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Compatibility of U-67590A Sterile Solution with Normal Saline, Dextrose Solution, and Solita-T No. 3 with or without Aminophylline Injectable

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ABSTRACT

The compatibility of U-67590A sterile solution (SS) with three drip fluids (normal saline, 5% dextrose solution, and Solita-T No. 3) and Neophylline^R (an aminophylline injectable) was studied. The entire volume of U-67590A SS in an ampoule was transferred into each of the drip fluid containers (500 mL fill). The container was laid under fluorescent light (ca. 1,200 lux) at room temperature. The physical appearance (color and precipitates), pH, U-67590A concentration, and degradation impurity levels were examined for 24 hr. The chemical and physical stability of U-67590A in these drip fluids was adequate enough so that any one of the three can be used for U-67590A administration. However, the stability was reduced when aminophylline co-existed in the admixtures because of increased pH levels. In this case, the stability depended on the strength of U-67590A SS added and the drip fluid used. In terms of chemical stability, Solita-T No. 3 was most favorable when admixed with aminophylline injectable. The fastest degradation was observed in normal saline containing aminophylline. This instability was caused by the higher ionic strength. Dextrose did not seem to affect the stability of U-67590A significantly. The Solita-T No. 3 admixture had the lowest pH, thereby overcoming the negative effect of ionic strength and giving the highest stability among the three admixtures. The stability of U-67590A in direct admixture with Neophylline^R also

depended on the strength of U-67590A; the stability increased with strength of U-67590A.

INTRODUCTION

U-67590A (sodium (+)-11β,17-dihydroxy-6α-methyl-21-[[8-[methyl(2-sulfoethyl)amino]-1,8-dioxooctyl]oxy]-1,4-pregnadiene-3,20-dione) sterile solution (SS) is being developed for the treatment of nephrosis and nephrotic syndrome, systemic lupus erythematosus, asthma, and renal transplant rejection. U-67590A SS is to be administered after mixing it with a drip fluid, such as normal saline (NS) or 5%-dextrose solution (D5W). Solita-T No. 3 (ST3), an electrolyte solution for infusion to supply water, is most commonly used with asthma patients in Japan and will be our choice with U-67590A SS. In the therapy of severe asthma, corticosteroids, such as U-67590A, are administered intravenously with aminophylline (1).

In this study, we examined the chemical and physical stability of U-67590A in the three drip fluids with or without aminophylline. The effects of the three drip fluids and Neophylline^R on the stability of U-67590A are discussed on the basis of pH, ionic strength, and self-micellization. In this report, "U-67590A strength" is given as the concentration in methylprednisolone equivalents, while "U-67590A concentration" denotes the concentration of U-67590A itself.

MATERIALS AND METHODS

Materials: U-67590A SS was supplied by The Upjohn Company, Kalamazoo, MI. The strengths (methylprednisolone equivalents) of U-67590A SS used were 50 mg/1 mL (Lot ZB134A), 100 mg/1 mL (Lot ZB140D), 200 mg/2 mL (Lot ZB140C), 400 mg/4 mL (Lot ZB140A), and 800 mg/8 mL (Lot ZB137B). Neophylline^R, an aminophylline injectable containing 250 mg of aminophylline and 11 mg of ethylenediamine in 10 mL (Eisai Ltd., Japan; Lot 2401 and Lot 2605) was purchased commercially. The following drip fluids (500-mL each) were examined: normal saline (NS) (Ohtsuka Pharmaceuticals Ltd., Japan) in a plastic bag (pH was 6.40, Lot M1L75) and in a plastic bottle (pH was 6.33, Lot K2D83); 5%-dextrose solution (D5W) (Ohtsuka Pharmaceuticals Ltd.) in a plastic bag (pH was 5.11, Lot

M2A92) and in a plastic bottle (pH was 5.33 Lot K2B73); and Solita-T No. 3 (ST3) (pH was 5.15, Shimizu Pharmaceuticals Ltd., Japan; Lot 20284 and Lot 20883). The ingredients of Solita-T No. 3 were (w/v%): 0.090% NaCl, 0.149% KCl, 0.224% sodium lactate, and 4.3% dextrose. The concentrations of Na^+, K^+, Cl^-, and L-lactate were 35, 20, 35, and 20 mEq/L, respectively.

