Stability Prediction of Nafamostat Mesilate in an Intravenous Admixture Containing Sodium Bisulfite[1)]

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The hydrolysis of nafamostat mesilate (NM) in aqueous solution was found to be accelerated by sodium bisulfite (SBS) using high-performance liquid chromatographic assay. The hydrolysis of NM catalyzed by SBS was pseudo-first-order and was considered to be a reaction between nafamostat cation (N^+) and sulfite ion. The effects of SBS concentration and temperature on the hydrolysis of NM in buffer solution were examined. From the findings obtained, we estimated the compatible pH range of the intravenous admixture (mixed infusion) of NM after a constant storage time at a constant SBS concentration and temperature employing a simulated pH-profile for the mixed infusion. In order to evaluate the compatibility of the prescribed mixed infusion, the method of pH estimation for the mixed infusion was also investigated using the pH titration curve of each preparation.

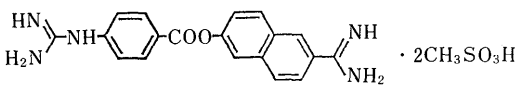
Keywords sodium bisulfite; nafamostat mesilate; degradation; intravenous admixture compatibility; pH estimation; stability estimation

Prediction of the stability of a drug in an intravenous admixture (mixed infusion) is very important for accurate and safe drug therapy. Generally, as reported by Koshiro and Fujita,[2)] the stability of a drug in a mixed infusion can be predicted from the pH-profile and Arrhenius equation of the degradation rate constants, if the temperature and pH of the test solution are given.

Sodium bisulfite (SBS), as a stabilizer in injectable preparations, is known to degrade various drugs including thiamine[3,4)] and fursultiamine.[4)] We previously applied the method reported by Koshiro *et al.*[2)] to estimate the stability of gabexate mesilate (GM) in the presence of SBS, and produced a nomograph for evaluation of the mixed infusion of GM containing SBS.[5)] Since the curves of the nomograph were drawn at restricted SBS concentrations and temperatures, full details could not be given.

In the present study, we examined the degradation of nafamostat mesilate (NM) (Chart 1), a protease inhibitor, due to catalytic hydrolysis by SBS. Kinetic studies of the degradation were also carried out. From the findings obtained, after a constant storage time, the compatible pH range of mixed infusion of NM was estimated from the simulated pH-profile of such infusion at a constant SBS concentration and temperature.

The method of pH prediction for the mixed infusions was then investigated in order to evaluate the compatibility of the mixed infusions. Employing the pH characteristic curve[6)] (PHC curve) (a kind of pH titration curve, see Fig. 6), a pH estimation method has been reported by Hirouchi *et al.*[7)] based on computer simulation. However, the theoretical equations described by Hirouchi *et al.* were derived from the model preparations for injection and ignored the influence of dilution with titrant. The PHC curve of the practical preparation could not therefore be fitted to the equations of Hirouchi *et al.*, and the PHC curve needed to be drawn from the data of many observed values after painstaking and time consuming work. In the present study, a theoretical and simple pH estimation method for the mixed infusion was also studied using the fitted PHC curve and curve simulation by a computer.



nafamostat mesilate

Chart 1

From the findings obtained, a computer program was developed to evaluate the compatibility of the mixed infusion of NM after a constant storage time at a constant SBS concentration and temperature, and to identify more stable conditions for the mixed infusions containing SBS.

Experimental

Materials NM was supplied by Torii Pharmaceutical Co., Ltd. SBS, 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), and other reagents were commercial products of special grade.

As buffer solutions, 0.05 M acetate buffer (pH 5.0—6.0) and 0.05 M 3,3-dimethylglutamate (pH 6.5—7.5) were adjusted to an ionic strength of 0.5 with sodium chloride. Distilled water which had been boiled and saturated with nitrogen gas was used.

NM for injection 10 mg (Futhan®, Torii Pharmaceutical Co., Ltd., Lot No. FMK06B), EL-Solution No. 3® (glucose and electrolyte infusion, Morishita Pharmaceutical Co., Ltd., Lot No. SK02A) and other commercial injections and infusions were employed as injectable preparations.

Kinetic Procedures The stability of the NM solution (7.4×10^{-5} M) buffered at pH 5.0—7.5 in the presence of SBS (3.7×10^{-3} M) was examined at 25 °C under a nitrogen atmosphere. On the other hand, the stability of the NM solution (7.4×10^{-5} M) buffered at pH 5.0—7.5 in the absence of SBS was studied by the isothermal method at various temperatures under a nitrogen atmosphere.

