AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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An evaluation of polycarbophil coated liposomes and nicardipine HCl oral sustained-release formulations are detailed and explained. Polycarbophil coated liposomes were characterized for their drug release, loss of entrapped drug, and membrane permeation. Weights of liposomes during incubation with polycarbophil increased as a function of time. The three model drugs entrapped in liposomes were insulin, dyphylline, and hydrocortisone. Rates of drug release from liposomes were not significantly controlled by the polycarbophil coating. Loss of the entrapped insulin (high MW) was reduced when 1-1.5% polycarbophil solution was applied as coating over the liposomes. In contrast, loss of the entrapped dyphylline and hydrocortisone (low MW) was not affected by polycarbophil coating. Low amounts of insulin, dyphylline, or hydrocortisone were transported across an ethylenevinylacetate membrane in membrane

permeation studies. The amounts of drug, entrapped in liposomes, penetrated through the membrane were too low to detect. Polycarbophil coated liposomes may be a promising drug carrier for topical application.

Nicardipine HCl sustained-release products were formulated and evaluated *in vitro* and *in vivo*. Appropriate methods and dissolution media for *in vitro* dissolution testing were investigated and selected. Both enzyme-free simulated gastric and intestinal fluids were required for dissolution testing of sustained-release drug products. Release rates of nicardipine HCl using USP basket or paddle at 50 RPM were comparable to Bio-Dis® at 5 or 10 DPM. Bio-Dis® was the most convenient method, and was therefore selected for product evaluation.

Nicardipine HCl sustained-release products consisted of 75% sustained-release beads and 25% immediate-release powder. Rates of drug release from the beads were controlled by percentages of ethylcellulose used in a spray layering process, but not significantly affected by incorporation of PVP at 10-15%. Rates of drug release were retarded by overcoating with ethylcellulose. Diluent incorporated in immediate-release powder had an influence on flow properties of powder.

A newly developed nicardipine HCl product was tested for bioequivalence with Cardene® SR. Statistical two one-sided t-test indicated that the two products could not be concluded as being bioequivalent. *In vitro/in vivo* correlation of percentages of drug release was found after the *in vitro* time scale was corrected.

(1) Evaluation of Polycarbophil Coated Liposomes and Membrane Permeation of Free and Liposomal Drugs, (2) *In Vitro-In Vivo* Evaluation of Nicardipine HCl Sustained-Release Formulations.

by

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CONTRIBUTION OF AUTHORS

Dr. Jacqueline Wardrop was involved in formulation development of nicardipine HCl sustained-release formulations.

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DEDICATION

This thesis is dedicated to my parents, Dr. Songwut and Dr. Acharee Sorasuchart, and my brother, Sawanit Sorasuchart, whose constant love and encouragement has ever been of inestimable support.

(1) Evaluation of Polycarbophil Coated Liposomes and Membrane Permeation of Free and Liposomal Drugs, (2) *In Vitro-In Vivo* Evaluation of Nicardipine HCl Sustained-Release Formulations.

CHAPTER I

INTRODUCTION

INTRODUCTION

Pharmaceutical products are available in various dosage forms, each of which is usually appropriate for a certain route of administration. This thesis describes two distinctive pharmaceutical dosage forms: liposomes and sustained-release oral dosage forms. A number of formulations of both products were evaluated *in vitro* for their further applications.

Chapter II presents formulations and methods used to produce liposomes. A formulation with good entrapment efficiency was selected for incubation with polycarbophil, a mucoadhesive polymer, to obtain mucoadhesive liposomes. Weight increase of the liposomes when incubating with polycarbophil was determined. The three model drugs used in this study were insulin, dyphylline, and hydrocortisone. Polycarbophil coated liposomes were characterized for rates of drug release and loss of entrapped drug from uncoated or polycarbophil coated liposomes. The stability of the three drugs dissolved in phosphate buffer saline at pH 7.0 was also examined. Penetration of dissolved and liposomal formulated drugs across ethylenevinylacetate membrane was also evaluated to determine potential for topical application of drug products.

Chapters III-V depict formulations and *in vitro-in vivo* evaluation of a sustained-release oral dosage form. *In vivo* evaluation is costly and time consuming; therefore, suitable *in vitro* testing is required to minimize the needs of *in vivo* product evaluation. Furthermore, a good *in vitro/in vivo* correlation is necessary for prediction of *in vivo* drug release from *in vitro* data.

In Chapter III, effects of pH of dissolution media and dissolution methods on rates of drug release were evaluated. Release rates of three different drugs, with different solubilities and degrees of ionization, from drug containing beads coated with ethylcellulose were compared in different pH dissolution media. Three USP dissolution methods (USP apparatuses I, II, and III) were applied for comparison of dissolution testing of drug products.

Formulation factors for nicardipine HCl sustained-release dosage forms which included sustained-release beads and immediate-release powder are specified in Chapter IV. Ingredients used as a binder/release controller in bead formulations are described and their effects on drug release investigated. Influences of diluents, including starch, talc, and magnesium stearate, on flow property and dissolution extent of powder formulation are also explained. Results of *in vitro* dissolution testing of beads, powder, and a combination of both are illustrated.

Nicardipine HCl sustained-release drug products were tested in healthy human subjects, as described in Chapter V. A test product, which was produced in laboratory scale at College of Pharmacy, Oregon State University, was tested for bioequivalence against the reference product, Cardene® SR. Pharmacokinetic parameters were obtained from plasma drug concentration-time curves. Bioequivalence testing of 2 products was statistically performed using the two one-sided t-test. *In vivo* drug release was mathematically generated by deconvolution of plasma drug concentration-time profiles and drug elimination function. The percentages of *in vivo* drug release were then correlated with those of *in vitro* drug release.

CHAPTER II

Evaluation of Polycarbophil Coated Liposomes and Membrane Permeation of Free and Liposomal Drugs

Waranush Sorasuchart and J. Mark Christensen

ABSTRACT

The lipid components and preparation technique for preparing liposomes with good entrapment efficiency were selected. Liposomes coated with polycarbophil were prepared by incubating liposomes with 0.5-2.0% polycarbophil solutions; and weight increase of liposomes was characterized. A comparison of drug release and loss of entrapped drug from uncoated and polycarbophil coated liposomes were made between three model drugs, including insulin (hydrophilic, high MW), dyphylline (hydrophilic, low MW), and hydrocortisone (hydrophobic). It was found that weights of liposomes incubated with polycarbophil generally increased as a function of time. Release rates of the three drugs from uncoated and polycarbophil coated liposomes were similar. However, the release rates of hydrophilic drugs were slightly faster when higher percentages of polycarbophil coated the liposomes than with lower percentages of coating, but the reverse order was observed for hydrophobic drug. The coating of polycarbophil on liposomes reduced loss of entrapped insulin from liposomes at room temperature and 4°C compared to liposomes without coating. In contrast, loss of entrapped dyphylline and hydrocortisone from liposomes was not decreased by the polycarbophil coating. Membrane permeation study revealed that low amounts of all test drugs dissolved in phosphate buffer saline diffused across an ethylenevinylacetate membrane. Hydrophilic drugs penetrated across the membrane at a higher rate than the hydrophobic drug. The amounts of drug transported across the membrane from the liposomes were too low to detect. In conclusion, distearoyl phosphatidylcholine and cholesterol at a mole ratio of 1:3.3 using the reverse phase evaporation method to obtain

liposomes gave good drug entrapment efficiency. Drug release from uncoated and polycarbophil coated liposomes at 37°C was almost complete within 8 hours. Liposomes coated with the mucoadhesive polycarbophil were a stable drug carrier for insulin. Dissolved drug and liposomal drugs barely permeated across the membrane indicating good tendency for topical application.

INTRODUCTION

Recently, significant increase of drug penetration across membranes from liposomes has been reported (1, 2, 3, 4). Liposomal triamcinolone acetonide penetration *in vitro* through the oral mucosa of hamster was significantly increased compared to nonliposomal drug in the ointment form (1). Likewise, pulmonary and nasal absorption of insulin were improved when it was delivered by liposomes (2, 3, 4). It was also reported that a variety of drug loaded liposomes, including triamcinolone acetonide, retinoids, lidocaine, methotrexate, econazole, and minoxidyl, delivered higher drug concentrations to the epidermis and dermis than the conventional drug products (5). Therefore, the improved adherence of liposomes to the local site of application by bioadhesive/mucoadhesive should enhance topical or transdermal drug delivery.

Polycarbophil, a polyacrylic polymer, has been widely known as being bioadhesive/mucoadhesive (6, 7). The mucoadhesive polymer is often used as an ingredient in making tablets or ocular inserts (8, 9). In these drug dosage forms the polymer tends to bind to gastrointestinal, buccal, or ocular membranes which prolongs and improves contact of the drug to the drug absorption site, maintaining drug absorption. In addition, polycarbophil acts as intestinal penetration enhancer and inhibits proteolytic degradation *in vitro* (10).

A new application may be accomplished by incubating polycarbophil polymer with liposomes. The polymer should bind to the outer portion of the liposome's membrane resulting in mucoadhesive liposomes which should be able to localize and be

retained at the specific site of application and allow the liposomes to be utilized as a drug carrier for buccal, nasal, pulmonary, oral, or topical (skin/wound) drug administration.

Previous approaches taken in creating bioadhesive liposomes included modification of liposome surface by the covalent attachment of bioadhesive ligand, *i.e.*, collagen and hyaluronic acid (11, 12, 13). The modification process was quite complicated and phosphatidylethanolamine was required for the chemical link.

Coating liposomes with polycarbophil was a simple incubation process. The use of costly phosphatidylethanolamine is not required. Most importantly, polycarbophil is a very effective mucoadhesive synthetic polymer of reasonable cost.

Three model drugs were included in the study. Insulin was selected because many efforts have been made to find an alternative route of administration other than injection. Insulin is a large water-soluble polypeptide, and is destroyed when administered orally and unlikely to penetrate through biological membranes. A study reported that blood glucose levels of rats were not altered significantly after buccal administration of insulin entrapped liposomes (1). However, as discussed earlier, pulmonary and nasal absorption of insulin were improved after being entrapped in liposomes (2, 3, 4). Included for a comparison in this study with insulin were dyphylline, a representative of hydrophilic drug with relatively small molecular weight and hydrocortisone, a representative of hydrophobic drug.

The objectives of this study were to search for a liposome formulation that provided good entrapment efficiency (high percentage of drug entrapment), to characterize weight increase of liposomes being incubated with polycarbophil solution, to evaluate release rates of drugs with different solubilities and molecular weights from

polycarbophil coated liposomes in comparison with uncoated liposomes, and to determine loss of entrapped drug from the uncoated and the polycarbophil coated liposomes at different temperatures over a period of time. Stability of the three model drugs used in the study was also measured. Furthermore, *in vitro* membrane permeation of dissolved and liposomal formulated drugs was investigated using ethylenevinylacetate (EVA) membrane.

MATERIALS AND METHODS

Chemicals

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC, MW 790.15) was purchased from Avanti Polar-Lipids, Inc, Alabaster, AL. Cholesterol (CHO, MW 386.7) and dicetyl phosphate (DCP, MW 546.9) were purchased from Aldrich, Milwauki, WI. Insulin (from bovine pancreas, anhydrous, MW 5777.6) was purchased from Sigma, St. Louis, MO. Dyphylline (MW 254.2) was received from Biocraft, Fairfield, NJ. Hydrocortisone (MW 362.15) was obtained from The Upjohn Company, Kalamazoo, MI. Noveon® AA1 (Polycarbophils) was provided by BF Goodrich, Cleveland, OH. Other chemicals were of reagent grade. Ethylenevinylacetate membrane (EVA, CoTran®) was generously provided by 3M, St.Paul, MN.

Methods

Preparation of Insulin Entrapped Liposomes

Preparation of Phosphate Buffer Saline (PBS, pH 7.0)

PBS was prepared by dissolving all ingredients provided in Table II.1 in 500 mL of distilled deionized water and adjusting the pH to 7.0 with concentrated HCl.

Table II.1 Ingredients in PBS

Ingredient	Amount (g)
NaCl	4.0031
KCl	0.0969
Na_2HPO_4	0.4544
KH_2PO_4	0.0953

Preparation of Insulin Solution

In a 25.0 mL volumetric flask, 25 mg of insulin powder was dissolved in 2 mL of 0.1 N HCl. Then, volume was adjusted to 25.0 mL with PBS.

Formulations of Insulin Entrapped Liposomes

Insulin entrapped liposomes were prepared based upon formulations and methods listed in Table II.2. Each preparation method is also discussed.

Table II.2 Ingredients used in liposome formulations

Formulation	DSPC (g)	CHO (g)	DCP (g)	$CHCl_3$ (mL)	INS (mL)	Method
1	0.1106	0.0541	0.0020	15	5.0	1
2	0.0553	0.0270	0.0191	5	2.5	1
3	0.0800	0.0130	0.0092	5	2.5	1
4	0.0553	0.0270	-	5	2.5	1
5	0.0553	0.0270	-	4	2.5	2
6	0.1124	0.0550	-	4	2.8	2
7	0.1007	0.0493	-	4	2.5	1
8	0.1007	0.0493	-	4	2.5	3
9	0.1007	0.0493	-	4	2.5	2
10	0.1007	0.0044	0.0063	5	5.0	3
11	0.1000	0.0200	-	4	2.5*	2
12	0.0830	0.0130	-	5	5.0	2
13	0.1740	0.0260	-	5	5.0	2

Note: DSPC represents distearoyl phosphatidylcholine. CHO represents cholesterol. DCP represents dicetyl phosphate. $CHCl_3$ is chloroform. INS represents 1 mg/mL insulin in PBS. Method 1 is thin film method. Method 2 is reversed phase evaporation method. Method 3 is freeze-thaw method. Note that * in formulation 11 represents 0.7 mg/mL insulin solution containing 1% v/v Tween® 80.

Thin Film Method

Lipid ingredients were completely dissolved in chloroform in a round bottom flask. Chloroform was removed under vacuum at 37 °C using a rotovapor (Flash Evaporator®, Buchler Instruments, Fort Lee, NJ). The thin lipid film obtained was then

hydrated with PBS (pH 7.0) containing insulin (1 mg/mL) and mixed in the rotovapor at 66 °C for 1 hour. The liposome suspension was vortexed, filtered through a 1.2 μm Acrodisc® (Gelman Sciences, Ann Arbor, MI), and then extruded 21 times though 1000 nm polycarbonate filter at room temperature using an extrusion device (Liposofast®, Avestin, Inc., Ottawa, ON, Canada).

Reversed Phase Evaporation Method

In a round bottom flask, lipid ingredients were dissolved in chloroform until a clear solution was obtained. The volume listed in Table II.2 of PBS containing insulin was added to the organic solution and subjected to vortexing, forming an emulsion. The emulsion was then sonicated for 10 minutes and vortexed for 1 minute. This process was repeated 3 times before removing the chloroform under vacuum with the rotovapor at 37 °C. The final processes of vortexing, filtration, and extrusion of the thin film method described previously were performed to finish making the liposomes.

Freeze-Thaw Method

The freeze-thaw method was modified from Kato Y., *et. al.* (14). Liposomes prepared by the reversed phase evaporation method were frozen in -20°C freezer and thawed in 40°C water bath. The freeze-thaw process was repeated 5 times before the final vortexing, filtration, and extrusion of the thin film method were performed.

Assay of Insulin Entrapped in Liposomes

An amount of insulin entrapped in liposomes from each formulation was determined to select the formulation and method that provided good entrapment efficiency. The selected formulation and method were used for further study.

HPLC System

Amount of insulin in each formulation was detected by a HPLC system, which consisted of a pump (Waters Model 590), an autoinjector (710 Wisp, Waters Associates, Milford, MA), an HPLC column (Microsorb® MV C18, 5 μm, 100 A, 4.6 mm ID x 25 cm L, Rainin Instrument Co., Inc., Emeryville, CA), a UV detector (Model 441, Waters Associates, Milford, MA), and an integrator (Shimadzu CR 501 Chromatopac, Shimadzu Corp., Kyoto, Japan). The HPLC mobile phase was a 2: 4: 9 mixture of isopropyl alcohol, acetonitrile, and phosphate buffer (0.01 M KH_2PO_4) containing 0.025 M Na_2SO_4, adjusted to a final pH of 3.20 with phosphoric acid. The mobile phase was pumped through the HPLC system at a flow rate of 0.8 mL/min. Benzocaine (0.005 mg/mL) was used as an internal standard. The UV absorbances were detected at 229 nm using the UV spectrophotometer (Waters Model 441, Water Associates, Milford, MA). Retention times of insulin and benzocaine were 4 and 7 minutes, respectively.

Sample Preparation

The liposomes were washed 3 times with PBS to remove unentrapped insulin before assaying the entrapped insulin. Washing of the liposomes went as follows; 50 μL of the liposome suspension was transferred to a 2 mL-centrifuge tube and centrifuged at a rate of 14,000 RPM (approximately 10,746g) for 5 minutes using an ultracentrifuge (Eppendorf® Centrifuge 5415 C). The supernatant was removed and 100 μL of fresh PBS was added and vortexed. The procedures of centrifugation, supernatant removal and addition of fresh PBS were repeated 3 times. Liposome pellets containing entrapped insulin were obtained for assay. To assay insulin entrapped in liposome pellets, the pellets were ruptured by adding 50 μL of isopropyl alcohol and subjecting to vigorous vortexing. A 100 μL of PBS and 50 μL of an internal standard solution were added into the tube, mixed, and centrifuged. The supernatant was removed, of which 25 μL was injected into the HPLC system.

Standard Preparation

A series of standard solutions of insulin was prepared by serial dilution to produce a range of insulin concentrations of 7 to 125 μg/mL. Blank liposomes were prepared by the same method as the insulin entrapped liposomes except that PBS containing no insulin was used. The same amount of blank liposomes as insulin liposomes was centrifuged and the supernatant was removed. To the blank liposome pellets, 50 μL of a standard solution of insulin was added and mixed before adding 50 μL of isopropyl alcohol and vortexing vigorously to disrupt the liposome pellets. For blank standard (no

insulin present), PBS was added instead of a standard solution of insulin. Into the disrupted liposomes, 50 μL of PBS and 50 μL of internal standard solution were added, vortex mixed, and centrifuged. The supernatant was removed, of which 25 μL was injected into the HPLC system.

Insulin concentrations of the samples were calculated from the linear regression relationship between peak area ratios of insulin to benzocaine and standard insulin concentrations. Correlation (R^2) of the standard insulin (0.007-0.125 mg/mL) was of 0.9951. Accuracy was in the range of 82.66-109.76% (average 97.32).

Preparation of Dyphylline and Hydrocortisone Entrapped Liposomes

Using formulation 13 and reversed phase evaporation, liposomes with dyphylline entrapped were prepared as previously described using PBS containing dyphylline at a concentration of 1 mg/mL.

Liposomes entrapped with hydrocortisone, a hydrophobic drug, were prepared differently from hydrophilic drugs. The hydrocortisone was dissolved with DSPC and CHO in the organic solvent (chloroform) prior to starting the preparation of the liposomes. Then using formulation 13 and the reversed phase evaporation technique, hydrocortisone entrapped liposomes were prepared. At the step where insulin or dyphylline were introduced into the liposomes, a solution of PBS (no drug present) was substituted.

Polycarbophil Coated Liposomes

Preparation of Polycarbophil Solution

PBS used in liposome preparation was not suitable for making polycarbophil solution. The polymer clumped and did not disperse well. To avoid these problems, phosphate buffer saline containing no KCl and KH_2PO_4, called NPBS (Table II.3), was used. To make NPBS, all ingredients were dissolved in water and then adjusted to pH of 7.0 with hydrochloric acid. 2% of a polycarbophil stock solution was prepared by dispersing polycarbophil 2 g in 100 mL of heated NPBS with stirring. After cooling, a series of polycarbophil solutions with a concentration of 0.5, 0.75, 1.0, 1.25, and 1.5 % was diluted from the 2% stock solution.

Table II.3 NPBS Formulation for Polycarbophil Solution

Ingredient	Amount (g)
Na_2HPO_4	0.7100
NaCl	0.6941
Deionized distilled water	100 mL

Coating Liposomes with Polycarbophil Solution

Formulations 12 and 13 of insulin entrapped liposomes were prepared and mixed with 0.5, 0.75, 1.0, 1.5, and 2.0% of polycarbophil solutions at a volume ratio of 1:1. The mixtures were incubated at 4°C for 48 hours.

Determination of Weight Increase of Insulin Entrapped Liposomes being Incubated with Polycarbophil

Each 100 μL of liposomes (formulations 12 and 13) freshly mixed with polycarbophil solution was transferred to a known weight (previously weighed) centrifuged tube. The samples were centrifuged and the supernatant was temporarily removed to weigh the tubes containing liposome pellets. The weight of the liposome pellets was obtained by subtraction of the weight of the tubes with the liposome pellets from the weight of the empty tube. The supernatant was added back to the tubes and the incubation at 4°C continued. At 0.5, 1, 2, 4, 6, 8, 24, and 48 hours, the weight of the liposome pellets in each sample was measured using the same technique. The ratios of the weights of the liposome pellets after incubation to those before incubation were made and ploted against time, indicating "ratios of weight increase to initial weight" of liposomes.

Drug Release from Polycarbophil Coated Liposomes

Release of Insulin from Polycarbophil Coated Liposomes

Formulations 12 and 13 were used in evaluating insulin release from liposomes. Insulin entrapped liposomes (200 μL), including uncoated and polycarbophil coated liposomes, were transferred into a centrifuge tube. The study was performed in triplicate. The liposomes were washed 3 times before being used. Each tube contained liposome pellets being dispersed in 100 μL of fresh PBS and was shaken in a water bath at 37°C. At the following times 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, and 48 hours, all supernatant was

removed and 100 μL of fresh PBS was added to the tubes wherein the tubes were continued to be shaken at 37°C. From the collected supernatant 50 μL was transferred to a 200 μL-sample tube where a 25 μL of 0.005 mg/mL benzocaine solution (internal standard) was added and mixed, of which 25 μL of the mixture was injected into the HPLC system. Standard solutions were prepared by serial dilution of 1 mg/mL insulin solution to create a range of 7 to 125 μg/mL concentrations of insulin.

After the last sample was collected, the assay for insulin still entrapped in the liposome pellets was performed to detect amounts of unreleased insulin in the liposomes. Percentage of drug released from liposomes was determined as,

$$\% \text{ drug released} = \frac{(A - A_0)}{(A_T - A_0)} \times 100,$$

where A_0 is the initial amount of drug in the supernatant at time 0, A_T is the total amount of drug entrapped in the liposomes, and A is the cumulative amount of drug released into the supernatant (modified from Reference 15).

Release of Dyphylline and Hydrocortisone Entrapped Liposomes

The study of the release of dyphylline and hydrocortisone entrapped in liposomes (formulation 13 only) were performed under the same condition as insulin entrapped liposomes. The sampling times and methods of collection of supernatant for determination of drug release was also the same. Amounts of drug released and entrapped in the liposomes were determined using the HPLC systems described as follows.

HPLC System for Dyphylline

All instrumental components of the HPLC system were as previously described for insulin, except the HPLC mobile phase. HPLC mobile phase for dyphylline, whose final pH was adjusted to 2.78 with phosphoric acid, was composed of 7: 14: 79 isopropyl alcohol: acetonitrile: distilled deionized water. The flow rate was 1.0 mL/min. Methyl paraben at a concentration of 0.02 mg/mL was used as an internal standard. Retention times for dyphylline and methyl paraben were 3 and 6 minutes, respectively.

Linear regression between dyphylline concentrations (0.0025-0.025 mg/mL) and peak area ratios of dyphylline to methyl paraben was performed for determination of dyphylline concentrations of the samples. Correlation (R^2) of the regression was of 0.9988 and accuracy was in the range of 93.94-113.23% (average 101.52).

HPLC System for Hydrocortisone

The instrumental components of HPLC system for hydrocortisone analysis were as previously described. The mobile phase was a mixture of 2: 4: 9 isopropyl alcohol: acetonitrile: distilled deionized water, with a final pH adjusted to 3.20 by phosphoric acid. The flow rate was maintained at 0.8 mL/min. β-hydroxypropyl theophylline was used as an internal standard at a concentration of 0.005 mg/mL. The retention times of β-hydroxypropyl theophylline and hydrocortisone were 4 and 7 minutes, respectively.

A correlation (R^2)of linear regression between hydrocortisone concentrations (0.0013-0.0518 mg/mL) and peak area ratios of hydrocortisone to β-hydroxypropyl

theophylline was of 0.9919. Accuracy was in the range of 92.73-105.45 % (average 99.09).

Loss of Encapsulated Drug from Liposomes

Drugs encapsulated in liposomes may leak from the vesicles into the extraliposomal compartment. To characterize the stability of polycarbophil coated liposomes in comparison with uncoated liposomes, liposome vesicles were stored at room temperature (22°C) and 4°C. Amounts of drug remaining in liposomes were detected at time 0, 3 days, 1, 2, and 3 weeks, 1, 2, 3, and 6 months after preparation of the liposome vesicles. Assay of drug entrapped in liposomes was performed as previously described.

Stability of Insulin, Dyphylline, and Hydrocortisone

As a control, stability of solution of insulin, dyphylline, and hydrocortisone in PBS (pH 7.0) at room temperature (22°C) and 4°C were evaluated to determine if the loss of drug from liposome vesicles was due to drug leakage or drug degradation. Insulin solution was prepared as previously described. Dyphylline solution was obtained by dissolving dyphylline and adjusting the volume with PBS. Hydrocortisone was dissolved in isopropyl alcohol and the volume was adjusted with PBS. Each solution was diluted to a concentration of 25 μg/mL. UV absorbances (day 0) of insulin, dyphylline, and hydrocortisone solution were measured at a wavelengths of 204, 274, and 250 nm, respectively. Drug solution was then separately stored at room temperature (22°C) and

4°C. Measurement of UV absorbances was performed after 3 days, 1, 2, and 3 weeks, 1, 2, 3, and 6 months. Drug concentration was determined from the UV absorbance using a linear regression between UV absorbances and a series of drug concentrations (3-50 μg/mL), diluted from stock drug solutions. Correlations (R^2) of linear regression relationship of standard insulin, dyphylline, and hydrocortisone were of 0.9908, 0.9980, and 0.9980, respectively. Accuracy of those drugs was in the range of 63.04-124.92%, 98.22-108.2%, and 92.73-105.44%, respectively.

Membrane Permeation

As discussed earlier, liposomes have in many instances improved drug delivery for topical or transdermal administration. Furthermore, entrapment of irritant drugs in liposomes for topical or transdermal administration may avoid or decrease the direct contact of drugs to the skin (5); thus, may reduce irritation. It was important to evaluate the membrane permeation of drugs in solution to the drugs being entrapped in polycarbophil liposomes in order to determine possible applications.

Membrane Permeation of Insulin, Dyphylline, and Hydrocortisone Solutions

Membrane permation study was performed using Franze diffusion cells (Crown Glass Co.). A solution containing insulin, dyphylline, or hydrocortisone in PBS (pH 7.0) at a concentration of 2 mg/mL, 20 mg/mL, and 4 mg/mL, respectively, was applied to the donor comparment. To prepare the insulin solution, insulin powder was dissolved in 0.1 N HCl before adjusting the volume with PBS. Dyphylline solution was prepared by

dissolving dyphylline directly into PBS. Hydrocortisone was dissolved in isopropyl alcohol before adjusting the volume with PBS.

Each receiver compartment was filled with calibrated volume (13-15 mL) of degassed distilled deionized water. The ethylenevinylacetate (EVA) membrane was placed in between the donor and the receiver compartments, which had a crossectional area of 3.3006 cm^2. The receptor solution was constantly stirred by means of a Teflon-coated magnetic stirring bar. The receptor solution was equilibrated with the membrane at 37°C for 15 minutes before placing the drug solutions in the donor cells. The openings of the donor and the receptor compartments were covered with parafilm and then with aluminum foil. From the middle of the receptor compartment, a 0.5 mL of the receptor solution was drawn via a sampling port with subsequent replacement of fresh PBS at times 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 8 hours after adding drug solutions to the donor compartment. Bubbles in the receptor compartment were avoided by using degassed receptor solution and transferring the solution to and from the receptor comparment gently. A syringe with extended tube connected to the needle was applied for transferring the receptor solution. A volume of fresh receptor solution was measured using the syringe where the bubbles were chased to the top and removed before volume measurement. Assay of drug concentrations in the receptor solution was performed using the HPLC systems as previously described.

Membrane Permeation Study of Polycarbophil Coated or Uncoated Liposomes

All conditions and collection of samples were performed as previously described, except the uncoated or polycarbophil liposomes loaded with insulin, dyphylline, or hydrocortisone were placed in the donor cells instead of solutions of the drugs.

RESULTS AND DISCUSSION

Entrapment of Insulin in Liposomes

Amounts of insulin entrapped in liposomes varied with different formulations and methods (Table II.4). Cholesterol was included to reduce the permeability of "fluid-crystalline state" bilayers (22), which improved rigidity and stability of the liposome membranes. Dicetyl phosphate (DCP), negative charge lipid, was used to provide a negative charge on the surface of the liposomes and prevent coagulation of the liposome particles (5). However, it was found in this study that inclusion of DCP lowered the percentage of insulin entrapped in liposomes compared to those without DCP. Tween® 80, a nonionic surfactant, is usually added into the lipid bilayer of liposomes to improve stability of the liposome vesicles (16). The efficiency and amount of insulin entrapped in liposomes was improved when Tween® 80 was included in the formulation (formulation 11).

A few techniques for liposome preparation were used in this study in order to select the most suitable preparation method for further studies. The reversed phase evaporation method, the fastest method among the three, provided the highest percentage

Table II.4 A summary of mole ratios of lipids, total lipids in grams and mmole, lipid concentrations ("Lipid Conc") in mM, and methods of preparation of formulations 1 to 13.

Formulation	Preparation method	Mole Ratio			Total Lipid (g)	Total Lipid (mmole)	Lipid Conc (mM)	% INS entrapment	INS:lipid weight ratio
		DSPC	CHO	DCP					
1	1	1	1	0.026	0.1667	0.2835	56.71	4.08	1.22×10^{-3}
2	1	1	1	0.5	0.1014	0.1747	69.89	3.47	8.55×10^{-4}
3	1	3	1	0.5	0.1022	0.1519	60.67	1.28	3.13×10^{-4}
4	1	1	1	-	0.0823	0.1398	55.92	11.46	3.48×10^{-3}
5	2	1	1	-	0.0823	0.1398	55.60	4.57	1.38×10^{-3}
6	1	1	1	-	0.1674	0.2845	101.61	12.65	2.11×10^{-3}
7	3	1	1	-	0.1500	0.2549	101.96	9.19	1.53×10^{-3}
8	2	1	1	-	0.1500	0.2549	101.96	11.41	1.90×10^{-3}
9	2	1	1	-	0.1500	0.2549	101.96	22.16	3.69×10^{-3}
10	3	1.33	1	0.12	0.1114	0.1503	30.06	2.93	1.30×10^{-3}
11*	2	1	2.45	-	0.1200	0.1783	71.32	23.02	4.79×10^{-3}
12	2	1	3.3	-	0.0960	0.1387	27.74	19.87	9.96×10^{-3}
13	2	1	3.3	-	0.2000	0.2874	57.48	19.65	4.91×10^{-3}

Note: DSPC represents distearoyl phophatidylcholine. CHO represents cholesterol. DCP represents dicetyl phosphate. INS represents insulin. * Formulation 11 contained 1% v/v Tween® 80.

of drug entrapment, nonetheless, this method needed careful attention and experience during the evaporation of organic solvent. However, unlike the thin film method, no lipid was lost by the lipid film adhering to the glass wall using the reversed phase evaporation method. Particularly in this study, the time consuming freeze-thaw technique could not improve the percentage of insulin entrapment. Thus, the reversed phase evaporation method was selected as the method of choice. Liposomes produced by reversed phase evaporation method , called reverse phase evaporation vesicles (REV), are unilamellar or oligolamellar (17, 22). Formulations 12 and 13 which contained DSPC:CHO at a mole ratio of 1: 3.3 were chosen for further study of polycarbophil coated liposomes because they provided a high percentage of entrapment and fairly high insulin: lipid weight to weight ratio compared to the other formulations and methods of liposome preparations.

Incubation of Liposomes with Polycarbophil and Weight Increase of Liposomes after Incubation

It was assumed that polycarbophil adhered to the outer side of the liposome membrane due to its mucoadhesive property. The polycarbophil solution was stickier at the higher concentrations, thus, probably adhered more effectively to the liposomes. When incubating with 0.5% and 0.75% polycarbophil solutions, the liposomes were not visably different from liposomes containing no polycarbophil. However, after adding polycarbophil solution at a concentration of 1% or higher, the liposome pellets aggregated, forming larger particles, but were dispersible after vortexing. The aggregation, which resulted in increase in liposome size, may be a serious problem for

parenteral injection application, but should not be a problem for topical administration. It should be noted that the size and lipid composition of the liposomes may affect some drug skin permeation and accumulation behavior, *i.e.*, caffeine (5).

The ratios of weight increase to initial weight of liposomes (formulation 12), when incubated with 0.5, 1.0, 1.5, and 2.0% polycarbophil solutions, increased significantly within 24 hour period (Fig. II.1, Table A.1). The results were different for formulation 13, where the ratios increased significantly only when liposomes were incubated with 1.25 and 1.5% polycarbophil solutions (Fig. II.2, Table A.2). Containing the same amount of PBS, formulation 12 included half as much lipids as formulation 13, however, the ratios of formulation 12 seemed to increase at a higher extent. It was unlikely to explain the reason of this difference, but it might be a result of higher water uptake of formulation 12 compared to that of formulation 13, due to higher ratio of aqueous phase to the lipid contents.

