This is to certify that the

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DISSOLUTION SHELF-LIFE OF UNPACKAGED SOLID ORAL DRUG PRODUCTS

presented by

Sheau-shya Wu

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of the requirements for

MASTER'S degree in PACKAGING

Hugh E. Lockhart
Major professor

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DISSOLUTION SHELF-LIFE OF UNPACKAGED SOLID ORAL DRUG PRODUCTS

By

Sheau-shya Wu

A THESIS

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ABSTRACT

DISSOLUTION SHELF-LIFE OF UNPACKAGED SOLID ORAL DRUG PRODUCTS

By

Sheau-shya Wu

If the relationship between drug dissolution, storage temperature and humidity were known, it would be easy to select the best materials for stability testing and marketing. The dissolution characteristics of one drug product have been determined. This information will allow selection of packages for the drug product. The drug product was Axid (Nizatidine capsules) provided by Eli Lilly.

The Axid capsules were stored at three temperatures (18°C, 28°C and 38°C) with nine relative humidities (12, 22, 33, 44, 50, 63, 75, 80, and 90% RH). The capsules were stored unpackaged, in open dishes. Samples were taken at 6 days and then at 30 day intervals for dissolution testing. Dissolution vs. relative humidity isotherms were obtained for Axid stored at the three temperatures and nine relative humidities for ninety days. Several dissolution failure points were identified, and a shelf life for unpackaged Axid was determined.

The dissolution stability of Axid (Nizatidine) was greatly reduced when capsules were stored at 38°C and around 75% RH. Therefore, the critical relative humidity inside the package could be identified as 75% RH. The dissolution stability was found to be reduced at 28°C, 80 and 90% RH as well.

This thesis is dedicated to my lovely parents

Meng-hua Wu and Zing- tsu Wu

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Many people provided guidance and encouragement to me over the last two years as the work in the thesis progressed. I would like to take this opportunity to acknowledge some of them.

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In addition, I would like to thank Dr. Sue Selke and Dr. Gililland for their prompt and thoughtful discussion of this thesis. Each individual provided expertise and valuable input regarding the research required for this area of study. The many students, both past and present, have provided much help to me during my time at Michigan State. Specifically, I want to thank X. Qian, M. Kokitar, K. Tanprasert and K. T. Feng for their academic advice and friendship over the years. Jay Lyle Chick deserves special thanks.

He has had no choice but to provide answers as I chased him around the department with my persistent questions for the last six months.

It was a privilege to work with the Center for Food and Pharmaceutical Packaging Research at MSU. I am grateful for the financial support and continued interest in this project. I deeply appreciated all of the materials and information supplied by Eli Lilly Company, as well, for use in this research area.

Finally, and most importantly, I want to thank Mr. and Mrs. Wu, my father and mother. Throughout my life, their encouragement and guidance have always served to point me in the right direction. And I would like to thank my family for their continued love and support during my prolonged absence from Taiwan.

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Chapter 1

INTRODUCTION

Currently, in the pharmaceutical industry, a major concern lies in the dissolution shelf life of solid oral drug products. To determine which packaging materials will prevent moisture interactions with product from adversely affecting a product throughout its shelf life is very important. It is necessary to know the expiration date of a drug product, and the expiration date is based on chemical, and physical characteristics of the drug product as well as upon estimates of the bioavailability of the active ingredient.

Product development research includes the estimation of expiration date based upon early research, and storage under accelerated conditions. This estimation is based on information about the kinetics of the chemical reaction or reactions that lead to degradation of the drug product. These predictions measure the loss, or change in concentration, of the active ingredient in the drug product.

Bioavailability of the active ingredient in the drug product has become a major factor in evaluation of the expiration date. Bioavailability refers to the ability of the drug product to release the active ingredient into the gastro-intestinal tract after ingestion. Bioavailability is affected not only by chemical changes in the drug, but also by physical changes in the drug product that affect the release of the active ingredient. For tablets, hardness, disintegration, and dissolution are drug product characteristics that affect the

degree of bioavailability of the active ingredient from the drug product. For capsules, solubility of capsule, disintegration of capsule contents and dissolution affect bioavailability. Changes in these characteristics are physical changes. They are not described by the kinetic equations used for the chemical properties of the drug.

Dissolution has been accepted by the United States Pharmacopeial Convention as a measure of bioavailability and as a stability indicating parameter for solid oral drug products. It is measured by a standard method published in the United States Pharmacopoeia (U.S.P.) and dissolution limits are specified in the U.S.P. monographs for most solid oral drug products. The US Food and Drug Administration (FDA) requires that any drug product on the market must at all times meet the requirements of the USP monograph. If it fails to do so, the product is subject to recall action. During the last year there have been 16 recalls of solid oral drug products because of failure to meet USP dissolution requirements.

The specific objectives of this study are listed below :

1. To measure the dissolution value of a solid oral product using USP standard methods for several time periods at different storage conditions
2. Plot the values for dissolution at the 30 minute time point in the dissolution profile, because the 30 minutes dissolution value is the USP requirement for the drug product under study.
3. To use statistical methods to analyze for significant differences in dissolution for capsules stored at three different temperatures and nine humidities.
4. To use the above information to generate dissolution isotherms for the product.

5. To identify dissolution failure points on the isotherm.

6. To determine if dissolution as an endpoint indicator of shelf life can be used in shelf life prediction for solid oral drug products.

Chapter 2

LITERATURE REVIEW

2.1 Dissolution

Many drug products should be protected from temperature and humidity changes to maintain acceptable product quality throughout their shelf life. There has been a considerable increase in the interest in physical aging of capsules, or dissolution value of capsules (Hoblitzell et al., 1985)

Dissolution stability of a drug product may be defined as the maintenance of the dissolution characteristics of the product from the time of manufacture up to the expiration date. Release of a drug from the dosage form into the gastrointestinal fluids is an essential first step in drug absorption and bioavailability, so dissolution is a critical parameter in determining the performance and defining the quality control, regulatory compliance, and impact on the bioavailability of solid oral products. Significant changes in the in-vitro release profiles of a drug product during storage may indicate a change in bioavailability. Formulation components (active drug, excipients, and coating materials), processing parameters, storage conditions, and packaging are all factors that can alter the dissolution stability of a product during aging.

A drug product may undergo changes in physico-chemical characteristics that can alter the bioavailability of the dosage form during storage. Important physico-chemical

parameters that decide the quality of the preparation and that are susceptible to change during aging include appearance, chemical assay, degradation product level, moisture content, dissolution rates, and for tablets, hardness, friability (and disintegration times). The dissolution profiles of an oral solid product can impact the rate and the amount of drug available for absorption and therefore may affect the therapeutic efficacy of the product. When a product is stored under the conditions specified on the label, it is expected that the product will retain its initial dissolution profiles throughout its shelf-life. One of the reasons for product recalls is failure to meet dissolution specifications during storage (Murthy and Ghebre-Sellassie, 1993).

2.2 Factors Influencing the Dissolution Stability of Solid Oral products

Primary among these are the six factors; manufacturing processes, formulation variables (e.g., physico-chemical properties of the active and inactive components), in-process controls, dissolution method, storage conditions, and packaging. In nearly all situations, the result is controlled by more than one factor. Therefore, the effects are difficult to outline by the particular factors alone. For instance, the effect of adverse storage conditions on drug release may be intensified by poor packaging. On the other hand, effective protective packaging can improve the stability of a product during aging. Any one of the four factors, formulation variables, processing factors, packaging, and storage conditions acting alone or in concert can influence the product shelf-life.

2.2.1 Formulation Variables

During storage, dissolution stability of a solid oral drug product is closely connected to the qualitative and quantitative composition of the formulation. There are some analytical parameters such as the solubility, hygroscopicity, and thermal properties of the active component and excipients, including coating materials, which have apparent impact on the result of dissolution stability. For example, the active drug may dissolve and recrystallize and in the process change the release properties of tablets during storage under high humidity conditions. Furthermore, a tablet may absorb or lose moisture and harden, thus changing the disintegration properties of the dosage form, depending on the storage conditions. The effect of moisture and heat on the integrity of the coating on tablets mainly affects the dissolution stability of the product. Generally, enteric- and sugar-coated products are more sensitive to the effects of humidity than uncoated or film-coated products. Consequently, formulation variables, fillers, disintegrants, binders, and coating materials are decisive to the maintenance of the dissolution stability of the dosage form.

2.2.2 Binders and Disintegrants Factors

Binders and disintegrants have opposing roles in a tablet formulation. Since a binder in a tablet formulation is to provide the necessary adhesion and bonding between particles, disintegrants are added to facilitate the dividing of the tablet mass, to increase the surface area, and thus promote dissolution. These two components of a tablet formulation should be chosen together because decrease in the dissolution rate of the product during storage due to binder effect can sometimes be overcome by the inclusion of a proper disintegrant.

