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Dissolution of bupivacaine 3-hydroxy-2-naphthoate into phosphate buffers

NAOMI I. NAKANO* AND AKIKO SETOYA

Physical Pharmacy Laboratories, Hokkaido Institute of Pharmaceutical Sciences, Otaru 047-02, Japan

Dissolution behaviour of bupivacaine 3-hydroxy-2-naphthoate (BUPNH) into phosphate buffers, pH 7·4 was investigated at 25° and 37°C. Although the dissolution pattern of the salt at 25°C was normal, i.e. the concentrations of the acid and base component agreed, during the entire dissolution period, that at 37°C was anomalous. The dissolution consisted of two phases, i.e. an initial normal phase was followed by a second slower phase in which the concentration of the acid became, at equilibrium, markedly higher than that of the base. This unusual dissolution behaviour at 37°C was shown to be due to the precipitation of the base, which, in turn, is attributed to the unusual temperature dependency of the solubility of the base in phosphate buffers, i.e. decrease in solubility with increasing temperature.

Bupivacaine 3-hydroxy-2-naphthoate (BUPNH) has been prepared in our laboratories, together with the 3-hydroxy-2-naphthoates of lignocaine and mepivacaine, with the intention of prolonging the duration of action of these local anaesthetics through their sparingly soluble salt form (Nakano et al 1978). During the course of dissolution studies of these 3-hydroxy-2-naphthoates at 37° C in 0·7 M phosphate buffer, pH. 7·46, we have noted an unusual behaviour only with the bupivacaine salt. When the dissolution of BUPNH was followed by the increase in concentration of 3-hydroxy-2-naphthoate ion (N^-) with time, the existence of two stages in the dissolution curve has been observed, i.e. within about 20 min an initial plateau was obtained and that was followed by a second slower equilibration period extending over 2 to 6 h.

The purpose of the present study was to elucidate this unusual dissolution behaviour of the bupivacaine salt. We have examined this problem by (1) determining the concomitant concentrations of both the base and the acid component of the salt during dissolution, and (2) studying the effects of buffer concentration, stirring rate, temperature, a common ion, and an additive. The results of these studies unambiguously support the unusual dissolution behaviour of BUPNH at 37° C to be due to the precipitation of the base, which, in turn, is attributed to the unusual temperature dependency of the solubility of the base.

MATERIALS AND METHODS

Materials. Bupivacaine hydrochloride, a gift from Yoshitomi Pharmaceutical Inds., Osaka, Japan was converted to the base for solubility study. Mepivacaine base was also supplied by Yoshitomi Pharmaceutical Inds. Bupivacaine 3-hydroxy-2-naphthoate (BUPNH) was prepared in our laboratories (Nakano et al 1978). Dichloromethane of g.l.c. grade and methylcellulose (500 cps) were purchased from Wako Pure Chemical Inds., Osaka, Japan. Buffer salts and all other chemicals were of reagent grade. Water was deionized and double distilled, with the second distillation performed in all glass apparatus.

Dissolution and solubility study. Dissolution study was carried out as described previously (Nakano et al 1978) employing, as the medium, 40 ml of pH 7·4 phosphate buffers at two concentrations. The composition of 0·5 M buffer at the time of preparation was 0·4267 M Na_2HPO_4 and 0·0710 M NaH_2PO_4, the pH values of which were 7·40 ± 0·01 at 25° C and 7·38 ± 0·01 at 37° C, and that of 0·7 M buffer was either 0·6076 M Na_2HPO_4 and 0·0924 M NaH_2PO_4 (pH = 7·40 ± 0·01 and 7·39 ± 0·01 at 25° and 37° C, respectively) or 0·6143 M Na_2HPO_4 and 0·0854 M NaH_2PO_4 (pH = 7·48 ± 0·01 and 7·46 ± 0·01 at 25° and 37° C, respectively). Measurement of pH was made using a Corning Digital 112 Research pH meter. Unless otherwise mentioned the stirring rate was fixed at 360 rev min^{-1}. Dissolution media containing added N^- were prepared by dissolving 3-hydroxy-2-naphthoic acid (NH) in the 0·5 M phosphate buffer and adjusting the pH value of the resultant solution to 7·40 with 2 M NaOH at room temperature (20° C). The media containing less N^- were prepared by serial dilution of this solution with the 0·5 M phosphate buffer. The pH of the filtrate at equilibrium was found to have remained constant.

* Correspondence.

Equilibrium solubility was obtained either from the dissolution study or by equilibrating an excess amount of the solid with an appropriate buffer for 24 to 48 h.

