RESEARCH ARTICLE

SOLVENT EFFECT ON STABILIZATION OF METFORMIN MICRO PARTICLES

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ABSTRACT:
Synthesis of micron size Metformin was carried out by antisolvent synthesis with low power ultrasonic agitation method. The organic solvents were found to play an important role in solubility of the drug particles because the supersaturation could be varied by using different solvents and the physicochemical characteristics of the suspension are also altered, which affects stability. In this study, we present the effect of solvents on particle formation and stability of micron scale Metformin formation in aqueous media. All three solvents were found to be quite effective in the anti-solvent synthesis under ultrasonic agitation. The crystal morphology did not affect and change significantly with the solvent. Average diameters of particles in suspension were smaller when DMSO used as a solvent. DMSO showed the highest long term stability the average size was found to be 200±30nm.

Keywords: Metformin hydrochloride, antisolvent synthesis, nano particles

INTRODUCTION

It is widely known that a high percentage of newly pharmaceutical ingredients or active pharmaceutical ingredients (API) have poor solubility in water. This leads to lower dissolution rate and poor bioavailability which can affect the efficacy of the drugs. Usually, the dissolution is often the rate-limiting step and various approaches and parameters, such as the reduction in particle size and complexes with hydrophilic carriers and/or surfactants have been used to address this issue. Therefore, particle size is critical parameter in drug formulation, and its reduction is of great interest. Conventional methods like milling and halogenation for the synthesis of micron and nano particles employed in order to reduce the particle size. However, controlling of size, morphology, surface properties and electrostatic charge is often difficult. Various precipitation processes that form particles directly may provide more control of these parameters.

In recent years, bottom-up approached in the size reduction such as emulsification and antisolvent precipitation methods have come up as methods of choice for the synthesis of particles of nano, micro or sub micro size from the liquids. Antisolvent precipitation of hydrophobic drugs involves contact of an aqueous phase with an organic solution containing the drug. In this study, we employed methods like sonication assisted anti-solvent methods for the synthesis of submicron and nano size particles of insoluble drug entities. Here micro and nano scale drug particles are formed by precipitation from an organic solution when the latter is micro mixed in an aqueous phase under ultrasonic agitation. The three basic steps involved in such a process is supersaturation, nucleation, and particle growth. Supersaturation is the driving force for nucleation/growth and can be achieved either by increasing solute concentration or decreasing the solubility using methods such as solvent evaporation, dissolution of a metastable solid phase, cooling, alternate antisolvents, and, salting out. The key to this approach is the stabilization of the colloid by reducing the size of APIs. An unstable suspension could lead to large dose variations; therefore, long-term stability is very important in pharmaceutical industry. Hydrophilic polymers have been known to contribute to steric stability as well via a thickening action. Recently we have reported the use of combination of surfactants and celluloses as effective means of suspension stabilization. In the antisolvent method, supersaturation can be varied by using different solvents.

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Moreover, the physicochemical parameters of the suspension are also altered, which alters suspension stability. Therefore, the combination of the appropriate and optimum solvent along with ceased growth by polymers and surfactants is a powerful approach to control the formation of micron and submicron size particles in an aqueous phase. The objective of this study is to study the effect of solvent on suspension stability.

2. EXPERIMENTAL

2.1. Materials

Metformin (98% purity) (Fig.1) was purchased from Sun Pharmaceutical Ltd., INDIA. Hydroxypropyl methyl cellulose (molecular weight of 10,000, and viscosity of 5 cP) used 2 wt. % in H2O and sodium dodecyl sulphate or SDS was obtained from Venus Chemical, Delhi. DMSO (Dimethyl Sulfoxide), Acetone, and Ethanol were purchased from ALCHEM of analytical grade were used as received. The purified water used in all the experiments which was purified with a Millipore filter (Milli-Q Plus) system. The melting points of HPMC and SDS are reported to be 230 °C and 206 °C respectively. Whereas, the glass transition temperature of HPMC is found to be 151 °C.

