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STABILIZATION OF EPTIFIBATIDE BY COSOLVENTS

by

Luwei Zhao

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GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Luwei Zhao

entitled Stabilization of Eptifibatide by Cosolvents

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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Michael Mayersohn, Ph.D.	8/10/2000 **Date**
Sherry Chow, Ph.D.	8-10-00 **Date**
	Date
	Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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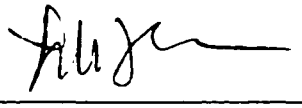
SIGNED: 

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To My Mother Pan Jia Hui

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Page

LIST OF TABLES

Page

ABSTRACT

Eptifibatide is a potent and highly specific inhibitor of platelet receptor glycoprotein IIb/IIIa and is indicated in the treatment of acute coronary syndrome. The commercial product Integrilin® (eptifibatide) Injection requires a cold/refrigerator storage condition. In an effort to improve the drug stability for room temperature storage and transportation, this study proposed a semi-aqueous formulation that contains 2 mg/ml dose, 10% ethanol, 40% propylene glycol and 50% 0.025 M citrate buffer. A carefully designed stability study was conducted in the pH range 4.25-6.25 under accelerated temperatures: 48°C, 60°C, 72.5°C. The results indicate that the proposed semi-aqueous vehicles greatly increased eptifibatide stability in comparison with aqueous vehicles. The pH-rate profiles of eptifibatide are V-shaped with the curves for semi-aqueous vehicles lower all over the test pH range. The pH of drug maximum stability is 5.25 in the aqueous vehicle, and it is shifted to 5.75 in the semi-aqueous vehicle. Studies indicate that eptifibatide degradation may involve a few different mechanisms: the specific acid catalyzed hydrolysis which is dominant in the acidic region, and a pH-dependent oxidation which is likely to be dominant in the basic region of the test pH range. The predicted drug shelf-life T_{90} at 25°C shows that an almost 2-fold increase can be achieved by formulating eptifibatide in the semi-aqueous vehicle, which is 60 months at its maximum stability pH 5.75 as opposed to the 33 months in the aqueous vehicle at its maximum stability pH 5.25.

CHAPTER 1 INTRODUCTION

1.1. Background for Eptifibatide

1.1.1. Thrombosis

Platelet-mediated thrombosis is a primary underlying mechanism that leads to life-threatening cardiac complications: myocardial infarction, angina, and stroke. At the site of atherosclerotic plaque rupture, platelet aggregation often initiates thrombus formation and intravascular coagulation which are mediated by the specific platelet adhesion receptor complex, i.e., glycoprotein (GP) IIb/IIIa (Scarborough, 1998). This receptor complex, which in its active form, serves to cross-link platelets through the binding of adhesive proteins such as fibrinogen and von Willebrand factor, plays a central role in thrombosis (Scarborough, 1998).

1.1.2. Eptifibatide and Integrilin®

A peptidic sequence that contains three amino acid residues arginine-glycine-aspartic acid, represented as RGD, is found to be effective in inhibiting the adhesive function of platelet GP IIb/IIIa (Bittl, 1996). Over the years research has been focused on more potent, short-acting and specific inhibitors of GP IIb/IIIa, which are either peptides or non-peptidic mimetics of the RGD sequence.

A cyclic peptidic compound, drug eptifibatide contains a modified RGD sequence. Its molecular design was based on an active pharmacophore of the disintegrin inhibitor, barbourin, found in the venom of the southeastern pigmy rattlesnake, *sistrurus milarus barbouri* (Scarborough et. al., 1991, 1993). Clinical studies indicate that eptifibatide reversibly inhibits platelet aggregation and thrombus formation by preventing the binding of fibrinogen, von Willebrand factor, and other adhesive ligands to platelet receptor GP IIb/IIIa (Scarborough, 1998). It is of note that platelet aggregation inhibition is reversible following cessation of the eptifibatide infusion, a likely result from dissociation of eptifibatide from the platelet (Description for Integrilin® Injection, 1998).

Eptifibatide was discovered and developed by Cor Therapeutics Inc. (South San Francisco, CA). Together with Key Pharmaceuticals Inc. (Kenilworth, NJ), the company launched its commercial product Integrilin® (eptifibatide) Injection in 1998 (Description for Integrilin® Injection, 1998). Food and Drug Administration approved the product for the treatment of acute coronary syndromes (unstable angina or non-Q-wave myocardial infarction) and percutaneous coronary intervention (angioplasty or atherectomy). The Integrilin® Injection is a clear, colorless, sterile and non-pyrogenic solution for intravenous (IV) use and is co-administered as 2-96 hours infusion concomitantly with aspirin and intravenous heparin (Description for Integrilin® Injection, 1998). Each 10-ml vial contains 2 mg/ml of eptifibatide in 0.025 M citrate buffer with a pH value of 5.25 (Description for Integrilin® Injection, 1998).

1.1.3. Other Drugs

Other marketed platelet GP IIb/IIIa antogonists include Abciximab (ReoPro -- Lilly) and Tirofiban (Aggrastar -- Merck) (Medical Letter, 1998). Abciximab is a monoclonal antibody and is widely used for coronary angioplasty. The drug was approved in 1995 for treating unstable angina when angioplasty or atherectomy is planned within 24 hours. Tirofiban was approved in 1998 for treating acute coronary syndromes, but not for angioplasty without an acute coronary syndrome. Like Integrilin®, both drugs are given intravenously (IV), generally with aspirin and low dose of heparin.

1.1.4. Chemistry

Chemically, eptifibatide contains six amino acid residues and one mercaptopropionyl (des-amino cysteinyl) residue (Figure 1-1, Scarborough, 1998). A disulfide bridge is formed between the cysteine amide and the mercaptopropionyl moiety. The chemical name of eptifibatide is N^6-Aminoiminomethyl)-N^2-(3-mercapto-1-oxopropyl)-L-lysyl-glycyl-L-α-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide cyclic (1→6)-disulfide. Similar to other small peptides, eptifibatide can be prepared by conventional solid-phase synthesis or by fragment synthesis in solution, and purified by preparative reverse-phase liquid chromatography (Scarborough, 1998).

1.1.5. Pharmacokinetics

The pharmacokinetics of eptifibatide is linear and dose-proportional for bolus doses ranging from 90-250 μg/ml and infusion rates from 0.5 to 3 μg/kg/min (Description for Integrilin® Injection, 1998). Plasma terminal half-life is approximately 2.5 hours. The recommended regimen of a bolus dose and an infusion produced an early peak level, followed by a small decline with attainment of steady state within 4-6 hours. The extent of eptifibatide binding to human plasma protein is about 25% (Description for Integrilin® Injection, 1998).

Clearance in patients with coronary artery disease is 55-58 ml/kg/h. In healthy subjects, renal clearance accounts for approximately 50% of total body clearance, with the majority of the drug excreted in the urine as eptifibatide, deamidated eptifibatide, and other more polar metabolites. But there were no major metabolites that were detected in human plasma (Description for Integrilin® Injection, 1998). Clinical studies have included 2418 patients with serum creatinine between 1 and 2 mg/dl (for the 180 μg/kg bolus and the 2 μg/kg/min infusion) and 7 patients with serum creatinine between 2 and 4 mg/dl (for the 135 μg/kg bolus and the 0.5 μg/kg/min infusion), without dose adjustment (Description for Integrilin® Injection, 1998).

1.2. Background for Drug Stability in Aqueous Solution

1.2.1. Chemical Degradation Routes

Many drugs experience various degrees and various routes of chemical degradation in almost all dosage forms: solutions, semi-solids, and solids. The direct consequences of the drug degradation are reduced or diminished pharmacological potency, and in many cases, the undesired effects of the degradants. In this section, the emphasis of this section is placed on drug chemical degradations that occur in aqueous solution.

There are many route of chemical degradation: hydrolysis, oxidation, isomerisation, poymerization, photolysis, dehydration, decarboxylation, deamination, etc. Among them, hydrolysis is probably the most important and the most commonly encountered. The reaction is seen for drugs containing functional groups that are susceptible to hydrolytic cleavage. These include esters, amides, lactams, etc. (Table 1). The reaction is frequently catalyzed by hydrogen ions (specific acid catalysis) or hydroxyl ions (specific base catalysis), as well as buffer components. The buffer catalyzed reaction is also refereed to as general acid-base catalysis.

1.2.2. Important Factors in Drug Degradation

There are many factors that affect both the rate and the order of drug chemical degradation in solution. This includes solution pH, temperature, medium, and ionic strength. This section only describes the important aspects of the pH and temperature in related drug stability issues in solution.

1.2.2.1. pH

A simplified expression is shown in Eq. 1-1 that accounts for the drug degradation, mostly hydrolysis, catalyzed by specific acid, specific base, and water.

$$-d[D]/dt = k_1[D][H^+] + k_2[D][OH^-] + k_3[D] \qquad (1\text{-}1)$$

The [D] is the concentration of the drug undergoing hydrolysis and k_1, k_2 and k_3 are the rate constants for acid catalysis, base catalysis, and water catalysis (solvent catalysis), respectively. The hydrolysis of drugs procaine and tetracaine are illustrative in showing the pH dependence of log k, or the V-shaped pH-rate profile, for a drug undergoing both specific acid and specific base catalyzed hydrolysis (Anschel et. al., 1972). In solutions of low pH, the k_1 term is greater than either k_2 and k_3 and dictates the hydrolysis rate. Thus, as pH is increased in acidic solutions, the hydrolysis rate gradually decreases. In solutions of high pH, the k_2 term influences the hydrolysis rate and thus the rate increases with increasing [OH^-]. The rate where minimum hydrolysis occurs depends on the relative magnitude of k_1 and k_2.

The pH-rate profiles are routinely determined in preformulation studies on drugs that are susceptible to hydrolysis. Although it helps identify the pH of maximum stability, it may not always be possible to formulate the drug at the desired pH due to solubility problems

or clinical acceptability. In such cases a compromise must be sought between the opposing effects.

Buffers are often used in pharmaceutical solutions to maintain a targeted pH. Often, in addition to the effect of pH on the reaction rate, there may be catalysis by one or more species of the buffer components. The degradation reaction is then said to be subject to general acid or general base catalysis, depending, respectively, on whether the catalytic components are acidic or basic. Therefore Eq. 1-1 can be rewritten as Eq. 1-2:

$$-d[D]/dt = k_1[D][H^+] + k_2[D][OH^-] + k_3[D] + k_4[D][Acid] + k_5[D][Base] \quad (1\text{-}2)$$

1.2.2.2. Temperature

Increasing temperature often causes an appreciable increase in the drug degradation rate. Empirically, for many reactions the increase in k is of the order of two to three times for a 10^0C rise in temperature. A quantitative relation between temperature and the rate of decomposition is established as the Arrhenius equation shown in Eq. 1-3,

$$k = Ae^{-Ea/RT} \quad (1\text{-}3)$$

where k is the specific rate of degradation of the pertinent component, A is the pre-exponential factor which is a constant associated with the entropy of the reaction and/or collision factors, the activation energy E_a is the energy which must be exceeded if the collision of two reactant molecules is to lead to reaction, and R is the gas constant (1.987 cal/mole/deg.). The natural logarithmic form of Eq. 1-3 can be written as Eq. 1-4.

$$\text{Ln } k = \text{Ln } A - E_a/RT \qquad (1\text{-}4)$$

Eq. 1-4 indicates that a plot of Ln k as a function of reciprocal temperature should be linear with a slope of $-E_a/R$ from which E_a may be calculated. If such a relation among several k values determined at elevated temperatures and the line is reasonably linear, the prediction of stability at shelf temperatures such as 25°C should be practical. The time taken in conducting stability tests is considerably reduced compared to that for a simple experiment in which the product is maintained at the required storage temperature and sampled over a period corresponding to the normal storage time.

1.2.3. Approaches to Drug Stabilization

For solution dosage form, there are a number of approaches that can be used in improving drug stability, especially when the drug is susceptible to acid-base catalyzed hydrolysis:

a) control of pH. The product is formulated at a pH where the drug maintains its maximum stability. The pH-rate profile generated from kinetic experiments over a range of pH values provides the basis for this approach;

b) use of cosolvents to alter solvent composition. The often used cosolvents include ethanol, propylene glycol, polyethylene glycol 400, glycerin, etc.;

c) formation of drug-ligand complex with cyclodextrins. For example, hydroxypropyl-β-cyclodextrin (HPβCD) was reported in increasing drug stability, e.g., penicillin G, thymopentin, and erythropoieten (Hedges, 1998). Other types of complexation formations include caffeine with benzocaine, or procaine, or tetracaine in aqueous solution, which decreases the base-catalyzed hydrolysis of these local anesthetics (Vadas, 1990);

d) formation of micelles by surfactants. Like the formation of drug-ligand complex, the drug incorporated in the micelles may experience decreased rate of degradation. For example, the half-life of benzocaine was increased 18 times by the addition of surfactant sodium lauryl sulfate (Vadas, 1990);

e) preparation of dried powder so that the drug can be completely away from water. Methods include freeze-drying (lyophilization), and spray-drying. The former is to freeze the solution at low temperature, e.g., minus 50^{0}C, and then vacuum-dry it in the frozen state; the latter is to make the solution to be in contact with hot, sterile gas, and to evaporate the solvent, allowing the drug to be carried as a powder;

f) decrease in drug solubility. The rationale is that it is only the fraction of the drug in solution that hydrolyzes;

g) modification of chemical structures. This includes pro-drug approach to retard drug hydrolysis;

h) low temperature for storage and transportation of the product.

1.3. Aims of the study

1.3.1. Background

Though Integrilin® Injection is already on the market, the product requires a cold/refrigerator condition (2-8^{0}C or 36-46^{0}F) for its storage and transportation (Scarborough, 1998). This study intends to propose a formulation that does not substantially alter the existing formulation, but with a greatly improved drug stability profile, so that the product can be conveniently handled and stored at room temperature with much convenience and sound economy. The controlled room temperature in the United States Pharmacopoeia is defined as 25^{0}C with excursions permitted between 15-30^{0}C.

Small peptides such as eptifibatide are susceptible to various degradation routes, and amide hydrolysis is often likely in aqueous solutions. This is supported by eptifibatide degradation studies by Cor Therapeutics Inc. (Van Gorp and Sluzky, 1999). By using LC/MS it was found that the drug undergoes a variety of degradation routes: hydrolysis, isomerizatin, oxidation, dimerization (Figure 1-2, Van Gorp and Sluzky, 1999). It was also found that the amide hydrolysis seems to be the most important degradation pathway, and the Asp-clipped eptifibatide (Figure 1-2) is the major degradant in the acidic

condition, a direct result of hydrolysis of amide bond between the aspartic acid and tryptophan residues. This seems to be true with regard to both the amount generated and the degree of sensitivity to the solution pH (Van Gorp and Sluzky, 1999). There are some other clipped peptidic fragments as well as further degradants that are based on the amide hydrolysis such as Trp-Pro-diketopiperazines (Figure 1-2, Van Gorp and Sluzky, 1999), which is likely to be formed when the free amino group of tryptophan in Asp-clipped eptifibatide reacts with the carboxyl group of the adjacent proline residue.

