ORIGINAL ARTICLE

A study of the formation of magnetically active solid dispersions of phenacetin using atomic and magnetic force microscopy

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ABSTRACT

A lot of pharmaceutical substances have a poor solubility that limits their absorption and distribution to the targeted sites to elicit the desired action without causing untoward effects on healthy cells or tissues. For such drugs, new modes of delivery have to be developed for efficient and effective delivery of the drug to the target site. Formation of magnetically active solid dispersion of such drugs could be a useful approach to addressing this problem because they combine targeted delivery and good solubility. In this work, the distribution of superparamagnetic nanoparticles in the solid dispersion of polyethylene glycol with average molecular weight 950–1050 g/mol and phenacetin was studied using atomic force and magnetic force microscopy. The distribution of nanoparticles was found to be uniform in studied composites. Magnetically active solid dispersions may find application in the production of the capsulated drug delivery systems with enhanced solubility parameters.

Key words: Atomic force microscopy, magnetic force microscopy, magnetic nanoparticles, phenacetin, polyethylene glycol, solid dispersion

INTRODUCTION

Magnetic nanoparticles are a subject of high interest in the field of medicine because such particles themselves, as well as systems containing such particles, can be controlled remotely by an external magnetic field. A wide range of magnetic nanoparticles is produced based on metals, iron oxides, ferrites as well as CoPt, FePt, MnAl, $SmCo_5$, $Fe_{14}Nd_2B$.[1,2] Oxide-based nanoparticles have weaker magnetic properties compared to metal-based nanoparticles; however, they are more resistant to the oxidation. Particles based on iron oxide found the most use in biomedicine because of stability of magnetic properties and low toxicity.[3,4]

Nanoparticles in solutions have a pronounced tendency for aggregation; thus, the application of nanoparticle-containing solutions is critically dependent on the stabilization of particles (coating of the magnetic core, addition of stabilizing agents, choice of solvent, etc.). Coatings used for the stabilization of nanoparticles might be organic-based (surfactants and polymers)[5,6] and inorganic-based (silica, carbon, noble metals).[7,8] The most widely used organic coatings are dextran, polyethylene glycol (PEG), starch, polyvinyl alcohol, heparin, medium- and long-chain fatty acids.[9-12]

Surface modification using ethylene glycol prevents macrophage consumption of particles and promotes

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membrane transport due to the combination of polar and nonpolar groups of PEG.[13]

In the same time, the problem of the solubility enhancement for improved bioavailability is also important. Solid dispersions of hydrophobic drugs with different polymers (including PEG) were found to be useful.[14-19] However, such systems do not provide the targeted delivery.

It was shown that PEG with relatively low molecular mass can form solid dispersions with hydrophobic drugs including phenacetin, sulfanilamide, and dipyridamole.[20-23] Thermodynamic parameters of the solution process of the solid dispersions of phenacetin with biocompatible polymers as well as intermolecular interaction energies in the composites based on them were also studied. It was shown that the optimal matrix for solid dispersions of phenacetin is PEG with the average weight 1400.[24] However, melting temperature of PEG with average weight 1000 is close to the physiological temperature,[21] which allows to create a wider range of drug systems based on it and use it in hyperthermia therapy. A combination of solution and thermophysical properties makes PEG with the molecular weight 950–1050 g/mol, the most interesting candidate for the production of solid dispersions with hydrophobic drugs. Inclusion of magnetic nanoparticles in such solid dispersions would allow drug formulations with a targeted delivery.

In the present work, the feasibility of inclusion of magnetic nanoparticles in solid dispersion of PEG with molecular mass 950–1050 g/mol with phenacetin was demonstrated.

MATERIALS AND METHODS

PEG molecular weight 950–1050 (PEG-1000) (Aldrich) and phenacetin, 98% (Aldrich) were used as received. Superparamagnetic nanoparticles fluidMAG-UC/C (Chemicell) was used for the modification. Absolute ethanol was used as a solvent.

