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International Journal of Pharmaceutics 218 (2001) 43–56

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Stabilization of eptifibatide by cosolvents

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Received 11 October 2000; received in revised form 29 January 2001; accepted 30 January 2001

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 eptifibatide, 10% ethanol, 40% propylene glycol and 50% 0.025 M citrate buffer. The stability study was conducted in the pH range 4.25–6.25 under accelerated temperatures: 48, 60, 72.5°C. The results indicate that the proposed semi-aqueous vehicles substantially increased the drug stability in comparison with aqueous vehicles. The predicted drug shelf-life T_{90} at 25°C shows that an almost twofold increase can be achieved by formulating eptifibatide in the semi-aqueous vehicle, which is 60 months at its maximum stability of pH 5.75, as opposed to the 33 months in the aqueous vehicle at its maximum stability of pH 5.25.

Keywords: Eptifibatide; Integrilin; Cosolvents; Stability

1. Introduction

Eptifibatide is a peptidic compound that contains a slightly modified RGD (arginine–glycine–aspartic acid) sequence, and is cyclized by a disulfide bridge between cysteine residue and mercaptopropionyl (des-amino cysteinyl) residue. Its chemical name: 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 (see Fig. 1, Scarborough, 1998). The drug reversibly inhibits platelet aggregation and thrombosis formation by preventing the binding of fibrinogen, von Willebrand factor, and other adhesive ligands to platelet receptor complex glycoprotein (GP) IIb/IIIa. Clinically, eptifibatide is indicated in the treatment of acute coronary syndrome and percutaneous coronary intervention (Scarborough, 1998).

Integrilin® (eptifibatide) Injection was launched by Cor Therapeutics Inc. and Key Pharmaceutical Inc. in 1998 (Description for Integrilin® Injection, 1998). The product is a clear and sterile solution

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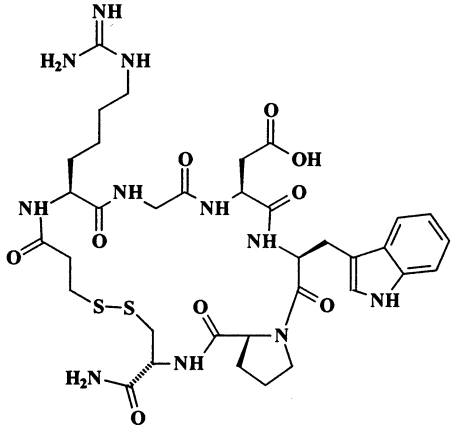
0378-5173/01/$ - see front matter
PII: S0378-5173(01)00618-4

for intravenous (IV) use. It contains 2 mg/ml eptifibatide in 0.025 M citrate buffer at pH 5.25 (Description for Integrilin® Injection, 1998). The product has a cold/refrigerator storage requirement (2–8°C or 36–46°F). In an effort to increase eptifibatide stability for room temperature storage and transport, this study proposes a semi-aqueous formulation that contains the same dose (2 mg/ml), 10% ethanol, 40% propylene glycol and 50% 0.025 M citrate buffer. Note that the controlled room temperature in the United States Pharmacopoeia is defined as 25°C with excursions permitted between 15 and 30°C.

The rationale for the proposal is that the semi-aqueous medium may reduce eptifibatide degradation by reducing the drug amide hydrolysis as well as other degradation. 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). It was found that the drug undergoes a variety of degradation routes: hydrolysis, isomerization, oxidation, dimerization (Table 1, 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 (see Table 1) is the major degradant in acidic conditions, 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-diketopiperazine (Table 1), 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.



