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Modification of the aqueous solubility and stability of progabide

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Summary

The intrinsic dissolution rate of progabide was determined using compressed non-disintegrating disks. The rate was very low especially at pH 5–7 (1.7×10^{-3} mg · min^{-1} · cm^{-2}). The effect of some surface active agents, cyclodextrins and other macromolecules on the solubility of progabide was examined. Tween 80, sodium deoxycholate, α-cyclodextrin, and β-cyclodextrin enhanced the solubility whereas polyvinylpyrrolidone-40 and urea slightly decreased it. Those agents that showed promise were further studied for their effect on the stability of the drug in aqueous solutions. With all the surfactants examined, the stability of progabide was improved above the critical micelle concentration. Further, β-cyclodextrin was found to enhance the stability in neutral media but not in acidic media.

Introduction

Progabide is a γ-aminobutyric acid agonist which has undergone clinical trials for the long-term treatment of epilepsy (Musch et al., 1987). The chemical name and structure of this prodrug appeared in a previous publication (Farraj et al., 1987).

It is likely that, in vivo, progabide will display dissolution-controlled absorption because of its low solubility (Farraj et al., 1988a). Hamlin et al. (1965) determined the initial dissolution rate for a large number of compounds and found it to be directly proportional to the solubilities of the compounds over four orders of magnitude. Normally, a minimum solubility of 1% is required in the pH range 1–7 to ensure a solubility-independent absorption (Smyth and Hottendorf, 1980). Although solubility is important, the rate of dissolution is the critical factor to consider; a compound may have a solubility of 1% but may dissolve so slowly that its dissolution becomes the rate-limiting step (Smyth and Hottendorf, 1980). In general, if the dissolution rate is greater than 1 mg/(min · cm^2), there is usually no problem in absorption (Kaplan, 1972). For this reason, we investigated the intrinsic dissolution rate of progabide from non-disintegrating compressed disks. Further to that, studies were performed to examine the effect of various complexing agents on the solubility and stability of this drug. Generally, surfactants are re-

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nowned for their ability, above the critical micelle concentration (c.m.c.), to form micelles which can solubilise poorly water-soluble compounds. Surfactants can be subdivided into two categories: the endogenous surface-active agents such as bile salts and the exogenous ones such as non-ionic surfactants. In the latter category, ionic surfactants can be included but these have limited oral use because of their potential toxicity to gastrointestinal membranes (Reynolds and Prasad, 1982). Another method of enhancing the aqueous solubility is by use of cyclodextrins (Hamada et al., 1975; Saenger, 1980). These form soluble inclusion complexes with smaller molecules which fit, whole or in part, into their 0.5–0.8 nm cavity (Saenger, 1980). Molecular complexes may also be formed with macromolecules such as gums, cellulose derivatives and high molecular polyols (Cadwallader and Madan, 1981). The removal of drug molecules into the micellar phase of the surfactant system or into the hydrophobic interior of the host molecule may impart a better overall stability on the system. Thus, the potential exists for enhancing the solubility and stability of progabide by use of these systems.

Materials and Methods

Progabide micronised powder (lot 11129) and coarse powder (lot 11040) were used as received from L.E.R.S., Paris, France. Hexadecyltrimethylammonium bromide (Fluka AG), sodium dodecyl sulphate (SDS) of special pure grade (B.D.H.), Tween 80 (Honeywill-Atlas), sodium deoxycholate (Sigma), polyvinylpyrrolidone-40 (PVP-40, Sigma), α-cyclodextrin (Sigma), β-cyclodextrin (Sigma), and urea (BDH) were used without further purification.

Instrumentation

The apparatus used for the dissolution rate measurement consisted of a 50 ml jacketed beaker with an over-head paddle-type (25 mm) glass stirrer driven by a Heidolph RZRl stirrer motor. A digital revolution counter (Compact Instruments, Barnet, U.K.) was used to calibrate the stirrer speed. The dissolution medium was circulated, by an LKB 2115 peristaltic pump, to and back from a 75 μl stream analysis cell in a Cecil CE292 spectrophotometer. The connecting tubing (0.74 mm i.d.) was made of PTFE except inside the pump where silicone tubing (1.3 mm i.d.) was used. Both the analysis cell and the dissolution beaker were maintained at 37 °C by a Grant FH15 thermocirculator.

