4. SUMMARY

Lysozyme release from in situ forming delivery systems – methodical aspects

An in vitro dissolution methodology for the release of hen egg-white lysozyme from in situ forming biodegradable drug delivery systems was developed, which provided complete recovery of fully active material from protein solutions in a pH 5 acetate buffer, which were stored up to 70 days at body temperature. However, HPLC analysis of the aqueous solutions revealed a time- and temperature-dependent change of the protein, which was probably due to a chemical modification during incubation. Loss of protein during incubation of aqueous lysozyme solutions in presence of the water-insoluble PLGA could be attributed to adsorption to the surface of the biodegradable polymer. Thereby, re-dissolution of adsorbed protein upon erosion of the polymer particles was observed. Higher protein recoveries were generally obtained with gentle agitation of the incubated samples (vertical test tube position). An effect of the slightly acidic medium pH on the degradation of PLGA could be excluded. The degradation rates showed a good correlation with previously reported in vivo degradation rates of the polymer.

An extraction methodology was developed, which facilitated complete recovery of fully active protein from organic solvents, PLGA solutions and in situ microparticle emulsions as well as from dried implants (with and without residual water). Deleterious conditions like water-organic solvent mixtures or interfaces were completely avoided. Non-aqueous precipitation of organic lysozyme solutions in DMSO showed that apparently native, reversible aggregates formed during the precipitation. The extraction of in situ microparticle formulations indicated, that the emulsification process, which was applied during manufacturing of oil-in-oil ISM, did not alter the protein integrity.

Formulation parameters of in situ forming drug delivery systems

<u>In situ systems based on water-immiscible polymer solvent</u>

The PLGA concentration of in situ implants affected only the initial release phase, whereas lysozyme release, which occurred after the usually reported 1-month release interval for the used PLGA grade was independent of the polymer concentration. Total protein recoveries during release testing did not exceed 90 %. The release of lysozyme from in situ implants based on triacetin could be significantly accelerated by dispersing small amounts of sesame oil in the drug-loaded polymer solutions (with paragraph 3.3.2.), wherein the protein

was in the dispersed state. A phase separation-assisted co-transport of dispersed drug particles was suggested as release mechanism.

In situ systems based on water-miscible polymer solvents

Lysozyme was unstable in NMP. The protein aggregated upon dilution of lysozyme solutions in NMP with aqueous medium. HPLC analysis revealed incubation-time dependent changes of the protein during storage at ambient temperature in NMP. The analytical characteristics of lysozyme were similar to the results obtained upon storage in aqueous solutions, where a chemical degradation was indicated. However, the rate of change was about 8-times faster in NMP at ambient temperature compared with the aqueous solutions at 37°C.

DMSO was the better solvent for lysozyme compared to NMP. Homogenous incorporation of lysozyme into DMSO-based PLGA solutions was achieved only, if lysozyme was dissolved prior to PLGA addition. Both compounds were phase-incompatible in DMSO. Liquid-liquid phase separation occurred.

No aggregation was observed upon dilution of lysozyme solutions in DMSO with aqueous medium. Dissolution results obtained with in situ implant formulations suggested a complex, four-phasic release characteristic consisting of an initial diffusion-controlled phase followed by an erosion-controlled release phase, a phase with marginal release and a second erosion-controlled phase. The changes of the release pattern resulting from a variation of the lysozyme loading of the formulation suggested a three-compartment model of the total drug distribution.

Accordingly, a mathematical description of the drug release behavior was derived, which combined a (semi-)empirical description of the diffusion-controlled drug release phase with a novel theoretical description of the PLGA erosion process. This composite model facilitated a good approximation of the actual lysozyme release characteristics and might be applicable to other drugs, which are preferentially released through degradative erosion of the polymer matrix. An uncertainty in the model was the stability (total recovery) of the drug. Although working at the stability maximum of the drug in aqueous solution, the release was incomplete. Therefore, a selection of additives was screened for a positive effect on the recovery of lysozyme. Co-incorporation of PVP and protamine sulfate into in situ implant formulations resulted in an increase of the lysozyme fraction, recoverable during in vitro release testing. Both compounds appeared to compete with lysozyme for adsorption to PLGA, which, in case of the auxiliary protein protamine, resulted in a preferential release of the model protein.

In situ systems based on blends of water-miscible and water-immiscible polymer solvents

Dissolution of lysozyme in DMSO and subsequent precipitation with PLGA and ethyl acetate or triacetin facilitated incorporation of finely dispersed lysozyme into in-situ forming implant formulations. Similarly to the developed extraction method, recovered precipitated lysozyme showed full biological activity. Application as an in-situ precipitation step during manufacturing could avoid costly or harmful protein micronization methods.

Lysozyme separated from DMSO / triacetin solvent blends displayed good storage stability in the non-aqueous systems at ambient temperature independent of whether it was dissolved in DMSO, precipitated from DMSO solution or dispersed in DMSO / triacetin or triacetin. However, apparently inactive covalent aggregates formed on storage of lysozyme solution in DMSO at elevated temperature (40°C). The storage stability in in-situ forming implants (PLGA solutions) depended on the physical state of lysozyme. Protein aggregation proceeded faster with more lysozyme dissolved (solvated). A sustained dissolution of the aggregates upon incubation in aqueous medium indicated that they were at least partially reversible.

External phase composition of ISM

The ejection of unstabilized in situ forming microparticle (ISM) emulsions from syringes equipped with hypodermic needles required comparable peak forces compared with the corresponding highly viscous in situ implant formulations. A rapid phase separation in the emulsions probably resulted in the formation of polymer solution droplets with a size exceeding the inner needle diameter (lumps). Thus, peak forces could be attributed to temporary needle blockages. Thus, an improved injectability of ISM-emulsions compared to in situ implants requires a sufficient quality and stability of the ISM-emulsion. The addition of parenterally applicable phospholipid derivatives was found to be a promising stabilization approach for oil-in-oil ISM-emulsions based on DMSO as polymer solvent.