Solid dispersions preparation

Two polymer/drug compositions (6:1 and 1:1 by weight) were prepared. Accurately weighed amounts of PEG-1000 (36 and 21 mg) and phenacetin (6 and 21 mg), total weight 42 mg, were dissolved in 30 ml of ethanol. After complete dissolution, the solvent was evaporated at 60°C and atmospheric pressure. Subsequently, the solid mass was dried in vacuum (100 Pa). Prepared composites were stored in the desiccator over P_4O_{10} until use.

Modification of solid dispersions with nanoparticles

A solution of solid dispersion in ethanol (1 mg/ml) was used for nanoparticle inclusion. 1 μl of nanoparticle solution (25 mg/ml) was added to 1 ml of the solution of the solid dispersion, and the resultant solution was dried as was described in the previous section.

Atomic force microscopy of solid dispersion

Atomic force microscope (AFM) Solver P47 Pro (NT-MDT, Russia)[25,26] was used for studies of the morphology of films of initial substances and solid dispersions. One drop of the solid dispersion ethanol solution (1 mg/ml) was allowed to dry on a highly oriented pyrolytic graphite (HOPG) surface, and AFM images were obtained on the tapping mode. Standard silicon cantilevers NSG-11 (NT-MDT, Russia) were used. HOPG was freshly cleaved before use.

Magnetic force microscopy of solid dispersion

Magnetic force microscopy (MFM) measurements were performed using Smena A (NT-MDT, Russia) microscope coupled to an external magnet. All experiments were carried out under the external magnetic field of 2000 Oe. Probes with magnetic coating NSC-19/Co-Cr (MikroMasch, Estonia) with force constant 0.6 N/m were used in MFM studies.[27] The samples were prepared following the same procedure as for AFM measurements.

X-ray powder diffraction of samples

X-ray powder diffraction (XRPD) studies of polymers, phenacetin, and its composites were made using a MiniFlex 600 diffractometer (Rigaku, Japan) equipped with a D/teX Ultra detector. In this experiment, Cu Kα radiation (40 kV, 15 mA) was used, and data were collected at room temperature in the range of 2θ from 3° to 50° with a step of 0.02° and exposure time at each point of 0.24 s without sample rotation.[28,29]

RESULTS

Results of atomic force microscope analysis

Results of AFM analysis of PEG-1000, phenacetin, and their composites are presented in Figure 1. Two types of structure are observed on the film of PEG-1000 deposited on the HOPG [Figure 1a]: crystalline formations with smooth edges (3–5 nm height) and the second type of structure is in the form of nanoscale size discs with a diameter of 150–320 nm and 4–20 nm height. A mean square roughness of the surface on the 20 μm × 20 μm scan is 3.55 ± 0.02 nm.

Two types of crystallites are typically found on the thin films of phenacetin [Figure 1b]: flat rectangular plates with the length of 1–2 μm, width of 0.6–0.9 μm, and height of 10–30 nm and nanowire shape crystallites with the length of 0.35–1.5 μm, 100–350 nm with, and 10–65 nm height. The roughness of surface on the 10 μm × 10 μm scan is 16.7 ± 0.1 nm.

As evident from AFM images, rectangular crystallites of phenacetin are present on the surface of the thin

film of PEG-1000:phenacetin 1:1 composite [Figure 1c]. The majority of crystallites have a size around 10 μm × 5 μm × 0.8 μm (L × W × H). Crystallites of phenacetin are not found on the scans of the dispersion of 6:1 composition [Figure 1d], its roughness is 2.66 ± 0.02 nm.

On Figure 2, AFM images of fluidMAG-UC/C magnetic nanoparticles [Figure 2a] and thin film of 6:1 PEG-1000/phenacetin composition [Figure 2b] on the surface of HOPG are presented. As is evident from AFM image [Figure 2a], magnetic nanoparticles form agglomerates containing a large number of superparamagnetic particles on the HOPG surface. The average size of the individual particles forming agglomerate is 50 nm.

