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Bropirimine formulation: The dynamic testing of injections

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Summary

A flow system for the dynamic testing of injection formulations of bropirimine has been developed. This system is based upon that reported by Yalkowsky and co-workers (Yalkowsky and Valvani, *Drug Intell. Clin. Pharm.*, 11 (1977) 417–419; Yalkowsky et al., *J. Pharm. Sci.*, 72 (1983) 1014–1017) where a parenteral formulation is injected into an infusion fluid of 5% dextrose which is being pumped through the flow cell of an ultraviolet spectrophotometer. Precipitation is indicated by the appearance of an apparent absorbance due to dispersion of the incident beam by particles. This system worked well for test injections of diazepam in which solubility is enhanced with cosolvents. Phenytoin injection also provided a precipitate although both cosolvent and pH control are used to maintain solution in this case. In contrast, bropirimine injections, which also use both cosolvent and pH control, exhibited no precipitation. When the pH of the mixed effluent was monitored it was found to be very high, sufficient to maintain bropirimine in solution. The infusion fluid thus did not provide sufficient buffering capacity to mimic the in vivo situation. When an infusion fluid with a buffer capacity similar to that of human blood was chosen, pH control was effected and precipitation could then be used to assess the efficiency of the injection formulation.

Introduction

Bropirimine [2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone, ABPP] has shown potential antiviral, antitumour and immunostimulatory activity (Taggart et al., 1980; Stringfellow, 1981a,b). This compound was developed from the 6-methyl analogue to reduce dose-related crystal formation in kidney and bladder. Bropirimine exhibits a reduced incidence of crystal formation but displays an aqueous solubility of only 39 μg ml^{-1} and does not readily provide aqueous formulations suitable for injection. To overcome this problem, we have undertaken preformulation studies on bropirimine including the determination of the solubility of bropirimine in a variety of systems using both pH and cosolvent enhancement (Alpar et al., 1986; Irwin and Iqbal, 1988, 1991). To monitor the behaviour of potential bropirimine injection formulations we have used the dynamic flow system developed by Yalkowsky and co-workers (Yalkowsky and Valvani, 1977; Yalkowsky et al., 1983). In this system, an infusion fluid is pumped through the flow cell of an ultraviolet spectrometer which is continually monitored at a long, fixed wavelength. Potential formulations are injected into the infusion fluid

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downstream of the flow cell. Infusion flow rate, injection flow rate and the separation of the injection site from the flow cell are variables which may be adjusted. Due to the long detection wavelength, little absorbance is apparent if drug remains in solution. However, if precipitation occurs, the ensuing particulate matter causes dispersion of the incident beam and an apparent absorbance is observed. Thus, the efficiency of formulation and delivery rate may be assessed.

Experimental

Materials

Meglumine (*N*-methyl-D-glucamine) and *N*,*N*-dimethylacetamide (DMA) were purchased from Sigma (Poole) and bropirimine was synthesised (Brown and Stevens, 1975). DMA was neutralised with 0.1 M HCl prior to use. The diazepam injection was Valium 10 (10 mg in 2 ml, Roche), the phenytoin was Epanutin Ready Mixed Parenteral (250 mg phenytoin sodium in 5 ml, Parke Davis) and the 5% dextrose was Steriflex (Boots). Salts for Britton-Robinson (Mongay and Cerda, 1974), McIlvaine's (Elving et al., 1956) and Tris buffers (Lentner, 1981) were BP standard and solutions were prepared to pH 7.4 as described in the literature.