Drug Release from Polycarbophil Coated Liposomes

The release pattern of each drug from liposomes with various percentages of polycarbophil coating (0-2%) was similar. The percentages of insulin (formulation 12), insulin (formulation 13), dyphylline, and hydrocortisone released were described in Tables A.3, A.4, A.5 and A.6, respectively, and illustrated in Figs. II.3, II.4, II.5 and II.6, respectively. In general, the rates of drug release were not controlled by the level of polycarbophil coating. 70-90% of the drug was released within 8 hours. However, the release rates of insulin and dyphylline (hydrophilic drugs) were slightly faster from the

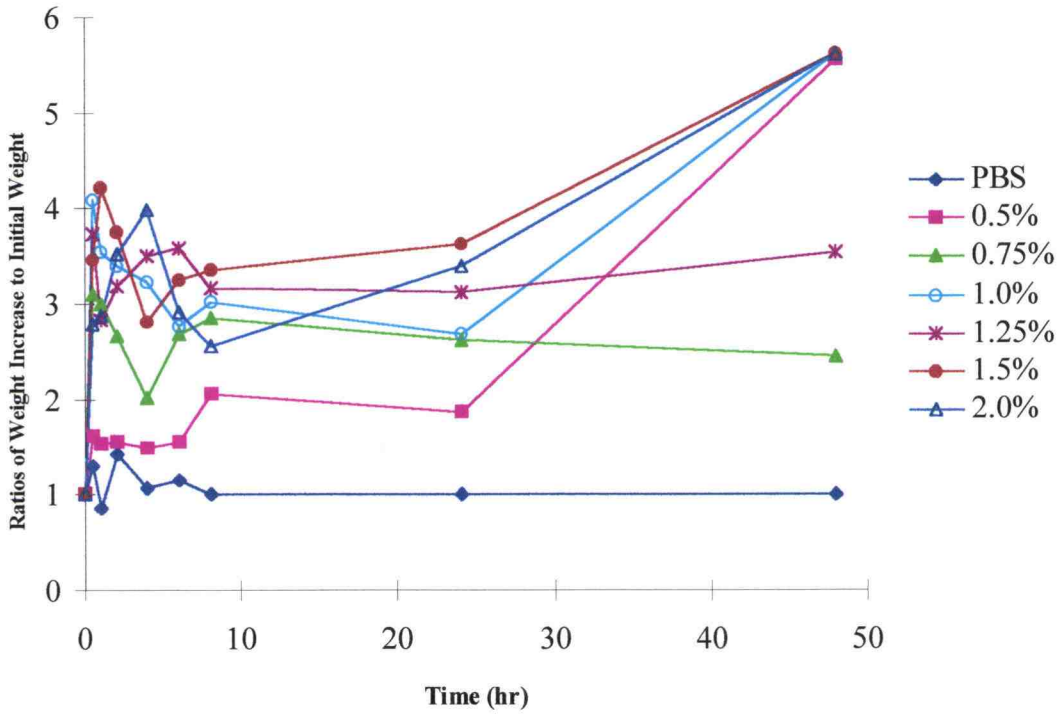

Fig. II.1 Ratios of weight increase to initial weight of liposomes (formulation 12) incubated with polycarbophil solution, plotted against time. Key: PBS represents incubation of liposomes with PBS (no polycarbophil). 0.5 to 2.0% indicate percentages of polycarbophil solution used for incubation with liposomes.

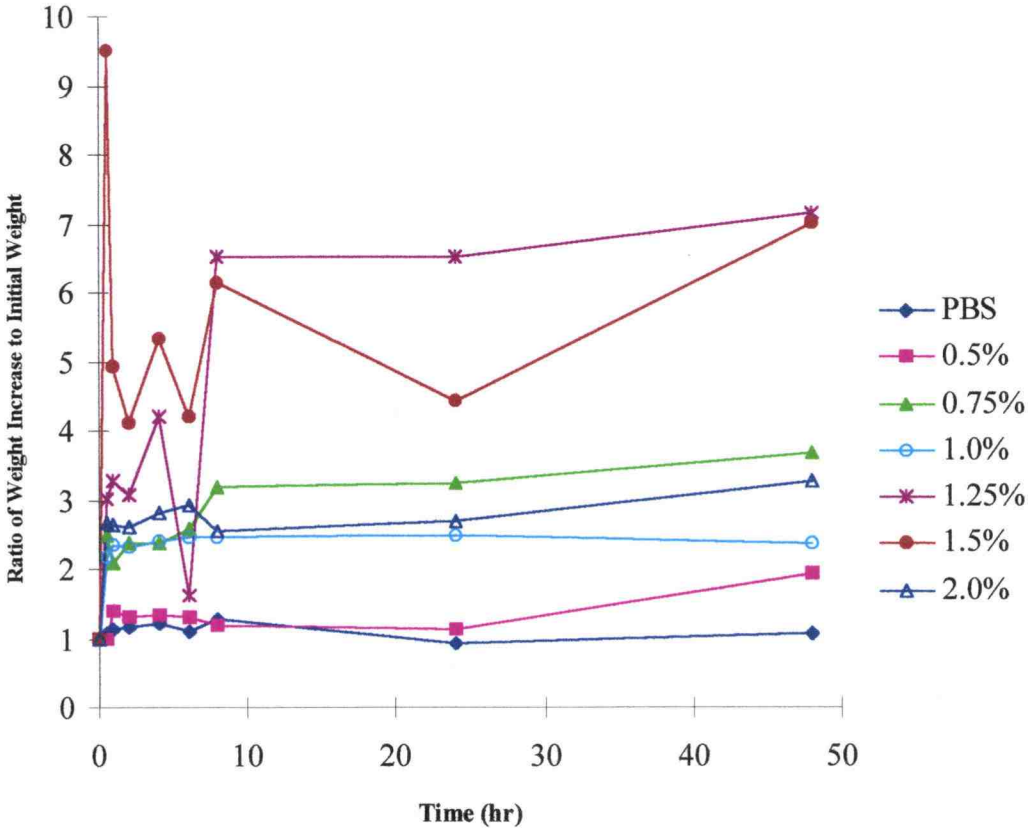

Fig. II.2 Ratios of weight increase to initial weight of liposomes (formulation 13) incubated with polycarbophil solution, plotted against time. Key: PBS represents incubation of liposomes with PBS (no polycarbophil). 0.5 to 2.0% indicate percentages of polycarbophil solution used for incubation with liposomes.

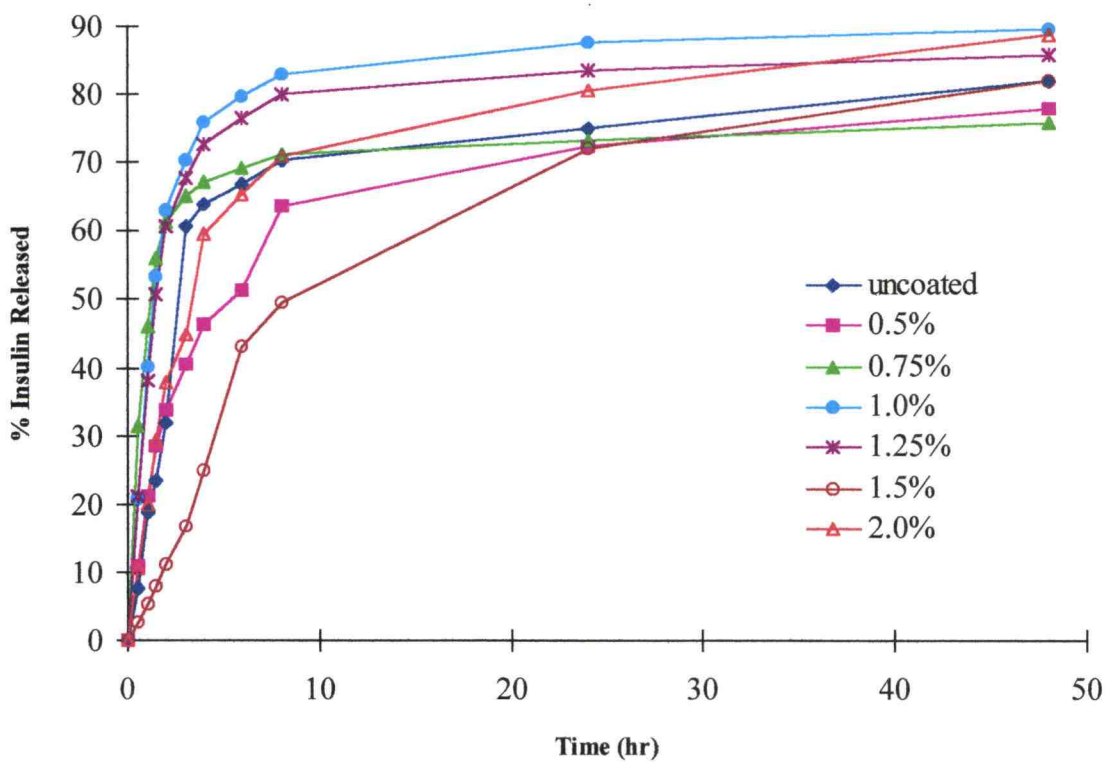

Fig. II.3 Insulin released from uncoated or polycarbophil coated liposomes (formulation 12). Key: "Uncoated" represents uncoated liposomes. "0.5-2.0%" indicate percentages of polycarbophil solution used for incubation with liposomes.

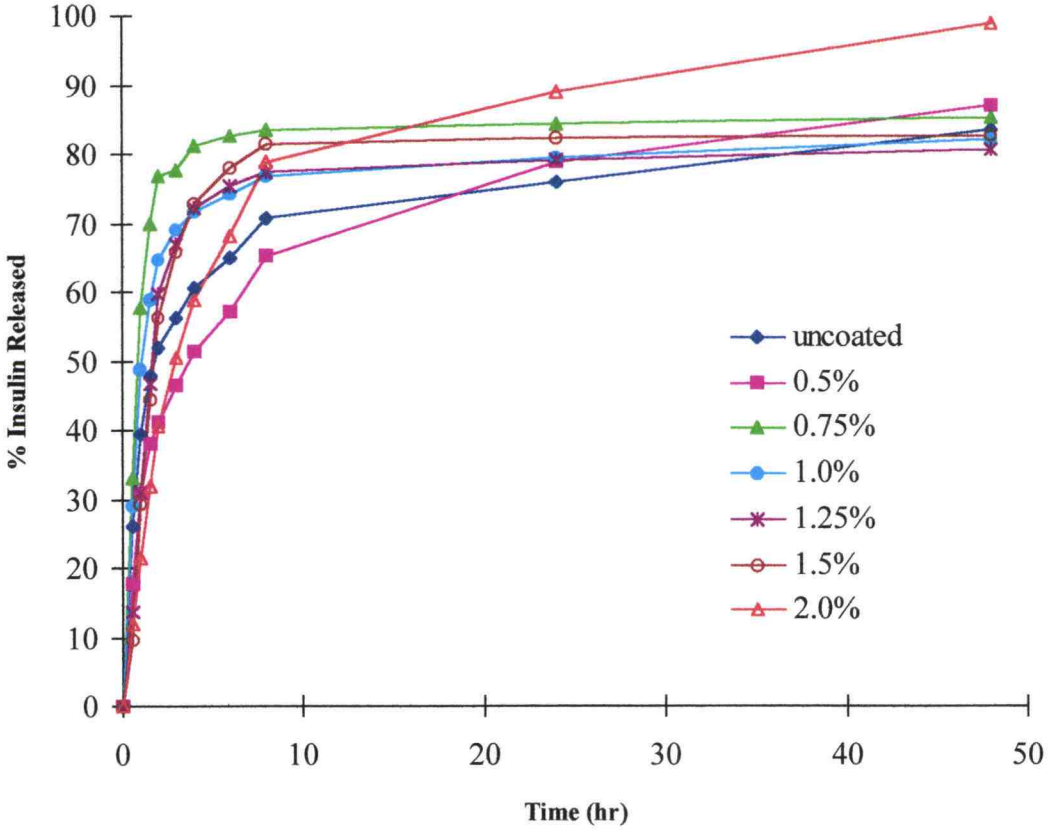

Fig. II.4 Insulin released from uncoated or polycarbophil coated liposomes (formulation 13). Key: "Uncoated" represents uncoated liposomes. "0.5-2.0%" indicate percentages of polycarbophil solution used for incubation with liposomes.

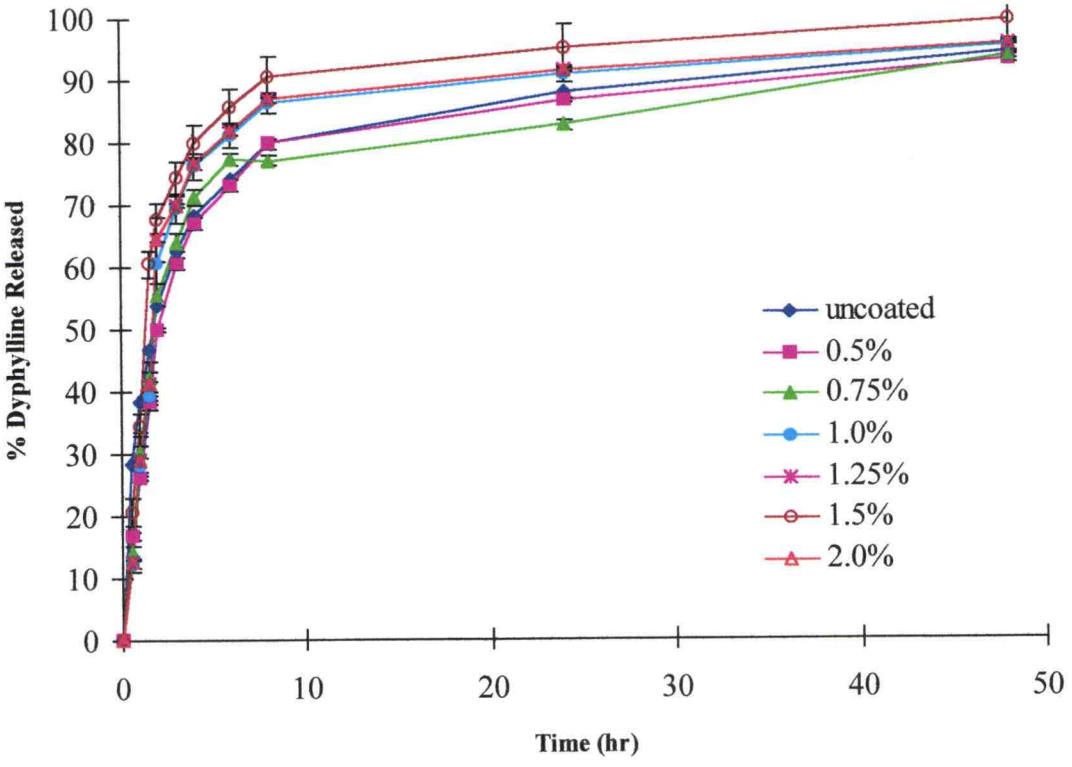

Fig. II.5 Dyphylline released from uncoated and polycarbophil coated liposomes. Error bars represent standard deviations. Key: "Uncoated" represents uncoated liposomes. "0.5-2.0%" indicate percentages of polycarbophil solution used for incubation with liposomes.

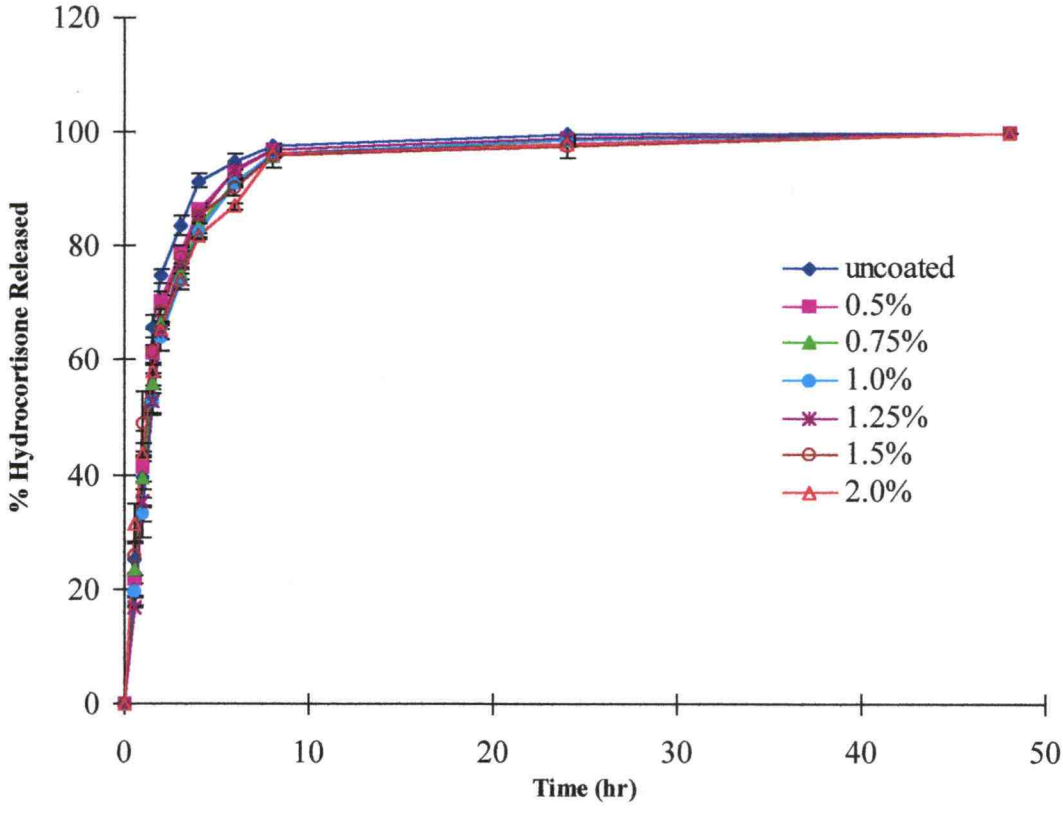

Fig. II.6 Hydrocortisone released from uncoated and polycarbophil coated liposomes. Error bars represent standard deviations. Key: "Uncoated" represents uncoated liposomes. "0.5-2.0%" indicate percentages of polycarbophil solution used for incubation with liposomes.

higher percentages (*i.e.*, 1.5%, 2%) of polycarbophil coated liposomes. In contrast, the release rates of hydrocortisone (hydrophobic drug) from liposomes were slower from the higher percentages (*i.e.*, 1.5%, 2%) of polycarbophil coated liposomes. Thus, an increase in polycarbophil coating slightly accelerated the release of hydrophilic drugs, but slightly retarded the release of hydrophobic drug from liposomes.

Loss of Entrapped Drug from Liposomes and Drug Stability

At room temperature (22°C) and 4°C drug concentrations in uncoated liposomes decreased over time as determined by the lower percentages of drug remaining entrapped, illustrated in Figs. II.7-II.12 and presented in Tables A.7, A.8, and A.9. To identify if the drug loss was due to drug being degraded over time, solutions of drug in PBS (pH 7.0) were stored at the same conditions as liposomes. Insulin dissolved in PBS degraded over time at room temperature, but was stable at 4°C. Both dyphylline and hydrocortisone were stable at room temperature or 4°C (Fig. II.13, Table A.10). Loss of insulin entrapped in liposomes stored at room temperature was due to drug degradation as well as drug leakage. Except for insulin stored at room temperature, loss of entrapped drug at both temperatures was due to drug leakage from liposomes. The loss of entrapped insulin was reduced by polycarbophil coating. The polycarbophil coated liposomes, previously incubated with 1.0-1.5% polycarbophil solutions, were the most stable, losing only small amounts of drug.

Loss of the entrapped dyphylline or hydrocortisone was not reduced by polycarbophil coating. Coated and uncoated liposomes entrapped with hydrocortisone

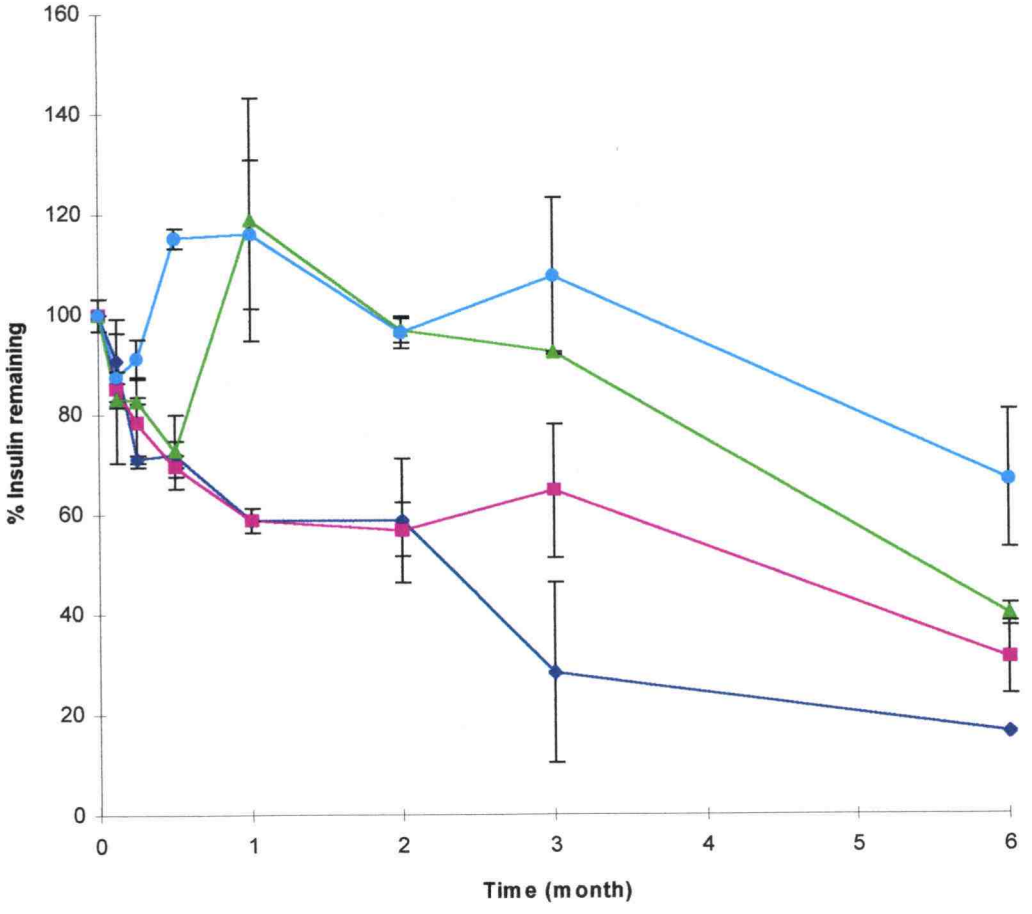

Fig.II.7 Percentages of insulin remaining in uncoated and polycarbophil coated liposomes over 6 months at room temperature. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

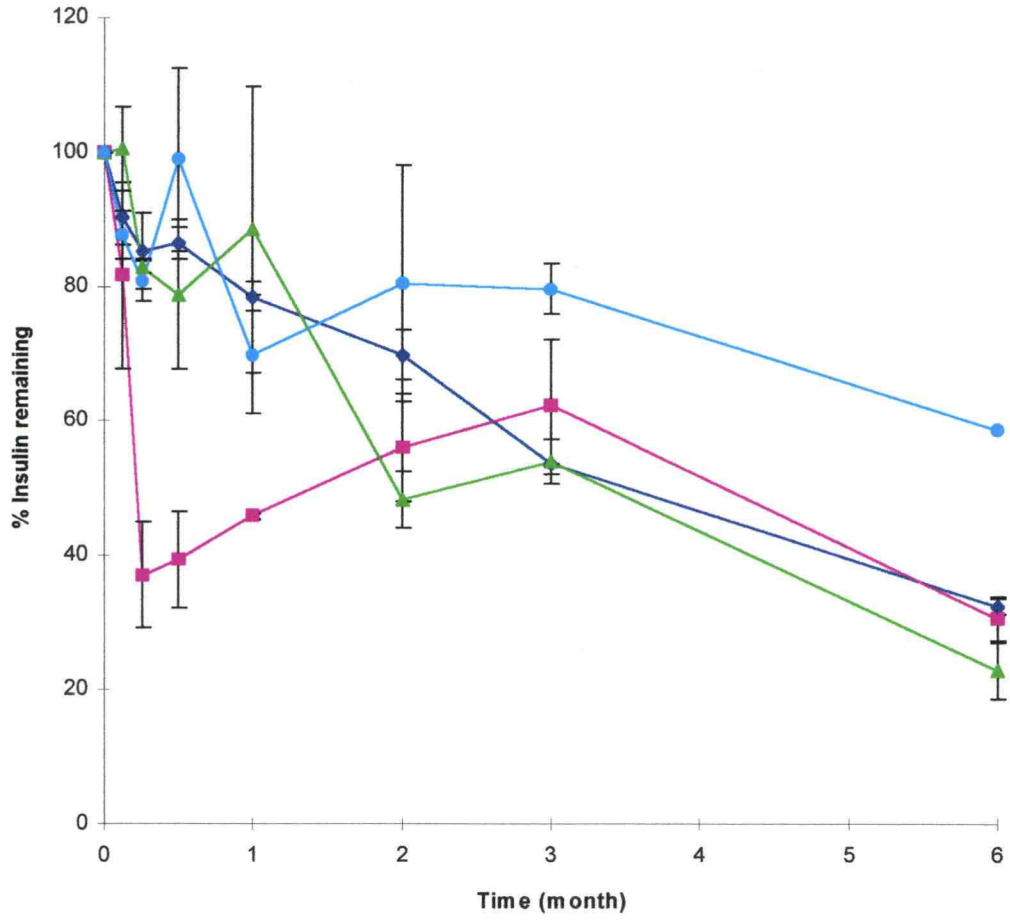

Fig.II.8 Percentages of insulin remaining in uncoated and polycarbophil coated liposomes over 6 months at 4°C. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

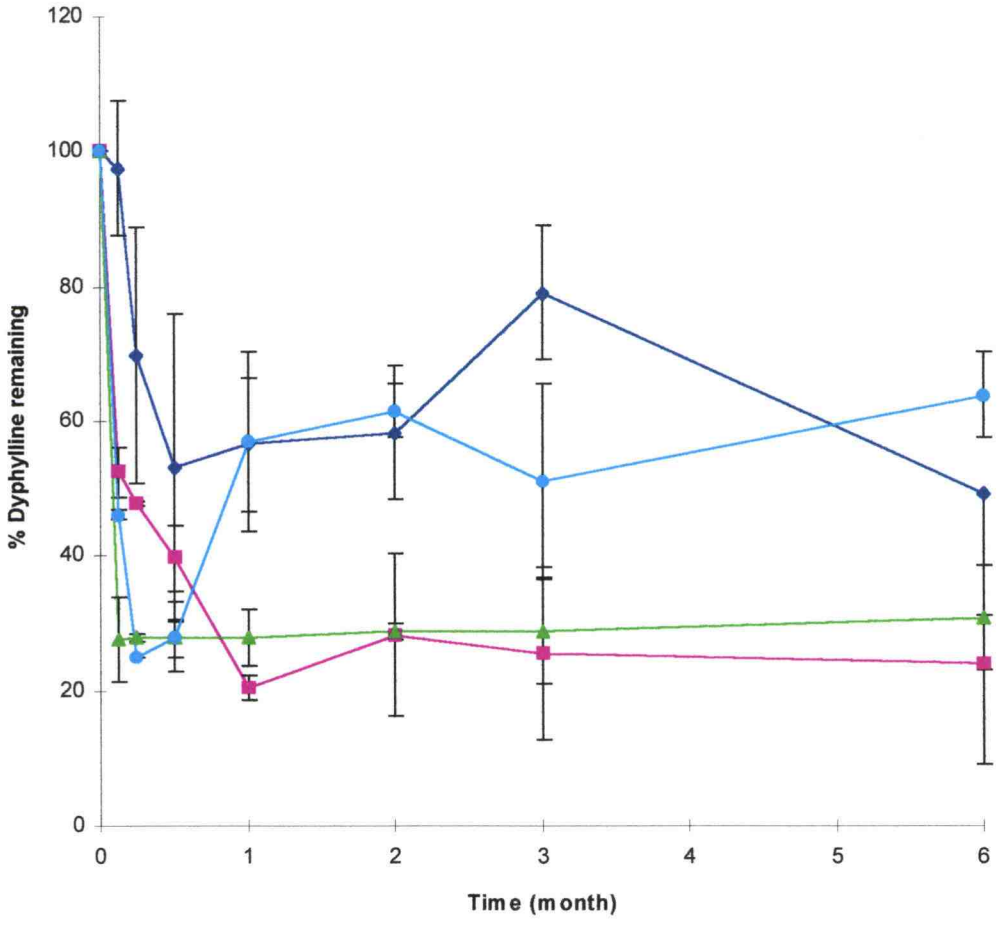

Fig.II.9 Percentages of dyphylline remaining in uncoated and polycarbophil coated liposomes over 6 months at room temperature. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

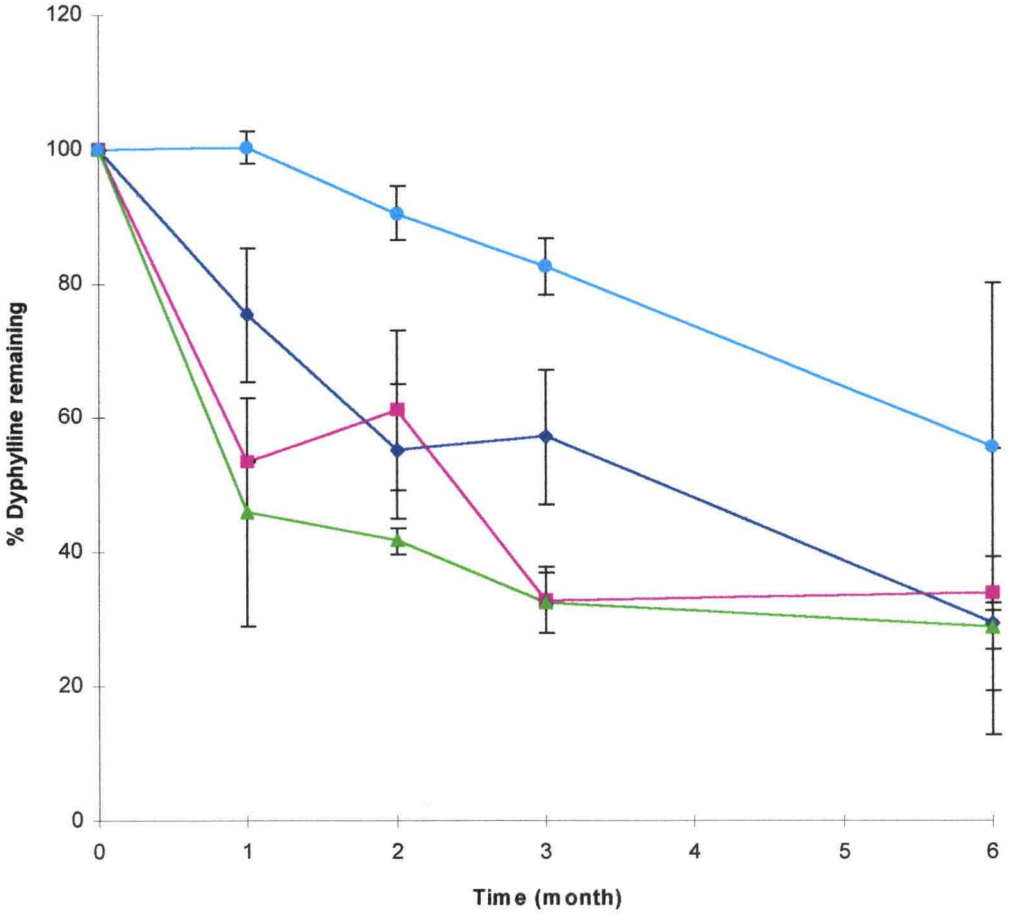

Fig.II.10 Percentages of dyphylline remaining in uncoated and polycarbophil coated liposomes over 6 months at 4°C. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

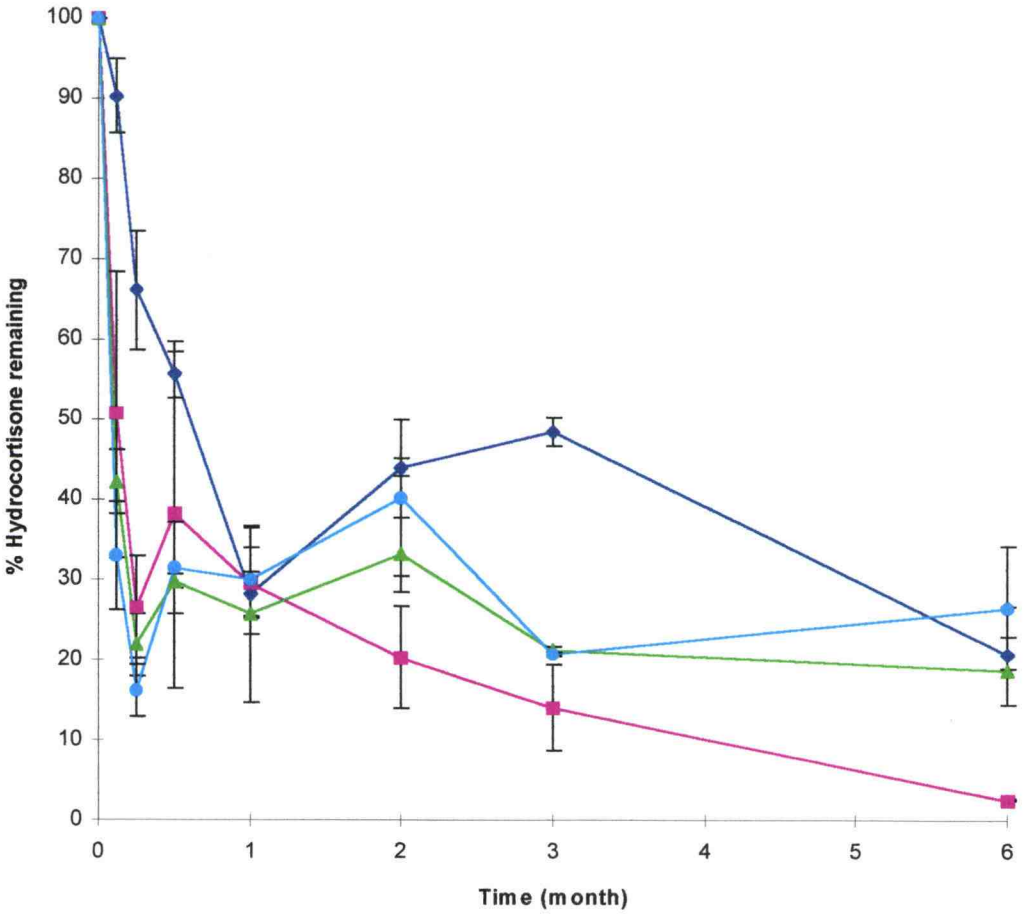

Fig. II.11 Percentages of hydrocortisone remaining in uncoated and polycarbophil coated liposomes at room temperature. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

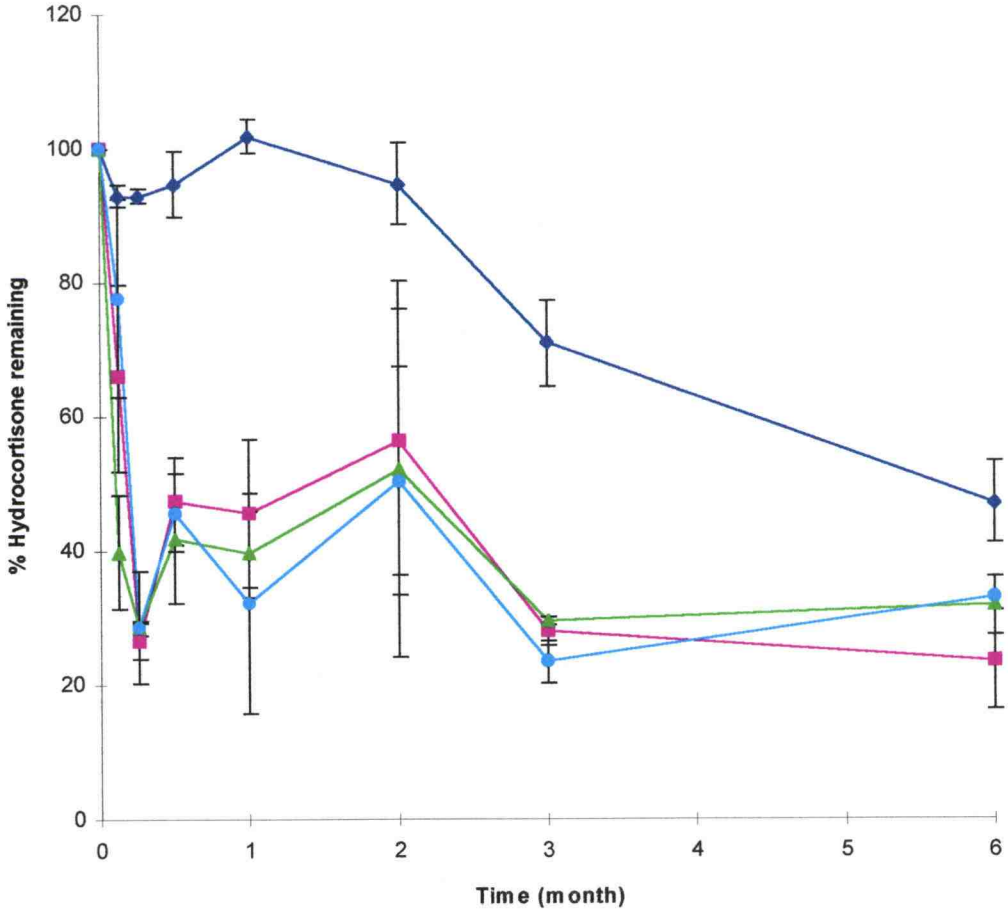

Fig.II.12 Percentages of hydrocortisone remaining in uncoated and polycarbophil coated liposomes over 6 months at 4°C. Error bars represent standard deviation. Key: (◆)represents uncoated liposomes; (■), (▲), and (●) represent liposomes incubated with 0.5%, 1.0%, and 1.5% polycarbophil solutions, respectively.