Though rare in conventional organic chemistry, amide hydrolysis is often reported in drug degradation studies. Examples include chloramphenicol (Higuchi and Bias, 1953), procainamide, nicotinamide (Connors, 1986), salicylamide, benzamide, N-substituted salicylamide and benzamide derivatives (Koshy, 1959), N-haloacetylphthalimides, and 1-acyl-3,5-dimethylpyrazoles (Stella and Higuchi, 1973).

1.3.2. Rationale

For eptifibatide, the pH-control is already in place, as the Integrilin® Injection has a citrate buffer solution at pH 5.25, presumably the maximum stability pH for the drug. Both cyclodextrins and surfactants are not particularly appealing, as there is no apparent molecular compatibility between eptifibatide and the excipients. The high solubility of the drug also prevents the use of these excipients (65 mg/ml in water). Freeze-drying might work but it is an expensive approach.

In considering all these options, and in particular the drug degradation information provided by the Cor Therapeutics Inc., the semi-aqueous medium seems to provide a sound choice in improving the drug stability. Thus, the whole rationale is that we intend to use the semi-aqueous medium to reduce the drug degradation by reducing its amide hydrolysis as well as other degradation.

Approximately more than 10% FDA approved injections (Sweetana and Akers, 1996) have used cosolvents to various extents. But they are mostly, if not exclusively, used for drug solubility enhancement. The cosolvent effect on drug hydrolysis was also observed in a number of case studies. For example, Hou and Poole (1969) found that at pH 1.2, the addition of ethanol decreases the ampicilin hydrolysis rate; ampicilin in 50% ethanol solution has a half-life twice than that in purely aqueous solution. Baker and Niazi (1983) studied aspirin decomposition in several media and they observed significant stability enhancement for the drug in water-propylene glycol 400 (4/1) solution. In the presence of increasing concentrations of ethanol in the solvent, aspirin degrades by an extra route, forming the ethyl ester of acetylsalicyclic acid (Garrett, 1961). Gu and Strickley (1990) reported that moexipril, an angiotensin-converting enzyme inhibitor, undergoes hydrolysis as well a cyclization reaction, leading to the formation of diketopiperazines. In mixed solvent system (75-90% ethanol) the hydrolysis reaction is suppressed, but the rate of the cyclization reaction increases by 5.5-fold to 29 fold. Yalkowsky et al. showed that aspartame stability is noticeably improved at pH 2 in the presence of 10% cosolvent PEG 400 in comparison with the aqueous solution (Yalkowsky, et al., 1993).

We believe that the proposed semi-aqueous vehicle for eptifibatide would be the first drug formulation that uses consolvents for the sole purpose of improving drug stability. This can have implications in the development of patenteral formulation of other hydrolysis-susceptible drugs.

1.3.3. Objective

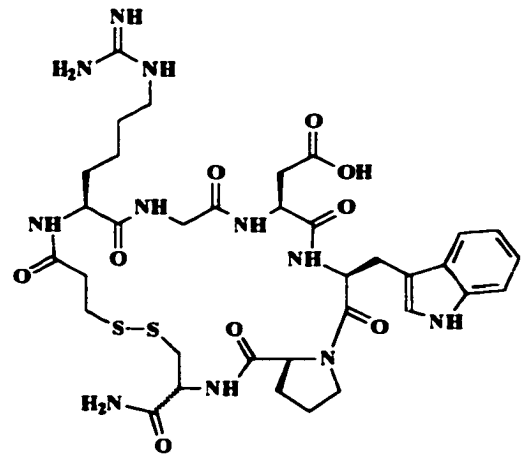
The study proposes a semi-aqueous vehicle that contains 2 mg/ml eptifibatide, 10% ethanol, 40% propylene glycol and 50% 0.025 M citrate buffer. The overall objective is to investigate the feasibility of using cosolvents to improve eptifibatide stability at room temperature. The reason for choosing such a mixed cosolvent system (10% ethanol and 40% propylene glycol) was primarily based on the fact that it is the most commonly used cosolvent system in FDA-approved parenteral formulations. Examples include injections of digoxin, phenytoin, pentobarbital, and diazepam (Sweetana and Akers, 1996).

The eptifibatide stability profile in the semi-aqueous vehicle will be constructed and compared with the stability profile in aqueous vehicle (2 mg/ml eptifibatide, 0.025 M citrate buffer) at pH 4.25-6.25 with 0.5 pH unit increments. It is of note that the prepared aqueous formulation at pH 5.25 is the same as the marketed Integrilin® Injection. The tested pH range is in the vicinity of the marketed formulation pH 5.25, which may also account for the possibility that the addition of cosolvents may cause slight variation in the pH of maximum stability. Accelerated stability testing will be conducted for all the

samples at three temperatures: 48^{0}C, 60^{0}C, and 72.5^{0}C. Drug degradation information at room temperature will be obtained by extrapolation from the Arrhenius plot.

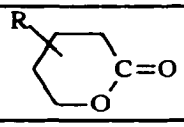
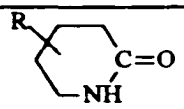
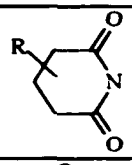
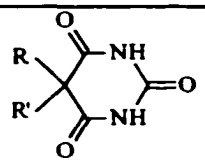
On the to-do list before the proposed eptifibatide stability studies are drug solubility, sealing appropriateness of sample vials, and HPLC assay development, all of which will be discussed in Chapter 2.

Figure 1-1 **Chemical Structure of Eptifibatide**



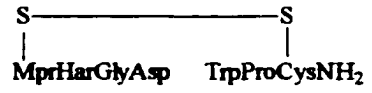
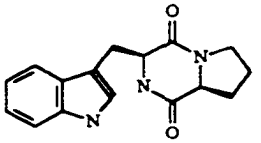
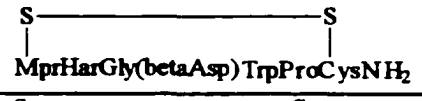
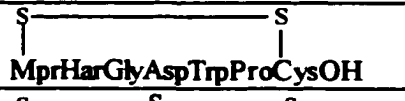
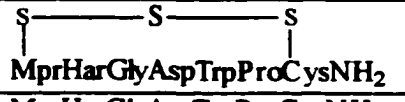
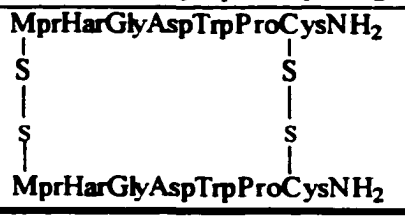
Formula:	$C_{35}H_{49}N_{11}O_9S_2$
Molecular weight:	831.96

Table 1-1 Functional Groups Subject to Hydrolysis

Drug type	Structure	Drug Examples
Esters	**RCOOR'**	**aspirin, alkaloids**
Lactones		**pilocarpine** **spironolactone**
Amides	**RCONR'R"**	**thiacinamide, chloramphenicol**
Lactams		**penicillins** **cephalosporins**
Oximes	**$R_2C = NOR'$**	**steroid oximes**
Imides		**glutethimides** **ethosuximide**
Malonic ureas		**barbiturates**

Source: Fung, H.L. Chemical Kinetics and Drug Stability. Modern Pharmaceutics. Ed. Banker, G.S.; Rhodes, C.T. Marcel Dekker, Inc. (1979)

Table 1-2 **Reported Decomposition Products of Eptifibatide**

Degradants	Structure	Degradation routes
Asp-eptifibatide		hydrolysis
Trp-Pro-diketopiperazine		hydrolysis
D-Har eptifibatide		isomerization
β-Asp eptifibatide		isomerization
D-Cys eptifibatide		isomerization
Deamidated eptifibatide		deamidation
Trisulfide eptifibatide		oxidation
Eptifibatide dimer		dimerization

Source: Van Gorp and Sluzky (1999)

CHAPTER 2 PRELIMINARY STUDIES

2.1. Introduction

This section describes a number of preliminary experiments including drug solubility, vial sealing test, assay development and validation.

2.2. Materials

Eptifibatide (lot N20494) was provided by Cor Therapeutics Inc. (South San Francisco, CA). It is a white, amorphous powder. Ethanol and propylene glycol were purchased from Sigma (St. Louis, MO). Citric acid, sodium citrate, trifluoroacetic acid (TFA), triethylamine (TEA) were purchased from Aldrich (Milwaukee, WI). Spectrophotometric grade acetonitrile (ACN) was purchased from Baxter (Muskegon, MI). The 0.1 N hydrochloric acid (HCl) and 0.1 N sodium hydroxide (NaOH) solutions were purchased from Fisher Scientific (Fairlawn, NJ). All other chemicals and reagents were analytical or HPLC grade.

Both sample vials and aluminum caps were purchased from National Scientific Company. The amber vial has a volume capacity of 4 ml (15×45 mm) with the Part Number C-4015-2W. The aluminum seal has the Part Number 73825-11.

2.3. Solubility

The solubility of eptifibatide in different solvents was determined by visual observation (see Table 2-1). It can be seen that solution pH greatly affects drug solubility. The aqueous solubility is approximately 65 mg/ml, while the solubility in 0.01 N HCl is more than 300 mg/ml. Eptifibatide has a high solubility in propylene glycol (>300 mg/ml). The solubility in the semi-aqueous vehicle (10% ethanol + 40% PG +50% 0.025 M citrate buffer) at pH 5.75 is also very high: > 200 mg/ml.

2.4. Sample Vial Sealing Test

It is important that the samples are contained in well-sealed vials so that the proposed accelerated stability testing can proceed properly. This study was designed to test the appropriateness of the vial sealing. The amber vials with a volume capacity of 4 ml were used and were crimped with aluminum caps (National Scientific Company). Mettler AE163 balance (Analytical & Precision Balance Company, Phoenix, AZ) was used for all sample weighing.

The procedure for the testing was as follows: a) add 1 ml solution in the vial, and crimp the vial with aluminum cap; b) place the crimped vial in the glass container immersed in the water-bath maintained at the specified temperature; c) assess the weight difference of the samples before and after the water-bath heating.

The results of weight fluctuation of various samples are listed in Table 2-2. If the solution density of 1 g/ml were assumed, the overall weight loss for all samples is in the range of 0.00029 to 0.00102 (g), or 0.029-0.102 % (w/w). Given the fact that the reproducibility of the balance is ±0.0001, such a small weight difference is considered to be negligible in future stability testing studies. In addition, since these results were obtained under very harsh conditions (73°C), it is likely that lower temperatures may reduce the weight loss difference to the balance error range.

In summary, the study indicated that the vial sealing by aluminum crimp was appropriate for future eptifibatide stability studies.

2.5. HPLC Assay Development and Validation

2.5.1. Ultraviolet Spectrum

The UV spectrum of eptifibatide was obtained over the wavelength range of 190-600 nm as seen in Figure 2-1. Eptifibatide has a maximum absorbance in the range of 210-220 nm. This is typical for peptidic compounds, as a result of $n \rightarrow \pi^*$ excitation on the amide bond. A broad but weak absorbance occurred in the range of 260-285 nm due to the $\pi \rightarrow \pi^*$ excitation of the conjugated system in the tryptophan residue.

2.5.2. HPLC Instrumentation and Chromatographic Conditions

A Beckman Gold HPLC system was used for all assays. The system was equipped with a model #167 detector. A wavelength of 220 nm was chosen for eptifibatide detection. The separation of eptifibatide from its degradation products was achieved by using a Pinnacle octyl amine (C8) column (5 μm, Dimension: 150×4.6 mm, Cat: 9183565, Serial #98040183P, Restek Corporation).

The HPLC mobile phase was composed of acetonitrile (ACN), and an aqueous solution that contained 0.1% trifluoroacetic acid (TFA) and 0.1% triethylamine (TEA). The combination of ACN and 0.1% TFA is a commonly used system that is considered to be applicable to a variety of organic compounds including small peptides. The TEA was added as it helped to reduce the tailing of eptifibatide peak.

The elution program started with constant 17% ACN for 15 minutes (isocratic), switched to gradient elution with ACN concentration up to 100% in 5 minutes, and then returned to equilibrium in 2 minutes at the original 17% ACN. The injection volume was 100 μl, and the flow rate was 1.0 ml/min.

2.5.3. Assay Discussion

A typical chromatogram of a standard solution that contains eptifibatide is shown in Figure 2-2. A three dimensional chromatogram of eptifibatide is provided in Figure 2-3. The retention time of eptifibatide is approximately 10.1±0.1 minutes at ambient

temperature. The diode array detector connected to the HPLC provided the verification of the peak homogeneity.

The HPLC system developed in this study can detect the major degradant, Asp-clipped eptifibatide (see Table 1-2 for structure), which is eluted at 8.3±0.1 minutes. This degradant can be rapidly generated in acidic conditions such as treatment of eptifibatide in 0.5 N HCl for 12-24 hours. Though structurally similar, the UV scans in the range of 200-600 nm display subtle difference between the parent eptifibatide and degradant Asp-clipped eptifibatide (Figures 2-1, 2-4). Eluants from HPLC were collected for mass spectroscopic measurement. Figures 2-5 and 2-6 show the $M+H^+$ and $M+2H^+$ for both eptifibatide and Asp-clipped eptifibatide, with the latter having an increase of 18 in molecular weight due to the hydrolysis. Figures 2-7 shows a representative chromatogram of eptifibatide degradation in 0.025 M citrate buffer at pH 4.25 at 60^0C after 53 days.

The current assay takes approximately 22 minutes. In comparison with the more sophisticated assay developed by Cor Therapeutics Inc., which took 70 minutes (personal communication with people at Cor Therapeutics Inc.), this assay meets the demand for analyzing a large number of samples required and the short time available for this study. The disadvantage is that the assay was not able to separate and quantitate many other degradants.

2.5.4. Standard Solution

Stock solutions for standard curves of eptifibatide in water were freshly prepared. The 17% ACN in water solution was used for all sample dilution purposes. The solution polarity was consistent with the HPLC starting mobile phase (17% ACN and 87% of aqueous solution containing 0.1% TFA and 0.1% TEA). The standard curves were determined from a series of eptifibatide solutions that covered a concentration range from 0.5 - 55 μg/ml. The standard curve was constructed by plotting eptifibatide peak area versus the drug concentration.

Each standard calibration curve of eptifibatide was found to be linear over the above mentioned concentration range. The correlation coefficient (r^2) values were greater than 0.999.