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

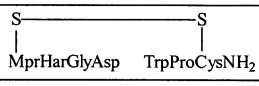
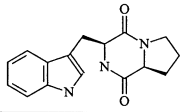
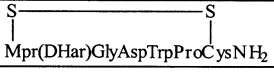
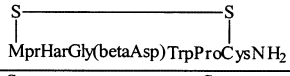
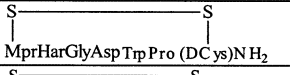
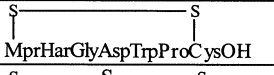
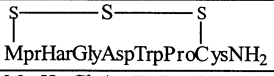
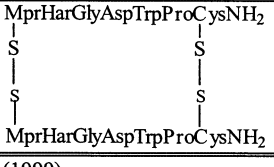
Fig. 1. Chemical Structure of Eptifibatide

Though rare in conventional organic chemistry, amide hydrolysis is often reported in drug degradation studies. Examples include chloramphenicol (Higuchi and Bias, 1953; Higuchi and Marcus, 1954), procainamide, nicotinamide (Connors et al., 1986), salicylamide, benzamide, *N*-substituted salicylamide and benzamide derivatives (Koshy, 1959), *N*-haloacetylphthalimides, and 1-acyl-3,5-dimethylpyrazoles (Stella and Higuchi, 1973), and klerval (Won et al., 1999).

Approximately more than 10% FDA approved injections use cosolvents to various extents (Sweetana and Akers, 1996). However, 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 that of purely aqueous solution. Bakar and Niazi (1983) studied aspirin decomposition in several media. They observed significant stability enhancement for the drug in water–propylene glycol 400 (4:1) solution. Gu and Strickley (1990) reported that moexipril, an angiotensin-converting enzyme inhibitor, undergoes hydrolysis as well as cyclization reaction, leading to the formation of diketopiperazines. In a 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. (1993) showed that aspartame stability is noticeably improved at pH 2

Table 1

Table 1 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)

in the presence of 10% cosolvent PEG 400 in comparison with the aqueous solution.

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

The study proposes a semi-aqueous vehicle that contains 2 mg/ml eptifibatide, 10% ethanol, 40% propylene glycol (PG) 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 such a combination (10% ethanol and 40% propylene glycol) was primarily based on the fact that it is the most often 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 are the same as the marketed Integrilin® Injection. The tested pH range is designed to be in the vicinity of the marketed formulation pH 5.25. Accelerated stability testing will be conducted for all the samples at three temperatures: 48°C, 60°C,

and 72.5°C. Drug degradation information at room temperature is obtained from Arrhenius plots by extrapolation, if that is possible.

2. Experimental

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). 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, Part Number: C-4015-2W). The aluminum seal has the Part Number 73825-11. Mettler AE163 balance (Analytical & Precision Balance Company, Phoenix, AZ) was used for all sample weighing.

2.2. Solubility

The aqueous solubility of eptifibatide was found to be 65 mg/ml in water. The drug solubility in other media was as follows: >300 mg/ml in 0.01 N HCl, 70 mg/ml in 0.025 M citrate buffer (pH 5.25), 25 mg/ml in methanol; >300 mg/ml in PG, and >200 mg/ml in the semi-aqueous vehicle (10% ethanol + 40% PG + 50% 0.025 M citrate buffer) at pH 5.75.

2.3. 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. No.: 9183565, Serial No. 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 elution program started with constant 17% ACN for 15 min (isocratic), switched to gradient elution with ACN concentration up to 100% in 5 min, and then returned to equilibrium in 2 min at the original 17% ACN. The injection volume was 100 μl, and the flow rate was 1.0 ml/min. The retention time of eptifibatide is approximately 10.1 ± 0.1 min at ambient temperature. The diode array detector connected to the HPLC provided the verification of peak homogeneity.