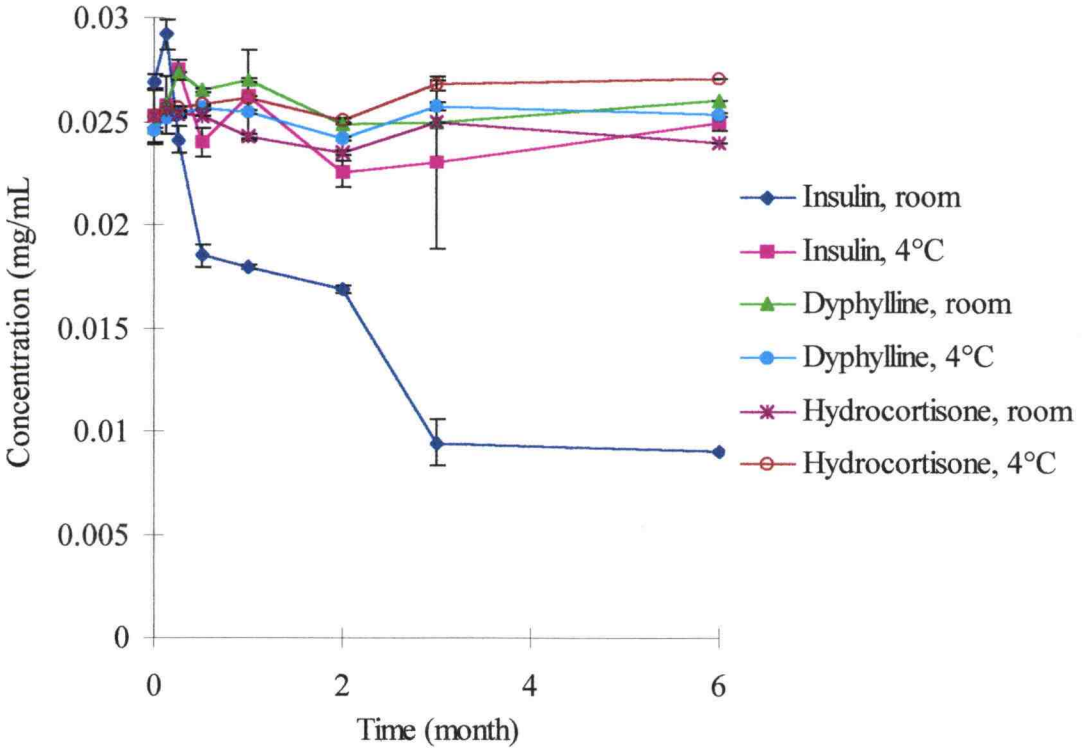

Fig.II.13 Percentages of insulin, dyphylline, and hydrocortisone in PBS (pH 7.0) remaining at room temperature and 4°C. Error bars represent standard deviations. Key: "room" represents room temperature.

were not very stable. Mostly, liposomes were more stable at 4°C than at room temperature.

Being entrapped in the aqueous phase of the liposomes, a large molecular drug like insulin diffused through the liposome membranes at the lower rate than small molecular drug like dyphylline. Thus, insulin entrapped liposomes were the most stable. Hydrocortisone entrapped liposomes, on the other hand, were the least stable because the drug (hydrophobic), being entrapped at the bilayer membrane, leaked easier compared to insulin and dyphylline (hydrophilic) which were entrapped in the aqueous phase.

Membrane Permeation

Amounts of insulin, dyphylline, and hydrocortisone diffusing across the EVA membrane were demonstrated in Table II.5. Very low amounts of all drugs diffused through the EVA membrane. Insulin, a high molecular weight polypeptide, which as expected, had difficulty diffusing through the membrane or transporting through small pores of membrane. However, insulin penetrated a higher percentage through than the other two drugs.

Dyphylline, a small water-soluble molecule, barely transported across the EVA membrane. It was reported that different vehicles affects the skin permeation of dyphylline (18). Dyphylline incorporated in polyethylene glycol (PEG) base had good skin partitioning and low transdermal delivery. The latter was also true for dyphylline in PBS (Table II.5), however, good membrane partitioning reported for dyphylline in PBS cannot be concluded from this study.

Table II.5 Permeation of insulin, dyphylline, and hydrocortisone in PBS across the EVA membrane

Drug	Cumulative Amount Permeated after 8 hrs (μg)		Permeation Rate [1] (μg/cm^2/h)		% Drug Permeated [2]	
	Mean	SD	Mean	SD	Mean	SD
INS	18.75	15.95	0.71	0.60	0.0195	0.0103
DY	8.39	7.85	0.32	0.30	0.0014	0.0070
HY	4.87	5.30	0.18	0.20	0.0121	0.0131

Note: INS represents insulin, DY represents dyphylline, and HY represents hydrocortisone. (1) Permeation rate described herein indicates cumulative amount of drug diffusing through after 8 hours divided by 8 hours and surface area of the membrane (3.3006 cm^2). Note that it is not the flux being presented which is a slope of the curve between cumulative amount permeated per unit area versus time. (2) "% drug permeated" is percentage of drug diffusing across the membrane relative to the total amount applied in the donor compartment.

A very low amount of hydrocortisone, a small lipophilic molecule, diffused through the EVA membrane. However, the results were similar to the the permeation of hydrocortisone in a mixture of 40% (v/v) polyethylene glycol (PEG) 400 in water diffusing across the intact abdominal hairless mouse skin, which had a permeation rate of 0.15±0.07 μg/cm^2/h (19).

The amounts of drugs transported across the EVA membrane was too low to detect when the drugs were formulated in liposomes. All drugs had very low membrane permeability and the amounts of drugs loaded in liposomes were very small; therefore,

drug concentrations in receptor solution were too low to detect with the instrumentation and assay method used in this study.

Drug transport through the membrane usually involves two different mechanisms: permeation of a drug through a solution-diffusion membrane and diffusion of a drug through membrane pores. Both mechanisms may be described by the following equations (20).

Solution-Diffusion Mechanism

A steady state permeation rate is mathematically described by Fick's first law (Eq. II.1),

$$\frac{dQ}{dt} = \frac{D_m \Delta C}{L} \qquad \text{Eq.II.1}$$

where D_m is the diffusion coefficient of a drug in the membrane. ΔC is the concentration gradient of the drug in the membrane, and L is the membrane thickness.

Porous Diffusion Mechanism

Diffusion of a drug through the membrane pores cannot be described by Fick's first law, but is expressed by Eq. II.2,

$$\frac{dQ}{dt} = \frac{D_v K' \varepsilon \, \Delta C}{\tau \, L} \qquad \text{Eq.II.2}$$

where D_v is the diffusion coefficient of the drug in the vehicle that fills in the membrane pore and K' is the partition coefficient of the drug between the bulk solvent and the

solvent in the membrane pore. ε is porosity, reflecting the volume fraction of pores in the membrane with a thickness of L. Tortuosity (τ) reflects a geometrically averaged path length in nonlinear pores.

Therefore, penetration of drug across the membrane depends on many factors related to chemical and physical properties of the drug, and types, nature, and thickness of the membrane. The permeation rate also relies on drug solubility in the donor compartment and a continuous sink condition of the receiver compartment maintained throughout the permeation. In this study, insulin penetrated through the membrane at a highest rate, while dyphylline penetrated at the higher rate than hydrocortisone. Nonetheless, percentage of dyphylline permeated was the lowest among the three, while that of hydrocortisone was very similar to insulin. It may be concluded that hydrophilic drugs permeated through the EVA membrane at a higher rate than the hydrophobic drug. The possibility that the membrane pores which were filled with the aqueous phase (hydrophilic in nature) were the major transport pathway of the drugs may explain the results. In addition, the EVA membrane may prefer hydrophilic drugs to diffuse through to hydrophobic drugs. The reason of higher permeation rate of insulin compared to dyphylline is unknown, however, it was possible that dyphylline has better membrane partitioning and tends to be retained on the membrane as previously reported (18).

The information obtained from this study implied that insulin, dyphylline, and hydrocortisone in PBS or in liposomes tend to localize on the membrane rather than penetrate through the membrane. Therefore, they may be more suitable for topical application in the liposome formulation rather than being used for transdermal administration.

However, the low permeation of drugs in this study might be due to the fact that the EVA membrane was impervious or not porous enough for the drugs to permeate through. Thus, to be able to evalute penetration of both dissolved and liposomal formulated drugs across the membrane, it is recommended that other synthetic membranes such as polydimethylsiloxane membrane which has low diffusional resistance (21) or animal skin such as hairless rat or mouse skin be used for further comparison.

If transdermal drug delivery is required, a suitable penetration enhancer may be incorporated to improve drug permeation through the membrane. Including Tween® 80 in the aqueous phase of liposomes may not only improve drug entrapment stability, but also enhance drug penetration through the membrane. Therefore, application of polycarbophil coated liposomes may be extended to various routes of drug administration.

CONCLUSIONS

Reversed phase evaporation method and lipid components of 1: 3.3 distearoyl phosphatidylcholine and cholesterol were selected for preparing liposomes in the study of polycarbophil coated liposomes.

Weights of liposomes generally increased as a function of time when being incubated with polycarbophil due to adhesion of polycarbophil on outer membrane of liposomes and, possibly allowing greater water uptake by the polycarbophil.

Drug release from polycarbophil coated liposomes was not controlled by increasing the percentages of polycarbophil in the incubating solution. Rates and patterns

of drug release were similar for all levels of polycarbophil. However, there was a tendency that the hydrophilic drugs were released slightly faster with higher percentages of polycarbophil, and *vice versa* for the hydrophobic drug.

Loss of entrapped insulin from liposomes was reduced considerably after coating with 1-1.5% polycarbophil solution. Therefore, in addition to being bioadhesive, liposomes coated with polycarbophil were a stable carrier for insulin.

Very low amounts of insulin, dyphylline, and hydrocortisone in PBS permeated across ethylenevinylacetate membrane. Hydrophilic drugs penetrated through the membrane at a higher rate than the hydrophobic drug. Interestingly, insulin which is a larger molecule, penetrated across the membrane to a greater extent than dyphylline. The amounts of drugs transported across EVA membrane from the liposomes were too low to be detected.

The information obtained from this study suggests that polycarbophil coated liposomes were suitable dosage form for topical application. Their mucoadhesiveness allows their application for drug administration at local sites, where they should be retained and localized.

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CHAPTER III

Drug Release from Spray Layered and Coated Drug Containing Beads: Effects of pH and Comparison of Different Dissolution Methods

Waranush Sorasuchart, Jacqueline Wardrop, and James W. Ayres

ABSTRACT

It is concluded that *in vitro* dissolution of oral controlled-release formulations should be performed in both gastric and intestinal media for ionizable drugs based on dissolution profiles of three model drugs on spray layered beads, with the same percentage of Aquacoat® coating. Ketoprofen (weak acid, pKa 4.8), nicardipine HCl (salt of weak organic base, pKa 8.6), and acetaminophen (very weak organic acid, pKa 9.7, not ionized at physiologic pH) provided different dissolution characteristics in different media: enzyme-free simulated gastric fluid (pH 1.4) and enzyme-free simulated intestinal fluid (pH 7.4), indicating that the rate of drug release was pH dependent and related to drug ionization even though the coating (ethylcellulose) solubility is pH independent. In acidic media, ketoprofen release was slower than nicardipine HCl and *vice versa* in basic media. Acetaminophen was released at approximately the same rate in both acidic and basic media. This information now allows prediction of site specific gastrointestinal drug release patterns for controlled release drug product formulations. A comparison of drug release profiles for nicardipine HCl nude beads was also investigated among three different dissolution methods: USP dissolution apparatus I (basket method, 50 RPM), USP dissolution apparatus II (paddle method, 50 RPM), and USP dissolution apparatus III (Bio-Dis®,Van-Kel Industries, 5 and 10 DPM). Release profiles obtained from all methods were similar, indicating that the three dissolution methods were comparable.

INTRODUCTION

Controlled-release drug products containing coated beads stay longer in dissolution media or biological systems than immediate release dosage forms; thus, scattering along the gastrointestinal tract and exposing drug beads to varying pH (pH 1 in fasting stomach as high as 8 in distal region of the intestine) (1). Therefore, pH has a major effect on drug release from these controlled-release formulations.

Dissolution testing is essential in designing and evaluating controlled-release dosage forms. Appropriate dissolution media should be carefully selected for particular drug and dosage form combinations.

Effects of pH of dissolution media on release rates of 3 model drugs with different solubilities and pKa's from Aquacoat® coated beads were investigated. Aquacoat® (FMC Corporation, Newark, DE) dispersion contains solid contents of 27% ethylcellulose and 3% sodium lauryl sulfate in water. Solubility of ethylcellulose is pH independent, however, it was previously reported that the release rates of theophylline (6), phenylpropanolamine HCl (6), and propranolol HCl (7) from Aquacoat® coated beads were pH dependent.

In dissolution testing of controlled-release dosage forms, gastric and intestinal media may be used to simulate the pH throughout the gastrointestinal tract. Nevertheless, the basket (USP apparatus I, Fig. III.1) and paddle (USP apparatus II, Fig. III.1 , III.2) are not convenient when a change of dissolution media is needed. Recently included in the United States Pharmacopeia (USP) XXIII (2), USP apparatus III (reciprocating cylinders, Bio-Dis®, Van-Kel Industries, Cary, NC, Fig. III.1, III.3) eliminates manual and tedious

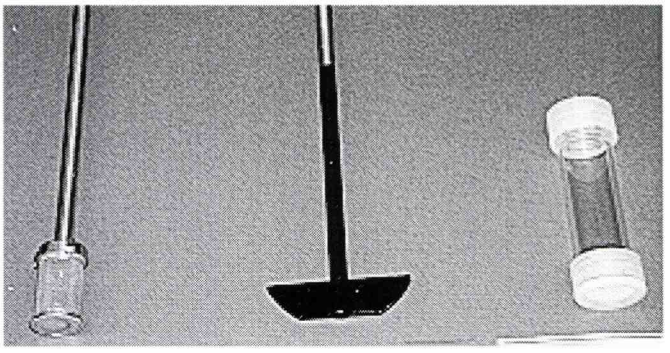

Fig. III.1 USP dissolution elements. From left to right; basket, paddle, and Bio-Dis® inner tube.

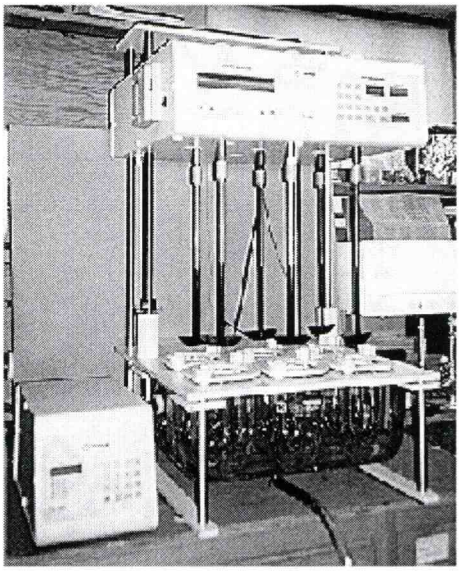

Fig. III.2 USP dissolution apparatus II (paddle)

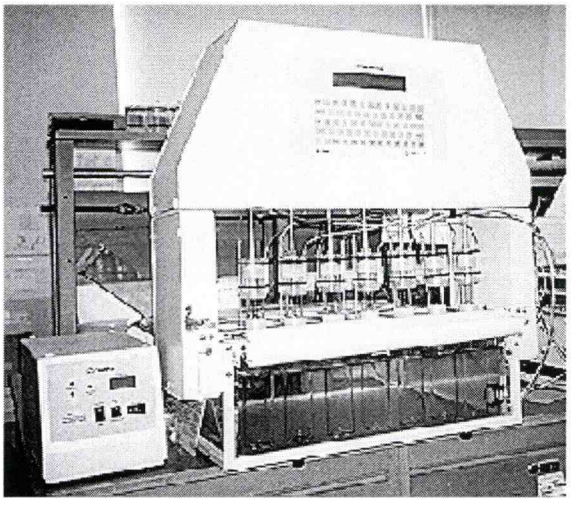

Fig. III.3 USP dissolution apparatus III (Bio-Dis®)

work in changing dissolution media providing an advantage when dissolution testing is performed in a pH step gradient. Drug release profiles obtained with the Bio-Dis® in pH step gradient dissolution media were comparable to those of the NF XIII (3) official bottle rotation method (4). Using an empirical equation to fit parameters for a specific formulation, Rohrs B. R. et. al. reported that dipping rates of 5-8 DPM of Bio-Dis® would be equivalent to 50 RPM paddle or 100 RPM basket (5). However, no publication of direct comparison of dips per minute (DPM) of Bio-Dis® which was equivalent to the standard rounds per minutes (RPM) of USP apparatuses I and II has been found. In this study comparative dissolution testing of dips per minute (Bio-Dis®) equivalent to standard rounds per minute (basket and paddle) was performed.

MATERIALS AND METHODS

Chemicals

Nicardipine HCl (Lot#4628) was supplied by Lemmon Company, Sellersville, PA. Ketoprofen was supplied by Biocraft, Fairfield, NJ. Acetaminophen was purchased from Sigma Chemical Co, St. Louis, MO. Other chemicals used included triethyl citrate 99% (TEC, Aldrich Chemical Company, Inc., Milwaukee, Wis), dibutyl sebacate (DBS, Sigma Chemical Co, St. Louis, MO), hydroxypropylcellulose (Klucel, Hercules Inc., Wilmington, Delaware), polyvinylpyrrolidinone K-30 (PVP, EM Science, Gibbstown, NJ), Aquacoat® (FMC Corporation, Newark, DE). Nonpareil sugar beads were purchased

from Crompton and Knowles Corp., Pennsauken, NJ. Other chemicals are of reagent grade.

Methods

Nude and Coated Beads Preparation

Each drug was sprayed layered onto 100 g of nonpareil sugar beads (25-30 mesh) in a coating chamber of a fluid-bed spray coater (Strea-1, Aeromatic Inc., Columbia, MD) containing a seven-inch Wurster column. The Wurster column was approximately 1 inch away from the bottom of screen of the coater, which was connected to a Lab-line/ P.R.L. High Speed Fluid Bed Dryer (Lab-line, Melrose Park, IL).

Spray layering was performed at 40°C. Air pressure was maintained at 10 psi and blower speed set at 80-90 % of full capacity to allow beads to move freely. Drug solution/ suspension was constantly delivered by a peristaltic pump (Rabbit® Peristaltic pump, Gilson Medical Electronics, Middleton, WI). During spray layering, the drug solution/suspension was kept stirring by a magnetic stirrer to ensure the homogeneity of solution/suspension. Drug layered beads were dried in the coating chamber for another 30 minutes at the same temperature and air flow before removing. Beads were then sieved to remove agglomerated and fine particles.

Drug spray layered beads (nude beads) were then overcoated with 3% (w/w) Aquacoat® that was previously diluted 1:1 with distilled deionized water and stirred with plasticizers (15% w/w TEC and 15% w/w DBS). Spray coating was performed using the fluid-bed spray coater. Other conditions were as previously described.

Dissolution Testing of Aquacoat® Coated Beads

Dissolution profiles of Aquacoat® coated drug layered beads were determined using the United States Pharmacopeia (USP) XXII apparatus II, paddle stirring method (VK 7000®, VanKel Industries, Inc., Edison, NJ). Dissolution media (filtered, degassed and maintained at 37.0 °C) included 900 mL of enzyme-free simulated gastric fluid (pH 1.4±0.1) for the first 2 hours and 900 mL of enzyme-free simulated intestinal fluid (pH 7.4±0.1) subsequently. In case of nicardipine HCl, the dissolution tank was protected from light with cardboard.

Dissolution tests of Aquacoat® coated drug containing beads were performed in triplicate. The beads were weighed and dropped in the dissolution vessels at time zero. Dissolution was studied at a paddle rotation speed of 50 rpm. Samples of 3 mL dissolution media were withdrawn without medium replacement at 0.25, 0.5, 0.75, 1, 1.5, 2 hours (in gastric fluid), 2.25, 2.5, 2.75, 3, 4, 5, 6, 8, 12, and 18 hours (in intestinal fluid) using an autosampler (Peristaltic Pump VK 810® connected to System Monitor VK 8000®, VanKel Industries, Inc., Edison, NJ). All samples were filtered through 5 μm Acrodisc® (Gelman Sciences, Ann Arbor, MI). At 2 hours, the gastric fluid containing beads was filtered. Beads were gently collected and transfered to intestinal fluid previously maintained at 37.0°C. Paddle rotation was continued at a rate of 50 RPM.

The amounts of ketoprofen, nicardipine HCl, or acetaminophen released were detected directly by UV spectrophotometer (Hewlett Packard 8452 A diode Array Spectrophotomer, Hewlett Packard GmbH, Waldbronn 2, Federal Republic of Germany)

at wavelength 258, 358, and 244 nm, respectively. Standard solutions were prepared by serial dilutions from 1 mg/mL stock solutions.

Dissolution profiles of ketoprofen and nicardipine HCl from coated beads were also obtained in intestinal fluid only (no gastric pretreatment) using the paddle method at the rate of 50 RPM. Samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours without replacement. Determination of amounts of drug released was as previously described.

Comparison of Dissolution Testing of Nicardipine HCl Nude Beads among Three USP Dissolution Methods

Dissolution profiles of Nicardipine HCl nude beads were compared among 3 USP dissolution methods. In each method, dissolution testing was performed in triplicate in citrate buffer (pH 4.5) or in enzyme-free simulated gastric-intestinal fluids as previously described. When the citrate buffer was used, samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 , 12, 18, and 24 hours without replacement. When the gastric-intestinal fluids were used, samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2 (in gastric fluid), 2.17, 2.33, 2.5, 2.75, 3, 3.5, and 4 hours (in intestinal fluid) without replacement. Samples were filtered through 5 μm Acrodisc®. Amounts of drug released were detected as previously described.

USP Apparatus I (Basket Method)

Beads were weighed and put in the baskets which were then placed in the dissolution medium at the same time. Baskets were rotated at 50 RPM. In case of dissolution testing in gastric-intestinal fluids, the baskets were drained and patted to remove excess solution before transferring to intestinal fluid.

USP Apparatus II (Paddle Method)

Beads were weighed and dropped in the dissolution medium at the time zero. Dissolution testing was performed at a paddle rotation rate of 50 RPM. Dissolution testing in the gastric-intestinal fluids was as previously described.

USP Apparatus III (Bio-Dis®)

Each of the dissolution vessels in the first row of the Bio-Dis® contained 250 mL of enzyme-free simulated gastric fluid for the first 2 hours and each of those in the second row contained 250 mL of enzyme-free simulated intestinal fluid. The beads were weighed and placed in the dipping tubes containing a bottom screen. Dipping was performed with the rate of 5 or 10 dips per minute. The first dip was held for 3 seconds. The dipping tubes were drained for 1 minute before moving to intestinal fluid.

Statistical Analysis

Linear regression analysis for correlations of percentages of drug releases between each dissolution method was performed using Microsoft Excel® 5.0.

RESULTS AND DISCUSSION

Table III.1 summarizes chemical characteristics of the three model drugs. Ratios of ionized to nonionized forms of drugs are described in Henderson and Hasselbalch's equations (Eqs. III.1 and III.2). Table III.2 describes the ratios of ionized to nonionized forms of each drug at pH 1.4 and 7.4.

Table III.1 Chemical characteristics of ketoprofen, nicardipine HCl, and acetaminophen.

Drug	Acid-base property	pKa	Drug Solubility in water
Ketoprofen	weak organic acid	4.8	less than 1:18,000 *
Nicardipine HCl	salt of weak organic base	8.6	1:850 *
Acetaminophen	very weak organic acid	9.7	1:70 **

Note: * Solubilities were obtained experimentally. ** Solubility was obtained from (8).

For weak acids (1), $\frac{\text{ionized}}{\text{nonionized}} = 10^{(pH-pKa)}$ Eq. III.1

For weak bases (1), $\frac{\text{nonionized}}{\text{ionized}} = 10^{(pH-pKa)}$ Eq. III.2

Table III.2 Ratios of ionized to nonionized forms

pH of Dissolution Media	Ratio of ionized to nonionized forms		
	Ketoprofen	Nicardipine HCl	Acetaminophen
1.4	$3.98 \times 10^{-4}/1$	$1.58 \times 10^{7}/1$	$5.01 \times 10^{-9}/1$
7.4	398/1	15.85/1	0.005/1

Effects of pH on rates of drug release are illustrated in Fig. III.4 and Fig. III.5. Even though the polymer in Aquacoat® (ethylcellulose) is pH independent, release rates of ketoprofen and nicardipine HCl were pH dependent. Ketoprofen (weak acid) and nicardipine HCl (salt of weak base) are slightly soluble in water (Table III.1). Drug solubilities of both compounds depend on degrees of drug ionization (Table III.2). Ketoprofen is more ionized in basic medium, thus released faster than in acidic medium. Nicardipine HCl was more ionized in acidic medium, thus released faster than in basic medium. Fig. III.5 demonstrates that in basic medium the release of ketoprofen was very fast while that of nicardipine HCl was very slow. On the other hand, acetaminophen (very weak acid) is relatively more soluble in water; therefore, the release rates were similar in both dissolution media and not afffected by degree of ionization. In acidic media, acetaminophen release was much slower than nicardipine HCl, but faster than ketoprofen.

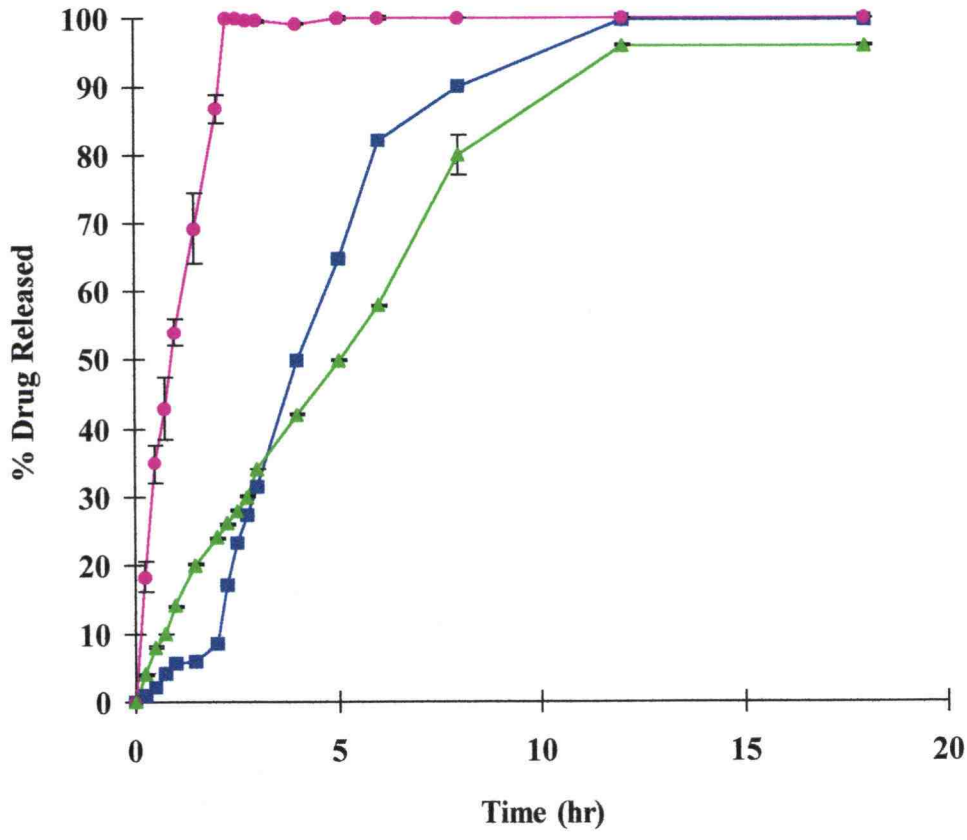

Fig. III.4 Dissolution profiles of model drugs in enzyme-free simulated gastric fluid (pH 1.4) for 2 hours and then in enzyme-free simulated intestinal fluid (pH 7.4) (paddle method). Error bar represents standard deviation. Key: (■) ketoprofen, (●) nicardipine HCl, and (▲) acetaminophen.

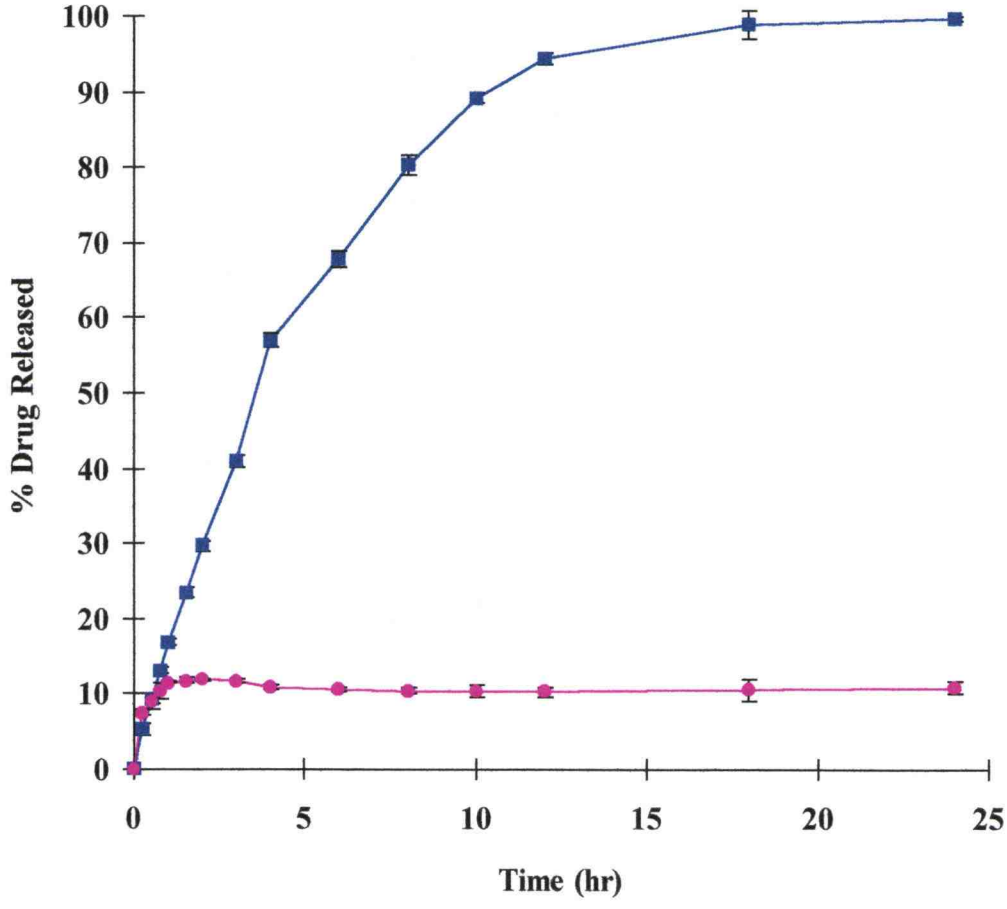

Fig. III.5 Dissolution profiles of ketoprofen and nicardipine HCl in enzyme-free simulated intestinal fluid (pH 7.4) (paddle method). Error bar represents starndard deviation. Key: (■) ketoprofen and (●) nicardipine HCl.