HPLC Assay of U-67590A and Degradation Impurities: The concentrations of U-67590A and its degradation impurities (Table 1) were determined by an HPLC assay. The HPLC system (Shimadzu, Japan) was composed of a system controller (SCL-10A), a pump (LC-10AD), an autoinjector (SIL-10A), a column oven (CTO-10A), a UV spectrophotometric detector (SPD-10A) operated at 254 nm, a degasser (DGU-4A), and an integrator (C-R7A). The mobile phase was 750-mL water containing 11.6 g of ammonium acetate and acetic acid q.s. (quantum sufficient) (pH 5.8), 210 mL of acetonitrile, and 40 mL of tetrahydrofuran at a flow rate of 1.6 mL/min. The column was Inertsil PH (4.6x150 mm, 5 μm) (GL Science, Japan) heated at 40°C. The external standard method was employed. The injection volume was 15 μL. The typical retention times of MP, 17-E, N-DM, U-67590A, and 11-K were 9, 11, 17, 20, and 22 min, respectively.

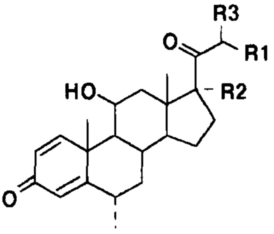
The concentration of U-67590A was calculated using the bulk drug [Lot (A2)7231-GR-155-J038] as a standard material. The levels of impurities, 17-E, N-DM, and 11-K, were obtained, based on the response factors, 1.0, 1.0, and 0.9, respectively. The concentration of MP was determined using the bulk drug (Lot 520KV) as a standard material.

Admixture Study of U-67590A SS with Drip Fluids with and without Aminophylline: The entire volume of U-67590A SS in an ampoule was transferred into each of the drip fluid containers (500-mL fill), using a disposable plastic syringe. The admixture was mixed gently, the physical appearance (color and precipitates) was observed, and an initial sample was removed to measure pH, U-67590A concentration, and degradation impurity levels. The container was laid on a desk in such a way that the admixture contacted the gum stopper under fluorescent light (ca. 1,200 lux) at room temperature. The admixture was observed for physical appearance and sampled after 1.5, 3, 6, and 24 hr. The fluorescent light was turned off during the night for approximately 12 hr.

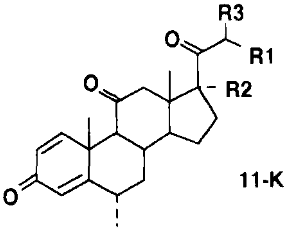
The compatibility of U-67590A with drip fluids with aminophylline injectable was examined in almost the same way. The entire volume of U-67590A SS was

TABLE 1

The Structures of U-67590A and Its Degradation Impurities.



U-67590A
17-E
N-DM
MP
21-17-MPH



11-K

	-R1	-R2	-R3
U-67590A	$-OCO(CH_2)_6CON(CH_3)CH_2CH_2SO_3Na$	-OH	-H
17-E	-OH	$-OCO(CH_2)_6CON(CH_3)CH_2CH_2SO_3Na$	-H
N-DM	$-OCO(CH_2)_6CONHCH_2CH_2SO_3Na$	-OH	-H
11-K	$-OCO(CH_2)_6CON(CH_3)CH_2CH_2SO_3Na$	-OH	-H
MP	-OH	-OH	-H
21-17-MPH	-OH	-H	-OH

introduced into each of the drip fluid containers containing Neophylline^R. The admixture was observed for physical appearance and sampled after 0, 1.5, 3, and 6 hr.

Stability Study of U-67590A in Various Sodium Chloride Solutions, Dextrose Solutions, and Solita-T No. 3 with Aminophylline: The effect of sodium chloride was investigated using NaCl solutions at various concentrations. NS was diluted with water for injection (WFI) to make 0.45% and 0.225% sodium chloride solutions. Aliquots (0.2 mL) of U-67590A SS (50 mg/mL) were transferred into 100-mL volumetric flasks. After adding 2 mL each of Neophylline^R into the flasks, the volumes were adjusted with WFI and 0.225%, 0.45%, and 0.9% NaCl solutions. The flasks were placed in a water bath thermostated at 25°C and protected from light. Sample solutions were removed after 0, 1.5, 3, 4.5, and 6 hr to determine U-67590A concentrations and the degradation impurity levels.