2.3 Effects of Temperature and Relative Humidity on Gelatin Capsules

The effects of storage time and relative humidity on the disintegration and in-vitro drug release of 4 brands of chloramphenicol capsules has been studied (Khalil et al., 1974). At 49% and 66% RH no obvious change in either disintegration or drug release occurred for storage periods up to 32 weeks. The drug suffered nearly total decrease in drug release due to the failure of the gelatin shell to disintegrate when stored at 80% RH. Drug release was slightly increased after storage at 100% RH. Nevertheless, the gelatin shells were rubbery, soft and difficult to handle.

A significant decrease in the amount of chloramphenicol released due to impaired disintegration was the result when the capsules were stored at 80% RH. In contact with the dissolution medium, the gelatin shell which was rubbery showed a considerable swelling and failed to dissolve within one hour. It is difficult to reflect the exact changes which might have occurred in the gelatin shell upon storage at 80% RH, but it is probable that cross linking occurred at this RH and caused changes in the dissolution property. This was given by Khalil et al.(1974) as the reason that the gelatin shell was rubbery and appeared to swell but did not dissolve.

2.4 Effects of Aging

Environmental conditions such as relative humidity have a considerable effect on the active substances in their various dosage forms, and this effect can be reflected in the bioavailability of the drug. During storage, this might be revealed as a decrease or loss in therapeutic effectiveness. The release rate determination of the active substance just after

preparation might not be an absolute reflection of the release pattern of the drug after aging storage (Akbuga et al., 1984).

2.5 Dissolution and Storage Conditions

Although formulation and process variables are mainly determinants of the in-vitro drug release of capsule products at the time of their manufacture, storage conditions significantly affect the stability of these preparations and influence their dissolution profiles during aging.

Gelatin is water-soluble at 37°C (Clark and Courts, 1977). The insolubility of gelatin that resulted from storing the capsules under stress conditions was probably caused by a polymerization process involving cross-linking and hydrogen bonding (Marks et al., 1968). A number of reagents that promote such cross-linking have been identified. These include aldehydes, sulfuric acids, hydrogen peroxide, and glucose. Specific interactions can occur between carboxyl and amino groups of gelatin and the various functional groups of the dyes involving ionic, hydrogen, and van der Waal's bonding, leading to an irreversible denaturation reaction. These reactions presumably are facilitated by UV or visible irradiation under high-humidity conditions. Dissolution changes were occurring in capsule products under accelerated conditions of storage depending on the storage conditions, the colorants present in the capsule shell, the aqueous solubility of the drug substance involved, and the test procedures used to measure drug dissolution.

Significant changes in drug-release patterns were observed with colored capsules only under the combined influence of high humidity and intense UV or visible radiation.

The plastic bottles and caps typically used to store and dispense pharmaceutical products would afford a high degree of protection against humidity and light. Nevertheless, the findings of this study have shown that storage conditions can play a significant role in the stability of these products and in their drug dissolution profiles over time.

Accelerated stability evaluation of hard-shell capsule products has shown that drug dissolution rates can be impaired by storage under high-stress conditions. For example, the denaturation of gelatin causes changes to occur in the conformational properties and solubility properties of the capsule shell (Murthy et al., 1989).

Another study found that the dissolution stability of hard gelatin capsule (HGC) products is decided by the moisture content of the shell, and this moisture content is related to the storage conditions. Gelatin is the main component of the capsule shell which is a heterogeneous mixture of polypeptides. The shells of HGC normally contain ~ 13.0-16.0% water and can be safely stored between 40 and 60% RH. Variations within the range 12.0-18.0% moisture do not seriously influence the integrity of the shell. Below ~12% water, the shell becomes brittle and is easily split. Above 18% moisture, the shell is moist, soft, and deformed with a propensity to transmit moisture to the capsule contents if the contents are hygroscopic (Murthy and Ghebre-Sellassie, 1993). Such a situation seems to prevail when the filled capsules are stored in high humidity environments. The contents will become moist and form a cake which may dissolve poorly.

During storage, changes in the dissolution characteristics of the capsule products occur due to either a physico-chemical change in the basic structure of the capsule shell or a change in the properties of the capsule contents. One of the effective ways of preventing

moisture transfer from the capsule shell to the capsule contents is to equilibrate each of the shell and the capsule blend, separately to the same relative humidity (e.g., 40-60% RH) so that no transfer of moisture happens to and from the shell (Murthy and Ghebre-Sellassie, 1993).

2.6 Effect of Packages on Dissolution

The role of packaging in dosage form design is to serve as a barrier to moisture transfer from the environment to the enclosed product and to protect the product from the effects of heat and oxygen. Packaging cannot protect the product from the effects of heat. Packaging has been accorded a lower priority relative to formulation activities and storage conditions in the design and development of dosage forms. Although packaging development is considered to lie outside the mainstream of product development, it is a main element that controls the result of dissolution stability during storage and should be treated as an integral part of developing stable and effective pharmaceutical products.

One consideration in the selection of packaging materials is the retention of identity, strength, quality, and purity of the given drug product for the duration of its shelf life. The formulation should retain the dissolution characteristics of the product during normal storage conditions. The use of proper packaging materials usually provides an extra element of protection for the product; the degree of protection afforded is dependent on the nature of the packaging materials used, including the permeation characteristics, and the environment in which the product is stored (Murthy and Ghebre-Sesllassie, 1993).

The effects of packaging and storage in multiple-unit and unit-dose containers on dissolution rate of model prednisone tablets were reported by Taborsky-Urdinola et

al.(1981). USP Prednisone Dissolution Calibrator Tablets were packaged in three multiple-unit and five unit-dose containers. Packaged tablets were stored for three to six months under three conditions: 40°C, 85% relative humidity (RH); 37°C, 75% RH; and 22°C, 75% RH. Dissolution rate was measured at pre-determined intervals during storage. For each condition tested, two separate runs of six tablets each were performed. Tablets in the least moisture-permeable containers were least affected by storage. The conditions of high heat and humidity caused the greatest change in dissolution rate. When stored at 22°C and 75% RH, little change in dissolution rate occurred in any packaged tablets (Taborsky-Urdinola et al., 1981).

The practical importance of packaging, and storage under controlled conditions was described. Proper packaging and storage are essential to maintain product integrity. The permeability of the container is critical in situations where the product is to be subjected to less desirable conditions, arbitrarily those in excess of 22°C and 75% RH. (Taborsky-Urdinola et al., 1981).

Chapter 3

EXPERIMENTAL MATERIALS AND METHODS

3.1 Materials and Equipment

1. Axid (Nizatidine) Capsules, 150 mg, Lot 7CT 31 from Eli Lilly
2. VK 7000 Dissolution Test Station (VanKel, VANKEL INDUSTRIES, INC.)
3. P-5000, 1 mL Pipetteman; 1 mL and 5 mL polypropylene pipette tips (from Rainin)
4. Cotton (from Target)
5. 5 mL disposable plastic syringe for sampling
6. Sampling tube- An 8 inch length of plastic tubing of 0.16 inch diameter. Conton is packed into about 1 inch length at one end of the tube to act as a filter. A 5 mL syringe is attached to the tube at the filter and of the tube to draw the sample from the dissolution vessel. The cotton filter serves to remove drug product particles that could interfere with the light beam in the spectrophotometer (Qian, 1996).
7. Spectrophotometer Lambda 3 (Perkin Elmer) for absorbance measurements
8. SAS system (Version 6.0) for statistical analysis

3.2 Dissolution Methodology

Dissolution treatment and sampling The testing was performed using a USP paddle apparatus in 900 mL of deionized, deaerated water at 37.3°C. The water is the dissolution

medium. The sample size for this experiment was three capsules for each condition. A vinyl coated paper clip was attached to a capsule (on the yellow cap) to allow the capsule to sink to the bottom of the vessel before rotation of the blade is started. The paddle rotation speed was 50 rpm. The procedure ran for 50 minutes. With the sampling tube and 5 mL syringe 2-3 mL sample was taken at 5, 10, 20, 30, 40, 50 minutes from the middle of each vessel. The sampling solution was filtered by the cotton in the sampling tube. So, the solution was transparant and clean. Then the sample was put into a plastic bowl. Using a 1 mL Pipetteman with 1 mL pipette tip, a 0.4 mL sample was drawn from the bowl. The filtered sample was diluted to 20 fold with deionized water, and the absorbance of the diluted sample at 314 nm in the spectrophotometer.