2.5.5. Assay Validation

For the eptifibatide HPLC assay, both intra-day and inter-day validation were performed. The procedures were as follows:

a) prepare stock solutions and dilute into a series of standard solutions or points with known concentrations;
b) obtain the area under the curve (AUC) by HPLC assay for each point;
c) triplicate each point for intra-day validation;
d) continue the study for three days for inter-day validation.

Table 2-3 shows the results for the intra-day assay validation. Both precision (represented by the coefficient of variation, CV %) and bias (represented by the standard deviation, S.D.) are less than 2%, which is well within the acceptable range (<10%). The results for inter-day assay validation are similar: both precision (CV%) and bias (S.D.) were less than 5%.

These studies indicate that the HPLC assay developed allows the quantitative and reproducible analysis of eptifibatide. The detection limit of eptifibatide is 0.5 mg/ml.

2.6. Eptifibatide Degradation in Various Medium

It is important to ensure that there is no drug degradation during the time from sample preparation to HPLC analysis. This study was designed to assess possible eptifibatide degradation in a few different solutions, which also covered the lowest and the highest solution pH to be used in the following eptifibatide stability studies. The solution pH was adjusted with 0.025 M citrate buffer. The testing time spanned 48 hours, which was long enough to cover the time needed for the sample preparation and HPLC analysis. Duplicate samples were used for all the data reported in Table 2-5.

No detectable degradation was found for both 17% ACN solution and 0.025 M citrate buffer at pH 6.25. At pH 4.25 citrate buffer, there was a very slight decrease (0.8%), though it could be in the measurement error range. Noticeable degradation was observed in the mobile phase after 24 hours. After 48 hours, the drug remaining decreased to

97.7%. It was, therefore, suggested that future sample dilution should use 17% ACN in water, which is close to the polarity of the mobile phase.

2.7. pH Adjustment

The apparent pH reading in the mixed solvent system can be adjusted by an experimentally obtained correction factor. The adjustment is based on a method developed by Van Uitert and Hass (1953) for dioxane-water mixtures and later validated by Bates et al. (1963) for use in alcohol-water system. It involved the measurement of solution pH of mixed solvents containing 1.00×10^{-3} M HCl. The difference between the observed reading and the expected value of 3.00 was used as the correction factor. The primary assumption in the use of the empirical correction factors is that strong electrolytes at dilute concentrations is completely ionized in all the solvents studied. This assumption is considered to be valid when the solvent dielectric constant is above 39 (Van Uitert and Hass, 1953). Corrections for changes in the activity coefficient of hydrogen ions in various solvents were considered negligible in these studies due to the low ionic strength and relatively high polarities of the solvents (Van Uitert and Hass, 1953).

In this study, it was found that the correction factor was in the range of 0.06-0.08 in the presence of 10% ethanol and 40% propylene glycol of mixed solution that contained 1.00×10^{-3} M HCl. The value is close to the pH correction factors given by Rubino and Berryhill (1986) for a variety of pure cosolvents at various percentages (0.04 for 10% ethanol; zero for 40% propylene glycol) (see Table 2-6).

Since the correction factors are fairly small (<0.1 pH unit), the proposed stability studies will continue to use the apparent pH reading. Literature search also found that, though published by Rubino and Berryhill (1986), there have been no reports employing these correction factors in cosolvent-related drug studies. It is likely that these corrections are considered to be small in general and they are often close to the error range of pH measurement.

Figure 2-1 **UV spectrum for eptifibatide obtained from the HPLC diode array detector**

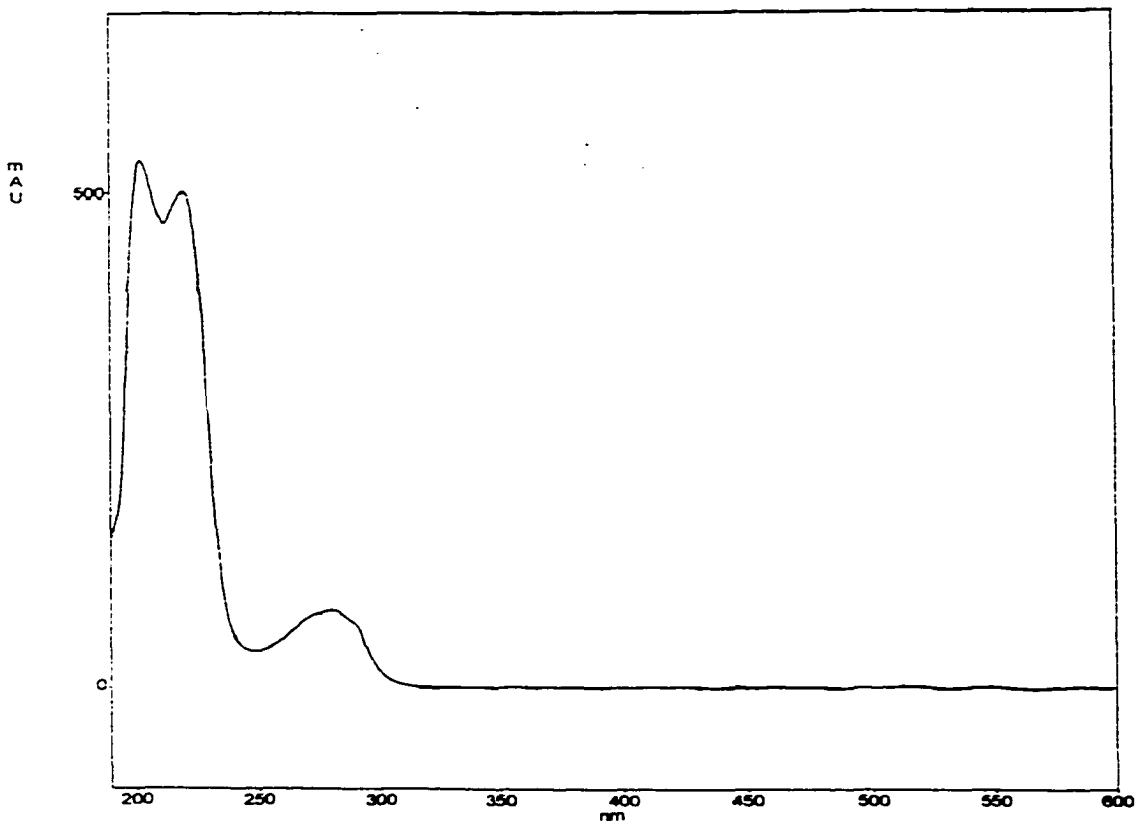


Figure 2-2 Chromatogram of eptifibatide in standard solution

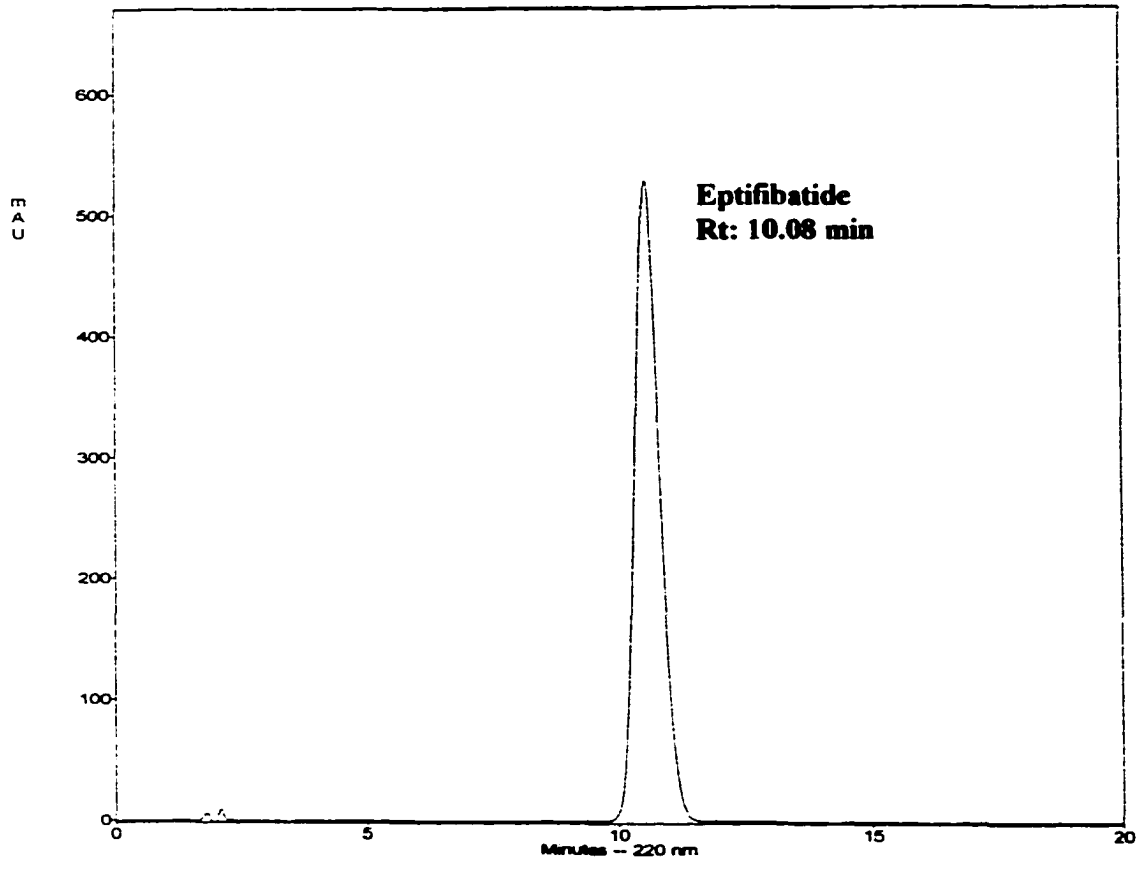


Figure 2-3 A three-dimensional chromatogram of eptifibatide

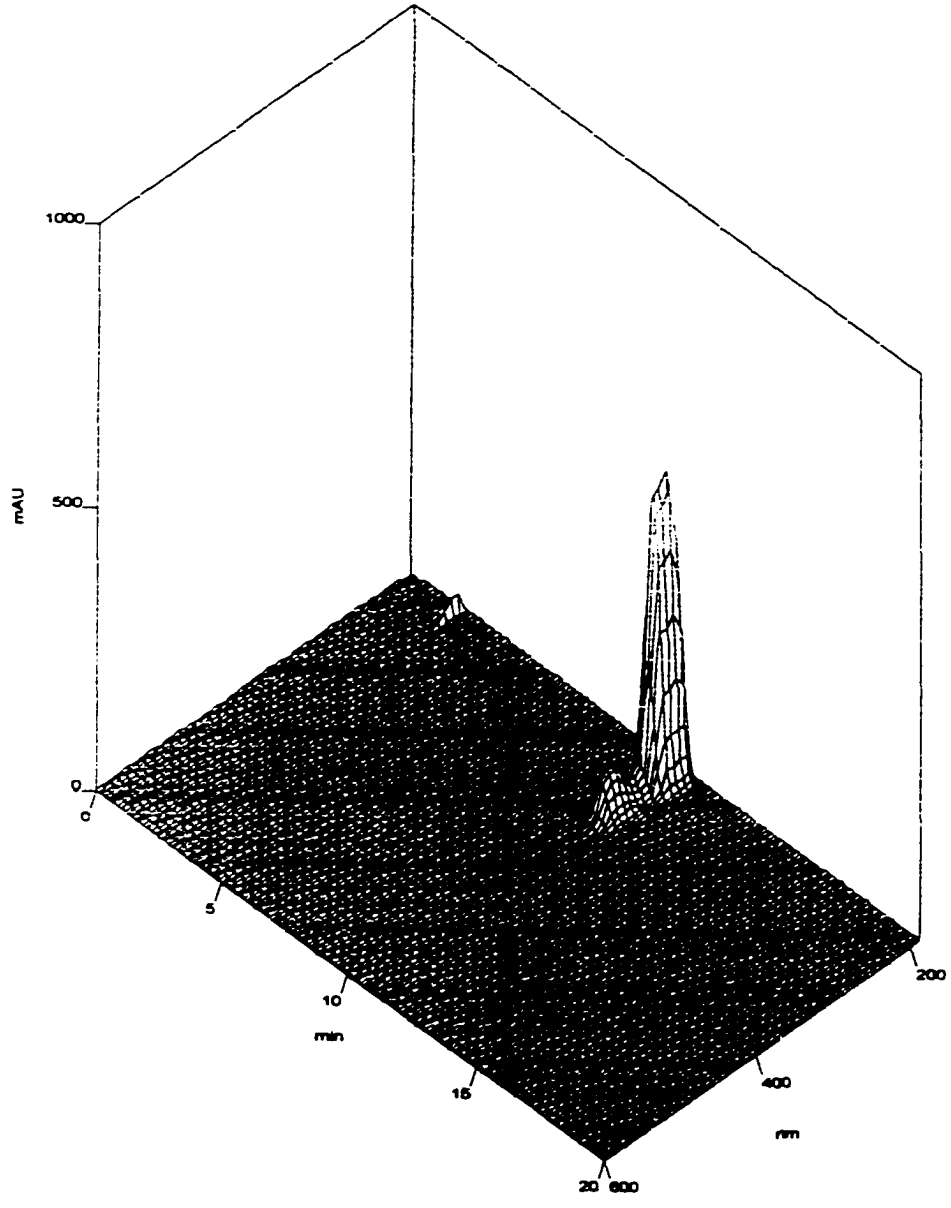


Figure 2-4 UV spectrum for Asp-clipped eptifibatide, the major degradant of eptifibatide, obtained from the HPLC diode array detector