2.2. Methods

In anisotropic precipitation technique, an API is dissolved in certain water miscible organic solvent is mixed with an aqueous solution containing a surfactant. After mixing, the supersaturated solution leads to nucleation and then it result growth of drug particles. All precipitation experiments were carried out at ambient temperature and ambient pressure. Firstly, Metformin was dissolved in three different solvents (i.e. Acetone, DMSO, ethanol) to form a clear and stable solution. The SDS and cellulose ethers were dissolved in Milli-Q water by constant stirring. The mixing ratio of drug, cellulose ether and SDS was kept 3:1:1. During sonication, the solution drug solution was added slowly dropwise into the aqueous phase. The final drug concentration in the suspension was 0.6 % w/w. The pH of the aqueous dispersions was kept approximately 7 by adding buffer..

Particle size and size distribution measurements of the bulk suspension were performed using static and dynamic light scattering. The light scattering which is static in nature gave an estimate of the particle size distribution of the stabilized suspension was carried out by employing particle size analyzer (Coulter Particle Size Analyzer LS230 ) (Fig.2). On the other hand, Beckman Coulter, N4 plus Submicron which is dynamic light scattering provided a selective picture of the relatively smaller particles that underwent significant Brownian motion. Dynamic light scattering was carried out using Beckman Coulter, N4 plus Submicron Particle Size Analyzer at 23° fixed detector angle. The micro particles nucleation and growth in the suspension was successfully monitored by measuring the change in size of particles as a function of time(minutes). Alongside, Zeta potential measurements were determined using a (Beckmann Coulter. The calculation of zeta potentials were obtained from the electrophoretic motilities of particles by applying the Smoluchowski equation which provide the motilities of particles. All measurements were performed at 25°C.

SEM (Scanning electron microscope LEO 1530VP) was employed for studying particle size and morphology (Fig. 2). The suspensions were kept in aluminum foil for overnight drying at room temperature. Then using the sputter coater (MED 020 HR) the samples were coated with carbon. In order to get the uniform representation of all the suspension of the particles, different zones of the suspensions were considered for sampling with SEM. Additionally, the raman spectra was measured by Nicolet Almege XR Dispersive Raman coupled with Olympus BX51 Confocal Microscope (Thermo Electron Corp.) using a neon laser beam at 532nm in imaging mode and recorded with a CCD camera. The samples were mounted on glass plate at 100x optical zoom.

Alongside, the sedimentation of the sample was monitored as a function of time by weighing the weight percentage of the solid settled in the suspension.

3. RESULTS AND DISCUSSION

The rate for homogeneous nucleation can be determined by the Gibbs, Volmer equation, where the variable parameters like viscosity, the degree of supersaturation; solubility, solid- liquid interfacial tension, and temperature play a role. Among all parameters, the parameters such as viscosity, supersaturation and solubility have strong dependence on the selection of the organic solvent. The solubility in equilibrium was measured experimentally (Table 2) and it was found to be significantly higher in DMSO as compared to acetone and ethanol. In addition to that DMSO also had the highest specific density, dielectric constant, viscosity, boiling point and pKa. (Table 1) For the same final Metformin concentration in DMSO offered the super saturation which is highest followed by acetone and DMSO. (Table 3)

![Figure 1: Metformin](image)

Particle Size Distribution

Light scattering of particles by Dynamic light scattering was employed to selectively monitor the growth of the smaller particles. It works by recording the velocity of the particles move under Brownian motion. Figure 4 shows the comparison of particle size as formed via anti solvent precipitation using the three solvents. It is interesting to observe that all the particle growth in all respective cases were significantly different. Over a period of 60 hours, the DCS measurements demonstrated a uniform particle size distribution observed with use of ethanol as solvent. In case of acetone, the particles increased in...
size and decreased quite dramatically. This decrease may be attributed to Oswald ripening. In case of DMSO, the particle’s growth was slow over a period of time. Based on the relatively higher degree of super saturation with ethanol, one may predict larger nucleation rate, agglomeration and higher fluctuation in particle size, however, that was not observed here. DMSO appeared to generate the smallest particles. It is evident that physico-chemical factors such as interfacial tension and viscosity may have played important roles here.