Infusion system

An Imed 960 Volumetric Infusion Pump (Abingdon, Oxford; infusion rate settings 200–800 ml h^{-1}) was connected, via a tube of 2.5 mm internal diameter, to a UV spectrometer (Cecil Instruments CE272), which in turn was connected to a chart recorder (Gallenkamp) run at a chart speed of 2 mm min^{-1}. The spectrometer was equipped with a 10 mm path length quartz flow cell (2 ml) and operated at a sensitivity of 2.0 AUFS. At a variable distance (*d*, 40–100 cm) from the flow cell, an injection port with a rubber septum was introduced into a tube of internal diameter 2.5 mm. The formulation under test was then injected into this port with a syringe pump (Sage Instruments, Orion Research Inc.), using a 20 ml syringe (delivery rate 0.1–1 ml min^{-1}) and a 20-gauge needle. Prior to each experiment, the infusion liquid was flushed through the system until there was no change in the absorbance reading. The vehicle was then used as the reference and the spectrometer was zeroed. The syringe pump was then switched on after setting the desired injection rate. Absorption within this system is due to dispersion caused by particulate matter and peaks subsequent to injection were attributed to the formation of precipitate. At greater wavelengths (> 500 nm), absorbance was principally due to dispersion from the precipitated particles rather than to vehicle effects. After each experiment, the whole system was flushed through once again with the vehicle to remove any remaining precipitate and the cycle was repeated with the next formulation.

Bropirimine injections

The vehicles used to prepare the various bropirimine injections are listed in Table 1. An

TABLE 1

Vehicles used for the preparation of bropirimine injections

Vehicle	Dimethyl acetamide (% v/v)	Meglumine (% w/v)	0.2 M sodium bicarbonate (% v/v)	Distilled water (% v/v)	Solubility of bropirimine (mg ml^{-1})	pH
A	5		to 100		14.0	10.05
B	10		to 100		18.84	10.40
C	10	5	to 100		32.44	10.64
D	10			to 100	0.18	8.15
E	10	5		to 100	18.1	10.33
F	50	2.5		to 100	15.0	10.13

appropriate amount of bropirimine was added to the vehicle which was vigorously shaken overnight. The suspension was then sonicated for 10 min and filtered through a 0.2 μm Nitrate Millipore filter to remove any suspended material. Solubilities were determined by suitable dilution and HPLC analysis used a mobile phase comprising aqueous acetonitrile (25% v/v, pH 2.0), containing diethylamine (0.1%) with 2-amino-5-bromo-6-(3-fluorophenyl)-pyrimidin-4(3H)-one (ABmFPP) as internal standard (Irwin and Iqbal, 1988). Unless stated otherwise, a 25 mg ml^{-1} solution of bropirimine was prepared in vehicle C and 10 mg ml^{-1} in vehicles B, C and F so that all concentrations were at least 20% below saturation. Buffer capacities were obtained by potentiometric titration of the buffer solutions (25 ml) with sodium hydroxide (0.1 M). The buffer capacity, β (mEq. l^{-1} pH^{-1}), was then calculated from Eqn 1:

$$\beta = \frac{1000MV_2}{\Delta pHV_1} \qquad (1)$$

where M is the molarity of base of volume V_2 ml which is added to V_1 ml of buffer to cause a change in pH of ΔpH.

Results and Discussion

An analytical wavelength was selected by infusing dextrose (5%) through the system at a flow rate of 400 ml h^{-1} while injecting the same solution at a rate of 0.51 ml min^{-1}. The effect of varying detector wavelengths is shown in Fig. 1. At wavelengths below 650 nm, the noise level due to vehicle mixing is high whereas wavelengths above this demonstrate that appreciably reduced and acceptable noise levels were obtained. Similar results were obtained with the other formulation vehicles. A working wavelength of 700 nm was thus chosen for subsequent experiments. With dextrose (5%, 400 ml h^{-1}) flowing through the system, an injection of bropirimine (25 mg ml^{-1}, 0.51 ml min^{-1}), formulated in DMA, meglumine and sodium bicarbonate (vehicle C), no absorption developed. Reducing or increasing the infusion flow rate and increasing the injection rate up to 1 ml min^{-1} had no effect. This implies that no precipitation of bropirimine is occurring under these conditions. This result is surprising because, when a formulation is cosolvent-depen-

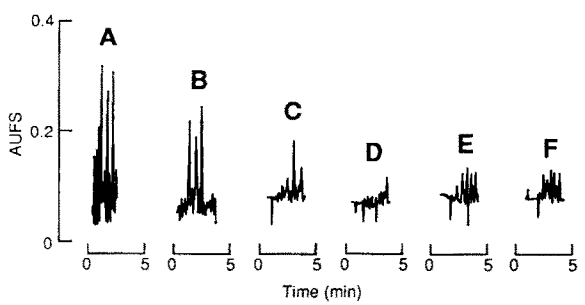

Fig. 1. Effect of wavelength on noise produced from an infusion of dextrose (5%, 400 ml h^{-1}) with injection of the same solution (0.51 ml min^{-1}). A, 500 nm; B, 590 nm; C, 650 nm; D, 700 nm; E, 750 nm; F, 800 nm.