The compressed disks were prepared using a punch and die assembly (Fig. 1), machined by the Engineering Faculty at Nottingham University, on a Specac hydraulic press.

The apparatus used for the solubility determination was described previously (Farraj et al., 1988a). The analysis of samples was performed by HPLC (Farraj et al., 1987) or by spectrophotometry (Farraj et al., 1988a).

Methods

Preparation of non-disintegrating disks. 750 mg of coarse powder was compressed in a steel die of 20 mm diameter and 2 mm depth (Fig. 1) at a pressure of 10 tons per square inch applied for 6 min to produce a smooth disk, level with the upper surface of the die. Use of the micronised powder or alteration of the above conditions resulted in disks which were brittle and which showed surface cracks.

Intrinsic dissolution rate. Buffer solutions (I = 0.1) were used as the dissolution media. The die was placed at the bottom of the dissolution beaker in the centre. The stirrer height was adjusted so that the paddle was always at 25 mm directly above the disk. 50 ml of the dissolution medium, warmed to 37 °C, was then added gently to the beaker, the sampling circuit started, and the solution stirred at 102 r.p.m. The forward and return lines of the sampling circuit were placed on opposite sides of the dissolution beaker. The absorbance of the dissolution medium was monitored at 273 nm (Farraj et al., 1988b) during the initial dissolution phase and a dissolution curve was constructed by plotting the concentration of progabide against time. Triplicate runs were performed for each pH examined, using new compressed disks.

Solubility determinations. The solubility of progabide at 37 °C in 1% w/v buffered solutions

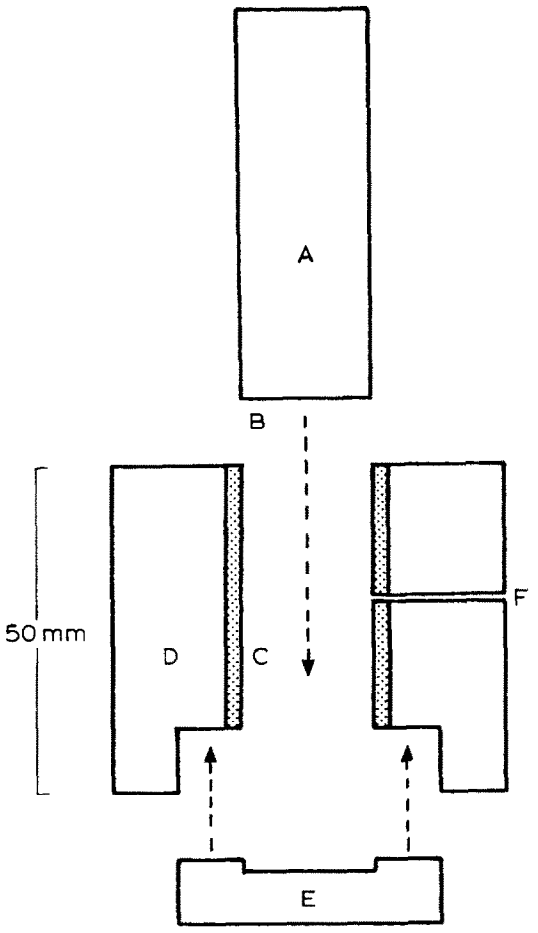


Fig. 1. Punch and die assembly used for the preparation of non-disintegrating disks: A, stainless-steel plunger; B, polished surface; C, phosphor-bronze lining; D, stainless-steel holder; E, stainless-steel mould; F, air vent.

(pH 7) of Tween 80, PVP-40, α-cyclodextrin, β-cyclodextrin, urea, and sodium deoxycholate (pH 7.4) was determined, in triplicate, by the procedure reported earlier (Farraj et al., 1988a). On the basis of the results obtained, the effect of the concentration of Tween 80, β-cyclodextrin, and sodium deoxycholate (SDC), on progabide solubility was investigated. The SDC solutions had to be buffered to pH 7.4 as they appeared cloudy at pH 7.