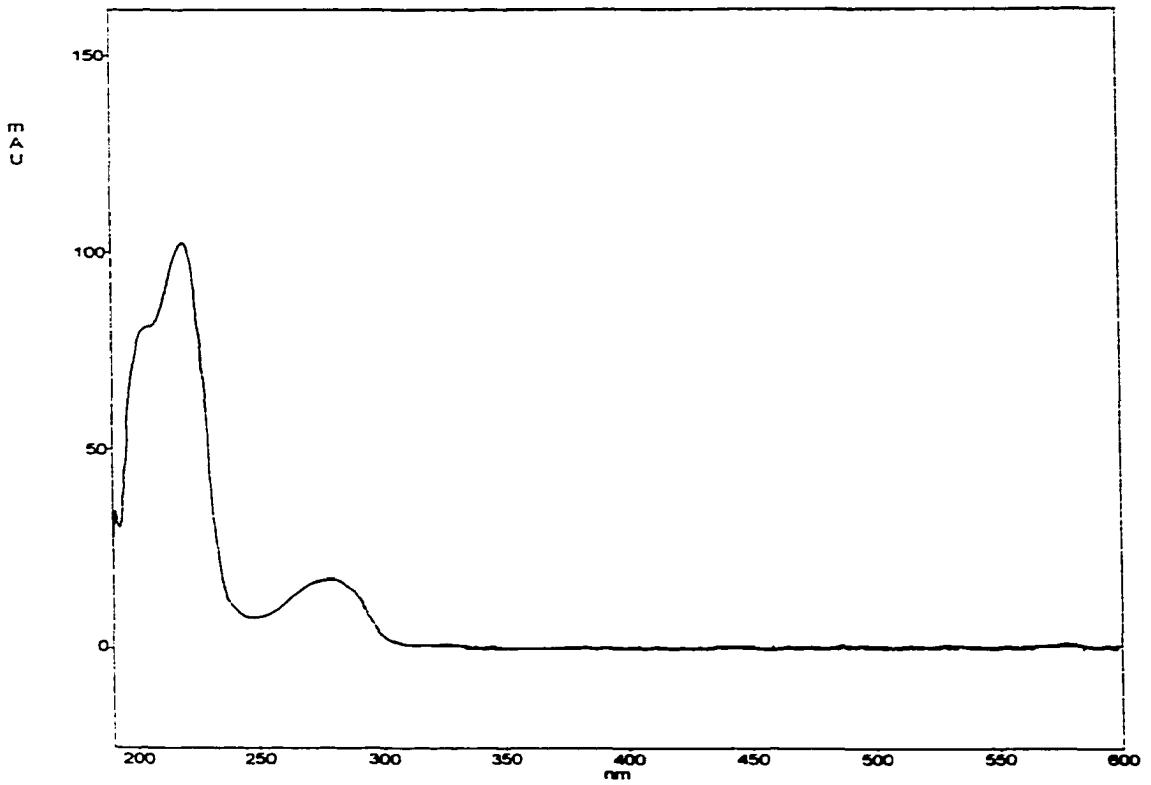
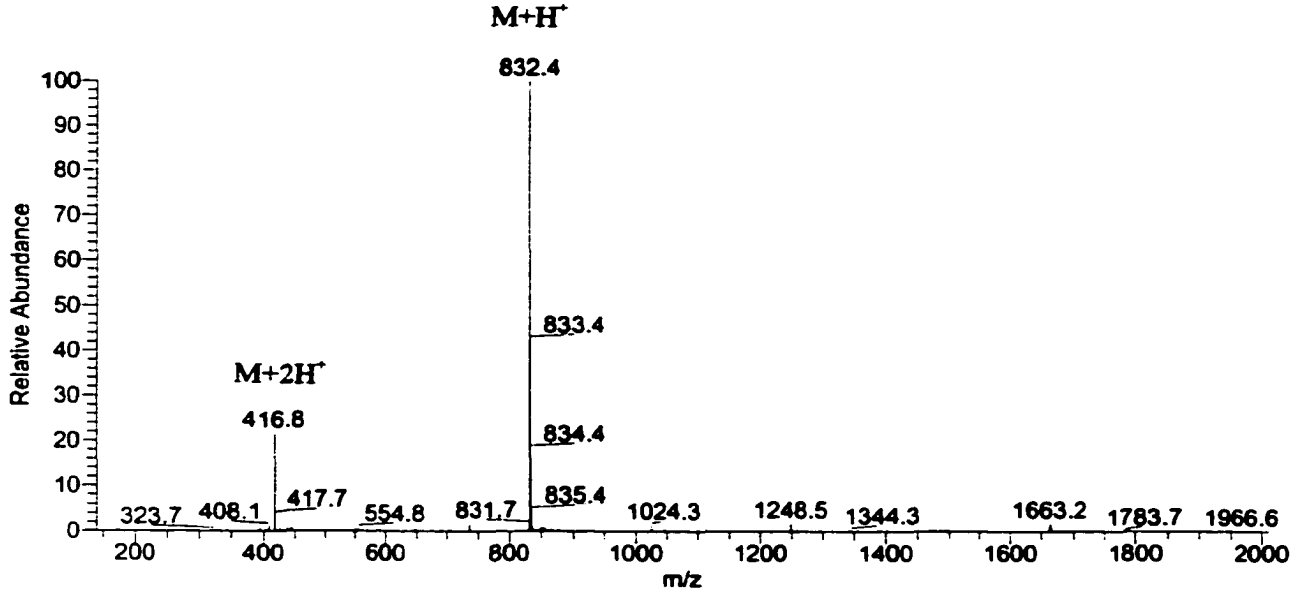
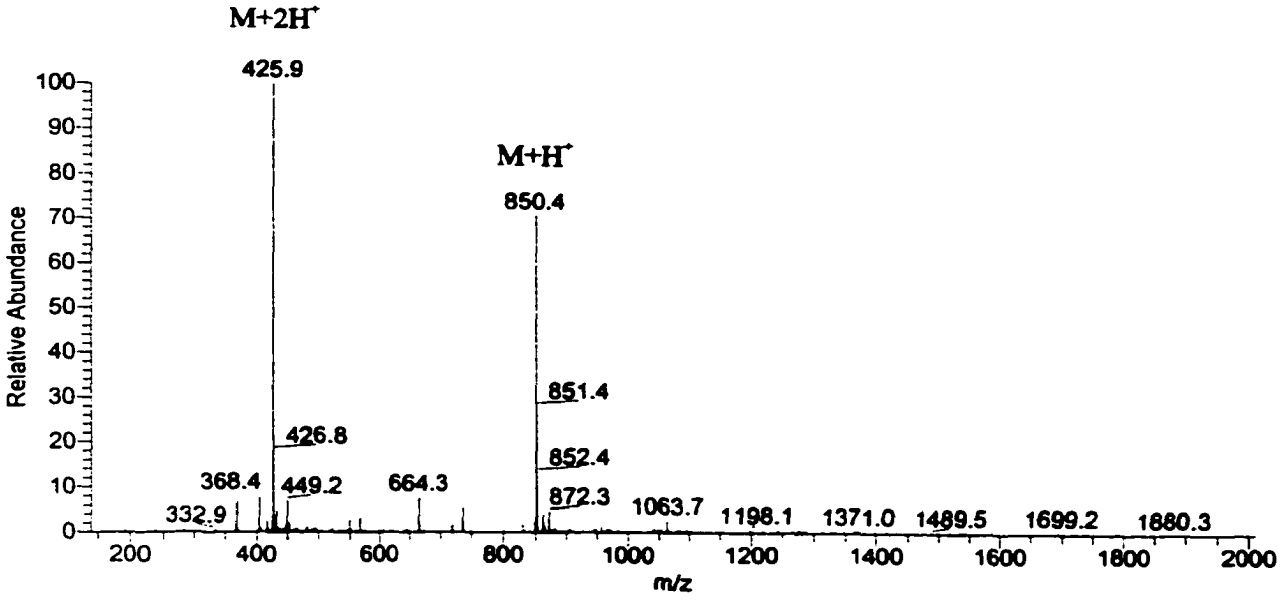


Figure 2-5 **Mass spectrum of eptifiatide**



Note: The spectrum was recorded on LCQ ion-trap mass spectrometer with an electrospray ionization source (Finnigan Corp., San Jose, CA). The ionization potential was 4.5 kV.

Figure 2-6 Mass spectrum of Asp-clipped eptifiatide



Note: The spectrum was recorded on LCQ ion-trap mass spectrometer with an electrospray ionization source (Finnigan Corp., San Jose, CA). The ionization potential was 4.5 kV.

Figure 2-7 Chromatogram of eptifibatide degradation at 60^{0}C after 53 days

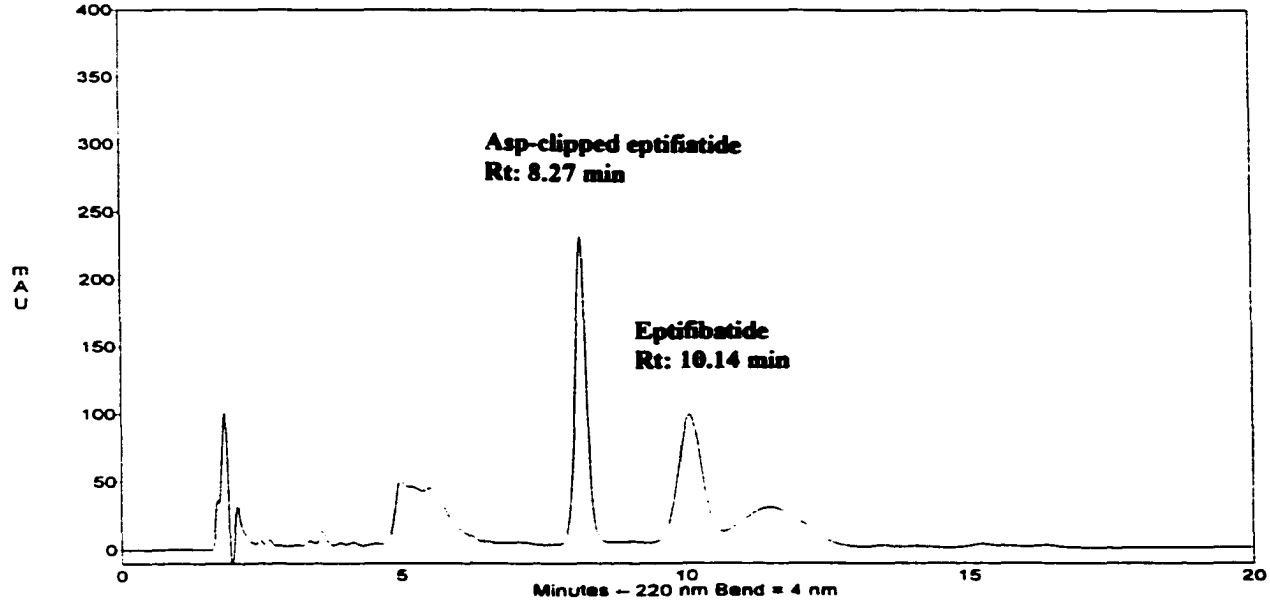


Table 2-1 Eptifibatide Solubility by Visual Observation after 24 Hours

Medium	Solubility (mg/ml)*
Water	65
0.01 N HCl	>300
0.025 M Citrate buffer (pH 5.25)	70
0.5 M Citrate buffer (pH 3)	50
0.5 M Phosphate buffer (pH 7)	15
Methanol	25
Ethanol	15
Propylene glycol (PG)	>300
Polyethylene glycol 400 (PEG400)	60
20% Ethanol + 80% PG	>200
10% Ethanol + 40% PG +50% 0.025 M Citrate buffer (pH 5.75)	>200

*: Each data point is the average of duplicate readings.

Table 2-2 Vial Sealing Test in Various Media at 73°C

Samples	Vials	Weight loss after 2 days	Average weight loss	Vials	Weight loss after 8 days	Average weight loss
0.025 M citrate buffer (pH 5.25)	1	0.00031	0.00029	1	0.00154	0.00054
	2	-0.00062		2	-0.00031	
	3	0.00074		3	0.00081	
	4	0.00073		4	0.00014	
10% ethanol + 40% propylene glycol + 0.025 M citrate buffer (pH 5.25)	1	0.00081	0.00092	1	0.00042	0.00102
	2	0.00102		2	0.00113	
	3	0.00082		3	0.00114	
	4	0.00104		4	0.00140	

Note: weight loss = original weight – current weight

Table 2-3 Intra-Day Reproducibility for Eptifibatide HPLC Assay

Original Conc. (μg/ml)	Times	Precision				Bias (accuracy)		
		Pred. Conc. (μg/ml)	Mean	S.D.	CV%	Dev. %	Mean	S.D.
6.76	1	6.87	6.78	0.08	1.21	1.64	0.24	1.21
6.76	2	6.72				-0.55		
6.76	3	6.74				-0.36		
13.53	1	13.70	13.83	0.24	1.79	1.26	2.27	1.83
13.53	2	13.68				1.17		
13.53	3	14.12				4.39		
24.80	1	24.26	24.51	0.47	1.94	-2.19	-1.16	1.92
24.80	2	25.06				1.05		
24.80	3	24.22				-2.35		
42.52	1	41.97	42.15	0.54	1.30	-1.28	-0.86	1.29
42.52	2	41.72				-1.88		
42.52	3	42.77				0.59		
55.81	1	55.77	56.14	0.51	0.91	-0.07	0.59	0.92
55.81	2	56.73				1.64		
55.81	3	55.92				0.20		

Note: For each concentration point, three samples were taken within one day. The predicted eptifibatide concentration was calculated from the regression equation ($y = 285885 x - 34157$ where x = predicted concentration and y = area under the curve; $r^2 = 0.9994$); Dev. (deviation) % = [(pred.-orig.)/orig.]*100%; S.D.: standard deviation; C.V.%: coefficient of variation % = (S.D./mean)*100%.

Table 2-4 Inter-Day Reproducibility for Eptifibatide HPLC Assay

Original Conc. (μg/ml)	Days	Precision				Bias (accuracy)		
		Pred. Conc.	Mean	S.D.	C.V.%	Dev. %	Mean	S.D.
6.76	1	6.78	6.66	0.29	4.43	0.24	-1.49	4.36
6.76	2	6.33				-6.46		
6.76	3	6.88				1.73		
13.53	1	13.83	13.77	0.16	1.15	2.27	1.76	1.17
13.53	2	13.88				2.60		
13.53	3	13.58				0.42		
24.80	1	24.51	24.65	0.58	2.35	-1.16	-0.62	2.34
24.80	2	25.28				1.93		
24.80	3	24.14				-2.65		
42.52	1	42.15	42.50	0.83	1.95	-0.86	-0.05	1.94
42.52	2	41.89				-1.47		
42.52	3	43.44				2.16		
55.81	1	56.14	55.85	0.41	0.74	0.59	0.07	0.74
55.81	2	56.04				0.41		
55.81	3	55.37				-0.78		

Note: For each concentration point, three samples were taken each day for three consecutive days. The predicted concentration is the average value (n = 3). The regression equations for day 1: y = 285885 x –34157 where x = predicted concentration and y = area under the curve, r^2= 0.9994; day 2: y = 282274 x + 80523, r^2 = 0.9994; day 3: y = 275938 x + 66692, r^2 = 0.9991; Dev. (deviation) % = [(pred.-orig.)/orig.]* 100%; Mean: average predicted concentration (n = 3); S.D.: standard deviation; C.V.%: coefficient of variation % = (S.D./mean)*100%.

Table 2-5 **Stability of Eptifibatide (2 mg/ml) in Various Media at 25°C**

Time (hours)	Eptifibatide Percentage Remaining*			
	17% ACN in water	Mobile phase: 17% ACN + 87% aqueous (0.1% TFA + 0.1%TEA)	pH 4.25 (0.025 M citrate buffer)	pH 6.25 (0.025 M citrate buffer)
0	100.0%	100.0%	100.0%	100.0%
12	99.5%	99.6%	100.2%	99.9%
24	100.2%	98.2%	99.6%	100.3%
48	99.7%	97.7%	99.2%	99.8%

*: mean values of duplicate samples.