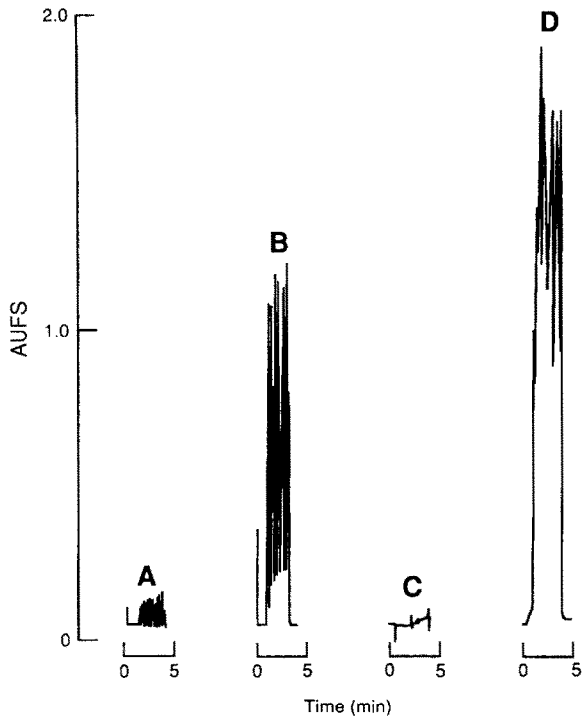

Fig. 2. Precipitation of diazepam and phenytoin injections dependent upon injection rate into an infusion of dextrose (5%, 400 ml h^{-1}). A, diazepam (0.51 ml min^{-1}); B, diazepam (1 ml min^{-1}); C, phenytoin (0.1 ml min^{-1}); D, phenytoin (0.24 ml min^{-1}).

dent, precipitation on injection might be expected as the solubility of the drug in the mixed solvent usually decreases exponentially with a reduction in cosolvent concentration (Yalkowsky and Valvani, 1977). As a check on this system, an injection of diazepam was tested (Newton et al., 1981). This material has an aqueous solubility of 8 μg ml^{-1} but may be formulated as a solution for injection (5 mg ml^{-1}) due the presence of propylene glycol (40%) and ethanol (10%) as cosolvents. At an infusion flow rate of 30 ml min^{-1}, Yalkowsky demonstrated that an injection of diazepam (2 ml min^{-1}), giving a dilution of 1:15, into a flow of either 5% dextrose or normal saline caused precipitation of the drug (Yalkowsky et al., 1983). With our equipment, this injection exhibited a small effect at a flow rate of 0.51 ml min^{-1}, but at 1.0 ml min^{-1} (dilution 1:6.7) precipitation, as indicated by significant absorption, was observed. Similarly, phenytoin has an aqueous solubility of 16 μg ml^{-1} but may be formulated as a solution for injection (50 mg ml^{-1}) in the same cosolvent system as diazepam injection (40% propylene glycol, 10% ethanol). In addition, however, phenytoin is a weak acid (pK_a 8.3 (Albert and Serjeant, 1984)), and the solution also requires strict pH control ($\geq$ 11.5) to ensure virtual complete ionisation of the drug (Newton and Kluza, 1980). Due to a low intrinsic solubility and weakly acidic properties, phenytoin solubility is

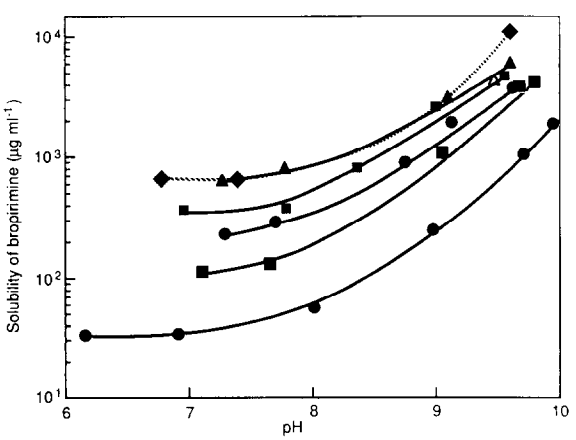

Fig. 3. Effect of dimethyl acetamide concentration and pH on the solubility of bropirimine. (●) 10%, (■) 20%, (▼) 30%, (▲) 40%, (▼) 50%.

