Inclusion Complex of Prednisolone with Skimmed Milk
Part I: Physicochemical Characterization

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Prednisolone is a safe antiinflammatory agent for the treatment of inflammatory diseases. To improve the aqueous solubility of the drug and dissolution rate, the complexation of prednisolone with skimmed milk was studied. A physical mixture and solid dispersion of prednisolone with skimmed milk were prepared. The lyophilization method was used to prepare the solid dispersion. Detection of inclusion complexes was performed in the solid state using differential scanning calorimetry (DSC), powder X-ray diffractometry, and scanning electron microscopy. The diffractogram of the complex differed from that of the physical mixture, where the characteristic peaks of prednisolone, particularly at 23.9°, 44.6°, and 72.2° (2 θ), nearly disappeared, indicating the formation of a true inclusion complex. These observations were in accordance with the results of the DSC analysis. Disappearance of the specific DSC peaks of the drug in the DSC curve of the solid dispersion showed that the drug interacts with the carrier.

Key words——prednisolone; solid dispersion; skimmed milk; X-ray powder diffraction analysis; differential scanning calorimetry; scanning electron microscopy

INTRODUCTION

Prednisolone [(8S,9S,10R,13S,14S,17R)−11,17−dihydroxy−17−(2−hydroxyacetyl)−10,13−dimethyl−7,8,9,11,12,13,14,15,16,17−decahydro−6H−cyclopenta[a]phenanthren−3(10H)−one] is a glucocorticosteroid used for the treatment of inflammatory diseases for many years (Fig. 1).[1,2] It possesses antiinflammatory and analgesic activity.[1] The mechanism its therapeutic action is rather complex. As with most other glucocorticosteroids, long-term use of prednisolone causes some undesirable effects on the cardiovascular system and bone metabolism.[3–11] Oral administration of prednisolone leads to gastric irritation.[10–13] Also, prednisolone is insoluble in aqueous solutions[12,13] and therefore the bioavailability of orally administered conventional dosage forms of the drug is limited. Several approaches have been proposed to solve these two problems.[14–21]

The preparation of solid dispersions with skimmed milk is one of the best approaches for modulating gastric irritation as well as improving the aqueous solubility of the drug.[21–24] In these systems, the drug is expected to form an inclusion complex with the skimmed milk due to the surface active agent and amino acid content of skimmed milk.[24–26] Various techniques are available to identify the formation of such complexes in solid dispersions.[27–34] Scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray powder diffraction analysis (XRD), and differential thermal analysis and thermal gravimetry have been used to obtain information on the interaction between the carrier and the plain drug upon formation of inclusion complexes in solid dispersions.[27–35]

Based upon a literature search, we could find no characterization of the solid dispersion of prednisolone prepared with skimmed milk. In this study, a
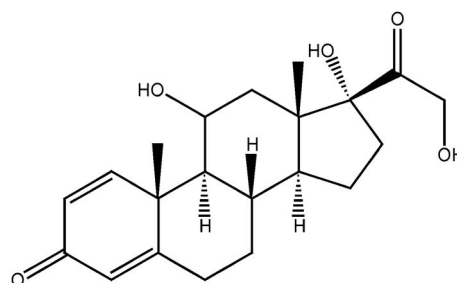
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Fig. 1. Structure of Prednisolone

solid dispersion of prednisolone was prepared using the lyophilization technique suggested by Topaloglu et al.[23] It is assumed that this method could result in an improved and more predictable dissolution rate along with reduced gastric side effects. In the first part of our study, the solid dispersion of prednisolone was characterized using DSC and XRD to confirm the formation of a solid dispersion with skimmed milk.

MATERIALS AND METHODS

Materials   Prednisolone was a gift from Mustafa Nevzat Pharm. Co., Turkey. Skimmed milk was used as purchased from Miss Dairy Products, Turkey. It was composed of 1% fat, 4.7% carbohydrate, and 3.3% protein. All other reagents and chemical substances were of analytical grade.

Preparation of Skimmed Milk Powder   Twenty-five milliliters of skimmed milk was freeze-dried (Lyovac GT 2-Leybold, Heraus) until the humidity of the sample was reduced to 3%. Based on preliminary studies, the duration of the lyophilization process was determined to be 72 h. The yield was 2.615 g for skimmed milk powder. The obtained product was sieved through 250-μm mesh.

