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The Effect of Freeze-Drying Parameters on the Cure Characteristics of Freeze-Dried BSA-Loaded Silicone Elastomer

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ABSTRACT: To formulate therapeutic proteins into polymeric devices the protein is typically in the solid state, which can be achieved by the process of freeze-drying. However, freeze-drying not only risks denaturing the protein but it can adversely affect the cure characteristics of protein-loaded silicone elastomers. This study demonstrates that a variation in the parameters of the freeze-dryer can significantly affect the residual moisture content of freeze-dried BSA, which in turn has an effect on the bulk density and flow properties of the BSA. The bulk density and flow properties of the BSA subsequently affect the cure characteristics of BSA-loaded silicone elastomers. An increase in the residual moisture content results in the freeze-dried BSA having a decreased bulk density and poor flow properties which can have a detrimental effect on the cure characteristics of a freeze-dried BSA-loaded silicone elastomer. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: silicone elastomer; freeze-drying; oscillatory rheology; protein delivery; sustained-release

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INTRODUCTION

In recent years, the potential for using protein molecules as HIV microbicides has generated great interest. Protein-based HIV microbicides have the advantages of specificity, potency, and a propensity to block an early step in the protein replication cycle, the binding of the gp120 envelope protein to the CD4 receptor. Protein based HIV microbicides have a number of disadvantages such as high production costs and instabilities during formulation, transport, and storage. However, recent advance in genetic engineering along with the use of various animal and plant cells to produce high yields of proteins based HIV microbicides at reduced costs may overcome some of these issues.[1–3]

Silicones are inert polymeric compounds that are hydrophobic, biocompatible, nonbiodegradable, and nontoxic.[4] They have been used in cosmetics and medicines for a number of years, for example as carriers for deodorants, transdermal patches, and breast implants.[5] Several silicone-based Controlled Release Dosage Forms (CDRFs) have been developed, which have the potential to be used to deliver therapeutic proteins, as they are generally prepared under mild conditions which do not tend to denature proteins (e.g., low temperature and absence of organic solvent). Furthermore, the protein is usually incorporated into the silicone elastomer delivery system in a solid form thereby, potentially increasing protein stability.

The development of a protein delivery device using silicone elastomer has been reported.[6] A matrix silicone rod was manufactured by mixing a model protein (interferon) and a release enhancing excipient (glycine) into a silicone elastomer mix then extruding it through a die and allowing it to cure. The study demonstrated that active protein could be released from silicone for up to 100 days. The inclusion of release enhancing excipients modified the release rate significantly.[6,7]

A further study by the same group of researchers demonstrated the potential of "covered-rod devices," wherein by coextruding a nonmedicated silicone sheath around the matrix rod a covered rod was created, which had a zero-order release rate.[8] By changing the various parameters, such as protein loading, excipient loading, and cross-sectional diameter, the release rate could be modified. By increasing the length of the covered rod, the duration of release could also be increased.[8] Maeda et al. demonstrated that the protein was only released from the uncovered ends of the covered rod device and that the protein release rate was dependent on the diffusion of the protein through the silicone elastomer and the solubility of the protein in the release media.[9]

Matrix and covered rods where used for the continuous delivery of an antigen into sheep demonstrating that a protein could be continuously delivered *in vivo* for up to 1 month using a matrix rod and several months using a covered rod.[10] A similar covered

Table I. Freeze-Drying Parameters Used for Each Freeze-Drying Method

	Method code Parameters	FD1	FD2	FD3	FD4	FD5	FD6	FD7
Freezing stage	Freezing temperature (°C)	−50	−40	−30	−50	−50	−50	−50
	Rate of cooling (°C/min)	0.47	0.43	0.39	0.47	0.47	0.47	0.47
	Length of time freeze dryer held at freezing temperature (min)	60	60	60	60	60	60	60
Drying stage	Drying temperature (°C)	30	30	30	30	30	20	10
	Rate of drying (°C/min)	0.44	0.44	0.44	0.67	0.89	0.44	0.44
	Length of time freeze dryer held at drying temperature (°C)	360	360	360	360	360	360	360

rod device was capable of providing controlled release of water-soluble low molecular weight drugs into rats after implantation in the brain.[11]

Previous publications[12–14] have demonstrated that a rod insert vaginal ring (RIVR) has the potential to deliver protein-based HIV microbicides to the vagina in a sustained fashion.