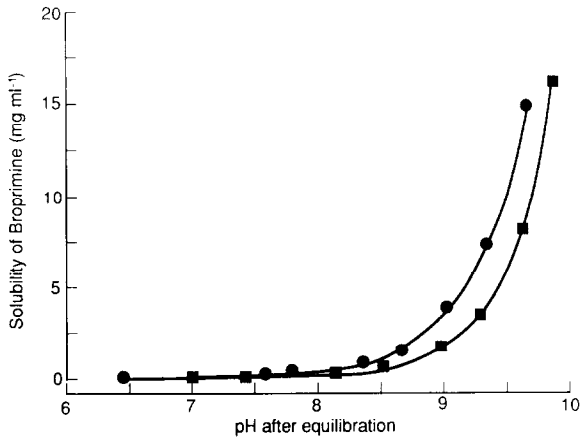

Fig. 4. Effect of pH and temperature on the solubility of bropirimine in vehicle A. (●) 21°C, (■) 6.4°C.

very sensitive to pH changes and rapid precipitation results when the pH is reduced to below 11.5 (Trissel, 1990). Because of this effect, phenytoin injection also shows evidence of precipitation when tested in the flow system (Fig. 2) with little effect noted at an injection rate of 0.1 ml min^{-1} but when this was increased to 0.24 ml min^{-1} (dilution 1:28) significant precipitation was observed. Although this confirms that extreme care should be exercised when injecting phenytoin to ensure a slow delivery, one further point is apparent. When the pH of the mixed injection and infusion fluids was monitored, the resultant solution displayed a value of 10.2. Although this is insufficient to maintain total dissolution of phenytoin, it is substantially higher than either the initial infusion pH (5.3) or the equilibrium pH of blood (7.4). This suggests that the system may not be mimicking the biological environment sufficiently with regard to buffer capacity and hence does not provide an appropriate model for such formulations.

The solubility of bropirimine is similarly dependent upon the level of cosolvent (Alpar et al., 1986) and thus a comparable precipitate was expected on dilution of the formulation into the infusion fluid. However, the solubility of bropirimine is also greatly enhanced at high pH values (Alpar et al., 1986). Fig. 3 shows the effect of DMA concentration on the solubility of bropirim-

ine as a function of pH. Increases in both DMA and pH result in an enhanced solubility of bropirimine. At the higher pH values, the enhanced solubility is due to ionisation, whereas at lower pH values, the increased solubility is largely cosolvent-driven. The solubilising effect of DMA thus becomes smaller with increasing pH. The solubility profiles of bropirimine at 21 and 6.4°C in vehicle C, adjusted to various pH values, are displayed in Fig. 4. In this case, the constant concentration of cosolvent shows that the exponential increase in the solubility of bropirimine is due solely to ionisation. Semilogarithmic plots are linear:

at 21°C: $\ln(S) = 2.064\text{pH} - 11.27$; $r = 0.997$; $n = 7$

at 6.4°C: $\ln(S) = 2.187\text{pH} - 13.21$; $r = 0.994$; $n = 7$

and the relationships may be used to give an indication of the concentration of bropirimine that would avoid precipitation. Formulations containing concentrations of bropirimine below the lines should maintain solution above the indicated temperature. It is suggested that, for cosolvent-dependent formulations, the concentration should be at least 20% below saturation to allow either for a fall in temperature or for dilution of the formulation (Howard and Gould, 1985).