Determination of the acid component. The samples were appropriately diluted and the concentration of the total acid component was spectrophotometrically determined at 350 nm on a Shimadzu UV 300 spectrophotometer.

Determination of the base component. The method of Berlin et al (1973) was generally adopted using a Shimadzu GC-4BM gas chromatograph equipped with a hydrogen flame ionization detector. The dual columns were 1·5 m, 3 mm i.d., silanized glass columns packed with 3% OV-17 coated onto 80/100-mesh Gas-Chrom Q. Temperatures were 265° C in the columns, 285° C in the injection port and detector. The flow rate of nitrogen was 31·6 ml min^{-1} and the flow rates of hydrogen and air were adjusted to give maximum recorder responses.

A 2 ml sample was placed in a 20 ml capacity glass-stoppered test tube together with 2 ml 2 M NaOH, 1 ml mepivacaine standard solution, and 2 ml water. To this mixture 2 ml of dichloromethane was added to extract the local anaesthetic bases. The organic layer (3 μl) was directly injected into the column. The concentration of bupivacaine (BUP) was determined from the peak-height-ratio of BUP to mepivacaine utilizing a previously established calibration curve.

RESULTS AND DISCUSSION

In Fig. 1a is presented the usual dissolution behaviour of BUPNH (concentrations of the acid and the base component agree in each sample) at 25° C in 0·5 M and 0·7 M phosphate buffer, pH 7·40. At 37° C, on the other hand, for both concentrations of the buffer, two-stage dissolution patterns shown in Fig. 1b were observed. In the initial stage which lasted for about 40 min under the experimental conditions, the concentrations of both the acid and the base component agreed, whereas in the second stage the concentrations of the total acid markedly exceeded those of the total base. This unusual two-stage dissolution pattern of BUPNH was first observed at 37° C in the 0·7 M buffer (Nakano et al 1978). Since similar behaviour was observed at both temperatures in the 0·5 M buffer as well, this unusual behaviour is considered to be independent of buffer concentration in this range. At both temperatures the solubility is lower for the 0·7 M buffer than for 0·5 M buffer. This is considered to be due to the salting-out effect of the buffer salts.

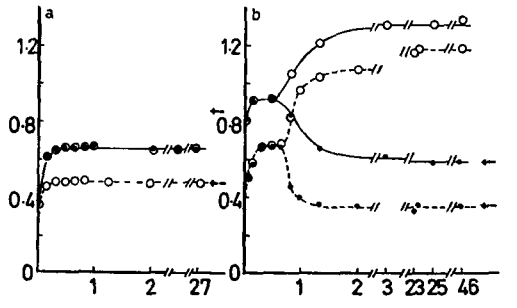

FIG. 1. Dissolution of BUPNH into phosphate buffers, pH 7·4, a: at 25°C and b: at 37°C. Analysis of the acid component (○); the base component (●). Buffer concentration—0·5 M, pH 7·40 (——); 0·7 M, pH 7·40 (— — —). The arrows indicate the solubilities of the BUP in these buffers. Ordinate: concentration (mM). Abscissa: time (h).

The fact that stirring rate markedly affects the initial stage of the dissolution pattern at 37° C is presented in Fig. 2. The initial plateau was not observable at the lower stirring rate. In fact, at the lower stirring rate, unless the base component is also analysed, the smooth dissolution curve drawn from the analysis of the acid may be mistaken as showing usual behaviour.

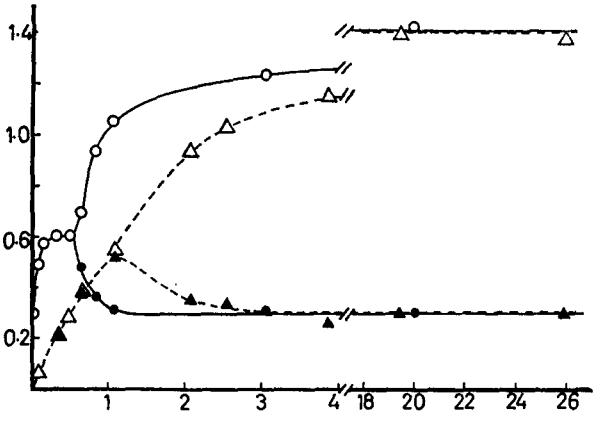

FIG. 2. Effect of stirring rate on the dissolution profile of BUPNH at 37°C. Dissolution medium—0·7 M phosphate buffer, pH 7·46. Stirring rate—360 rev min^{-1}: Analysis of the acid component (○—○), the base component (●—●); 150 rev min^{-1}: Analysis of the acid component (△ – – △), the base component (▲ – – ▲). Ordinate: concentration (mM). Abscissa: time (h).