The overall distribution of particle size or PSD was monitored using laser diffraction. The principle of laser diffraction is based upon particle size analysis as particles passing through a laser beam will scatter light at an angle that is directly related to their size. Typical particle size distribution in stabilized suspension for three solvent systems is shown in Figure 4. It is evident that beyond 12 microns, the particles settled rapidly. Ethanol produced the largest average particle size distribution, and DMSO is the smallest. The higher degree of supersaturation in case of ethanol might lead to higher nucleation rate and subsequently higher agglomeration. The opposite can be described true for DMSO.

Figure 2: SEM image of Drug Particles: (a) Acetone + GF (b) DMSO + Metformin (c) Ethanol + Metformin

Long Term stability as a function of time
The long term stability of a suspension was measured as a function of variation in time. The stability was studied for up to 50 hrs. The mean diameter or particle size was measured every 2.5 hrs without sonication. When DMSO was used as a solvent, it produced relatively smaller and more stable particles whose size did not change significantly for up to 50 hours. On the other hand Acetone produced suspensions that were not as stable. Particle growth over time depends on the metastable zone of a particular solvent–solute system. The metastable zone/width is the region between the solubility and super solubility curve. Nucleation rate depends on the metastable zone width 11. When this width exceeded, the nucleation rate increased rapidly and crystallization took place at a faster rate leading to larger particle size. To achieve high stability, the nucleation rate/particle growth needs to be controllable within the metastable zone. Solvents play a vital role in achieving this control because the nucleation rate is varied from solvent to solvent. As shown in Figure 5, DMSO produced the smallest particle size and slower nucleation rate compared to ethanol-GF, and acetone-GF systems. That is also evident from Figure 1.

Zeta potential measurements were used to study the stability of the drug suspension. The zeta potential values were recorded every 24 h after the preparation of the drug suspensions, which ranged from − 15 mV and + 10 mV (Table 3). These numbers predict the stability of suspension was not highly stable from an electrostatic consideration, but rather prone to agglomeration. In case of acetone, the measurements suggest zeta potentials were more negative, which is consistent with previous observations 15.
Sedimentation Rate

Sedimentation rate is an important parameter in determining long term stability. Typically, the particles grow or aggregate to a large size, which enhances the overall rate of setting. Rate of settling can be described using Stokes equation\(^1\).

Table 1: Physiochemical Properties of Solvents

<table>
<thead>
<tr>
<th>Physiochemical properties</th>
<th>Acetone</th>
<th>DMSO</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (gm/ml)</td>
<td>0.8</td>
<td>1.09</td>
<td>0.8</td>
</tr>
<tr>
<td>Viscosity</td>
<td>0.3</td>
<td>1.996</td>
<td>1.2</td>
</tr>
<tr>
<td>Dielectric constant</td>
<td>20.7</td>
<td>47.2</td>
<td>24.3</td>
</tr>
<tr>
<td>B.P((^{0})C)</td>
<td>56</td>
<td>189</td>
<td>78</td>
</tr>
<tr>
<td>Pka</td>
<td>19.3</td>
<td>28</td>
<td>16</td>
</tr>
</tbody>
</table>

Here the rate of settling is found to be proportional to the square of the density, diameter, difference between the particle and the medium, and inversely proportional to medium viscosity. Solvent affects both viscosity and density of the medium and are important parameters. The density and viscosity of the three systems is presented in Table 2. DMSO had the highest density and viscosity.

