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Computational and experimental approaches for development of methotrexate nanosuspensions by bottom-up nanoprecipitation

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ABSTRACT

Development of nanosuspensions offers a promising tool for formulations involving poorly water-soluble drugs. In this study, methotrexate (MTX) nanosuspensions were prepared using a bottom-up process based on acid-base neutralization reactions. Computational studies were performed to determine structural and electronic properties for isolated molecules and molecular clusters in order to evaluate the mechanism of MTX nanoparticle formation. Computational results indicated that the clusters in zwitterionic and cationic states presented larger dimensions and higher energies of interaction between MTX molecules, which favored aggregation. In contrast, the clusters in the anionic state exhibited lower energies of interaction, indicating aggregation was less likely to occur. Experimental results indicated that the higher the HCl proportion during drug precipitation, the greater the particle size, resulting in micrometric particles (2874–7308 nm) (cationic and zwitterionic forms). However, MTX nanoparticles ranging in size from 132 to 186 nm were formed using the lowest HCl proportion during drug precipitation (anionic form). In vitro release profiles indicated that the drug release rate from nanosuspension was increased (approximately 2.6 times) over that of the raw material. Overall, computational modeling and experimental analysis were complementary and assisted in the rational design of the nanosuspension based on acid-base reactions.

1. Introduction

Nanosuspensions are a relatively new trend in pharmaceutical technology because of the benefits of reducing the size of insoluble drug particles. Nano-ranged particles can lead to an increase in the dissolution rate and bioavailability of a drug, especially one with stability and solubility problems (Bose et al., 2012; George and Ghosh, 2013; Möschwitzer, 2013; Sinha et al., 2013). Additionally, particle size reduction can increase penetration through biological barriers and cell membranes, which can lead to an improved drug residence time in a specific tissue or organ (Alaei et al., 2016; Collnot et al., 2012; Rabinow, 2004). Additional advantages include dose and toxicity reduction, increased drug concentration in affected tissues or organs, and better adhesion of nanoparticles to mucosal surfaces (Das and Suresh, 2011; Jacobs and Müller, 2002; Tian et al., 2013). In addition, nanosuspensions can be administered by various routes, including oral (Xu et al., 2012), parenteral (Tian et al., 2013), nasal (Bhavna et al., 2014), ophthalmic (Pignatello et al., 2002), and pulmonary (Jacobs and Müller, 2002).

Nanosuspensions are submicron colloidal dispersions of pure drug particles, which are stabilized by surfactants, polymers, or a combination of both (Rabinow, 2004). A reduction in particle size leads to an increase in dissolution rate because of an increased surface area, according to the Noyes-Whitney equation (Noyes and Whitney, 1897), and an enhancement in saturation solubility of the drug, based on the Ostwald-Freundlich equation (Sinha et al., 2013; Xu et al., 2012).

Nanosuspensions can be prepared using top-down processes, which involve drug particle size reduction using various techniques, including media milling, microfluidization and high-pressure homogenization. The wet milling and high-pressure homogenization methods are widely exploited because they efficiently produce

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small particles and do not require organic solvents, making them more feasible for industry production. However, these techniques involve a high energy input, which generate heat, making it difficult to process thermolabile materials. In addition, the large amount of energy can produce amorphous particles and cause deformation of crystals (Chen et al., 2008; Rabinow, 2004; Sinha et al., 2013; Verma et al., 2009).

Another technique for making nanosuspensions is the bottom-up process, also known as the precipitation process, in which the particles are formed from a molecular state, based on the precipitation of particles from a supersaturated drug solution. The drug can be precipitated using methods such as solvent evaporation, supercritical fluid, antisolvent precipitation and chemical precipitation. For drugs with pH-dependent solubility, precipitation can be accomplished utilizing acid-base neutralization reactions. The bottom-up method requires a low energy input, making it useful for thermolabile materials, and is also simple and inexpensive (Chen et al., 2008; Mou et al., 2011; Verma et al., 2009).

Methotrexate (MTX) ((2S)-2-[[2,4-diaminopteridin-6-yl)methyl-methylamino]benzoyl]amino]pentanedioic acid) is a chemotherapeutic drug that, acts as a folic acid antagonist and interferes with the formation of DNA, RNA, and proteins. The aqueous solubility of MTX is pH-dependent ranging from 0.9 mM (pH 5) to 20 mM (pH 7). It has a log P of −1.85 (Chen et al., 2012; Hansch et al., 1995; Rubino, 2001).

It is widely used in the treatment of various diseases, including leukemia, osteosarcoma, non-Hodgkin's lymphoma, head and neck cancer, lung cancer, breast cancer, colorectal cancer, choriocarcinoma, as well as autoimmune diseases such as psoriasis and rheumatoid arthritis. However, MTX is poorly water-soluble and has a bioavailability limited by its poor solubility and slow dissolution rate. It also is associated with several side effects when administered at high doses (Chen et al., 2012; Pereira et al., 2014; Rubino, 2001).