Since the concentrations of total base at equilibrium in the second stage of dissolution are lower than those of the initial stage, the precipitation of the base may be suspected to be due to the solubility limitation of the base in the medium. Therefore,

we have measured the solubilities of the base in these buffers at different temperatures. The results are presented in Table 1 which shows that the solubility of the base increases with decreasing temperature. At 25° C, the solubility of the base exceeds the concentration of the total base dissolved out of BUPNH in these buffers, thus the usual dissolution patterns (Fig. 1a) resulted. At 37° C, on the other hand, the concentration of the total base dissolved (the initial plateau of Fig. 1b) in 0·5 M phosphate buffer is about 1·3 times the solubility in this medium. This may be the cause of the precipitation of the base in the latter equilibrium stage.

Table 1. Temperature-dependent solubility of bupivacaine base in phosphate buffers, pH 7·4.

Temp.* °C	Solubility (mM)** 0·5 M phosphate buffer	0·7 M phosphate buffer***
25·0	0·850 ± 0·020(3)	0·481 ± 0·001(2)
37·0	0·575 ± 0·008(3)	0·363 ± 0·003(2)
48·0	0·465 ± 0·010(2)	
54·8	0·374 ± 0·009(4)	

* Temperature control: ± 0·1°C.
** Average ± standard error (number of determinations).
*** Composition: 0·6076 M Na_2HPO_4 and 0·0924 M NaH_2PO_4.

The experimental observations presented above suggest that the unusual dissolution behaviour of BUPNH at 37° C is the consequence of the unusual temperature dependency of the solubility of the base in these buffers. Equilibrium reactions for the dissolution of BUPNH in these media could therefore be described as shown in Scheme 1, where the subscript s denotes solid phase. At 25 °C only solid phase I is present, whereas at 37° C both solid phases I and II may be present, at equilibrium.

$$\underset{\text{solid phase I}}{BUPNH_s} \rightleftharpoons BUPNH_0 \overset{K}{\rightleftharpoons} BUPH^+ + N^-$$

$$BUPH^+ \;\Updownarrow\; K_a \;\; BUP$$

$$BUP \;\Updownarrow\; BUP_s \quad \text{solid phase II}$$

Scheme 1

In this model, the presence of undissociated dissolved species denoted by $BUPNH_0$ is assumed, although good agreement between the equilibrium concentration of the total base in the dissolution study and the solubility of the base suggests that this species is not present in great amounts. For simplicity, if we use concentrations rather than activities, the following relationships can be written according to Scheme 1:

$$K = \frac{[BUPH^+]\,[N^-]}{[BUPNH_0]} \quad .. \quad .. \quad (1)$$

$$A_t = [BUPNH_0] + [N^-] \quad .. \quad (2)$$

$$B_t = [BUPNH_0] + [BUPH^+] + [BUP] \quad .. \quad (3)$$

where A_t and B_t are total concentrations of the acid and base, respectively. The dissociation constant K_a for $BUPH^+$ is given by:

$$K_a = \frac{[BUP]\,[H^+]}{[BUPH^+]} \quad .. \quad .. \quad .. \quad (4)$$

From equation (1), the solubility product K_{sp} may be expressed by:

$$K_{sp} = K\,[BUPNH_0] = [BUPH^+]\,[N^-] \quad .. \quad (5)$$

Equations (3), (4), and (5) yield equation (6):

$$B_t = [BUPNH_0] + \left(1 + \frac{K_a}{[H^+]}\right)\frac{K_{sp}}{[N^-]} \quad .. \quad (6)$$

Equation (6) relates B_t with $[N^-]$ which is obtainable from equation (2) if $[BUPNH_0]$ is known. The first estimation of $[BUPNH_0]$ was carried out according to the method employed by Swintosky et al (1956) for procaine penicillin by adding large excess amounts of N^- in the dissolution medium so that the dissociation of $BUPNH_0$ would be suppressed due to the common ion effect. Under the condition, the relationship $A_t \doteqdot [N^-] \doteqdot [N^-]_{added}$ would hold. Application of this treatment for data at 25° C is shown in Fig. 3a where B_t is plotted against $1/[N^-]_{added}$. From the ordinate intercept of the line, $[BUPNH_0]$ is estimated to be $1{\cdot}4 \times 10^{-5}$ M. Utilizing this approximate value, we have extended this treatment, by calculating $[N^-]$ from equation (2), to the data obtained from the dissolution and solubility studies in the presence of lower $[N^-]_{added}$ including the case where $[N^-]_{added} = 0$. The results are shown in Fig. 3b. The intercept on the ordinate of the straight line drawn by the least-squares method gave $[BUPNH_0] = 1{\cdot}0 \times 10^{-5}$ M. The slope which

