# The structure-function relationship of ovalbumin matrix as the result of protein denaturation (Short communication)

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## 1 Introduction

In the last years the use of biopolymers, such as gelatin, bovine albumin and ovalbumin, as drug carriers, received increased attention due to their high biocompatibility, safeness and low cost [1]. However, possibility of denaturation always takes place during processing and employment of sustained release devices. Dry heating is one of the main factors that induces protein denaturation during pharmaceutical production of pellets. Very little data was found on protein denaturation upon heating in the dry state [2, 3]. Present work was undertaken to evaluate the effect of dry heating on the ovalbumin (OVA) behavior as a drug carrier.

## 2 Materials and methods

Solid ovalbumin (OVA) with 11% of water was heated in non-closed conditions for different periods of time (1 h, overnight and 5 days at temperatures 80 °C and 100 °C). The dissolution rates of the drug were monitored using a tablet dissolution tester at 37 °C. Diclofenac sodium was used as a model drug. Surface hydrophobicity was determined with 1-anilino-8-naphthalenesulfonate (ANS) fluorescent probe as described in [4]. Secondary structure content was calculated using *Provencher* nonlinear least-squares curve-fitting program and the results of circular dichroism (CD) measurements.

## 3 Results and discussion

Dissolution rate of diclofenac sodium from OVA matrices is shown in Fig. 1. The release rate of the drug from OVA matrices heated in the dry state for different periods of time gradually decreased with an increase in heating time and temperature. Sustained release from hydrophilic polymer matrix is achieved owing to gel formation [5]. These gels differ from heat-induced gels that commonly are considered in literature [6], because at temperature 37 °C protein does not undergo denaturation. Nevertheless gel-like structure is formed during swelling of protein tablets in aqueous medium, and we will consider them further as gels. Gel properties of different OVA tablets, swelled in the water, as expressed by breaking stress and deformation at break were compared. These results indicate that the rheological properties of OVA tablets were affected by heat treatment. Clear tendency for increased gel hardness upon heating was observed, but these changes were gradual for native –100 °C overnight (ovnt) tablets. The rheological behavior of 80 °C ovnt and 100 °C ovnt tablets was similar one to another, but differed considerably from that of 100 °C 5d OVA tablets. The difference in ultimate properties between various OVA tablets as expressed by compression test behavior was the following: 100 °C 5d OVA tablets were broken at deformation that was about 3 times larger, and at a stress that was 28-fold larger, than that of the native OVA tablets. Also elasticity of 100 °C 5d OVA gels increased significantly, as expressed by large modulus value (0.0942 MPa) in contrast to 0.0062 MPa for the native one. Such increase in gel strength influences release rate of the drug.

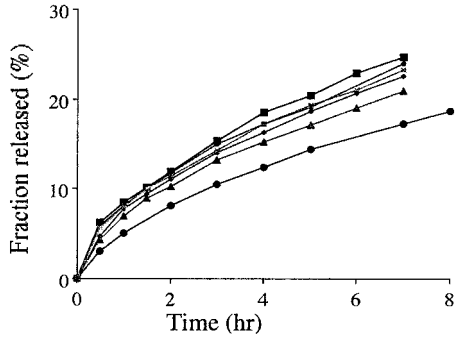


**Figure 1.** Effect of OVA denaturation on the dissolution rate of diclofenac sodium from different OVA matrices: ■ – native OVA; ● – OVA heated at 80 °C 1 h; x – OVA heated at 100 °C 1 h; ◆ – OVA heated at 80 °C ovnt; Δ – OVA heated at 100 °C ovnt; ● – OVA heated at 100 °C 5 days.

Electronmicroscopic studies (not shown) indicate heterogeneous aggregated structure for native tablets. However, for denatured matrices cellular homogeneous structure was found. These results agree with the results on heat-seted gels [7], where regular network structure was noted only for gels with high gelation temperature (higher than the temperature of their denaturation). Regular network structure is also considered to be responsible for a high gel strength and low value of syneresis.

Surface hydrophobicity of OVA was examined with ANS fluorescent probe that considered to be the measure of aromatic hydrophobicity. Fig. 2 demonstrates positive correlation between increase in surface hydrophobicity and time of OVA denaturaion. The sharp increase occurred at maximal time 5 days. Such increased hydrophobicity can be attributed to the altered, partially unfolded OVA conformation induced by dry heating.

CD analysis did not reveal destruction of the secondary structure. Denaturation and unfolding resulted in promoted interactions bet-

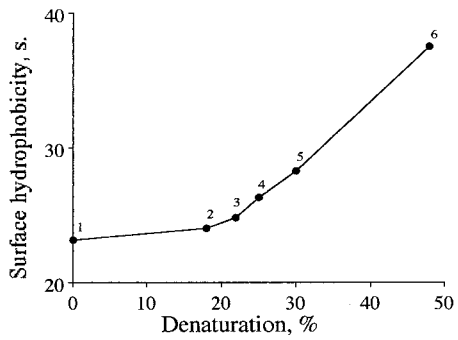


**Figure 2.** Effect of heat treatment on the surface hydrophobicity of OVA: 1 – native OVA; 2 – OVA heated at 80 °C 1 h; 3 – OVA heated at 100 °C 1 h; 4 – OVA heated at 80 °C ovnt; 5 – OVA heated at 100 °C ovnt; 6 – OVA heated at 100 °C 5 days.

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  0027-769X/98/0304-0252$17.50+.50/0

**Table 1.** Effect of dry heating on the secondary structure of OVA.

| | Fractions of secondary structure | | |
|---|---|---|---|
| Ovalbumin | α-Helix | β-Sheet | Remainder |
| Native | 0.32 | 0.17 | 0.51 |
| 100 °C, 1 h | 0.13 | 0.38 | 0.49 |
| 80 °C, overnight | 0.22 | 0.27 | 0.51 |
| 100 °C, overnight | 0.25 | 0.06 | 0.69 |
| 100 °C, 5d | 0.58 | 0.00 | 0.42 |

ween exposed functional groups which involved transconformations of α-helix, β- sheet and aperiodic structure. Dry heating brought to increase in α-helical content at expence of β-sheet structure (Tab. 1). These results contradict to previous reported data for protein solutions, which described increase in β-sheet content in sacrifice of α-helix during heating [8]. CD data indicate difference in the secondary structure reorganizations during heating of proteins in solutions and in the dry state.

## 4 Conclusions

OVA heating in the dry state brings to denaturation by the disruption of hydrogen bonds. Rearrengement of the protein structure causes significant increase of surface hydrophobicity. CD analysis for the first quantitatively described changes in secondary structure content of OVA upon dry heating, and increase of α-helix in sacrifice of β-sheet was observed in contrast to known data for protein solutions [8]. These partially unfolded molecules do not participate readily in intermolecular interactions and as the result balance between chain-chain and chain-solvent interactions exist. Consequently, matrix tablets have regular extended network structure that explains high gel strength and low value of syneresis. However, native OVA with stable globular structure and polar groups on its surface has strong intermolecular interactions and forms aggregates during hydration of matrix tablets (with participation of intermolecular β-sheet structure). Such strong interactions bring to granular heterogeneous network structure, and consequently to low gel strength, low swelling and high value of syneresis. Changes in the gel properties result in the decrease of the release rate of diclofenac sodium.

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Received: 2 September 1997.
Accepted: 9 February 1998.