For a protein to be successfully formulated into a silicone elastomer CRDF, it must first be in the solid state. Freeze-drying can be used to increase the stability of therapeutic proteins as well as for producing solid proteins.[15,16] The freeze-drying process consists of three distinct stages: (1) the freezing stage, were the aqueous buffer is frozen generally at temperatures below 0°C, (2) the primary drying stage, were the frozen material is slowly heated under vacuum to remove the frozen bulk free water via sublimation, and (3) the secondary drying stage, were the nonfrozen bound water is removed under vacuum, at temperatures above zero. However, it has been demonstrated that freeze-dried proteins, loaded into silicone elastomer systems, can adversely affect the elastomers cure characteristics, resulting in high tan (δ) values for the precured elastomers and increased cure time,[12] which can be detrimental to protein stability during manufacturing.

In this study we evaluate the effects of various freeze drying parameters (freezing temperature, drying rate, and drying temperature) on the residual water content, bulk density and flow properties of freeze dried BSA, as well as the cure characteristics of the freeze dried BSA-loaded silicone elastomer LSR90-9508-30.

EXPERIMENTAL SECTION

Materials

The medical-grade, low-consistency poly(dimethylsiloxane) elastomer system LSR90-9508-30 from NuSil Technology (Carpinteria, CA) was purchased from Polymer Systems Technology Ltd (High Wycombe, UK). Bovine serum albumin (BSA) fraction V lyophilized with a particle size of 110 μm ($\pm$3.45) was supplied by Kraeber GMBH (Schleswig-Holstein, Germany).

Preparation of BSA Solutions

BSA solutions where prepared by dissolving 5 g of BSA into 100 mL of HPLC grade water.

Freeze-Drying of the BSA Solutions

The BSA solutions were freeze-dried using the laboratory freeze-dryer VirTis adVantage. Table I summarizes the freeze-drying protocols that were used in this study. All freeze-drying studies had a starting shelf temperature of 20°C to ensure freezing rates where controlled.

Karl Fisher Titration

The freeze dried BSA was analyzed for its residual moisture content using a Karl Fischer titrator (701 KF Titrino, Metrohm Ion Analysis, Herisau, Switzerland). Totally, 5 mg of BSA was added to the titration vessel containing dried methanol, stirred for 3 min and titrated with Hydranal Composite 5 reagent.

Determination of the Bulk Density and Flow Properties of Freeze-Dried BSA

A known weight of freeze-dried BSA was placed into the measuring cylinder of a Pharma test PT-TD1 tapped density analyzer. The bulk density was calculated by dividing the weight (g) by the volume (mL). The cylinder was then tapped 50 times and the tapped density calculated by dividing the weight (g) by the tapped volume (mL). The Carr's index and Hausner ratio [calculated using eqs. (1) and (2), respectively] were used to determine the flow properties of the freeze-dried BSA.

$$\text{Carr's index} = 100 \times (1 - B/T) \quad (1)$$

$$\text{Hausner ratio} = T/B \quad (2)$$

Where B is the bulk density of the active mix and T is the tapped density of the active mix.

Determination of the Particle Size of Freeze-Dried BSA

Totally, 10 mg of each freeze-dried BSA powder was suspended in glycerol (Sigma Aldrich, Dorset, England). The suspensions were mixed in a Speed Mixer DAC 15FVZ-K (Synergy Devices) for 1 min at 3500 RPM to remove any air bubbles. BSA particle size was determined using a HELOS light diffracting particle size analyzer (Sympatec, Clausthal-Zellerfeld, Germany).

Rheological Evaluation of a 10% w/w Freeze-Dried BSA Silicone Elastomer Mix

Evaluation of the effect of varying the freeze-drying method on the initial tan (δ), curing rate and the final log10 G′ of a 10% w/w Freeze-dried BSA-loaded silicone elastomer mix was performed using oscillatory rheology. The percent w/w loading of BSA was kept the same, so that any changes in initial tan (δ), cure rate or final log10 G′ would be due to the freeze-drying method. A w/w loading of 10% was chosen so there would be sufficient BSA from each freeze-drying method to accommodate

Table II. The Effect of Various Freeze-Drying Methods on the Residual Moisture Content of Freeze-Dried BSA as well as the Initial Tan (^{TM}), Cure Rate, and Final log10 G′ Value of Silicone Elastomer Loaded with 10% w/w Freeze-Dried BSA