Uniformly distributed nano-size objects with the 150–300 nm diameter and 10–20 nm height are visible on the AFM images of the composite films [Figure 2b]. On the crystallographic steps of HOPG, agglomerates of up to 0.8 μm in diameter and 100 nm in height are present. The increase in size is probably due to the coating of superparamagnetic particle with the polymer matrix.

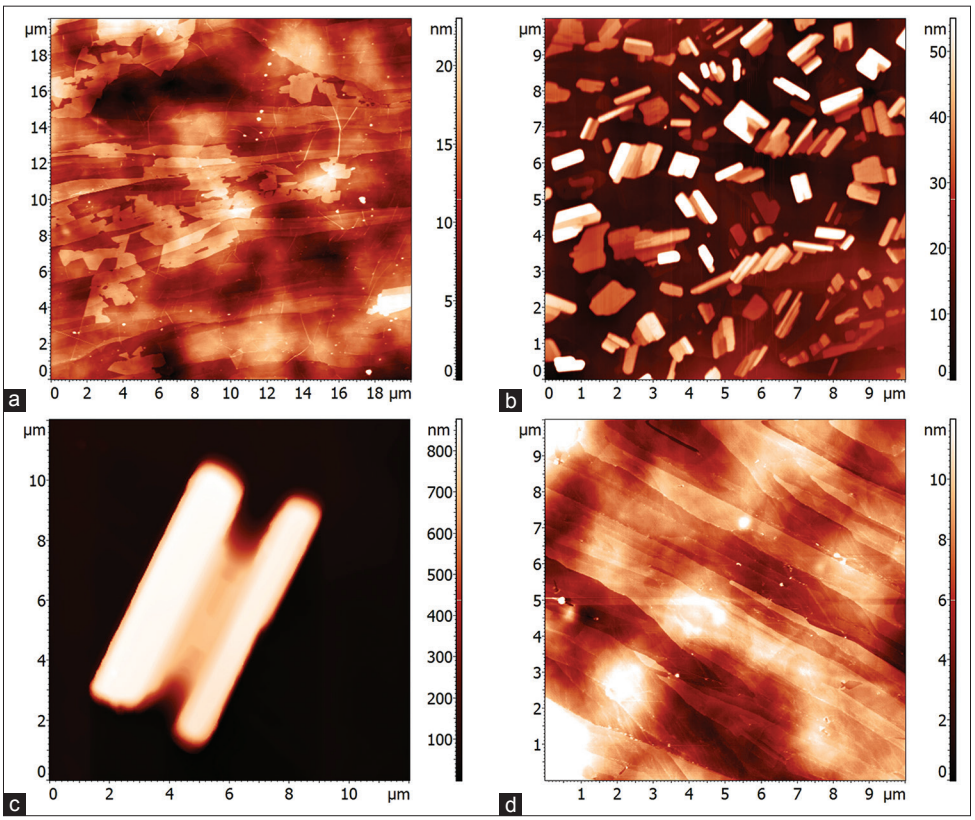

Figure 1: Atomic force microscope images of the thin film of polyethylene glycol-1000 (a), phenacetin (b) and polyethylene glycol-1000/phenacetin 1:1 (c) and 6:1 (d) compositions on the surface of highly oriented pyrolytic graphite