Table 2: Experimented values for different GF/Solvent systems

<table>
<thead>
<tr>
<th>Parameters MF with different solvents</th>
<th>Metformin(MF)/ Acetone</th>
<th>MF/ DMSO</th>
<th>MF/Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cp)</td>
<td>1.07</td>
<td>1.41</td>
<td>0.93</td>
</tr>
<tr>
<td>Density (gm/ml)</td>
<td>0.99</td>
<td>1.41</td>
<td>0.91</td>
</tr>
<tr>
<td>Zeta potential (mv)</td>
<td>-11.16</td>
<td>-1.24</td>
<td>9.23</td>
</tr>
<tr>
<td>Solubility (% wt/v)</td>
<td>4.56</td>
<td>11.05</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Most of the solid particles were evenly distributed in the suspension after the precipitation form anti solvent method. The quantity of the particles in solid form suspended in the suspension decreased with time as they start to settle in suspension. These are presented in Figure 5. The results can be explained by the Stokes equation that describes that the particles with smaller diameter have tendency for lower settling rate. However, the rate at which particles settled varied depending upon the solvent used. After eight hours, nearly 50% of the particles were suspended in suspension regardless of the solvents used. Although, ethanol showed a rather unusual trend, where initially the particles were settled down but eventually they were reverting back to the suspension. The settling rate was the maximum for ethanol followed by DMSO and acetone. As explained by Stokes equation, in the suspensions the density differential and viscosity play a critical roles, based on which we can expect DMSO to have the lowest settling rate, and this was observed in this study\(^1\). Figure 6 show the plot of experimental settling rate vs. theoretical settling rate which was calculated by Stokes. The measured data set did not indicate any trend, implying that stability of
suspension stability relied on other parameters that could include dielectric constant and hydrophobicity or lipophilicity. Also, stokes law is strictly applicable for dilute suspensions with uniformity in particle size and spherical shapes having less inter and intra particle collisions and devoid any affinity with the parent dispersion medium. Some of these assumptions were not valid in this case. Figure 7 shows the settling rate as a function of time for all solvent systems. It was clear that the settling rate initially increased rapidly within the first two hours and later dropped to 1-2%. The rate of settling was the highest in ethanol.

Particle Morphology

Fig. 2 shows the comparison of SEM images of drug particles synthesized from these three solvents. It was found that the crystal shape was identical for all the three systems. There were no apparent agglomerations in any of the systems. The Raman spectra of the particles produced from the three solvents showed similar peaks, indicating that both the similar chemical nature of the particles which are stabilized particles and the their polymorphs were identical (Fig.3).

The Raman spectra showed the C=O stretching of benzofuran ring of metformin in the region 1590-1800cm⁻¹ and C-H stretching in the region of 2800-3200 cm⁻¹ were observed in both pure MF and it stabilized analog. It is clear from the Raman scattering data that the crystals contained the respective drugs, and there was no apparent interaction observed between the drug, cellulose ethers and SDS.

### 4. CONCLUSIONS

Three solvents were used for anti-solvent precipitation of hydrophobic drug molecules and found to be was significantly effective under ultrasonic agitation. At the end of 1 hours, average diameters of particles in suspension were smallest when DMSO was used the solvent, while ethanol produced the largest particles (Fig.4).
Long term stability was higher in DMSO compare to other two systems. Raman Spectroscopy confirmed the presence of the drug molecule in these crystals (Fig.5). The long term stability of the metformin and solvent system is plotted in figure 4. It can inferred from the figure that the metformin has high stability in acetone.

The organic solvents were found to play an important role in solubility of the drug particles because the supersaturation could be varied by using different solvents and the physicochemical characteristics of the suspension are also altered, which affects stability. All three solvents were found to be quite effective in the anti-solvent synthesis under ultrasonic agitation. The crystal morphology did not affected and change significantly with the solvent. Average diameters of particles in suspension were smaller when DMSO used a solvent. DMSO showed the highest long term stability the average size was found to be 200±30nm.

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