Freeze-drying method	Residual moisture (%) (Std Dev)	Initial tan(δ) (Std Dev)	Cure rate[a] (Std Dev)	Final log10 G′ (Std Dev)
FD1	7.0 (+0.2)	2.6 (+0.1)	1.1 (+0.1)	5.7 (+0.2)
FD2	7.1 (+0.3)	2.5 (+0.2)	1.1 (+0.0)	5.6 (+0.2)
FD3	7.1 (+0.4)	2.4 (+0.2)	1.1 (+0.0)	5.6 (+0.2)
FD4	4.1 (+0.3)	3.2 (+0.2)	1.0 (+0.0)	4.8 (+0.2)
FD5	1.1 (+0.1)	4.8 (± 0.2)	0.7 (± 0.1)	4.2 (± 0.3)
FD6	8.5 (+0.5)	1.9 (+0.1)	1.3 (+0.1)	6.2 (+0.1)
FD7	10.4 (+0.7)	0.7 (+0.1)	1.4 (+0.1)	6.9 (+0.1)

[a]Equal to the initial gradient of the log10 G′ versus time rheograph.

all the rheological testing. A 3 g 10% w/w freeze-dried BSA silicone formulation was prepared by adding Part A and Part B of the medical-grade, low-consistency, platinum catalyzed, poly(dimethylsiloxane) elastomer system LR90-9508-30 in a 1 : 1 ratio and the appropriate amount of freeze-dried BSA to a sealed plastic container and mixing for 30 s at 3500 rpm using a Speed Mixer DAC 15FVZ-K (Synergy Devices Ltd.) The formulation was immediately placed onto the lower plate of a TA Instruments AR2000 Rheometer using a disposable plastic syringe. The upper 40 mm diameter crosshatch parallel plate was lowered to produce a gap between the plates of 1000 μm and the excess silicone mix removed before the oscillation experiment was begun. The linear viscoelastic region (LVR) was determined for the silicone by performing a stress sweep on part A of the elastomer. The stress of 8.5 Pa for subsequent cure analysis experiments was selected from the midpoint of the horizontal portion of a stress sweep. Each rheological evaluation was performed in triplicate at a frequency of 1 Hz.

Statistical Analysis

The effect of freeze drying parameters on the residual moisture content, bulk density and flow properties of freeze dried BSA, as well as the cure characteristics of the freeze dried BSA-loaded silicone elastomer LSR90-9508-30 were statistically compared

