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Low molecular weight silicones particularly facilitate human serum albumin denaturation

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ABSTRACT

There is a market trend towards the administration of therapeutic proteins using sterilized, pre-filled glass syringes lubricated with silicone oil. It has been widely reported that initially clear solutions of proteins can become turbid during transport and storage, with unclear outcomes with respect to bioefficacy. While the basic processes of interactions of proteins with hydrophobic entities, leading to denaturation and aggregation, are increasingly well understood, the apparently random occurrence of such processes in syringes is not. To better understand the parameters that may be responsible for this change, we report the systematic examination of a series of factors that can affect the behavior of the protein human serum albumin (HSA) when in contact with silicone oil in water. Fluorescence spectroscopy showed that greater mixing times and greater concentrations of silicones (polydimethylsiloxane (PDMS)), especially lower molecular weight hydrophobic silicones like octamethyltetracyclosiloxane (D_4), were associated with increased protein denaturation. The turbidity of HSA solutions, due to the formation both of silicone oil-in-water (O/W) emulsions and protein aggregates, was also facilitated by the presence of D_4. A series of mixtures of silicone oils, all of which exhibited a viscosity of 1000 cSt but which were comprised of different silicone constituents, clearly showed a correlation between the presence of lower molecular silicones and enhanced solution turbidity. While the addition of a non-ionic silicone-polyether surfactant led to greater turbidity by increasing the number of stabilized oil droplets, it was not accompanied by protein denaturation. These results are consistent with HSA denaturation and subsequent aggregation as a consequence of contact particularly with low molecular weight, hydrophobic silicones that are more mobile, leading to more efficient protein/silicone contact.

1. Introduction

The use of protein therapeutics has exploded in the last 30 years, with 165 new protein therapeutics introduced and approved by regulatory agencies [1]. Simultaneously, significant progress has been made in the development of tools for protein manufacture, isolation, purification, storage, transport and delivery to cells in active form [2]. However, harsh environments, including those used in protein production, like high concentrations, exposure to various organic solvents, or acidic or basic environments, can cause proteins to denature, typically via unfolding from the precise three-dimensional structures necessary to perform their biological function, and aggregate [3,4]. In addition to these triggers, protein aggregation can be caused by hydrophobic moieties, particularly dimethylsilicone polymers (PDMS-OTMS, Fig. 1) [5,6]: silicone oils are used, almost universally, as a lubricant in glass syringes that deliver therapeutic proteins [7–10].

The aggregation of proteins, as a consequence of the hydrophobic character of silicones, has been observed in silicone lubricated or treated glass and plastic syringes, cartridges, packaging and tubing that deliver or store protein therapeutics [2,8–10]. Observations of cloudiness have been reported after syringe filling, shipment or several months of the storage of therapeutic proteins [10]. Reports of aggregation of different proteins like the therapeutic protein Orencia [8], monoclonal antibodies, fibrinogen, fibronectin, α-chymotrypsin, alkaline phosphatase and glucose oxidase after contact with silicone have been discussed in Refs. [6,10–13]. The addition in commercial formulations of surfactants such as Polysorbate 20 [11] reduces, but does not completely resolve this problem.

A variety of researchers have made seminal contributions to our understanding of the mechanisms through which silicone polymers lead to protein denaturation and aggregation. With unmodified

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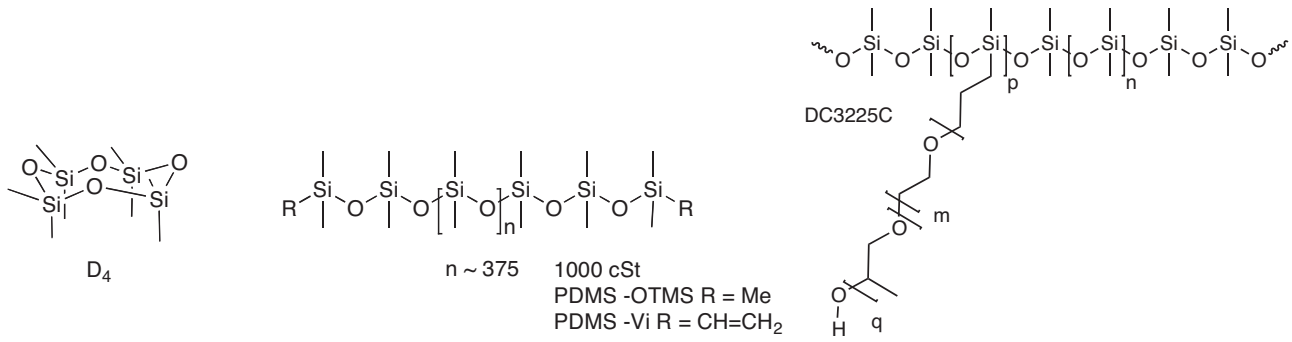

Fig. 1. Chemical structures of D_4, 1000 cSt PDMS, and the surfactant DC3225C.

silicone oils, the materials commonly used to lubricate syringes, adsorption of proteins on dispersed silicone oil droplets is understood to be a first step in the denaturation process [14], that can ultimately lead to aggregation [15]. Aggregation/denaturation processes are affected, both positively and negatively, by other excipients. The presence of surfactants, for example, leads to a reduction in the degree of protein adsorption to silicone oil droplets. Salt, by contrast, was affiliated with decreased emulsion stability and increased protein adsorption as shown with lysozyme dispersions [16,17].

