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Cover story

Prevention of nanoparticle aggregation during freeze-drying

Nanoparticle formulations have been widely used in the drug delivery field. The majority of published articles dealing with nanoparticle formulations have focused on the use of freshly prepared nanoparticles, but not many deal with scale-up production of nanoparticles and their long-term preservation by freeze-drying. To date, little information is available on an effective freeze-drying process. Lyophilization or freeze drying enables the preparation of nano formulations in the dry state, which permits their long-term storage and ease of transport. Removal of water improves the stability of materials susceptible to denaturation and prevents premature drug release from the formulations. Lyophilization of nanoparticles, however, can induce severe aggregation and increase the particle size due to aggregation [1,2]. This can result in reduced therapeutic efficacy and undesirable side effects such as an embolism. Furthermore, redispersion of lyophilized formulations can be difficult without the use of high energy methods such as sonication.

Use of a cryoprotectant (also called a lyoprotectant), such as sucrose and trehalose, can mitigate freeze drying-induced aggregation [3]. The precise mechanisms underlying the effects of lyophilization-induced stress on nanoparticulate formulations and protection afforded by cryoprotectants are not well understood. It has been postulated that cryoprotectants shield particles from one another ('particle isolation' hypothesis) and thereby prevent aggregation [4]. However, there has been no direct experimental evidence to support this hypothesis. Visualization of the lyophilization process and its effect on nanoparticles has been challenging.

The paper by Professor Panyam and Dr. Niu in this issue shows a clear, causal relationship between freezing and particle aggregation by employing real-time dynamic visualization and super-resolution imaging techniques for frozen systems [5]. Interestingly, particle aggregation appears to occur primarily during the freezing step. The extent of freezing-induced aggregation was dependent on the cooling rate; however, even with flash freezing under liquid nitrogen, an 18% increase in the hydrodynamic diameter of polymeric nanoparticles was observed after thawing. Slower cooling rates can result in 200-300% increase in particle size. Microscopy studies show that freezing results in the concentration of nanoparticles at ice-ice, ice-air and ice-container interfaces, generating freezing stress and aggregation. Thawing of these frozen dispersions generated fiber-like aggregates, which are reminiscent of fibers typically observed in nanoparticle formulations that have been lyophilized. In the presence of a cryoprotectant, concentration of nanoparticles at the interfaces still occurs; however, cryoprotectants provide a buffer zone for the particles, creating a relatively large inter-particle distance. This observation provides direct visual evidence to support the particle isolation hypothesis for cryoprotection of nanoparticles.

Further, based on the relationship between freeze-concentrated particles and void fraction within a confined interfacial space and using various sphere packing models, the Panyam team was able to define the boundary condition of the minimal 'cryoprotectant to particle ratio' required for effective design space of particle isolation and cryoprotection. Of the various space packing models evaluated, the random close packing model was successful in correctly predicting the minimal amount of the cryoprotective agent needed for complete protection against lyophilization-induced aggregation. Other models either predicted lower amounts of cryoprotectant that were not fully protective (Kepler dense packing model) or higher amounts than experimentally needed (caged sphere model).

The findings by the Panyam team clearly demonstrate the utility of visualization techniques and modeling in elucidating the mechanism of freezing stress and protection, providing guiding tools to the rational design of cryoprotectant-containing nano formulations and processes. These studies were performed using model polymeric nanoparticles made of poly(D,L-lactide-*co*-glycolide) or polystyrene. It would be interesting to determine whether the particle isolation model applies to other soft and inorganic nanoparticle systems. In addition, the ability of the cryoprotectant to preserve the native properties of nanoparticles in the presence of additional moieties on the surface (such as an antibody for targeting) would also be of interest.

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Kinam Park
Purdue University
Departments of Biomedical Engineering and Pharmaceutics
West Lafayette, IN 47907, USA
E-mail address: kpark@purdue.edu

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