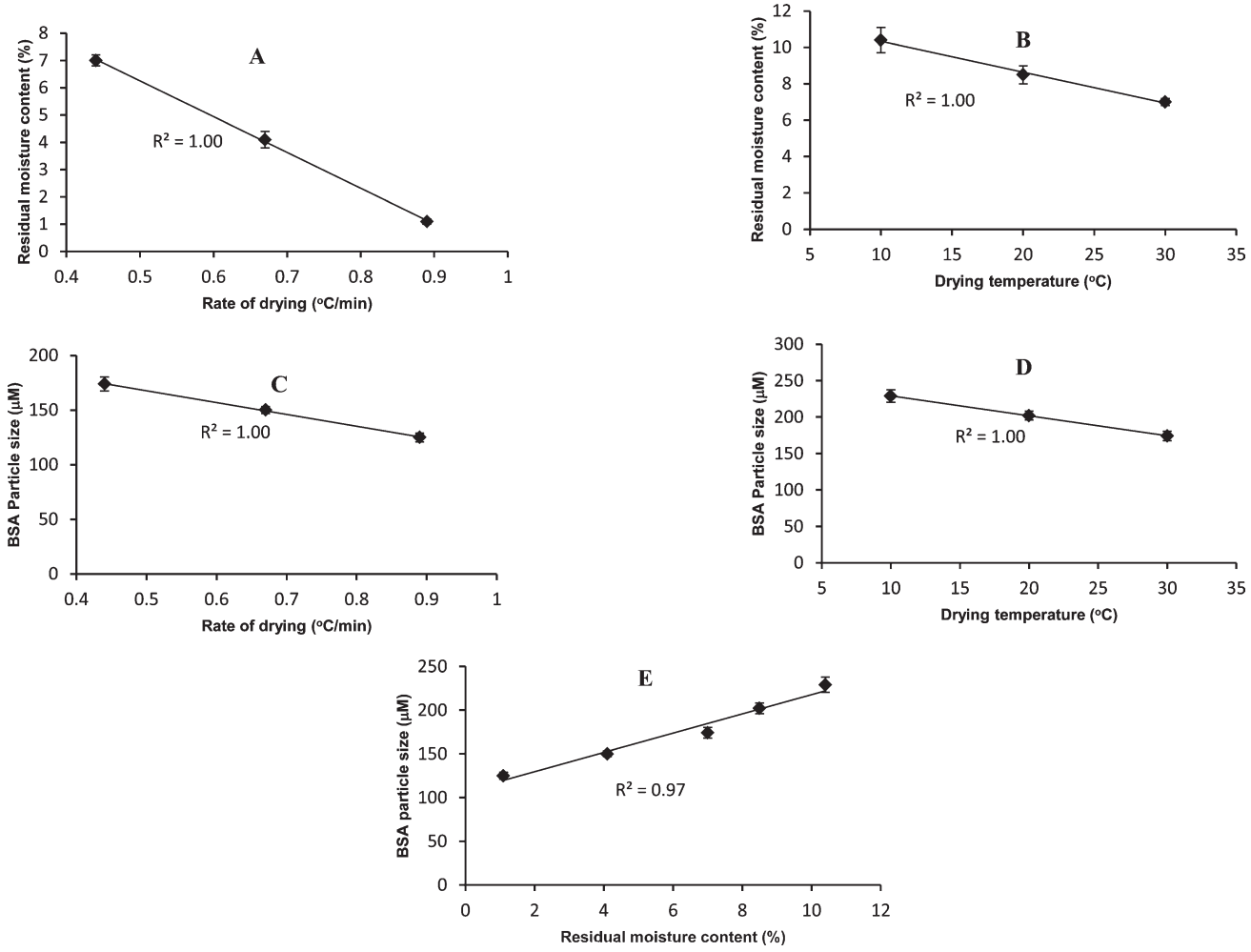

Figure 1. The effect of rate of drying and drying temperature on the residual moisture content (A and B) and particle size (C and D) of freeze-dried BSA and the effect of residual moisture content on the particle size of BSA (E).

Table III. The Effect of Various Freeze-Drying Methods on the Bulk Density Carr's Index and Hausner Ratio of Freeze-Dried BSA

Freeze-drying method	Bulk density (Std dev)	Carr's index (Std dev)	Hausner ratio (Std dev)	Particle size (Std dev)
FD1	0.28 (±0.02)	33.33 (±0.7)	1.50 (±0.04)	174 (±6.32)
FD2	0.26 (±0.03)	32.90 (±0.4)	1.53 (±0.06)	178 (±7.52)
FD3	0.27 (±0.02)	33.17 (±0.3)	1.50 (±0.05)	173 (±6.08)
FD4	0.33 (±0.01)	26.67 (±0.3)	1.36 (±0.01)	150 (±3.17)
FD5	0.38 (±0.01)	23.08 (±0.5)	1.30 (±0.01)	125 (±3.91)
FD6	0.21 (±0.02)	37.50 (±0.9)	1.60 (±0.03)	202 (±6.27)
FD7	0.17 (±0.01)	44.83 (±1.0)	1.81 (±0.07)	229 (±8.64)

using a one-way ANOVA (Statview, Abacus Concepts, CA). Post-hoc comparisons of the means were performed using Tukey's Honestly Significance Difference test. A significance level of $P < 0.05$ was accepted to denote significance in all cases.

RESULTS AND DISCUSSION

Effect of Freeze-Drying Parameters on the Residual Moisture and Particle Size of Freeze-Dried BSA

Karl fisher analysis was used to assess the effect of the freeze-drying method on the residual moisture content of the freeze-dried BSA (Table II). The data demonstrates that the freezing stage parameters have no significant effect on the residual moisture content or particle size of the freeze dried BSA as freeze drying methods FD1, FD2, and FD3 had similar values for the residual moisture content ($P = 0.13$ and 0.26). Only the freezing parameters of these methods where varied, the drying parameters remained the same. The freeze dried BSA prepared using methods FD4 and FD5 had significantly lower residual moisture contents and particle size compared with method FD1, with method FD5 having an even lower residual moisture