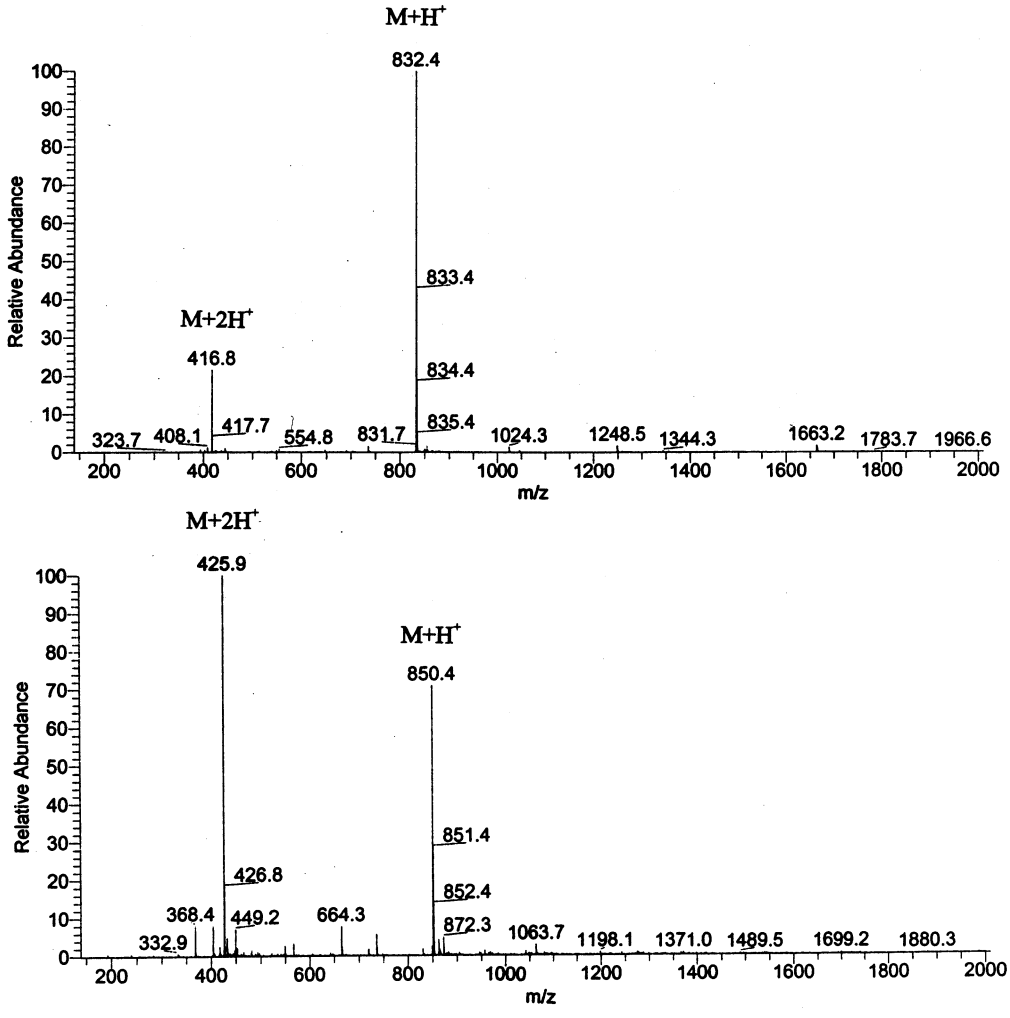
The HPLC system developed in this study can detect the major degradant, Asp-clipped eptifibatide which is eluted at 8.3 ± 0.1 min. This degradant can be rapidly generated in acidic conditions, e.g. storing eptifibatide in 0.5 N HCl for 24 h. Though structurally similar, the UV scans in the range of 200–600 nm display a subtle difference between the parent eptifibatide and degradant Asp-clipped eptifibatide: eptifibatide has an even double-peak at 200–220 nm while Asp-clipped eptifibatide shows a major peak in the same range. Fig. 2 shows the mass spectra for both eptifibatide and Asp-clipped eptifibatide with the degradant having an increase of 18 in molecular weight due to the hydrolysis. Fig. 3 shows a representative chromatogram of eptifibatide degradation in 0.025 M citrate buffer at pH 4.25 at 60°C after 53 days.

2.4. 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% 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 to 55.0 μg/ml. 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.

Eptifibatide HPLC assay validation: both precision (represented by the coefficient of variation, or CV%) and bias (represented by the standard deviation, or S.D.) in intra-day assay validation were less than 2%, which is well within the acceptable range (< 10%). Inter-day validation were similar: both precision (CV%) and bias (S.D.) were less than 5%.



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.

Fig. 2. Mass spectra of eptifibatide (top) and Asp-clipped eptifibatide (bottom).