Absorbance measurements To determine the amount of Nizatidine dissolved, absorbances at the wavelength of maximum absorbance for Nizatidine at about 314nm was used. The raw readings will use 48.96 ml/mg of USP Standard Nizatidine concentration (the slope of calibration curve) to calculate the concentration and dissolution value of samples. This information is then used to plot curves which represent the dissolution profile of a product.

Calculation equation

$$\% \text{ dissolved} = (Abs/S) \times n \times V/W \times 100$$

where S: slope of the calibration curve for Nizatidine, 48.96 ml/mg

n: dilution fold of the sample, 20 times

V: volume of the dissolution medium in the vessel, 900 ml

W: the amount of the Nizatidine in each capsule, 150 mg

Statistical analysis

The SAS system (Version 6.0) was used to analyze the dissolution data by 2-way and 3-way ANOVA (analysis of variance). The 30 min dissolution data reported in Tables 7-9 were obtained by averaging the dissolution results of three separate capsules. Then, the 30 min dissolution value was utilized to run the General Linear Models Procedure and results were summarized in an ANOVA table. Significant differences (p-values < 0.05) were detected for storage times, humidity, and temperature; which indicated the dissolution rate of capsules is a function of these parameters (SAS/STAT User's Guideline).

3.3 Storage Conditions

The three temperatures used were 18°C, 28°C, and 38°C in order to cover the entire temperature range the product is likely to encounter. At each temperature, nine humidity levels were used, sampling from 12% RH to 93% RH. The humidities are shown in Table 1. The humidity buckets were prepared by placing nine different saturated salt solutions into tightly closed 5 gal plastic containers. Calibrated hygrometer sensors were installed in each bucket to monitor the humidity values. Humidities were measured at 0, 3, and 6 months. The dissolution test was determined at four different storage times - 6, 30, 60 and 90 days.

3.4 Storage Exposure

The capsules were stored in open dishes in the buckets. They were not in any package. Therefore this experiment found the response of the capsule product to the humidity at each temperature.

3.5 Sampling Intervals

Samples of stored capsules were removed for dissolution testing at intervals of 6, 30, 60 and 90 days.

3.6 Dissolution Isotherm

Because of the open dish exposure of the capsules, the resulting data represent dissolution/RH isotherms for the Axid capsules. This is analogous to the moisture isotherm used for moisture sensitive foods and other products.

Table 1. Equilibrium relative humidities for saturated salt solutions at three temperatures

Saturated Salt Solution	Percent Relative Humidity at Stated Temperatures 18°C	28°C	38°C
Lithium Chloride $LiCl \cdot H_2O$	16.10	12.10	14.20
Potassium Acetate $KC_2H_3O_2$	22.50	22.00	23.60
Magnesium Chloride $MgCl \cdot 6H_2O$	36.75	32.50	35.25
Potassium Carbonate $K_2CO_3 \cdot 2H_2O$	45.75	43.75	44.50
Magnesium Nitrate $Mg(NO_3)_2 \cdot 6H_2O$	55.00	51.50	53.75
Sodium Nitrite $NaNO_2$	67.00	64.00	63.25
Sodium Chloride NaCl	74.70	75.20	75.70
Ammonium Sulfate $(NH_4)_2SO_2$	79.20	80.00	80.20
Potassium Nitrate KNO_3	92.80	91.00	89.80

Note: The humidity values in the table are the actual values measured, verified after 30 and 60 days storage. In reporting, these values may be rounded to 2 places, and grouped into categories.

Chapter 4

RESULTS

Figures 1 and 2 show typical dissolution profiles. Figure 1 is the profile for Axid capsules as manufactured, and for any time during their shelf life when no deleterious changes have taken place. This profile was generated for fresh capsules stored at room temperature in a high density polyethylene bottle in a desiccator. Drug release was quick from the fresh capsules, and almost all of the drug was in solution within 30 min. The differences in the amount of drug dissolved at the initial sampling time of 5 min. were caused by small differences in the dissolution times of the capsules. Figure 2 shows a dramatic change in the profile that has taken place after 32-34 days at equilibrium with 75% RH and 38°C. Note that the 30 minute dissolution has been reduced from 95% dissolved to about 50% dissolved. USP requires 75% dissolved, so this product is headed for a shelf life problem.

Dissolution Isotherms

Figures 3, 6, and 7 describe the dissolution isotherms for Axid capsules at three temperatures and four different time intervals. The 30 min dissolution is used because it is the one specified in the USP monograph for Nizatidine (Axid). The SAS system analysis (2-way and 3-way ANOVA) results show that temperature, storage time, and relative humidity will affect the dissolution values (p-values < 0.05) (Table 10). The percent drug

dissolved has significant differences for four different storage time intervals at high temperature (38°C) and high relative humidities (75% and 80% RH). At 28°C and 75% RH, there is a significant difference in drug dissolved after 90 days. At three different temperatures, the dissolution values have small, but significant differences at 6 and 90 days storage in different relative humidities.

Dissolution at 18°C

Figures 4 and 5 show the time effect for dissolution of Axid at 18°C. The 30 min. dissolution values did not change much over the whole testing range of relative humidities during 90 days storage. There is a significant difference (p-values < 0.05) in dissolution value of capsules after storage at 93% RH for 90 days. The dissolution value decreased from about 100% to 90%. The information has helped establish the length of time that capsule products can be exposed at 93% RH before detectable changes occur in their dissolution rates. There is no change in dissolution of the product until the 90th day. So, the result showed that the time effect may occur at a higher humidity, and after a longer time. There is no change in appearance of capsules at 18°C.

Dissolution at 28°C

Figure 6 describes the dissolution isotherm for Axid at 28°C at four time intervals. Capsules stored at higher relative humidity (75%-90% RH) for 30 days are soft and sticky. They would fail on that without doing a dissolution test. Note that there is no loss of dissolution at any relative humidity through 60 days. By 90 days, note the progressive loss in dissolution at 75% RH. The product has failed sometimes between 60 and 90 days.

When coming into contact with the dissolution medium (deionized water), the aged capsules swelled and formed a partially insoluble gelatin shell. A swollen, rubbery, gelatin shell which enveloped the encapsulated powder, was observed during the dissolution testing. The capsule shell broke up so slowly, that powder release through this gelatin shell was rate-limiting for the dissolution process. This effect was apparent in the capsules tested at the end of three months of storage at 75% RH (Figure 6). Drug dissolution was reduced under this storage condition; only about 65% of the drug was in solution after 30 min.

Dissolution at 38°C

The combined effect of high temperature and humidity on the dissolution performance of stored capsules is exhibited in Figure 7. Note the progressive loss in dissolution at 75% RH. The product has failed before 30 days. The capsules became soft and sticky when they were stored at high humidity (75-90% RH). So, they are failed samples. Capsules stored at 75% and 80% RH, were rubbery, forming a big bubble during the dissolution testing. The bubble trapped the powder, and this ballooning phenomenon resulted in a lower dissolution value.

Notice the behavior at 90% RH. The product seems to lose dissolution, then regain it. However, this does not mean the product becomes usable again. At 90% RH, the capsules were sticky, soft, and fell apart because the gelatin has absorbed so much water. The crucial roles played by moisture and heat in affecting the conformational properties of gelatin and rendering it insoluble are clearly evident from the 30, 60 and 90 day points on the graph at 75% RH and 80% RH. About 62% of the drug was dissolved at 75% RH and about 68% of drug was dissolved at 80% RH (Table 7). Delayed disintegration of the

capsule shell was the primary reason for the slow release of drugs. As time goes on, the dissolution value decreases under storage at 75% RH and 80% RH. After storage for three months, only about 15% of drug dissolved at 75% RH and 49% of drug dissolved at 80% RH (Table 7). The aged capsules showed a huge effect on drug dissolution (p-values < 0.05) when stored at 75% RH at the high temperature (Table 6). There was some delay in the disintegration of these capsules, and a big, insoluble gelatin film was observed during the dissolution experiments, suggesting that heat and humidity accelerates the reaction leading to the denaturation and cross-linking of gelatin.

Figures 8 and 9 present the results of the effect of storage time on the dissolution profile of the capsules after storage at 38°C. The dissolution value decreases with longer storage time at higher relative humidities. Figure 8 shows dissolution after storage below 60% RH at 38°C. There are no significant changes in dissolution over time. Figure 9 presents the effect of storage time on the dissolution profile of capsules after storage above 60% RH at 38°C. There were apparent effects on the dissolution profile of stored capsules at 75% RH and 80% RH (p-values < 0.05) (Table 6). When capsules were stored at 90% RH and high temperature (38°C) for three months, there was no apparent change from initial dissolution patterns. The aged capsule shell dissolved rapidly and exposed the powder bed, which subsequently broke up, resulting in rapid drug dissolution (Figure 7). However, the capsules stored at 90% RH were not usable because they were sticky and soft. They had failed before doing the dissolution test. Therefore the appearance of "return to initial dissolution" is a false positive.