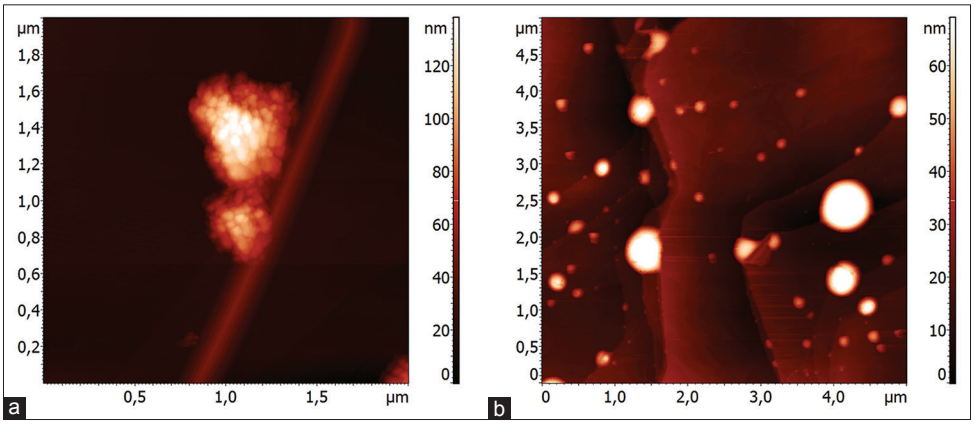

Figure 2: Atomic force microscope images of fluidMAG-UC/C magnetic nanoparticles (a) and thin film of dispersion of polyethylene glycol-1000 and phenacetin with 6:1 composition containing magnetic nanoparticles (b) on the surface of highly oriented pyrolytic graphite

Results of magnetic force microscopy analysis

MFM was employed for unambiguous identification of magnetic nanoparticles in the solid dispersion phase. AFM and MFM images of the thin film of PEG-1000 and phenacetin with 6:1 composition containing magnetic nanoparticles on the HOPG surface are presented in Figure 3.

The measurements were performed in the magnetic field with 2000 Oe magnitude. The field direction was along the surface of the surface horizontally (parallel to the surface of the sample), in this case the MFM probe magnetization lies in the direction of the external magnetic field. This explains the observed magnetic contrast from the particles [Figure 3b].[27] A film with nano-size objects is observed on the AFM-image in topography mode [Figure 3a]. It is evident from the MFM-image [Figure 3b] that at least a single object which contains magnetic nanoparticles produces a magnetic signal in the external magnetic field.

X-ray powder diffraction result

XRPD results of PEG-1000 and phenacetin samples, as well as their compositions (1:1 and 6:1 by weight), are presented in Figure 4.

As can be seen from diffractograms, same types of reflections are produced by phenacetin [Figure 4b] and PEG-1000/phenacetin 1:1 mixture [Figure 4c]. At the same time, reflection characteristics of phenacetin are absent in the diffractogram of PEG-1000/phenacetin 6:1 composition [Figure 4d], which corresponds to the lack of crystalline phase of drug in this mixture. For PEG-1000 [Figure 4a] were observed only low-intensive reflexes.

DISCUSSION

AFM is widely used for studying of thin polymer and oligopeptides films, drugs and other crystalline compounds to determine the size and shape of crystallites.[30-33] Results obtained in the present work are in agreement with the earlier results for different PEGs which showed a low roughness of the surface of the films of this polymer.[34,35] Low roughness indicates a smoothness of the individual polymer film which allows determining the presence and sizes of crystallites and nanoparticles in the PEG-1000 film. Thus, AFM might be employed as an alternative to the physicochemical methods such as X-ray diffraction and differential scanning calorimetry (DSC) currently used for determining of the formation of the solid drug dispersions. The criterion for the solid dispersion formation from AFM data is the homogeneity of the thin film of composite.[36] The feasibility of the study of the amorphization of the drug surface using AFM was demonstrated for zanamivir.[37]

AFM data for pure phenacetin and PEG-1000/phenacetin 1:1 (w/w) composite indicate the presence of crystalline phase. The comparable roughness of pure PEG-1000 and PEG-1000/phenacetin 6:1 (w/w) composite may correspond to the formation of the solid dispersion. This result is in agreement with DSC data[21] as well as X-ray diffraction which show the formation of solid dispersions of PEG-1000 with phenacetin with composition ratios >5:1.

