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RESEARCH PAPER

Evaluating the Dissolution Behavior of Zinc-Complexed Protein Suspensions by Computer Modeling and Simulation

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ABSTRACT

In vitro dissolution of zinc insulin suspensions can be promoted by the complexation of zinc with an ionic species for which the zinc ion has a greater affinity. Studies conducted by our group have previously shown that the rate-limiting steps that govern the dissolution of zinc-complexed insulin suspension may be (1) chemical complexation (surface reaction) and (2) subsequent drug mass transport (diffusion and solubility). The purpose of this work was to use a computer simulation model to predict the dissolution behavior of zinc-complexed insulin suspensions and determine the influence of the above rate-limiting steps on the overall process of dissolution. A quasi-steady-state model was chosen which included the effects of a shrinking particle radius, the drug's solubility, and a convective mass transfer term. Based on this model, the computer simulation program evaluated dissolution behaviors of various model drugs, including zinc insulin suspensions. The experimental data obtained from actual dissolution experiments were superimposed on computer-generated profiles that incorporated quantitative values to key terms, namely the α (diffusion resistance) and β (surface reaction resistance) values. Results demonstrated that the computer simulations could be used to predict the dissolution behavior of zinc-complexed protein suspensions by manipulating the α and β values. Overall, the computer

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simulations indicated the involvement of both the surface reaction and the diffusion rate-limiting steps in zinc insulin dissolution, which was consistent with the results obtained from actual experimental studies.

Key Words: *Computer simulation models; Diffusion and solubility; Dissolution; Surface reaction; Zinc insulin suspensions*

INTRODUCTION

The importance of zinc for the crystallization of insulin in the rhombohedral form has been recognized extensively from early studies (1). Addition of small amounts of zinc ions resulted in insulin preparations with prolonged effect. Also, the degree of prolongation at the same zinc concentration depended on the physical state of the suspended insulin particles, amorphous insulin particles having a shorter period of activity than crystalline insulin particles (2). The presence of zinc ions either in the bound or unbound state in equilibrium with each other within the crystal lattice most critically affects insulin solution properties. Zinc promotes a more physically stable crystal, one which is less prone to dissolve. While the behavior of zinc insulin preparations post-crystallization has been well characterized (3), the process of dissolution of these solids is not as clearly understood.

Early studies identified the dissolution process as the rate-limiting factor in the absorption of subcutaneously injected zinc insulin (4). Indeed, if the dissolution step in some way controls the release of drug and therapeutic availability, then an understanding of the mechanism by which zinc insulin suspensions dissolve, and the factors which influence the kinetics of this process, may be significant. In vitro dissolution studies of zinc-complexed insulin suspensions have established a fundamental understanding of the rate-limiting steps in zinc insulin dissolution (5,6). Our group has previously shown that complexation of the zinc with an ionic species with which the zinc has a greater affinity than it does for insulin promotes zinc insulin dissolution (7). This occurrence is due to a zinc concentration gradient effect and loss of crystal stability upon loss of the zinc ion. Therefore, the steps which seem to be controlling the release kinetics of zinc insulin are (1) loss of zinc from the crystal lattice (surface reaction via chemical complexation) and (2) insulin transport (diffusion and solubility).

The objective of the present study was to develop a computer simulation model to characterize the dissolution behavior of zinc insulin suspensions under the influence of each of the rate-limiting steps outlined above. Assessment was based on the calculation of quantitative values from superimposed experimental and simulated dissolution profiles obtained from the computer simulation program. Several parameters were incorporated into the computer program, such as a quasi-steady-state diffusion model in which the mechanism assumed sink conditions, a spherical particle shape, a diffusion coefficient independent of concentration, and an average particle size. Based on these parameters, the computer simulation program was used to test various model drugs, including zinc insulin suspensions for the prediction of their dissolution behavior. As mentioned earlier, previous experimental studies in this laboratory have shown a significant involvement of both surface reaction via complexation and diffusion steps in the dissolution of zinc insulins (5). The results obtained from the computer simulation model in this study were used to demonstrate the consistency of results obtained from previously conducted experimental dissolution studies.

EXPERIMENTAL

Materials

Human ultralente insulin suspension samples (25 μm) were donated by Eli Lilly and Company, Indianapolis, IN and used as received. Acetylsulfisoxazole, USP (ACSS) and prednisolone acetate (PA) were purchased from Roche Laboratories, Nutley, NJ (Code RO2-6222, Lot # A-25) and Sigma Chemicals, St. Louis, MO, respectively. Sodium lauryl sulfate surfactant, citric acid monohydrate, and other buffer salts were purchased from Fisher Scientific Company, Fair Lawn, NJ. The computer simulation program was developed for this project by the Department of Chemical Engineering at West Virginia University (WVU), Morgantown, WV.