Determination of NM Nafamostat mesilate was measured by high-performance liquid chromatography (HPLC) according to the method reported by Kyakui *et al.*[8)] with some modifications.

1) Equipment A Hitachi 655-11 high-performance liquid chromatograph, a Hitachi 655A UV detector, and a Shimadzu C-R3A chromatopack were used.

2) Measurement Conditions Column, Lichrosorb RP-8 5 μm (4.0 mm i.d. × 250 mm); mobile phase, 0.1 M acetate buffer (pH 3.0, containing 0.05 M 1-heptanesulfonate)–acetonitrile (65 : 35); flow rate, 1.0 ml/min; column temperature, 40 °C; and detection wavelength, 240 nm. As an internal standard, papaverine hydrochloride was employed, and a 10 μl aliquot was injected into the HPLC.

Estimation of the pH of Mixed Infusions In order to estimate the pH of the mixed infusion, many observed values of the pH titration were necessary to draw the PHC curve of each preparation. Based on the general equation for the PHC curves, the PHC curve of each preparation was fitted from some of the observed values. Using the PHC curve of each preparation, the pH of the mixed infusion was estimated from the simulated PHC curve of the mixed infusion.

1) pH Titration Various commercial injections were diluted to 500 ml with distilled water and titrated with 0.1 N HCl and NaOH. Similarly, 500 ml aliquots of various commercial infusions were titrated with 0.1 N HCl and NaOH.

2) Fitting of the PHC Curve of the Injectable Preparation Injectable drugs are generally classified as weak acids, weak bases or their salts. First, taking a preparation of a weak acid of which the total concentration is C_{a1}, the ionization of the weak acid may be represented as $HA \rightleftarrows H^+ + A^-$, where HA is the non-ionized acid, A^- is the ionized acid and H^+ is hydrogen ion. Applying the law of mass action, material balance and charge balance to the equilibrium of this weak acid, the general equation of the PHC curve for the preparation can be expressed as in Eq. 1. Similarly, taking a preparation containing two kinds of weak acids of which the total concentrations are C_{a1} and C_{a2}, the general equation for the preparation can be expressed as in Eq. 2. If a preparation contains n kinds of weak acids, the general equation of the PHC curve for the preparation can be expressed as in Eq. 3.

In order to improve solubility, the preparation of a weak base often contains a strong acid as an excipient. The preparation of the weak base can therefore be assumed to represent a preparation of the conjugated weak acid. Accordingly, the general equation of the PHC curve for the preparation of the weak base can be expressed as in Eq. 1, and the PHC curve of the preparation of n kinds of weak bases can be represented as in Eq. 3.

On this basis, the general equation of the PHC curve of injectable preparations can for practical purposes be represented as Eqs. 1, 2 or 3. The influence of dilution with titrants was corrected by Eqs. 4—6.

The parameters of the general equations of the PHC curve were fitted with some of the observed values. The parameters of the PHC curve (*i.e.* concentrations and pK_s of weak acids, and concentration of strong acid or base) were obtained by solving the simultaneous equations of Eq. 2. Next, the parameters except pK_s were fitted by the nonlinear least-squares method (simplex method[9]). The PHC curve simulated by these parameters and the observed titration values was drawn graphically. If the PHC curve partially fitted, the parameters of the PHC curve were modified by the simultaneous equations of Eq. 1 and the simplex method.

$$[H^+]=\frac{C_{a1}\cdot K_1}{[H^+]+K_1}+\frac{K_w}{[H^+]}+C+C_t \tag{1}$$

$$[H^+]=\frac{C_{a1}\cdot K_1}{[H^+]+K_1}+\frac{C_{a2}\cdot K_2}{[H^+]+K_2}+\frac{K_w}{[H^+]}+C+C_t \tag{2}$$

$$[H^+]=\sum_{i=1}^{n}\frac{C_{ai}\cdot K_i}{[H^+]+K_i}+\frac{K_w}{[H^+]}+C+C_t \tag{3}$$