A low content of magnetic nanoparticles in the polymer phase hinders their determination using diffraction methods and precludes assessment of their magnetic properties. In the same time, combination of AFM and MFM allows not only to study the distribution of particles but also to unambiguously prove the existence of the magnetic moment.[38] In the present work, a combination of microscopy methods was used to demonstrate first the sufficient uniformity of nanoparticle distribution in the drug-polymer dispersion and second a simple method for the introduction of nanoparticles in the polymer phase allows the production of magnetically active drug formulations.

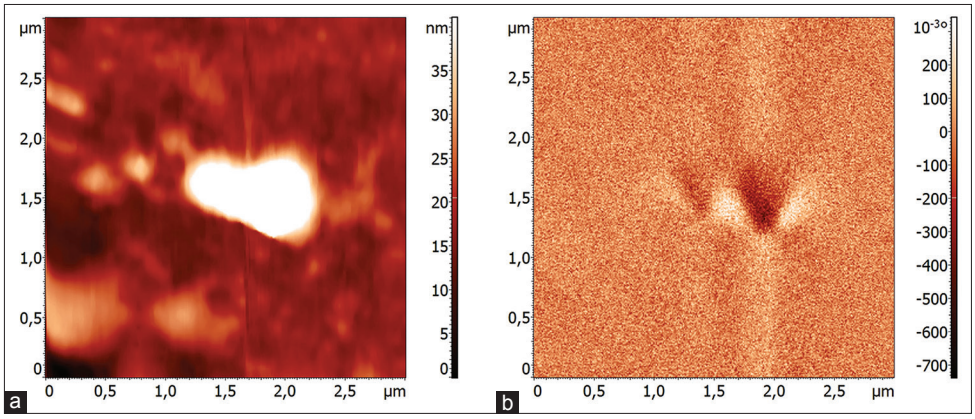

Figure 3: Atomic force microscope image of sample topology (a), magnetic force microscopy image of the same sample on the same location (b)

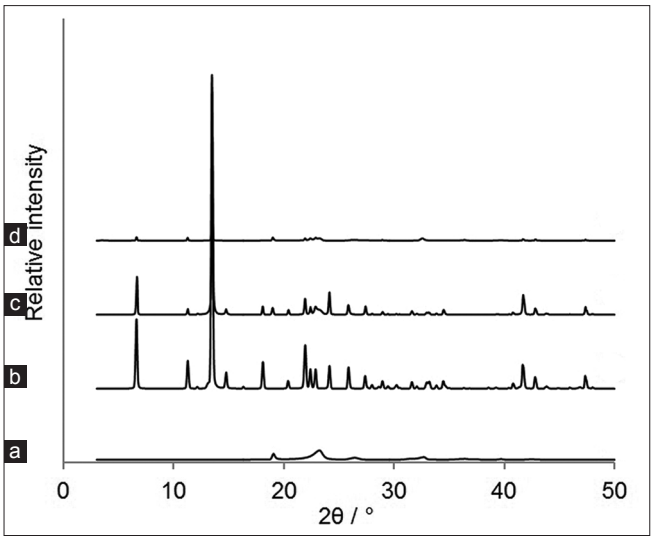

Figure 4: X-ray powder diffractograms of polyethylene glycol-1000 (a), phenacetin (b), and their mixtures 1:1 (c) and 6:1 (d)