The effect of dextrose was studied in the same manner as that of NaCl using WFI and 1.25%, 2.5%, and 5% dextrose solutions.

ST3 containing U-67590A SS and aminophylline had a lower pH than NS and D5W containing the same (see RESULTS). The degradation of U-67590A SS in ST3 at a higher pH was examined by increasing the pH to approximately 8.7 with a 0.1N-NaOH solution. The mixture of U-67590A SS (10 mg/0.2 mL) and Neophylline[R] (2 mL) was diluted with the ST3 solution to 100 mL. The U-67590A concentration and the degradation impurity levels were monitored as mentioned above.

Admixture Study of U-67590A SS with Aminophylline: The compatibility of U-67590A with Neophylline[R] was examined by mixing the entire volume of U-67590A SS and Neophylline[R] directly in a 50-mL glass tube at room temperature under fluorescent light. The measurements were done after 0, 1.5, 3, and 6 hr.

RESULTS

Admixture Study of U-67590A SS with Drip Fluids with and without Aminophylline: In the admixture study with drip fluids without aminophylline (data not shown), no change in physical appearance was observed. The pH values of both NS and D5W admixtures were approximately 6, while those of ST3 admixtures were nearly 5 regardless of the U-67590A SS strength. No systemic variation of pH with time was observed. The concentrations of U-67590A in the admixtures were more than 99.0% of the initials at 24 hr, showing no significant degradation. The major impurities detected were MP, 17-E, N-DM, and 11-K. The impurity levels did not change throughout the 24-hour experiment.

Table 2 summarizes the results of the admixture study with the drip fluids with aminophylline. No change in physical appearance was observed throughout the 6-hr test. The pH levels of admixtures rose after the addition of Neophylline[R] because of ethylenediamine contained in Neophylline[R]. The initial pH levels of NS and D5W admixtures ranged from 8.5 to 8.8, whereas those of the ST3 admixtures were lower, in a range of 7.6 to 8.2. The initial pH levels were higher when the strength of U-67590A added was smaller. The pH was apt to decrease during the experiment. The decrease in the U-67590A concentration was apparent. The maximum degradation was observed in the NS admixtures while the ST3 admixtures showed the minimal potency loss. The strength of U-67590A SS admixed affected the degradation velocity; the higher strength showed favorable

TABLE 2

Stability of U-67590A SS in Drip Fluids with Neophylline[R] (Average of Two Experiments).

Admixture	U-67590A (%)				pH			
(hr)	0	1.5	3	6	0	1.5	3	6
50mg/NS[a]	100	92.7	85.9	76.5	8.73	8.71	8.71	8.76
50mg/D5W[a]	100	96.2	92.7	87.2	8.78	8.74	8.64	8.68
50mg/ST3[a]	100	98.8	97.6	94.3	8.08	7.79	7.94	7.88
100mg/NS[a,c]	100	91.9	85.2	75.6	8.71	8.72	8.73	8.66
100mg/D5W[a]	100	96.3	93.1	87.6	8.82	8.71	8.68	8.65
100mg/ST3[a]	100	97.1	96.2	93.8	8.11	7.96	7.94	7.91
200mg/NS[a]	100	92.1	86.8	78.2	8.75	8.75	8.66	8.62
200mg/D5W[a]	100	96.5	93.5	88.5	8.74	8.68	8.70	8.62
200mg/ST3[a,c]	100	99.1	98.3	95.8	8.04	7.88	7.86	7.85
400mg/NS[a]	100	93.7	89.5	82.1	8.67	8.67	8.56	8.61
400mg/D5W[a]	100	97.1	94.5	90.3	8.77	8.62	8.56	8.54
400mg/ST3[a]	100	99.1	97.8	96.5	7.99	7.84	7.80	7.70
800mg/NS[b]	100	97.9	95.1	90.9	8.53	8.50	8.45	8.33
800mg/D5W[b]	100	98.2	96.2	93.4	8.57	8.42	8.36	8.37
800mg/ST3[b,c]	100	99.0	98.9	98.3	7.74	7.61	7.56	7.47

[a] The solutions were clear and colorless throughout the experiments.
[b] The solutions were clear and slightly yellow throughout the experiments.
[c] One of the two measurements was done at 6.5 hr instead of 6 hr.

stability. Two of the major impurities, MP and 17-E, increased with time, while the levels of the other two, N-DM and 11-K, were almost plateau or decreased slightly (data not shown).