In spite of our increased understanding of the phenomena leading to protein denaturation and aggregation in the presence of silicone oil (lubricants), only some protein formulations (i.e., only certain manufactured lots) undergo denaturation/aggregation. There are thus factors, not currently understood, that facilitate the destabilization of proteins in some solutions in contact with hydrophobic entities. To better understand the role that silicone structure, particularly molecular weight, may have on protein denaturation we have undertaken a study of human serum albumin (HSA) denaturation and aggregation as a function of agitation intensity, silicone type (cyclic vs. linear, hydrophobic vs. surface active), protein concentration and, in particular, the molecular weights and molecular weight distributions of the silicone oils. HSA is a particularly convenient protein to study, as we and others have shown, because of the changes in fluorescence maxima that accompany denaturation [13,18–21].

2. Experimental

2.1. Materials

Human serum albumin (HSA) (product # A1653) was purchased from Sigma–Aldrich. Tris(hydroxymethyl)aminomethane (TRIS, 99.9% purity) (Aldrich), disodium ethylenediaminetetraacetate (EDTA) and sodium chloride (Caledon Laboratories) were used as received. Octamethylcyclotetrasiloxane (D_4, Aldrich), vinyldimethylsiloxy-terminated polydimethylsiloxane (PDMS-Vi, 1000 centistokes cSt [22,23], MW ~ 28,000 g/mol), trimethylsiloxy-terminated polydimethylsiloxane (PDMS-OTMS, 100 cSt, MW ~ 5970 g/mol, United Chemical Technologies) and 200, 5000, 60,000 cSt PDMS-OTMS (MW, 9430, 17,250, 116,500 g/mol, respectively, Hüls America) and the surfactant 3225C Formulation Aid (Dow Corning), a rake-type surfactant with pendant oligoethylene oxide/propylene oxide side chains (Fig. 1) [21], were used as received [24]. Most experiments were undertaken with PDMS-OTMS. However, as noted below, some experiments were done with PDMS-Vi instead of PDMS-OTMS. It was judged that essentially no change in hydrophobicity, viscosity or the interaction of the silicone polymer with proteins would arise when the vinyl replaced a methyl group at the silicone chain termini. Sodium hydroxide pellets were purchased from EMD Chemicals and anhydrous copper (II) sulfate powder from J.T. Baker.

2.2. Methods

2.2.1. Buffer solution

A buffer solution, used for dissolution of HSA, was prepared by dissolving TRIS (6.05 g, 50.00 mmol), EDTA (1.86 g, 5.00 mmol) and sodium chloride (8.77 g, 150.00 mmol) in Milli-Q water (1.00 L). Silicone/protein solutions were prepared by dissolving HSA (0.00670 g, 0.100 μmol) in 10.00 mL buffer and then adding the requisite amount of silicone (see below).

2.2.2. Silicones

Pure silicones of different molecular weights were used in the emulsification experiments. These included D_4 and PDMS-Vi (1000 cSt (unimodal molecular weight distribution)). In addition, mixtures of silicones having a viscosity of 1000 cSt were prepared by mixing various quantities of 100, 200, 5000 and 60,000 cSt PDMS-OTMS, respectively. An ATS RheoSystems STRESSTECH rheometer with a cone and plate geometry was used to adjust the final viscosity of the *unimodal*, *bimodal*, *trimodal*, and *broad* distributions (Table 1). The RheoExplorer software package was used for data analysis.

2.2.3. Emulsification and denaturation as a function of mixing time and added silicone concentrations

Two different modes were used to mix protein/buffer/silicones, which differed considerably in the amount of shear generated. Magnetic stirrers with 20 mm × 6 mm cylindrical stirring bars were used at 90 rpm (low shear). Higher shear mixing was achieved with a DREMEL tool (Multi PRO, Model 395, Type 5, 442 ½ inch carbon steel brush) at 5000 rpm for different time periods.

2.2.3.1. D_4, 1000 cSt PDMS-Vi and 3225C. D_4 (52.1, 72.9, or 104.2 μL corresponding to 0.5, 0.7 and 1.0 wt%, respectively) was added using a micropipette to the HSA buffer solution (10 mL; total formulation volume for 1.0 wt% D4, for example, was 10.1 mL). Volumes of 1000 cSt PDMS-Vi and 3225C formulation aid were added in the

Table 1
Total volume in mL and (volume % compositions) of different PDMS-OTMS (recorded in top row) that were mixed to make silicone blends with 1000 cSt viscosity.

Blend name	Component viscosity (cSt)				
	100	200	1000	5000	60,000
Broad	1 (8)	3 (23)	7 (54)	5 (15)	
Bimodal	8 (80)				2 (20)
Trimodal		5 (36)	6 (43)	3 (21)	
Unimodal			5 (100)		