$$C_{an}=\frac{C'_{an}\cdot V_0}{V_0+V_t} \tag{4}$$

$$C=\frac{C'\cdot V_0}{V_0+V_t} \tag{5}$$

$$C_t=\frac{C'_t\cdot V_t}{V_0+V_t} \tag{6}$$

where C_{a1}, C_{a2}, $C_{a3}\cdots C_{an}$ are the concentrations of weak acids 1, 2, $3\cdots n$ in the sample solution, and K_1, K_2, $K_3\cdots K_n$ are the dissociation constants of the weak acids 1, 2, $3\cdots n$, respectively, C is the concentration of the strong acid (represented as a positive value) or the base (negative value) in the sample solution contained as an excipient and C_t is the concentration of HCl (represented as a positive value) or NaOH (negative value) in the sample solution added as the titrant. C'_{an} and C' are the initial concentration of the weak acid and that of the strong acid or base in the sample solution, respectively. C'_t is the concentration of HCl or NaOH added to the sample solution as the titrant. V_0 is the initial volume of the titration sample (500 ml) and V_t is the volume of the titrant added to the sample. $[H^+]$ is the hydrogen ion concentration. K_w is the ion product of water. In this paper, for practical purposes, $[H^+]$ and K_w at 25 °C were regarded as $10^{-pH_{obs}}$ and 1.0×10^{-14}, respectively.[10]

3) Simulation of the PHC Curve of Mixed Infusions The effect of dilution was an important factor when the parameters of the PHC curve were fitted to the titration data. Nevertheless, once these parameters were obtained, the PHC curves of the preparations could be treated simply according to Eqs. 1, 2 or 3 without Eqs. 4—6. Therefore, assuming the general equations of the PHC curves for an injection, an infusion, a mixed infusion of these preparations and water to be Eqs. 7, 8, 9 and 10, respectively, the relationship Eq. 9 = Eq. 7 + Eq. 8 − Eq. 10 held at a constant hydrogen ion concentration. Each of the terms except C_{tA}, C_{tB}, C_{tw} and C_{tM} could then be eliminated from Eqs. 7—10 at a constant hydrogen ion concentration, and Eq. 11 was obtained. Since each PHC curve is represented as the relation between the pH (*i.e.* hydrogen ion concentration) and the volume of the titrants (*i.e.* C_{tn}), the PHC curves of mixed infusions could be simulated by applying Eq. 11 at all pHs. If n kinds of preparations were mixed, the PHC curve of the mixed infusion could be simulated from the PHC curves of $n-1$ kinds of preparations and another additive injection.

$$[H^+]=\frac{C_{aA}\cdot K_A}{[H^+]+K_A}+\frac{K_w}{[H^+]}+C_A+C_{tA} \tag{7}$$

$$[H^+]=\frac{C_{aB}\cdot K_B}{[H^+]+K_B}+\frac{K_w}{[H^+]}+C_B+C_{tB} \tag{8}$$

$$[H^+]=\frac{C_{aA}\cdot K_A}{[H^+]+K_A}+\frac{C_{aB}\cdot K_B}{[H^+]+K_B}+\frac{K_w}{[H^+]}+C_A+C_B+C_{tM} \tag{9}$$

$$[H^+]=\frac{K_w}{[H^+]}+C_{tw} \tag{10}$$

$$C_{tM}=C_{tA}+C_{tB}-C_{tw} \tag{11}$$

4) Revision of the PHC Curve for Increase of Infusion Volume The influence of dilution with additive injections was omitted in Eq. 11. If the infusion volume was increased with additive injections, the PHC curves of the mixed infusion must be corrected by Eq. 12. This equation was obtained by following the same treatment as for Eq. 11.

$$C_{tC}=r\cdot C_t-(r-1)\cdot C_{tw} \tag{12}$$

where r is the concentration ratio (initial infusion volume (500 ml)/increased infusion volume with additives), C_{tC} is the corrected concentration of HCl or NaOH (added as the titrant) in the mixed infusion at a constant pH, C_t is the concentration of HCl or NaOH in a 500 ml aliquot of the mixed infusion at the same pH, and C_{tw} is the concentration of HCl or NaOH in a 500 ml aliquot of distilled water at the same pH. At C_t and C_{tw}, the influence of dilution with additive injections was omitted.

5) Simulation of the PHC Curve of a Mixed Infusion According to Eqs. 11 and 12, the PHC curve of the mixed infusion could be simulated from the PHC curve of each preparation.

6) Estimated pH of a Mixed Infusion The pHs of the injections (diluted to 500 ml with distilled water) or infusions are represented by the pHs at the origin of the PHC curves of the preparations. The estimated pH of the mixed infusion was thus obtained as the pH at the origin of the simulated PHC curve.