The stability of aminophylline in NS and D5W with U-67590A was examined in a preliminary study. Up to 15% concentration loss of U-67590A was observed for 3 hr, while the aminophylline concentration remained 100% (data not shown).

Stability Study of U-67590A in Various Sodium Chloride Solutions, Dextrose Solutions, and Solita-T No. 3 with Aminophylline: The stability of U-67590A SS in 0, 0.225, 0.45, and 0.9% NaCl solutions with aminophylline were investigated to assess the effect of NaCl (Table 3). The degradation of U-67590A was accelerated with an increase in NaCl concentration. The major impurities, MP and 17-E,

TABLE 3

Stability of U-67590A SS in NaCl Solutions with Neophylline^{R}.

NaCl (%)	U-67590A (%)					pH				
(hr)	0	1.5	3	4.5	6	0	1.5	3	4.5	6
0	100	94.5	89.6	85.7	82.0	9.01	8.99	8.98	8.98	8.98
0.225[a]	100	93.6	87.9	83.1	75.5	8.87	8.88	8.86	8.86	8.85
0.45	100	93.2	87.5	82.4	78.3	8.85	8.85	8.85	8.80	8.82
0.9	100	91.8	85.6	80.6	75.7	8.82	8.78	8.77	8.79	8.81

[a] The measurements were done at 6.5 hr instead of 6 hr.

appeared faster with higher NaCl concentrations (data not shown), concurring with the potency loss.

The effect of dextrose was investigated in the same way (Table 4). The pH was lower with higher dextrose concentration. The potency loss and formation of degradation impurities showed that the higher dextrose concentration affects the stability of U-67590A favorably.

ST3 had higher stability than the other two drip fluids (Table 2) when aminophylline co-existed. Table 5 summarizes the stability of U-67590A in ST3 with aminophylline at pH raised close to 9 with 0.1N-NaOH. The degradation of U-67590A was faster, compared to the data shown in Table 2 (50mg/ST3).

Admixture Study of U-67590A SS with Aminophylline: Table 6 shows the compatibility study results of U-67590A with Neophylline^{R}. The pH levels of the admixtures were beyond 9. The degradation of U-67590A was higher when the U-67590A concentration was lower. The major degradation impurities were MP and 17-E (data not shown). In this study, an additional peak was observed on the HPLC chromatograms, which had a retention time corresponding to 21-17-MPH (Table 1). A further study is required to identify this peak.

DISCUSSION

Many factors, such as pH, temperature, light, buffer composition, ionic strength, and additives, affect solution stability. Self-micellization is also known to

TABLE 4

Stability of U-67590A SS in Dextrose Solutions with Neophylline^{R}.

Dextrose (%)	U-67590A (%)					pH				
(hr)	0	1.5	3	4.5	6	0	1.5	3	4.5	6
0	100	94.4	90.0	85.8	82.1	9.00	8.97	8.98	8.98	8.98
1.25	100	95.1	90.8	86.9	83.6	8.92	8.92	8.90	8.89	8.89
2.5	100	95.6	91.7	88.5	85.5	8.92	8.86	8.84	8.82	8.81
5	100	96.2	93.0	90.2	87.9	8.84	8.74	8.71	8.70	8.68

TABLE 5

Stability of U-67590A SS in Solita-T No.3 (Higher pH) with Neophylline (Average of Two Experiments).

	U-67590A (%)					pH				
(hr)	0	1.5	3	4.5	6	0	1.5	3	4.5	6
	100	93.5	89.0	84.5	81.1	8.84	8.79	8.79	8.74	8.71

TABLE 6

Stability of U-67590A SS in Admixture with Neophylline^{R}.

