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SOME FACTORS INFLUENCING THE IN VITRO RELEASE OF PHENYTOIN FROM FORMULATIONS

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SUMMARY

Using a stirred flask method the dissolution behaviour of formulations containing sodium phenytoin or phenytoin acid have been compared. In general the release pattern from capsules is more complex than that from tablets. The pH of the dissolution medium requires to be specified with accuracy. Formulation variables which affect release are particle size, packing of the capsule and the nature of the diluent. The formation of insoluble calcium or magnesium salts from formulation adjuvants is believed to occur and may be a factor which should receive further attention both in vitro and in vivo.

INTRODUCTION

Although phenytoin is a drug of first choice in the treatment of major epilepsy it has several important disadvantages, in particular a narrow therapeutic ratio (Richens and Dunlop, 1975). Phenytoin itself is a weak acid, practically insoluble in water, and differences in bioavailability from various formulations might be anticipated (Alvan et al., 1975). Despite the widespread use of the soluble sodium salt the bioavailability of the drug has been subject to question and close scrutiny. This increased following an outbreak in toxicity in Australia (Tyrer et al., 1970, 1971; Bochner et al., 1972a and b). Initially the outbreak was attributed to a change in formulation of the capsules of the sodium salt which involved replacing the original diluent, calcium sulphate dihydrate, with lactose. This apparently produced a rapid increase in the release rate of the drug in vivo. Further investigations have shown that the absorption of the drug in vivo is a more complex phenomenon. Tyrer et al. (1970), Appleton et al. (1972) and Bochner et al. (1972b) noted that the calcium sulphate itself may have been involved in suppressing the toxicity of the drug or reducing absorption. The observation that the presence of calcium sulphate results in an increased faecal phenytoin excretion (Tyrer et al., 1970), may be significant. In this connection Glazko and Chang (1972) observed

that phenytoin was capable of forming a chelate with copper but not with calcium, magnesium or ferrous ions. However, these authors do not appear to have taken into account the possible formation of salts. A calcium salt of phenytoin, Phenhydan, is mentioned in the Merck Index (9th ed., 1976).

The absorption of the sodium salt is non-uniform (Albert et al., 1974) and more recently, by newer non-linear pharmacokinetic techniques, has been shown to be prolonged over a period of at least two days (Jusko et al., 1976). Owing to protein binding and other factors the half-life has been estimated to be 22 ± 9 hr (Arnold and Gerber, 1970). Since the sodium salt is likely to be precipitated as the insoluble free acid in the stomach a number of investigations of the use of the free acid itself have been undertaken. Bochner et al. (1972a) found that there was no advantage to be gained in using the acid as a delayed action product since it was absorbed at about the same rate as the sodium salt. Lund (1974) claimed that the sodium salt was more completely absorbed than the acid, but the results may have been confused by the use of a formulation of the latter which, on further examination, proved to have poor bioavailability (Tammisto et al., 1976). This group of workers, in fact, supported the idea that the acid is adequately absorbed and gives better control over the epileptic patient than formulations of the sodium salt (Dill et al., 1956).

Other formulation variables include the use of tablets or capsules as delivery systems (Manson et al., 1975), and the particle size of the drug (Johansen, 1972; Johansen and Wiese, 1970; Neuvonen et al., 1977). Arnold et al. (1970) demonstrated that the pH of the dissolution medium may influence results in vitro and Neuvonen et al. (1977) have recently shown that the in vitro dissolution rate at pH 9.0 gave good correlation with absorption when the drug was administered to volunteers. This may appear to be rather surprising since the pH of intestinal contents rarely, if ever, approaches this level.

This present study was initiated in order to determine if there were other factors involved in vitro which might be related to any future in vivo studies.

MATERIALS AND METHODS

Materials

Formulations of sodium phenytoin were obtained from a pharmacy in order to be representative of currently available commercial material.

Capsules (Parke Davis): 50 mg, batch no. 071086A; 100 mg, batch no. 473106A.

Tablets (Thomas Kerfoot): 50 mg, batch no. 01 LDL; 100 mg, batch no. 02 LAL.

Sodium phenytoin BP, supplied by Parke Davis and Company Ltd., batch no. 1460.

Phenytoin, supplied by Parke Davis and Company Ltd., batch no. 3357.

All other materials and reagents were of laboratory grade. Empty hard gelatin capsules were Size 2 Lockcaps supplied by the Elanco Division of Eli Lilly and Company. The pH 9.0 buffer was the Korthoff borax-phosphate system (Documenta Geigy Scientific Tables, 6th ed.) adjusted using a glass electrode pH meter.

