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Note

Light scattering investigations on freeze-dried glucocorticoids in aqueous solution

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Abstract

Aqueous solutions of three glucocorticoid drug substances were investigated using dynamic light scattering. Evidence of micelle formation was found. The identified diffusion coefficients, micellar radii and the dependence of these parameters on the concentration of the solution as well as on the molecular structure of the drugs are discussed. It is postulated that the micellar character of the solutions is one reason for the ease of lyophilization of such molecules without additional substances.

Keywords: Micelles; Prednisolone; Dynamic light scattering

1. Introduction

Freeze-dried glucocorticoids like prednisolone esters are well-known as stable pro-drugs. These drug substances often exist in several crystalline and amorphous forms, including so-called solvates. The type if crystal form is important with respect to its physical and chemical stability (Claußen et al., 2001).

The stabilizing process of initially wet material (in aqueous solutions or suspensions) involves freezing, then subliming the ice while simultaneously desorbing some of the bound moisture (primary drying). Following the disappearance of the ice, desorption may be prolonged (secondary drying). This process is carried out under vacuum. Usually the chemically or biologically active component is present in a low concentration, so that cryoprotective substances like mannitol or sucrose are added to establish physical stability (Franks, 1998). However, these excipients are not necessary for the freeze drying of glucocorticoids such as prednisolone hemi-succinate (Prednisolut®). This statement indicates the multitude of complex and interacting problems, relating the chemical composition of the product resulting in freeze-dried product (Franks, 1998; Bruttini et al., 1986).

The objective of this work was to characterize the solutions of freeze-dried prednisolone hemi-succinate (Prednisolut®), methylprednisolone hemi-succinate (Urbason® soluble forte) and hydrocortisone hemi-succinate (Hydrocortison Rotexmedica®) by dynamic light scattering. This showed that these drugs form micelles in aqueous environments, and we postulate that this physical state influences the behavior of the systems during freeze drying.

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2. Dynamic light scattering

The dynamic light scattering (DLS) detects the fluctuations of the scattering intensity $I(t)$ (mostly of a liquid sample) caused by concentration changes in the scattering volume due to the Brownian motion of particles (Berne and Pecora, 1976). The smaller the dissolved particles the more rapid are the fluctuations. These fluctuations can be analyzed by correlation techniques. The autocorrelation function of the electric field g_1 in relation to the scattered intensity by Eq. (1) and directly proportional to a time-dependent exponential function of the diffusion coefficient D (Janich et al., 1998):

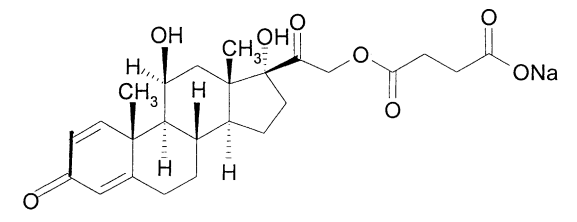
$$g_1(\tau)=\sqrt{1-\frac{\sum_i I(t_i)I(t_i+\tau)}{\sum_i (I(t_i))^2}} \propto \exp[-q^2 D\tau] \qquad (1)$$

where $q = 4\pi n/\lambda^* \sin(\theta/2)$ is the scattering vector, n the refractive index of solution, $\lambda = 532$ nm the wavelength, and θ is the scattering angle. For small particles, the diffusion coefficient is a function only of the concentration of the solution. This due to the interparticle interaction hindering the free motion in the solvent (Janich et al., 1998). In order to obtain the free diffusion coefficient D_0 one extrapolates the measured values to zero concentration. From that result one can evaluate the size of particles using the well-known equation relation from Stokes–Einstein (Eq. (2)) yielding the hydrodynamic radius R_h:

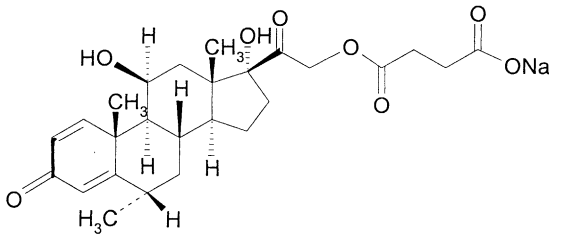
$$R_h = \frac{KT}{6\pi\eta D_0} \qquad (2)$$

where K is Boltzmann's constant, T the absolute temperature and η is the solvent viscosity.