Preparation of the Physical Mixture   Physical mixtures of prednisolone were prepared by simple mixing. Micronized prednisolone (500 mg) was uniformly mixed with lyophilized skimmed milk (2.615 g) using an agate mortar and pestle. The resultant mixtures were kept in a desiccator over calcium chloride at room temperature.

Preparation of the Solid Dispersion   Solid dispersions of prednisolone were prepared with the technique of Topaloglu et al.[23] Prednisolone (500 mg) was suspended in 25 ml of skimmed milk. The suspension was mixed with a magnetic stirrer in a water bath at 50℃ until a homogenous mixture was formed. Subsequently, it was frozen in a fluid nitrogen bath and lyophilized (Lyovac GT 2-Leybold, Heraus). The resultant solid dispersion of prednisolone was sieved through 250-μm mesh.

Validation of the Method of Preparing Solid Dispersions   In formulation studies, the procedures should meet proper standards of accuracy and reliability.[36,37] Method validation is the process of demonstrating that the manufacturing procedure used for formulation studies is suitable for the intended use. Validation of the method of preparing solid dispersions of prednisolone includes the accuracy test

run for the content of solid dispersions prepared. The prednisolone content of each solid dispersion formulation was determined spectrophotometrically. First, the regression equation and correlation coefficient (r) were calculated for six series of formulations. Next, the amount of prednisolone in each formulation was calculated using this regression equation and then the relative standard deviation was found for each formulation. The test was repeated six times.

Differential Scanning Calorimetry   DSC analysis of the drug, carrier, solid dispersion formulation, and its physical mixture were carried out on the samples using a Perkin Elmer DSC-2. Samples (3–5 mg) were heated under a nitrogen atmosphere on an aluminum pan at a rate of 10℃/min over the temperature range of 30° to 300℃.

Powder X-ray Diffraction Analysis   XRD patterns were traced employing an X-ray diffractometer (Philips PW 1710) for the samples, using Ni-filtered Cu K$_\alpha$ radiation ($\lambda = 1.54060$), voltage of 40 kV, current of 30 mA, and receiving slit of 0.2 inches. The samples were analyzed over 2θ range of 5–75° with scan step size of 0.040° (2θ) and scan step time of 1 s.

Scanning Electron Microscopy   Scanning electron micrographs of skimmed milk, prednisolone, physical mixture, and solid dispersion of prednisolone were taken. Each sample was mounted on stubs using conductive double-sided carbon tape and spatter-coated with gold/palladium in a spatter coater (Polaron SC7620, UK) for 90 s at 9 mA. The samples were examined and digital images captured using a JEOL JSM-5500 (Tokyo, Japan) scanning electron microscope at an accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

In this study, skimmed milk was chosen as a carrier for the preparation of solid dispersions of prednisolone to improve its aqueous solubility. Milk can be described as a colloidal suspension of casein micelles, globular proteins, and lipoprotein particles.[38] The principal casein fractions are alpha (s1) and alpha (s2)-caseins, beta-casein, and kappa-casein. The caseins are conjugated proteins, most with phosphate groups esterified to serine residues. These phosphate groups are important to the structure of casein micelles. Beta-casein is very amphiphilic and acts like a detergent molecule with surface activity.[38,39]

Most of the casein proteins exist in a colloidal particle known as the casein micelle.[38–41] It is assumed that

these micelles entrap hydrophobic prednisolone inside and with its surface active property improves its solubility in gastric media when administered orally. It has a porous structure, which may play an important role in the release of entrapped drug molecules from the inclusion complex into water (*i.e.*, dissolution media). The casein submicelle model was also suggested.[38,39] In this model, it is thought there are small aggregates of whole casein, containing 10–100 casein molecules, called submicelles. It is assumed that there are two different kinds of submicelle: with and without kappa-casein. These submicelles contain a hydrophobic core and are covered by a hydrophilic coat that is at least partly comprised of the polar moieties of kappa-casein. Submicelles rich in kappa-casein occupy a surface position and exhibit surface activity.