Assay

Although Arnold et al. (1970) measured the UV absorbance of aqueous solutions of phenytoin from dissolution tests directly at 230 nm, it was found that the procedure due

to Wallace (1966), modified by Wallace and Hamilton (1974). was to be preferred. This involves oxidation of the phenytoin to benzophenone, extraction with *n*-heptane and determination of the benzophenone at 247 nm. A sample volume of 3 cm^3 was added to 4 cm^3 5 N sodium hydroxide and 250 mg potassium permanganate in a stoppered 25 cm^3 conical flask painted matt black to protect the contents from light. The flask was immersed in a boiling water bath with continuous shaking for 15–20 min. It was then allowed to cool to room temperature before extracting with a total of 3 cm^3 *n*-heptane. The heptane solution was filtered through paper and the absorbance determined at 247 nm in a 1 cm quartz cell using an Unicam SP 800 spectrophotometer. A calibration curve obtained from solutions of crystalline sodium phenytoin showed that Beers Law was obeyed, with a slope of 0.032 (correlation coefficient 0.99) and passing through the origin. Repeating the experiment with 20 mg lactose in each sample displaced the curve by an amount equivalent to 4.8 μg phenytoin, the slope being 0.031 (correlation coefficient 0.99). Since other oxidizable materials will clearly affect the result in a similar fashion and may be present in commercial formulations to an unknown degree, most dissolution data given here are reported in terms of the percentage mass of the total amount of drug detected.

Solubility

The method used was similar to that of Dill et al. (1956) in that samples were shaken overnight at ambient room temperature (20–23°C), filtered through 0.45 μm pore diameter membrane filters and assayed directly. Results are given in Table 1.

Particle size alterations

A sample of sodium phenytoin was allowed to recrystallize slowly from absolute ethanol. After standing overnight the crystals were collected, washed and air dried. They were then stored over anhydrous calcium chloride under vacuum for up to 48 hr. Samples were classified by sieving and ground in a Fritsch agate vibrating ball mill to provide a total of 4 different size ranges. Each sample was sized by optical microscopy using liquid paraffin as a dispersion medium. Since the particles were elongated acicular crystals the weight distribution was calculated from the square root of the product of the length and breadth. The weight distribution was then converted to a volume-surface mean diameter

TABLE 1

SOLUBILITY OF PHENYTOIN AND SODIUM PHENYTOIN IN AQUEOUS MEDIA AT AMBIENT ROOM TEMPERATURE (20–23°C)

Medium	pH	Solubility $\mu g\ cm^{-3}$	
		Phenytoin	Sodium phenytoin
Hydrochloric acid 0.1 M	1	10	19
Water	6–7	95	236
Borax/phosphate	9.0	160	480
Sodium hydroxide 0.1 M	10	945	2250

($d_{vs} = \Sigma nd^3/\Sigma nd^2$). The effect of particle size on dissolution rate was tested by weighing 100 mg of the sodium phenytoin sample and 150 mg lactose, mixing gently with a spatula and hand-filling into a gelatin capsule. Whilst tedious this process did ensure that there was minimum attrition on the power sample and was considered to be sufficiently accurate for the purpose of the test.

Dissolution test

The test used was similar to that described by Groves (1973), and consisted of stirring 1 dm^3 of the appropriate dissolution medium in a 3-necked round bottomed 2 dm^3 flask with a 2 cm PTFE flat blade positioned 1.5 cm below the liquid meniscus using a constant speed motor adjusted to 50 rev^{-1}. The flask and its contents were maintained at 37 ± 0.5°C using a water bath. Samples were admitted through a side-neck, capsules being wound with a stainless steel spiral to prevent floating. Assay samples were removed at timed intervals and filtered through a 0.45 μm pore diameter membrane filter. Each sample was replaced by an equal volume of fresh dissolution medium at the temperature of the water bath. Results are given as dissolution profiles of percentage mass released vs time or as the log time vs probit plots which often linearize dissolution data obtained from tablets (Wagner, 1971).

RESULTS

Dissolution tests on the commercial tablets are shown in Fig. 1 and on the commercial capsules under different conditions in Fig. 2. It should be noted that, under conditions of acidic pH, no drug could be detected in the dissolution medium. Each line is the mean of 5 replicates. The marked bimodal character of the release profile for the capsules made direct comparison with the tablet behaviour somewhat difficult. However, the times for 50% of drug to be released, t_{50}, might suggest that the tablets released their contents rather more rapidly than the capsules in water since the t_{50} for the 100 mg tablets and capsules were 20 and 24 min, respectively. The differences were more marked for the 50 mg products, being 12 and 32 min for the tablets and the capsules, respectively. Increasing the pH of the medium produced a decrease in the t_{50} values for the capsules from 38 min at pH 9 to 28 min at pH 10, but when compared to the value of 24 min in water and the generally involved behaviour shown in Fig. 2 these differences could not be said to be significant.