Results and Discussion

Degradation Rate of NM in the Presence and Absence of SBS NM is known to be hydrolyzed to 4-guanidinobenzoic acid and 6-amino-2-naphthol in the absence of SBS.[11] HPLC of NM in the presence of SBS revealed peaks of the same degradation products (Fig. 1). The degradation kinetics of NM in the presence and absence of SBS were examined at pH 5.0—7.5. Since NM in the absence of SBS was rather stable at pH 5.0—7.5, the degradation rate of NM alone (k_0) was measured by the isothermal method at various temperatures. The degradation rate constant of NM in the presence of SBS (k_{obs}) was measured at 25 °C. The data in Fig. 2 show that the hydrolysis of NM in both the presence and absence of SBS obeys pseudo-first-order kinetics.

Effects of SBS Concentrations The degradation of NM at an initial concentration of 7.4×10^{-5} M by SBS at various concentrations was evaluated at 25 °C and pH 6.5. Typical plots for SBS concentration *vs.* degradation rate constants of NM yielded a straight line as shown in Fig. 3. The rate constant of the catalytic hydrolysis of SBS (k_{SBS}) obtained from the slope of the line was $323.0\,M^{-1}h^{-1}$, and the intercept of this line on the y axis was $8.71\times10^{-4}h^{-1}$,

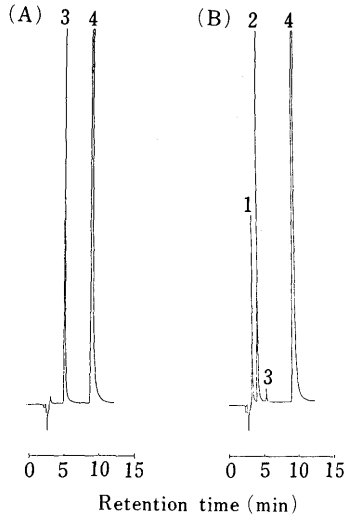


Fig. 1. HPLC of NM

(A) NM standard; (B) NM (7.4×10^{-5} M) in the presence of SBS (3.7×10^{-3} M) in 0.05 M 3,3-dimethylglutamate buffer (pH 7.5) at 25 °C and $\mu=0.5$ (1 h incubation). Peak 1, *p*-guanidinobenzoic acid; peak 2, 6-amidino-2-naphthol; peak 3, NM; peak 4, internal standard (papaverine hydrochloride).

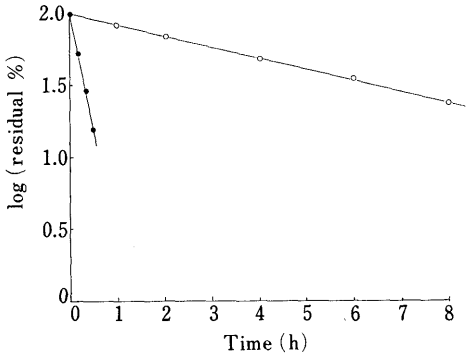


Fig. 2. First-Order Plots for the Degradation of NM in the Presence and Absence of SBS in 0.05 M 3,3-Dimethylglutamate Buffer (pH 7.5) at $\mu=0.5$

Initial concentration of NM, 7.4×10^{-5} M. ○, $[SBS]_{total}=0$ M at 50 °C; ●, $[SBS]_{total}=3.7\times10^{-3}$ M at 25 °C.

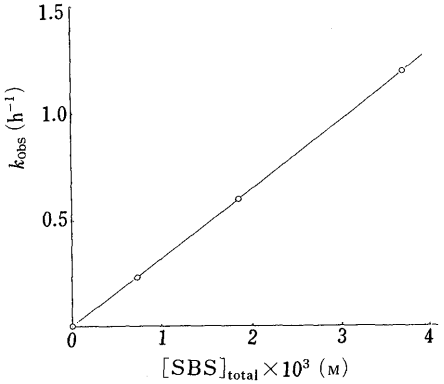


Fig. 3. Relationship between the Total Concentration of SBS and the Pseudo-First-Order Rate Constant (k_{obs}) for the Degradation of NM by SBS in 0.05 M 3,3-Dimethylglutamate Buffer (pH 6.5) at 25 °C and $\mu=0.5$

Initial concentration of NM, 7.4×10^{-5} M.