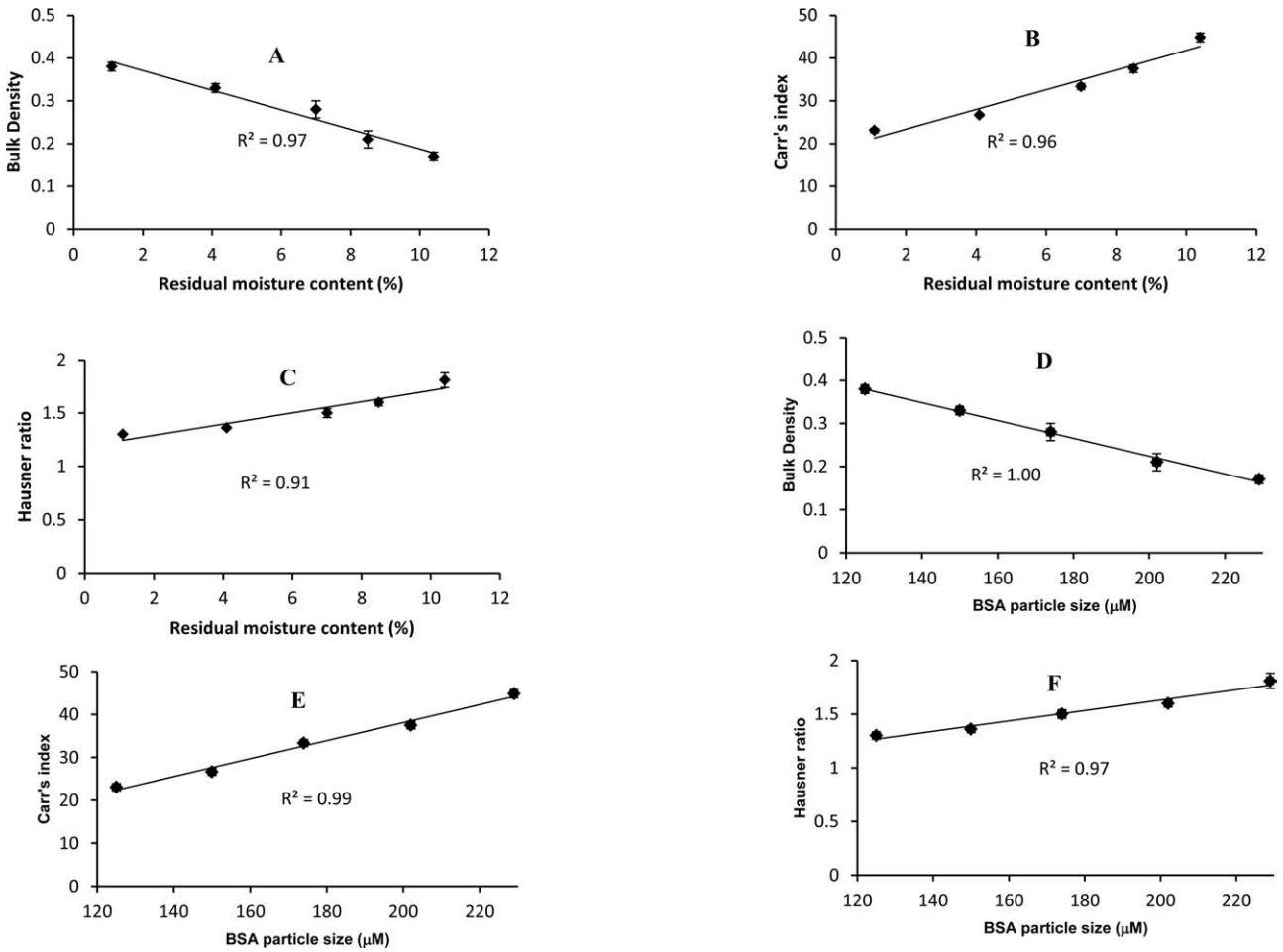

Figure 2. The effect of residual moisture content and particle size on the bulk density (A and D), Carr's index (B and E), and the Hausner ratio (C and F) of freeze-dried BSA.