CONCLUSION

The present study demonstrates a possibility of inclusion of superparamagnetic particles into solid dispersions of PEG with a molecular weight of 950–1050 and phenacetin using a combination of microscopy methods. A composite with relatively uniform distribution of superparamagnetic particles was produced which can find application in the production of capsule drugs, which would combine targeted delivery due to the presence of magnetic nanoparticles with improved solubility.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gu H, Xu K, Xu C, Xu B. Biofunctional magnetic nanoparticles for protein separation and pathogen detection. Chem Commun (Camb) 2006;9:941-9.
2. Weller D, Moser A. Thermal effect limits in ultrahigh density magnetic recording. IEEE Trans Magn 1999;35:4423-39.
3. Berry C, Curtis A. Functionalisation of magnetic nanoparticles for applications in biomedicine. J Phys D Appl Phys 2003;36:R198-206.
4. Lu AH, Salabas EL, Schuth F. Magnetic nanoparticles: Synthesis, protection, functionalization, and application. Angew Chem Int Ed 2007;46:1222-44.
5. Molday RS, MacKenzie D. Immunospecific ferromagnetic iron-dextran reagents for the labeling and magnetic separation of cells. J Immunol Methods 1982;52:353-67.
6. Pardoe H, Chua-Anusorn W, St. Pierre TG, Dobson J. Structural and magnetic properties of nanoscale iron oxide particles synthesized in the presence of dextran or polyvinyl alcohol. J Magn Magn Mater 2001;225:41-6.
7. Hong J, Gong P, Xu D, Dong L, Yao S. Stabilization of alpha-chymotrypsin by covalent immobilization on amine-functionalized superparamagnetic nanogel. J Biotechnol 2007;128:597-605.
8. Tan W, Wang K, He X, Zhao XJ, Drake T, Wang L, *et al.* Bionanotechnology based on silica nanoparticles. Med Res Rev 2004;24:621-38.
9. Koneracká M, Kopčanský P, Timko M, Ramchand CN. Direct binding procedure of proteins and enzymes to fine magnetic particles. J Magn Magn Mater 2002;252:409-11.
10. Lacava LM, Lacava ZG, Da Silva MF, Silva O, Chaves SB, Azevedo RB, *et al.* Magnetic resonance of a dextran-coated magnetic fluid intravenously administered in mice. Biophys J 2001;80:2483-6.
11. Tartaj P, Serna CJ. Synthesis of monodisperse superparamagnetic Fe/silica nanospherical composites. J Am Chem Soc 2003;125:15754-5.
12. Portet D, Denizot B, Rump E, Lejeune JJ, Jallet P. Nonpolymeric coatings of iron oxide colloids for biological use as magnetic resonance imaging contrast agents. J Colloid Interface Sci 2001;238:37-42.
13. Zhang Y, Kohler N, Zhang M. Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. Biomaterials 2002;23:1553-61.
14. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today 2007;12:1068-75.
15. Jenkins M. Biomedical Polymers. Cambridge: Woodhead Publishing Limited; 2007.
16. Vippagunta SR, Maul KA, Tallavajhala S, Grant DJ. Solid-state characterization of nifedipine solid dispersions. Int J Pharm 2002;236:111-23.
17. Chen Y, Zhang GG, Neilly J, Marsh K, Mawhinney D, Sanzgiri YD. Enhancing the bioavailability of ABT-963 using solid dispersion containing Pluronic F-68. Int J Pharm 2004;286:69-80.
18. Bley H, Fussnegger B, Bodmeier R. Characterization and stability of solid dispersions based on PEG/polymer blends. Int J Pharm 2010;390:165-73.
19. Sharma A, Jain CP. Preparation and characterization of solid dispersions of carvedilol with PVP K30. Res Pharm Sci 2010;5:49-56.
20. Gerasimov AV, Ziganshin MA, Gorbatchuk VV. A calorimetric study of the formation of phenacetin solid dispersions with PEG-1400 and Pluronic F127. World Appl Sci J 2013;24:920-7.
21. Gerasimov AV, Ziganshin MA, Gorbatchuk VV, Usmanova LS. Formation of solid dispersion of PEG-1000 with phenacetin according to differential scanning calorimetry. Pharm Chem 2013;5:149-55.
22. Gerasimov AV, Ziganshin MA, Gorbatchuk VV, Usmanova LS. Low molecular weight polyethylene glycols as matrix to obtain solid dispersions of sulfanilamide. Int J Pharm Pharm Sci 2014;6:372-7.
23. Gerasimov AV, Ziganshin MA, Gorbatchuk VV, Usmanova LS. Increasing the solubility of dipyridamole using polyethylene glycols. Int J Pharm Pharm Sci 2014;6:244-7.
24. Gerasimov AV, Varfolomeev MA, Ziganshin MA, Gorbatchuk VV, Rakipov IT, Klimovitskii AE, *et al.* Thermodynamics of dissolution and infrared-spectroscopy of solid dispersions of phenacetin. J Adv Pharm Technol Res 2016;7:6-12.