Whey proteins of milk are globular and more water soluble than caseins. The lyophilization procedure to prepare solid dispersions provides protection against heat denaturation of these protein molecules. The principle fractions of whey proteins are beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, and immunoglobulins, which are all surface-active molecules.[38–41]

Although we have not determined which substance in skimmed milk contributes more to the formation of an inclusion complex to prepare solid dispersions, we assume that casein micelles and whey proteins are involved as they possess significant surface activity to solubilize prednisolone entrapped in submicelles and interacting with hydrophilic whey protein fractions.

Solid dispersions of prednisolone were prepared with skimmed milk utilizing the lyophilization method. For the validation of the method of preparing solid dispersions of prednisolone, the accuracy test was carried out. For this purpose, the amount of prednisolone in solid dispersions was determined spectrophotometrically. First, the standard curve for prednisolone was plotted and the regression equation was determined along with the correlation coefficient ($r = 0.9993$). Then, using this equation, the concentration of drug in each formulation was calculated (Table 1). Finally, the relative standard deviation value for accuracy was found to be 0.93%. The result indicates the efficacy of the method of preparing solid dispersions.

As part of preformulation studies in pharmaceuticals, a physicochemical characterization study was carried out. To determine the interaction between prednisolone and skimmed milk, DSC, and XRD analyses were performed on solid dispersions and individual components.

XRD Studies   XRD is a useful method for the detection of prednisolone complexation in powdered or microcrystalline states. The diffraction pattern of the complex should be clearly distinct from that of the superimposition of each of the components if a true inclusion complex has been formed. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary systems with those of a reference. The relationship used for the calculation of crystallinity was the relative degree of crystallinity (RDC);

$$\text{RDC} = \frac{I_\text{sample}}{I_\text{reference}}$$

where I_sample is the peak height of the sample under investigation and $I_\text{reference}$ is the peak height at the same angle for the reference with the highest intensity.[42] The pure drug peak at 13.7° (2θ) was used for calculating the RDC of the solid dispersion and physical mixture. The RDC values of the solid dispersion and physical mixture were 0.272 and 0.495, respectively.

The XRD pattern of pure prednisolone exhibits its characteristic diffraction peaks at various diffraction angles, indicating the presence of crystallinity (Fig. 2), whereas skimmed milk exhibits a diffraction spectrum typical of mostly amorphous material, not showing any detectable diffraction peaks.

In the diffraction patterns obtained with the prednisolone-skimmed milk physical mixture, all the principal peaks of prednisolone and skimmed milk were observed. Overlap of the drug peaks by the skimmed milk peaks was also observed. The diffractograms of the solid dispersion of prednisolone differed from the corresponding physical mixture, where the charac-

Table 1. Results of the Accuracy Test for Validation of the Method of Preparing Solid Dispersions

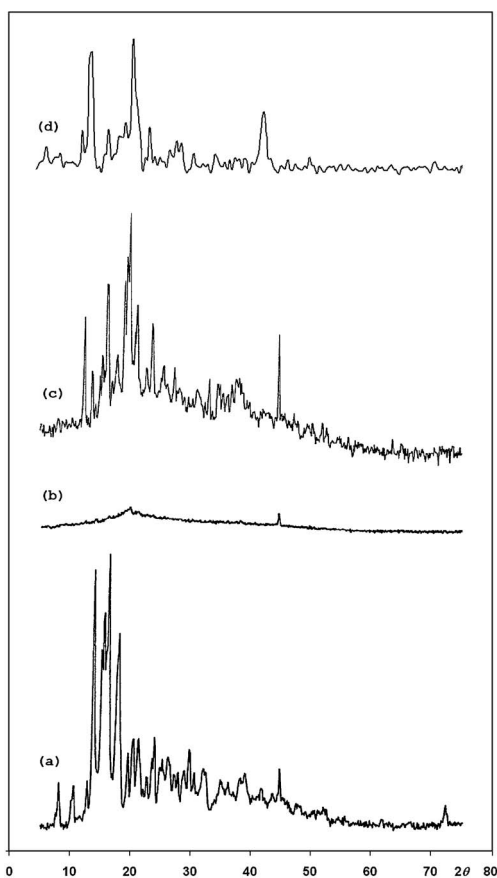| Concentration, mg/ml	Standard deviation	Relative standard deviation, %
18.21	0.18	0.98
18.66	0.17	0.91
18.82	0.19	0.93
19.23	0.18	0.93
19.31	0.17	0.88
19.60	0.19	0.96