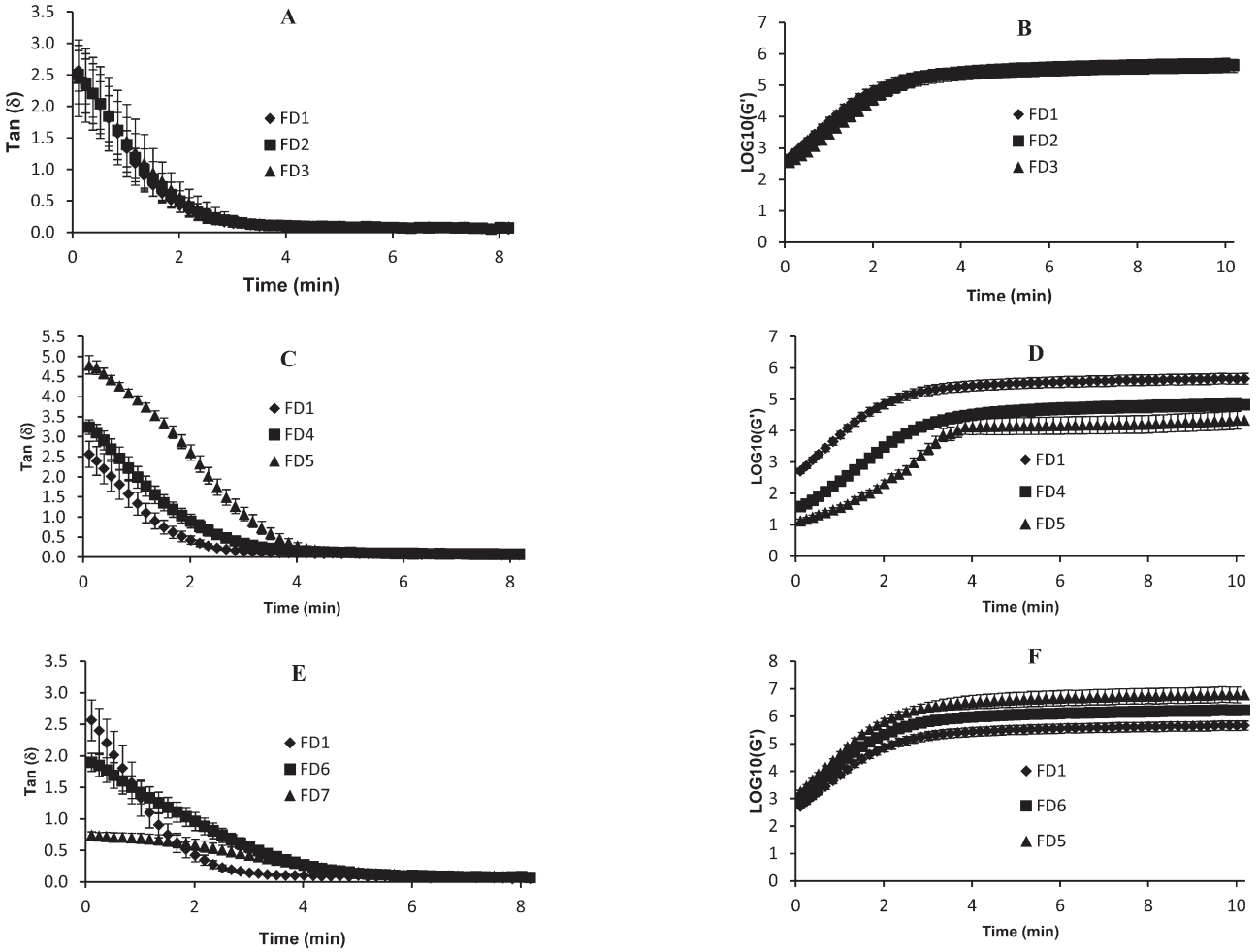

Figure 3. The effect of the freezing temperature, drying rate, and drying temperature on the log10 G′ versus time profile (A,C, and E) and the tan (δ) versus time profile (B,D, and F) of 10% freeze-dried BSA-loaded silicone elatomer.

content and particle size than FD4 ($P = 0.006$ and 0.002). This demonstrates that the rate of drying significantly effects the residual moisture content and particle size as these methods only differed in their rate of drying with FD1 having the lowest rate of drying and FD5 the highest. Figure 1(A) demonstrates that residual moisture content has a linear relationship with the rate of drying (r^2 1.00), with residual moisture content decreasing as the rate of drying is increased. Table II also demonstrates that the drying temperature has a significant effect on residual moisture content as methods FD5 and FD6 have significantly higher residual moisture contents than method FD1, with FD6 having a high residual moisture content than FD5 ($P = 0.002$). These methods only differ in their drying temperature with method FD1 having the highest drying temperature and FD6 the lowest. Residual moisture content also has a linear relationship with drying temperature [Figure 1(B), r^2 1.00], as drying temperature is increased the residual moisture content decreases.

There is also a linear relationship between BSA particle size and both drying rate and drying temperature [Figure 1(C, D)], with the BSA particle size also decreasing as the drying rate and drying temperature is increased. This would suggest that as the residual moisture content of the freeze-dried BSA increases so does its particle size, as demonstrated by Figure 1(E) and Table II.