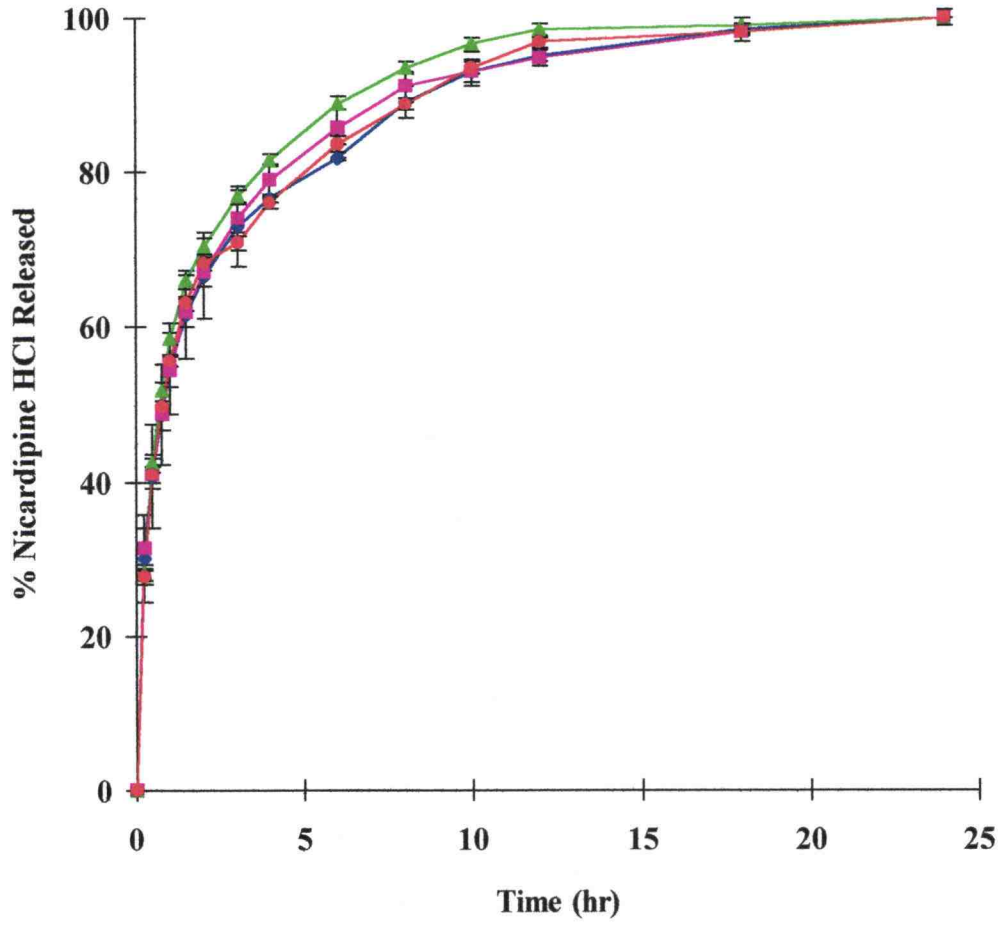

Fig. III.6 Dissolution profiles of nicardipine HCl nude beads in citrate buffer (pH 4.5). Error bar represents standard diviation. Key: (◆) Basket 50 RPM, (■) paddle 50 RPM, (▲) Bio-Dis® 5 DPM, and (●) Bio-Dis® 10 DPM.

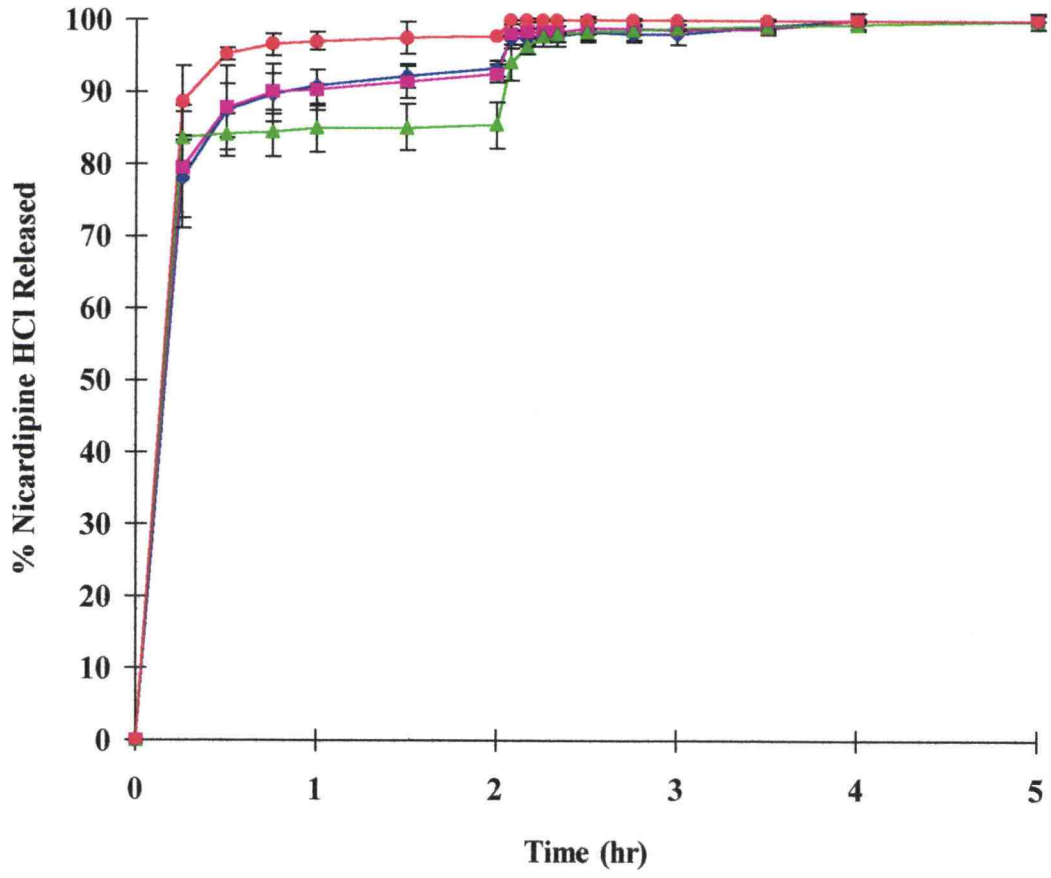

Fig. III.7 Dissolution profiles of nicardipine HCl nude beads in gastric fluid (pH 1.4) for 2 hours and then in intestinal fluid (pH 7.4). Error bar represents standard diviation. Key: (◆) Basket 50 RPM, (■) paddle 50 RPM, (▲) Bio-Dis® 5 DPM, and (●) Bio-Dis® 10 DPM.

Figs. III.8-a-e Correlations of percentages of drug released in citrate buffer (pH 4.5)

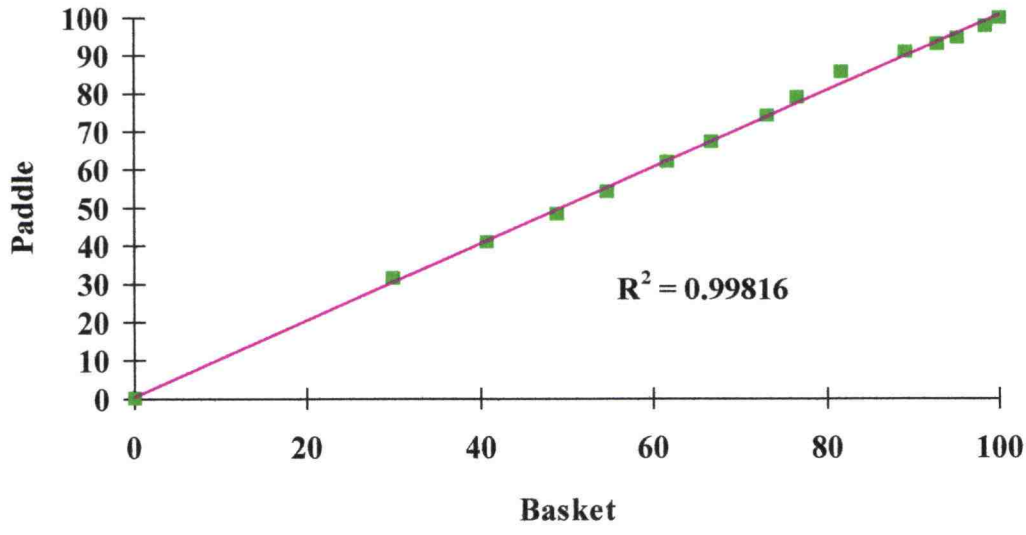

Fig. III.8-a Correlation of percentages of drug released between basket (50 RPM) and paddle (50 RPM) methods

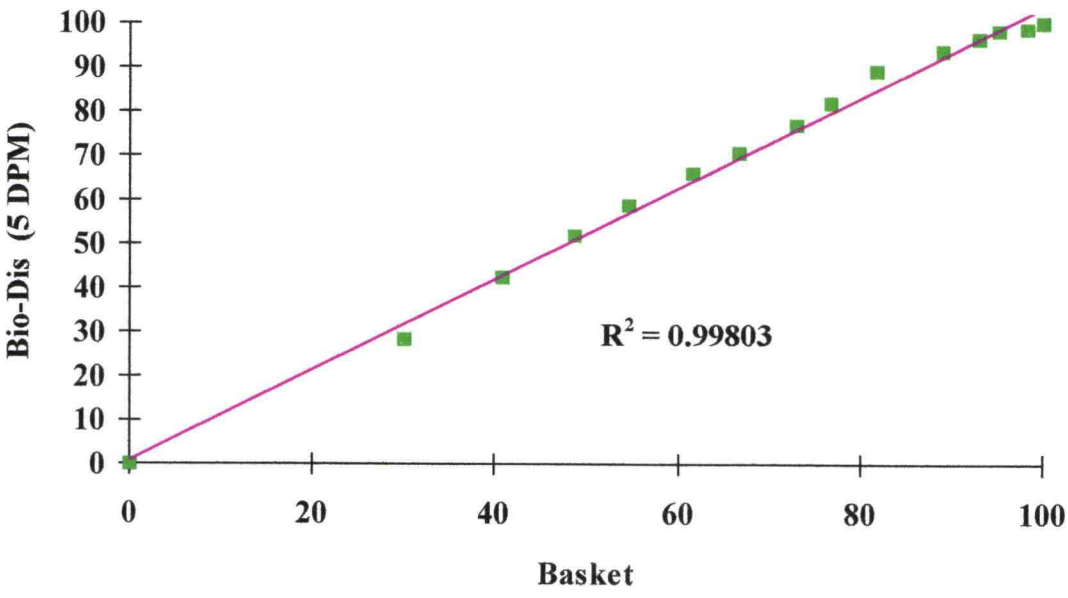

Fig. III.8-b Correlation of percentages of drug released between basket (50 RPM) and Bio-Dis® (5 DPM) methods

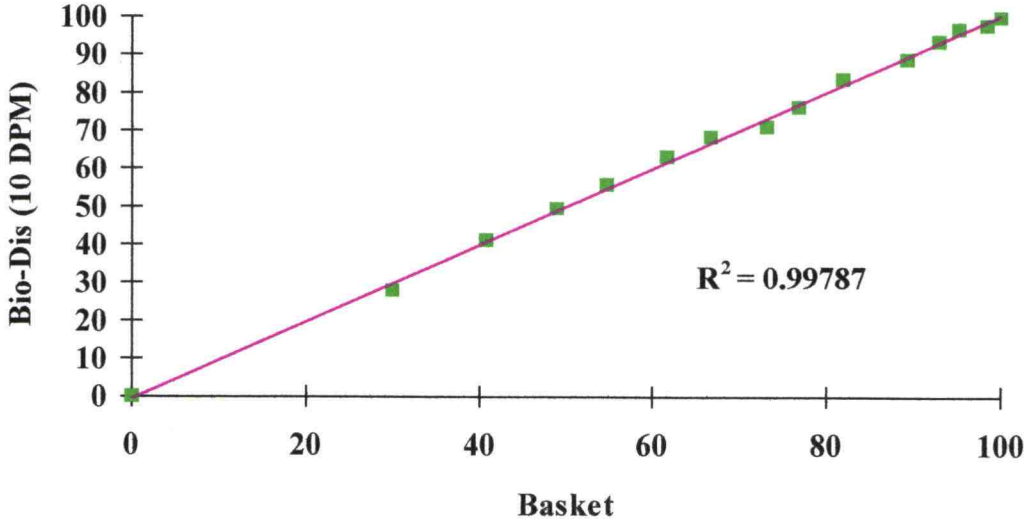

Fig. III.8-c Correlation of percentages of drug released between basket (50 RPM) and Bio-Dis® (10 DPM) methods

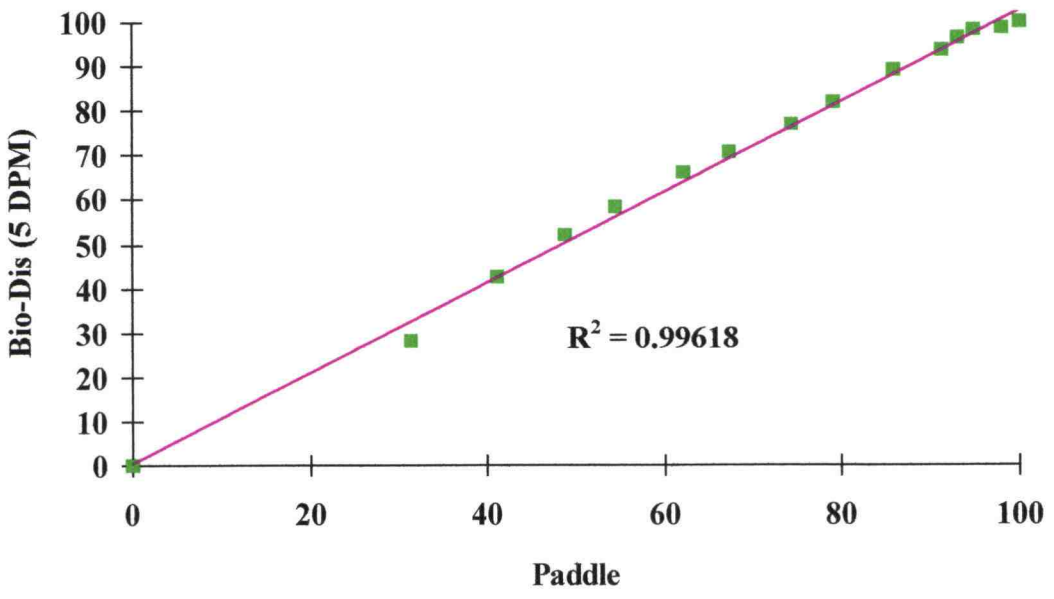

Fig. III.8-d Correlation of percentages of drug released between paddle (50 RPM) and Bio-Dis® (5 DPM) methods

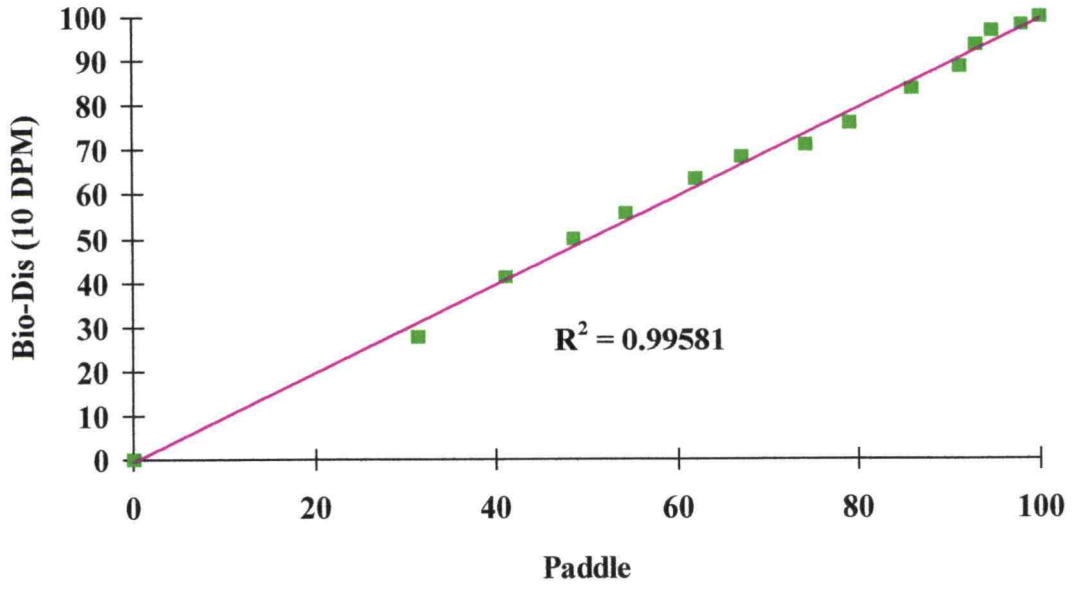

Fig. III.8-e Correlation of percentages of drug released between paddle (50 RPM) and Bio-Dis® (10 DPM) methods

Figs. III.9-a-e Correlations of percentages of drug released in enzyme-free simulated gastric and intestinal fluids

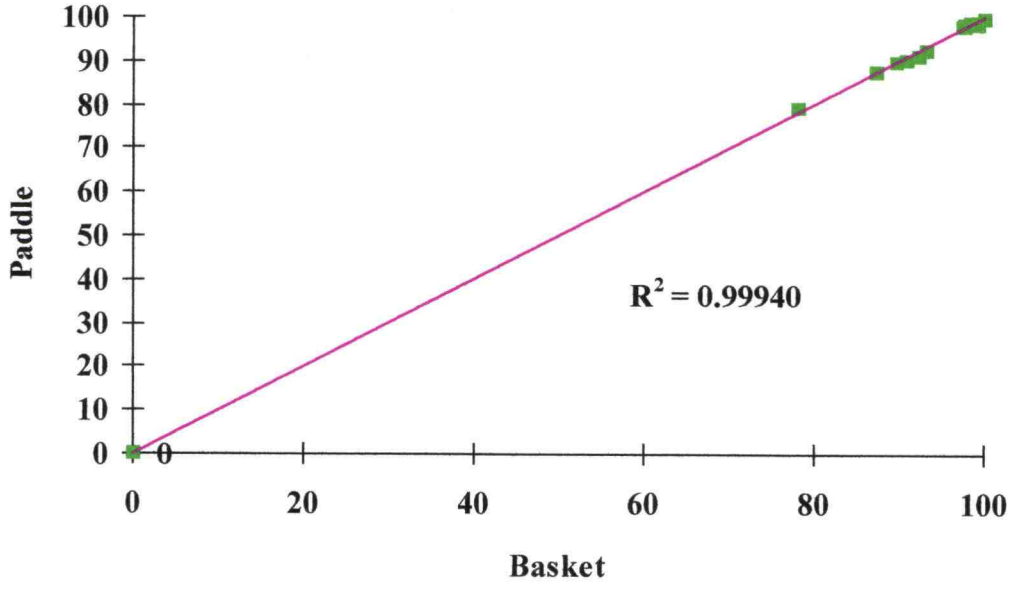

Fig. III.9-a Correlation of percentages of drug released between basket (50 RPM) and paddle (50 RPM) methods

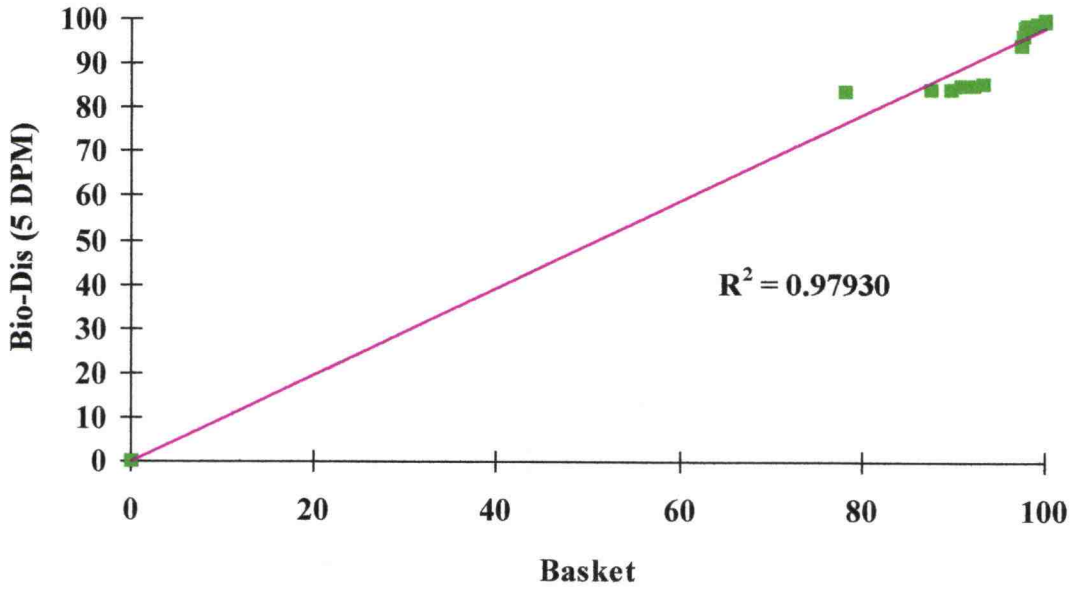

Fig. III.9-b Correlation of percentages of drug released between basket (50 RPM) and Bio-Dis® (5 DPM) methods

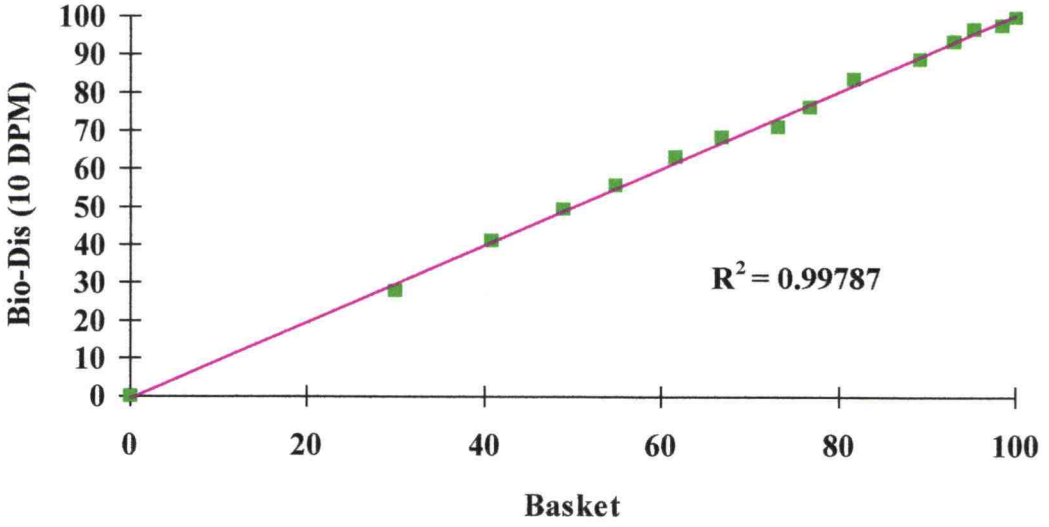

Fig. III.9-c Correlation of percentages of drug released between basket (50 RPM) and Bio-Dis® (10 DPM) methods

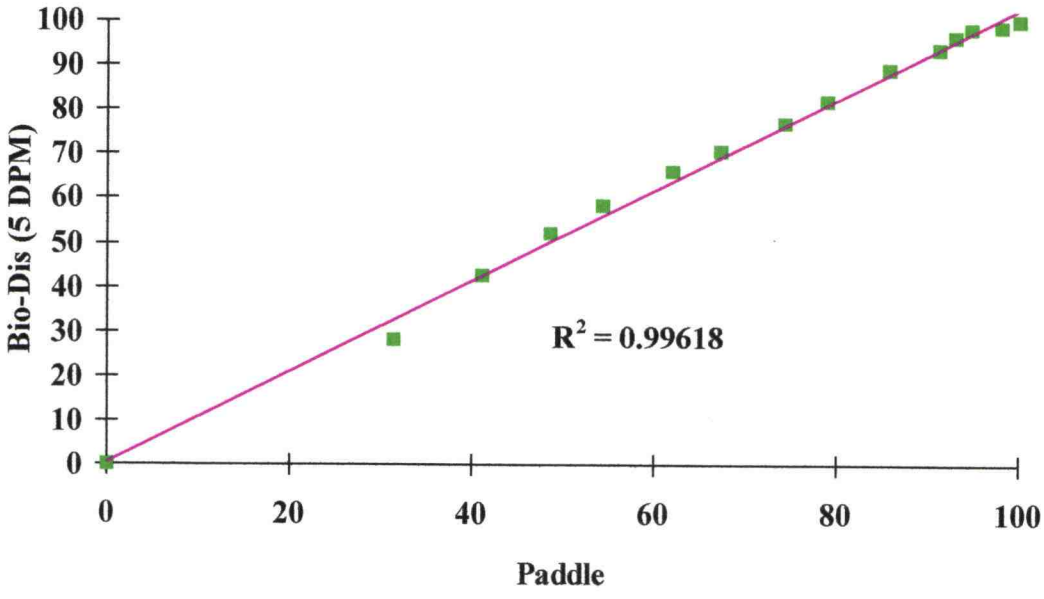

Fig. III.9-d Correlation of percentages of drug released between paddle (50 RPM) and Bio-Dis® (5 DPM) methods

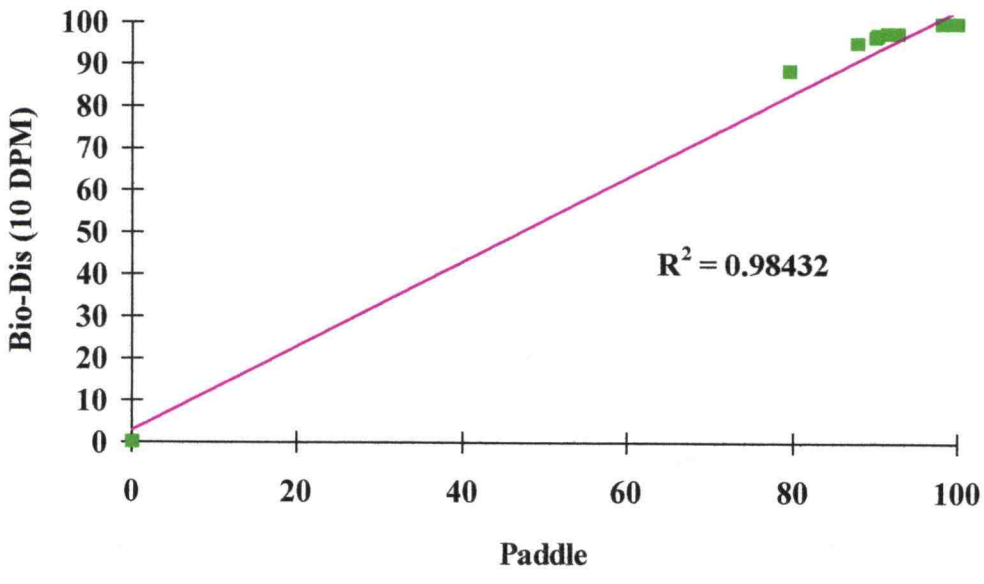

Fig. III.9-e Correlation of percentages of drug released between paddle (50 RPM) and Bio-Dis® (10 DPM) methods

In basic media, acetaminophen release was much slower than ketoprofen, but faster than nicardipine HCl.

Comparisons of three USP dissolution methods are illustrated in Fig. III.6 (citrate buffer as dissolution medium) and Fig. III.7 (gastric and intestinal fluids as dissolution media). In both citrate buffer and gastric-intestinal fluids, the release profiles of nicardipine HCl obtained from basket method (50 RPM), paddle method (50 RPM) and Bio-Dis® (5 and 10 DPM) were similar. A linear correlation between methods in both dissolution media was found as shown in Figs. III.8-a-e and Figs. III.9-a-e (R^2 of 0.97-0.99). Therefore, all methods applied in this study were comparable.

CONCLUSIONS

Rate of drug release from Aquacoat® coated beads was pH dependent even though the coating polymer solubility (ethylcellulose) is pH independent. Drug release was faster when the drug was more ionized. Thus, to formulate gastrointestinal controlled-release drug products, drug solubility or ionization should be considered. Furthermore, *in vitro* dissolution of controlled release formulations should be performed in both gastric and intestinal media for molecules which ionize anywhere in the gastrointestinal tract.

Drug release profiles obtained from three USP dissolution methods were similar, indicating that the three methods were comparable. Therefore, Bio-Dis® method at dipping rate of 5 or 10 DPM was equivalent to paddle or basket method at the rate of 50 RPM. Bio-Dis® method is preferred when more than one dissolution medium is utilized.

ACKNOWLEDGMENTS

Thanks are due to Dr. Kris Holt for ketoprofen beads and their dissolution results in gastric-intestinal fluids. Acetaminophen release rates were obtained from Mohammad Hossain's Ph.D. thesis (9). Final thanks to Marcus Cox, Matthew Brazer, and Shi-Gyong Jeong for their technical assistance.

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CHAPTER IV

Nicardipine HCl Sustained-Release Formulations: Binders/Release Controllers and Inactive Ingredients as Formulation Factors for Prediction of Drug Release Rates

Waranush Sorasuchart, Jacqueline Wardrop, and James W. Ayres.

ABSTRACT

Nicardipine HCl sustained-release product, consisted of sustained-release beads and immediate-release powder, was formulated and evaluated *in vitro*. A comparison of rates and extents of drug release or dissolved from various formulations was made. Rates of drug release from spray layered beads were slowed down when Eudragit® L, an enteric coat polymer, was used as a binder/release controller, however, drug dissolution was not completely protected in acidic dissolution media. Nicardipine HCl spray layered beads containing Aquacoat® (ethylcellulose) was pH dependent even though solubility of ethylcellulose is not pH dependent. Rates of drug release were controlled either by spray layering or overcoating the beads with Aquacoat®. Drug release rates from the beads containing no polyvinylpyrrolidone (PVP) remained the same when 10 or 15% PVP was added. Percentages of magnesium stearate and talc affected flow property of powder. Furthermore, extents of drug dissolved from a mixture of powder containing various types of starch were different. Suitable amounts or types of inactive ingredients for powder formulation were selected. Combination of sustained-release beads and immediate-release powder yielded fast dissolution and prolonged nicardipine HCl release. The information from this study provided prediction of drug release from spray layered and coated beads containing Eudragit® L or Aquacoat®; therefore, a desired rate of drug release may be obtained.

INTRODUCTION

Nicardipine HCl is a 1,4 dihydropyridine-derivative calcium-channel blocking agent (1) structurally shown in Fig. IV.1. It is used orally in the management of hypertension, 3 times daily as a conventional capsule or twice daily as an extended-release capsule (1). Nicardipine HCl has a short half-life (2-4 hours) which requires frequent oral administration (2). The extended-release formulation prolongs therapeutic levels of drug in plasma with minimal fluctuation which provides less frequency of drug intake, less side effects and, therefore, better patient compliance. Nicardipine HCl is currently on the market as 20 and 30 mg capsules (Cardene®, Roche Laboratories, Nutley, NJ) and as 30, 45, and 60 mg extended-release capsules (Cardene® SR, Roche Laboratories, Nutley, NJ).

Many 1,4-dihydropyridine derivatives (nifedipine in particular) are photosensitive (3,4). It was reported that 27% of nicardipine HCl in 50:50 MeOH:water degraded over 1 hour (4). However, no significant difference of nicardipine HCl concentration was found after 24 hour in a mixture of 74: 13: 120 acetonitrile: MeOH: phosphate buffer (0.05 M KH_2PO_4, pH 4.8), which was used as mobile phase in a HPLC system (5). Additional stability studies at various pH levels protected from or exposed to light were performed in this research. It was found that nicardipine HCl was stable for 24 hours even when exposed to light, except at pH above or equal to 6.2.

An objective of this research is to evaluate spray layering process and drug release rates for new spray layered bead formulation as compared to matrix controlled drug release from extruded and marumerized granule formulations (Cardene® SR). The spray

layering process applied in this research was unique, performed by spray layering nicardipine HCl together with a binder/release controller (Eudragit® L 30 D or Aquacoat®) in ethanol/aqueous or aqueous system onto nonpareil sugar beads.

Eudragit® L 30 D is a 30% aqueous dispersion of methacrylic acid copolymer type C, which is soluble in a neutral to weakly alkaline aqueous solvent (above pH 5.5). It is commonly used for enteric film coating, *i.e.*, not soluble in gastric fluid, but soluble in intestinal fluid (6). Aquacoat® is a trade name of an aqueous dispersion of 27% ethylcellulose mixed with sodium lauryl sulfate and cetyl alcohol producing a final solids content of 30% (7).

Rates of drug release from the spray layered beads using Eudragit® L 30 D or Aquacoat® as a binder/controler were evaluated and compared to those of spheroidal granules in Cardene® SR. Dissolution profiles of the spray layered beads overcoated with various levels of Aquacoat® were also investigated.

Effects of polyvinylpyrrolidone (PVP) on rate of drug release were also investigated by a comparison of dissolution profiles of Aquacoat® spray layered beads without PVP and with 10 and 15% PVP.

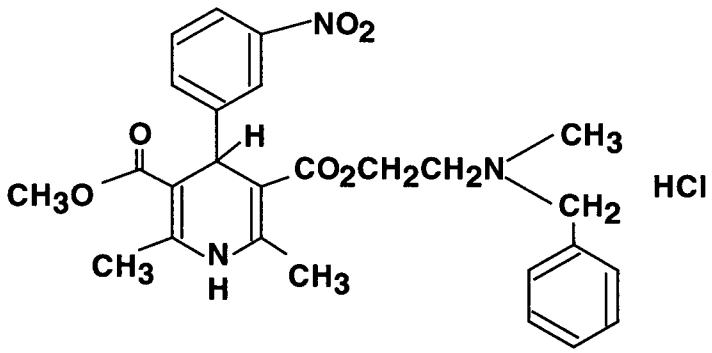

Fig. IV.1: Chemical structure of nicardipine HCl

MATERIALS AND METHODS

Chemicals

Nicardipine HCl (lot# 4628) was supplied by Lemmon Company, Sellerville, PA. Aquacoat® was purchased from FMC Corporation, Newark, DE. Eudragit® L 30D was from Rohm GmbH, Chemische Fabrik, Potfach, Germany. Nonpareil sugar beads were purchased from Crompton and Knowles Corp., Pennsauken, NJ. Starch was from A. E. Staley Manufacturing Company, Decatur, IL. Soluble starch was from Difco Laboratories, Detroit, MI. Pregelatinized starch (starch 1500, lot# 7620) was provided by Lemmon Company, Sellerville, PA. Magnesium stearate (lot# 4236) was received from Biocraft, Fairfield, NJ. Talc was from Mallinckrodt Chemical Works, St. Louis, MO. Other ingredients included triethyl citrate 99% (TEC, Aldrich Chemical Company, Inc., Milwaukee, Wis), dibutyl sebacate (sebacic acid dibutyl ester, DBS, Sigma Chemical Co., St. Louis, MO), and polyvinylpyrrolidone K-30 (PVP) from EM Science, Gibbstown, NJ. Other chemicals were of reagent grade.

Methods

Nicardipine HCl Stability Study

Stability of Nicardipine HCl in HPLC Mobile Phase

A stability study of nicardipine HCl in the HPLC mobile phase of 74: 13: 120 acetonitrile: MeOH: phosphate buffer (0.02 M KH_2PO_4), pH 4.8 (modified from reference 5) was performed. Various concentrations of nicardipine HCl of 1.56, 3.12, 6.25, 12.5, and 25 μg/mL, protected from light, were determined during 8 hour storage at room temperature.