Figure 10 describes the effect of temperature on the dissolution profile of capsules after storage at 75% RH. The dissolution value decreased more when capsules were stored at 38°C for longer time. The lower temperature (18°C) does not seem to affect the dissolution value of stored capsules. There was no apparent change from initial dissolution patterns to be seen during the dissolution experiments. Capsules stored at 90% RH, at all three temperatures, stuck together, and some of them were already broken before doing dissolution testing. These are failed samples even before doing the dissolution test.

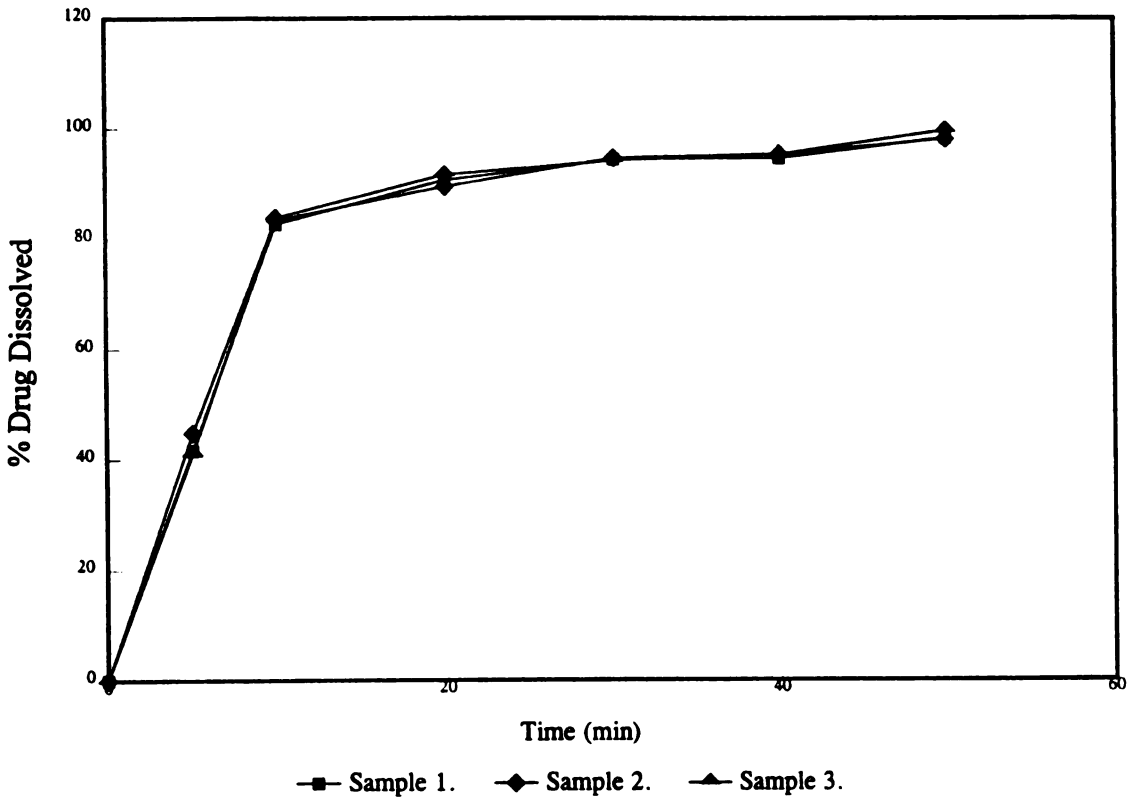

Figure 1. Initial dissolution profile of Axid capsules. It is the typical dissolution profile for three Axid capsules as manufactures, and for any time during their shelf life when no deleterious changes have taken place. Drug release was quick from the fresh capsules, and almost all of the drug was in solution within 30 min.

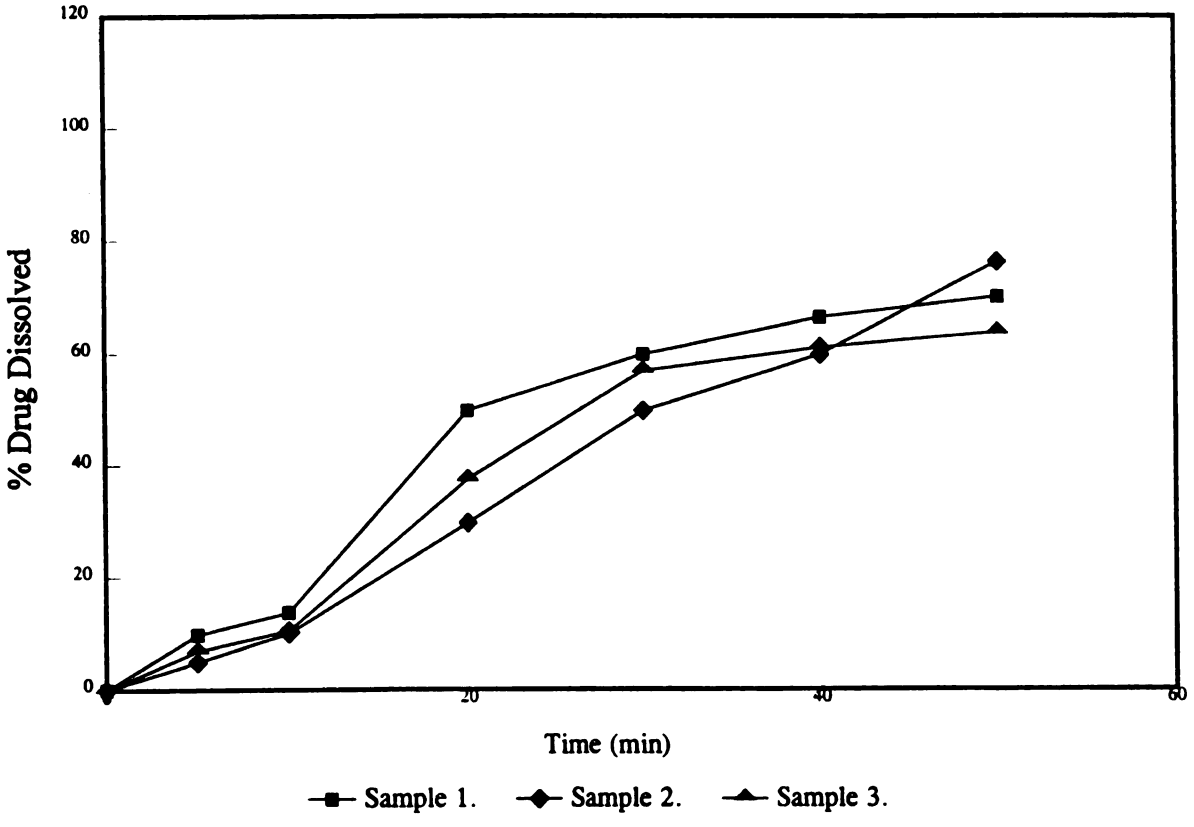

Figure 2. Dissolution profile of capsules after storage at 75% RH and 38°C for 32-34 days. Notice that the 30 min dissolution has been reduced from 95% dissolved to 50% dissolved.