U-67590A (mg)	U-67590A (%)				pH			
(hr)	0	1.5	3	6	0	1.5	3	6
50	100	91.1	85.5	78.1	9.19	n.d.[a]	n.d.	9.22
100	100	98.1	92.4	87.1	9.25	n.d.	n.d.	9.23
200	100	96.7	94.0	91.8	9.23	n.d.	n.d.	9.23
400	100	96.2	92.9	90.9	9.17	n.d.	n.d.	9.15
800	100	99.8	97.2	96.5	9.02	n.d.	n.d.	8.98

[a] Not determined.

enhance the stability of molecules such as those of U-67590A (2). The degradation of U-67590A is pH-dependent. The pH-rate minimum for diluted U-67590A solutions is 4.8, while that for solutions concentrated enough to form self-micelle is 5.5 (2). The admixtures without aminophylline had pH levels close to the pH minimum and no degradation was observed. By contrast, the pH levels of the admixtures with aminophylline shifted to the alkaline region and the degradation was accelerated. Table 2 indicates that the higher the U-67590A concentration in admixture, the lower the pH, and the admixture at a lower pH suppressed the degradation to some extent. As many corticoid prodrugs, U-67590A degrades by OH^- catalysis in an alkaline solution to form MP (hydrolysis of the side chain) and 17-E (migration of the 21-side chain) (3). In this study, the fact that MP and 17-E were the major degradation impurities suggested that the degradation was catalyzed by OH^- ion in the admixtures with aminophylline. The discussion below is focused on the different degradation velocities in the admixtures containing aminophylline.

The apparent first order rate constants k1 and k3, which stand for the formation of MP and 17-E, respectively, were calculated by the appearance rates (molar/hour) of MP and 17-E for the first 1.5 hr divided by the initial concentration of U-67590A (molar). In this study performed at pH approximately 9, the formation of MP and 17-E is considered to have been catalyzed by OH^- ions, and an increase in pH by 1 unit would result in 10 times as fast degradation (2). To cancel the effect of pH on the calculated rate constants (k_{obs}), the rate constants were converted to the "estimated rate constants at pH 9 (k_{pH9})" by the following equation:

$$k_{pH9} = k_{obs} \times 10^{9-pH} \qquad (1)$$

In Figure 1, the $k1_{pH9}$ and $k3_{pH9}$ estimated by the first 1.5-hr degradation (Table 2) were plotted as a function of U-67590A SS strength.

It has been reported that the stability of U-67590A is not affected by its concentration when the concentration is below cmc (critical micelle concentration), while the stability increases when the concentration increases above cmc because of self-micellization (4). The cmc of U-67590A has been reported to be 1.0-2.0 mM (0.67-1.3 mg/mL) in a solution of ionic strength (I) of 0.5 M (2) and 6.0 mg/mL when I=0.05 M (4). The cmc tends to be lower when the ionic strength is higher. The ionic strengths of NS, D5W, and ST3 are about 0.154, 0, and 0.055 M, respectively.

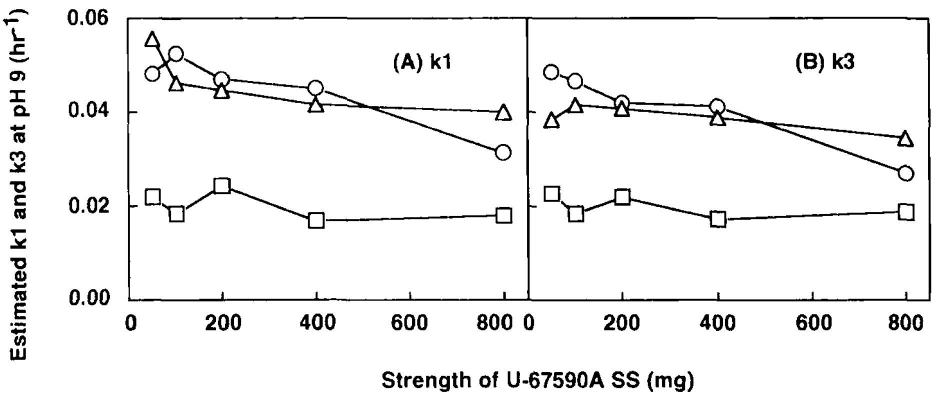


FIGURE 1

Effect of Strength of U-67590A SS on Its Degradation in Drip Fluids. ○: Saline, □: 5% Dextrose Solution, and △: Solita-T No.3. Mean of Two Experiments.