Spectral studies of the complexes. The change in the u.v./visible spectrum of 54.8×10^{-6} M or a 51.3×10^{-6} M solution of progabide, produced by a change in the concentration of Tween 80, β-cyclodextrin or SDC, was determined against appropriate blank solutions at 23° C.

Stability studies. 9.0×10^{-5} M solutions of progabide were prepared freshly in pH 7 buffer at varying concentrations of SDS, hexadecyltrimethylammonium bromide (C_{16}TAB) or Tween 80 both above and below their c.m.c.'s. The solutions were incubated at 37° C and samples were removed at suitable intervals for spectrophotometric analysis. The first-order degradation rate constants were computed, for the triplicate runs, as given previously (Farraj et al., 1988a).

Progabide stability, at the same concentration given above, was further examined at pH 7 and pH 1.5 in 1% w/v solutions of β-cyclodextrin at 37° C. The samples were analysed by HPLC and the first-order rate constant of degradation was calculated from the triplicate runs.

Results and Discussion

The Noyes-Whitney equation,

$$dm/dt = (D/h) \cdot A \cdot [S_t - C] \quad (1)$$

describes the dissolution of drugs which obey the diffusion layer model where dm/dt is the dissolution rate, A is the surface area of the solid, D is the diffusion coefficient of the solute across the diffusion layer of thickness h, C is the uniform solute concentration in the bulk of the solution and S_t is its saturation solubility achieved at the solid-liquid interface. If the concentration C approaches zero and the surface area, A, is held constant, as in non-disintegrating disks, then the intrinsic dissolution rate, I, can be expressed as:

$$I = (D/h) \cdot S_t = (dm/dt)/A$$

By integration,

$$m/A = I \cdot t \quad (2)$$

and a plot of m/A against time will allow the direct determination of the intrinsic dissolution rate. Experimentally, the initial phase of the dissolution curve was found to obey zero-order kinet-

ics in accordance with Eqn. 2 and was used in calculating the intrinsic dissolution rate. This avoids two problems. First, because of the low aqueous solubility of progabide (Farraj et al., 1988a), the accumulating drug in solution would soon affect the dissolution process as predicted by Eqn. 1 with the result that the assumptions of Eqn. 2 would be invalidated. Second, because of progabide instability, a concomitant process other than dissolution was taking place in the system. As the degradation rate is first-order (Farraj et al., 1988a), its effect would have been more pronounced at later times. Hence, the rationale for using the slope of the initial dissolution curve.

The intrinsic dissolution rate-pH relationship is presented in Fig. 2. The striking similarity between this profile and that of the solubility-pH profile (Farraj et al., 1988a) is a clear indication of the controlling influence of solubility on the rate of dissolution. At pH 5–7, the intrinsic dissolution rate was only 1.7×10^{-3} mg · min^{-1} · cm^{-2}. In fact, all the data points of Fig. 2 were well below the lower limit of intrinsic dissolution rate suggested by Kaplan (1972) for dissolution-independent absorption. However, because of the logarithmic relationship between solubility and pH (Farraj et al., 1988a), it is expected that the dissolution rate will approach this limit as the pH is decreased further. No experimental data points were obtained below pH 1.8 because the disk integrity and constant surface area could not be maintained as a result of the high solubility of progabide in that pH region. Nevertheless, it is anticipated that dissolution may prove to be rate-limiting in the in vivo situation.