Table 2-6 Polarity Indexes and pH Correction Factors of Solvents

Solvent	Dielectric constant: ε	HBA	pH Correction Factors (volume fraction cosolvents)				
			0.1	0.2	0.3	0.4	0.5
Water	78.5	23.4	-	-	-	-	-
Ethyl alcohol	24.3	12.7	0.04	0.03	0.07	0.14	-.23
Propylene glycol	32.0	12.6	0.00	-0.04	-0.04	0.00	0.00
Glycerol	42.5	17.7	-0.05	-0.09	-0.16	-0.21	-0.28
Polyethylene glycol 400	13.6	11.3	0.03	0.13	0.27	0.50	0.81
Dimethylacetamide	37.8	10.8	0.27	0.44	0.61	0.78	0.95
Demethyl sulfoxide	46.7	12.0	0.08	0.12	0.24	0.36	0.50

Source: Physical properties of Some Organic Solvents. Eastman Kodak Co. Rochester, NY (1995); Rubino and Berryhill (1986)

CHAPTER 3 STABILITY KINETICS OF EPTIFIBATIDE

3.1. Introduction

This section describes various aspects of eptifibatide stability studies including the pH-rate profile, degradation, and possible mechanism for cosolvent effect.

3.2. Experimental

3.2.1. Materials

Eptifibatide (lot N20494) was provided by Cor Therapeutics Inc. (South San Francisco, CA). It is a white, amorphous powder. Ethanol and propylene glycol were purchased from Sigma (St. Louis, MO). Citric acid, sodium citrate, trifluoroacetic acid (TFA), triethylamine (TEA) were purchased from Aldrich (Milwaukee, WI). Spectrophotometric grade acetonitrile (ACN) was purchased from Baxter (Muskegon, MI). 0.1 N hydrochloric acid (HCl) and 0.1 N sodium hydroxide (NaOH) solutions were purchased from Fisher Scientific (Fairlawn, NJ). All other chemicals and reagents were analytical or HPLC grade.

Both vials and aluminum caps were purchased from National Scientific Company. The amber vial has a volume capacity of 4 ml (15×45 mm) with the Part Number C-4015-2W. The aluminum seal has the Part Number 73825-11.

3.2.2. HPLC Instrumentation and Chromatographic Conditions

A Beckman Gold System equipped with a model #167 detector at 220 nm was used for all assays. The separation of eptifibatide from its degradation products was achieved by using a Pinnacle octyl amine (C8) column (5 μm, Dimension: 150*4.6 mm, Cat: 9183565, Serial #98040183P, Restek Corporation).

The mobile phase used a combination of organic solvent and aqueous solution. The organic solvent was ACN, and the aqueous solution contained 0.1% TFA and 0.1% TEA. The elution program started with constant 17% ACN for 15 minutes (isocratic), switched to gradient elution with ACN concentration increasing up to 100% in 5 minutes, and finally returned to equilibrium at the original 17% ACN. The injection volume was 100 μl, and the flow rate was 1.0 ml/min. The retention time for eptifibatide was 10.1±0.1 minutes at ambient temperature.

3.2.3. Formulations

The aqueous vehicle contained 0.025 M citrate buffer; the semi-aqueous vehicle contained 10% ethanol, 40% propylene glycol and 50% 0.025 M citrate buffer, which yielded a buffer concentration of 0.0125 M. The eptifibatide concentration was 2±0.1 mg/ml, or 0.0024 M, for all formulations.

3.2.4. Procedures for Sample Preparation

Stock solutions of 0.025 M sodium citrate and 0.025 M citric acid were mixed to obtain a citrate buffer stock solution at approximately pH 3.5-3.8 to which 2.0 ±0.1 mg/ml drug was added. The solution was then adjusted with 0.5 N NaOH to pH 4.25, 4.75, 5.25, 5.75 and 6.25, respectively. The preparation of the semi-aqueous vehicle was similar: the aqueous stock solution was mixed with an equal volume of the cosolvents (20% ethanol, 80% propylene glycol; mixed by volume) to which 2.0 ± 0.1 mg/ml drug was added. The 0.1 N NaOH was used to adjust the semi-aqueous solutions to the desired pH. All samples prepared were clear, colorless liquid.

The aseptically filtered sample solutions were distributed to sterile amber vials, and were crimped with aluminum caps. The sample vials were incubated in thermostatically controlled water-baths (Precision Microprocessor Controlled 280 Series, Jouan Inc.) and protected from light. The temperatures for the kinetics studies were 48^{0}C, 60^{0}C and 72.5^{0}C and were maintained at ±0.2^{0}C. The pH of buffered solutions was measured by Corning pH meter (model# 140). The pH meter was standardized by buffer solutions (VWR Scientific company, West Chester, PA). Samples were removed from the water-bath at the pre-set time. Triplicate samples were diluted with 70% ACN in water and assayed within 24 hours. The observed eptifibatide degradation rate constant (k_{obs}) used for all calculations was as an average of three experimental k_{obs} values. Approximately 8-10 time points were collected for each kinetic study.

3.3. Results and Discussion

3.3.1. Eptifibatide Degradation Kinetics

The degradation profiles of eptifibatide under all conditions and compositions are consistent with apparent 1st order kinetics. Typical degradation profiles are shown in Figures 3-1. There is a linear relationship between natural logarithm of the drug remaining and time as shown in Eq. 3-1 from which the observed degradation rate constants K_{obs} were derived.

$$\text{Ln } [C] = \text{Ln } [C_0] - K_{obs}t \qquad (3\text{-}1)$$

The $[C_0]$ and $[C]$ are the initial and time (t)-dependent concentrations of eptifibatide, respectively. Table 3-1 lists all K_{obs} data for eptifibatide degradation at various investigated pH, cosolvent, and temperature conditions, as well as statistical significance indicators.

From Table 3-1, it is clear that the K_{obs} data are markedly dependent on temperature, pH, and the vehicle used. The cosolvent effect is of particular interest: the K_{obs} is smaller in semi-aqueous vehicles than in respective aqueous vehicles under all investigated conditions.

3.3.2. pH-Rate Profile

Figure 3-2 shows the pH-rate profiles for eptifibatide in both aqueous and semi-aqueous vehicles. The aqueous vehicles are characterized by a pH of maximum stability of 5.25 and increased degradation rates in both more acidic and more basic conditions. The semi-aqueous vehicles show similar results, but the degradation rate is lower all over in an almost parallel fashion and the pH of maximum stability is shifted to 5.75. The V-shaped pH-rate profiles of eptifibatide are similar to those of drugs that undergo hydrolysis catalyzed by specific acid (hydronium ion) and specific base (hydroxide ion) (Connors et. al., 1986). Interestingly, the V-shape of eptifibatide is fairly flat with a slope in the range of -0.4 to -0.6 for left side and 0.4-0.6 for the right side. This indicates that either other components such as buffer species or other degradation mechanism may contribute to eptifibatide degradation (Martin, 1993). Apparently, the rate equation can not be simply written as Eq. 1-1.

3.3.3. Eptifibatide Degradation

3.3.3.1. Degradation in Aqueous Vehicle

There are a number of routes for eptifibatide degradation. Amide hydrolysis is the major degradation pathway, and Asp-clipped eptifibatide is the most important degradation product with regard to both the amount generated and the degree of sensitivity to the solution pH (Van Gorp and Sluzky, 1999).

At acidic conditions, the rapid increment of Asp-clipped eptifibatide in the aqueous vehicles supports specific acid catalyzed hydrolysis: the lower the pH, the more Asp-clipped eptifibatide generated. Figure 3-3 shows the percentage of Asp-clipped eptifibatide produced in aqueous and semi-aqueous vehicles in the pH range of 4.25-6.25 at 60^0C after 53 days. Similar patterns for Asp-clipped eptifibatide were observed under other days and other temperatures, though with different magnitude and proportions. The amount of Asp-clipped eptifibatide increased with temperature at any pH.

Interestingly, the rate of hydrolysis decreases in the aqueous vehicle as the pH increases, becoming negligible above pH 5.25. There are two possible explanations for the reduced rate of Asp-clipped eptifibatide production:

a) the Asp-clipped eptifibatide may be generated, but at basic conditions it quickly decomposes to other peptidic fragments or related degradants such as Trp-Pro-diketopiperazine (see Table 1-2 for structure);
b) which is more likely, that other degradation patterns such as dimerization, aggregation and oxidation may take over.

The reported major degradants in alkaline pH (≥5.5) are trisulfide eptifibatide, which comes from oxidation, and eptifibatide dimer, which comes from dimerization (Table 1-2, Van Gorp and Sluzky, 1999). Unfortunately, the current assay was not able to separate or quantitate these products. An assessment of peak area percentage of many HPLC

chromatograms indicates that in the basic pH, as Asp-clipped eptifibatide percentage decreased, the percentages of other impurity peaks noticeably increased. This confirms the postulated change of mechanism with pH.

The oxidative degradation of some drugs has been reported to be pH-dependent. This is often a consequence of the pH effect on the oxidation or reduction potential of the system (Florence and Attwood, 1988). Both hydronium and hydroxyl ions catalyze oxidative reactions. The rate of degradation for epinephrine (or adrenaline), for example, is more rapid in a neutral to alkaline solution with maximum stability (minimum oxidative degradation) at pH 3.4 (Schroeter and Higuchi, 1958; Sokoloski and Higuchi, 1962). Another example, morphine, undergoes oxidative degradation rapidly in neutral to alkaline pH following apparent first order kinetics while the drug is fairly stable at acidic solutions (Yeh and Lach, 1961). Other drugs including some antibiotics and vitamines were also reported that their oxidation degradation were pH-dependent (Florence and Attwood, 1988).

At this point, it is suggested that the effect of pH on eptifibatide is not restricted entirely to specific acid and specific base catalyzed hydrolysis. Other mechanisms may contribute to the drug degradation. Whatever the degradation mechanism or a combination of these mechanisms, however, it ought to be either pH-related or pH-dependent. The end results are the steady upward movement of eptifibatide degradation rate in the basic pH range.

3.3.3.2. Degradation in Semi-aqueous Vehicle

As mentioned above, the presence of cosolvent greatly reduces Asp-clipped eptifibatide generation, especially at more acidic pH solutions such as pH 4.25. Figures 3-4 presents the data generated at 60^{0}C after 53 days. It shows that eptifibatide percentage remaining is related to both solution pH and the vehicle used.

3.3.4. Mechanism for Eptifibatide Degradation

In this section, we attempt to understand the dynamics of the cosolvent effect on eptifibatide degradation.

3.3.4.1. Collision Theory

The addition of cosolvent has partially replaced water that is necessary for drug hydrolysis, i.e., it reduces the collision probability between a water molecule and a drug molecule. In a semi-aqueous medium, both concentrations of hydronium and hydroxide ion remain the same at specified pH, but the water concentration is greatly reduced from 55.5 M to 22.7 M in 50% cosolvent solution. Thus the water catalysis rate constant k_3, which is the product of k_{H2O} and $[H_2O]$, reduces to half of its value. The water concentration effect seems to be able to explain the reduced rate of drug degradation in the semi-aqueous medium, but not the fact that the maximum stability pH is shifted from 5.25 in aqueous medium to 5.75 in semi-aqueous medium. This indicates that there may exist some other factors that affect the drug degradation. The water concentration may be part of it, but it certainly is not the full explanation.

3.3.4.2. Transition State Theory

As an alternative to classical collision theory, transition state theory assumes that there is an equilibrium existing between normal reactant molecules and an activated complex of these molecules, or transition state. As qualitatively depicted in Figure 3-5, the transition state is of higher energy than either the initial or final states. It is unstable and its decomposition leads to the product. The reaction coordinate axis gives a rough measure of the extent of progress of the system from reactants to the product via the transition state. For an elementary bimolecular process, the reaction can also be written as

$$A + B \Leftrightarrow (A...B)^* \rightarrow \text{Product} \qquad (3\text{-}2)$$

According to transition state theory, the rate of reaction is determined by the concentration of the transition state species, or $(A...B)^*$, and this concentration is controlled by the assumed equilibrium between the initial and transition states. Thus, the relative polarity of the initial and transition states becomes critical. If the transition state is more polar than the initial state, the addition of cosolvent such as ethanol or propylene glycol, which reduces the medium polarity (Table 3-2), would destablize or increase the energy of, the transition state, and thereby decrease the reaction rate. If, on the other hand, the transition state is less polar than the initial state, the addition of cosolvent would

stabilize, or lower the energy of the transition state, and, therefore, increase the reaction rate.

Transition state theory was proposed to explain simple reactions for small molecules (Ingold, 1969; Connors, et. al., 1986). There have been no reports on large and complex molecules such as eptifibatide with multiple charges. In order for the theory to work, we must further assume that the more charges the drug molecule carries, the more polar it becomes. For example, a transition state that carries both one negative charge and one positive charge would be more charged than a neutral form of the molecule, and thus be less favored in the semi-aqueous medium.

Eptifibatide contains two functional groups that can be ionized in pH range 4.25-6.25: guanidino group on the homoarginine residue and carboxylic acid group on the aspartic acid residue. The guanidino group is an extremely strong base. Its pK_a is around 12.48 (Loudon, 1988), and it should be protonated over the entire pH range. The carboxylic acid group has a pK_a around 3.87 (Loudon, 1988; Dean, 1992), which makes it ionized in the test pH range. By using the Henderson-Hasselbalch equation (3-3), the carboxylic acid group is calculated to be approximately 70.6% ionized at the lowest pH 4.25, As the pH increases, the ionized percentage also increases. Therefore, the eptifibatide drug molecule is predominantly ionized at both the carboxylic acid group and the guanidino group over the entire test pH range 4.25-6.25. In other words, the drug species is mostly

a zwitterion (M±) with a small percentage of protonated species (on guanidino group) at the lower end of the test pH range.

It is well known that amide hydrolysis, catalyzed by either acid or base, undergoes a charged transition state as illustrated in Figure 3-6 (Ingold, 1969). It can be reasoned that when hydronium ion or hydroxyl ion reacts with eptifibatide, either M+ or M±, the transition state is always more charged than the initial state. The addition of the cosolvent decreases the medium polarity, which favors the destabilization of the transition state. The end result is that drug degradation is decreased.

It needs to be mentioned that transition state theory is only qualitative in establishing the relationship between the cosolvent effect and the reaction rate constant. It has no appreciation of molecular spatial arrangement and spatial interactions among functional groups, which are critical in determining the reaction rate in conventional organic chemistry. Neither is it able to explain other degradation routes that are pH-dependent such as oxidation. Most importantly, the transition state theory needs to be further tested and evaluated on a greater variety of drug molecules, and especially on large and complex molecules.