Fig. 2. X-ray Powder Diffraction Spectra of Prednisolone (a), Skimmed Milk (b), Physical Mixture (c), and Solid Dispersion of Prednisolone with Skimmed Milk Powder (d)
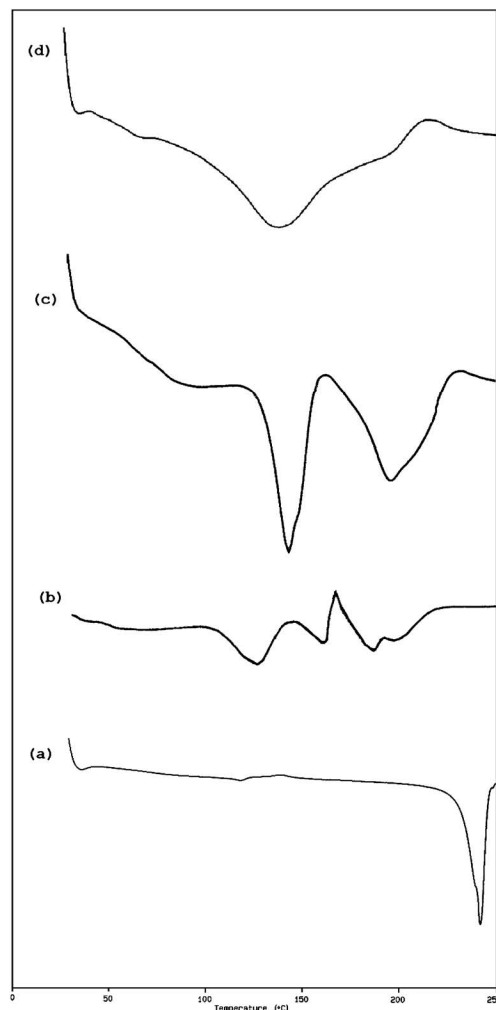

Fig. 3. DSC Thermograms of Prednisolone (a), Skimmed Milk (b), Physical Mixture (c), and Solid Dispersion of Prednisolone with Skimmed Milk Powder (d)

teristic peaks of prednisolone, particularly at 23.9°, 44.6°, and 72.2° (2θ), disappeared, indicating the formation of inclusion complexes in these systems. These observations were in accordance with the results of the DSC studies. In the case of the diffraction patterns of the prednisolone-skimmed milk physical mixture, all the principal peaks of prednisolone and skimmed milk are present, although with a lower intensity than with the prednisolone and skimmed milk. This difference in peak intensities in the physical mixture is due to the amount of drug and carrier. The decline in the crystallinity of the physical mixtures (as evidenced by peak heights) compared with pure prednisolone was due to their composition since this was a pure substance being compared with a physical mixture of two substances with different diffraction patterns.

DSC Studies　　DSC has been shown to be a powerful analytical tool in the characterization of solid-state interactions between drugs and skimmed milk. Thermograms were analyzed qualitatively by

examination of both the peak temperature and the endothermic transition contour. The prednisolone thermal curve (Fig. 3) is typical of crystalline anhydrous substances and is characterized by a sharp endothermic effect (peak temperature at 239.5°C), assigned to its melting point, whereas the skimmed milk endothermic peaks are at 139, 165, and 205°C. The thermal curve of the complex (Fig. 3) is similar to that of skimmed milk and dissimilar to the physical mixture.

The DSC plot of solid dispersion showed only one broad peak at about 195°C. Since the DSC peak value of prednisolone is far from the melting point of prednisolone,[43] it could be concluded that the formation of an interaction between prednisolone and skimmed milk occurred. Disappearance of the specific peak of the drug indicated that the drug has interacted with the carrier. These observations indicate the

formation of an amorphous solid dispersion, the prednisolone molecule inside the skimmed milk cavity, and formation of a true inclusion complex.