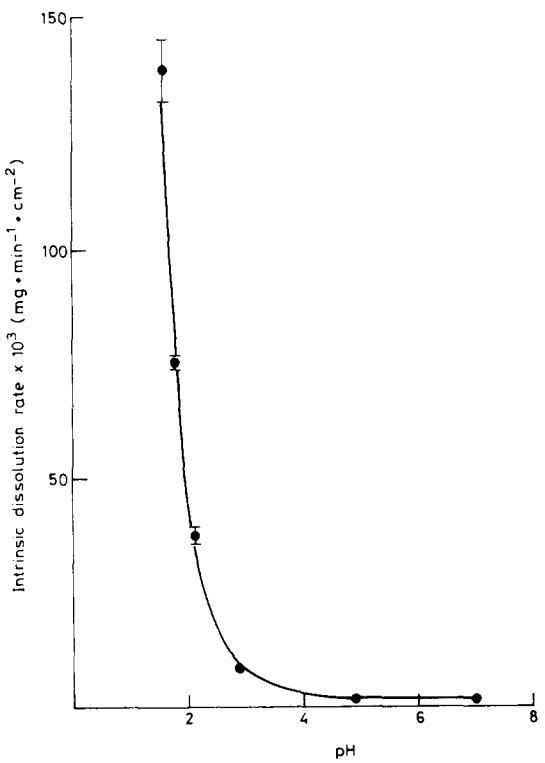


Fig. 2. Variation of the intrinsic dissolution rate of progabide with pH. Each point represents the mean ± S.D.

TABLE 1

Effect of various complexing species on the solubility of progabide in buffered solutions (pH 7)

Potential complexing species	$[S_t \pm$ S.D.$] \times 10^6$ (M)
None	111.0 ± 0.1
Tween 80	1505.1 ± 4.5 **
Polyvinylpyrrolidone-40	107.2 ± 0.9 *
α-Cyclodextrin	252.5 ± 6.3 **
β-Cyclodextrin	420.0 ± 6.6 **
Urea	94.9 ± 1.0 **
Sodium deoxycholate (pH 7.4)	950.0 ± 6.0 **

Significance of difference by Student's t-test: * $P < 0.002$, ** $P < 0.001$.

For the second part of the study, the c.m.c. values were taken as 8.5×10^{-3} M and 9.9×10^{-4} M for SDS and C_{16}TAB respectively (Mukerjee and Mysels, 1971), and 1.07×10^{-5} M for Tween 80 (Wan and Lee, 1974). The molecular weight of Tween 80 was taken as 1308 (Krasowska, 1976). The solubility of progabide at pH 7 was previously determined as 1.11×10^{-4} M (Farraj et al., 1988a). The solubility data obtained (Table 1) indicated that Tween 80, α-cyclodextrin, and β-cyclodextrin enhanced the solubility of progabide whereas PVP-40 and urea slightly decreased it.

Variation of Tween 80, β-cyclodextrin, and SDC concentrations affected the solubility of progabide as shown in Fig. 3. By comparison, it can be seen that the solubilising power of Tween 80 is much greater than that of SDC. This is probably because bile salts, such as SDC, differ significantly