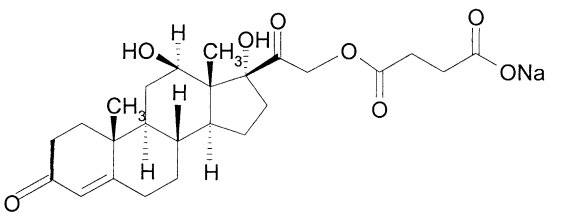
Prednisolut® (B.N.: 01034) was obtained from Jenapharm GmbH & Co., Urbason® soluble forte 1000 (B.N.: H097) from Hoechst Marion Roussel and Hydrocortison 100-Rotexmedica® (B.N.: 80103) from Rotexmedica GmbH. The chemical formulae of the drug molecules are depicted in Fig. 1, which shows that they consist of hydrophilic (ionic head group, some hydroxyl groups) and hydrophobic parts (hydrocarbon skeleton) similar to amphiphilic bile acids. All three medicinal substances are similar in their chemical structure and are used to treat allergic conditions, inflammatory disorders, autoimmune diseases, and some cancers. They differ in that prednisolone



prednisolone hemi-succinate



methylprednisolone hemi-succinate



hydrocortisone hemi-succinate

Fig. 1. Structural formulae of prednisolone hemi-succinate, methylprednisolone hemi-succinate and hydrocortisone hemi-succinate.

hemi-succinate has an additional methyl group in the C6-position and hydrocortisone hemi-succinate has no double bond at C1–C2. Functional groups essential for a glucocorticoid are a C21 hydroxyl and C11 ketone or hydroxyl.

First a buffer of 108.6 mM $NaHCO_3$, 35.5 mM Na_2HPO_4 and 35.5 mM NaH_2PO_4 yielding a pH-value of 7.0 as prepared. 500 and 1000 mg of methylprednisolone hemi-succinate (Urbason® soluble forte) and hydrocortisone hemi-succinate (Hydrocortison Rotexmedica®) and 600, 800 and 1000 mg if prednisolone hemi-succinate (Prednisolut®) were

dissolved in 10 ml of this buffer. The resulting solutions were passed though cellulose nitrate filters with a 0.2 μm pore size into dust-cleaned sample cells made by HELLMA/Germany (Cat. No. 540.111-QS). The samples were kept at room temperature for about 15–60 min and measured immediately after, in order to prevent degradation of the drug molecules. The DLS measurements were performed at 25 °C with an ALV light scattering goniometer (ALV-GmbH Langen, Germany) equipped with a frequency-doubled 200 mW Nd:YAG laser (532 nm wavelength), a single-mode fiber/PMT detection system and an ALV-5000 multiple tau digital correlator with a sampling time of 12.5 ns. The autocorrelation function of each sample was measured at five angles from 50 to 70° in steps of 5°. Each curve was analyzed by an exponential fit and the resulting diffusion coefficients were averaged because of the angular independence of the diffusion coefficient of small particles. No dependence on the scattering angle was in fact detected.

Fig. 2 shows the measured diffusion coefficients for the three substances at different concentrations. Straight lines were fitted and used for the extrapolation to infinite dilution, providing the free diffusion coefficients. These are shown in Table 1 together with the calculated hydrodynamic radii and their errors. The sizes of the aggregates are exactly in the range of micelles formed by similar amphiphiles like such as bile acids (Janich et al., 1998; Nair and Kritchevsky, 1971). The small differences between the chemical structures leads to a size difference of their aggregates of about 25%. Prednisolone hemi-succinate and hydrocortisone hemi-succinate micelles have statistically the same particle size. The replacement of only one hydrogen atom with a methyl group results in a more hydrophobic molecule in methylprednisolone and this leads to a significant growth in micelle size.