Stability of Nicardipine HCl in Dissolution Medium

Stability of nicardipine HCl in dissolution medium (citrate buffer, pH 4.5) was studied over a 24 hour period with effects of temperature and light. Nicardipine HCl solution of 6.5 μg/mL was kept at room temperature and at 37°C and was exposed to and protected from light.

Stability of Nicardipine HCl in Solution as a Function of pH

Concentrations of nicardipine HCl in buffer at pH of 1.41, 4.50, 4.80, 6.20, 7.40, 8.51, and 9.52, protected from light and stored at room temperature, were evaluated over 24 hours. Table IV.1 shows compositions of the buffer.

Table IV.1 Compositions of Buffer for Nicardipine HCl Stability Study

pH	Buffer	Compositions
1.4	Enzyme-free simulated gastric fluid	0.1 M HCl acid and 34.22 mM NaCl
4.5	Citrate buffer	27.12 mM citric acid, adjusted pH with 10 N NaOH
4.8	HPLC mobile phase	74: 13: 120 of acetonitrile: MeOH: phosphate buffer (0.02 M KH_2PO_4)
6.2	Phosphate buffer	8.9: 1 of 0.1 M KH_2PO_4: 0.1 N NaOH
7.4	Enzyme-free simulated intestinal fluid	1.7: 1 of 0.1 M KH_2PO_4: 0.1 N NaOH
8.5	Borate buffer	2.4: 1 of 0.025 M sodium tetraborate: 0.1 M HCl
9.5	Borate buffer	10.8: 1 of 0.25 M sodium tetraborate: 0.1 M HCl

Note: All ingredients were dissolved in deionized distilled water. Buffer was pH adjusted with 0.1 N HCl or 0.1 N NaOH unless indicated.

HPLC Analysis

Concentrations of nicardipine HCl in solution for stability study were detected by HPLC system modified from reference (8). The mobile phase was 74: 13: 120 (v/v/v) acetonitrile:MeOH:phosphate buffer (0.02 M KH_2PO_4), adjusted to a final pH of 4.80 with phosphoric acid, filtered and degassed before use. The HPLC column was

Microsorb-MV® C8 5 μm 110 A° 15 cm (Rainin Instrument Company, Inc., Woburn, MA). The flow rate was 1.5 mL/min. Propyl paraben was used as an internal standard at a concentration of 10 μg/mL. UV absorbance was recorded at 229 nm (UV detector Waters Model 441, Waters Associates, Milford, MA). Other instruments in the HPLC system included a delivery pump (Waters Model 590 Solvent Delivery System, Waters Associates, Milford, MA), and automatic sample injector (Waters WISP Model 712, Waters Associates, Milford, MA), and an integrator (CR 501 Chromatopac, Shimadzu Corp., Kyoto, Japan).

Formulations and *In Vitro* Dissolution Testing of Sustained-Release Nicardipine HCl Beads

Preparation of Nicardipine HCl Spray Layering Beads

Nicardipine HCl and binders were mixed and stirred in distilled deionized water (aqueous suspension) or a mixture of 95% ethanol and water (ethanol/aqueous solution). Percentages of nicardipine HCl, binders, and nonpareil sugar beads in each formulation are listed in Table IV.2.

Solution or suspension of nicardipine HCl was spray layered onto 200 g of nonpareil sugar beads (25-30 mesh) in a fluid-bed spray coater containing a seven-inch Wurster column (Strea-1, Aeromatic Inc., Columbia, MD). The Wurster column was approximately 1 inch away from the bottom screen of the coater, which was connected to a Lab-line/P.R.L. High Speed Fluid Bed Dryer (Lab-line, Melrose Park, IL).

Table IV.2: Percentages of dry solid contents in the final bead formulations

Formulation	Percentages						
	NC (1)	ED (2)	AQ (3)	PVP (4)	Sugar core (5)	Solvent	Delivery rate (mL/min)
1	19	11	-	-	70	ethanol/water	1.1
2	20	5	-	-	75	ethanol/water	1.6
3	21	-	10	-	69	ethanol/water	2.2
4	21	-	8	-	71	ethanol/water	2.2
5	21	-	4	-	75	ethanol/water	1.2
6	21	-	4	10	65	ethanol/water	1.6
7	21	-	10	-	69	water	1.45
8	21	-	10	10	59	water	1.5, 2.4 *
9	21	-	10	15	54	water	1.5, 2.4 *

Into each formulation (3-9), 20% triethylcitrate (TC) of solid content of Aquacoat® was added. (1) NC represents nicardipine HCl (2) ED represents Eudragit® L 30 D; (3) AQ represents Aquacoat®; (4) PVP represents polyvinylpyrrolidone; (5) sugar core was nonpareil sugar beads (25-30 mesh). * Delivery rates were 1.5 mL/min in the first 2 hours and 2.4 mL/min subsequently.

Spray layering was performed at 40°C in case of aqueous suspension or at room temperature in case of ethanol/aqueous solution. A small nozzle (1.2 mm) was used for spray layering of ethanol/aqueous solution. A big nozzle (4 mm), specially designed and manufactured locally at College of Pharmacy, Oregon State University, was used for spray layering of aqueous suspension. Air pressure was maintained at 10 psi and the blower speed set at 80-90% of full capacity to allow beads to move freely. Nicardipine HCl suspension/solution was constantly delivered by a peristaltic pump (Rabbit® Peristaltic Pump, Gilson Medical Electronics, Middleton, WI). Delivery rates were controlled for a specific formulation (Table IV.2). During the spray layering process the nicardipine HCl solution/suspension was kept stirring by a magnetic stirrer to ensure homogeneity. Drug spray layered beads were dried in the coating chamber for another 30 minutes at the same temperature and air flow before removing. Beads were then sieved (mesh size# 10 and 60) to remove agglomerated and fine particles.

Preparation of Nicardipine HCl Coated Beads

Nicardipine HCl spray layered beads (formulation 9) were overcoated with 1.0, 1.5 and 3.0% (w/w) of Aquacoat® solids (formulations 9A, 9B, and 9C, respectively). An equal amount of distilled deionized water was added to Aquacoat® which was being stirred with plasticizers (15% (w/w) TEC and 15% (w/w) DBS) at least 1 hour prior to spray coating. The fluid-bed spray coater was used as previously described. All parts in the spray coating chamber were the same as in the spray layering process. A nozzle with an opening of 1.2 mm diameter was utilized.

100 g of spray layered drug containing beads was placed in the bottom of the coating chamber. The spray coating was conducted at 40°C. Pressure was maintained at 10 psi and the blower speed was set at 70-80% of full capacity to allow beads to move freely. Aquacoat® coating suspension was constantly delivered by a peristaltic pump controlled at 20 RPM (0.55 mL/min). The Aquacoat® coating suspension was stirred using a magnetic stirrer during the spray coating process, which was complete in about 3 hours. Polymer coated beads were dried in the coating chamber for another 30 minutes at the same temperature and air flow before removing.

Drug Loading Assay

Nicardipine HCl spray layered beads or polymer coated beads were ground into a fine powder. An exact amount of the powder (0.01 g) was weighed in triplicate and transferred to 50.0 mL volumetric flasks. Samples were dissolved in 2 mL methanol and sonicated for 30 minutes. Enzyme-free simulated gastric fluid (pH 1.4) was added to adjust volume to 50.0 mL. The solution was mixed well and then filtered through 5 μm Acrodisc® (Gelman Sciences, Ann Arbor, MI). Standard nicardipine HCl stock solution was prepared by dissolving 10 mg nicardipine HCl in 2 mL methanol and adjusting volume to 50.0 mL with the gastric fluid. A series of standard solutions with a concentration of 1 to 30 μg/mL was prepared from the stock solution by serial dilution. Blank solution was prepared by adding gastric fluid to 2 mL methanol in the volumetric flask to make 50.0 mL. UV absorbance of samples and standard solutions was measured at 358 nm.

Dissolution of Nicardipine HCl Beads

USP Apparatus II (Paddle Method)

Dissolution profiles of nicardipine HCl beads were studied using the United States Pharmacopeia (USP) XXIII apparatus II, paddle stirring method (VK 7000®, VanKel Industries, Inc., Cary, NC). Each dissolution vessel was filled with 900 mL of citrate buffer pH 4.5 (filtered and degassed) and maintained at 37°C. Citrate buffer was prepared by dissolving 28 g citric acid in 4 L of deionized distilled water and the pH was adjusted to 4.50±0.01 using 10 N NaOH. The dissolution tank was protected from light with cardboard.

Dissolution testing of nicardipine HCl beads produced in the laboratory (or so-called laboratory beads) was performed in triplicate and compared with Cardene® SR granules. Each 60 mg capsule of Cardene® SR was opened to separate granules and powder. The granules (equivalent to 45 mg nicardipine HCl) were then weighed and used in dissolution testing. Approximately the same amount of laboratory beads equivalent to 45 mg nicardipine HCl (0.22 g) were weighed and dropped in the appropriate dissolution vessels at the same time as the Cardene® SR granules. Dissolution was studied at a paddle rotation speed of 50 RPM. Samples of 3 mL dissolution medium were withdrawn without medium replacement at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hours using an autosampler machine (Peristaltic Pump VK 810® connected to System Monitor VK 8000®, VanKel Industries, Inc., Cary, NC). All samples were filtered through 5 μm Acrodisc®.

Amounts of nicardipine HCl released were detected by UV spectrophotometer at wavelength 358 nm. Nicardipine HCl standard solutions (6-50 μg/mL) were prepared by serial dilutions with citrate buffer pH 4.5 from 1 mg/mL nicardipine HCl stock solution.

USP Dissolution Apparatus III (Bio-Dis®)

Dissolution testing of nicardipine HCl beads was performed in triplicate using Bio-Dis® (VanKel Industries, Cary, NC). Each of the dissolution vesels in the first row of the Bio-Dis® contained 250 mL of enzyme-free simulated gastric fluid (pH 1.4±0.1) and each of those in the second row contained 250 mL of enzyme-free simulated intestinal fluid (pH 7.4±0.1). Each 30 mg capsule of Cardene® SR was opened to separate granules and powder. The granules were then weighed and used in dissolution testing. Approximately the same amount of the laboratory beads (0.11 g) equivalent to 22.5 mg nicardipine HCl were weighed. Both Cardene® SR granules and the laboratory beads were placed in the dipping tubes containing a bottom screen. Dipping was performed at the rate of 5 dips per minute (DPM) for 2 hours in gastric fluid and subsequently in intestinal fluid. The first dip was held for 3 seconds. The dipping tubes were drained for 1 minute before moving to the intestinal fluid. 3 mL samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2 hours (in gastric fluid) and 2.17, 2.33, 2.5, 2.75, 3, 3.5 and 4 hours (in intestinal fluid) without replacement using the autosampler and filtered through 5 μm Acrodisc®. Amounts of nicardipine HCl released were detected as previously described.

Effects of Polyvinylpyrrolidone (PVP) on Nicardipine HCl Release Rates

Dissolution testing of spray layered beads, containing 10% Aquacoat® without PVP, with 10% and 15% PVP (formulations 6, 7, and 8, respectively), were performed in enzyme-free simulated gastric and intestinal fluids using the Bio-Dis as previously described.

Formulations and Dissolution Testing of Immediate-Release Powder of Nicardipine HCl

Formulations of Immediate-Release Powder of Nicardipine HCl

The powder component of 30, 45, and 60 mg nicardipine HCl sustained release capsule contains 7.5, 11.25, and 15 mg of nicardipine HCl, respectively, which is difficult to accurately weigh and fill in capsules. To reduce the technical and individual error of weighing and transfering small amounts of the drug, nicardipine HCl was mixed with inactive ingredients. Formulations of nicardipine HCl immediate release powder are listed in Table IV.3. Starch served as a diluent, while magnesium stearate or talc was a lubricant used to facilitate the flow of drug-fill into the capsules (4). All ingredients were weighed and mixed well in a bottle.

Measurements of Angles of Repose (9)

Powder formulations tested were poured through a fixed glass funnel onto a piece of paper placed on a flat horizontal surface to create a cone shaped pile of powder. A height and a diameter of the cone were then measured (Fig. IV.2). The process and

Table IV.3: Formulations of Nicardipine HCl Immediate Released Powder

Powder Formulation	% magnesium stearate or talcum	Nicardipine HCl (g)	Starch (g)	Magnesium stearate (g)	Talc (g)	Angle of Repose (degree)	Amount of powder equivalent to 7.5 mg NC*(g)	Amount of NC * dissolved (g)
A	0.55	0.1035	0.3975 (1)	0.0028	-	N/A	0.0365	8.85
B	0.25	0.7500	9.2259 (1)	0.0260	-	N/A	0.1000	8.12
C	0	0.7508	9.2507 (2)	-	-	N/A	0.0999	7.26
D	0	3.75	46.25 (3)	-	-	37.11	N/A	N/A
E	0.25	3.75	46.125 (3)	0.125	-	39.13	N/A	N/A
F	0.50	3.75	46.00 (3)	0.25	-	35.11	N/A	N/A
G	1.0	1.50	18.30 (3)	0.20	-	30.79	0.0998	8.12
H	0.5	1.50	18.40 (3)	-	0.10	33.41	0.1000	7.63
I	1.0	1.50	18.30 (3)	-	0.20	34.45	N/A	N/A
J	2.0	0.75	9.05 (3)	-	0.20	34.03	N/A	N/A
K	1.0	1.50	13.35 (3)	-	0.15	38.10	N/A	N/A
L	1.0	1.50	6.42 (3)	-	0.08	42.20	N/A	N/A
Cardene®	N/A	N/A	N/A	N/A	N/A	N/A	0.1487	9.25

Note:* NC represents nicardipine HCl, (1) represents starch, (2) represents soluble starch, and (3) represents pregelatinized starch.
N/A: not applicable

measurement were quadrupled. Angle of repose was calculated using Eq. IV.1, where θ represents angle of repose and radius is the diameter divided by 2.

$$\tan\theta = \frac{height}{radius} \qquad \text{(Eq. IV.1)}$$

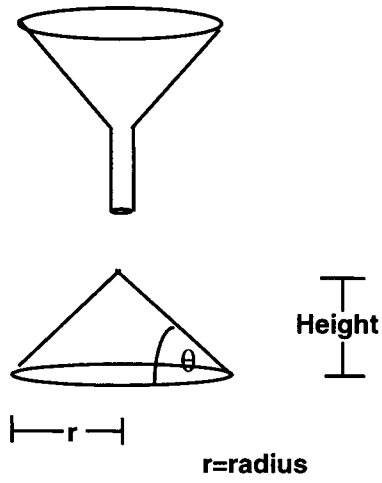

Fig. IV.2: Cone shaped pile of powder for measurements of angles of repose

Dissolution of Nicardipine HCl Powder

Exact amounts of the powder listed in Table III.3 were weighed within an error of 0.0005 g and filled in #2 capsules in triplicate. Dissolution testing was performed in comparison with powder in Cardene® SR capsules. Each 30 mg capsule of Cardene® SR was opened to separate granules and powder. The powder was then weighed, filled back into the capsule shell, and used in dissolution testing. The laboratory and Cardene® SR capsules were placed in the Bio-Dis® dipping tubes. Dipping was performed at the rate of 5 DPM for 1.5 hours in the gastric fluid. The first dip was held for 3 seconds. Samples of 3 mL were collected at 5, 10, 20, 30, 45, 60 and 90 minutes without replacement using

the autosampler and filtered through 5 μm Acrodisc®. Amounts of nicardipine HCl dissolved were detected as previously described. Total amounts of nicardipine HCl dissolved from the powder are listed in Table IV.3.

Table IV.4: Components of powder and beads in capsules and sizes of the capsules

Capsule	Powder		Beads	Capsule size
	Formulation	Amount (g)	Amount (g)	
30 mg	G	0.0998	0.1101	2
45 mg	K	0.1125	0.1651	2
60 mg	L	0.0800	0.2202	1

Formulations and Dissolution Testing of Nicardipine HCl Sustained Released Capsules

Nicardipine HCl beads and powder were combined in the same capsule. The amount of beads equivalent to 22.5, 33.75, and 45 mg of nicardipine HCl was calculated based on the total amount of drug released from the beads in the dissolution studies. The amount of powder equivalent to 7.5, 11.25, and 15 mg nicardipine HCl was calculated based on the combined amounts of nicardipine HCl and its diluent. Powder formulations G, K, and L were applied in the sustained release capsule formulations. A summary of capsule formulations for 30, 45, and 60 mg nicardipine HCl sustained-release capsules is described in Table IV.4. Note that each dose contained the same bead formulation, but different powder formulations. All components were weighed within an error of 0.0005 g.

Dissolution testings of all formulations compared to Cardene® SR capsules were performed in the simulated gastric and intestinal fluids as previously described.

RESULTS AND DISCUSSION

Nicardipine HCl Stability Study

Stability study indicated relatively good stability of nicardipine HCl in the HPLC mobile phase (pH 4.8) as shown in Fig. IV.3. In citrate buffer (pH 4.5), nicardipine HCl was stable at room temperature as well as at 37°C either protected from or exposed to light (Fig. IV.4). The stability study of nicardipine HCl at various pH values when protected from light indicated good stability except at pH higher than or equal to 6.2 (Fig. IV.5).

Nicardipine HCl Sustained-Release Beads

Results of drug loading assay are shown in Table IV.5. Most beads contained approximately 21% nicardipine HCl as expected.

Nicardipine HCl and Eudragit L 30 D® were dispersed and mixed in water for spray layering. However, the suspension was clay-like and viscous resulting in plugging of the inlet side of the spray nozzle (1.2 mm). Ethanol was included as a cosolvent to obtain a solution which was sprayed without problems. For formulation 2, instead of obtaining the the spray layered beads containing 5% Eudragit® L 30D and 20%

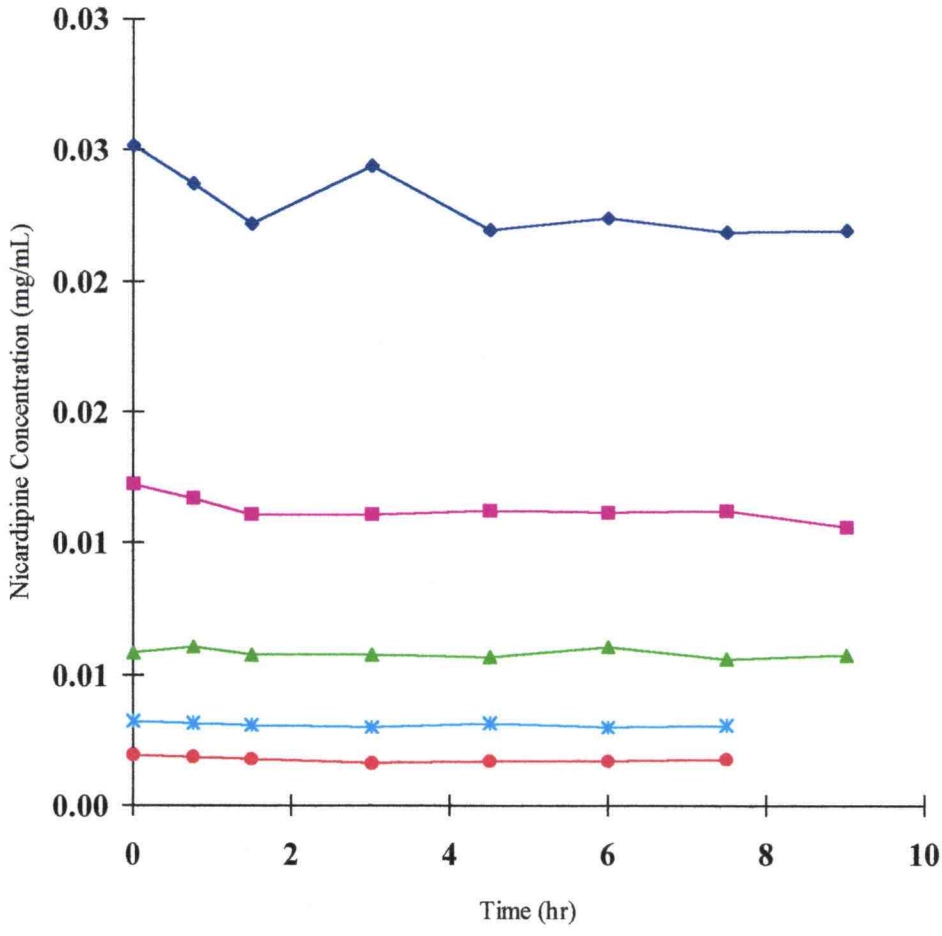

Fig. IV.3 Stability of nicardipine HCl at pH 4.80, protected from light and stored at room temperature. Key: (◆) 0.025 mg/mL, (■) 0.0125 mg/mL, (▲) 0.00625 mg/mL, (✳) 0.00312 mg/mL, and (●) 0.00156 mg/mL.

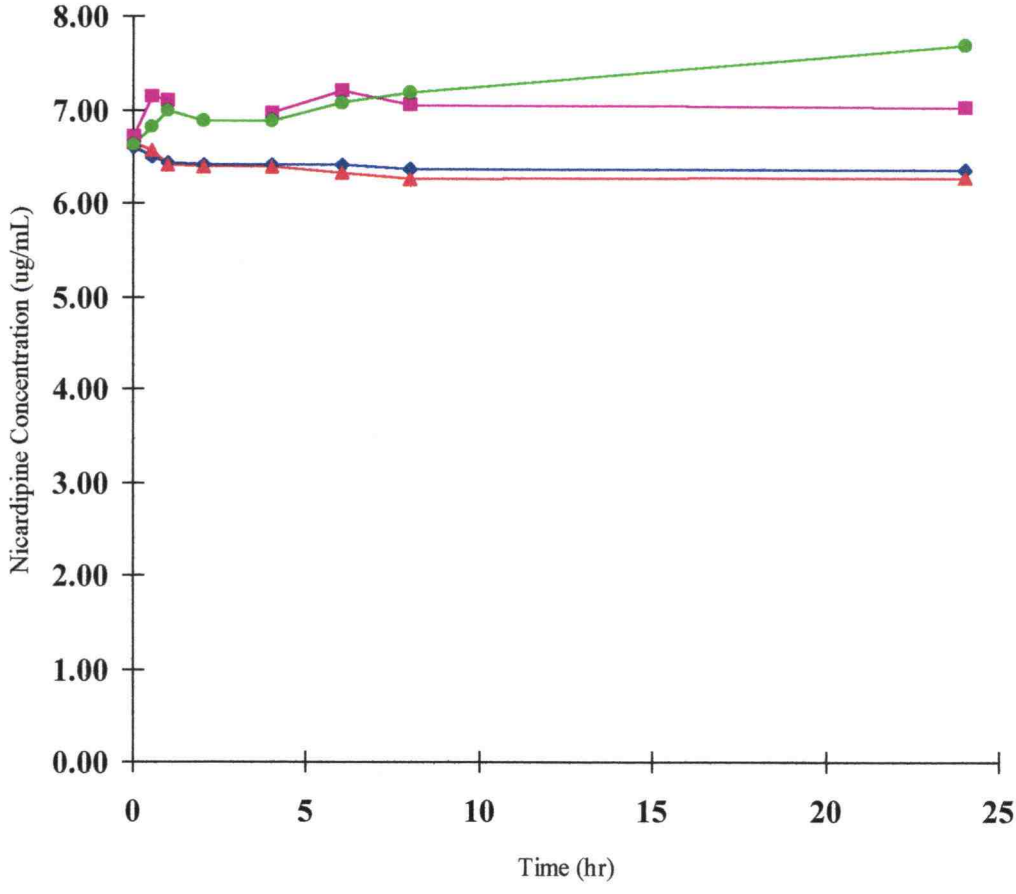

Fig. IV.4 Stability of Nicardipine HCl at pH 4.50, exposed or protected from light. Key: (◆) protected from light, room temperature, (■) ,protected from light, 37°C (▲) exposed to light, room temperature, and (●) exposed to light, 37°C.

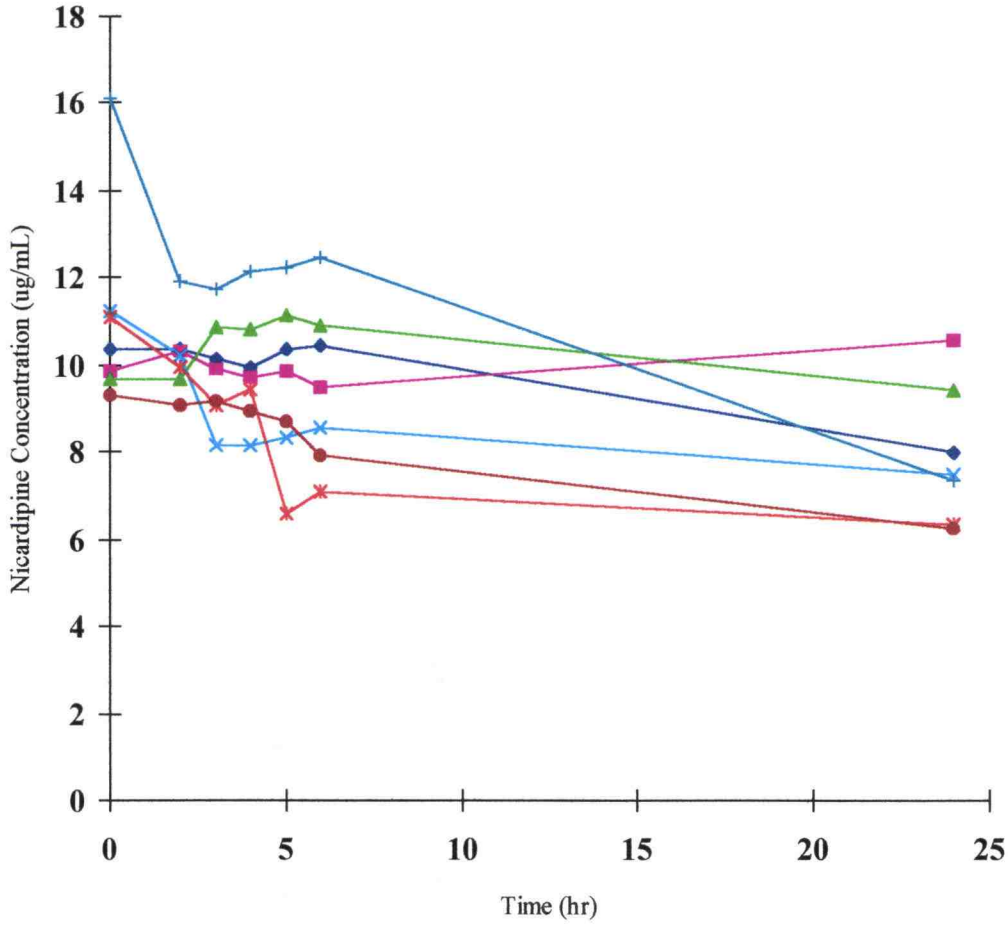

Fig. IV.5 Stability of Nicardipine HCl at various pH levels, protected from light and stored at room temperature. Key: (◆) pH 1.4, (■) pH 4.5, (▲) pH 4.8, (×) pH 6.2, (✳) pH 7.4, (●) pH 8.6, (+) pH 9.5.

nicardipine HCl after spray layering the solution onto the nonpareil sugar beads, the final beads contained 1.09% Eudragit® L 30D and 4.35% of nicardipine HCl due to a large amount of drug lost during the spray layering process which was a result of inadequate quantity of binder.

Table IV.5: Percentages of nicardipine HCl loading in spray layered beads

Formulation	% Nicardipine HCl loading	SD
1	21.45	0.0115
2	4.35	0.1253
3	15.76	0.0101
4	15.10	0.0022
5	16.48	0.0065
6	20.27	0.0046
7	19.48	0.5186
8	19.33	0.1562
9	21.33	0.0666
9A (1.0% coated)	21.35	0.0159
9B (1.5% coated)	21.37	0.4842
9C (3.0% coated)	21.48	0.6270

Fig. IV.6 illustrates dissolution profiles in citrate buffer (pH 4.5) of nicardipine HCl beads prepared with Eudragit® L 30 D as a binder/controller in comparison with Cardene® SR granules. Release rate of the beads containing 1.09% Eudragit® L 30 D was faster than that of the beads containing 11% Eudragit® L 30 D. However, the release rate of the beads containing either 1.09 or 11 % Eudragit® L 30 D was much slower than Cardene® SR granules.

Dissolution testing was also performed in simulated gastric (pH 1.4) and intestinal (pH 7.4) fluids. Results are shown in Fig. IV.7. Nicardipine HCl release rate from the

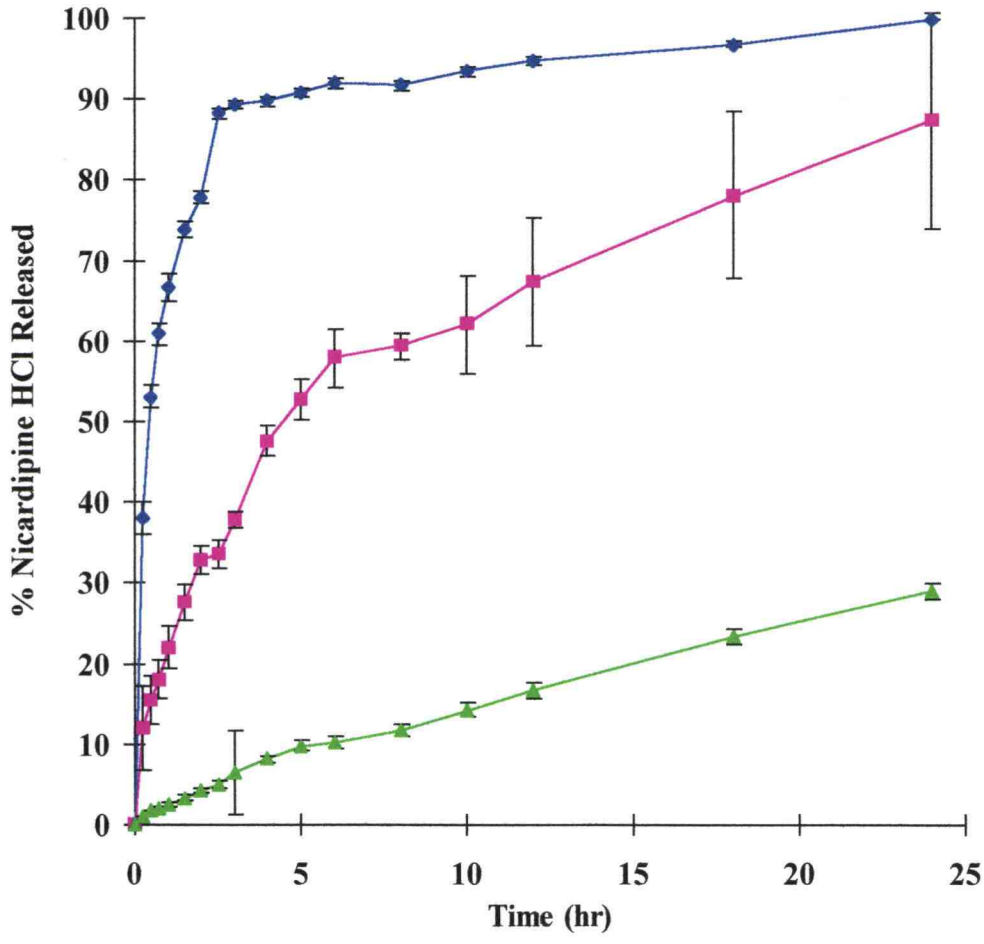

Fig. IV.6 Dissolution profiles of nicardipine HCl containing Eudragit L 30 D® in citrate buffer (pH 4.5). Error bars represent standard deviation. Key: (◆) Cardene® SR granules, (■) 1.09% Eudragit® L 30 D (formulation 2) and (▲) 11% Eudragit® L 30 D (formulation 1).

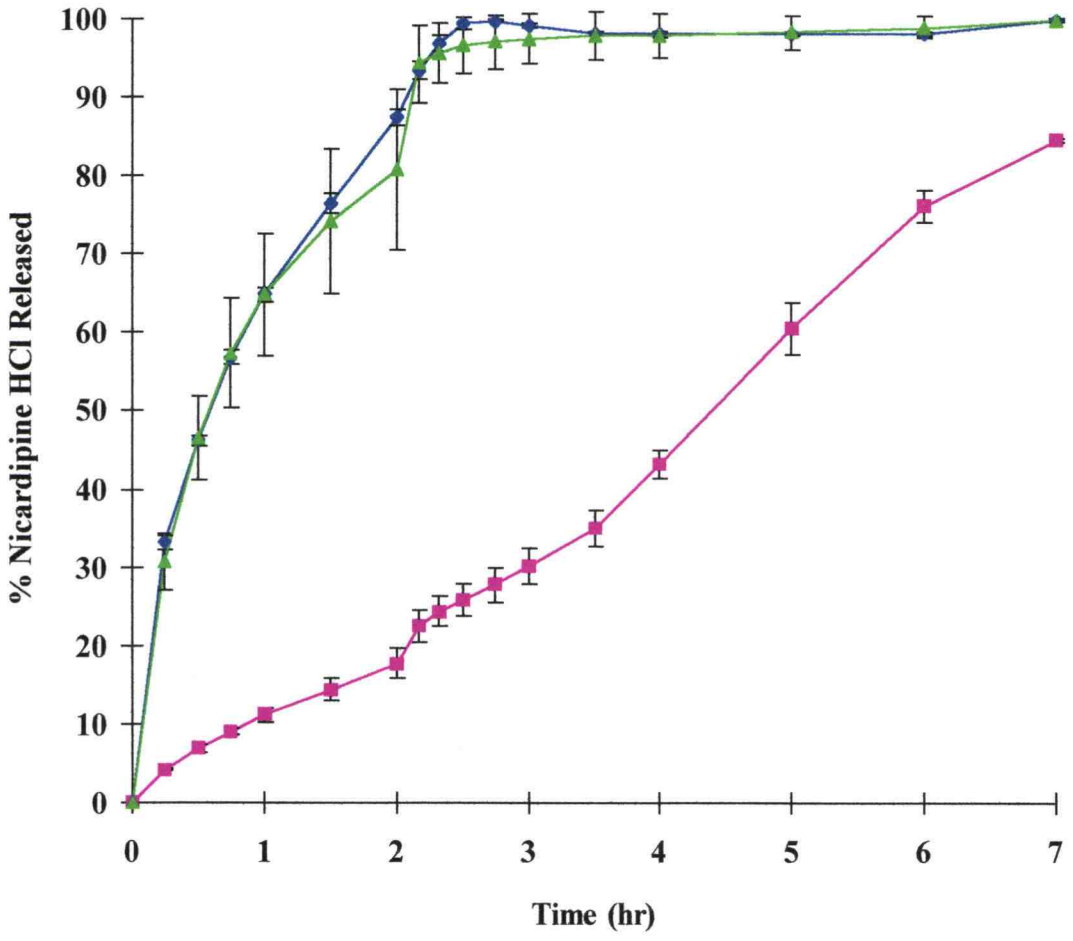

Fig. IV.7 Dissolution profiles of nicardipine HCl beads in gastric fluid (pH 1.4) for 2 hours and then in intestinal fluid (pH 7.4). Error bars represent standard deviation. Key: (◆) Cardene® SR granules, (▲) 1.09% Eudragit® L30 D (formulation 2), and (■) 11% Eudragit® L 30 D (formulation 1).

beads containing 11% Eudragit® L 30D was very slow in gastric fluid, but the release rate increased when the beads were transfered to the higher pH of intestinal fluid. The spray layered beads containing 1.09% Eudragit® L 30D provided faster release rates than those containing 11% Eudragit® L 30D and also increased under these conditions of higher pH, but to a much lesser extent. Dissolution profiles of Cardene® SR granules which contained methacrylic acid copolymer type C (9, 10) were similar to those containing 1.09% Eudragit® L 30D; therefore, the lower pH effect may be a result of a low percentage of the polymer in Cardene® SR.

