Pollen-Protein Interaction in Formulations: Effect of Pollens on Protein Stability
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**Purpose**
In recent times there has been a rapid increase in the development and use of protein and peptide-based drugs due to the various advantages they offer. A number of novel systems have been developed for their delivery. However, these systems suffer from instability of the protein in formulation because when proteins in solution come in contact with a solid surface, the interaction between them can adversely affect the structure and function of the protein. Hence, whenever a new delivery system is introduced it is important to study its interaction with the protein of interest, determine whether there are any stability issues and develop an optimal formulation. Pollen grains (PGs) as novel biomaterials have been successfully used for oral vaccination in our previous work. The formulation of this vaccine involves an intimate interaction between the pollen surface and the antigen. Thus, it is important to study the effect of pollen grains on the stability of protein antigens in vaccine formulations.

**Methods**
Pollen vaccine formulations were prepared by mixing 500μg of protein with 5mg of Lycopodium clavatum spores (LSs). Three model protein antigens viz. hen egg lysozyme (HEL), ovalbumin (OVA), bovine alkaline phosphatase (ALP) were used. After mixing, the formulations were subjected to overnight vacuum. Post-filtration, the clear protein solutions were obtained and the amount of protein adsorbed was determined using SDS-PAGE and Bicinchoninic acid (BCA) protein assay. The structural stability was analyzed using i) circular dichroism spectroscopy (secondary structure), ii) intrinsic fluorescence (tertiary structure and conformational stability), and iii) native PAGE. The activity of the three proteins was determined using different activity assays.

**Results**
Most of the protein in the formulation was found to exist in a dynamic equilibrium with the pollen surface engaging in weak adsorption or encapsulation at any point of time. This protein could be easily recovered by filtration. A small amount of protein was found to be irreversibly adsorbed and was irrecoverable. The stability of the recovered proteins was analyzed and it was found that interaction with pollen surface did not affect the stability of any of the three model proteins at any level (secondary or tertiary). Moreover, the conformation of the proteins was also found to be minimally disturbed. All the three proteins retained their activity in spite of a close interaction with LSs.

**Conclusion**
Pollen vaccines were formulated by mixing model proteins and LSs and their structure and activity were analyzed. It was found that proteins adsorb on and encapsulate in PGs and that these close interactions do not adversely affect the structure and function of the proteins. This study demonstrates the potential of PGs to be used in the formulation of proteins without an unfavorable effect on the protein of interest.