which corresponded to the rate constant of NM in the absence of SBS (k_0). The rate constant of NM in the presence of SBS (k_{obs}) can thus be represented as in Eq. 13.

$$k_{obs}=k_0+k_{SBS}\cdot[SBS]_{total} \quad (13)$$

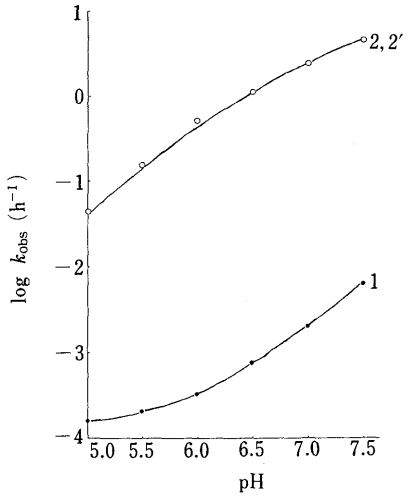


Fig. 4. pH-Profiles of the Degradation of NM in the Presence and Absence of SBS at 25 °C and $\mu=0.5$

Initial concentration of NM, 7.4×10^{-5} M. ●, $[SBS]_{total}=0$ M; ○, $[SBS]_{total}=3.7\times10^{-3}$ M. 1, theoretical curve calculated from Eq. 14; 2, curve for SBS-dependent degradation of NM in NM–SBS solution ($k_{obs}-k_0$); 2′, theoretical curve calculated from Eq. 16.

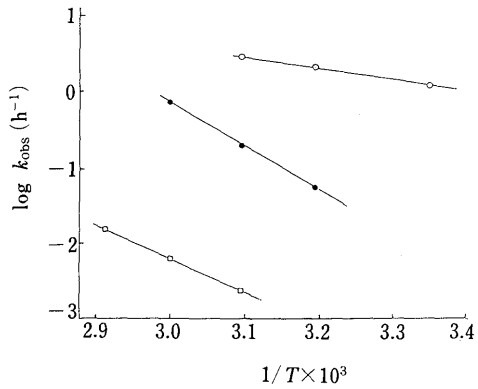


Fig. 5. Arrhenius-Type Relationship between the Degradation Rate Constant of NM and Temperature in the Presence (pH 6.5) and Absence (pH 5.0 and 7.5) of SBS in Buffer Solutions at $\mu=0.5$

Initial concentration of NM, 7.4×10^{-5} M. ○, $[SBS]_{total}=3.7\times10^{-3}$ M in 0.05 M 3,3-dimethylglutamate buffer (pH 6.5); ●, $[SBS]_{total}=0$ M in 0.05 M 3,3-dimethylglutamate buffer (pH 7.5); □, $[SBS]_{total}=0$ M in 0.05 M acetate buffer (pH 5.0).

pH-Profiles At various pHs, the degradation of NM at 7.4×10^{-5} M in the presence (3.7×10^{-3} M) or absence of SBS was studied at 25 °C or by the isothermal method at various temperatures, respectively. The pH-profiles of NM degradation at 25 °C in the presence and absence of SBS are shown in Fig. 4, where the rate constants are expressed on a logarithmic scale and the rate constants of NM at 25 °C in the absence of SBS were obtained from Arrhenius plots (see Fig. 5 as an example). Closed circles represent the rate constants of NM degradation in the absence of SBS (k_0), and open circles represent the rate constants of NM degradation in the presence of SBS (k_{obs}).

The concentration of nafamostat cation (N^+) was considered to be equal to the total NM concentration based on the assumption that NM is present in the form of N^+ in the pH range of 5.0—7.5.[11] It was concluded that water-catalyzed and specific hydroxide ion-catalyzed degradations were proceeding at pH 5.0—7.5 from the pH-profile of NM in the absence of SBS. The pseudo-first-order rate

constant of NM degradation in the absence of SBS can therefore be expressed as in Eq. 14.

$$k_0 = k_{H_2O} + k_{OH} \cdot \frac{K_w}{[H^+]} \quad (14)$$

where k_{H_2O} is the first-order rate constant for water-catalyzed degradation, k_{OH} is the second-order rate constant for hydroxide ion-catalyzed degradation. These fractional rate constants were estimated by the simplex method,[9] and values of $k_{H_2O} = 1.181 \times 10^{-4}\,h^{-1}$ and $k_{OH} = 22390\,M^{-1}\,h^{-1}$ were obtained. The theoretical curve for k_0 (curve 1) obtained with these values fitted well to the measured values of k_0.