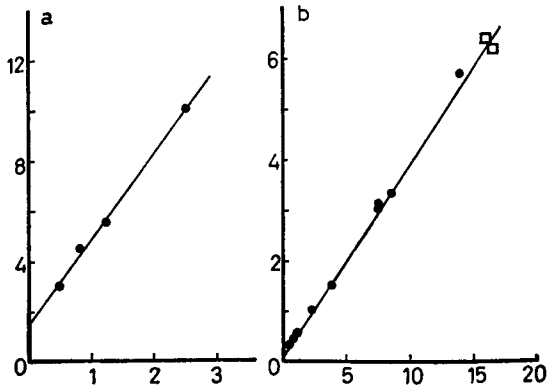

FIG. 3. Effect of $[N^-]_{added}$ on the solubility of BUPNH at 25°C in 0·5 M phosphate buffer, pH 7·40. a: Solubility of BUPNH in the presence of excess $[N^-]_{added}$. Ordinate: B_t (M × 10^5). Abscissa: $1/[N^-]_{added}$ (M^{-1} × 10^{-2}). b: Solubility of BUPNH in the presence (●) and absence (□) of $[N^-]_{added}$. Ordinate: B_t (M × 10^4). Abscissa: $1/[N^-]$ (M^{-1} × 10^{-2}), $[N^-]$ is calculated using equation (3) (see text for details).

corresponds to $(1 + K_a/[H^+])\ K_{sp}$ is determined to be $3{\cdot}9 \times 10^{-7}$ M². Further, if $pK_a = 8{\cdot}1$ at 23° C (Friberger & Aberg 1971) is used, this system at 25° C gives $K_{sp} = 3{\cdot}2 \times 10^{-7}$ M².

Similar treatment of equilibrium data at 37° C shown in Fig. 4a also indicates the presence of $BUPNH_0$ and in Fig. 4b the line is drawn for all equlibrium data subject to the above treatment.

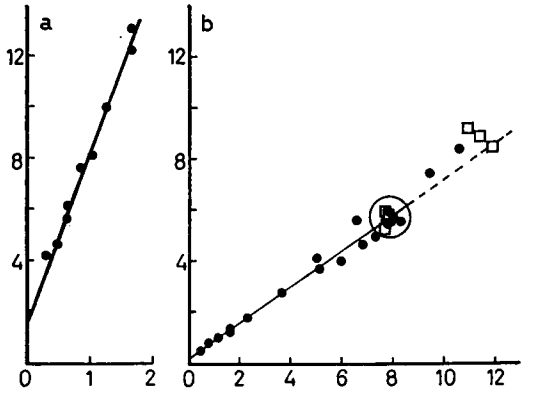

FIG. 4. Effect of $[N^-]_{added}$ on the equilibrium and metastable solubilities of BUPNH in the presence and absence of $[N^-]_{added}$. a: Equilibrium solubility of BUPNH in the presence of excess $[N^-]_{added}$ (only solid phase I is present). Ordinate: B_t (M × 10^5). Abscissa: $1/[N^-]_{added}$ (M^{-1} × 10^{-2}). b: Equilibrium and metastable solubilities in the presence (●) and absence (□) of $[N^-]_{added}$. Data enclosed in the large circle represent equilibrium data (shown in Table 2) in the presence of solid phases 1 & II. Data below the circle are at equilibrium and those above the circle are at metastable state in the presence of solid phase I only. Ordinate: B_t (M × 10^4). Abscissa: $1/[N^-]$ (M^{-1} × 10^{-2}), $[N^{-1}]$ is calculated using equation (3) (see text for details).

From the straight line $[BUPNH_0]$ and the slope are determined to be $1{\cdot}4 \times 10^{-5}$ M and $7{\cdot}0 \times 10^{-7}$ M², respectively.