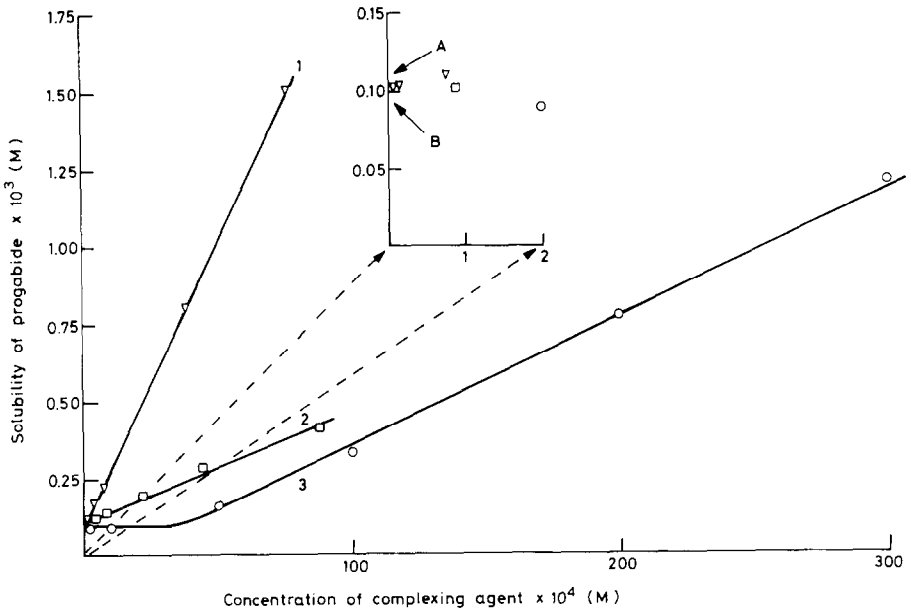


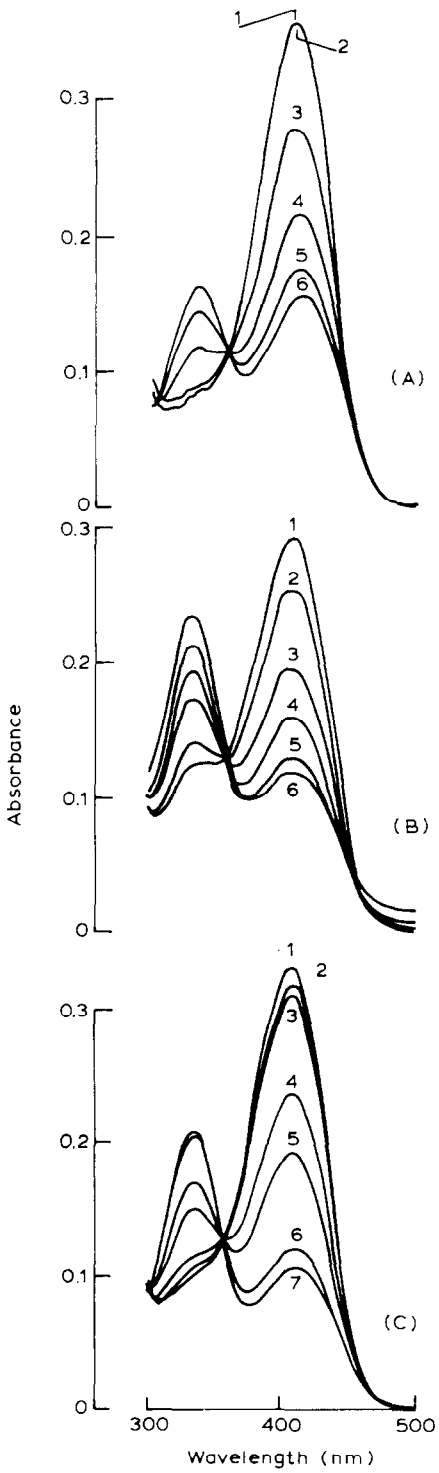
Fig. 3. Variation of the solubility of progabide with the concentration of complexing agents. The pH was 7.0 for Tween 80 (1) and β-cyclodextrin (2) but 7.4 for SDC (3). A and B indicate progabide solubility in the buffers of pH 7 and 7.4, respectively.

from ordinary aliphatic detergents, such as Tween 80, in that they are restrained to a rigid structure. They form no liquid-crystal phases and form relatively small micelles. In contrast, long-chain aliphatic detergents form liquid-crystal phases and form large micelles (Small, 1968). For both surfactants above their c.m.c. values, the linear increase produced in the solubility of progabide (Fig. 3) indicates that the average number of solubilisate molecules per micelle and the concentration of the drug in the aqueous phase remain constant, otherwise, a linear relationship between the solubility and surfactant concentration would not result (Moroi et al., 1982). This is substantiated further by the lack of change in the position of the isosbestic points of Fig. 4A, C.

The solubility of progabide also increased with the addition of β-cyclodextrin (Fig. 3) in an almost linear fashion which indicates the formation of a 1 : 1 complex (Miyahara and Takahashi, 1982). Further evidence for this type of association was again obtained from the invaried position of the isosbestic point in Fig. 4B. Assuming a 1 : 1 complex, the stability constant, K_{11}, can be calculated (Higuchi and Kristiansen, 1970) from the equation:

$$K_{11} = S/[S_t \cdot (1 - S)] \quad (3)$$

where S is the slope of the plot of the solubility of progabide vs β-cyclodextrin concentration and S_t is the drug solubility in the absence of β-cyclodextrin. The value of K_{11} obtained using the above equation was 353 M^{-1}. Szejtle (1981) in his evaluation of the effect of β-cyclodextrin on the bioavailability of drugs pointed out that the ideal stability constant should be between 100 and 1000 M^{-1}. Smaller stability constants give too weak an interaction to improve the solubility whereas larger values than the optimum range hinder the absorption process by reducing the thermodynamic activity of the drug. It is clear that the stability constant of the progabide/β-cyclodextrin lies well within the required range. It is interesting to note that if Eqn. 3 is used for the micellar solubilisation of progabide then the value of the computed as-