Methods

Manufacture of Acetylsulfisoxazole and Prednisolone Acetate Suspensions

Both ACSS and PA suspensions were manufactured in the laboratory as per the accepted procedure (8). Briefly, saturated solutions of both ACSS and PA were prepared separately, filtered, and assayed at wavelengths of 285 nm for ACSS and 247 nm for PA using an ultraviolet (UV)–vis spectrophotometer (Beckman DU-65, Philadelphia, PA). A measured amount of ACSS and PA (10 mg each) was placed in separate glass mortars to which a suspending agent, sodium lauryl sulfate (SLS, 0.01 mg), was added. This was followed by the addition of 1 mL of saturated solution of either ACSS or PA, respectively. The wetted mass was transferred into a volumetric container and the required volume (100 mL) was made up with saturated ACSS and PA solutions.

Computer Modeling and Simulation

The appropriateness of an elementary transport model for describing zinc insulin dissolution can be tested by using the following assumptions applied to the model: (1) a surface reaction, whereby zinc ions complex with buffer ions for which they have a greater affinity than for insulin, leading to the removal of the metal ion and (2) diffusion of the protein from the surface of the solid into solution. Mathematically, a quasi-steady-state model (9) was used to represent the decrease in mass of the dissolving crystals. Here, the rate of loss of mass is equal to the steady-state flux:

$$\mathrm{d}M/\mathrm{d}t = -AJ \tag{1}$$

where $\mathrm{d}M/\mathrm{d}t$ is the dissolution rate, A is the surface area, and J is the flux. The steady-state flux, J, may be represented as:

$$J = \Delta c/(R_\mathrm{r} + R_\mathrm{d}) \tag{2}$$

where flux (J) is equal to the driving force for dissolution (Δc for drug particle) divided by the total resistance ($R_\mathrm{r} + R_\mathrm{d}$). The driving force for the drug's release (Δc) is the concentration difference between the surface and solution represented as ($c^* - c$). It is assumed that the concentration at the interface between two phases is at equilibrium and that the concentration at the solid surface is the solute equilibrium solubility. Assuming linear resistance, the denominator term is the sum of the individual resistances in series. It is common to refer to the reciprocal of the total resistance as overall mass transfer coefficient, K, which is defined here as:

$$1/K = R_\mathrm{r} + R_\mathrm{d} = 1/k_\mathrm{r} + 1/k_\mathrm{d} \tag{3}$$

where k_r and k_d are mass transfer coefficients for the surface reaction and diffusion step, respectively. Hence, the reciprocals of constants, $1/k_\mathrm{r}$ and $1/k_\mathrm{d}$, represent surface reaction resistance and diffusion resistance, respectively. Therefore, the flux expression can be written as:

$$J = \frac{c^* - c}{1/k_\mathrm{r} + 1/k_\mathrm{d}} = K(c^* - c) \tag{4}$$

The advantage of the above model is that the equilibrium and rate effects are separated. All of the rate effects are contained within the overall mass transfer coefficient, and the equilibrium effects are contained within the driving force. The above relationship was used to assess the influence of either resistance term on the dissolution of zinc-complexed insulin suspensions.

While the overall resistance to dissolution ($1/K$) was measured experimentally, in order to estimate the value of the surface reaction resistance ($1/k_\mathrm{r}$), the diffusion resistance ($1/k_\mathrm{d}$) was first determined. An expression was obtained for the mass transfer coefficient for diffusion (k_d) from a freely suspended spherical particle moving with fluid streamlines (10,11), assuming the following relationship:

$$Sh = \beta + \alpha Re^{1/2}\, Sc^{1/2} \tag{5}$$

where Sh is the Sherwood number (relating convection to diffusion transport), Re is the Reynolds number (characterizing flow), Sc is the Schmidt number (relating momentum transfer to mass transfer), and α and β are constants representing diffusion and surface reaction resistances, respectively. The three dimensionless terms (Sh, Re, and Sc) are further defined mathematically as:

$$Sh = kd/D = k(2R)/D \tag{6}$$

$$Re = dv\rho/\mu = (2R)v\rho/\mu \tag{7}$$

$$Sc = \mu/\rho D \tag{8}$$

where d is the particle diameter, v is the particle velocity, ρ is the solvent density, μ is the solvent viscosity,

D is the diffusivity, and k is the mass transfer coefficient.

As such, the approach of using dimensionless numbers to describe dissolution behavior is common in the field of chemical engineering. Previous studies have used this approach to describe the dissolution of benzoic acid spheres in moving streams of water (12,13). Specifically, the Reynolds number describes the ratio of momentum forces to viscous forces in a moving fluid, whereas the Schmidt number is the ratio of kinetic viscosity to molecular diffusivity. The last term, the Sherwood number, is the ratio of mass diffusivity to molecular diffusivity.