In the presence of solid phases I and II, the equilibrium reactions of Scheme 1 demand the concentrations of all the species in the solution phase be fixed. This is experimentally verified (Table 2), i.e. for variation of $[N^-]_{added}$ from 0 to $5{\cdot}99 \times 10^{-4}$ M, A_t and B_t at equilibrium remained essentially constant. These data correspond to points enclosed in a large circle in Fig. 4b. The points below the circle correspond to the systems in which solid phase II is absent since $[N^-]_{added}$ was

Table 2. Effect of $[N^-]_{added}$ on the metastable and equilibrium concentrations of total acid (A_t) and base (B_t) in the dissolution of bupivacaine 3-hydroxy-2-naphthoate in 0·5 M phosphate buffer, pH 7·4, at 37°C.

$[N^-]$added (M × 10^4)	Equilibrium concn (mM)		Metastable concn (mM)	
	A_t	B_t	A_t	B_t
0	1·30 ±0·01(3)*	0·567 ±0·023(3)*	0·895 ± 0·017(4)*	
0·847	1·21	0·555	—	—
1·04	1·26	0·570	—	*M*
1·08	1·29	0·590	0·597	0·840
2·45	1·27	0·545	—	—
3·33	1·30	0·560	1·07	0·754
4·95	1·28	0·550	—	—
5·99	1·29	0·550	—	—
0·847–5·99	1·27 ±0·01(7)*	0·560 ±0·006(7)*		

* Average ± standard error (number of determination).

enough to suppress the dissociation of $BUPNH_0$ so that the concentrations of $BUPH^+$ and BUP originated from $BUPNH_S$ did not exceed the solubility of the base. The extension of the straight line to the region of metastable plateau data (presented in Table 2 and shown in Fig. 4b above the circle) shows that the fit is not unreasonable. Thus, the theory that the initial plateau is close to the equilibrium concentration, had the base not precipitated, is most likely to be held.

According to the present interpretation, the precipitation of BUP should be a fairly time-consuming process. This is in fact shown to be the case. When 1 ml of a solution containing 16 mg of bupivacaine hydrochloride in water was added to 40 ml of 0·5 M phosphate buffer at 37° C (final concentration = 1·15 mM and the solubility of BUP = 0·58 mM) under the same high stirring rate as to observe the plateau shown in Fig. 1b, precipitation did not begin during first 45 min and was not complete even after about 7 h. The precipitation was further delayed when 0·002% methylcellulose was added to the buffer. Methylcellulose is known to delay precipitation (Ebian et al 1975). When the

dissolution of the salt was carried out in the presence of 0·002% methylcellulose, the metastable state was extended from 40 ∿ 45 min to over 2 h, suggesting the delay of the precipitation of the precipitation of the base (Fig. 5).

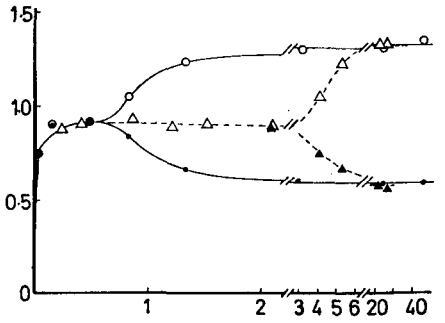

FIG. 5. Effect of 0·002% methylcellulose on the dissolution characteristics of BUPNH at 37°C in 0·5 M phosphate buffer, pH 7·40. The concentration of the acid component (○—○) and base component (●—●) in the absence of methylcellulose and that of the acid component (△ - - △) and base component (▲ - - ▲) in the presence of 0·002% methylcellulose. Ordinate: concentration of the acid or base component (mM). Abscissa: time (h).

An additional piece of evidence which supports the dissolution behaviour of BUPNH into the phosphate buffers at 37° C to conform to the series of equilibria presented in Scheme 1, was obtained when solid BUP was equilibrated in the presence of an excess amount of N^-. The solid phase collected after equilibration had a melting point of 165° C, corresponding to that of the 3-hydroxy-2-naphthoate (165–168° C) and analysis for the acid and base showed that the both components are present in the ratio of 1:1.

Our preliminary investigation showed lignocaine and mepivacaine also exhibit an increase in solubility with decreasing temperature in these phosphate buffers. However, since the solubilities of the bases are higher than those of the 3-hydroxy-2-naphthoates (Nakano et al 1978), they do not show the same unusual dissolution pattern as does BUPNH. The concentration of the acid and base at the metastable equilibrium indicates that the solubility of BUPNH would be about 0·9 mM in 0·5 M phosphate buffer at 37° C, had the base precipitation not occured, whereas that of the base is about 0·6 mM (see Tables 1, 2).

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