Nicardipine HCl and Aquacoat® mixture in an aquous system was also too viscous to spray using a small nozzle (1.2 mm). Ethanol was added as a cosolvent to obtain sprayable suspension. Nicardipine HCl beads using ethylcellulose (Aquacoat®) as a binder/controller demonstrated faster release rates than when Eudragit® L 30 D was applied. Dissolution profiles in citrate buffer (pH 4.5) of the nicardipine HCl beads containing 4, 8, and 10% of Aquacoat® are illustrated in Fig. IV.8. Release profiles of the beads containing 8 and 10% Aquacoat® are the same, but much slower than from those containing 4% Aquacoat®. It was demonstrated that the release rates of the beads containing 4% Aquacoat® without PVP (formulation 5) or with 10% PVP (formulation 6) were not different, and both are also similar to Cardene® SR granules (Fig. IV.8). However, in simulated gastric fluid (pH 1.4), the release rate of 4% Aquacoat® beads was much faster than Cardene® SR granules (Fig. IV.9), indicating that release from the beads is pH dependent.

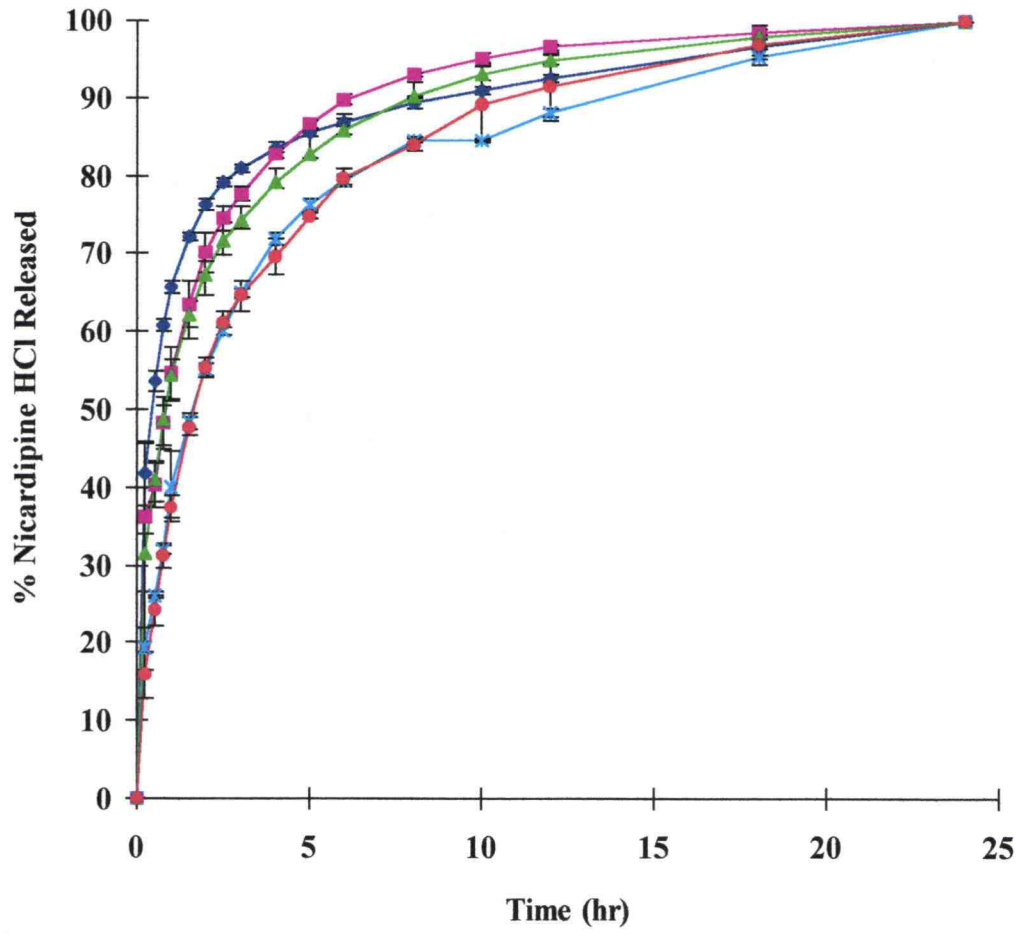

Fig. IV.8 Dissolution profiles of nicardipine HCl beads in citrate buffer (pH 4.5). Error bars represent standard deviations. Key: (●) 10% Aquacoat® (formulation 3), (*) 8% Aquacoat® (formulation 4), (■) 4% Aquacoat® without PVP (formulation 5), (▲) 4% Aquacoat® with 10% PVP (formulation 6), and (◆) Cardene® SR granules.

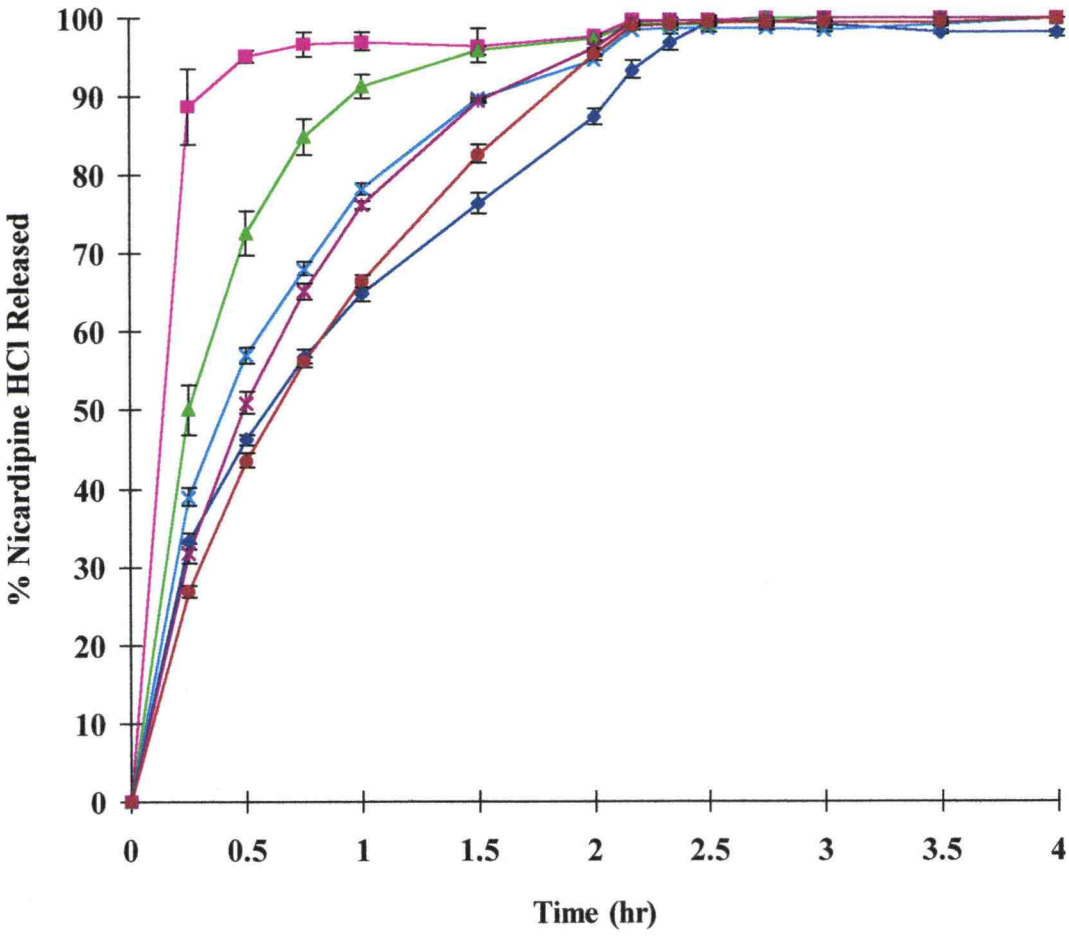

Fig. IV.9 Dissolution profiles of nicardipine HCl beads in enzyme-free simulated gastric fluid (pH 1.4) for 2 hours and then in enzyme-free simulated intestinal fluid (pH 7.4). Error bars represent standard deviations. Key: (■) 4% Aquacoat® with 10% PVP (formulation 6), (▲) 10% Aquacoat® (formulation 9), (×) 1% Aquacoat® coated (formulation 9A), (✳) 1.5% Aquacoat® coated (formulation 9B), (●) 3% Aquacoat® coated (formulation 9C), and (◆) Cardene® SR granules.

Aqueous suspension of nicardipine HCl, Aquacoat® (and PVP) (formulations 7-9) was successfully sprayable with use of a bigger nozzle (4 mm), specially designed and locally manufactured. The spray layered beads (formulation 9) were then overcoated with 1, 1.5, and 3% of Aquacoat® (formulation 9A, 9B, and 9C, respectively). Dissolution profiles of uncoated and overcoated beads, demonstrated in Fig. IV.9, indicate that the drug release rate from the beads containing 10% Aquacoat® was slower than from the beads containing 4% Aquacoat®. In addition, overcoating the beads with Aquacoat® further slowed the rate of drug release. The release rate was slower with the higher percentages of Aquacoat® overcoating, as expected.

The beads overcoated with 3% Aquacoat® provided similar release profile to Cardene® SR granules; therefore, were selected for further formulation testing.

Adding PVP in the formulation improved the binding quality of drug and Aquacoat® onto the sugar beads. Dissolution testing of the spray layered beads containing no PVP, 10% and 15% PVP in citrate buffer indicated that PVP (0-15%) had no effect on rates of drug release (Fig. IV.10). However, PVP may decrease the release rate if higher percentages are applied.

Nicardipine HCl Immediate-Release Powder

Angles of repose of powder formulations shown in Table IV.3 were means of four measurements. Most of them were above 30 degrees, but less than 40 degrees. Theoretically (8), powder flows freely when an angle of repose is equal to or less than 30

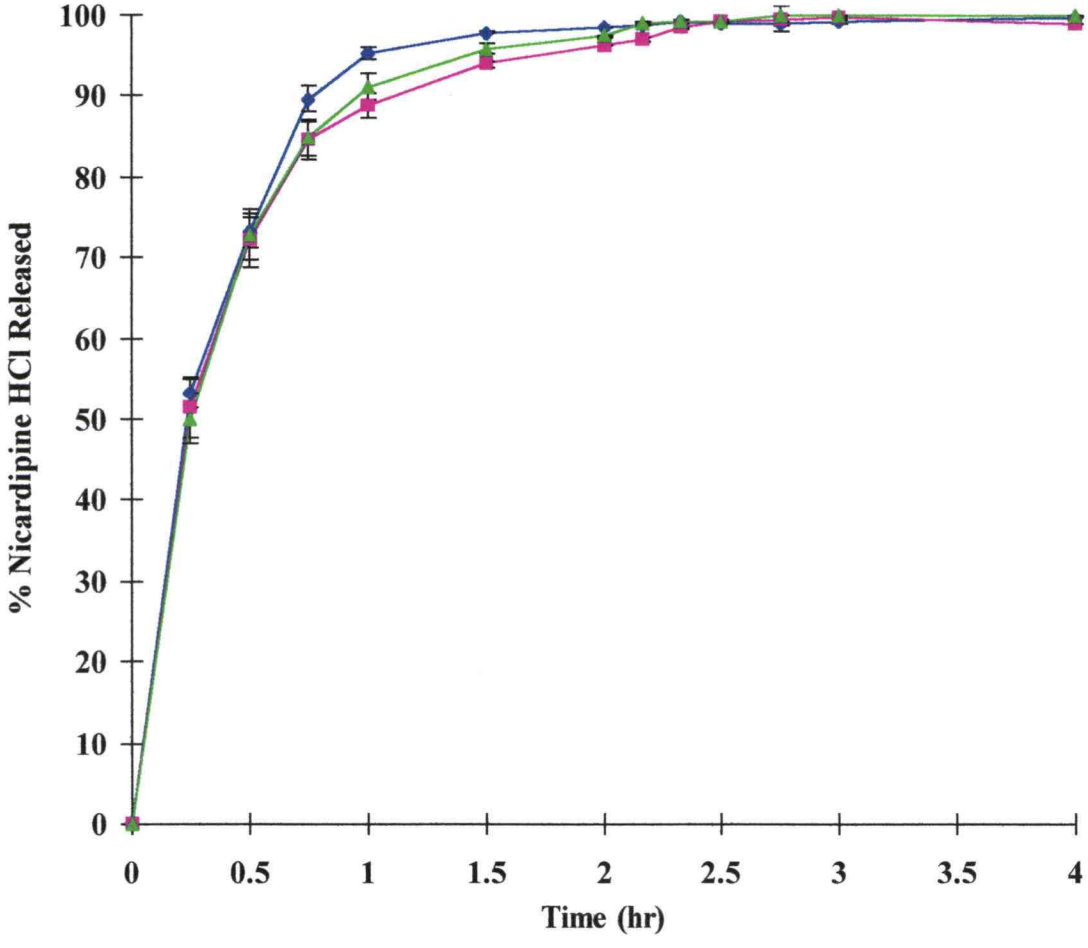

Fig. IV.10 Dissolution profiles of nicardipine HCl spray layered beads containing 10% Aquacoat® in enzyme-free simulated gastric fluid (pH 1.4) for 2 hours and then in enzyme-free intestinal fluid (pH 7.4). Error bars represent standard deviations. Key: (◆) without PVP (formulation 7), (■) with 10% PVP (formulation 8), and (▲) with 15% PVP (formulation 9).

degrees. In contrast, powder with an angle of repose above 40 degrees flows poorly. Powder formulations G and H were selected for further formulation of 30 mg capsules since means of the angles of repose were of 30.79 and 33.41, respectively.

Dissolution profiles of nicardipine HCl powder formulations are shown in Fig. IV.11. Drug dissolved from the powder formulation was very fast and complete in 30 minutes. Amounts of drug dissolved from formulations containing magnesium stearate and starch (lipophilic) (formulations A and B) were a little lower than that dissolved from Cardene® SR powder. It was possible that magnesium stearate (as a lubricant) and starch (as a diluent) were insoluble in water and thus may obstruct water penetration and delay dissolution of the drug (11). Powder formulation C, containing soluble starch and no magnesium stearate, was used as a control where drug solubility was not affected by the insolubility of the inactive ingredients. The amount of drug dissolved from formulation C was lower than those from formulations A and B; therefore, the amounts of drug dissolved may not relate to the type of starch. The smaller amounts of drug dissolved may be a result of drug lost during transferring process or drug degradation. Amounts of drug dissolved from formulations containing pregelatinized starch (formulations D-J) were similar to formulations A and B. Formulation G (nicardipine HCl, pregelatinized starch, and 1% magnesium stearate) was selected for further formulation of 30 mg capsules because of its good flow property and its similar dissolution pattern to Cardene® SR powder. Powder formulations K and L were specially designed for 45 and 60 mg capsules, respectively. Both formulations contained 1% magnesium stearate, but the amounts of pregelatinized starch varied so that the total amounts of powder would fit in the capsules.

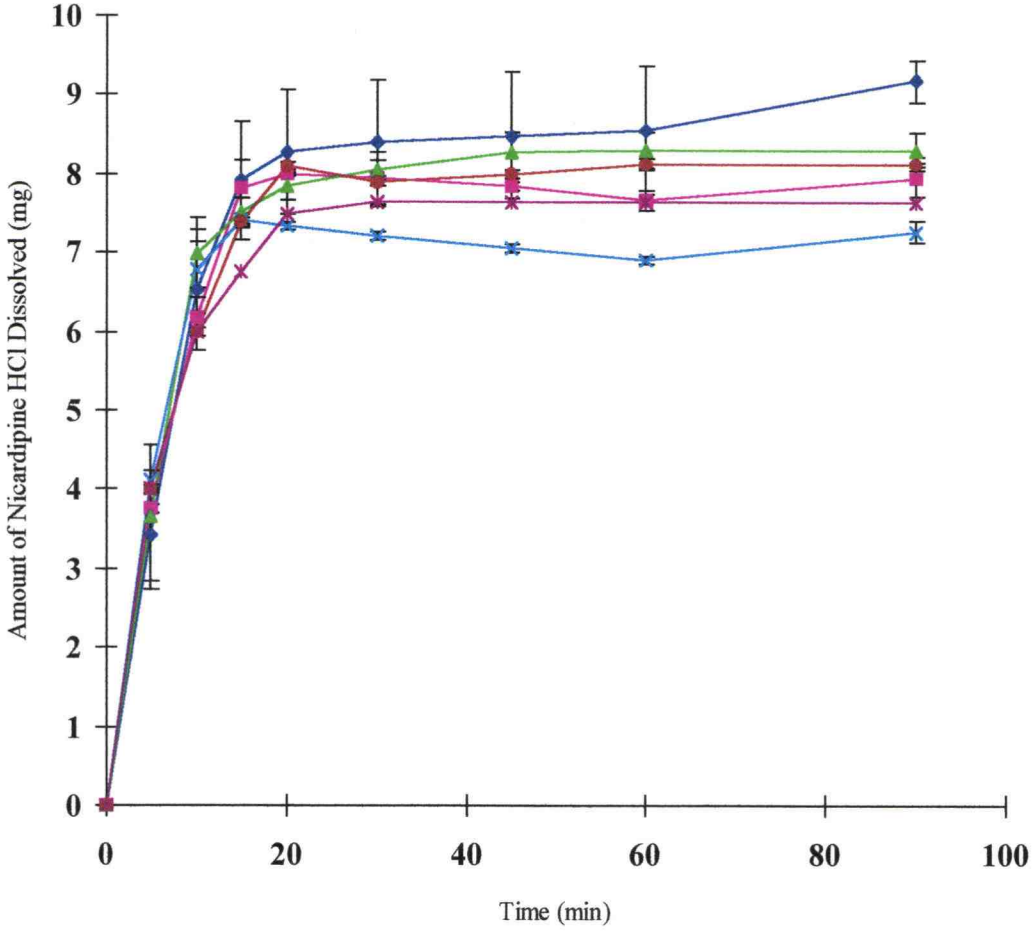

Fig. IV.11 Dissolution profiles of nicardipine HCl powder in enzyme-free simulated gastric fluid (pH 1.4). Error bars represent standard deviations. Key: (◆) Cardene® SR powder, (■) powder A, (▲) powder B, (×) powder C, (●) powder G, and (✳) powder H.

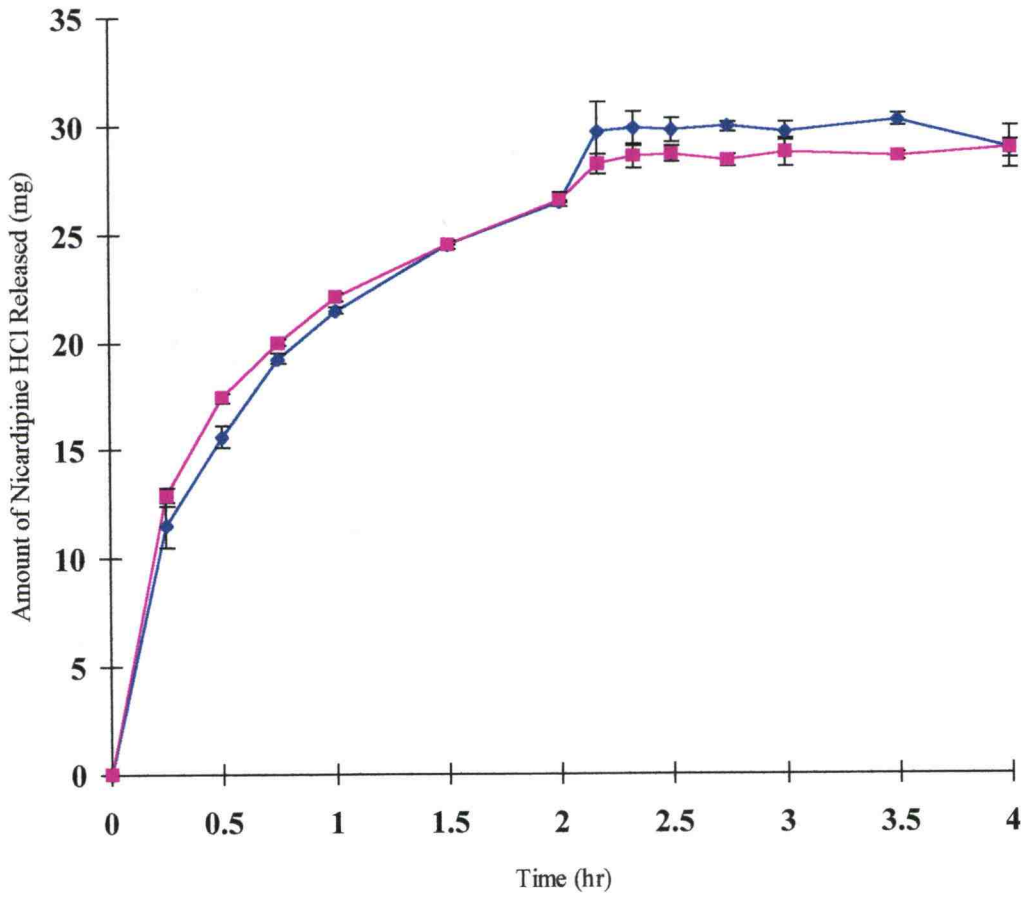

Fig. IV.12 Dissolution profiles of nicardipine HCl 30 mg capsules. Key: (◆) Cardene® SR and (■) Lab formulation.

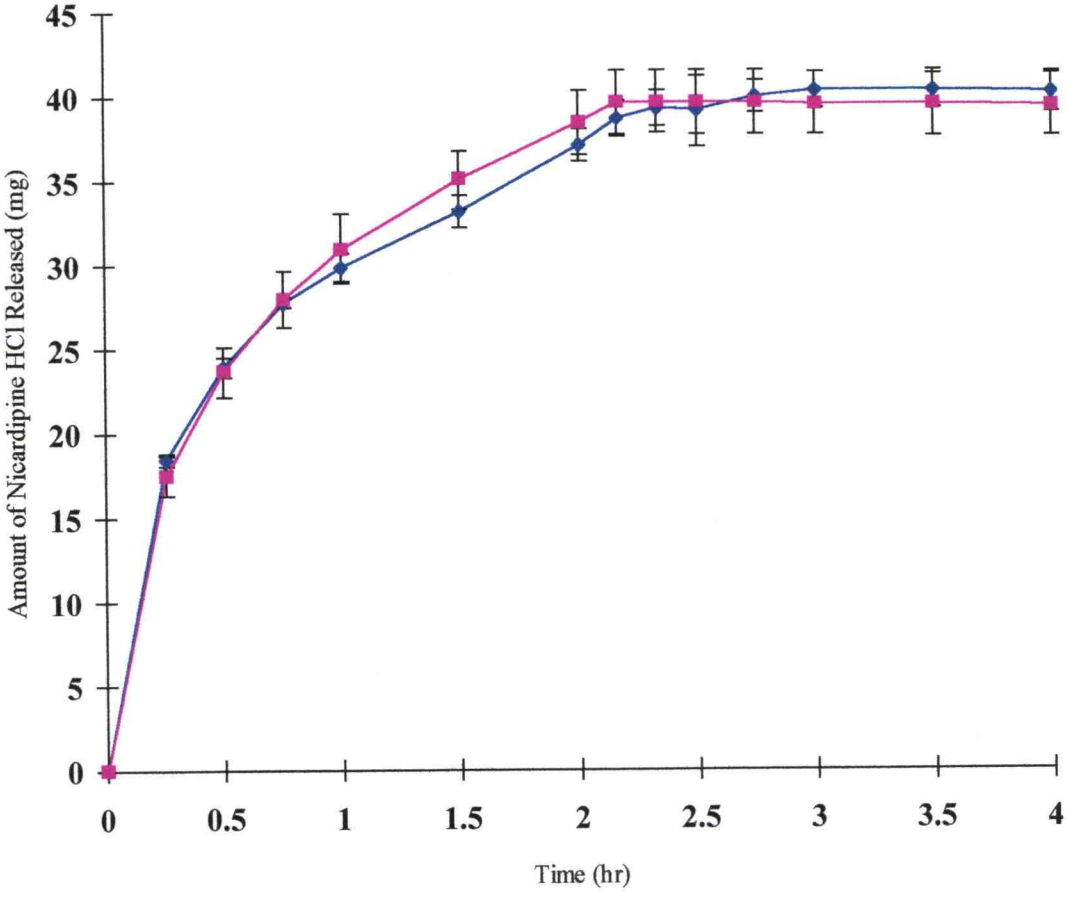

Fig. IV.13 Dissolution profiles of nicardipine HCl 45 mg capsules. Key: (◆) Cardene® SR and (■) Lab formulation.

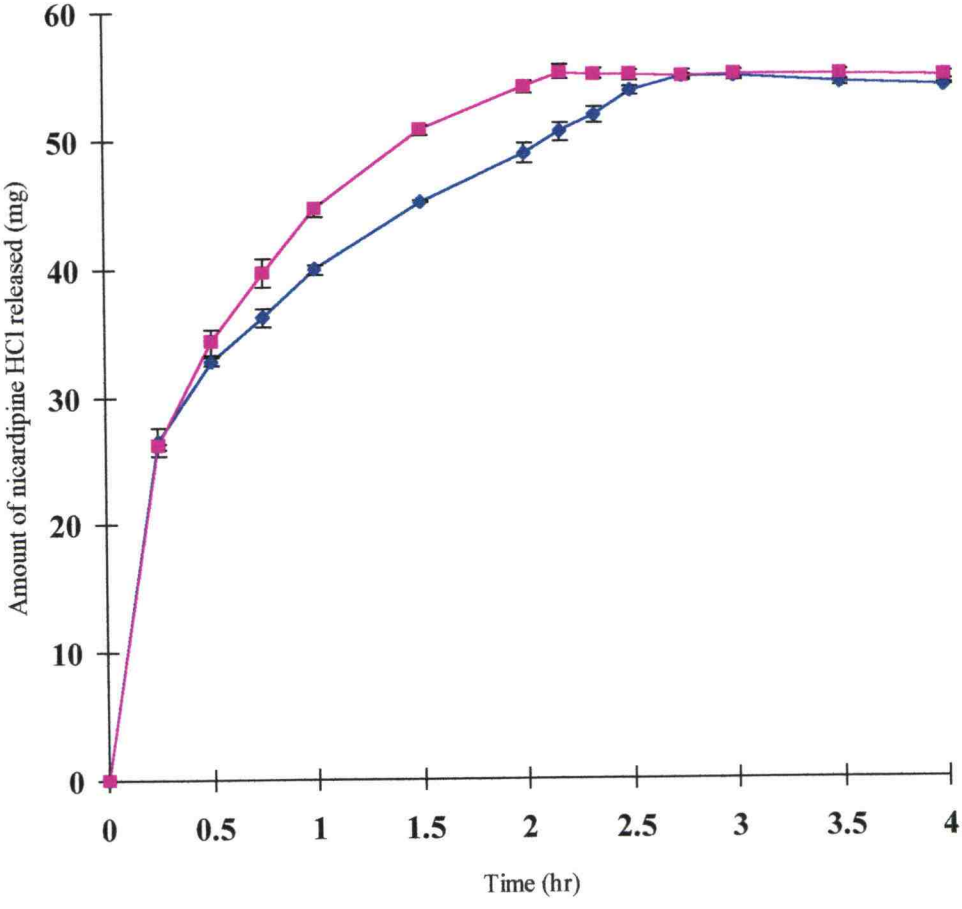

Fig. IV.14 Dissolution profiles of nicardipine HCl 60 mg capsules. Key: (◆) Cardene® SR and (■) Lab formulation.

Nicardipine HCl Sustained-Release Capsules

Dissolution profiles of nicardipine HCl sustained-release capsules produced in the lab provided similar results compared to Cardene® SR capsules at all doses shown in Fig. IV.12, IV.13, and IV.14.

CONCLUSIONS

Nicardipine HCl in the HPLC mobile phase (pH 4.8) and in dissolution media of citrate buffer (pH 4.5) or simulated gastric fluid (pH 1.4) was stable enough to analyze within 24 hours when protected from light. However, nicardipine HCl in simulated intestinal fluid (pH 7.4) degraded quite fast; therefore, it is recommended that the drug analysis be performed immediately.

Eudragit® L 30 D, when applied in spray layered beads at 1.09 and 11% (w/w), could not absolutely protect nicardipine HCl dissolution at low pH (1.4 or 4.5), but at these pH levels the drug release rate was slow compared to that at higher pH (7.4). In addition, the release rate of nicardipine HCl decreased with the increased percentages of the polymer. Rates of drug release from extruded or marumerized granules (matrix) depended on pH of the dissolution media at a much lesser extent compared to the spray layered beads containing the same ingredient (methacrylic acid copolymer type C).

Nicardipine HCl spray layered beads containing Aquacoat® was pH dependent. Rate of drug release was controlled either by spray layered binders or overcoat (with Aquacoat®). The higher the percentages of Aquacoat® in the spray layered beads, the

slower the rate of drug release. Furthermore, Aquacoat® coating decreased rates of drug release dramatically. Release rates were slower when higher percentages of coating polymer were applied. The desired dissolution profiles of nicardipine HCl may be obtained by adjustment of percentages of Aquacoat® as a binder/controller or a coating polymer.

Adding PVP in the formulation improved binding quality of drug onto the sugar beads with no significant effects on rates of nicardipine HCl release. The dissolution profiles of the drug with 10 and 15% PVP were similar to those without PVP.

Amounts of magnesium stearate may affect dissolution of nicardipine HCl and also affect flow property of the powder. Use of starch (lipophilic), soluble starch, and pregelatinized starch resulted in similar patterns of drug dissolution.

Combination of sustained-release beads and immediate-release powder resulted in both fast dissolution and prolonged nicardipine HCl release; therefore, immediate and sustained action of the dosage form *in vivo* is expected.

Information obtained from this study allows prediction of drug release in the gastrointestinal tract. Pharmacokinetic study of the dosage form in human is required to correlate *in vitro* and *in vivo* release rates of drug.

ACKNOWLEDGMENTS

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CHAPTER V

Preliminary Bioequivalence Testing of Two Nicardipine HCl Sustained-Release Formulations with *In Vitro/In Vivo* Correlations

Waranush Sorasuchart and James W. Ayres

ABSTRACT

New nicardipine HCl oral sustained-release dosage form was evaluated for bioequivalence in comparison with a reference product, Cardene® SR. *In vitro* dissolution profiles of both formulations were tested before the *in vivo* study in human volunteers. Six healthy subjects, fasted overnight, were enrolled in a single-dose, open-label, randomized, and two-way crossover study. Blood samples were collected over a 12 hour period. Nicardipine HCl plasma concentrations were analyzed from plasma samples. Pharmacokinetic parameters, including C_{max}, t_{max}, and AUC, were obtained from drug plasma concentration-time curve and pharmacokinetic analysis using WinNonlin®. The two one-sided t-test was applied in statistical analysis for comparison of the pharmacokinetic parameters between the two products. There was no convincing evidence that nicardipine HCl test formulation and Cardene® SR were bioequivalent. Amounts of nicardipine HCl release *in vivo* was mathematically obtained by deconvoluting plasma concentration-time data after oral sustained-release dosage form administration and those after IV bolus injection. Plots of percentages of drug release *in vitro* against percentages of drug release *in vivo* illustrated triphasic curves. The *in vitro* time scale was corrected by mutiplying with a correction factor before plotting against *in vivo* data. The plots of corrected scale provided a polynomial relationship (R^2 of 0.9920 and 0.9954). The *in vitro/in vivo* correlation may be useful in adjusting rates of drug release for this particular test formulation to obtain a product with the *in vivo* release rate similar to Cardene® SR.

INTRODUCTION

Nicardipine HCl is a calcium ion influx inhibitor which is indicated for treatment of hypertension (1). Given orally, it is absorbed rapidly and completely, but is extensively metabolized by saturable first-pass metabolism resulting in nonlinear pharmacokinetics, and relatively low and variable bioavailability (F = 0.15-0.45) (2). Having a short half-life (2-4 hours), nicardipine HCl is usually administered 3 times daily as an immediate-release oral dosage form or twice daily as a sustained-release dosage form (1,3). Cardene® SR (Roche Laboratories, Nutley, NJ), currently on the market, contains 25% of nicardipine HCl immediate-release powder and 75% of nicardipine HCl sustained-release granules (4), which provides fast action and prolong therapeutic levels of the drug in plasma with minimal fluctuation resulting in less frequency of drug intake, less side effects and, therefore, better patient compliance.

An objective of this research project is to evaluate a new oral sustained-release formulation of nicardipine HCl which is expected to be bioequivalent to Cardene® SR. Commercial availability of such a bioequivalent product may save patients approximately 30% on their prescription costs.

Nicardipine HCl sustained-release formulation produced at College of Pharmacy, Oregon State University (or so-called "test formulation") was evaluated *in vitro* (dissolution testing) and *in vivo* as a test product using Cardene® SR as a reference product. Furthermore, drug release from two products *in vitro* and *in vivo* were correlated,

which will be informative for adjusting the formulation and rate of *in vitro* drug release from the test formulation if necessary to modify *in vivo* performance.