SEM Studies   Microscopic investigations were undertaken on the skimmed milk, plain drug, physical mixture and solid dispersion using SEM. Micrographs revealed that the particle size of prednisolone in the physical mixture is similar to that of the pure drug. In contrast, the size of the particles of inclusion complex is smaller than that of the pure drug. The smaller the particle size, greater the wetted surface area, and hence the better the solubility. Furthermore, the surface morphology of the plain drug, its physical mixture, and the inclusion complex was investigated with SEM (Fig. 4). Micrographs indicate that the pure drug is in crystalline form whereas physical mixture possesses mostly amorphous particles and some crystals of the drug. In the case of the solid dispersion, the drug particles are almost in an amorphous state, confirming the formation of an inclusion complex.

Based on these findings, it is expected that the solubility of the solid dispersion may be greater than that of the physical mixture and the pure drug. Taking this into account, the further aim of this study was to investigate the solubility and *in vitro* dissolution behavior of the physical mixture and solid dispersion in comparison with the pure drug in the second part of this project.

CONCLUSIONS

An inclusion complex was prepared successfully with the prednisolone and the water-soluble complexing agent skimmed milk. It can be concluded that skimmed milk is a good carrier to prepare solid dispersions of prednisolone for oral administration. The properties of the prednisolone-skimmed milk complex were characterized using DSC, SEM, and XRD techniques. Comparison of DSC diagrams, XRD patterns, and SEM micrographs indicates that a chemical interaction takes place between skimmed milk and prednisolone while forming the inclusion complex for solid dispersion. SEM micrographs of the pure drug, physical mixture, and solid dispersion support this finding and also show evidence for the amorphous state and reduced particle size of the solid dispersion, which might improve the solubility, *in vitro* dissolution, and hence the bioavailability of the drug when administered by the oral route.
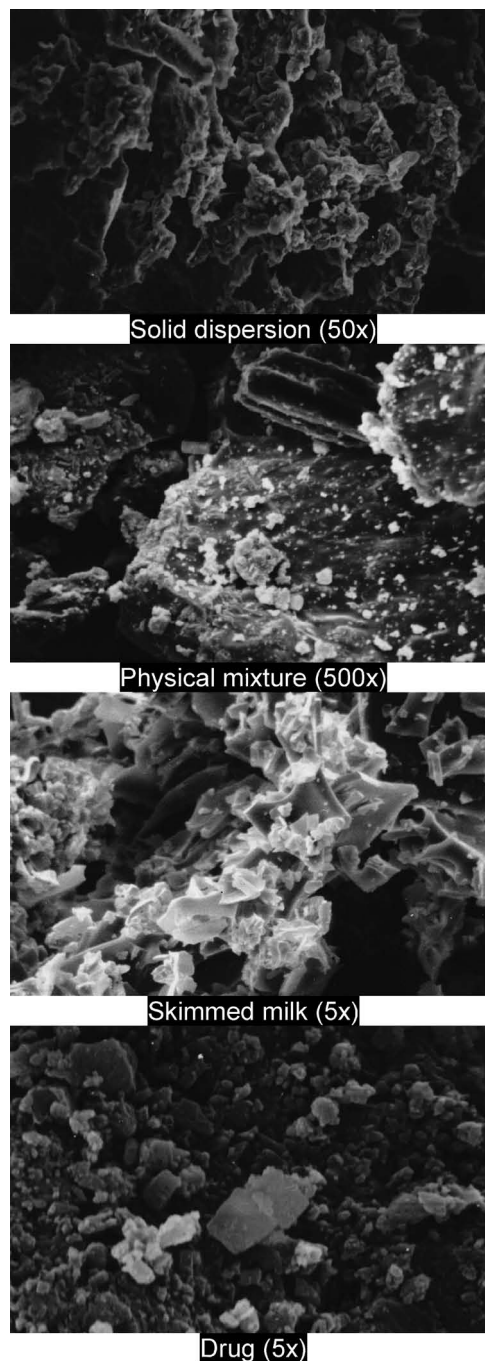

Fig. 4.   SEM of Prednisolone, Skimmed Milk, Physical Mixture, and Solid Dispersion

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