sociation constant, K_{ij}, is 457 M^{-1} for SDC and 2122 M^{-1} for Tween 80. The use of the symbol K_{ij} indicates that i molecules of progabide are associated with j molecules of the surfactant.

Table 2 is a summary of the stability data obtained showing the apparent first-order degradation rate constants. Comparison with the stability of progabide at pH 7 reveal that, below the c.m.c., C_{16}TAB and SDS improved the stability of the drug whereas Tween 80 slightly decreased it. Above the c.m.c., all the surfactants examined improved the stability of progabide. An insight into the mechanism of stabilisation cannot be gained without further studies that involve varying the drug concentration and pH. However, what appears to happen is that the molecules of progabide are removed from the aqueous phase into the micellar phase where they are protected to varying degrees from hydrolytic degradation depending on the surfactant in question. With β-cyclodextrin, the results obtained (Table 2) indicated that the stability of progabide was improved at pH 7 but worsened at pH 1.5. This is probably caused by a better accommodation of the progabide molecule in the β-cyclodextrin cavity at pH 7 where the drug is unionised than at pH 1.5 where the drug is in its ionised form (Farraj et al., 1988b). This combined with the fact that cyclodextrins can (Bender and Komiyama, 1978) catalyse a number of reactions, including hydrolysis, by acting as enzyme substrates help explain the observed effect of β-cyclodextrin.

Overall, the implication of the data gathered is that progabide cannot be formulated readily as an aqueous dosage form with acceptable shelf-life using conventional techniques. However, the stability improvement and solubility enhancement achieved by micellisation or molecular encapsulation might, if applied correctly, improve the oral

Fig. 4. Effect of the concentration of the complexing species on the absorption spectrum of progabide, present at 51.3×10^{-6} M for A and at 54.8×10^{-6} M for B and C. A: 1–6 represent 2, 10, 50, 100, 200, and 300×10^{-4} M sodium deoxycholate at pH 7.4. B: 1–6 represent 0.88, 4.4, 8.8, 22.0, 44.1, and 88.1×10^{-4} M β-cyclodextrin at pH 7.0. C: 1–7 represent 0.08, 0.38, 0.76, 3.8, 7.6, 38.2, and 76.4×10^{-4} M Tween 80 at pH 7.0.

TABLE 2

Effect of various complexing agents on the aqueous stability of progabide

Agent	Concentration of agent (M)	$[k' \pm \text{S.D.}] \times 10^3$ (min^{-1})	$t_{1/2}$ (min)
None [a]	–	5.042 ± 0.085	138
None [b]	–	39.145 ± 0.521	18
SDS [a]	1.39×10^{-3}	4.786 ± 0.035 **	145
	20.81×10^{-3}	1.442 ± 0.010 ***	481
C_{16}TAB [a]	4.15×10^{-4}	3.454 ± 0.027 ***	201
	16.57×10^{-4}	1.102 ± 0.005 ***	629
Tween 80 [a]	0.34×10^{-5}	5.266 ± 0.018 *	132
	338.7×10^{-5}	4.692 ± 0.058 **	148
	763.5×10^{-5}	0.858 ± 0.012 ***	808
	3817.7×10^{-5}	0.574 ± 0.010 ***	1 208
	7635.3×10^{-5}	0.537 ± 0.006 ***	1 291
β-Cyclodextrin [a]	88.1×10^{-4}	2.282 ± 0.027 ***	304
β-Cyclodextrin [b]	88.1×10^{-4}	52.768 ± 1.4 ***	13

k' denotes the first-order degradation rate constant. Significance of difference by Student's t-test: * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$.

[a] pH = 7.0, [b] pH = 1.5.

bioavailability of the solid dosage forms of this drug.

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