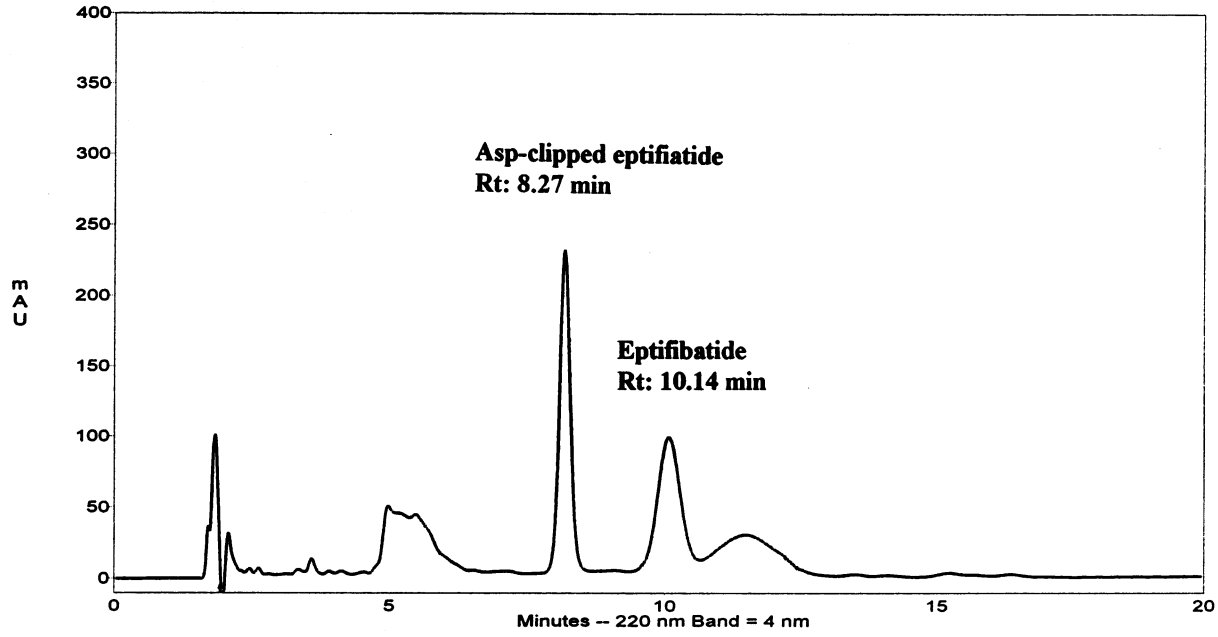


Fig. 3. HPLC chromatogram of eptifibatide degradation: 0.025 M citrate buffer, pH 4.25, temperature 60°C and after 53 days.

2.5. pH adjustment

The proposed study will use the apparent pH reading for all the semi-aqueous vehicles, though the correction factor was obtained experimentally and was found to be too small (< 0.1 pH units). The method was first developed by Van Uitert and Hass (1953) for dioxane–water mixtures and later validated by Bates et al. (1963) for use in an 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 are completely ionized in all the solvents studied. This assumption is considered to be valid when the solvent dielectric constant of the mixed solvent is above 39 (Van Uitert and Hass, 1953). It was found that the correction factor was in the range of 0.06–0.08 in 10% ethanol and 40% PG 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% PG).

2.6. 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.

2.7. 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 liquids.

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°C, 60°C and 72.5°C and were maintained at ± 0.2°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 h according to the studies in Section 2.6. The observed eptifibatide degradation rate constant (k_{obs}) used for all calculations was an average of three measurements. Approximately 8–10 time points were collected for each kinetic study.

2.8. Sample vial sealing test

The appropriateness of the vial sealing was tested to ensure that the proposed accelerated stability testing proceeds properly. The procedure 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 weight loss for samples containing 10% ethanol and 40% PG (four samples tested) was in the range of 0.00042–0.00140 (g), or 0.042%–0.140% (w/w) at 73°C over an 8-day period. Given the fact that the reproducibility of the balance is ± 0.0001 (g), the weight difference is considered to be negligible. Since these results were obtained under very harsh conditions (73°C), lower temperatures may further reduce the weight loss difference into the balance error range.

3. Results and discussion

3.1. Eptifibatide degradation kinetics

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

$$\ln[C] = \ln[C_0] - k_{obs}t \quad (1)$$

$[C_0]$ and $[C]$ are the initial and time (t)-dependent concentrations of eptifibatide, respectively.