The cmc of U-67590A in NS, D5W, and ST3 may be speculated to be <6.0, >6.0, and ≈6.0 mg/mL, respectively. The U-67590A concentrations in the admixtures with the drip fluids were approximately 0.17, 0.34, 0.72, 1.5, and 2.9 mg/mL when the strengths of added U-67590A SS were 50, 100, 200, 400, and 800 mg, respectively.

The $k1_{pH9}$ and $k3_{pH9}$ for D5W were independent of the U-67590A strength (Figure 1), consistent with the estimation that the cmc in D5W is higher than the solution concentrations. This suggests that the stability of U-67590A in D5W with aminophylline was higher when the strength of U-67590A was higher because of the lowered pH and not because of self-micellization. The rate constants for NS containing 800 mg of U-67590A were smaller than the other saline admixtures (Figure 1), because the U-67590A concentration likely exceeded its cmc. In the case of saline, both pH and self-micellization seem to have acted in determining the U-67590A stability. The self-micellization does not seem to have affected the stability of U-67590A in the ST3 admixtures.

In Figure 2, the estimated $k1_{pH9}$ and $k3_{pH9}$ for the direct admixtures of U-67590A and Neophylline^R were plotted against the initial concentration of U-67590A. The degradation of U-67590A was suppressed as the U-67590A concentration increased. The initial concentrations were 9 to 80 mg/mL, which may be above cmc; thus, self-micellization would greatly affect its stability.

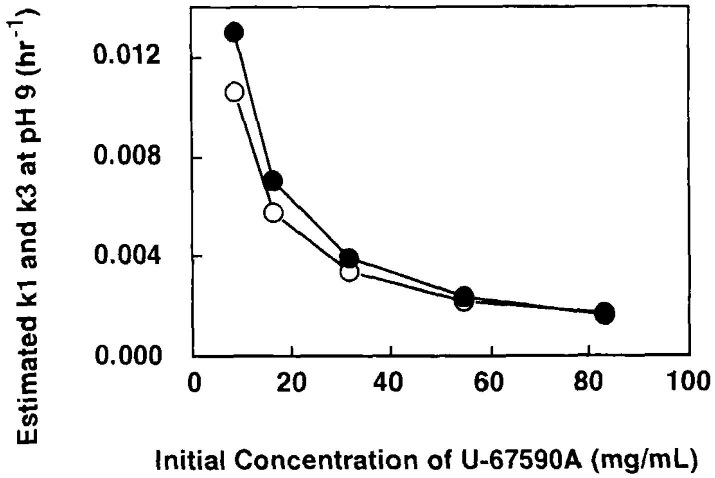


FIGURE 2

The Relationship between U-67590A Initial Concentration and Its Degradation in Direct Admixture of U-67590A SS and Neophylline. ○: Estimated k1 at pH9 and ●: Estimated k3 at pH9.

The $k1_{pH9}$ and $k3_{pH9}$ estimated for data in Table 4 were independent of the dextrose concentration (Figure 3), suggesting dextrose did not affect the U-67590A stability directly, and the stability of the dextrose solution was dominantly determined by the solution pH.

The reaction rate constants of electrolytes are known to be the function of the ionic strength of the reaction solution. For solutions of ionic strength (I) up to 0.1 M, the reaction rate constant is expressed as:

$$\log k = \log k_0 + 1.02 \cdot Z_A Z_B \frac{I^{1/2}}{1 + I^{1/2}} \qquad (2)$$

where Z_A and Z_B are the valence of reacting species (5). In the present study, Z_A and Z_B are -1 (U-67590A anion and hydroxy ion). When NaCl solutions were used, the ionic strength was attributed mainly to NaCl (0 to 0.154 M) and the contribution of the other electrolytes was small enough to be disregarded. Figure 4 illustrates the plots for NaCl solutions (data from Table 3) and ST3 (data from Table 5)