Based on the dissociation constants of SBS ($k_1 = 1.72 \times 10^{-2}$ and $K_2 = 6.24 \times 10^{-8}$),[12] SBS in the pH range of 5.0—7.5 was considered to be present as bisulfite ion (HSO_3^-) and sulfite ion (SO_3^{2-}). From the hydrolysis of NM by SBS alone (curve 2), NM was thought to be catalyzed mainly by sulfite ion. From these findings, assuming that $k_{obs} - k_0$ (curve 2) is dependent on the concentration of sulfite ion, Eq. 15 can be obtained from Eq. 13 and the dissociation equilibrium of SBS.

$$k_{obs} - k_0 = k_{SO_3^{2-}} \cdot \frac{[SBS]_{total} \cdot K_1 \cdot K_2}{[H^+]^2 + K_1 \cdot [H^+] + K_1 \cdot K_2} \quad (15)$$

where $k_{SO_3^{2-}}$ is the rate constant for the catalytic hydrolysis of sulfite ion. Equation 15 was solved by the least-squares method, and $k_{SO_3^{2-}} = 1520\,M^{-1}\,h^{-1}$ was obtained. The theoretical curve of $k_{obs} - k_0$ (curve 2′) obtained by applying this value to Eq. 15 was almost equal to the curve of $k_{obs} - k_0$ (curve 2). These findings show that the hydrolysis of NM in the presence of SBS in the pH range of 5.0—7.5 can be represented practically as in Eq. 16.

$$k_{obs} = k_{H_2O} + k_{OH} \cdot \frac{K_w}{[H^+]} + k_{SO_3^{2-}} \cdot \frac{[SBS]_{total} \cdot K_1 \cdot K_2}{[H^+]^2 + K_1 \cdot [H^+] + K_1 \cdot K_2} \quad (16)$$

Influence of Temperature on Degradation Rate As shown in Fig. 5, the Arrhenius plots revealed good linearity in the presence and absence of SBS. The values for the activation energy (E_a) of NM degradation in the presence or absence of SBS were evaluated from the slope of the line. The E_a values of NM degradation in the presence (pH 6.5) and absence (pH 5.0 and 7.5) of SBS were 6.81, 20.6 and 24.8 kcal mol^{-1}, respectively. The E_a value in the presence of SBS was about 1/3—1/4 times that in the absence of SBS. These findings reflect the catalytic action of SBS.

Based on the E_a values in the absence of SBS, the E_a values of k_{H_2O} and k_{OH} were estimated to be 20.6 and 24.8 kcal mol^{-1}, respectively. Although the rate constant k_{obs} in the presence of SBS was as described in Eq. 16, the influence of $k_{SO_3^{2-}}$ was much larger than those of k_{H_2O} and k_{OH}. The E_a value in the presence of SBS could thus be approximated to that of $k_{SO_3^{2-}}$. In other words, the E_a value in the presence of SBS at pH 6.5 was considered to represent that of the catalytic hydrolysis of NM by sulfite ion. By application of Arrhenius' law, one of the fractional rate constants can be represented as in Eq. 17.[2]

$$k_2(T_2) = k_1(T_1) \cdot \exp(-E_{a1}/R) \cdot \frac{T_1 - T_2}{T_1 \cdot T_2} \quad (17)$$

where $k_1(T_1)$ and $k_2(T_2)$ are the fractional rate constants at two temperatures, E_{a1} is the activation energy of the fractional reaction, R is the gas constant and T_1 and T_2 are the absolute temperatures. From Eqs. 16 and 17 and the E_a values obtained, k_{obs} of NM in the presence of SBS at temperature T_2 may be expressed as in Eq. 18 over the relatively small range of pH (5.0—7.5).

$$\begin{aligned} k_{obs}(T_2) = {} & k_{H_2O}(T_1) \cdot \exp(-E_{a1}/R) \cdot \frac{T_1 - T_2}{T_1 \cdot T_2} \\ & + k_{OH}(T_1) \cdot \exp(-E_{a2}/R) \cdot \frac{K_w}{[H^+]} \cdot \frac{T_1 - T_2}{T_1 \cdot T_2} \\ & + k_{SO_3^{2-}}(T_1) \cdot \frac{[SBS]_{total} \cdot K_1 \cdot K_2}{[H^+]^2 + K_1 \cdot [H^+] + K_1 \cdot K_2} \\ & \times \exp(-E_{a3}/R) \cdot \frac{T_1 - T_2}{T_1 \cdot T_2} \end{aligned} \quad (18)$$

where the value of E_{a1} (20.6 kcal mol^{-1}) represent that of water-catalyzed degradation, the value of E_{a2} (24.8 kcal mol^{-1}) represents that of specific hydroxide ion-catalyzed degradation, and the value of E_{a3} (6.81 kcal mol^{-1}) represents that of sulfite ion-catalyzed degradation.