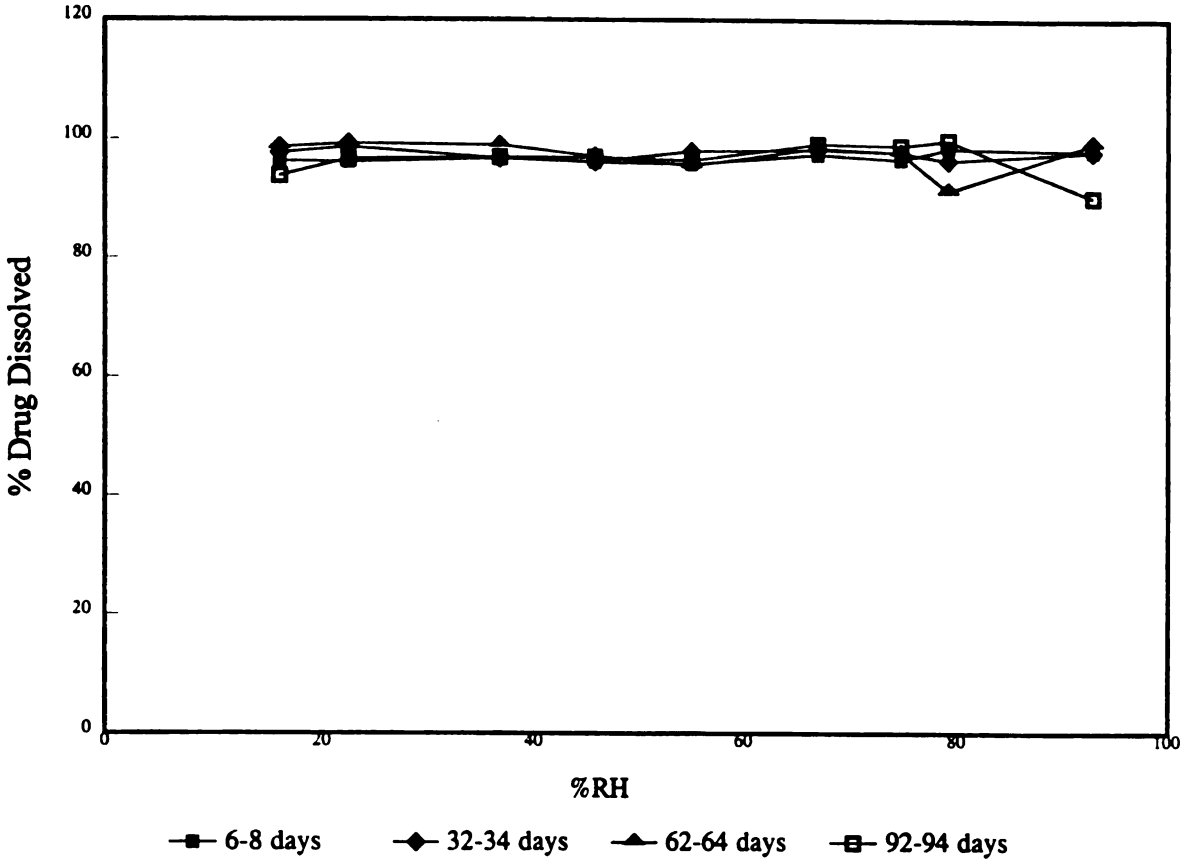

Figure 3. The effect of RH on 30 min dissolution of capsules after storage at 18°C. There is no change in appearance of capsules at 18°C. The 30 min dissolution values did not change much over the whole testing range of RH during 90 days storage.

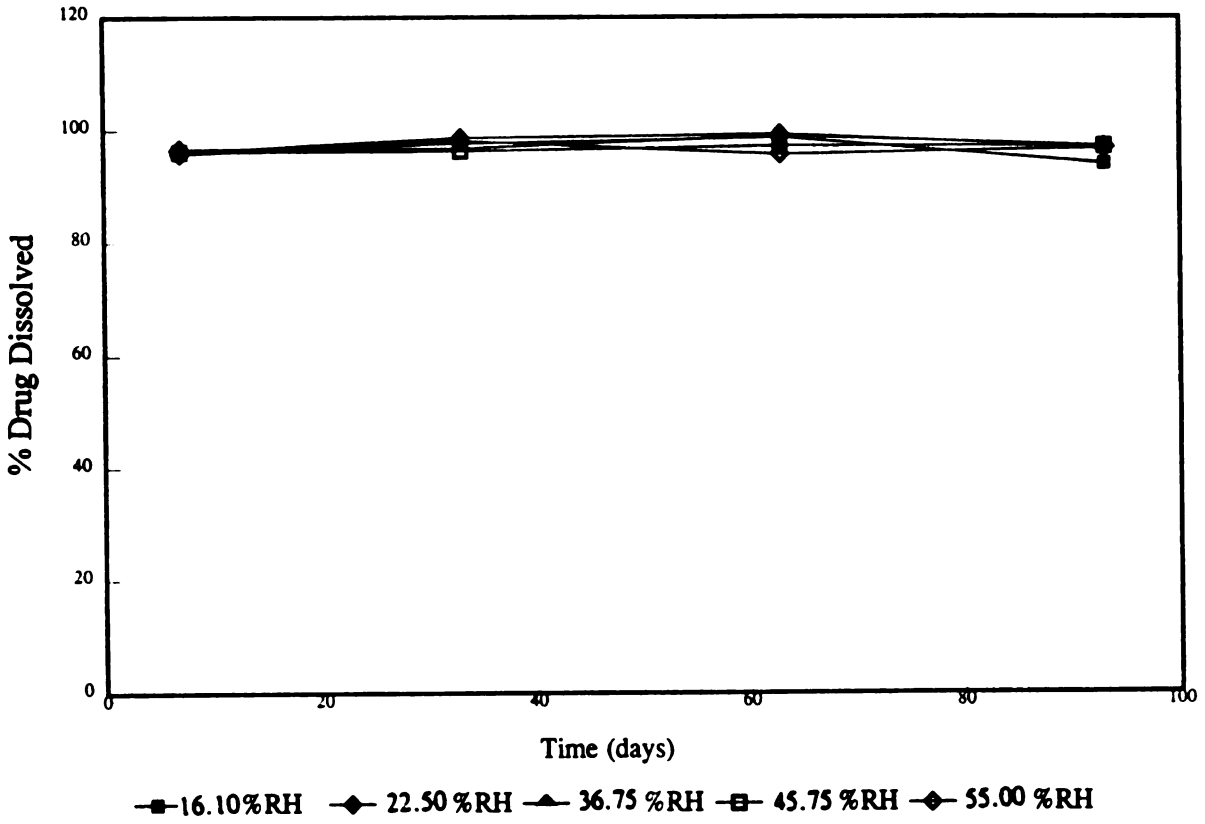

Figure 4. The effect of storage time on 30 min dissolution of capsules after storage at 18°C (below 60% RH).

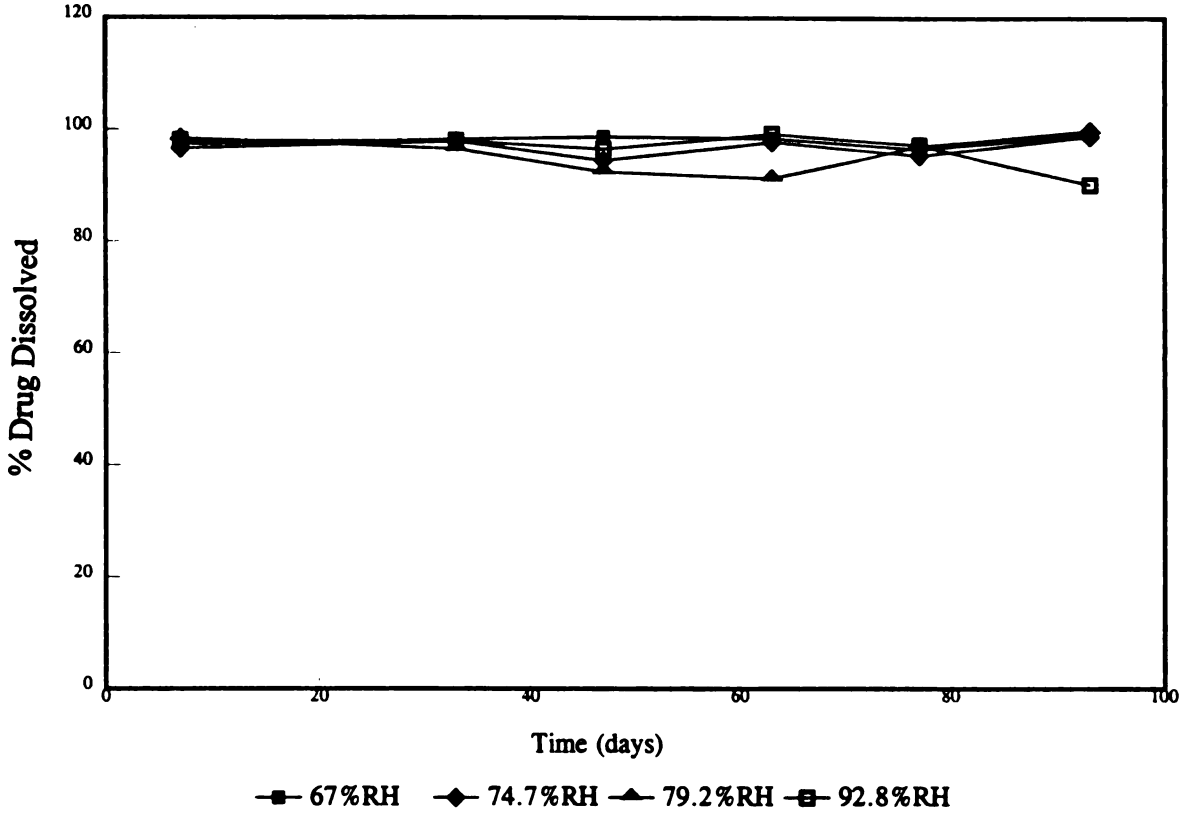

Figure 5. The effect of storage time on 30 min dissolution of capsules after storage at 18°C (above 60% RH).