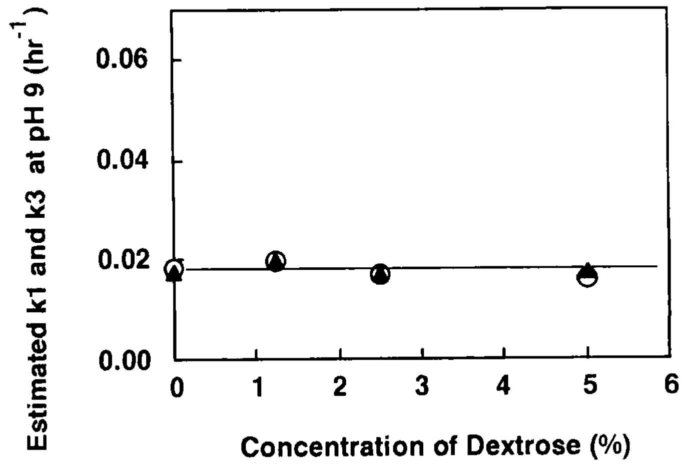


FIGURE 3

Effect of Dextrose on U-67590A Degradation. ○: Estimated k1 at pH9 and ▲: Estimated k3 at pH9.

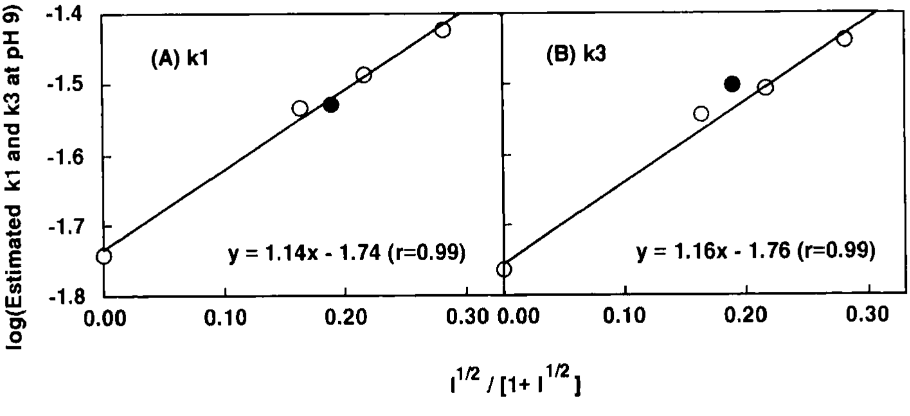


FIGURE 4

Effect of Ionic Strength (I) on U-67590A Degradation. ○: NaCl Solutions with Various Concentrations and ●: Solita-T No.3 Solution (Average of Two Experiments). The Regression Lines Were Obtained with Data of NaCl Solutions.

according to eq. (2) for k1 and k3 estimated at pH 9. The plots show fairly good correlations with slopes near 1. These plots suggest that the degradation in NS occurred faster than in other two drip fluids because of its higher ionic strength.

The ionic strength of ST3 solution was calculated to be 0.055 M, ranging between the 0.225% NaCl solution (I=0.038 M) and 0.45% NaCl solution (I=0.077 M). The plots based on eq. (2) revealed that the degradation rate in ST3 would be the same as that of the NaCl solution at the same pH and ionic strength (Figure 4), and would exceed that of D5W at the same pH. However, when aminophylline co-existed, the ST3 admixtures had lower pH levels than the D5W admixtures by an approximately 0.7 pH unit (Table 2). The positive effect of pH on the stability, thereby overcoming the negative effect of ionic strength, resulted in the highest stability in the ST3 admixtures.

CONCLUSIONS

1. The stability of U-67590A is adequate enough in NS, D5W, and ST3 so that any one of these three can be used for U-67590A administration.
2. The stability decreases when aminophylline co-exists in the admixture because of the increased pH, yet dependent on the strength of U-67590A and the drip fluid used.
3. The stability of U-67590A in NS is adversely affected by increasing ionic strength.
4. Dextrose does not seem to affect the stability of U-67590A significantly.
5. When U-67590A is co-mixed with aminophylline, the ST3 admixture has the lowest pH, among the three admixtures studied, thereby overcoming the negative effect of ionic strength and giving the maximum stability.

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