3.3.5. General Acid and General Base Catalysis

One concern has been, if the buffer used would have any catalytic effect on the drug degradation, i.e., the general acid and general base catalysis. As there is a small

concentration difference in citrate buffer in aqueous vehicles (0.025 M) and in semi-aqueous vehicles (0.0125M), it is necessary to assess if such a difference would have an impact on eptifibatide degradation.

The experimental procedures were set as follows:

a) use citrate buffer concentrations at 0.01 M and 0.05 M;

b) conduct the test for both the aqueous and the semi-aqueous vehicles;

c) keep the solution ionic strength at 0.2 μ with use of sodium chloride NaCl;

d) set the test temperature at 72.5^{0}C.

Table 3-3 shows that there is no appreciable difference in K_{obs} (<2%) when the citrate buffer concentration is the only variable. This indicates that the citrate species does not contribute to the eptifibatide degradation under the test conditions: 0.01-0.05 M in pH 4.25-6.25. It also ensures that the small difference of citrate buffer in aqueous (0.025 M) and in semi-aqueous vehicle (0.0125 M) is too small to have meaningful impact on eptifibatide degradation.

3.3.6. Ionic Strength Effect

This study investigated the effect of ionic strength on eptifibatide degradation. Table 3-4 lists the effect of sodium chloride (NaCl) at 1% (w/v), or 0.17 M, in different solutions.

It is clear that the K_{obs} value is approximately the same with and without the addition of 1% NaCl. This indicates that the change in ionic strength in such a small range has virtually no detectable effect on drug degradation. A number of drug degradation studies also indicated that ionic strength at low range has no consistent effects on the drug degradation (Connors et. al., 1986).

3.3.7. Arrhenius Plot and Shelf-life Prediction

Figure 3-7 presents the Arrhenius plots for eptifibatide in both aqueous and semi-aqueous vehicles. The apparent linearity at different pH solutions (r^2 = 0.989-0.999) suggests that the extrapolation of k_{obs} to 25^{0}C is appropriate. It is worth mentioning that initially the eptifibatide kinetics study was set under four temperatures: 37^{0}C, 48^{0}C, 60^{0}C, 72.5^{0}C. However, the degradation data at 37^{0}C were not sufficient for reliable generation of a degradation rate constant, most samples had only 5-20% drug loss after 90 days. Thus, the 37^{0}C data series were not included in all kinetic calculations.

Table 3-5 lists activation energy (E_a) and the frequency factor (A). The E_a is in the range of 23.8-26.3 kcal/mol, which is consistent with E_a values of hydrolysis degradation of other drugs (Connors et al., 1986).

Figure 3-8 provides the predicted shelf-life (T_{90}) of eptifibatide at 25^{0}C. The T_{90} is a commonly used drug shelf-life indicator, which refers to the point at which the potency reaches 90% of the label claim. Figure 3-8 shows that eptifibatide T_{90} at 25^{0}C is 33

months for the aqueous vehicle at maximum stability pH 5.25. The T_{90} is increased almost 2-fold (60 months) for the semi-aqueous vehicle at maximum stability pH 5.75.

3.3.8. Degradation in Pure Solvents

Eptifibatide is more stable in either pure ethanol or pure propylene glycol, than in all aforementioned aqueous and semi-aqueous vehicles. Table 3-6 presents the results for drug degradation in both propylene glycol and ethanol. Figure 3-9 compares the degradation data in pure cosolvents with other aqueous and semi-aqueous vehicles, again at 60^0C after 53 days. The eptifibatide percentage remaining follows the order of ethanol, propylene glycol, semi-aqueous vehicle at maximum stability pH 5.75, and aqueous vehicle at maximum stability pH 5.25. The fact that eptifibatide undergoes degradation in pure ethanol and propylene glycol indicates that solvolysis, though small, does contribute to the total drug degradation.

3.3.9. Conclusions

The proposed semi-aqueous vehicle that contained 10% ethanol and 40% propylene glycol substantially increased eptifibatide stability in comparison with the aqueous vehicle over the test pH range 4.25-6.25, most likely a result of reduced hydrolysis-related degradation in the presence of cosolvents.

The degradation of eptifibatide is complex. It may involve a few different mechanisms: the specific acid catalyzed hydrolysis, which is dominant in the acidic region, and a pH-

dependent oxidation, which is likely to be dominant in the basic region of the test pH range.

The fact that cosolvents can be used to increase eptifibatide stability has important implications in future parenteral formulation development of other hydrolysis-susceptible drugs.

Figure 3-1 Eptifibatide Degradation in Aqueous and Semi-aqueous Vehicles at 72.5°C

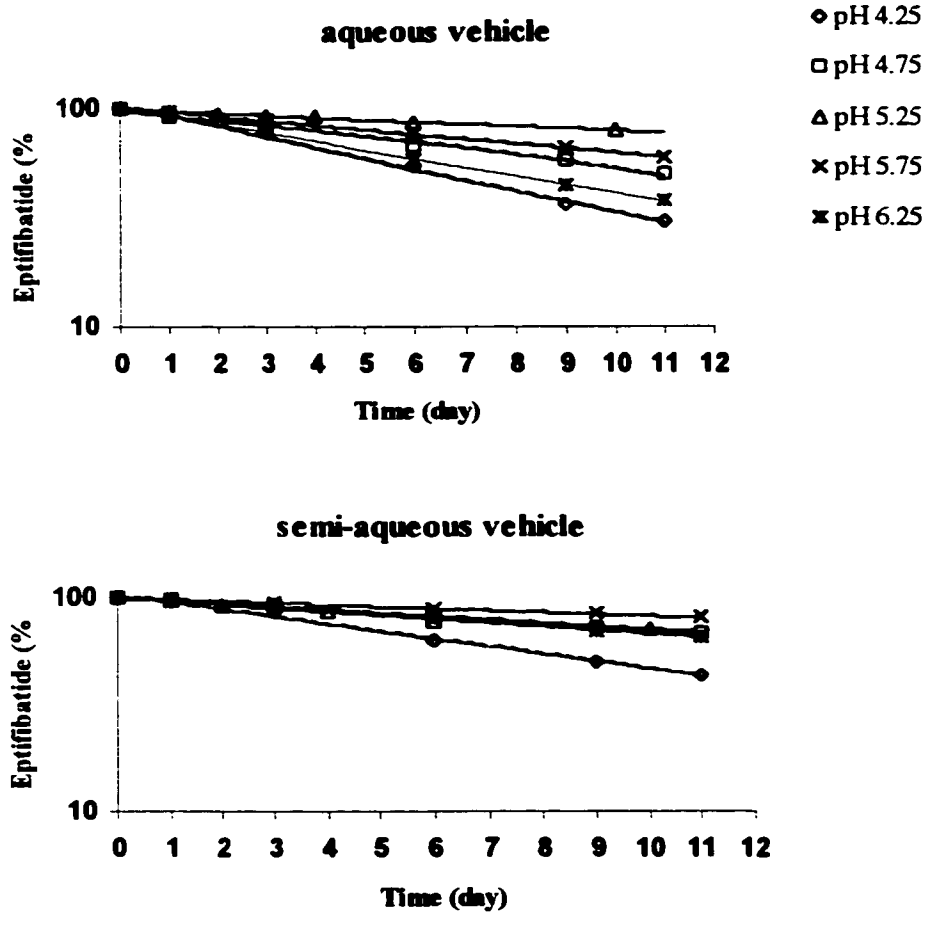


Figure 3-2 pH-Rate Profiles of Eptifibatide (k_{obs}: day^{-1})