Effect of Freeze-Drying Parameters on the Bulk Density and Flow Properties of Freeze-Dried BSA

Bulk and tapped density was used to determine the effect of freeze-drying parameters on the bulk density and flow properties of the freeze-dried BSA (Table III). A variation in the freezing temperature (methods FD1, FD2, and FD3) has no significant effect on the bulk density ($P = 0.09$) or the flow properties of the freeze-dried BSA. However, increasing the drying rate (methods FD1, FD4, and FD5) results in an increase in the bulk density ($P = 0.01$) while improving the flowability of the freeze-dried BSA powder, which is represented by a decrease in both the Carr's and Hausner ratio. A similar trend is observed for an increase in the drying temperature (methods FD1, FD6, and FD7) (Table III). These observations are a result of the lower residual moisture content and smaller particle size which is associated with both an increase in drying rate and drying temperature. This is demonstrated by the linear relationship when both residual moisture content and particle size are

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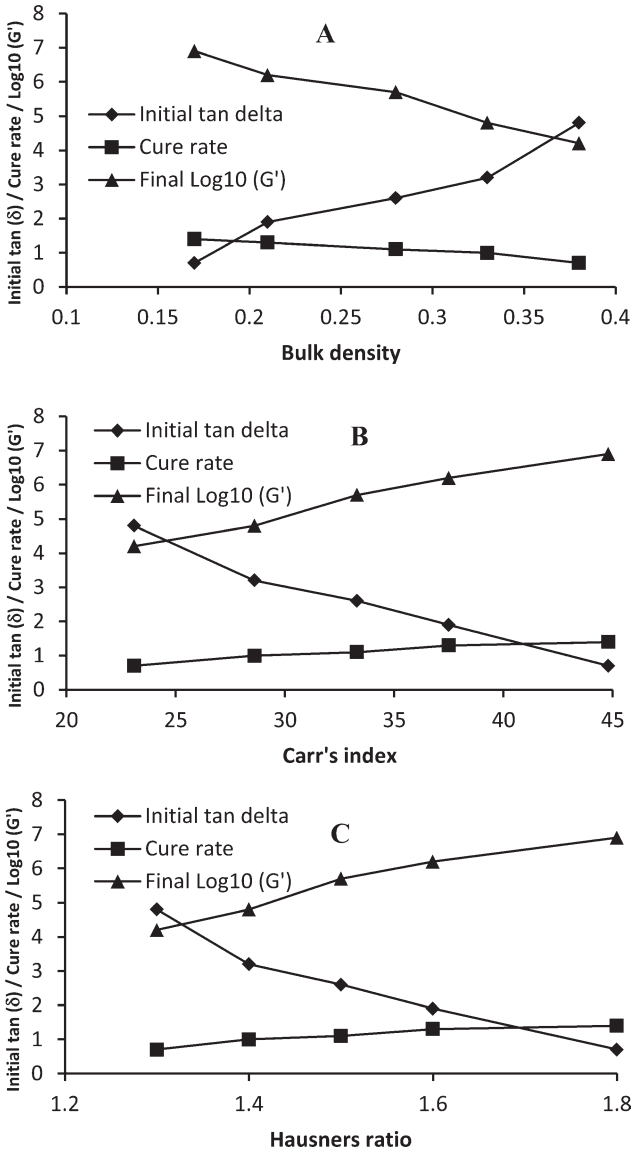

Figure 4. The effect of bulk density (A), Carr's index (B) and Hausner ratio (C) on the initial tan (δ), cure rate and final log10 G′ of 10% freeze-dried BSA-loaded silicone elatomer.

plotted against bulk density ($r^2 = 0.97$ and 1.00), Carr's ratio ($r^2 = 0.96$ and 0.99) and Hausner ratio ($r^2 = 0.91$ and 0.97) [Figure 2(A–F)].

Effect of Freeze-Drying Parameters on the Cure Characteristics of the Silicone Elastomer LSR90-9508-30 Loaded with 10% w/w Freeze-Dried BSA

To investigate the effects of freeze-drying parameters on the cure characteristics of a 10% w/w loading of freeze-dried BSA in the silicone elastomer LSR90-9508-30 oscillatory rheology was used. Table I contains the parameters for the freeze-drying methods used in this study. The rheological parameters evaluated where Initial tan (δ), cure rate and the final 1og10 G'.