Table 2 lists all k_{obs} data for eptifibatide degradation at various investigated pH values, cosolvent, and temperature conditions, as well as statistical significance indicators. 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.

3.2. pH rate profile

Fig. 4 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 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 over the tested pH range 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 pH rate profile is fairly flat with a slope of $\pm$ 0.4–0.6. It is possible that other degradation mechanisms may contribute to eptifibatide degradation (Martin, 1993).

3.3. Eptifibatide degradation

Under 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 is generated. Fig. 5 shows the peak area percentage of Asp-clipped eptifibatide produced in aqueous and semi-aqueous vehicles at pH 4.25–6.25 and 60°C after 53 days. Similar patterns for Asp-clipped eptifibatide were observed under different conditions, though with different magnitudes and proportions.

Interestingly, the rate of formation of Asp-

Table 2
Observed eptifibatide degradation rate constants (k_{obs}: /day^{-1}) in aqueous and semi-aqueous media

PH	Temp.	Aqueous			Semi-aqueous		
		k_{obs} (day^{-1})[a]	S.D.	r^2	k_{obs} (day^{-1})[a]	S.D.	r^2
4.25	72.5°C	0.1175	0.00870	0.9828	0.0802	0.00162	0.9886
4.75	72.5°C	0.0600	0.00992	0.9965	0.0352	0.00259	0.9970
5.25	72.5°C	0.0351	0.00190	0.9887	0.0225	0.00151	0.9816
5.75	72.5°C	0.0475	0.00249	0.9982	0.0190	0.00041	0.9969
6.25	72.5°C	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	60°C	0.0157	0.00043	0.9868	0.0090	0.00017	0.9607
5.25	60°C	0.0085	0.00034	0.9534	0.0048	0.00015	0.9812
5.75	60°C	0.0124	0.00070	0.9466	0.0049	0.00045	0.9758
6.25	60°C	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	48°C	0.0040	0.00011	0.9613	0.0022	0.00024	0.9612
5.25	48°C	0.0022	0.00016	0.9464	0.0016	0.00013	0.8470
5.75	48°C	0.0028	0.00011	0.8480	0.0012	0.00015	0.8685
6.25	48°C	0.0057	0.00013	0.9906	0.0021	0.00011	0.8312

[a] Average value ($n = 3$); S.D., standard deviation; r, regression coefficient.

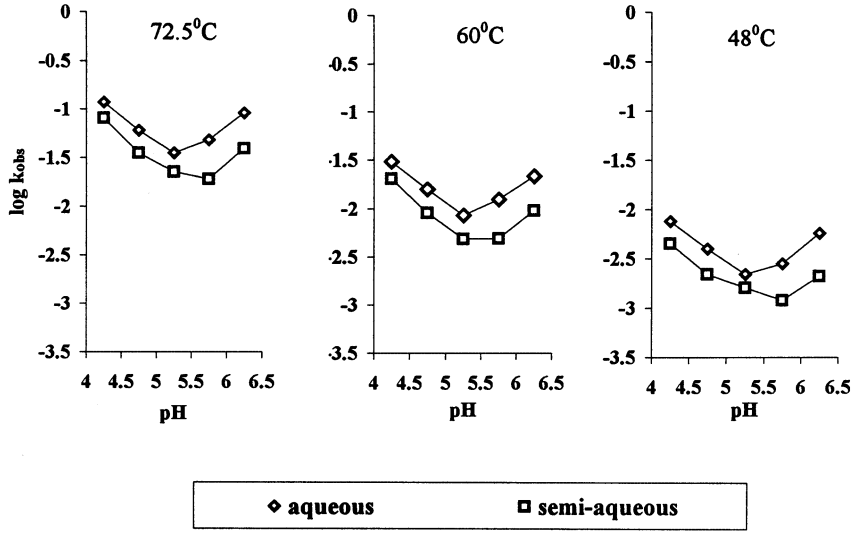


Fig. 4. pH rate profiles of eptifibatide (k_{obs}: (day^{-1}).