Substituting Eq. (6), (7), and (8) into Eq. (5) and solving for the mass transfer coefficient for diffusion (k_d), the following expression was obtained:

$$k_d = \beta(D/2R) + \alpha(Dv/2R)^{1/2} \quad (9)$$

However, in the absence of a surface reaction term (β) due to the absence of metal ion complexation, a simpler expression for the mass transfer coefficient [Eq. (9)] was obtained:

$$k_d = \alpha(Dv/2R)^{1/2} \quad (10)$$

The above Eqs. (9) and (10) were used as the operating equations for the computer simulation program.

The computer simulation program was developed by the Department of Chemical Engineering, West Virginia University using fourth-order Runge–Kutta technique to solve simultaneous equations for the particle radius (R) and concentration in the solvent (c) with time (14). Essentially, the program was a modification of a basic program for solving ordinary differential equations (ODEs). Using this technique, the dissolution data obtained from the experimental runs were first incorporated into the simulation program. Secondly, manipulation of the three terms (Reynolds, Schmidt, and Sherwood numbers) yielded the mass transfer expression from which the constants, α (diffusion resistance) and β (surface reaction resistance) values, were derived. These are constants that represent the resistance to mass transfer (diffusion) and surface reaction (complexation), respectively.

The significance of the α term is reflected in cases where it is assumed that the model chosen is diffusion-based, whereas the β term signifies a surface reaction-based model. By varying the values of α and β a computer-simulated curve was generated which was then superimposed over the experimentally generated dissolution curve. To counter the differences observed during superimposition of both the curves, adjustments were continued until a minimal sum of differences of the computer-generated curve to that of experimental data was obtained. Thus, α and β terms obtained from the operating equations [Eqs. (9) and (10)] could be manipulated to provide the line of best fit to the experimental data.

Dissolution Kinetics Experiments

Dissolution studies were conducted to generate experimental data for inclusion into the computer program. All studies were performed using a citrate-buffered aqueous medium (pH 7.4, 25±0.5°C) using the spin-filter dissolution apparatus, previously shown to be appropriate for formulation testing of suspensions (15). A measured amount of sample (10 mL) of each suspension (acetylsulfisoxazole, prednisolone acetate, and human ultralente insulin) was introduced separately into dissolution flasks containing 180 mL of the dissolution medium. Prior to the injection of samples, the dissolution apparatus was set up to assay samples on a continuous basis. For this, a peristaltic pump was connected to a flow-through UV spectrophotometric cell and placed inside a UV–vis spectrophotometer (Beckman Instruments, Philadelphia, PA). The pumping rate was maintained at 15 mL/min while the speed of stirring was set at 300 rpm. At predetermined intervals, samples from the flask were analyzed in the spectrophotometer at wavelengths of 285 nm for ACSS, 247 nm for PA, and 280 nm for human ultralente insulin. All studies were performed in triplicate. The dissolution profiles generated from these experiments were entered into the computer simulation program for further analysis and comparison.

RESULTS AND DISCUSSION

Although the overall resistance to dissolution can be measured experimentally, the knowledge of diffusion resistance (α) is essential in determining the influence of the surface reaction resistance term (β). Hence, the computer program was first calibrated with only the diffusion resistance term. To achieve this, model drug suspensions, ACSS and PA, were used, which unlike human zinc insulin have no surface reaction term (β). This is due to the lack of complexation of the zinc metal ion ligand to ACSS

and PA. Hence, β has little effect upon the dissolution profile and may be set at zero value in the computer program. After calibrating the computer model, the simulation was then employed to assess the influence of the two possible rate-limiting steps on zinc insulin dissolution.

The sensitivity of the simulations was first demonstrated by incorporating several values of α (diffusion resistance) into the computer program to determine the line of best fit. As shown in Fig. 1, using the model drug suspension of ACSS, the influence of increasing values of α provided simulated curves that extended beyond the range of the experimental data. As mentioned earlier, the surface reaction resistance term (β) was set to zero, since the drug ACSS is not complexed with a metal ion, and thus lacks a resistance at the surface of the solid. As the α values increased from 1 to 8, corresponding shifts in the positions of the simulated curves were observed. The importance in calculating the value of the α term is best illustrated when it is necessary to predict the diffusion-related behavior of a drug molecule.

Subsequently, another model drug, PA, was tested prior to final testing with zinc insulin suspensions. Dissolution data generated from PA was fitted into the computer simulation to generate the line of best fit. Again, there was no involvement of the surface reaction resistance term (β) since metal ions were not complexed with the drug molecule. As seen from Fig. 2, the α value was calculated to be 0.145, at which the computer-simulated graph provided a line of best fit with that of the dissolution data. Hence, the influence of diffusional resistance was evident from these profiles.