G' is known as the storage modulus and is representative of the elastic character of the silicone elastomer. Tan (δ) is equivalent to the loss modulus (G''), which is representative of the liquid nature of the silicone elastomer, divided by the storage modulus (G''/G'). Therefore, a higher initial tan (δ) would suggest that the silicone elastomer formulation is predominantly liquid in nature during the early stages of cure. This parameter is important for the manufacture of silicone elastomer drug delivery devices, which are usually manufactured by either injection molding or extrusion. If the silicone elastomer formulation has a low tan (δ) and therefore increased elastic characteristics, then the force required to either injection mould or extrude the formulation will be greatly increased, which will in turn increase the stress placed on the protein within the elastomer, possibly causing it to lose some of its activity. Alternatively, if the initial tan (δ) is too high, the silicone elastomer formulation may to be too liquid in nature during the early stages of cure causing the formulation to leak out of the injection molder or extruder. During the cure process, the tan (δ) values decrease while the log10 G' values increase as the silicone elatomer formulation moves from predominantly liquid to elastic. However if the final log G' value is too high then the silicone elatomer formulation can become quite brittle, resulting in it cracking or breaking during use. Cure rate was investigated due to both injection molding and extrusion being high temperature processes. If the formulation takes too long to cure then the protein is exposed to high temperatures for longer periods of time which is not conducive to protein stability.

Varying the different freeze-drying parameters can have varying effects on the cure characteristics of a silicone elastomer loaded with 10% w/w freeze-dried BSA (Table II). For example, the freezing temperature (methods FD1, FD2, and FD3) has no significant effect on the initial tan (δ) ($P = 0.11$), cure rate ($P = 0.17$) or the final G' value ($P = 0.14$) of a 10% w/w freeze-dried BSA-loaded silicone elastomer. The tan (δ) and log10 G' versus time profiles for freeze drying methods FD1, FD2, and FD3 are superimposed on top of each other [Figure 3(A,B)]. However, increasing the drying rate (methods FD1, FD4, and FD5) results in a significant increase in the initial tan (δ) value ($P < 0.01$) (decrease in initial viscosity) while decreasing both the cure rate ($P = 0.02$) and the final G' value ($P = 0.01$). Figures 3(C) and 4(D) demonstrate that an increase in the drying rate results in an increase in the tan (δ) versus time profile and a decrease in the log10 G' versus time profile during the early stages of curing, which translates to a lower viscosity for the BSA-loaded silicone elastomer system. Similar trends are observed for the initial tan (δ), cure rate, final log10 G' value, tan (δ) and log10 G' versus time profiles when the drying temperature is increased (methods FD1, FD6, and FD7) [Table II, Figures 3(E) and 3(F)]. The varying effects of the freeze-dried BSA on the cure characteristics of the silicone elastomer appear to be related to the BSA's residual moisture content and particle size after freeze-drying and the effect they have on the bulk density and the flow properties of the freeze-dried BSA. Figure 4(A) demonstrates that as the bulk density increases the final log10 G' and the cure rate decrease while the initial tan (δ) increases. Figure 4(B,C) show that a decrease in the flowability of the freeze-dried BSA, which is represented by an increase in the Carr's index

and the Hausner ratio, results in an increase in the final log10 G' and the cure rate, while the initial tan (δ) value is decreased. Therefore, the above rheological trends are as a result of the physical properties of the freeze-dried BSA powder, rather than a chemical effect of the BSA powder on the silicone elatomer cure chemistry. The increased cure rate associated with increased residual moisture content and particle size of the freeze-dried BSA (Table II) is actually caused by a decrease in the bulk density and flowability of the freeze-dried BSA (a result of an increase in the residual moisture content and particle size) resulting in higher log10 G' values during the early stages of cure, which suggests an increase in the silicone elatomer cure rate.

CONCLUSIONS

Sustained-release strategies for protein-based therapeutics from polymeric devices are of considerable interest in a wide range of clinical applications. However, in order to formulate these proteins into polymeric devices they are usually in the solid state, which can be achieved by the process of freeze-drying. Freeze-drying cannot only result in the denaturization of the protein it can produce a protein powder which has such poor flow properties that it has a detrimental effect on the cure characteristics of silicone elastomers, thus limiting the amount of protein that can be loaded into such polymeric drug delivery devices. This study demonstrates that by varying the parameters of the freeze-dryer it is possible to produce a freeze-dried BSA powder with flow properties that allow it to be formulated into a silicone elastomer without having any adverse effects on its cure characteristics.

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