MATERIALS AND METHODS

Chemicals

Cardene® SR (30 mg) capsules were manufactured by Roche Laboratories, Nutley, NJ. Lab capsules (30 mg) were produced at College of Pharmacy, Oregon State University, Corvallis, OR. Nicardipine HCl was provided by Teva Pharmaceuticals, USA, Sellersville, PA. n-Hexane (Chrom AR HPLC) was supplied from Mallinckrodt, Paris, KY. Acetonitrile (Fisher Chemicals, Lawn, NJ) and methanol (EM Sciences, Gibbstown, NJ) were of HPLC grade. Chlorpromazine HCl (CH) was from Sigma, St. Louis, MO. Dehydrated alcohol was from Midwest Grain Products of Illinois, Pekin, IL. Other chemicals included sodium citrate dihydrate (Mathecon Coleman and Bell Manufacturing Chemists, Norwood, OH), potassium phosphate monobasic, and sodium hydroxide (Aldrich, Milwaukee, Wis).

Supplies

1. Catheter 18 guage, 1.16", 1.3x30 mm, 105 mL/min (Insyte-W, Becton Dickinson Infusion Therapy Systems, Inc., Sandy, Utah 84070)

2. Deseret PRN Adapter-Luer_Lok, Fluid Capacity 0.1 mL (Becton Dickinson Vascular Access, Sandy, Utah 84070)
3. IV Start Kit #5500 w/Tegaderm Dressing contains Tourniquet, alcohol prep pad (medium), iodophor PVP swabstick, 2"x2" cotton guaze sponges, Tegaderm dressing, 1/2"x4" tape strips, 1"x4" tape strips, and dressing change label (The Clinipad Corporation, Guilford, CT 06437)
4. 10 mL syringe
5. Precision Glide Needle 19 Gl (Becton Dickinson & Co, Franklin Lakes, NJ 07417-1884)

Methods

Study Design and Subjects

The study was a single-dose, open-label, randomized, and two-way crossover design. The proposal of the study was reviewed and approved by the OSU Institutional Review Board (IRB) for the protection of human subjects. Six healthy male and female adults aged 18 to 37 were enrolled in the study which involved one day of receiving Cardene® SR (30 mg) and another day of receiving nicardipine HCl test formulation (30 mg) separated by a 3 day washout period. All subjects read and signed the informed consent document prior to enrollment.

Blood Sample Collection and Treatment

All subjects were fasted overnight and arrived at College of Pharmacy at 7 am. A blood sample of 10 mL was collected using an indwelling catheter prior to receiving a dose of nicardipine HCl. Then, 10 mL of blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours. 200 mL of water were provided to each subject every time after blood collection. The subjects were not allowed to eat during the first 2 hours. Standard breakfast (Burger King® sausage and egg biscuit, hashbrown potatoes, and 250 mL of orange juice) was provided at 2 hours after the dose. Blood samples were transferred from 10 mL syringe to a 15 mL polypropylene centrifuge tube containing 1 mL of 4% sodium citrate dihydrate solution as an anticoagulant. The blood samples were kept in the ice cooler before being centrifuged at 10°C and 3000 rpm for 10 minutes. Plasma was transferred to another 15 mL centrifuge tube and stored at -20°C until drug assay.

Drug Assay Method

Chromatographic Conditions

Concentrations of nicardipine HCl after extraction were detected by HPLC using chlorpromazine HCl (CH) as an internal standard. The mobile phase, modified from reference (5), was 67:13:127 acetronitrile:methanol:phosphate buffer (0.02 M KH_2PO_4), adjusted to the final pH of 4.80 with phosphoric acid and degassed before use. The HPLC column was Microsorb-MV® C8 5 µm 110 A° 15 cm (Rainin Instrument Company, Inc.,

Woburn, MA). The flow rate was 1.5 mL/min. The UV absorbance was detected at 254 nm (Waters Model 440). Other instruments in the HPLC system included a delivery pump (Waters 550 Solvent Delivery System, Waters Associates, Milford, MA), an automatic sample injector (Waters WISP Model 712 B, Waters Associates, Milford, MA) and an integrator (C-R3A Chromatopac, Shimadzu Corp., Kyoto, Japan).

Standard Solutions

Nicardipine HCl standard stock solution (1 mg/mL) was prepared by dissolving in a small amount of methanol (1% w/v) and adjusting the volume with phosphate buffer (pH 4.80). Standard solutions containing 400, 300, 200, 100, 75, and 50 ng/mL nicardipine HCl were prepared by serial dilution from stock solution. Chlorpromazine HCl (CH) stock solution at a concentration of 1 mg/mL was prepared by dissolving in phosphate buffer (pH 4.80). A solution of 0.1 μg/mL CH was prepared by diluting the stock solution.

Sample Preparation

Liquid-liquid extraction method, used for extraction of drug from plasma, was modified from Kobayashi S-I (6). 1.1 mL of each plasma sample was transferred into a centrifuge tube and another 1.1 mL of the same plasma sample was transferred to another tube. 100 μL of 0.1 μg/mL CH was added into each tube and then vortex mixed before adding 1 mL of a mixture of phosphate buffer (pH 7.4) and absolute alcohol (20:1). The mixture was vortex mixed for 10 seconds. After adding 6 mL n-hexane, the mixture was

vortexed gently for 3 minutes and centrifuged for 5 minutes at 1000 rpm. The organic layer of both tubes was transferred and combined into a 10 mL glass tube and evaporated to dryness in a vacuum chamber. The residue was reconstitued with 50 μL of HPLC mobile phase, and 30 μL of the solution were injected onto the HPLC column.

Standard Curve

100 μL of each nicardipine HCl standard solution was added to 1 mL blank human plasma in a 15 mL centrifuge tube (2 tubes for each standard concentration) and then processed as described above. A standard curve was constructed by plotting the peak-height ratios of nicardipine HCl to CH against nicardipine HCl concentrations in plasma. Sensitivity of the assay was as low as 5 ng/mL with linear relationship between peak-height ratio and nicardipine HCl concentrations at 5 to 40 ng/mL (R^2 of 0.9958). Accuracy of the assay was between 91.35-114.49%.

Pharmacokinetic Analysis

Significant pharmacokinetic parameters used for bioequivalence testing including peak plasma concentration (C_{max}), area under the curve from zero to the last measurable concentration (AUC_t) and area under the curve from zero to infinity (AUC_∞) were obtained from individual data. C_{max} was obtained directly from the plasma drug concentration-time curve. AUC_t and AUC_∞ were estimated by noncompartmental analysis using a computer program, WinNonlin® (Scientific Consulting, Inc., Cary, NC).

Statistical Analysis

To test bioequivalence of two products, it is recommended by the Division of Bioequivalence, US FDA that the log transformed pharmacokinetic parameters (*i.e.* C_{max} and AUC) of the test product be within 80% to 125% of the reference product using the 90% confidence interval (7).

The two one-sided tests were applied for statistical analysis of bioequivalence testing with the null and alternative hypotheses (H_0 and H_1, respectively) as follows (8), where μ_T and μ_R are the true test and reference means, respectively.

$$H_{01}: \mu_T - \mu_R \leq \theta_1$$

$$H_{11}: \mu_T - \mu_R \geq \theta_1$$

and

$$H_{02}: \mu_T - \mu_R \geq \theta_2$$

$$H_{12}: \mu_T - \mu_R \leq \theta_2$$

With the -20% and +25% criteria, $\theta_1 = -0.20\mu_R$ and $\theta_2 = 0.25\mu_R$ and the interval hypotheses would be stated as

$$H_0: \mu_T - \mu_R \leq -0.20\mu_R \text{ or } \mu_T - \mu_R \geq 0.25\mu_R$$

$$H_1: -0.20\mu_R < \mu_T - \mu_R < 0.25\mu_R$$

which, if $\mu_R > 0$, may be restated as

$$H_0: \mu_T/\mu_R \leq 0.80 \text{ or } \mu_T/\mu_R \geq 1.25$$

$$H_1: 0.80 < \mu_T/\mu_R < 1.25$$

To appropriately assume the statistical assumption of normality and homogeneous variance, variables are then log transformed. The hypotheses for -20% and +25% criteria, may be restated as

$$H_0 : \eta_T - \eta_R \leq \ln(0.8) \text{ or } \eta_T - \eta_R \geq \ln(1.25)$$

$$H_1 : \ln(0.8) < \eta_T - \eta_R < \ln(1.25)$$

η_T and η_R are the true test and reference means, respectively, of the natural log transformed variables. The two one-sided tests procedure consists of rejecting the null hypothesis (H_0), and thus concluding bioequivalence of η_T and η_R (8).

C_{max}, AUC_t, and AUC_∞ were log transformed before the analysis of variance (ANOVA) for crossover study was performed (9). A square root of error mean square (S) from the ANOVA was then used to calculate 100(1-α)% confidence interval (CI) for the mean difference of log transformed variables (η_T-η_R) as follows (10, 11).

$$100(1-\alpha)\%\ \text{CI} = (\eta_T - \eta_R) \pm t_{1-\alpha/2(\nu)}\left(S\sqrt{\frac{2}{n}}\right) \qquad \text{Eq. V.1}$$

where α is 0.1 for 90% confidence interval, ν is a number of degrees of freedom associated with the error mean square (n-2), and n is number of subjects.

The null hypothesis is rejected if the confidence interval falls in between ln (0.8) and ln (1.25):

$$\ln(0.8) < \eta_T - \eta_R < \ln(1.25)$$

or

$$-0.22314 < \eta_T - \eta_R < 0.22314$$

***In Vitro/In Vivo* Correlations (IVIVC)**

Numerical deconvolution technique was applied to the mean plasma concentration-time data of sustained-release dosage forms (input response, c(t)) to estimate the *in vivo* cumulative amounts of drug release (f(u)) of the two products using the mean plasma concentration-time data from IV bolus injection (12) as an unit impulse response, $c_\delta(t-u)$. The deconvolution was performed using computer software, PCDCON. The input-response relationship may be described according to the convolution integral as follows (13).

$$c(t) = (c_\delta * f)(t) = \int_0^t c_\delta(t-u) f(u) du \qquad \text{Eq. V.2}$$

Percentages of nicardipine HCl released *in vitro* and in vivo were correlated by plotting the mean percentages of drug released *in vivo* at time t against those *in vitro* at time t×k, where k is a correction factor calculated from the ratio of the maximum time of drug release obtained from *in vivo* data over time at complete *in vitro* dissolution.

RESULTS AND DISCUSSION

Pharmacokinetic Analysis and Bioequivalence Testing

Pharmacokinetic parameters of individual subjects after oral administration of Cardene® SR and test formulation as estimated by noncompartmental analysis are shown in Table V.1 and Table V.2.

Table V.1 Pharmacokinetic parameters of individual subjects after oral administration of 30 mg Cardene® SR

Pharmacokinetic parameter	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
t_{max} (hr)	1.5	2	1	1.5	1.5	1.5
C_{max} (ng/mL)	20.66	35.97	41.11	25.47	22.92	25.87
$AUC_{t-\infty}$ (ng.hr/mL)	92.87	127.39	132.31	93.91	116.83	83.00
AUC_{∞} (ng.hr/mL)	215.95	150.51	152.07	104.06	176.84	103.50
$AUMC_t$ (ng/mL)	340.25	369.94	433.25	279.50	425.87	290.63
$AUMC_{\infty}$ (ng/mL)	2648.40	605.87	625.45	397.69	1168.69	503.92
MRT_t (hr)	3.66	2.90	3.27	2.98	3.65	3.50
MRT_{∞} (hr)	12.26	4.02	4.11	3.82	6.61	4.90
k_e (1/hr)	0.09	0.31	0.58	0.27	0.23	0.39
$t_{1/2}$ (hr)	7.45	2.22	1.20	2.53	3.03	1.77
Cl/F (μg/hr)	164.63	199.32	197.28	280.91	197.01	305.57
V_{ss}/F (L)	1770.35	638.68	340.96	1023.73	862.55	779.22

Table V.2 Pharmacokinetic parameters of individual subjects after oral administration of 30 mg test formulation

Pharmacokinetic parameter	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
t_{max} (hr)	1.5	1.5	1.5	1.5	1	1
C_{max} (ng/mL)	27.70	22.79	24.72	23.56	23.06	15.12
AUC_t (ng.hr/mL)	102.90	99.41	99.41	83.51	118.60	48.00
AUC_{∞} (ng.hr/mL)	127.02	241.47	111.56	92.26	166.58	57.44
$AUMC_t$ (ng/mL)	339.16	330.67	334.54	252.92	406.14	150.54
$AUMC_{\infty}$ (ng/mL)	598.78	3176.29	451.22	338.23	1007.99	247.24
MRT_t (hr)	3.30	3.33	3.36	3.03	3.42	3.14
MRT_{∞} (hr)	4.71	13.15	4.04	3.67	6.05	4.30
k_e (1/hr)	0.36	0.08	0.63	0.57	0.22	0.44
$t_{1/2}$ (hr)	1.91	9.03	1.11	1.22	3.15	1.55
Cl/F (μg/hr)	236.18	117.91	287.53	332.65	180.09	522.27
V_{ss}/F (L)	652.65	1536.40	459.14	581.85	818.60	1171.56

Figs. V.1-a-f Individual nicardipine HCl plasma concentration-time curve

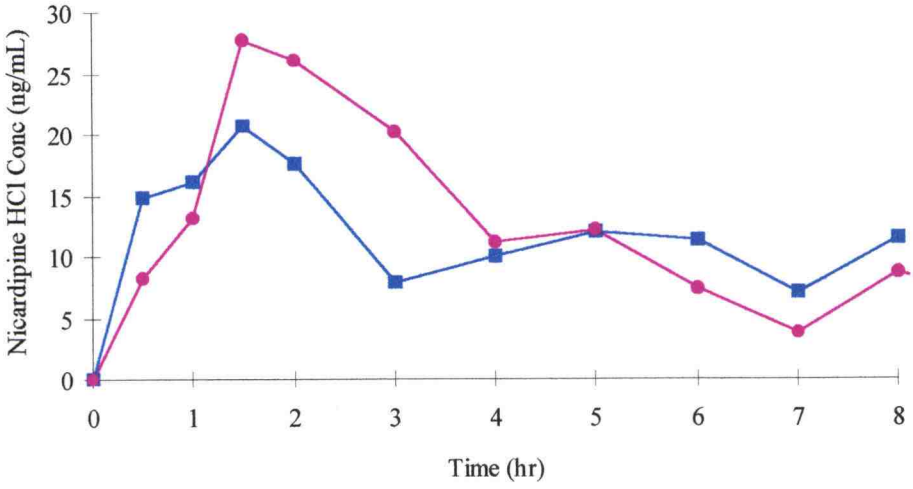

Fig. V.1-a Nicardipine HCl plasma concentration-time curve (subject A). Key: (■) Cardene® SR and (●) test formulation.

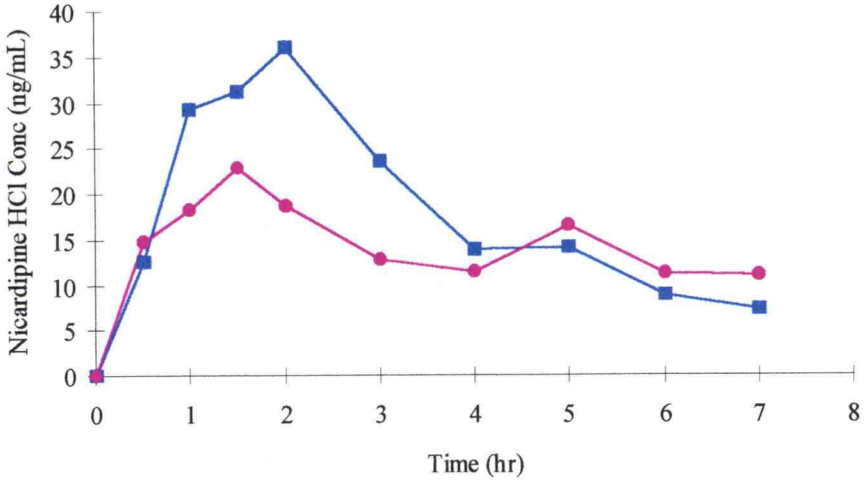

Fig. V.1-b Nicardipine HCl plasma concentration-time curve (subject B). Key: (■) Cardene® SR and (●) test formulation.

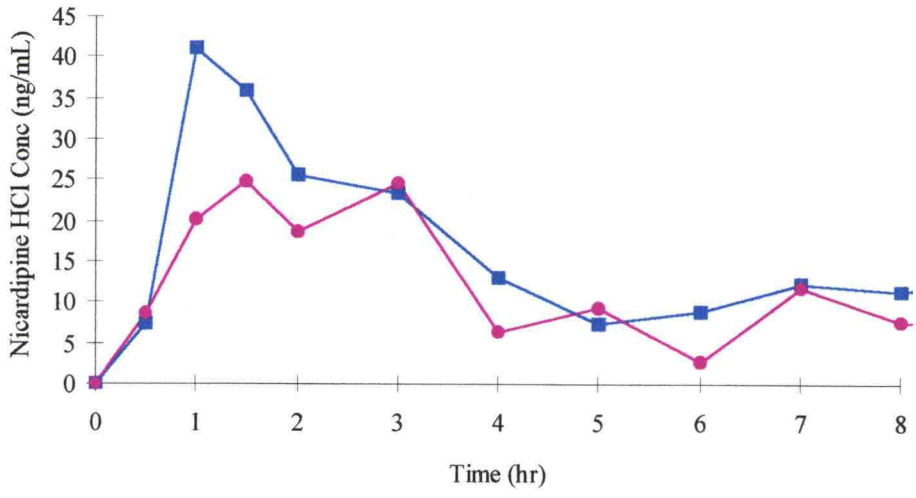

Fig. V.1-c Nicardipine HCl plasma concentration-time curve (subject C). Key: (■) Cardene® SR and (●) test formulation.

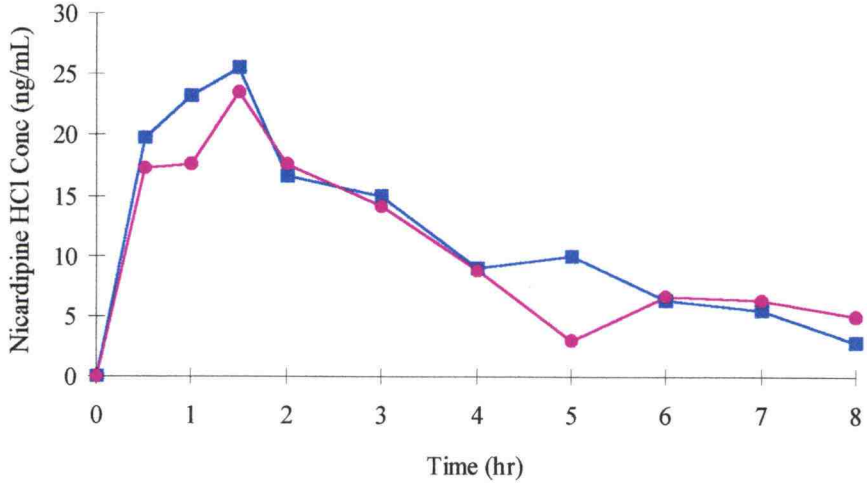

Fig. V.1-d Nicardipine HCl plasma concentration-time curve (subject D). Key: (■) Cardene® SR and (●) test formulation.

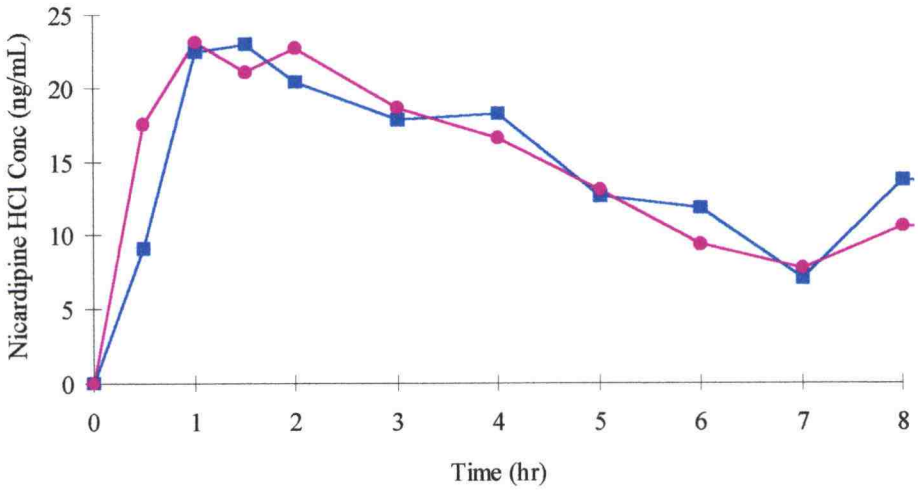

Fig. V.1-e Nicardipine HCl plasma concentration-time curve (subject E). Key: (■) Cardene® SR and (●) test formulation.

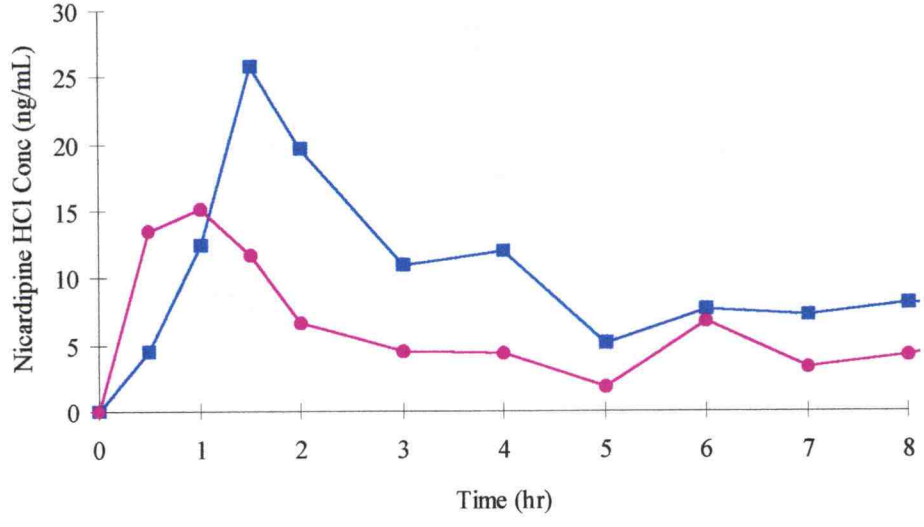

Fig. V.1-f Nicardipine HCl plasma concentration-time curve (subject F). Key: (■) Cardene® SR and (●) test formulation.

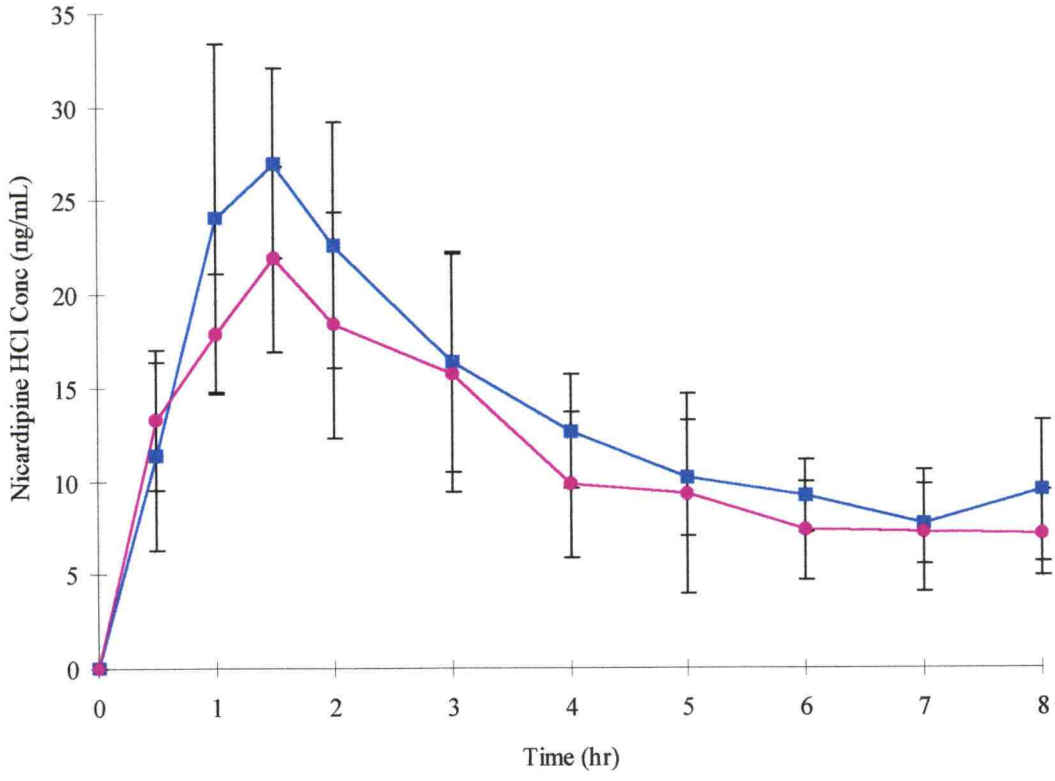

Fig. V.2 Nicardipine HCl plasma concentration-time curve, average of 6 subjects. Error bars represent standard deviation. Key: (■) Cardene® SR and (●) test formulation.

Individual plasma concentration-time curves are illustrated in Figs. V.1-a-f. Averages of plasma concentration-time curves indicated that the plasma concentrations of test product (lab formulation) were somewhat lower than Cardene® SR (Fig. V.2). Average time of maximum concentration (t_{max}) was approximately 1.5 hours for both formulations. Average C_{max} for Cardene® SR and test formulation were 27.79 and 22.83 ng/mL, respectively. Tables V.3, V.4, and V.5 are the ANOVA table for C_{max}, AUC_t and AUC_∞, respectively. Means of the pharmacokinetic parameters are listed in Table V.6 and means of natural log transformed pharmacokinetic parameters and their 90% confidence intervals are listed in Table V.7. The 90% confidence intervals of all parameters are not in the range of ln (0.8) to ln (1.25); thus, the null hypothesis cannot be rejected. There was no convincing evidence that the two nicardipine HCl formulations were bioequivalent, using the two one-sided t-test for statistical analysis. However, this was a preliminary study involving only 6 subjects which was a small number; therefore, it cannot be concluded that the two products were not bioequivalent.

Table V.3 ANOVA table of ln (C_{max})

Source of Variation	d.f.	SS	MS	P
Subjects	5	0.2244	0.0448	
Period	1	0.0831	0.0831	F1,4 = 1.9456
Treatment	1	0.1092	0.1092	F1,4 = 2.5563
Error	4	0.1709	0.0427	
Total	11	0.5877		

Table V.4 ANOVA table of ln (AUC_t)

Source of Variation	d.f.	SS	MS	P
Subjects	5	0.5611	0.1122	
Period	1	0.0462	0.0462	F1,4 = 2.0520
Treatment	1	0.0976	0.0976	F1,4 = 4.3358
Error	4	0.0900	0.0225	
Total	11	0.7949		

Table V.5 ANOVA table of ln (AUC_∞)

Source of Variation	d.f.	SS	MS	P
Subjects	5	1.1353	0.2270	
Period	1	0.0613	0.0613	F1,4 = 0.7770
Treatment	1	0.0797	0.0797	F1,4 = 1.0112
Error	4	0.3154	0.0788	
Total	11	1.5917		

Table V.6 Means and standard deviations of pharmacokinetic parameters after oral administration of Cardene® SR and test formulation (30 mg)

		Cardene® SR	Test formulation
C_{max}	(ng/mL)	27.79±6.56	22.83±4.18
AUCt	(ng.hr/mL)	107.72±20.48	91.97±24.27
AUC_∞	(ng.hr/mL)	150.49±43.23	134.74±61.12
T_{max}	(hr)	1.58±0.20	1.50±0.32

Table V.7 Means of natural log transformed pharmacokinetic parameters after oral administration of Cardene® SR and test formulation (30 mg) and their statistical results

	Cardene® SR	Test Formulation	Difference (η_T-η_R)	90% Confidence Interval
ln (C_{max})	3.3022	3.1116	-0.1906	ln (0.69) - ln(0.98)
ln (AUC_t)	4.6642	4.4840	-0.1802	ln (0.73) - ln (0.95)
ln (AUC_∞)	4.9789	4.8161	-0.1628	ln (0.66) - ln (1.08)

Determination of Sample Size

It is recommended by the FDA that at least 24 subjects be enrolled in a single dose fasting two-way crossover bioequivalence study (5). In this study, only 6 subjects were enrolled. This small number of subjects and high value of mean square of error due to intersubject variation produced a large confidence interval which means theres is only a small possibility of rejecting the null hypotheses. When a higher number of subjects are included, it is possible that the 90% confidence intervals of C_{max} and AUC will be smaller so that the null hypotheses can be rejected, and bioequivalence of the two products can be concluded.

For further study where the null hypothesis can possibly be rejected, a required sample size, n, may be calculated from the specified confidence interval (14, 15) using the relationship described earlier in Eq. V.1. Thus, half-width interval is as follows.

$$\text{Half-width interval} = t_{1-\alpha/2(\nu)}\left(S\sqrt{\frac{2}{n}}\right) \qquad \text{Eq. V.2}$$

50% power (1-β, where β = 0.5) is assumed for calculation of sample size (n) using this half-width interval. The required half-width interval depends on the value of η_T-η_R used in the calculation so that the 100(1-α)% confidence interval falls in the range of ln 0.8 and ln 1.25 (to conclude bioequivalence between two products). η_T-η_R can be assumed zero or obtained from a pilot study. From this study η_T-η_R of C_{max}, which was the biggest value among all parameters, was 0.1906 (Table V.7). Fig. V. 3 and Table V. 8 demonstrate the calculated sample sizes relative to η_T-η_R. In order to reject the null hypothesis and conclude bioequivalence between the two products, the sample size needs to be bigger when η_T-η_R is further away from 0. When η_T-η_R is approaching the edge of the "rejection range" (-0.223 or 0.223), the sample size is approaching infinity (Fig. V.3).

Table V.8 Sample sizes calculated using "specified confidence interval" (50% power) and various values of η_T-η_R, where a required half-width is 0.223.

η_T-η_R	Sample size
0	16
0.02	17
0.05	23
0.08	32
0.1	45
0.15	115
0.1906*	221

Note: * The value of 0.1906 was the difference between test and reference ln (Cmax)'s obtained experimentally.

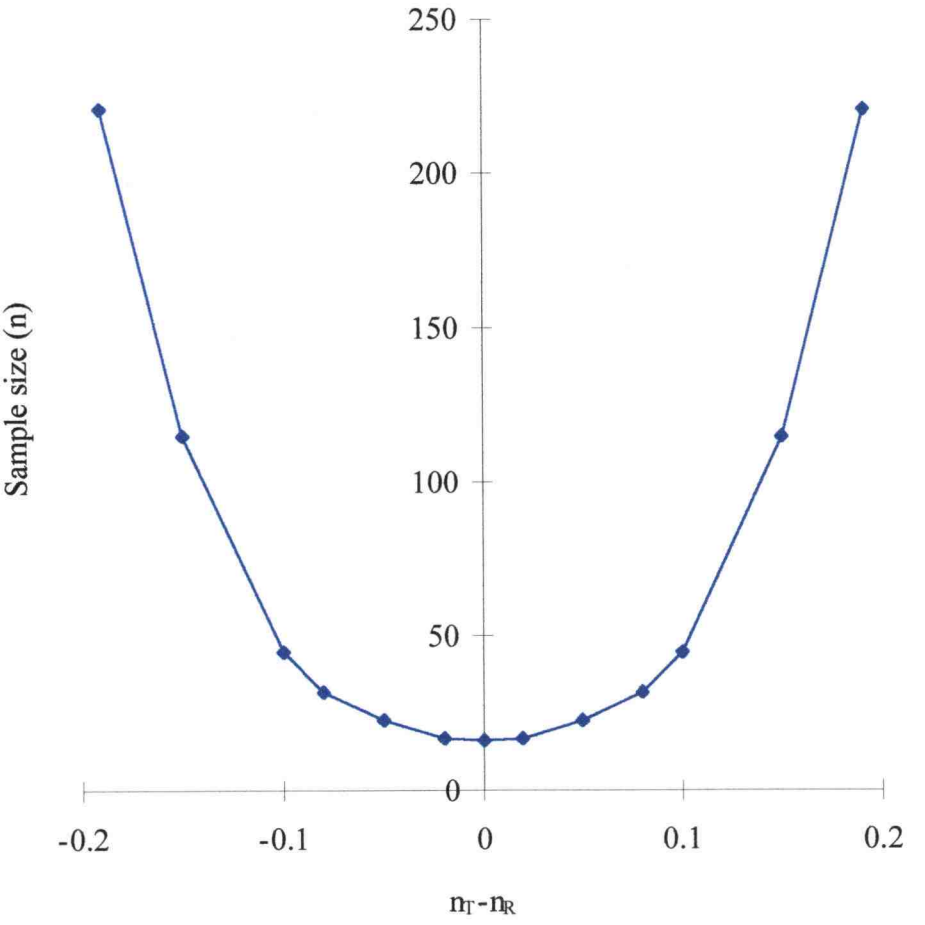

Fig. V.3 Relationship between calculated sample size and η_T-η_R

The sample size can also be calculated for a required power using Eq. V.3 (14), where Z_α and Z_β are critical values of standard normal curve and can be obtained from statistical tables. Z_α is $t_{1-\alpha/2,\,\nu}$, where α is the significance level and ν is n-2. Δ is "practical significance," which is 20% difference between test and reference means. For log transformed data, Δ is log of ratio of the test and reference means that makes 20% difference between the two values. Therefore, Δ is ln 0.8 (-0.223) or ln 1.25 (0.223). $2S^2$ is substituted for σ^2, where S^2 is mean square error from ANOVA. For this equation, it was assumed that η_T-η_R is zero. A few numbers of n and $t_{1-\alpha/2,\,\nu}$ are substituted in Eq. V.3 and Δ is calculated. The smallest n that provides Δ of 0.223 or lower is the required sample size. Calculated sample size of 29 is found for 80% power and 5% level of statistical test as shown in Table V.9. The sample sizes for different levels of power are listed in Table V.10. As expected, the required sample size is smaller when the power of statistical test is lower if the level of statistical test remains the same. Note that at 50% power ($Z_\beta = 0$) the calculated sample size is similar to that calculated using the "specified confidence interval" when η_T-η_R is assumed zero.

$$n = \left(\frac{\sigma}{\Delta}\right)^2 \left(Z_\alpha + Z_\beta\right)^2 + 0.5\left(Z_\alpha\right)^2 \qquad \text{Eq. V.3}$$

The sample size calculated using "specified confidence interval" can be changed depending on selection of η_T-η_R, but the power of the test remains at 50%. In contrast, the sample size may be calculated by varying the power of the test (usually 80-95%), but η_T-η_R is always assumed zero. Calculation based on the power is preferred because power of the test can be assigned to find the appropriate number of subjects. In addition, the test

and reference products are usually expected to be the same in bioequivalence testing. Moreover, the number of subjects calculated using this method is more reasonable and practical.