Table 1
Diffusion coefficient D_0 and hydrodynamic radius R_h of prednisolone, methylprednisolone and hydrocortisone as the hemi-succinates

	D_0 ($\times 10^{-8}$ cm^2/s)	R_h (nm)
Prednisolone	200.64 ± 5.2	1.22 ± 0.04
Methylprednisolone	159.76 ± 3.52	1.53 ± 0.03
Hydrocortisone	204.92 ± 8.39	1.19 ± 0.05

Another interesting result is the slope of the lines (Fig. 2) indicating a repulsive force between the aggregates for positive values and an attractive interaction for negative values. Normally, one should expect a repulsive force due to the Coulomb inter-

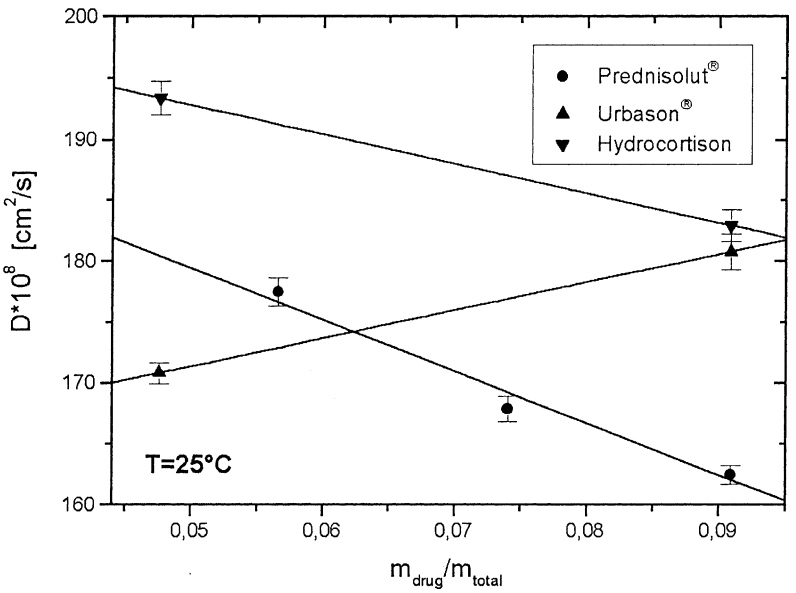


Fig. 2. Measured diffusion coefficients for (●) prednisolone hemi-succinate; (▲) methylprednisolone hemi-succinate and (▼) hydrocortisone hemi-succinate at different concentrations and at 25 °C.

action of charged particles. This behavior is seen for methylprednisolone hemi-succinate. On the other hand, attractive forces due to hydrogen bonds at the positions of the hydroxyl groups are predictable. Probably these hydrogen bonds act as interparticle attraction forces only for small micelles and overcome the repulsion. One can imagine that the hydroxyl groups are located on the surfaces of micelles together with ionic groups. In the case of the large micelles formed by methylprednisolone hemi-succinate, the hydroxyl groups are inside the aggregates, acting as intraparticle forces. The surfaces are mostly occupied by ionic head groups leading to an interparticle repulsion.

The statement that micelle-forming medicinal substances like the glucocorticoids tested here can be lyophilized without adding structure-forming substances, makes the determination of the micelle parameters and their influence on the final product pharmaceutically interesting. Therefore, the connection between chemical structure and micelle-forming properties should be regarded as an essential factor in preformulating the pharmaceutical form of such medicinal substances. Stable freeze-drying glucocorticoids could be designed as optimum product formulations with associated ability to form micelles in aqueous environments for the freeze drying of glucocorticoids without excipients.

It was possible to characterize aqueous solutions of prednisolone hemi-succinate, methylprednisolone hemi-succinate and hydrocortisone hemi-succinate as micellar systems. The diffusion coefficients and hydrodynamic radii were measured and discussed with respect to the chemical structure of drug molecules used. It is predicted that micelle-forming drug molecules can be lyophilized without excipients.

The effect of ionic strength, pH value and concentration on the micelle formation and stability of prednisolone hemi-succinate will be examined in further studies.

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