Prediction of the Stability of NM in Mixed Infusions Employing Eq. 18, the pH-profile of NM in mixed infusion containing SBS at a constant temperature could be simulated with the computer. In addition, the degradation rate constant (k_t) at which NM degrades to 90% of the initial concentration (utilizable concentration) after incubation for a time interval t could be represented by Eq. 19.

$$k_t = \frac{-\log 0.9 \cdot 2.303}{t} \quad (19)$$

In accordance with the Eq. 19, the degradation rate constant k_t at a constant storage time was plotted on the curve of the estimated pH-profile for the mixed infusion of NM (Fig. 6). From the curve in Fig. 6, the mixed infusion of NM was considered to be compatible at the pH range of the 5.0-plotted value. Thus, the compatible pH of each mixed infusion of NM after a constant storage time at a constant SBS concentration and temperature could be readily estimated.

pH Estimation Method of Mixed Infusion Since NM is attacked by sulfite ion and the pK_2 of sulfite ion is 7.2,[12] pH is a most important factor practically for the compatibility of the mixed infusion of NM at a constant SBS concentration. The pH estimation method of the mixed in-

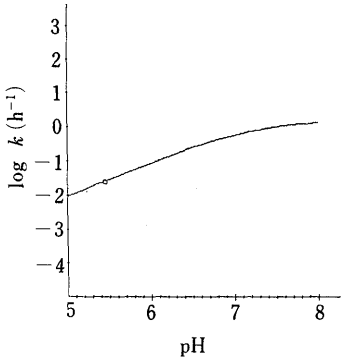


Fig. 6. Estimation of the Compatible pH Range of NM in Mixed Infusion at 1.30×10^{-3} M of SBS at 18 °C and the Conditions of 4 h Storage

Initial concentration of NM, 7.4×10^{-5} M. —, simulated pH-profile of the degradation of NM; ○, pseudo-first-order rate constant of NM at which NM degrades to 90% residual after 4 h; estimated compatible pH range (5.0—5.45).

TABLE I. Parameters of PHC Curves of Commercial Preparations for Injection

	Sample[a] (Brand name)	$C^{b)}$	pK_1	$C_1^{c)}$	pK_2	$C_2^{c)}$	pK_3	$C_3^{c)}$
A	Bisulase (10 mg)	−1.45	2.03	1.46	12.2	21.4		
B	Futhan (10 mg)	−4.93	2.73	4.99	7.42	0.0934	12.2	3.46
C	Lasix (20 mg)	−0.239	2.98	0.240	12.3	59.5		
D	Pydoxal (10 mg)	−5.79	2.52	5.31	7.14	0.988	11.0	4.23
E	Vitacimin (500 mg)	−6.31	4.45	6.39	11.0	4.79		
F	EL-Solution No. 3	−11.9	4.04	12.1	6.62	6.31	7.61	2.83
G	Lactec	−21.8	3.81	21.7	8.45	0.273	10.3	0.123
H	Lactec G	−18.3	3.85	18.3	9.10	0.110	13.2	403
I	KN Solution 3B	−21.6	3.69	21.9	12.4	28.4		
J	Low Molecular Dextran L	−28.7	3.72	29.2	12.5	113		
K	Physiosol-3	−44.3	3.31	48.3	4.91	2.95	10.2	9.00
L	Solita-T No. 2	−5.01	3.73	5.23	6.83	2.18	10.3	1.16
M	Solita-T No. 3	−19.9	3.68	20.6	7.36	0.206	12.4	544

a) Samples A—E are injections, and samples F—M are infusions (500 ml). *b*) C is the concentration of strong acid (positive value) or base (negative value) (mM). *c*) C_1, C_2 and C_3 are the concentrations of weak acids (mM).