The development of nanosuspensions represents a promising strategy for drugs such as MTX that have poor water-solubility. This study aimed to develop nanosuspensions using a bottom-up technique because MTX exhibits a pH-dependent solubility and particles of MTX can be formed using acid-base neutralization. The effect of variables, such as the different acid and base proportions employed in the MTX solubilization and precipitation, were evaluated regarding particle formation and size. The parameters investigated were size, polydispersity index (PDI), zeta potential, Fourier transform infrared spectroscopy (FTIR), drug content, and *in vitro* particle dissolution rate.

Computational modeling studies, especially quantum chemistry calculations, can be used in pharmaceutical nanotechnology together with experimental studies to advance the understanding of the formation, mechanism of action, interaction, mechanical properties, characterization, and stability of drug delivery systems (Bunker et al., 2016; De Souza et al., 2016; Ramezanpour et al., 2016). In this study, the interaction energies of the particles were evaluated with computational calculations (quantum chemistry calculations) using density functional theory (DFT) to obtain various properties, including the equilibrium geometry for each molecular system, Mulliken charges, orbital energies, and molecular orbitals. Computational modeling and experimental analysis were combined synergistically for the rational design of MTX nanosuspensions and to understand the experimental methods.

2. Materials and methods

2.1. Computational study

Quantum chemistry calculations were performed using DFT as implemented in the GAUSSIAN 09W package (Frisch et al., 2009) because of their sufficient accuracy and lower computational cost when compared to *ab initio* Hartree-Fock, as observed in other studies (Becke, 1988; Dennington et al., 2009; Frisch et al., 2009; Lewars, 2003; Rassolov et al., 2001). Generalized gradient approximation (GGA) was used with the B3LYP (Becke, 1988) exchange correlation functional, along with the 6-31G(d,p) basis set (Rassolov et al., 2001). This basis functions were chosen due of its additional polarization and diffuse functions that have advantage to describe better equilibrium geometry, ground state energies, charges, and electronic densities for second and third row atoms.

In this study, it was used DFT with an implicit water solvation PCM model (Tomasi et al., 2005). The PCM (Polarizable Continuum Model) calculates the free energy of solvation by attempting to sum over three different terms (Eq. (1)):

$$G_{solvatation} = G_{electrostatic} + G_{\text{dispersion}-\text{repulsion}} + G_{\text{cavitation}} \quad (1)$$

The cavity used in the PCM is generated by a series of overlapping spheres normally defined by the van der Waals radii of the individual atoms (Fig. 1). It is possible in Gaussian to customize the spherical radii.

The mathematical formalism for the integral equation formalism PCM (IEF-PCM) model (this is the model that was employed by Gaussian) is presented below. The complete Hamiltonian of the solute molecule can be written as (Eq. (2)):

$$H = H^0 + V_{MS} + V'(t) \quad (2)$$

Where H^0 is the Hamiltonian in vacuo, V_{MS} is the solute-solvent molecule interaction, and the V'(t) component is the time-dependent perturbation on the solute molecule. The V_{MS} component is further defined as (Eq. (3)):

$$V_{MS} = \int_{\Sigma} V(s)\left[\sigma^N(s) + \sigma^e(s)(\rho;s)\right]ds \quad (3)$$

Here, the surface charge density is broken into two parts for the nuclei ($\sigma^N(s)$) and the electrons ($\sigma^e(\rho;s)$) for the solute. The V(s) component is the electrostatic potential of the solute molecule calculated on the cavity surface, Σ. The last element to the

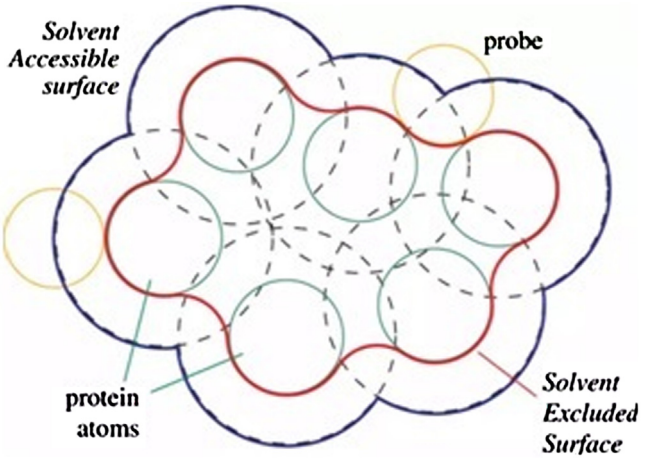

Fig. 1. Illustration of cavity of solute-solvent interaction. Solvent accessible surface (SAS) traced out by the center of the probe representing a solvent molecule (blue line). The solvent excluded surface (SES) is the topological boundary of the union of all possible probes that do not overlay with the molecule (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)