Table V.9 Sample sizes and values of Δ calculated from the sample sizes

n	ν	$t_{1-\alpha/2,\,\nu}$	Δ
26	24	2.06439	0.2360
27	25	2.06003	0.2293
28	26	2.05602	0.2259
29	**27**	**2.05232**	**0.2214**
30	28	2.04889	0.2171

Table V.10 Calculated sample sizes for various levels of power for statistical test

Power	Sample size (n)
50	17
80	29
90	37
95	61

This calculation of sample size suggests that at least 29 subjects are required for a bioequivalence study with 80% power and 5% significance level to conclude bioequivalence of the two products. Therefore, no final conclusion for the products studied can be made until the bioequivalence study with a higher number of subjects is performed. Nevertheless, if bioequivalence cannot be concluded based on 29 subjects, it

is recommended that drug release rate of the test formulation be adjusted using the information obtained from the *in vitro/in vivo* correlations discussed in the following section. It is highly recommended that at least 24 subjects be included in any future bioequivalence study with Cardene® SR and new test formulations.

In Vitro/In Vivo Correlations of Drug Released

In vitro dissolution profiles of Cardene® SR and lab formulation in enzyme-free simulated gastric fluid (pH 1.4) for 2 hours and then in enzyme-free simulated intestinal fluid (pH 7.4) are displayed in Fig. V.4. The *in vivo* cumulative amounts of drug release obtained from deconvolution of plasma drug concentration-time profiles for both products are illustrated in Fig. V.5. The cumulative amounts of drug release *in vivo* were very low compared to the dose given because the plasma drug concentrations which were used for deconvolution were very low. As mentioned earlier, nicardipine HCl is extensively metabolized by a hepatic first-pass effect, resulting in relatively low bioavailablity. Therefore, to be more specific, these cumulative amounts of drug release actually represent the cumulative amounts of drug delivered into the systemic circulation after the first-pass metabolism, and before the distribution phase.

Without correction of *in vitro* time scale, the relationship between percentages of drug release *in vitro* and *in vivo* of both formulations produced triphasic curves (Figs. V.6-a-b). The last phase of the curve is almost vertical since the *in vitro* drug release is approaching completion, while the *in vivo* drug release still occurs at a steady rate. The correction of the *in vitro* time scale was made by multiplying by a correction factor (k) of

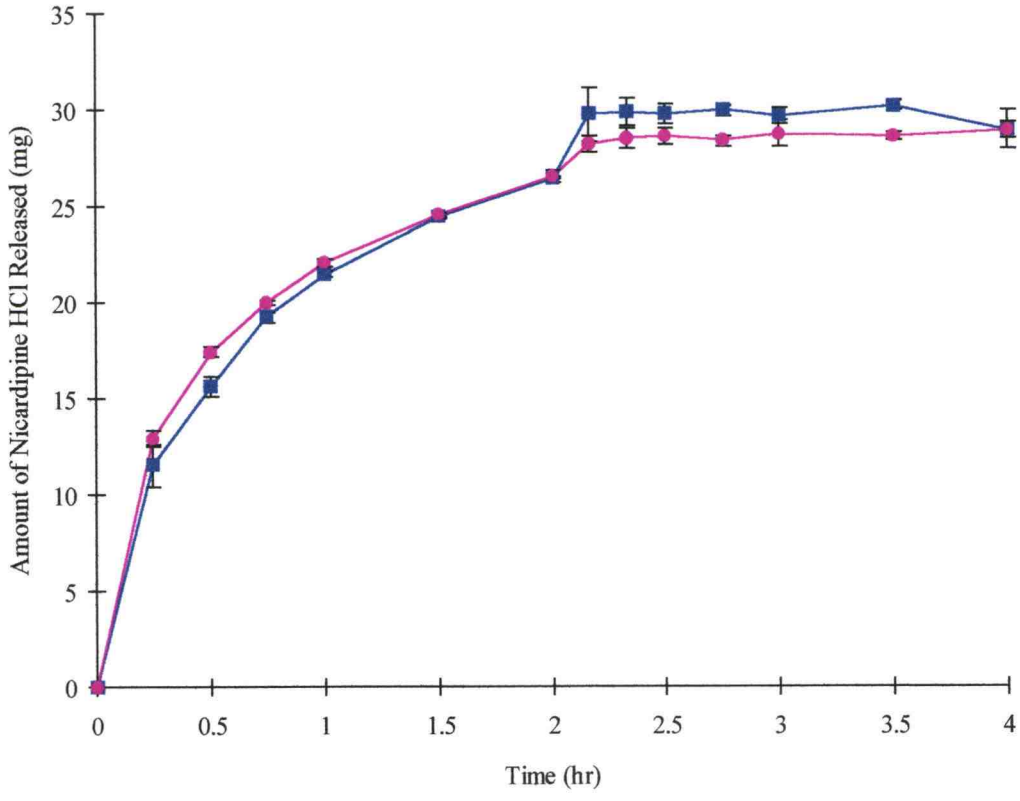

Fig. V.4 *In vitro* dissolution profiles of nicardipine HCl sustained-release formulations in enzyme-free simulated gastric fluid (pH 1.4) for 2 hours and then in enzyme-free simulated intestinal fluid (pH 7.4). Error bars represent standard deviations. Key: (■) Cardene® SR and (●) test formulation.

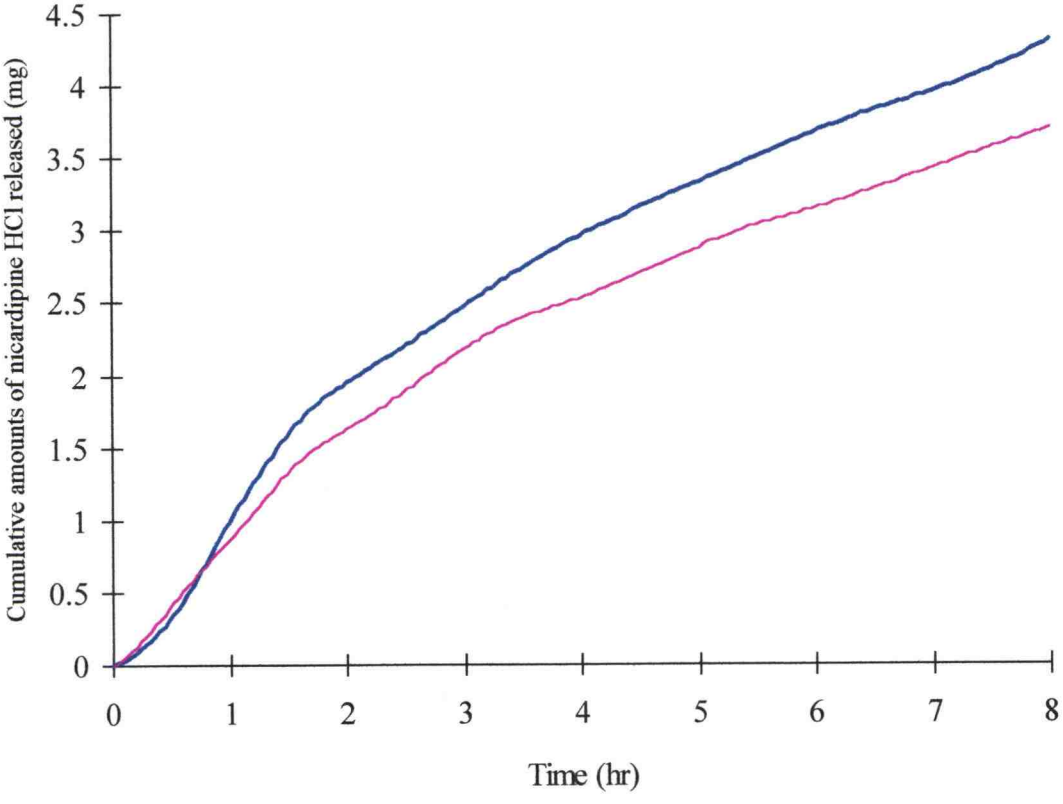

Fig. V.5 *In vivo* nicardipine HCl release from Cardene® SR (thick line) and test formulation (thin line), obtained from deconvolution of plasma drug concentration-time profiles of both formulations.

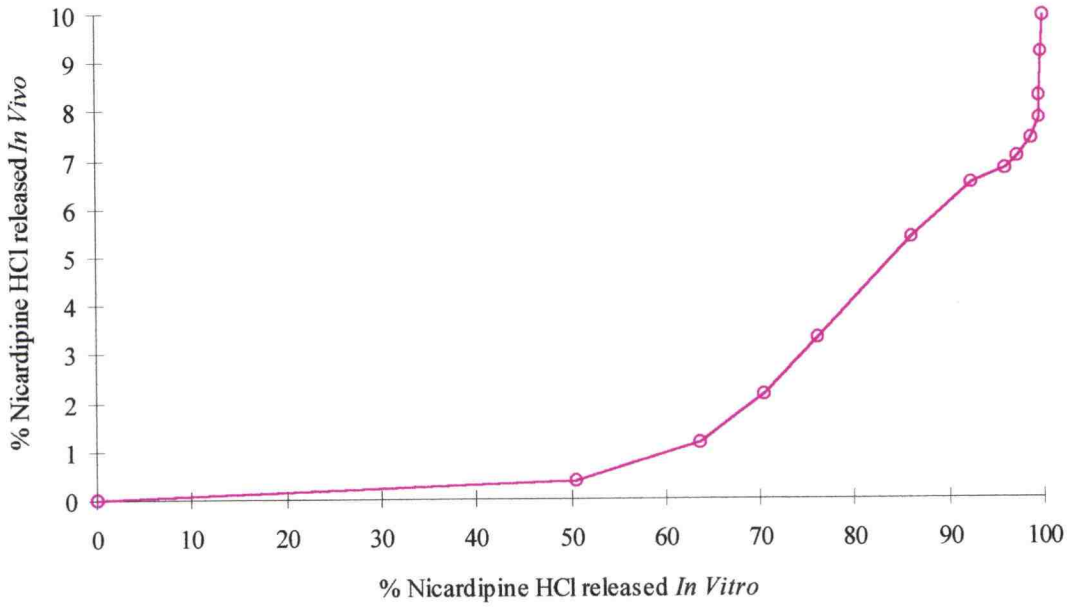

Fig. V.6-a Percentages of nicardipine HCl release from Cardene® SR *in vivo* against *in vitro*, plotted in uncorrected *in vitro* scale.

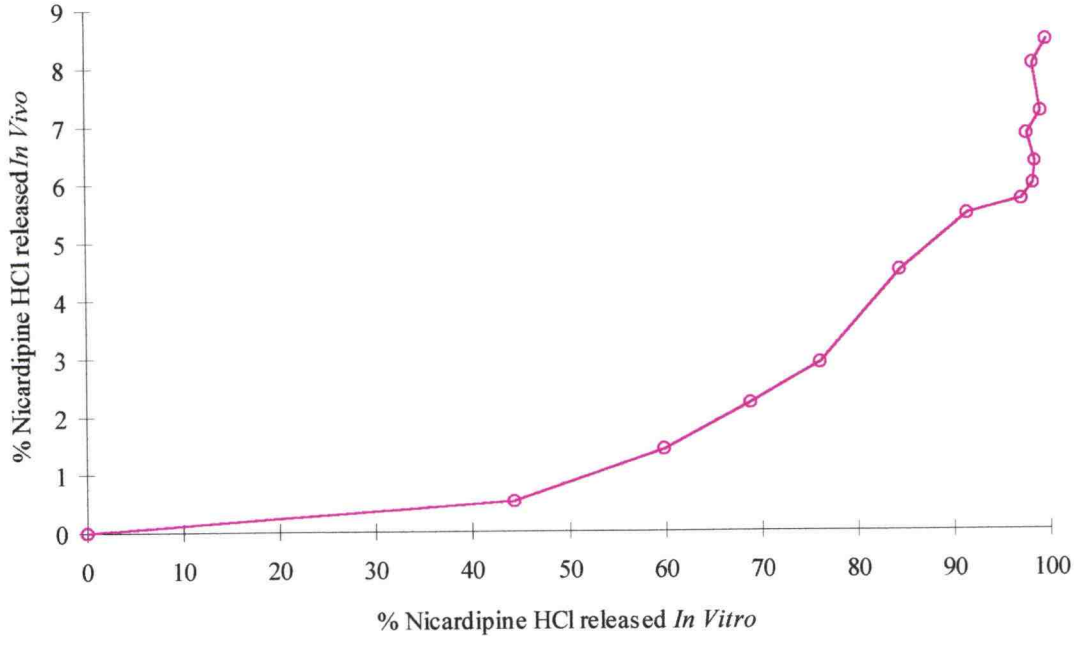

Fig. V.6-b Percentages of nicardipine HCl release from test formulation *in vivo* against *in vitro*, plotted in uncorrected *in vitro* scale.

8/2.33, where 8 is the maximum time obtained from the deconvolved data and 2.33 is the time of complete *in vitro* dissolution. The percentages of drug release *in vitro* (at the corrected scale) and *in vivo* were plotted against time from 0 to 8 hours as shown in Figs. V.7-a-b. Furthermore, the percentages of drug release *in vitro* of corrected scale and *in vivo* were plotted against each other, illustrated in Fig. V.8. The relationship after time scale corrected was fitted by a polynomial of degree 2 (Microsoft® Excel). R^2 of *in vitro/in vivo* correlations of Cardene® SR and lab formulation were of 0.992 and 0.9954, respectively.

Even though the *in vitro/in vivo* correlations indicated good relationship between drug release *in vitro* and *in vivo*, it is almost impossible to predict the *in vivo* absorption of nicardipine HCl using this relationship. As stated earlier, nicardipine HCl is highly metabolized by hepatic first-pass metabolism. A shift in release rates of drug would alter the extent of drug metabolism, and thus change the bioavailability of the drug. However, the relationship is informative enough to predict the *in vivo* drug release from the *in vitro* drug release when the release rates and patterns are relatively similar which is then useful for adjusting the release rates of this particular test formulation to achieve the goal of obtaining a pharmaceutical bioequivalent product. It is suggested that the initial burst of drug release from the test product should be reduced, while the rate of drug release after the burst should be increased so that the release rate *in vivo* will be closer to Cardene® SR.

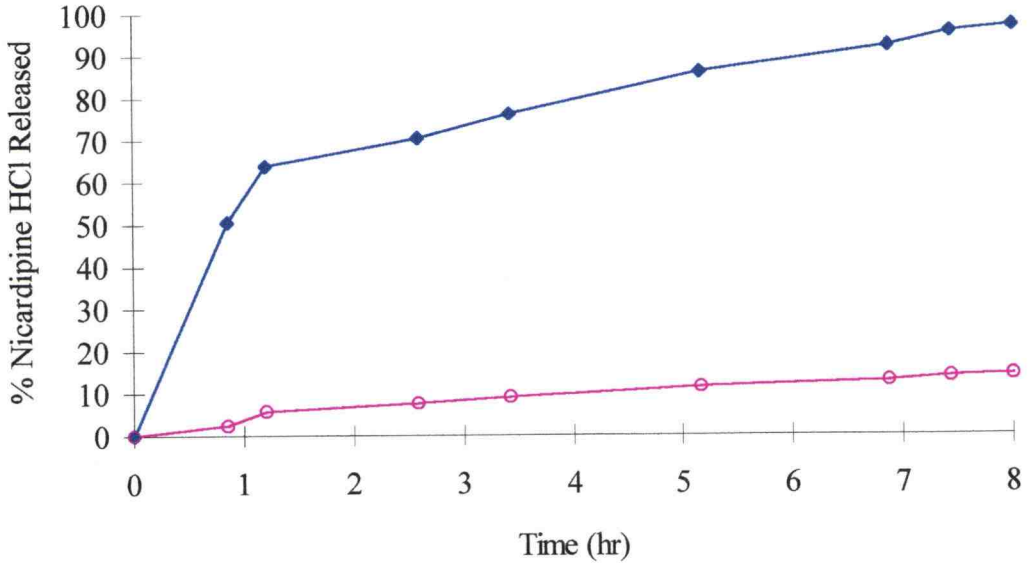

Fig. V.7-a Percentages of nicardipine HCl release from Cardene® SR *in vivo* (O) and *in vitro* (◆) after scale corrected.

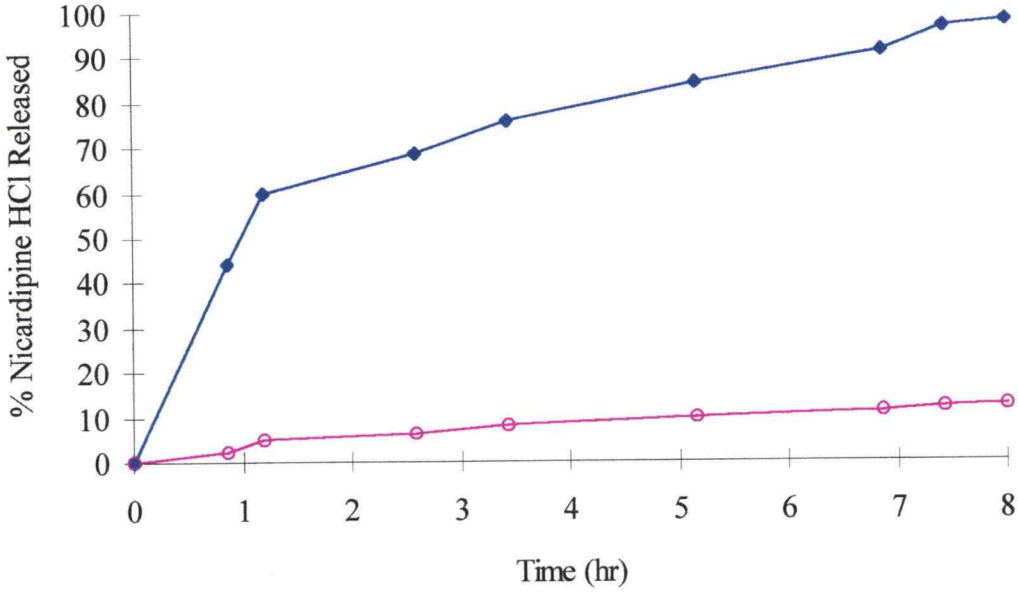

Fig. V.7-b Percentages of nicardipine HCl release from test formulation *in vivo* (O) and *in vitro* (◆) after scale corrected.

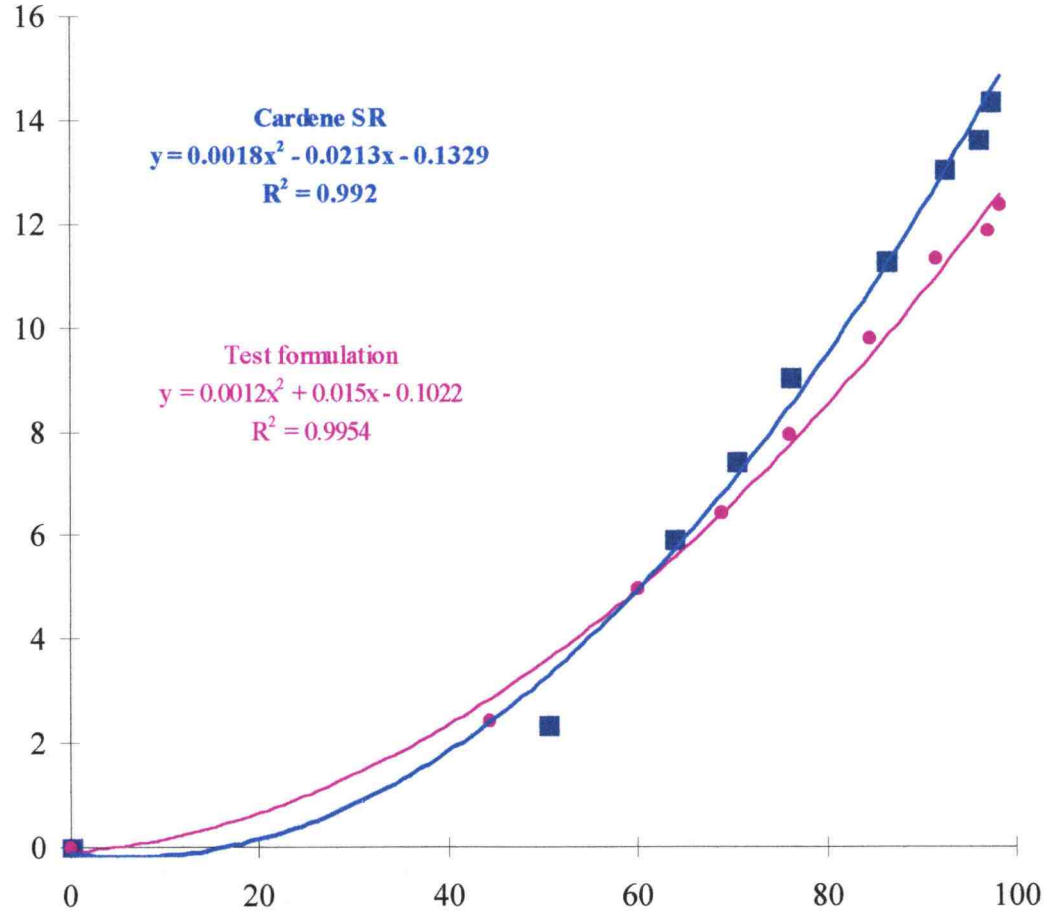

Fig. V.8 Percentages of nicardipine HCl release *in vivo* against *in vitro*, plotted in corrected *in vitro* scale. Key: (■, thick line) Cardene® SR and (●, thin line) test formulation.

CONCLUSIONS

Pharmacokinetic parameters including C_{max}, AUC_t, and AUC_∞ obtained from individual nicardipine HCl plasma concentration data were log transformed and statistically analyzed using two one-sided t-tests. There was no convincing evidence that nicardipine HCl test formulation and Cardene® SR at a dose of 30 mg were bioequivalent. However, the range of 90% confidence intervals were large because of relatively big differences between log of test and reference means, high value of square root of mean square error (MSE), and small numbers of subjects involved. After calculation of sample size for statistical test with 80 % power and 5 % significance level, it was proposed that both products may be bioequivalent when at least 29 subjects are enrolled in the study.

Percentages of drug release *in vitro* and *in vivo* are correlated when the *in vitro* time scale was corrected; hence, the *in vitro-in vivo* relationship may be useful in adjusting rates of drug release for this particular test formulation to achieve a generic product that is bioequivalent to Cardene® SR.

ACKNOWLEDGMENT

Thanks are due to Dr. David Thomas, Department of Statistics, Oregon State University for his valuable time in review and discussion of statistical analysis. Thanks to Dr. Kris Holt for his advice on deconvolution. Special thanks to Carol Moldowan, Matthew Brazer, Lupita Olson, and John Eskander for their technical assistance.

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CHAPTER VI

CONCLUSIONS

CONCLUSIONS

As high as 19 to 23% of insulin was entrapped using the reversed phase evaporation method. When incubating with polycarbophil, weights of liposomes increased as a function of time. Rates of drug release from liposomes were not significantly controlled by levels of polycarbophil coating. However, release rates of hydrophilic drugs were slightly faster as the percentages of polycarbophil used for coating liposomes were higher, and *vice versa* for hydrophobic drug. Loss of entrapped insulin from liposomes was reduced after coating with polycarbophil, especially when 1-1.5% polycarbophil solution was used for incubation. Loss of entrapped dyphylline and hydrocortisone was not changed upon polycarbophil coating. Therefore, polycarbophil coated liposomes were a stable drug carrier for insulin. Membrane permeation study indicated that insulin, dyphylline, and hydrocortisone barely penetrated across the ethylenevinylacetate membrane. In addition, amounts of the drugs entrapped in uncoated or polycarbophil coated liposomes diffused through the membrane were too low to detect. It was concluded that polycarbophil coated liposomes were a promising drug carrier for topical application.

Rates of drug release from spray layered and ethylcellulose coated drug containing beads were pH dependent even though the solubility of ethylcellulose is pH independent. Release rates of drugs with different solubilities and degrees of ionization were distinct even at the same pH. Drugs that were more soluble or more ionized dissolved better and were released faster than those that were less soluble or less ionized. Comparison of drug

release using three USP methods indicated that basket and paddle methods at 50 RPM were comparable to Bio-Dis® at 5 or 10 DPM.

Spray layering of Eudragit® L along with nicardipine HCl could not completely protect drug release in dissolution media at pH 1.4 or 4.5. Rates of nicardipine HCl release from the beads were controlled by ethylcellulose in spray layered beads. The higher the percentages of ethylcellulose, the slower the rates of drug release. However, the release rates were not affected by incorporation of 10-15% of PVP as a binder in the spray layering process. Overcoating the spray layered beads with ethylcellulose slowed down the rates of drug release.

Flow of nicardipine HCl and starch powder mixture depended on percentages of magnesium stearate or talc. The flow property was at its best when 1% magnesium stearate was included. Formulation that included pregelatinized starch provided satisfactory results in terms of extent of drug dissolved. A combination of selected nicardipine HCl beads and powder provided the same dissolution profiles as Cardene® SR.

Even though *in vitro* drug release of nicardipine HCl test formulation was the same as Cardene® SR, it could not be concluded that the two formulations were bioequivalent. The *in vitro/in vivo* correlation of nicardipine HCl release was found after the *in vitro* time scale was corrected. It is highly recommended that the test formulation be changed so that the *in vitro* release rate is faster at the very beginning and slower afterwards, as compared to the new formulation used in the bioequivalence study.

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APPENDIX

Table A.1 Ratios of weight increase to initial weight of liposomes incubated with polycarbophil (formulation 12)

Time	Weight ratio *						
(hr)	uncoated	0.5%**	0.75%**	1.0%**	1.25%**	1.5%**	2.0%**
0	1	1	1	1	1	1	1
0.5	1.31	1.61	3.11	4.09	3.73	3.47	2.79
1	0.86	1.52	2.99	3.55	2.83	4.22	2.87
2	1.42	1.55	2.66	3.40	3.18	3.76	3.52
4	1.07	1.48	2.02	3.23	3.50	2.81	3.98
6	1.16	1.55	2.69	2.76	3.58	3.24	2.91
8	1	2.05	2.85	3.02	3.16	3.35	2.55
24	1	1.87	2.63	2.69	3.13	3.63	3.39
48	1	5.56	2.46	5.62	3.54	5.62	5.62

* Weight ratio is a ratio of weight of liposomes at time t to the initial weight (time 0).

** % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.2 Ratios of weight increase to initial weight of liposomes incubated with polycarbophil (formulation 13)

Time	Weight ratio *						
(hr)	uncoated	0.5%**	0.75%**	1.0%**	1.25%**	1.5%**	2.0%**
0	1	1	1	1	1	1	1
0.5	1.06	1	2.49	2.19	3.02	9.51	2.66
1	1.12	1.39	2.08	2.36	3.27	4.92	2.65
2	1.15	1.29	2.38	2.33	3.07	4.11	2.61
4	1.22	1.32	2.38	2.41	4.21	5.33	2.81
6	1.10	1.31	2.59	2.47	1.61	4.21	2.93
8	1.26	1.18	3.18	2.47	6.53	6.14	2.54
24	0.93	1.14	3.24	2.50	6.53	4.44	2.71
48	1.08	1.94	3.67	2.38	7.17	7.00	3.26

* Weight ratio is a ratio of weight of liposomes at time t to the initial weight (time 0).

** % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.3 Percentages of insulin released from liposomes (formulation 12)

Time	% Insulin Released						
(hr)	uncoated	0.5%*	0.75%*	1.0%*	1.25%*	1.5%*	2%*
0	0	0	0	0	0	0	0
0.5	7.76	10.9	31.32	20.77	21.12	2.55	10.5
1	18.73	21.26	46.02	40.14	38.23	5.38	20.08
1.5	23.52	28.51	55.91	53.25	50.77	7.9	29.44
2	32.08	33.81	61.13	62.89	60.81	11.13	37.75
3	60.63	40.35	64.97	70.41	67.74	16.78	44.93
4	63.86	46.22	67.28	75.96	72.61	24.99	59.37
6	66.81	51.30	69.32	79.86	76.46	43.00	65.46
8	70.50	63.49	71.36	82.96	80.02	49.47	70.82
24	75.10	72.48	73.36	87.76	83.58	72.22	80.67
48	82.23	77.92	75.81	89.79	86.02	82	88.84

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.4 Percentages of insulin released from liposomes (formulation 13)

Time	% Insulin Released						
(hr)	uncoated	0.5%*	0.75%*	1.0%*	1.25%*	1.5%*	2%*
0	0	0	0	0	0	0	0
0.5	26.06	17.69	33.03	29.07	13.59	9.51	11.80
1	39.28	30.75	57.69	48.80	31.08	29.22	21.38
1.5	47.69	37.85	69.98	58.73	46.62	44.41	31.97
2	51.88	41.29	76.86	64.75	59.71	56.14	40.72
3	56.35	46.44	77.61	68.88	66.82	65.71	50.38
4	60.67	51.30	81.26	71.56	72.13	72.69	58.76
6	64.91	57.19	82.59	74.06	75.22	78.03	68.25
8	70.59	65.21	83.61	76.77	77.44	81.40	78.82
24	75.83	78.76	84.48	79.31	79.17	82.38	88.85
48	83.59	86.87	85.33	82.16	80.53	82.70	98.85

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.5 Percentages of dyphylline released from liposomes

Time	% Dyphylline Released						
(hr)	uncoated	0.5%*	0.75%*	1.0%*	1.25%*	1.5%*	2%*
0	0	0	0	0	0	0	0
0.5	28.48	16.80	14.05	12.16	12.47	20.59	12.47
1	38.46	26.14	30.37	27.95	29.13	34.67	29.13
1.5	46.88	38.51	42.34	39.32	41.34	60.56	41.34
2	53.89	50.06	55.62	60.8	64.57	67.82	64.51
3	62.61	60.57	63.89	69.56	70.06	74.42	70.06
4	68.23	67.08	71.28	76.37	76.79	80.13	76.79
6	74.21	73.21	77.28	81.35	82.06	85.69	82.06
8	80.03	79.99	77.23	86.35	87.09	90.62	87.09
24	88.11	86.82	82.81	90.98	91.60	95.21	91.60
48	94.54	93.24	93.76	95.39	95.81	99.83	95.81

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.6 Percentages of hydrocortisone released from liposomes

Time	% Hydrocortisone Released						
(hr)	uncoated	0.5%*	0.75%*	1.0%*	1.25%*	1.5%*	2%*
0	0	0	0	0	0	0	0
0.5	25.14	21.75	23.43	19.65	16.89	25.94	31.56
1	39.66	41.13	39.39	33.32	35.26	48.83	43.87
1.5	65.87	61.12	56.06	52.89	52.86	61.48	57.97
2	74.71	70.35	66.61	63.99	65.21	68.72	65.25
3	83.62	78.59	75.15	73.66	77.06	78.01	74.20
4	91.34	86.35	73.53	82.69	85.24	85.24	81.76
6	94.96	92.99	91.30	91.00	93.38	90.27	87.03
8	97.57	96.89	95.85	96.18	96.77	95.94	96.36
24	99.61	99.03	98.52	98.78	99.07	97.69	97.83
48	100	100	100	100	100	100	100

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.7 Percentages of insulin remaining in liposomes stored at room temperature (22°C)and 4°C over 6 months

Time (month)	Percentages of insulin remaining in liposomes							
	Room temperature (22°C)				4°C			
	uncoated	0.5%*	1%*	1.5%*	uncoated	0.5%*	1%*	1.5%*
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.12	90.87	85.11	83.39	87.75	90.35	81.71	100.72	87.75
0.25	71.20	78.56	82.98	91.12	85.36	37.10	82.93	81.00
0.5	72.17	69.71	72.63	115.09	86.59	39.28	78.92	99.02
1	58.92	58.92	118.93	116.00	78.56	45.83	88.63	70.00
2	58.83	56.96	96.81	96.46	69.98	56.08	48.35	80.63
3	28.49	64.70	92.23	107.49	53.65	62.28	53.98	79.82
6	16.53	31.12	39.83	66.93	32.67	30.65	22.90	58.83

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.8 Percentages of dyphylline remaining in liposomes stored at room temperature (22°C)and 4°C over 6 months

Time (month)	Percentages of dyphylline remaining in liposomes							
	Room temperature (22°C)				4°C			
	uncoated	0.5%*	1%*	1.5%*	uncoated	0.5%*	1%*	1.5%*
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.12	97.57	52.49	27.62	46.06	102.78	57.28	34.84	72.26
0.25	69.76	47.89	27.98	24.94	82.87	53.64	27.44	50.89
0.5	53.19	39.89	27.80	27.99	69.52	62.84	39.35	54.45
1	56.66	20.50	27.98	57.00	75.43	53.45	46.03	100.25
2	58.29	28.35	28.88	61.58	55.16	61.11	41.70	90.59
3	79.14	25.48	28.88	51.15	57.24	32.95	32.49	82.70
6	49.25	23.95	30.87	63.87	29.43	34.10	29.06	55.73

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.9 Percentages of hydrocortisone remaining in liposomes stored at room temperature (22°C)and 4°C over 6 months

Time (month)	Percentages of hydrocortisone remaining in liposomes							
	Room temperature (22°C)				4°C			
	uncoated	0.5%*	1%*	1.5%*	uncoated	0.5%*	1%*	1.5%*
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.12	90.37	50.64	42.22	32.99	92.96	65.86	39.74	77.76
0.25	66.14	26.57	21.89	16.22	92.96	26.57	28.56	28.73
0.5	55.64	38.18	29.85	31.51	94.75	47.46	41.94	45.78
1	28.22	29.58	25.64	30.03	101.86	45.74	39.56	32.25
2	44.09	20.30	33.15	40.32	94.69	56.32	52.20	50.51
3	48.47	14.10	21.34	20.76	70.92	28.03	29.58	23.45
6	20.72	2.58	18.68	26.60	47.21	23.65	31.87	33.27

* % indicates percentage of polycarbophil solution incubated with liposomes.

Table A.10 Percentages of insulin, dyphylline, and hydrocortisone remaining in phosphate buffer saline (pH 7.0) stored at room temperature (22°C)and 4°C over 6 months

Time (month)	Percentages of drug remaining					
	Room temperature (22°C)			4°C		
	INS	DY	HY	INS	DY	HY
0	100.00	100.00	100.00	100.00	100.00	100.00
0.12	108.38	104.87	101.23	102.10	102.22	101.55
0.25	89.53	110.86	100.28	108.76	102.91	101.83
0.5	68.69	107.52	99.96	94.92	104.08	102.62
1	66.83	109.50	96.11	104.01	103.32	103.61
2	62.60	100.99	93.14	89.12	98.23	99.25
3	35.05	101.39	99.09	91.13	104.69	106.11
6	33.41	105.45	95.12	99.05	102.99	107.30