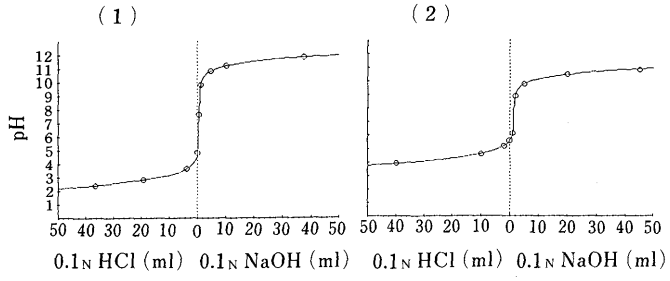


Fig. 7. Fitted PHC Curve of Commercial Preparations

(1) Futhan® (Nafamostat Mesilate, 10 mg) diluted to 500 ml with distilled water, (2) KN Solution 3B®, 500 ml.

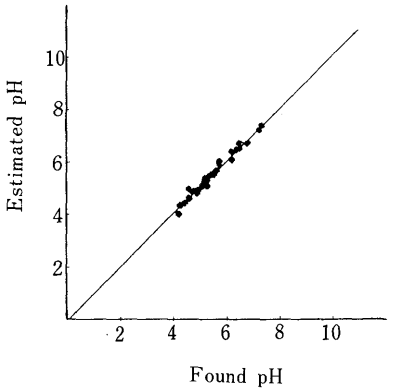


Fig. 8. Correlation between the Estimated and the Found pH Values in Mixed Infusions of an Injection and an Infusion as in Table I ($n=40$)

$Y=1.02X-0.0834$; $r=0.991$.

fusion described in Experimental was investigated. Figure 7 presents the PHC curves for NM injection (Futhan®) and glucose and electrolyte infusion (KN Solution 3B®). Open circles indicate observed values, and the curves represent the fitted PHC curves of each preparation obtained by that method. The PHC curve of each preparation showed a simple fit, and the parameters of the PHC curves for the object preparations are listed in Table I. The pH of each mixed infusion could be estimated correctly using these PHC curves (Fig. 8).

Stability Prediction of NM in Practical Mixed Infusions Containing SBS The residual ratios of NM in mixed infusions involving NM injection (Futhan®) were studied practically. The mixed infusions of Futhan® (one vial) and 8 kinds of infusions (500 ml) included in Table I were incubated for 4 h at 25 °C, and the residual ratios of NM were measured. In the 7 kinds of mixed infusions of Futhan® (initial pHs 4.5—6.5) other than that of Futhan® and EL-Solution No. 3®, NM decreased to 99.1—99.9% after 4 h, and NM was considered to be rather stable in these mixed infusions. Nevertheless, the remaining ratio of NM in the mixed infusion (initial pH 5.35) of Futhan® and EL-Solution No. 3® after 4 h decreased to 90.7% and the pH value changed to 5.25.

On the other hand, the SBS concentration in the mixed infusion of Futhan® and EL-Solution No. 3® was estimated to be 1.30×10^{-3} M by the DTNB method.[13] SBS could not be detected in the other infusions. The difference in stabilities of NM in these mixed infusions was therefore considered to be related to the SBS concentration. The residual ratio of NM in the presence of SBS (1.30×10^{-3} M) at pH 5.35 and 25 °C after 4 h was estimated to be 89.6% based on Eq. 18. The estimated residual percent of NM agreed well with the observed value of NM in the mixed infusion of Futhan® and EL-Solution No. 3®. On the other hand, with the stability of NM in the mixed infusions not containing SBS, the residual ratios of NM in the absence of SBS in the pH range of 4.5—6.2 at 25 °C after 4 h were estimated to be 99.7—99.9%. Based on these findings, the fundamental kinetic study of NM in the presence of SBS and the reported stability of NM in buffer solutions,[11] it was inferred that the influence of SBS on the stability of NM was generally much larger than that of ordinary weak acids and salts contained in the mixed infusions. In practical terms, the compatible pH range estimation of NM in the presence of SBS was considered to be feasible. Furthermore, the estimated pH of the mixed infusion of Futhan® and EL-Solution No. 3® (5.26) by this pH estimation method agreed well with the observed pH value (5.35).

The SBS concentration remaining in the injectable preparations is considered to be vary with the conditions of sterilization and storage.[14] Coordinated use of the compatible pH range estimation of NM and the pH estimation of the mixed infusion of NM is thus expected to contribute to the evaluation of the compatibility of the mixed infusion

of NM in the presence of SBS or to finding more stable conditions for that infusion.

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References and Notes

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