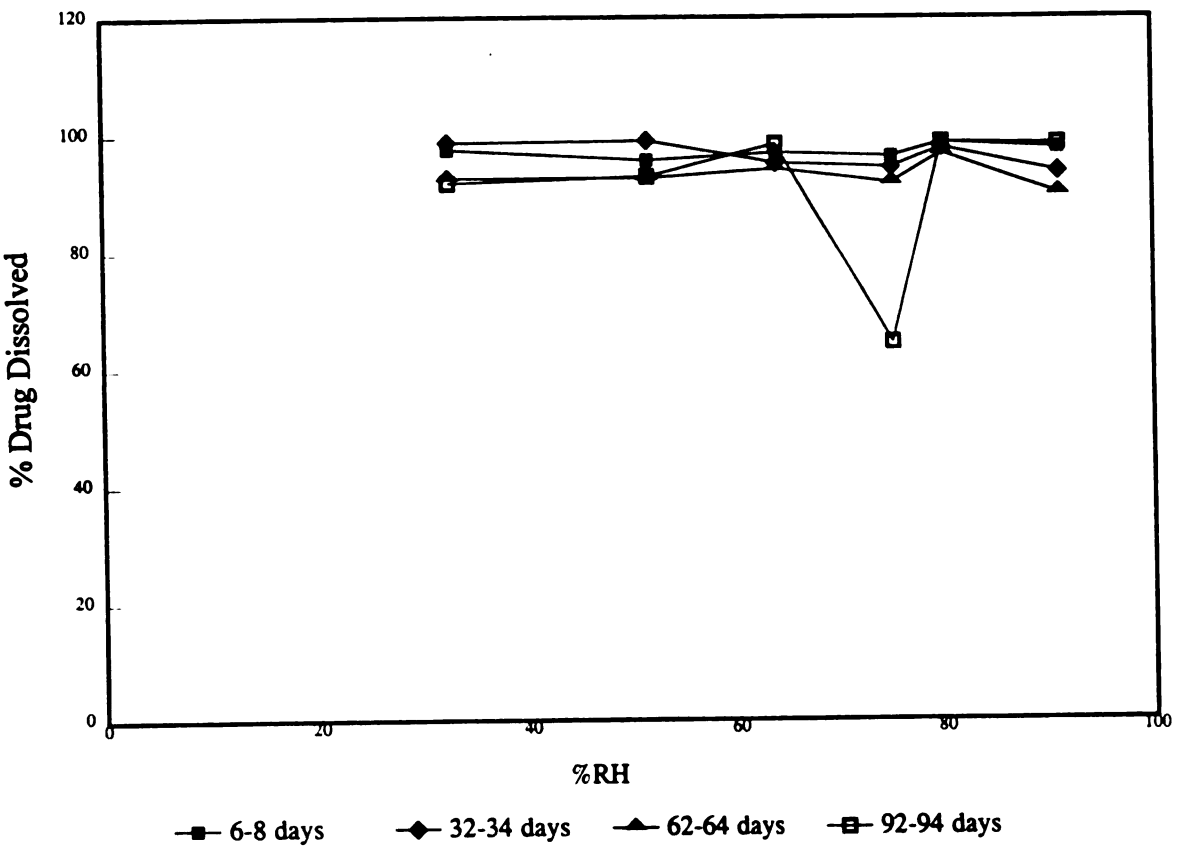

Figure 6. The effect of RH on 30 min dissolution of capsules after storage at 28°C.

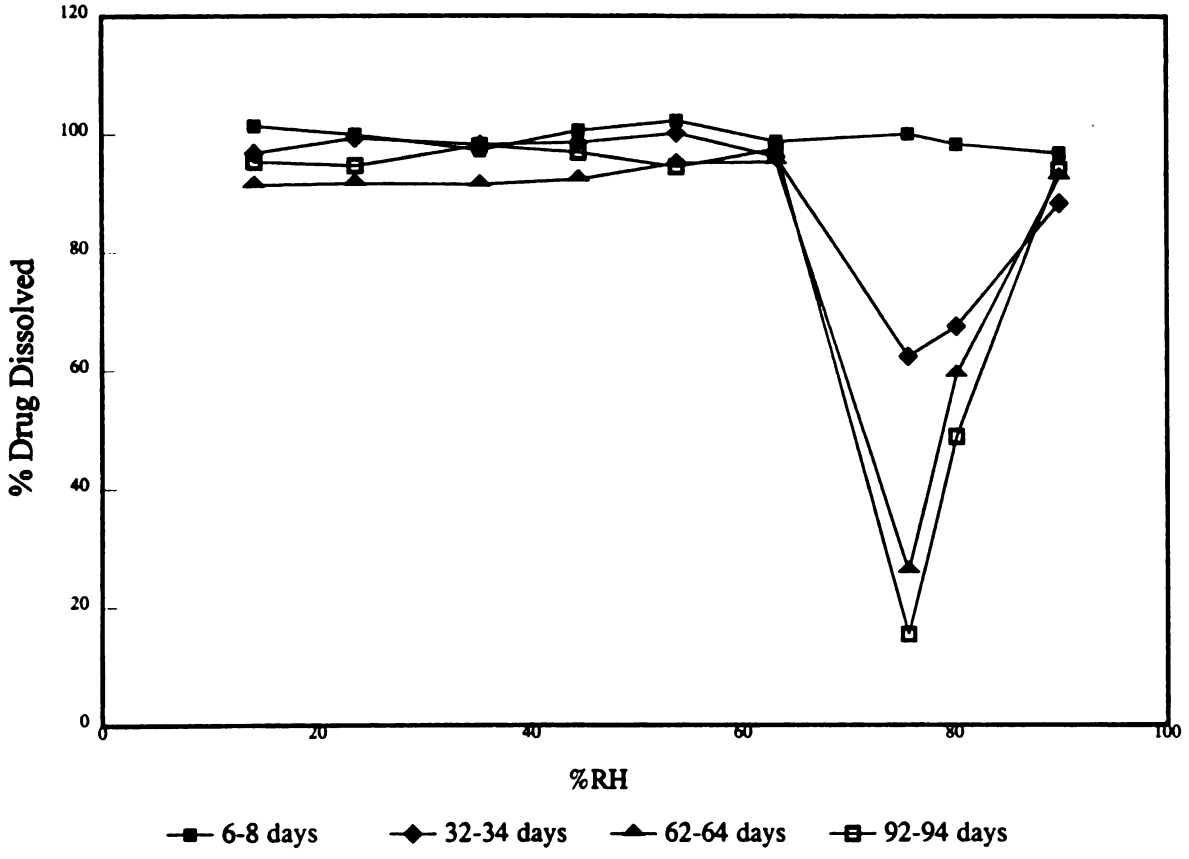

Figure 7. The effect of RH on 30 min dissolution of capsules after storage at 38°C. Note the progressive loss in dissolution at 75% RH. The product has failed before 30 days.