Application of the model to zinc insulin dissolution data using only the diffusion resistance term showed a difference in profiles of the simulated and experimental curves. As shown in Fig. 3, an α value equal to 0.04 and a β value equal to 0.00 showed a skewed appearance of the simulated curve. The surface reaction resistance term (β) was deliberately kept at a zero level initially to determine if changes in β could improve the fit of both curves. The deliberate lack of incorporating a surface reaction resistance term resulted in the simulated profile being placed considerably lower than the actual

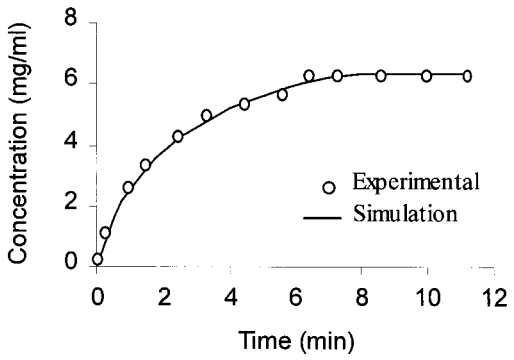

Figure 2. Model drug PA suspension dissolution simulation at calculated values of $\alpha = 0.145$ and $\beta = 0$.

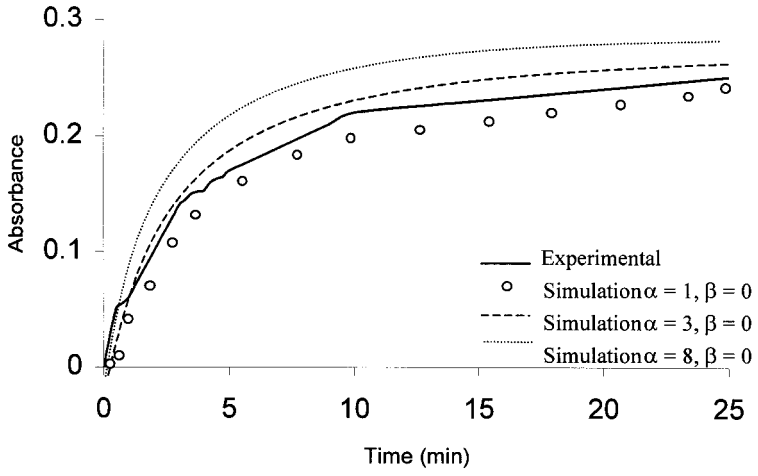

Figure 1. Sample dissolution simulations demonstrating sensitivity of diffusion resistance (α) term using model drug ACSS suspension.

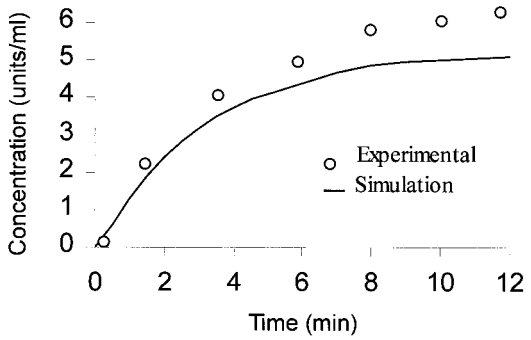

Figure 3. Human ultralente insulin dissolution simulation at calculated values of $\alpha = 0.04$ and $\beta = 0.00$.

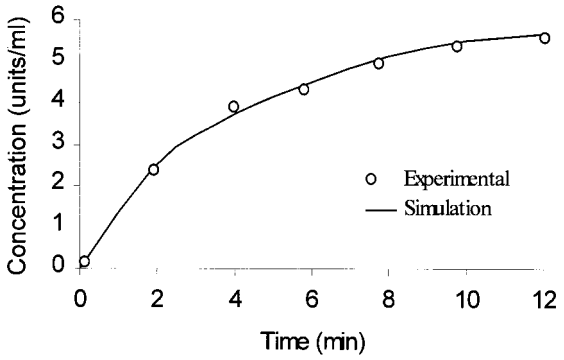

Figure 4. Human ultralente insulin dissolution simulation at calculated values of $\alpha = 0.04$ and $\beta = 0.002$.

experimental dissolution profile. It was determined that a better fit could be generated by the application of low calculated values of the β term. Thus, a minimal surface reaction resistance value of 0.002 was determined, as shown in Fig. 4. From this, it was clear that both the surface reaction and the diffusion term influenced the zinc insulin dissolution kinetics. These results are consistent with those obtained from experimental studies reported in a separate publication (7).

CONCLUSIONS

Results from these studies concluded that both steps (i.e., surface reaction and diffusion) influence the dissolution kinetics of zinc insulin suspensions. This was consistent with the data obtained during experimental studies showing the influence of both rate-limiting steps to be apparent (6,7). The computer model calculated representative α and β values to demonstrate the influence of resistance terms on the dissolution of zinc-complexed and non-complexed pharmaceutical suspensions.

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