry, T. S. Sabir, W. J. Livingston, J. R. Milligan, Q. Chen, V. Maskiewicz, D.S. Boskovic, Journal of Colloid and Interface Science 354 (2011) 662
[39] J. Jiang, C. Li, J. Lombardi, R.H. Colby, B. Rigas, M.H. Rafailovicha, J.C. Sokolov, Polymer 49 (2008) 3561
[40] J. Fang, H. Nakamura, H. Maeda,Adv. Drug Delivery Rev. 63 (2011)136
[41] S. M. Moghimi, S. S. Davis, Crit. Rev. Ther. Drug Carrier Syst. 11 (1994) 31
[42] Y. H. Li, J. Wang, M. G. Wientjes, J. L. S. Au, Adv. Drug Deliver. Rev. 64 (2012) 29