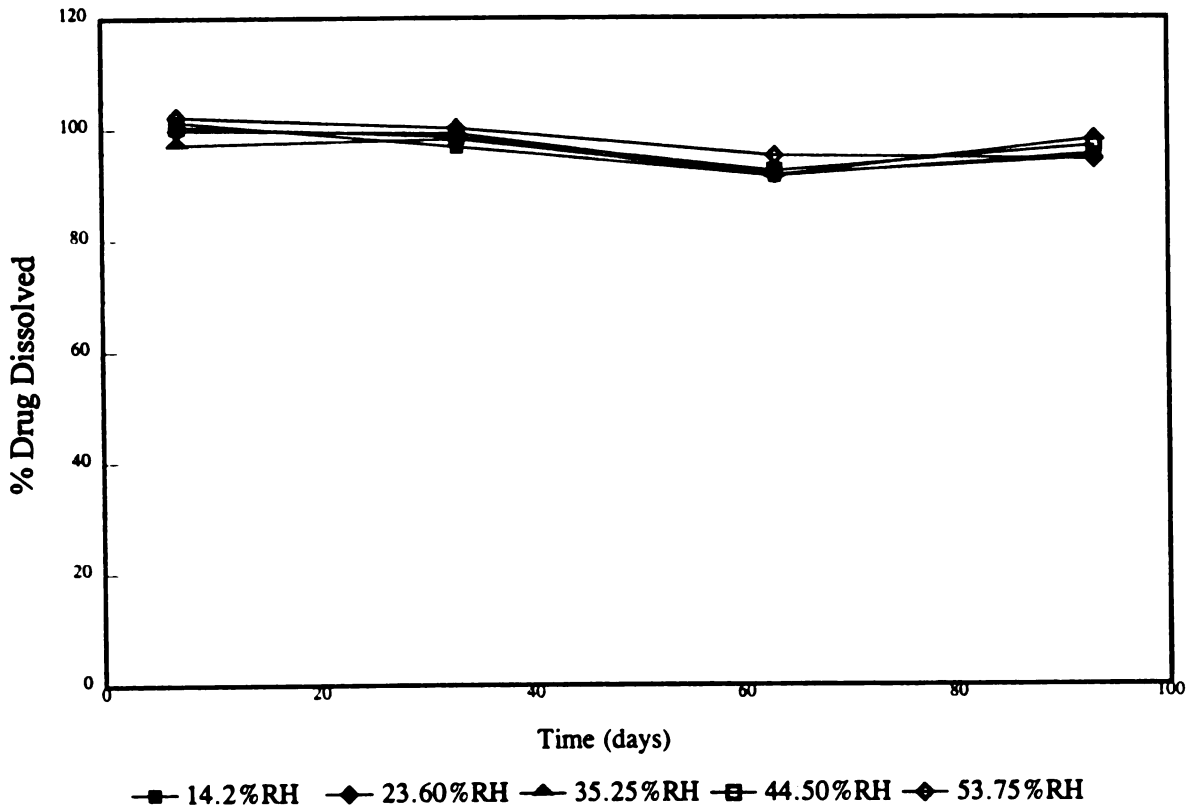

Figure 8. The effect of storage time on 30 min dissolution of capsules after storage at 38°C (below 60% RH).

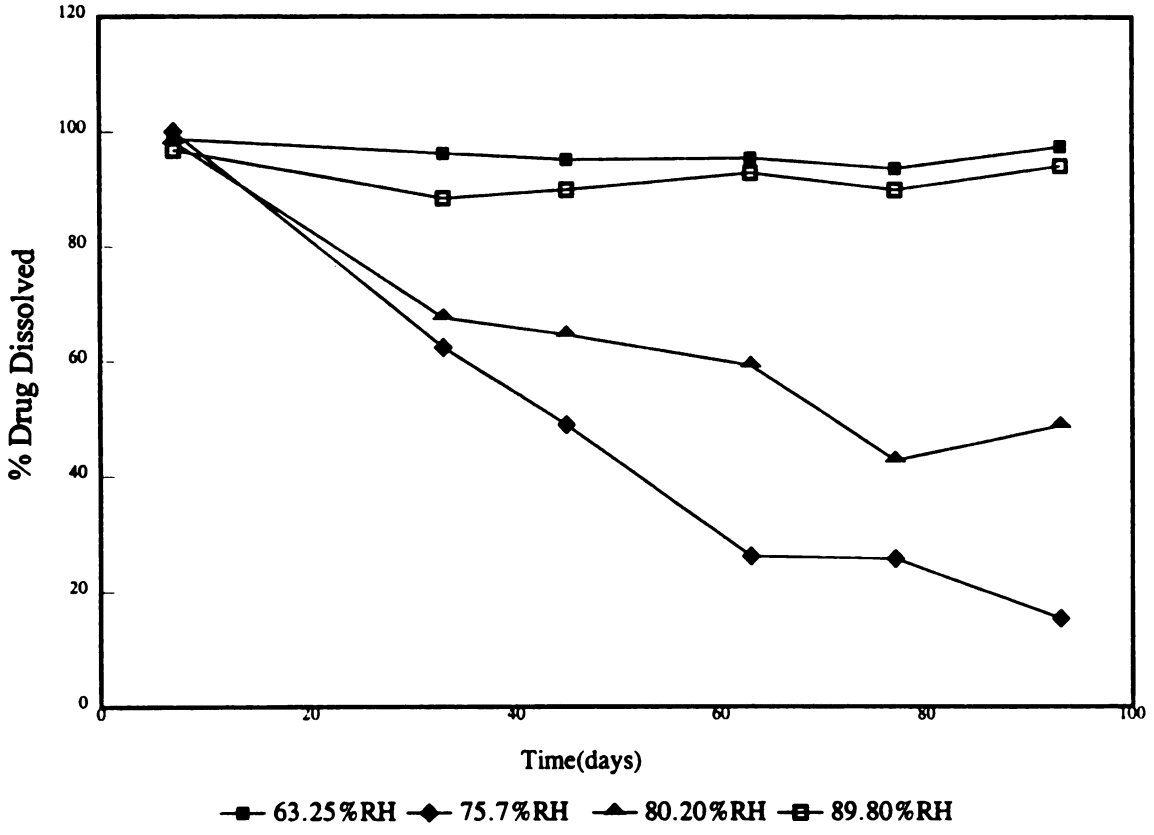

Figure 9. The effect of storage time on 30 min dissolution of capsules after storage at 38°C (above 60% RH). The capsules stored at 90% RH were not usable because they were sticky and soft. They were failed before doing the dissolution test.

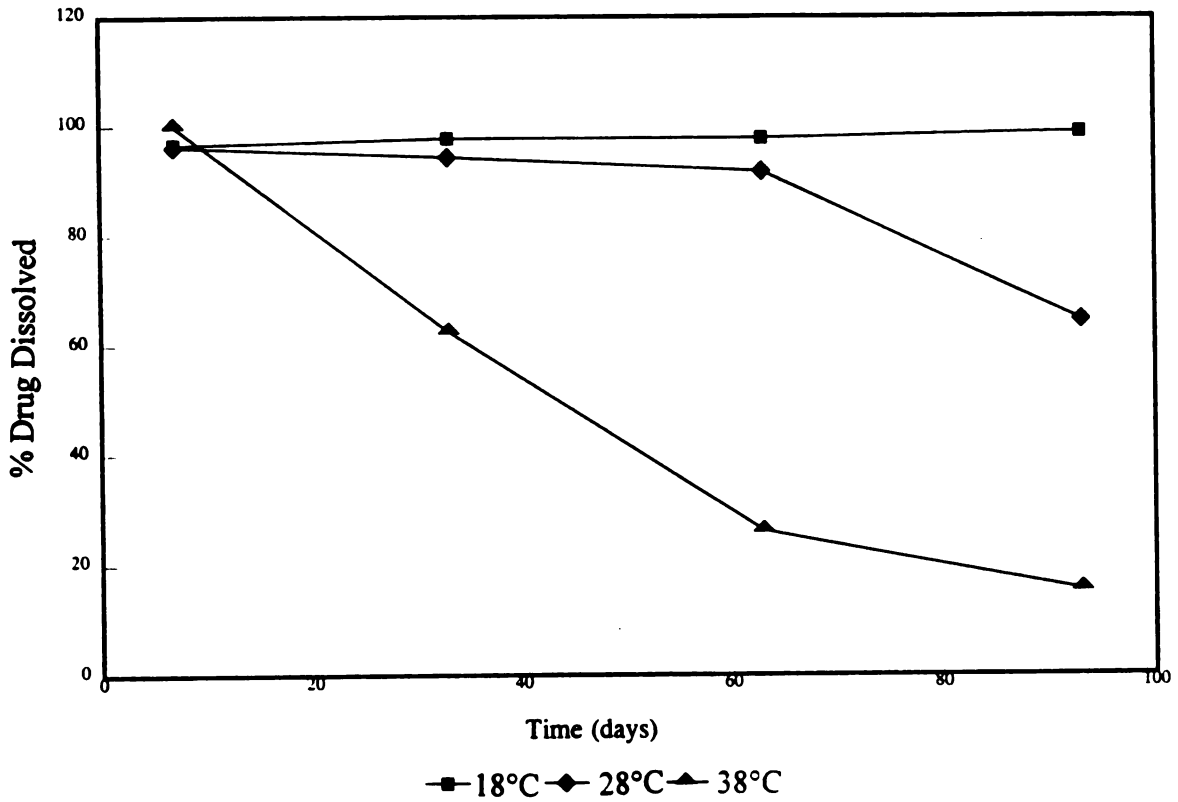

Figure 10. The effect of temperature on 30 min dissolution of capsules stored at 75% RH for 90 days. The lower temperature (18°C) does not seem to affect the dissolution value of stored capsules.

Chapter 5

CONCLUSIONS

Significant changes in drug-release patterns were observed only under the combined influence of high humidity, storage time and heat. Increased understanding of these factors should help in developing better capsule formulations and packaging that will maximize dissolution over the shelf life of the capsules, and thereby maximize the bioavailability of the medicament throughout the shelf life of the capsules.