As in the case with phenytoin injection, measurement of the pH of the mixed bropirimine injection and infusion fluids showed that the pH had increased from pH 5.3 to pH 10.0. This change indicates that the buffering capacity of the infusion fluid has been exceeded and that the final pH was sufficiently high to keep the bropirimine in solution. In common with phenytoin, bropirimine demonstrates weakly acidic properties and the two should behave similarly with regard to pH-dependent solubility. Bropirimine is a slightly stronger acid (pK_a 8.21) and has a somewhat higher intrinsic solubility (35 μg ml^{-1}). These properties, together with the somewhat lower concentration of bropirimine in the injection solution (25 mg ml^{-1}), corresponding to 78% of saturation, combine to ensure that the solubility of bropirimine is not exceeded in the flow-through model.

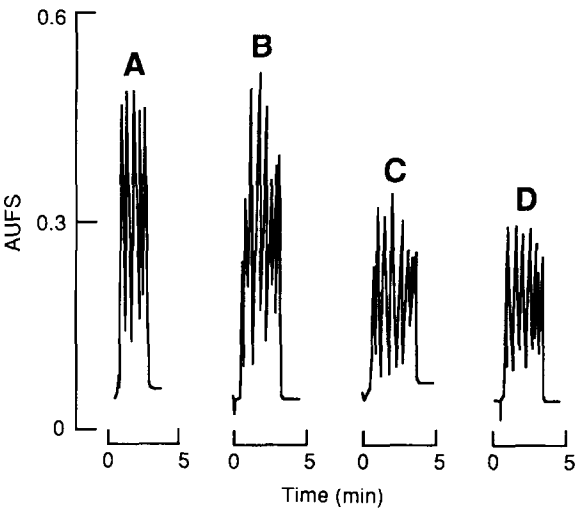

Fig. 5. Effect of the separation distance between injection port and detector on the precipitation of bropirimine from vehicle C (25 mg ml^{-1}; 0.24 ml min^{-1}) into Tris buffer (0.1 M; 400 ml h^{-1}). A, 100 cm; B, 80 cm; C, 60 cm; D, 40 cm.

TABLE 2

Buffering capacities of Tris buffer (mEq l^{-1} pH^{-1}) and blood (mEq kg^{-1} pH^{-1}) (Lentner, 1981)

Parameter	Tris buffer			Blood
	0.05 M	0.075 M	0.1 M	
β	15	21	25	18
β_{calc}	11.5	17.2	23.0	
β_{max}	28.8	43.2	57.6	

β, measured buffer capacity; β_{calc}, value calculated using the Henderson-Hasselbalch equation and a pK_a of 8.3 for Tris (Budavari, 1989); β_{max}, maximum buffer capacity (at pH 8.3) calculated from the equation of Van Slyke (1922) $\beta_{max} = 0.576C$, where C is the total buffer concentration.

Recent in vitro testing of injection formulations has used plasma or buffers (Darwish et al., 1989; Cox et al., 1991; Davio et al., 1991) and in an attempt to improve the infusion fluid, dextrose (5%) was replaced by Tris buffer (pH 7.4, 0.05 M). This fluid also failed to completely buffer the system, a final effluent pH of 9.5 was attained and again no bropirimine precipitation was evident. Further Tris buffers of one-and-a-half (0.075 M) and double-strength (0.1 M) were prepared

and titrated against 0.1 M sodium hydroxide to calculate the buffering capacity over the range pH 7.3–7.5. The results are shown in Table 2. It can be seen that 0.075 M Tris buffer has a similar buffering capacity to blood. When this buffer was substituted for dextrose, immediate precipitation of bropirimine resulted. This is indicated by the appearance of an irregular detector response which is indicative of inhomogeneity in the suspension passing through the flow cell. This result confirms that buffer capacity of the infusion fluid is an important parameter in the dynamic mod-

