CHAPTER V
CONCLUSIONS

Intranasal drug delivery system is a promising route for delivery wide range of substances from small to large molecules like peptides and proteins and for direct access of active substances to the brain. Among intranasal dosage forms, dry powder is interesting due to several advantages over liquid formulation. Therefore, the main purpose of this research was to formulate dry protein powder for intranasal delivery to brain. Dry protein powder was prepared from two main processes; matrix film preparation and then pulverization. A matrix film was prepared by powder casting method, a novel film preparing technique under low temperature and without water. Then the obtained films were pulverized by different milling machines such as JM, CM, and PBM.

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Matrix films were characterized by visual detection, physical examination, and SEM, while pulverized powders were characterized by visual detection, SEM, DSC, FT-IR, XRPD, Karl-Fischer for %moisture content, viscometers, and *in vitro* mucoadhesion. BSA was selected as a model protein, incorporated in selected formulations, and pulverized under optimized milling condition. Protein integrity including SDS-PAGE and CD, *in vitro* BSA release, and *in vitro* permeation studies on OLF and RES were performed. All findings would be concluded as followed:

1. By powder casting method, film forming polymers such as S, C, HK, EL, EE and combination of 2 film forming polymers could form a matrix film when mixed with PEG. The smoothness and characteristics of obtained films were varied depending on amount of PEG and film forming polymer type.
2. Films obtained from EL were sticky and transparent, while from the others were brittle.
3. Upon oven curing in hot air oven, weight loading on top of powder casting had no an impact on film characteristics.

4. A was able to be preferably used as a glidant in the formulation and also provided better film characteristics than T.
5. Proposed mechanisms of this film formation by powder casting method composed of melting of polymers and spreading over the substrate, growing of melted polymers into one unit, as well as flowing and leveling of film, which was close to mechanisms in solventless coating.
6. In JM process, more milling cycles resulted in smaller particle size with narrower size distribution and more roundish shape. However, increased milling cycles decreased production yield.
7. Higher amount of sample fed yielded higher production yield which could reach up to 80% in JM process.
8. JM could produce powder with median size ranging from 6.4-9.4 micron which was appropriate for intranasal drug delivery and lower risk to lung deposition.
9. There was no physicochemical interaction occurring after pulverization by JM because obtained DSC thermograms and FT-IR spectra showed no new peaks or shifted peaks observed and XRPD patterns still exhibited crystalline peak of PEG after milling.
10. In PBM, smaller particle size and narrower size distribution resulted from increasing of milling time.
11. There was peak intensity changing in XRPD pattern which may be assumed that transformation of crystalline to amorphous occurred due to heat generation during PBM process.
12. CM powder was too small size of less than 1 micron and had a tendency to agglomerate to be a bigger one.
13. There was a new endothermic peak occurring in DSC thermogram and a change in XRPD pattern of CM powder which originated from crystalline-amorphous transformation under cryogenic condition.
14. Powder formulations could stay on the mucosa for up to 60 minutes and formulations containing positively-charged polymer had a better tendency to attach to the mucosa than the others.

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15. One of the mucoadhesive mechanisms of C and EE was ionic interaction between positive charge of polymers and negative charge of sialic acid and sulphonic acid in mucin.
16. According to protein integrity study, primary and secondary structure of BSA could maintain under film preparation by powder casting process. However, in milling process, formulations had an influence on protein integrity except S-6 (S+60% w/w of PEG) formulation whose protein structure could be reserved in all milling processes.
17. The release kinetic of model protein, BSA, in powder formulations was Weibull model, according to goodness-of-fit parameters. Moreover, it was found that BSA release followed Fickian diffusion.
18. Powder formulation could permeate through OLF and RES similar to solution formulation without tissue damage. However, powder formulation could longer remain on the mucosa than liquid formulation. Therefore, it can be assumed that permeated amount of protein of dry powder was likely to be more than that of solution where MCC was active. These results may also imply that BSA could reach to the brain after permeation through the mucosae.
19. FITC-BSA dry powder was probably delivered through nasal mucosa via passive diffusion mechanism and/or additional with other mechanisms and enhanced with proteolytic enzyme inhibition by S.


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In conclusion, these overall findings suggest that dry powder prepared by the powder casting and the pulverization the obtained film by JM would be a promising and interesting powder formulation utilized for delivery of macromolecule from the nose. Furthermore, these novel techniques may be suitable and potential tools for drugs sensitive to heat and hydrolysis, as well.

However, there were some limitations in the present research that might have an impact on the completeness and interpretation of the findings as follow:

1. In evaluation of mucoadhesive properties, oscillating rheometer was more suitable than other types due to mimicking ciliary movement and no damage of polymeric-chain structure.
2. Aerodynamic mean diameter may be more suitable for particle size determination for nasal delivery system than volume mean diameter.
3. There is lack of information about risk of lung deposition and stability of powder formulations by this method.

Finally, this study should be further investigated in some aspects to improve efficiency of formulation and fulfill all requirements for intranasal dry powder system, such as adjustment of formulations which incorporate polymers with absorption enhancing effect, application of these technique to other therapeutic peptides, study on nasal deposition of powder when applied with suitable devices and risk of lung deposition, study on stability, and application to animal model for brain delivery.

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