The results obtained from the experiment allow us to draw the following three conclusion about Axid:

1) Axid has a critical relative humidity for dissolution that is between 60% RH and 75% RH. Dissolution failure is extensive at 75% RH, but minimal at 60-63% RH.
2) Axid has a critical temperature for dissolution that is between 18° C and 28°C. Dissolution failure has been observed at 28° C but not at 18° C. Dissolution failure is catastrophic at 38° C.
3) Dissolution response (time to failure) is affected by interactions among time, temperature and relative humidity.
    a) In this experiment, dissolution decreased with increasing time of exposure at both 75% and 80 % RH, and the rate of change was greater at 75% than at 80%. (See Figure 9).

b) At 75% RH and 38° C, dissolution failure occurred by the 30th day of storage, while at 75% and 28° C dissolution remained within USP limits (Figure 9).

c) At 75% RH, 28° C, dissolution decreased sharply from day 60 to day 90, while at 38°C the rate of dissolution loss was less. In fact, the slope of dissolution loss at 28° C between day 60 and 90 may be about the same as the slope for 38° C between day 6 and day 60. The 38° C slope from day 60 to day 90 is less than either of them (Figure 10).

The dissolution results obtained for Axid suggest that for solid oral drug products, there is a product specific dissolution function that can be related to temperature, relative humidity and time of exposure to a relative humidity at a temperature. The Axid evidence suggests that the time to dissolution failure goes down as temperature and relative humidity goes up. Furthermore, it seems the temperature and relative humidity interact to exacerbate the deleterious effect of either one alone.

If a dissolution/temperature/relative humidity function can be determined, then that function can be used in a mathematical model, to calculate the time to dissolution failure for packaged product. The dissolution function, along with permeability constants for the package and temperature and relative humidity of storage will be the input to the model, and time to dissolution failure will be the output.

Such calculations are not likely to replace stability testing. However, they can be used to select the packages in which to put product for stability test. The advantage would be: 1) rapid selection of package. 2) greater certainty that the package chosen will provide the protection needed.

For future work --

1. Use existing shelf life equations and computer models to predict the shelf life of Axid, modify as needed.
    a. Calculate dissolution rate, k, and dissolution rate reduction constant, K.
    b. Determine a mathematical function for dissolution similar to those used for moisture content.
    c. Determine permeability constants for candidate packaging materials.
2. Use the relationship among temperature, dissolution, and moisture content to construct a time-at-temperature and relative humidity equation to model the dissolution shelf life.

APPENDICES

APPENDIX A

Table 2. An example ANOVA table of capsules stored at 18°C and 38°C for six days by SAS system analysis. The RH value was 63%. From this result, the temperature will affect the dissolution value of capsules ($p = 0.001$). (p-values < 0.05 considered significant)

The SAS System

General Linear Models Procedure

Dependent Variable: DISSOLVE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	156.84117500	52.28039167	11.19	0.0001
Error	32	149.49910000	4.67184687		
Corrected Total	35	306.34027500			

R-Square	C.V.	Root MSE	DISSOLVE Mean
0.511984	2.206889	2.161444555	97.94083333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEP	1	145.08202500	145.08202500	31.05	0.0001
CAPSULE	2	11.75915000	5.87957500	1.26	0.2978

APPENDIX B

Table 3. Results of ANOVA for temperature effect on 30 minutes dissolution at 18°C and 38°C by SAS analysis (p-values < 0.05 considered significant). The RH value was 63%.

Source	Storage Time	p-values	Significant Difference
Temp	6	0.0831	N.S.
	30	0.3280	N.S.
	60	0.0551	N.S.
	90	0.0689	N.S.
Capsules	6	0.3636	N.S.
	30	0.0383	S.
	60	0.2061	N.S.
	90	0.2638	N.S.

Table 4. Results of ANOVA for RH effect on 30 minutes dissolution at 18°C by SAS analysis (p-values < 0.05 considered significant)

Source	Storage Time	p-values	Significant Difference
RH	6	0.0376	S.
	30	0.7658	N. S.
	60	0.0003	S.
	90	0.0001	S.
Capsules	6	0.0340	S.
	30	0.4300	N. S.
	60	0.5960	N. S.
	90	0.1069	N. S.

APPENDIX C

Table 5. Results of ANOVA for RH effect on 30 minutes dissolution at 28°C by SAS analysis (p-values < 0.05 considered significant)

Source	Storage Time	p-values	Significant Difference
RH	6	0.0001	S.
	90	0.0001	S.
Capsules	6	0.7832	N. S.
	90	0.5853	N. S.

Table 6. Results of ANOVA for RH effect on 30 minutes dissolution at 38°C by SAS analysis (p-values < 0.05 considered significant)

Source	Storage Time	p-values	Significant Difference
RH	6	0.0001	S.
	30	0.0001	S.
	60	0.0001	S.
	90	0.0001	S.
Capsules	6	0.8809	N. S.
	30	0.9351	N. S.
	60	0.9196	N. S.
	90	0.3264	N. S.

APPENDIX D

Table 7. The effect of storage conditions on 30 minutes dissolution of Axid capsules at 38°C (the dissolution value was the average of three capsules)

% RH	6 - 8 days	32 - 34 days	62 - 64 days	92 - 94 days
14.20	101.3	96.7	91.3	95.3
23.60	99.8	99.2	91.6	94.6
35.25	97.2	98.1	91.4	98.0
44.50	100.5	98.5	92.3	96.8
53.75	102.2	100.1	95.0	94.4
63.25	98.7	96.1	95.3	97.4
75.70	100.0	62.4	26.2	15.4
80.20	98.2	67.5	59.3	48.8
89.80	96.7	88.3	92.7	94.1

Table 8. The effect of storage conditions on 30 minutes dissolution of Axid capsules at 28°C (the dissolution value was the average of three capsules)

% RH	6 - 8 days	32 - 34 days	62 - 64 days	92 - 94 days
12.10	91.4	-	-	93.2
22.00	94.8	-	-	93.9
32.50	97.4	98.7	92.6	91.8
44.00	-	-	-	-
51.50	95.6	98.9	92.6	92.8
64.00	96.8	95.0	94.1	98.3
75.20	96.2	94.3	91.7	64.5
80.00	98.6	97.7	96.7	98.6
91.00	97.7	93.6	89.9	98.4

Note: - No sample from these conditions

APPENDIX E

Table 9. The effect of storage conditions on 30 minutes dissolution of Axid capsules at 18°C (the dissolution value was the average of three capsules)

% RH	6 - 8 days	32 - 34 days	62 - 64 days	92 - 94 days
16.10	96.3	97.6	98.6	93.8
22.50	96.1	98.6	99.2	96.6
36.75	96.7	96.7	98.9	96.9
45.75	96.1	96.2	97.1	96.9
55.00	95.8	98.0	95.6	96.5
67.00	97.4	98.2	98.5	99.2
74.70	96.6	97.8	97.8	98.9
79.20	98.4	96.5	91.3	99.8
92.80	98.0	97.9	99.2	90.2

Table 10. Results of ANOVA for RH and storage time effect on 30 minutes dissolution at three different temperatures by SAS analysis (p-values < 0.05 considered significant)

Source	p-values	Significant Difference
Temp	0.0001	S.
Day	0.0001	S.
RH	0.0001	S.
Temp*Day	0.0001	S.
Temp*RH	0.0001	S.
Temp*Day*RH	0.0001	S.

APPENDIX F

Nizatidine **(from USP monograph)**

$C_{12}H_{21}N_5O_2S_2$ 331.47

* Nizatidine contains not less than 98.0 percent and not more than 101.0 percent of $C_{12}H_{21}N_5O_2S_2$, calculated on the dried basis.

Packaging and storage - Preserve in tight, light-resistant containers.

USP Reference standards < 11 > - USP Nizatidine RS.

Nizatidine Capsules

* Nizatidine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of $C_{12}H_{21}N_5O_2S_2$.

Packaging and storage - Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards < 11 > - USP Nizatidine RS.

Identification -

A: Empty the contents of 2 Capsules into a beaker, add 20 mL of methanol, and swirl for approximately 2 minutes. Filter through a filter paper, and evaporate the methanol solution with a current of cool air to dryness : the infrared absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Nizatidine RS.

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation, both relative to the internal standard, as obtained in the Assay.

Dissolution < 711 > -

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Procedure-- Determine the amount of $C_{12}H_{21}N_5O_2S_2$ dissolved from ultraviolet absorbances at the wavelength of maximum absorbance at about 314 nm using filtered portions of the solution under test, diluted with water if necessary, in comparison with a Standard solution having a known concentration of USP Nizatidine RS in the same medium.

Tolerances-- Not less than 75% (Q) of the labeled amount of $C_{12}H_{21}N_5O_2S_2$ is dissolved in 30 minutes.

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