25. Ziganshin MA, Efimova IG, Gorbatchuk VV, Ziganshina SA, Chuklanov AP, Bukharaev AA, *et al.* Interaction of L-leucyl-L-leucyl-L-leucine thin film with water and organic vapors: Receptor properties and related morphology. J Pept Sci 2012;18:209-14.
26. Ziganshin MA, Bikmukhametova AA, Gerasimov AV, Gorbatchuk VV, Ziganshina SA, Bukharaev AA. The effect of substrate and air humidity on morphology of films of L-leucyl-L-leucine dipeptide. Prot Met Phys Chem Surf 2014;50:49-54.
27. Biziaev DA, Bukharaev AA, Borodin PA, Ovchinnikov DV. *In situ* magnetization reversal measurement of magnetic tips in a magnetic force microscope. Phys Low Dimens Struc 2004;1/2:153-8.
28. Ziganshin MA, Gerasimov AV, Ziganshina SA, Gubina NS, Abdullina GR, Klimovitskii AE, *et al.* Thermally induced diphenylalanine cyclization in solid phase. J Therm Anal Calorim 2016;125:905-12.
29. Galukhin AV, Khelkhal MA, Gerasimov AV, Biktagirov T, Gafurov MR, Rodionov A, *et al.* Mn-catalyzed oxydation of heavy oil in porous media: Kinetics and some aspects of mechanism. Energy Fuels 2016;30:7731-7.
30. Rasa M, Kuipers BW, Philipse AP. Atomic force microscopy and magnetic force microscopy study of model colloids. J Colloid Interface Sci 2002;250:303-15.
31. Shi HG, Farber L, Michaels JN, Dickey A, Thompson KC, Shelukar SD, *et al.* Characterization of crystalline drug nanoparticles using atomic force microscopy and complementary techniques. Pharm Res 2003;20:479-84.
32. Ziganshin MA, Gubina NS, Gerasimov AV, Gorbatchuk VV, Ziganshina SA, Chuklanov AP, *et al.* Interaction of L-alanyl-L-valine and L-valyl-L-alanine with organic vapors: Thermal stability of clathrates, sorption capacity and the change in the morphology of dipeptide films. Phys Chem Chem Phys 2015;17:20168-77.
33. Ziganshin MA, Ziganshina SA, Gubina NS, Gerasimov AV, Gorbatchuk VV, Bukharaev AA. Thermal stability, sorption properties and morphology of films of dipeptide and tripeptide based on L-glycine. Orient J Chem 2015;31:1977-84.
34. Alcantar NA, Aydil ES, Israelachvili JN. Polyethylene glycol-coated biocompatible surfaces. J Biomed Mater Res 2000;51:343-51.
35. Yang Z, Galloway JA, Yu H. Protein interactions with poly (ethylene glycol) self-assembled monolayers on glass substrates: Diffusion and adsorption. Langmuir 1999;15:8405-11.
36. Fule R, Dhamecha D, Maniruzzaman M, Khale A, Amin P. Development of hot melt co-formulated antimalarial solid dispersion system in fixed dose form (ARLUMELT): Evaluating amorphous state and *in vivo* performance. Int J Pharm 2015;496:137-56.
37. Bérard V, Lesniewska E, Andrès C, Pertuy D, Laroche C, Pourcelot Y. Affinity scale between a carrier and a drug in DPI studied by atomic force microscopy. Int J Pharm 2002;247:127-37.
38. Cordova G, Attwood S, Gaikwad R, Gu F, Leonenko Z. Magnetic force microscopy characterization of superparamagnetic iron oxide nanoparticles (SPIONs). Nano Biomed Eng 2014;6:31-9.