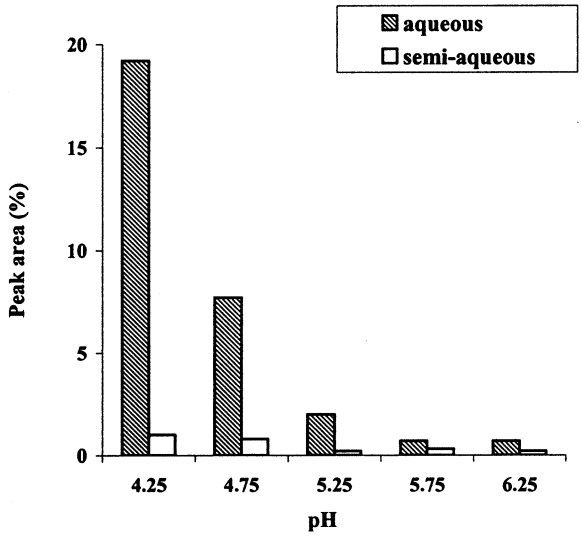


Fig. 5. Peak area (%) of Asp-clipped eptifibatide: pH 4.25–6.25, aqueous and semi-aqueous vehicles, temperature 60°C and after 53 days.

clipped eptifibatide decreases in the aqueous vehicle as the pH increases, becoming negligible above pH 5.25. There are two explanations: (a) the Asp-clipped eptifibatide may be generated, but at basic conditions it quickly decomposes to other peptidic fragments or further degradants such as Trp-Pro-diketopiperazine; and (b) other degradation routes such as oxidation, dimerization, aggregation may take over (more likely scenario).

The reported important degradants in alkaline pH (≥ 5.5) are trisulfide eptifibatide, which comes from oxidation, and eptifibatide dimer, which comes from dimerization (Van Gorp and Sluzky, 1999). Unfortunately, the current assay was not able to separate these degradation products. Assessment of peak area percentage of HPLC chromatograms indicates that in the basic pH, as Asp-clipped eptifibatide percentage decreased, the percentages of other impurity peaks, most of them overlapped, noticeably increased. This confirms the postulated change of drug degradation 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 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 vitamins also demonstrated that their oxidation degradation was pH-dependent (Florence and Attwood, 1988).

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

3.4. Possible mechanism for cosolvent effect

3.4.1. Collision theory

The addition of cosolvent partially replaces 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 a 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, which is the product of k_{H2O} and $[H_2O]$, reduces to half its value. The water concentration effect seems to be able to explain the reduced rate of drug degradation in 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.

3.4.2. Transition state theory

As an alternative to classical collision theory, transition state theory assumes that an equilibrium exists between normal reactant molecules and an activated complex of these molecules, or transition state. For an elementary bimolecular process, the reaction can also be written as follows:

$$A + B \rightleftharpoons (A\ldots B)^* \rightarrow \text{Product} \quad (2)$$

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, would destabilize 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 situation is reversed, i.e. the reaction rate is increased.

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 (carrying no charge), and thus be less favored in the semi-aqueous medium.

Eptifibatide contains two functional groups that can be ionized over the pH range of 4.25–6.25, i.e. a guanidino group on the homoarginine residue and a carboxylic acid group on the aspartic acid residue. The guanidino group is an extremely strong base. Its pK_a is approximately 12.48 (Loudon, 1988), and it should be protonated over the entire pH range. The carboxylic acid group has a pK_a of 3.87 (Loudon, 1988), which makes it ionized in the test pH range. By using the Henderson–Hasselbalch equation, the carboxylic acid group can be calculated as approximately 70.6% ionized at the lowest pH (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 pH range of 4.25–6.25. In other words, the drug species is mostly a zwitterion ($M\pm$) with a small percentage of protonated species (on the guanidino group) at the lower end of the test pH range.

It is known that amide hydrolysis, catalyzed by either acid or base, undergoes a charged transition state as illustrated in Fig. 6 (Ingold, 1969). It can be reasoned that when hydronium ion or hydroxyl ion reacts with eptifibatide, either $M+$ or $M\pm$, 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. Thus the 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. Nor 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.