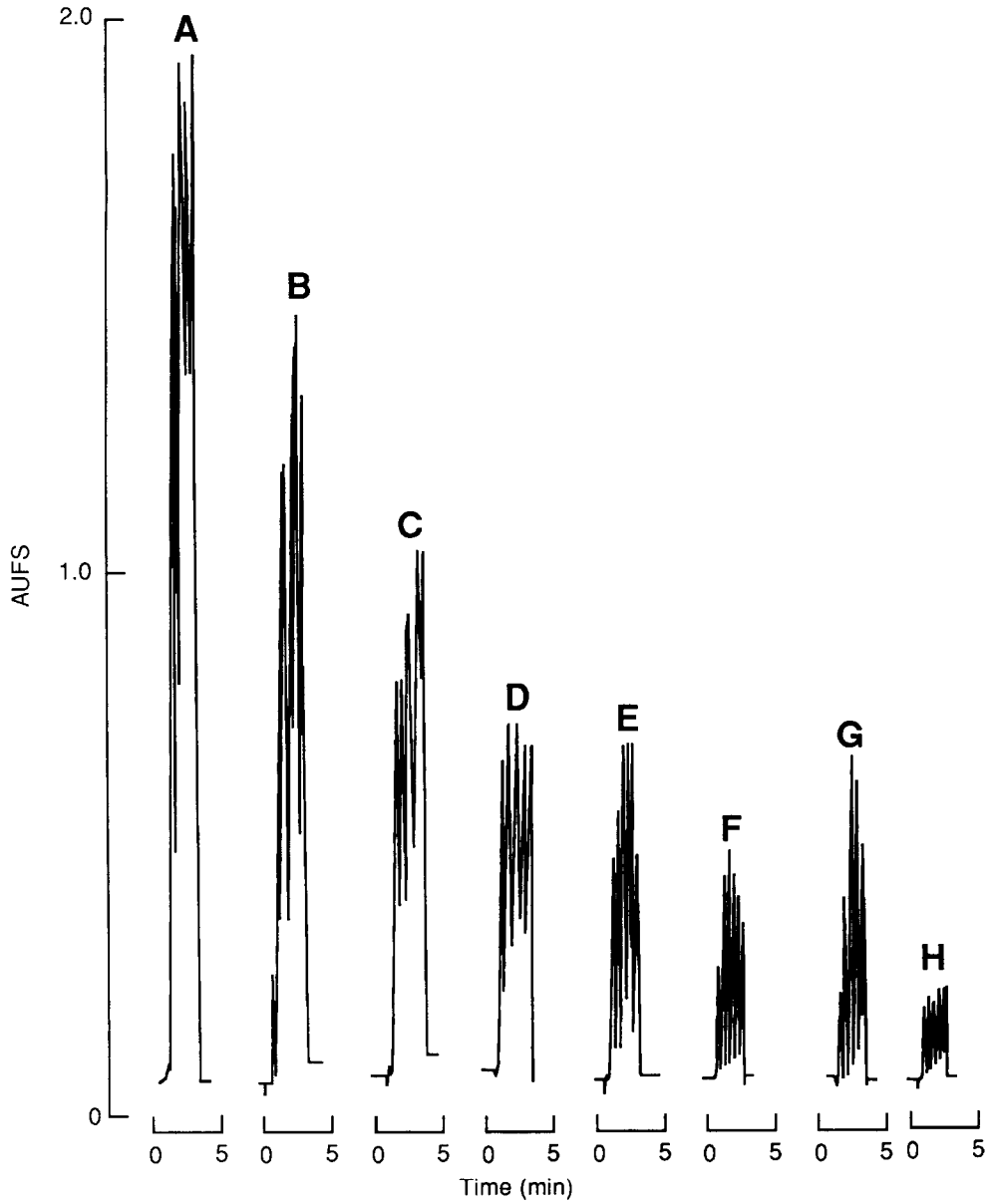

Fig. 6. Effect of infusion rate of Tris buffer (0.1 M) on the precipitation of bropirimine from vehicle C (25 mg ml^{-1}; 0.24 ml min^{-1}). A, 100 ml h^{-1}; B, 200 ml h^{-1}; C, 300 ml h^{-1}; D, 400 ml h^{-1}; E, 500 ml h^{-1}; F, 600 ml h^{-1}; G, 700 ml h^{-1}; H, 800 ml h^{-1}.

elling of injection formulations and, in order to demonstrate potential precipitation on injection, the buffering capacity of the infusion fluid must be maintained during each experiment and to simulate injection into the blood stream a value of at least 18 mEq. l^{-1} pH^{-1} should be maintained. To ensure that the buffering capacity of this phase is maintained, Tris double-strength (0.1 M) buffer was used for subsequent experiments.

