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

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J. Adv. Pharm. Technol. Res.

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, SmCo5, Fe14Nd2B.[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) 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

Access this article online

Quick Response Code:
Website: www.japtr.org
DOI: 10.4103/2231-4040.197331

How to cite this article: Usmanova LS, Ziganshin MA, Gorbachuk VV, Ziganshina SA, Bizyayev DA, Bukharaev AA, et al. A study of the formation of magnetically active solid dispersions of phenacetin using atomic and magnetic force microscopy. J Adv Pharm Technol Res 2017;8:2-7.

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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. 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 dipyriramidine. 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. However, melting temperature of PEG with average weight 1000 is close to the physiological temperature, 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) 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 P₂O₅ 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) 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. 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.

**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]. 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. 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. 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. The feasibility of the study of the amorphization of the drug surface using AFM was demonstrated for zanamivir.

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 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. 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)
Usmanova, et al.: Magnetically active solid dispersions

Journal of Advanced Pharmaceutical Technology & Research

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.

Acknowledgment
This work has been performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and supported by Scholarship of the President of the Russian Federation (SP-1423.2016.4). The work of Gorbatchuk V.V. was supported by grant №14. Y26.31.0019 from Ministry of Education and Science of Russian Federation.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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