3.5. General acid and general base catalysis

Of concern is whether 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 (0.0125 M), it is necessary to assess if such a difference would have an impact on eptifibatide degradation. Table 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 at 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 a meaningful impact on eptifibatide degradation.

3.6. Ionic strength effect

This study investigated the effect of ionic strength on eptifibatide degradation. Table 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.

3.7. Arrhenius plot and shelf-life prediction

Fig. 7 presents the Arrhenius plots for eptifibatide in both aqueous and semi-aqueous vehicles. Linearity at different pH solutions was observed ($r^2 = 0.989–0.999$). It is noted that initial eptifibatide studies were carried out at four temperatures: 37°C, 48°C, 60°C, and 72.5°C. However, the degradation data at 37°C were not

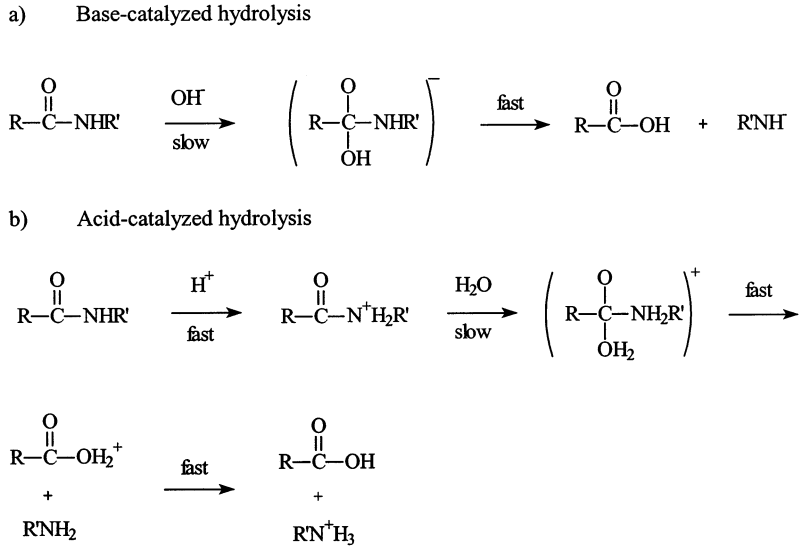


Fig. 6. Amide hydrolysis catalyzed by base and acid.

Table 3
Eptifibatide degradation and buffer catalysis

Citrate buffer	k_{obs}: $(day^{-1})^a$			
	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

[a] Mean values of duplicate readings; the ionic strength was kept at 0.2 μ.

Table 4
Eptifibatide degradation and ionic strength at 72.5°C

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

[a] Mean values of duplicate readings.
[b] Ionic strength (μ) was calculated from CHEMIST program (1998 version, MicroMath Research Inc., Salt Lake City, UT).

sufficient for reliable generation of a degradation rate constant; most samples had only 5–20% drug loss after 90 days. Thus, the 37°C data series were not included in all kinetic calculations.

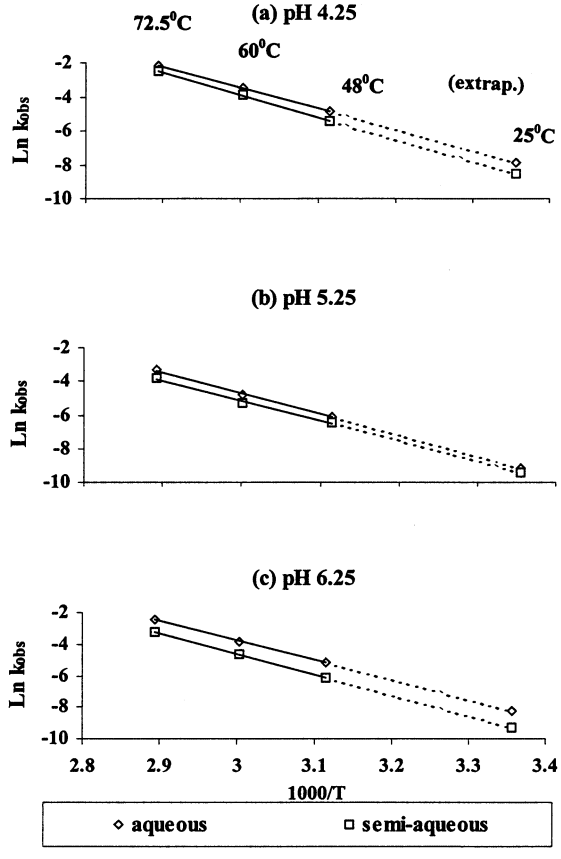


Fig. 7. Arrhenius plot for eptifibatide degradation (k_{obs}: (day^{-1}; T, Kelvin).