As seen in Fig. 5, changing the distance between the injection port and the flow cell (d) only marginally affected the detection of a precipitate. Although a shorter pathlength (40 cm) gave less detectable interference than a greater distance (100 cm) most of the precipitation occurs within the initial mixing zone. With shorter distances ($d < 15$ cm) peaks may be seen which are due to Schlieren patterns (Yalkowsky and Valvani, 1983). These form when there are differences in the refractive indices of the two partially mixed liquids flowing through the flow cell. Longer distances provide more time for the two components to mix and hence reduce the risk of this interference. The effect of varying the infusion rate is demonstrated in Fig. 6. An increase in this parameter leads to increased dilution such that at very high infusion flow rates (> 600 ml h^{-1}) negligible precipitate is detected. This flow rate represents a dilution of 1 : 42. A roughly exponential decrease in precipitation with increased flow rate is apparent and a semilogarithmic plot of peak height (h_{peak}) against the infusion rate (k_{inf}) reveals a linear relationship [$\ln(h_{peak}) = 5.025 - 3.969k_{inf}$; $r = 0.992$; $n = 8$] in the range k_{inf} = 100–800 ml h^{-1}.

The results of a variation in the rate of injection of a 25 mg ml^{-1} solution of bropirimine in vehicle C, at an infusion flow rate of 200 ml h^{-1}, are illustrated in Fig. 7. Little precipitation was detected at lower injection rates (< 0.24 ml

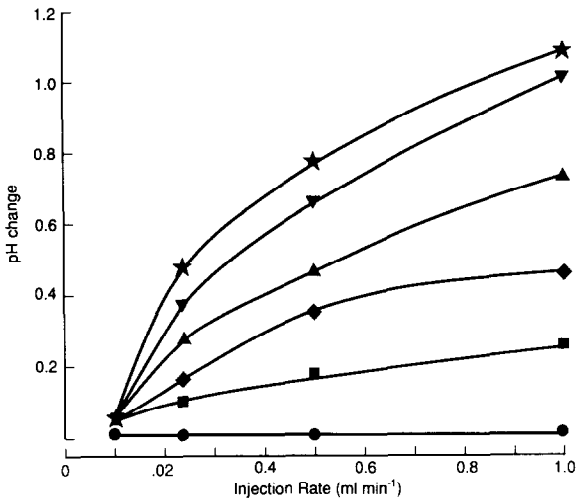

Fig. 8. Effect of injection rate of various formulations on the pH of Tris buffer (0.1 M; 400 ml h^{-1}). (●) Diazepam injection, (■) bropirimine in vehicle comprising meglumine (5%) in 0.2 M Na_2CO_3, (◆) bropirimine in vehicle F, (▲) phenytoin injection, (▼) bropirimine in vehicle comprising DMA (50%) in Na_2CO_3, (★) bropirimine in vehicle C.