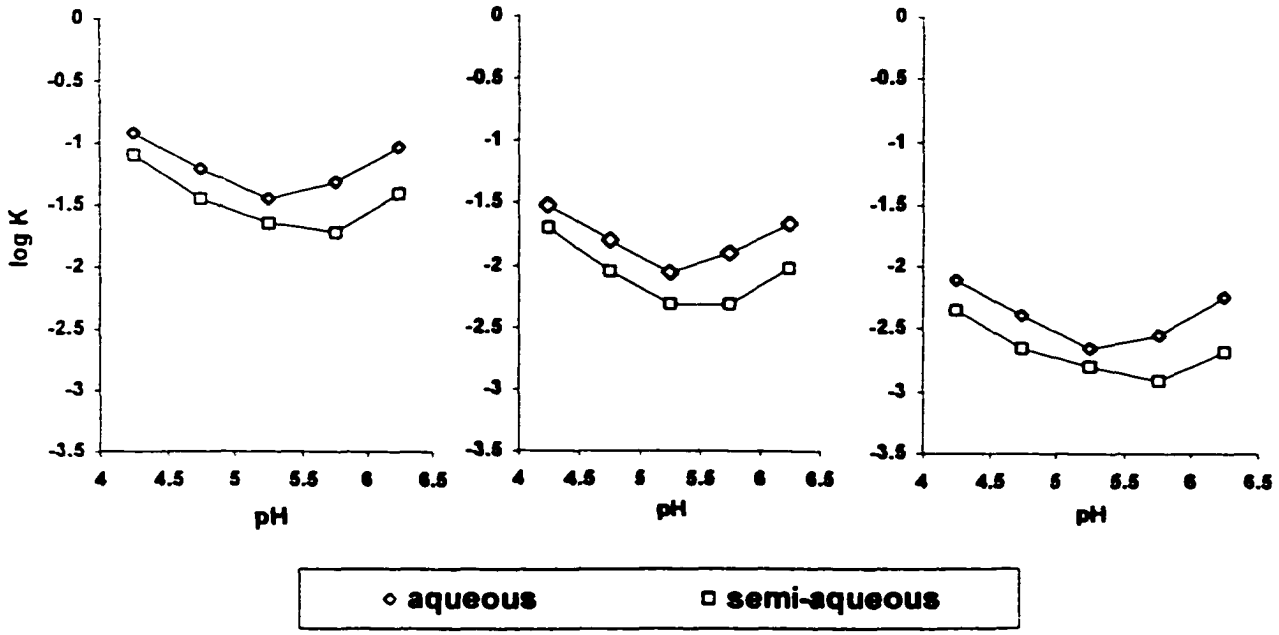


Figure 3-3 **Major Degradant Asp-clipped Eptifibatide at 60^{0}C after 53 days**

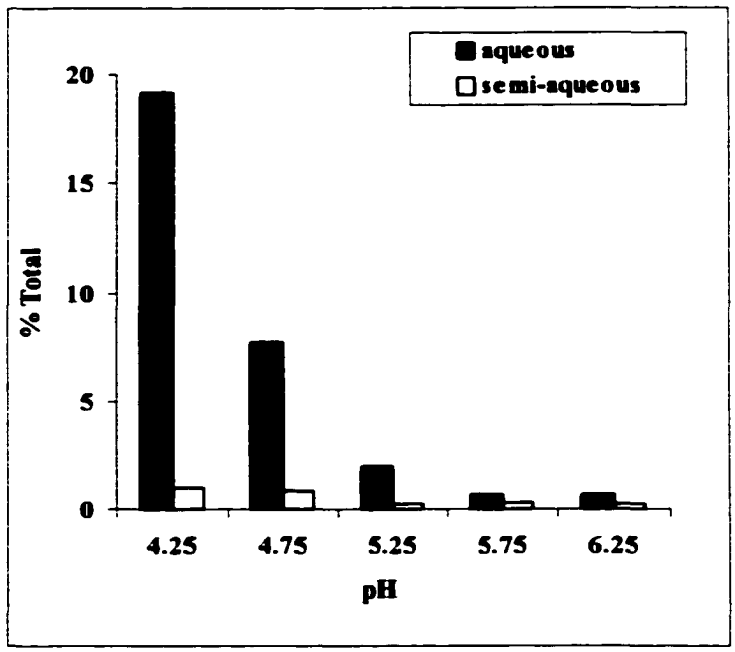


Figure 3-4 **pH Effect on Eptifibatide Degradation at 60°C After 53 Days**

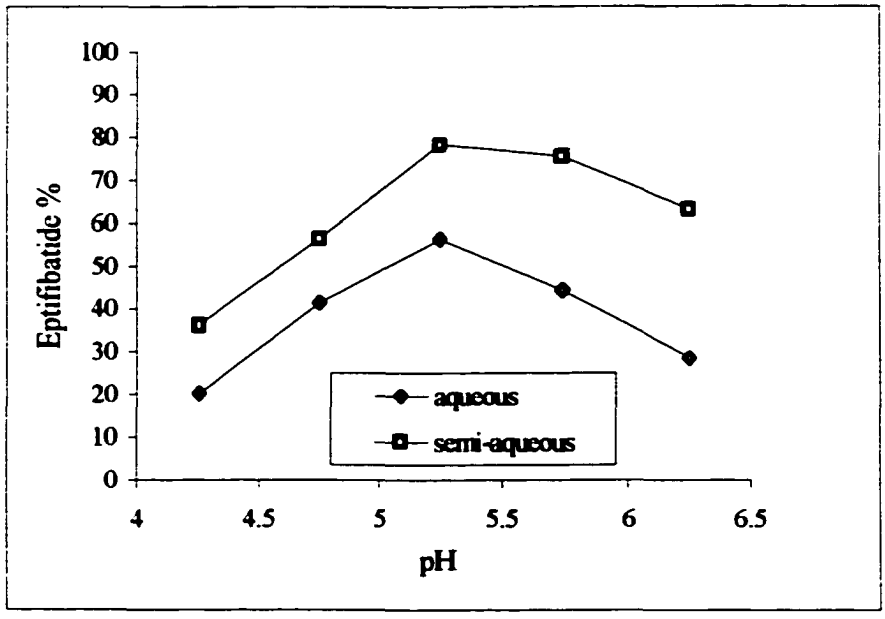


Figure 3-5 Hypothetical Reaction-Coordinate Diagram

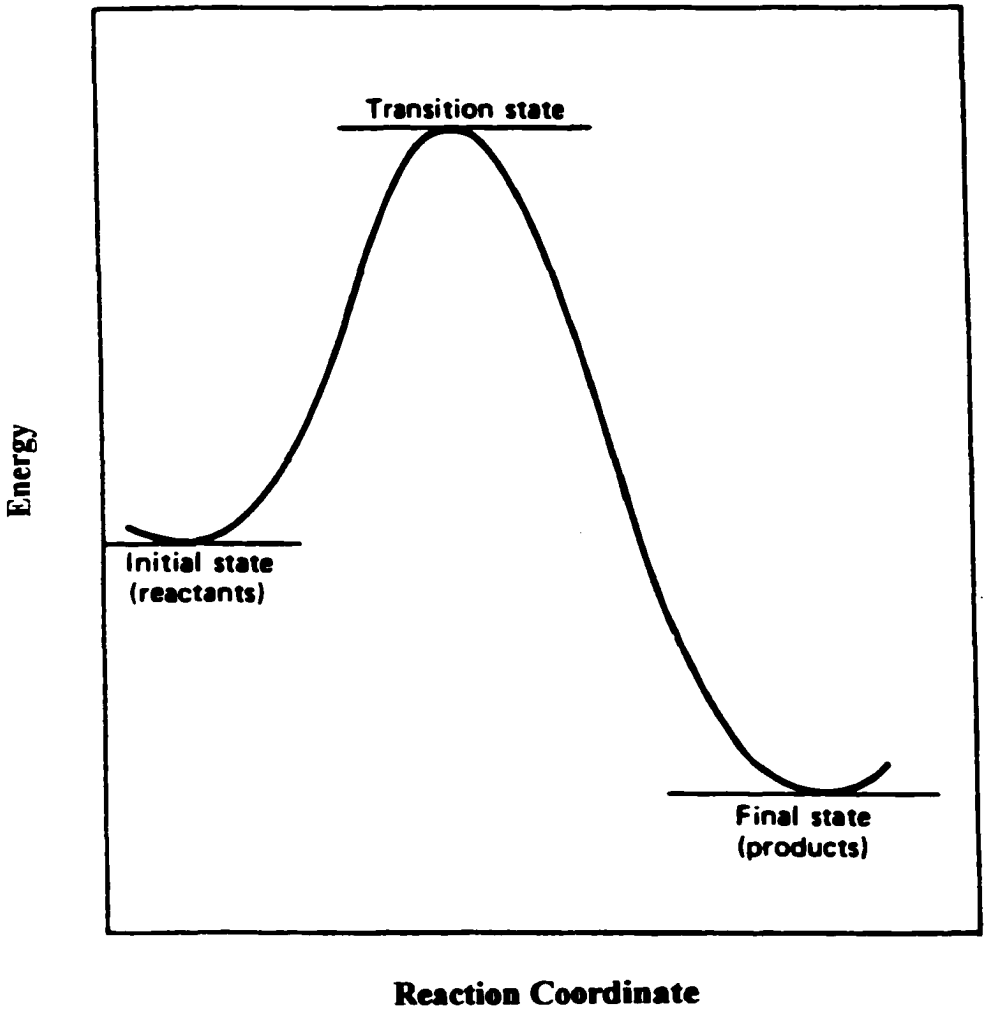
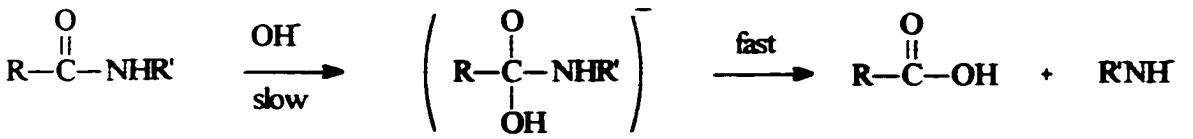
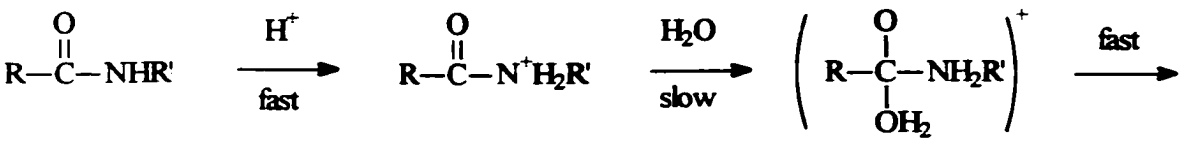


Figure 3-6 Amide Hydrolysis catalyzed by Base and Acid

a) base catalyzed hydrolysis



b) acid catalyzed hydrolysis



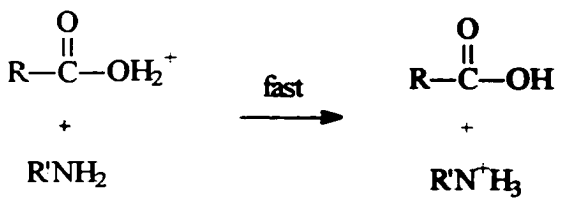


Figure 3-7 Eptifibatide Arrhenius Plot (k_{obs}: day^{-1}, T: Kelvin)

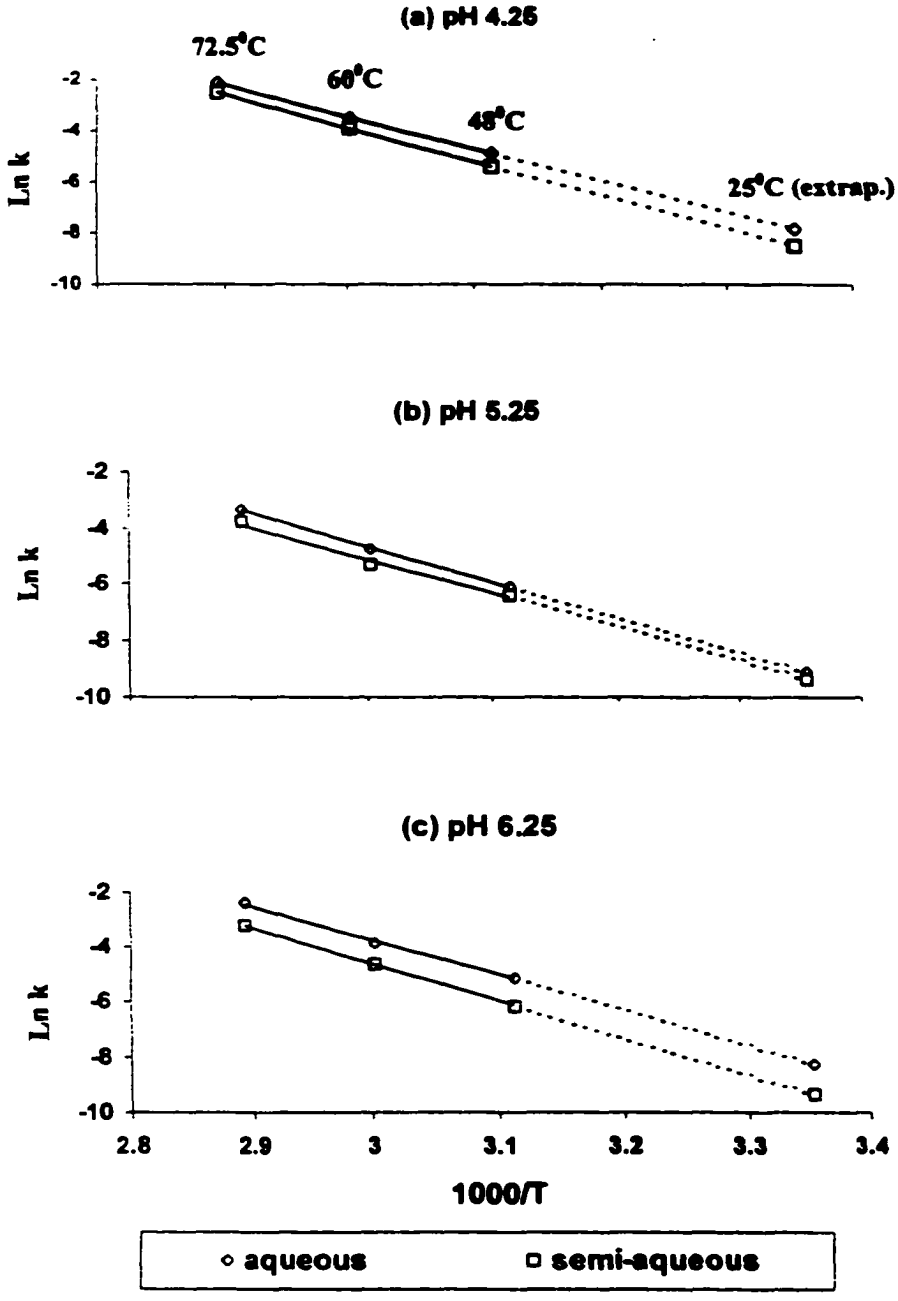


Figure 3-8 **Predicted Eptifibatide Shelf-Life T_{90} at 25°C**

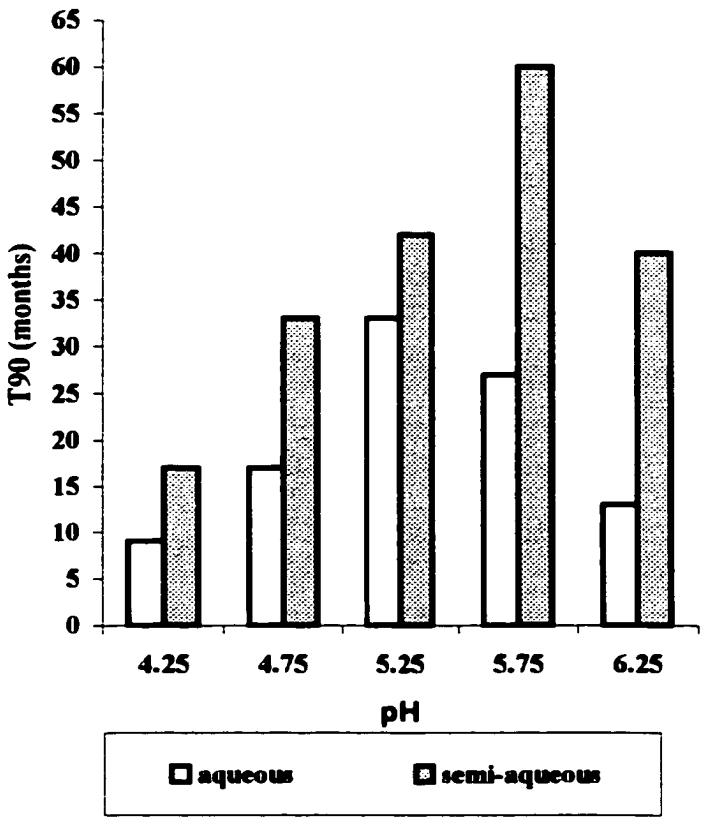


Figure 3-9 Eptifibatide (%) in Various Media at 60°C after 53 Days

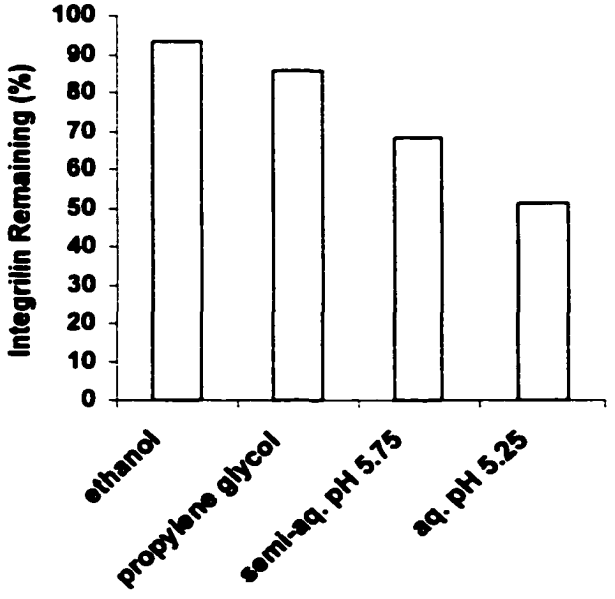


Table 3-1 Observed Eptifibatide Degradation Rate Constants: K_{obs}: day^{-1}

pH	Temp.	Aqueous			Semi-aqueous		
		k_{obs} (day^{-1})*	S.D.	r^2	k_{obs} (day^{-1})*	S.D.	r^2
4.25	72.5°C	0.1175	0.00870	0.9828	0.0802	0.00162	0.9886
4.75		0.0600	0.00992	0.9965	0.0352	0.00259	0.9970
5.25		0.0351	0.00190	0.9887	0.0225	0.00151	0.9816
5.75		0.0475	0.00249	0.9982	0.0190	0.00041	0.9969
6.25		0.0906	0.00347	0.9989	0.0389	0.00152	0.9985
4.25	60°C	0.0302	0.00274	0.9935	0.0202	0.00846	0.9948
4.75		0.0157	0.00043	0.9868	0.0090	0.00017	0.9607
5.25		0.0085	0.00034	0.9534	0.0048	0.00015	0.9812
5.75		0.0124	0.00070	0.9466	0.0049	0.00045	0.9758
6.25		0.0215	0.00105	0.9939	0.0095	0.00047	0.9860
4.25	48°C	0.0076	0.00029	0.9962	0.0045	0.00024	0.9907
4.75		0.0040	0.00011	0.9613	0.0022	0.00024	0.9612
5.25		0.0022	0.00016	0.9464	0.0016	0.00013	0.8470
5.75		0.0028	0.00011	0.8480	0.0012	0.00015	0.8685
6.25		0.0057	0.00013	0.9906	0.0021	0.00011	0.8312

* Average value (n = 3); S.D.: standard deviation

Table 3-2 Some Common Solvents Listed in Order of Increasing Polarity

Solvent	Dipole Moment (debyes)	Dielectric constant
Cyclohexane	0	2.0
Benzene	0	2.3
Chloroform	1.01	4.8
Polyethylene glycol 400		13.6
n-butanol	1.66	17.8
n-propanol	1.68	20.1
Acetone	2.88	20.7
Ethanol	1.69	24.3
Propylene glycol		32.0
Methanol	1.70	32.6
Ethylene glycol	2.28	37.0
Dimethylacetamide		37.8
Glycerol		42.5
Demethyl sulfoxide		46.7
Water	1.85	78.5

Source: a) Connors, et al. (1986); b) Rubino and Berryhill (1986)

Table 3-3 **Eptifibatide Degradation and Buffer Catalysis**

Citrate Buffer	k_{obs}: day^{-1}*			
	pH 4.25		pH 6.25	
	Aqueous	semi-aqueous	Aqueous	semi-aqueous
0.01 M	0.1152	0.0796	0.0912	0.0403
0.05 M	0.1185	0.0821	0.0904	0.0410

* mean values of duplicate readings; the ionic strength was kept at 0.2 μ.

Table 3-4 Eptifibatide Degradation and Ionic Strength at 72.5°C

pH	Addition of 1% NaCl	Aqueous		Semi-aqueous	
		K_{obs} (day^{-1}) *	Ionic strength **	K_{obs} (day^{-1})*	Ionic strength **
4.25	Yes	0.1212	0.21	0.0824	0.19
	No	0.1175	0.04	0.0802	0.02
6.25	Yes	0.0923	0.26	0.0390	0.22
	No	0.0906	0.09	0.0389	0.05

*: mean values of duplicate readings.

