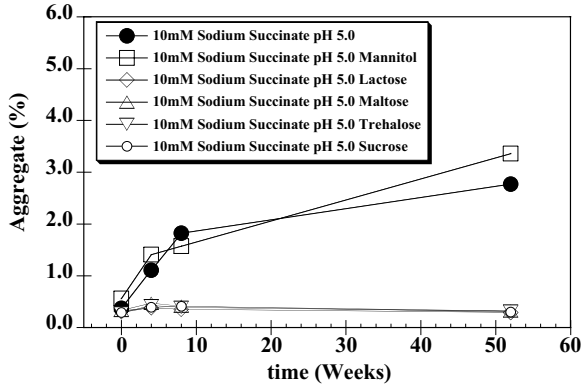

Figure 4.8 Generation of aggregate as determined by size exclusion chromatography for an IgG1 anti-IgE mAb formulated in 10 mM succinate buffer at pH 5 with different sugars at 275 mM.

Caveats when using sugars as lyoprotectants

It is well known that reducing sugars, such as glucose, can form adducts with free amino acid groups in a protein which may result in lysine–lysine covalent aggregates (Nagaraj, Shipanova, & Faust, 1996). This reaction, known as the Maillard reaction, proceeds during storage in the dried state resulting in a "browning" of the lyophilized cake. The modified protein also may be unstable and thus reducing sugars should be avoided (Carpenter, Chang, Garzon-Rodriques, & Randolph, 2002; Carpenter et al., 1997). Nonreducing disaccharides such as sucrose are susceptible to hydrolysis at low pH, and thus the solution conditions could result in generation of reducing sugars (Chambre, Iditoiu, & Szabo, 2007). As an example, an IgG_1 mAb was formulated at pH 5 with mannitol, lactose, trehalose, and sucrose and after lyophilization stored at 40 °C for 1 year. A substantial amount of aggregate formed when no lyoprotectant was added, as expected, and as noted previously, mannitol provided no protection because of its propensity to crystallize (Figure 4.9(a)). Good protection from aggregate formation was provided by lactose, maltose, and trehalose. Although lactose and maltose are reducing sugars, they did not impact formation of aggregate under these conditions. However, sucrose, a nonreducing disaccharide, can hydrolyze into the component reducing monosaccharides fructose and glucose under mildly acidic conditions (Edye & Clarke, 1998). Thus, at pH 5 and at 40 °C the IgG_1 lyophilized cakes shrunk and turned brown with a concomitant increase in aggregate. Although the presence of glucose was not ascertained in this study, it is likely that the hydrolysis of sucrose to glucose and fructose at pH 5 was responsible for the degradation. Interestingly, an increase of pH from 5 to 6 resulted in no aggregate formation after storage for 24 weeks at 40 °C, demonstrating the pH sensitivity of the hydrolysis reaction (Figure 4.9(b)). Even when formulating at pH >6 it is important to consider the buffer species that is chosen. As discussed previously, buffer salts such as sodium dibasic phosphate can easily crystallize on freezing resulting in a pH shift. A great example of this was a study by Deluca and colleagues where ribonuclease A was formulated in a 0.1 M sodium phosphate buffer system with sucrose as a lyoprotectant