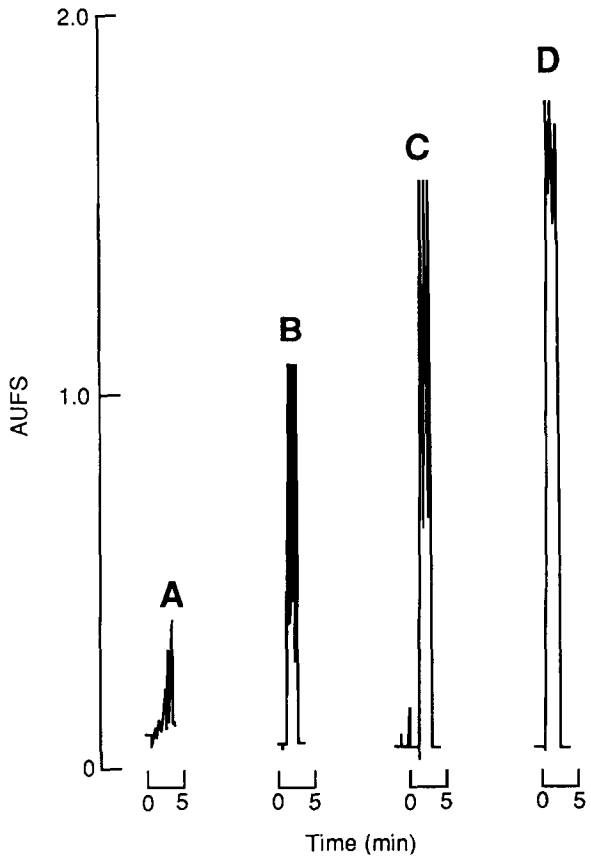

Fig. 7. Effect of injection rate on precipitation of bropirimine from vehicle C into Tris buffer (0.1 M, 200 ml h^{-1}). A, 0.1 ml min^{-1}; B, 0.24 ml min^{-1}; C, 0.51 ml min^{-1}; D, 1.0 ml min^{-1}.

min^{-1}) and a final pH of 7.40 compared favourably to that of the initial infusion fluid (7.40). When the injection rate was increased to 0.51 ml min^{-1} insufficient infusion fluid and time were available to maintain the bropirimine, now with reduced cosolvent and lower pH, in solution and immediate precipitation resulted. Indeed, this system produced so much precipitate that the tubing became blocked and the system was totally occluded (dilution ratio 6.5). The influence of the formulation may also be demonstrated. Fig. 8 shows the effect of injection rate on the pH of the mixed solvents when various formulations of bropirimine (10 mg ml^{-1}) were injected into an infusion of Tris buffer (0.1 M) flowing at 400 ml h^{-1}. With diazepam injection, which has no pH-controlled solubility, no effect on pH was observed. In all other instances, a pH increase after injection was detected and this increased with increasing injection rate. The magnitude of this effect paralleled the base-loading of the vehicle with greatest influence being exerted by the solvent containing the larger amount of meglumine in sodium carbonate solution.

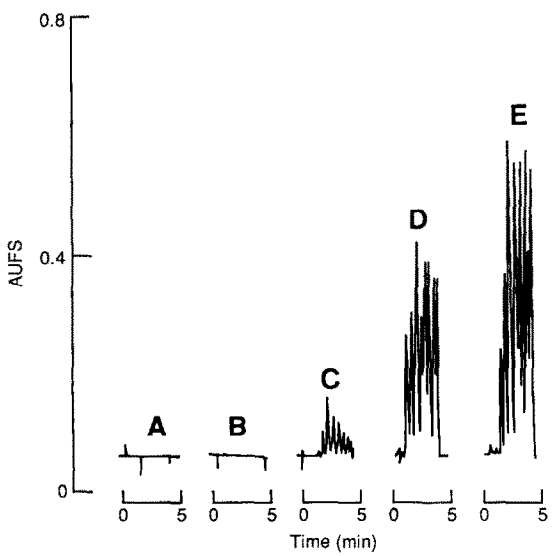

Fig. 9. Effect of bropirimine concentration in vehicle C, injected at 0.24 ml min^{-1}, on precipitation into Tris buffer (0.1 M; 400 ml h^{-1}). A, 5 mg ml^{-1}; B, 10 mg ml^{-1}; C, 15 mg ml^{-1}; D, 20 mg ml^{-1}; E, 25 mg ml^{-1}.

The concentration of the drug in the formulation also plays a significant role if precipitation is to be avoided. The effect on precipitation of five increasing concentrations of bropirimine in vehicle C is displayed in Fig. 9. Injection of concentrations below 20 mg ml^{-1}, at a rate of 0.24 ml min^{-1}, into an infusion fluid at a flow rate of 400 ml h^{-1} (dilution ratio 1:28) shows very little precipitate. In contrast, when concentrations exceed this value, precipitation is rapid. It may therefore be preferable to administer low concentrations of bropirimine at high injection rates rather than high concentrations over a long period of time. This work extends the dynamic in vitro testing of formulations developed by Yalkowsky and Valvani (1983) by introducing a buffered infusion fluid and suggests that slow injection rates (< 0.24 ml min^{-1}) or high infusion rates (> 400 ml h^{-1}) minimise the precipitation of bropirimine from injections.

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