**: ionic strength (μ) was calculated from CHEMIST program (1998 version, MicroMath Research Inc., Salt Lake, UT).

Table 3-5 Eptifibatide Activation Energy (E_a) and Frequency Factor (A)

pH	E_a (kcal/mol)		A (day^{-1})	
	Aqueous	Semi-Aqueous	Aqueous	Semi-Aqueous
4.25	24.6	25.9	4.5×10^{14}	2.0×10^{15}
4.75	24.4	24.9	1.5×10^{14}	2.1×10^{14}
5.25	24.9	23.8	2.0×10^{14}	2.2×10^{13}
5.75	25.5	24.8	6.3×10^{14}	9.9×10^{13}
6.25	24.9	26.3	4.8×10^{14}	1.6×10^{15}

Table 3-6 Eptifibatide Percentage Remaining in Cosolvents

Time (days)	Eptifibatide Remaining (%)*			
	60 °C	48 °C	60 °C	48 °C
	propylene glycol	ethanol	propylene glycol	ethanol
0	100.0	100.0	100.0	100.0
15	90.8	95.5	94.1	98.4
26	87.9	92.6	91.9	98.1
42			92.0	95.8
53	85.7	93.5		
84	80.4	92.0	94.6	96.2

* mean values of duplicate readings.

APPENDIX A ABSTRACTS OF POSTERS AND PUBLICATIONS

Formulation and Stability Evaluation of PEG-Coated Liposomes Containing Antitumor Agent 2-4'-Amino-3'-Methylphenyl Benzothiazole (AMPB)

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11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy
Amsterdam, Netherlands (November, 2000)

ABSTRACT

Purpose. This study investigates the formulation and stability of polyethylene glycol (PEG)-coated liposomes containing the antitumor agent 2-4'-amino-3'-methylphenyl benzothiazole (AMPB) for intravenous administration. **Methods**. The proposed liposomal formulation used egg phosphotidylcholine (EPC) and cholesterol (Chol) in equi-molar concentration as primary lipid components, and DSPE2000, or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-polyethylene glycol 2000 to provide the PEG-coating on the surface. A variety of AMPB-containing liposomes were prepared by thin-film hydration, sized by membrane extrusion, and physically evaluated. Both vesicle particle size and encapsulation efficiency were shown to be important indicators of the physical stability of the liposomal formulations. **Results**. It was found that: a) the particle size of all AMPB-containing liposomes increased over time (30 days) from 118-137 nm to 176-253 nm at 37^0C with high lipid concentration and high AMPB loading having greater increase; b) low temperature had a less pronounced effect on the particle size increment. The particle size of liposomes at 4^0C showed negligible change over 51 days; c) the encapsulation efficiency was high for all prepared liposomes and remained so for 9 days, from approximately 98±2% to 94±2% determined by HPLC assay. **Conclusions**. The study shows that formulation of AMPB 4 mg/ml with AMPB-lipid molar ratio 2/5/5/1 (AMPB/EPC/Chol/DSPE2000) is feasible. This formulation is high in dose loading, high in encapsulation efficiency while it is able to maintain its physical stability. **Acknowledgement**. This work was performed under contract NCI-CM-77109 from the Pharmaceutical Resources Branch, National Cancer Institute.

Solubilization of Cyclosporine A

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AAPS Meeting Abstract, Indianapolis, IN (October, 2000)

ABSTRACT

Purpose. This study investigates the solubilization of Cyclosporine A (CsA), a neutral undecapeptide used in organ transplantation, by using pH control, cosolvency and micellization. **Methods**. Table 1 lists the cosolvents and surfactants used. The pH of the citrate-phosphate buffer solutions ranged from 2.25-7.84. The equilibrium CsA solubility was measured by HPLC assay at elevated temperature (70^{0}C) after 7 days on a mechanical rotator at ambient temperature. **Results**. The solubility of CsA ranged from 25.99 ug/ml at pH 2.25 to 4.78 ug/ml at pH 7.84. Surfactants have noticeable effect in increasing the CsA solubility. Ten percent solutions of Tween 20, Tween 80 and Cremophor increased the solubility to 1109, 7264 and 1994 ug /ml respectively. This represents 40 to 270 increases in solubility. Cosolvents on the other hand did not increase the solubility as much as expected. The solubility of CsA in twenty- percent solutions of several cosolvents is given in Table 1. **Conclusion**. The nonpolar cyclic peptide drug-CsA could be efficiently solubilized by different surfactants, but the efficiency of the cosolvents was lower than expected from the ClogP value.

Table 1 Solubilization Parameters for Cyclosporine A

Excipient		Solubility in twenty percent solution of solubilizing agents
EtOH	(v/v)	134 μg/ml
PG	(w/v)	87 μg/ml
Tetraglycol	(w/v)	151 μg/ml
Glycerin	(w/v)	58 μg/ml
PEG-400	(w/v)	110 μg/ml
Tween 20	(w/v)	1,800 μg/ml
Tween 80	(w/v)	14,800 μg/ml
CremophorEL	(w/v)	3,900 μg/ml

Combined Effect of Cosolvent and Cyclodextrin on Solubilization of Non-polar Drugs

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The University of Arizona, Tucson, AZ 85721

Journal of Pharmaceutical Sciences, 88, 1107-1111 (1999)

ABSTRACT

Solubility enhancement has broad implications in parenteral formulation design. A simple mathematical model has been developed to describe the combined effect of cosolvency and complexation on non-polar drug solubilization. The total drug solubility is determined by the summation of three drug species present in the solution: free drug [D], drug-ligand binary complex [DL], and drug-ligand-cosolvent ternary complex [DLC]. The proposed model established the dependencies of these three species upon the intrinsic drug solubility, [Du], the cosolvent solubilizing power, σ, the binary and ternary intrinsic complexation constants, K_b^{int} and K_t^{int}, and the cosolvent destabilizing powers for the binary and the ternary complexes, ρ_b and ρ_t. A non-polar solute, fluasterone, is used to evaluate the newly generated equation. The model explains the decline in drug solubility produced by low cosolvent concentrations as well as the increase in the solubility produced by high cosolvent concentrations that are observed at all cyclodextrin concentrations.

A Combined Group Contribution and Molecular Geometry Approach for Predicting Melting Points of Aliphatic Compounds

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Industrial & Engineering Chemistry Research, 38, 3581-3584 (1999)

ABSTRACT

A combined approach that utilizes both group contribution and simple molecular geometric parameters is employed to predict normal melting points for a variety of aliphatic compounds. The melting points are estimated from the ratio of the enthalpy of melting and the entropy of melting. The former is calculated from the sum of enthalpic group contributions and correction factors while the latter is calculated using a modification of Walden's rule. Approximately 1,040 melting point data were compiled and analyzed by multiple regression. The root mean square error of the estimation is 34.4 K. This is relatively low given the complexity of melting and the diversity of the database used. A comparison of the proposed method with the method of Joback and Reid was performed on fifty aliphatic compounds that were not used in the training set. The average absolute error for this method is approximately 20% while that for the Joback and Reid is 34%. The higher prediction accuracy of the proposed method suggests that the melting point prediction can be better approached by using both group contribution (enthalpic) and simple molecular geometric parameters (entropic).

Solubilization of Fluasterone

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Journal of Pharmaceutical Sciences, 88, 967-969 (1999)

ABSTRACT

Solubilization of non-polar drugs constitutes one of the most important tasks in parenteral formulations design. This study investigates and assesses the solubility enhancement of fluasterone by various techniques including cosolvency, micellization, and complexation. Of the solubilizing agents used, the modified β-cyclodextrins were found to be the most effective. The solubility of fluasterone is 1.55×10^{-4} mM, 3.13 mM and 4.04 mM in water, 20% sulfobutyl ether-β-cyclodextrin (SBEβCD) and 20% hydroxypropyl-β-cyclodextrin (HPβCD), respectively.

Table 1 Solubilization Parameters for Fluasterone

Excipent	Concentration Range	Dependence of $[D_{tot}]$ on $[D_u]$ ([C]: excipient concentration***)
EtOH	0-80% (v/v)	$10^{5.8[C]}$
PG	0-80% (v/v)	$10^{4.1[C]}$
PEG400	0-80% (v/v)	$10^{4.9[C]}$
Glycerin	0-20% (v/v)*	$10^{1.1[C]}$
Tween 20	0-20% (v/v)	32,258 [C]
Tween 80	0-20% (v/v)	43,226 [C]
Sodium cholate	0-20% (w/v)	43,871 [C]
Sodium deoxycholate	0-20% (w/v)	55,484 [C]
Sodium taurocholate	0-10% (w/v)**	24,516 [C]
HPβCD	0-20% (w/v)	180,000 [C]
SBEβCD	0-20% (w/v)	216,129 [C]
αCD	0-14% (w/v)**	645 [C]
HPγCD	0-20% (w/v)	19,355 [C]

* Maximum concentration prepared due to the increased viscosity of cosolvent solution

** Maximum concentrations prepared due to the limited solubility of the excipients

***The [C] unit is % for cosolvents and mM for surfactants and cyclodextrins

A Modification of Trouton's Rule by Simple Molecular Parameters for Hydrocarbon Compounds

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ABSTRACT

The entropy of vaporization at the normal boiling point (ΔS_b) is an important property in the chemical engineering and environmental sciences. This paper presents a simple modification of Trouton's rule to estimate this property for a variety of hydrocarbon compounds including alkanes, alkenes, alkynes, dienes, cycloalkanes and alkylaromatics. The equation contains three easy-to-determine parameters that describe important molecular features: flexibility, symmetry and planarity. The entropy data were calculated from experimental enthalpy data of 477 different organic compounds at the normal boiling point. The average percentage error for the prediction is 1.30 by the proposed method, 2.79 by Trouton's rule, 1.62 by Kistiakowsky's method, 1.51 by Vetere's method, and 2.23 by the group contribution method of Ma and Zhao.

In Vitro Release of 17-Demethoxy-17-Allylamino Geldannamycin (17-AAG) from Seven of Its Ester Prodrugs

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ABSTRACT

Purpose. To evaluate the in vitro stability of seven ester prodrugs of anticancer agent 17-Demethoxy-17-allylaminogeldanamycin (17-AAG): 11-4'-aminobutanoate HCl, 11-2'-aminoacetate HCl, 11-2'-N,N-dimethylaminoacetate HCl, 11-3'-aminopropionate HCl, 11-3'-N,N-dimethylaminopropionate HCl, 11-5'-aminopentanoate HCl, and 11-4'-N,N-dimethylaminobutanoate. **Methods**. In vitro stability procedure was established for both standard curves and actual plasma samples. A small amount of acetonitrile was added as a cosolvent to help solublize the prodrugs. The plasma samples were incubated at 37 °C over a 24-hour watch period. Excess acetonitrile was used to stop possible enzymatic reactions in plasma at different preset time points. Each prodrug was studied individually by HPLC to monitor the concentrations of both the remaining prodrug and the accruing 17-AAG. **Results**. The stability of seven prodrugs was investigated in mouse plasma, recovered human plasma and fresh human plasma. It was found that each individual prodrug has a comparable degradation pattern in three different plasmas. The 17-AAG is the major degrading compound that was observed for all seven prodrugs. In all plasma samples the dimethylaminoacetate and the dimethylaminopropionate are the least stable. Approximately 50% of these two drugs are degraded to 17-AAG in the first five hours. Note that both aforementioned prodrugs have a tertiary amine on the end of acyl chain. Though the dimethylaminobutanoate is similarly structured, its degrading rate is far slower: the conversion to 17-AAG is only approximately 10% after 24 hours. It appears that the number of $-CH_2-$ group affects the degradation rate: there are three $-CH_2-$ groups on the acyl chain in the dimethylaminobutanoate while only one in the dimethylaminoacetate and two in the dimethylaminopropionate. It also appears that the prodrugs that have an amino group on the end of the acyl chain degrade to a lesser extent: the aminobutanoate almost has no degradation over 24 hours. In the first 6 hours the aminoacetate, the aminopropionate, and the aminopentanoate only degrade marginally. **Conclusions**. Stability profiles indicate that for each individual prodrug the degradation pattern is comparable in three different plasmas with 17-AAG as the major degrading compound. The dimethylaminoacetate and the dimethylaminopropionate are the least stable. The results also suggest that the structural differences in the acyl chain affect the degradation rate of prodrugs.

The Effect of Povidone on the Precipitation of Phenytoin Formulation

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ABSTRACT

Purpose. To evaluate the effect of the presence of povidone in the dilution media upon the extent of precipitation of the current commercial and high buffer capacity (β= 0.27) phenytoin formulations. **Methods**. High buffer capacity phenytoin formulations were prepared by modifying current commercial formulation (Na-Phenytoin: 50 mg/ml; Propylene glycol: 40%; Ethanol: 10%; Water: 50%; pH 12.0) by using 50% buffer instead of water. Isotonic Sorensen's phosphate buffer (ISPB) with or without water soluble polymers was used as the dilution medium. Polymer: povidone (from 0.03% to 1%), hydroxypropyl methylcellulose (HPMC) (1%), or carboxymethylcellulose sodium (CMC) (1%), was added into ISPB to make the dilution media. Precipitate was evaluated by means of one -to-one static series dilution and measured spectrophotometrically at 540 nm. **Results**. The results show that adding 1% povidone into ISPB dilution solution reduced precipitation by 50% for the current commercial formulations and completely eliminated precipitation for the high buffer capacity phenytoin formulations. Addition of 0.5%, 0.2%, 0.05%, and 0.03% povidone into ISPB respectively reduced the precipitate by about 94%, 92%, 85%, and 60% for the high buffer capacity formulations. Povidone gave the best results among the three tested polymers. **Conclusions.** Results demonstrated that adding 1% povidone into the dilution medium and using high buffer capacity phenytoin formulation completely eliminated precipitate on dilution. Povidone, which is an inert and non-toxic and reported to be used as drug carrier at the concentration up to 25%, could help reduce the precipitation of intravenous phenytoin injection.

Predicting the Entropy of Boiling for Organic Compounds

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ABSTRACT

This study aims to predict the entropy of boiling (ΔS_b) for organic compounds by considering parameters that are indicative of both molecular geometry and molecular association. The proposed method is a modification of Trouton's rule. It utilizes three geometric parameters σ, τ and ω that address molecular symmetry, flexibility and planarity, respectively, as well as a set of group contributors that focus on hydrogen bonding and strong dipolar interactions. The molecular geometry parameters are unique in that they reflect the ordering of molecules in the liquid that cannot be described by group contribution. The database of 903 organic compounds covers a broad array of structural categories. The root mean square error (RMSE) for the prediction is 2.12 J/mol·K, which is very low given the diversity of the database. An independent entropy dataset of 31 compounds was used to show that the proposed method is more accurate than four published methods. The success of this approach illustrates the need for non-additive structural parameters along with group additive parameters to characterize phase change entropy.

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