Fig. 8 provides the predicted shelf-life (T_{90}) of eptifibatide at 25°C. It shows that eptifibatide T_{90} is 33 months for the aqueous vehicle at maximum stability (pH 5.25), and it is increased almost twofold (60 months) for the semi-aqueous vehicle at maximum stability (pH 5.75).

3.8. Degradation in pure cosolvents

Eptifibatide is more stable in either pure ethanol or pure propylene glycol, than in all afore-

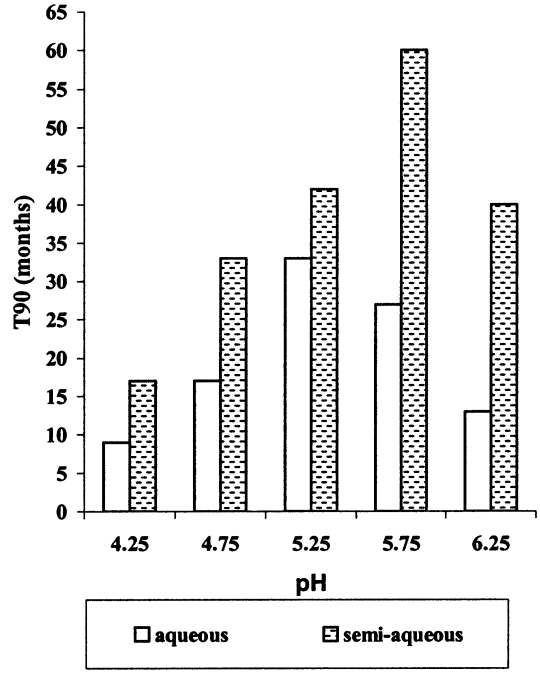


Fig. 8. Predicted eptifibatide shelf-life T_{90} at 25°C.

Table 5
Eptifibatide percentage remaining in cosolvents

Time	Eptifibatide (%)[a]			
(days)	60°C Propylene glycol	48°C Ethanol	60°C Propylene glycol	48°C 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

[a] Mean values of duplicate readings.

mentioned aqueous and semi-aqueous vehicles. Table 5 presents partial results for drug degradation in both propylene glycol and ethanol. Fig. 9 compares the degradation data in pure cosolvents with other aqueous and semi-aqueous vehicles, again at 60°C after 53 days. The fact that eptifibatide undergoes degradation in pure ethanol and propylene glycol indicates that solvolysis as well as other degradation pathways may contribute to the total drug degradation.

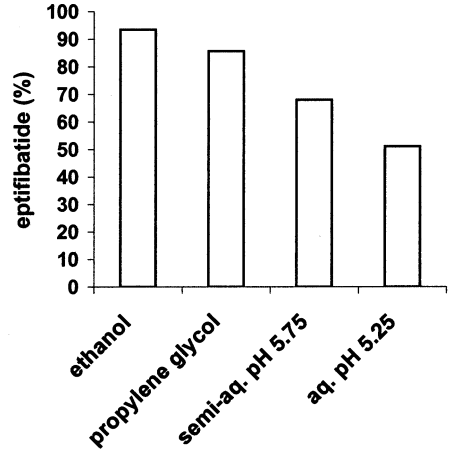


Fig. 9. Eptifibatide remaining (%) in various media at 60°C after 53 days.

4. 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 (pH 4.25–6.25), most likely a result of reduced hydrolysis-related degradation in the presence of cosolvents. The fact that cosolvents can be used to increase eptifibatide stability has important implications in future parenteral formulation development of other hydrolysis-susceptible drugs.

The degradation of eptifibatide is complex. It may involve several 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.

Acknowledgements

We would like to thank Cor Therapeutics Inc. for funding of this research.

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