To determine the effect of encapsulation 100 mg tablets were scraped to remove the sugar coat and the exposed cores crushed in a mortar and pestle before packing in a no. 2 capsule. These formulations were compared with capsule contents compressed in a 0.5 in. (12 mm) punch and die at a pressure of 0.5 tons (33 MPa) (Fig. 3). Although not shown here the log probability plots indicated that the tabletted capsules had a simple unimodal release pattern whereas the capsules made from tablets were bimodal. This strongly suggests that the characteristic bimodal behaviour of the capsules is due to the capsule shells.

Formulation variables tested in capsules did not generally show this bimodal behaviour, Figs. 4–7, although it was a common feature. The variable factors exmined are given in the captions to the figures and include packing (Fig. 4), particle size (Fig. 5) and various diluents and adjuvants (Figs. 6 and 7).

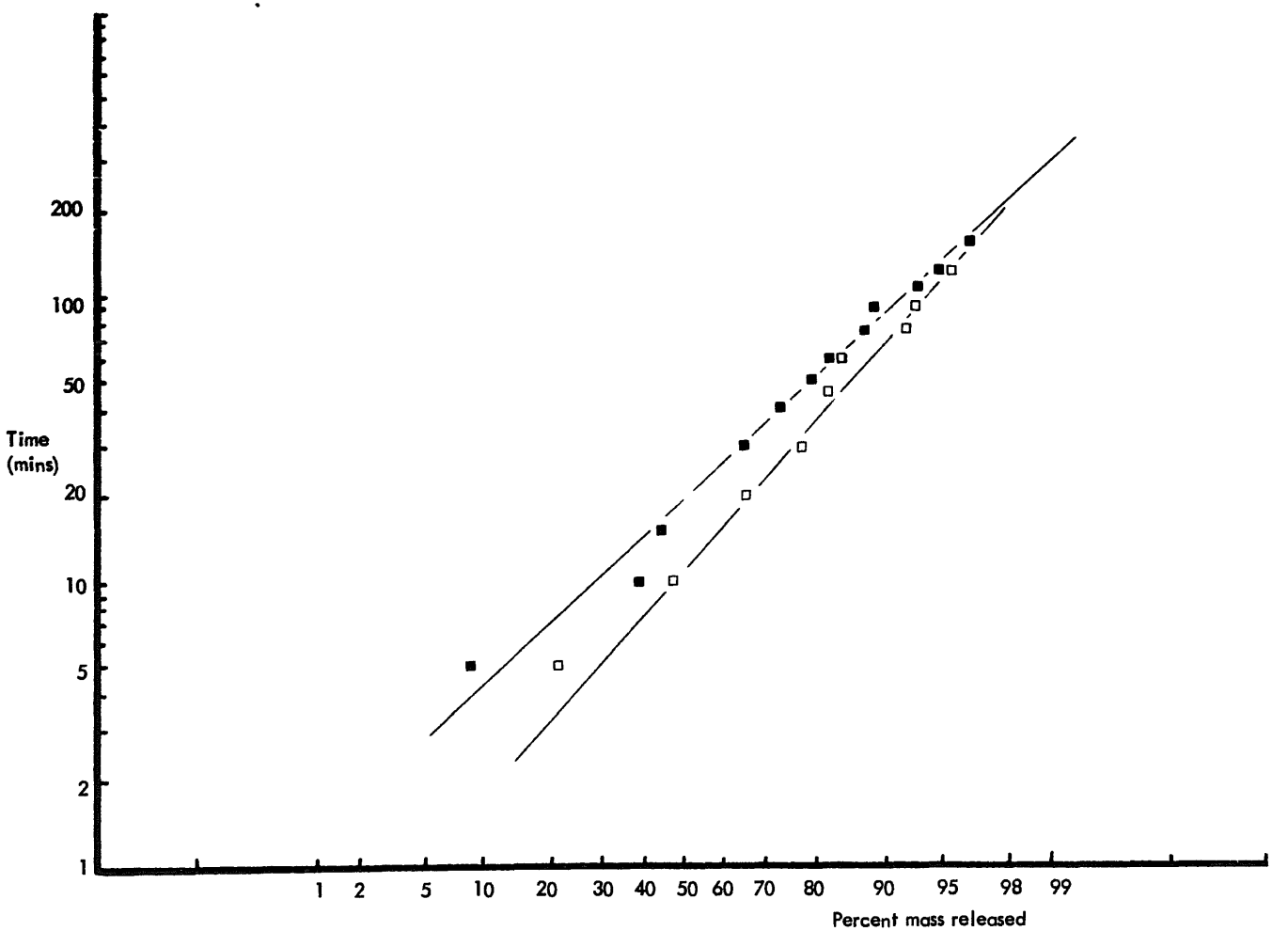

Fig. 1. The release of phenytoin from commercial tablets containing sodium phenytoin in water at 37°C (mean of 5). □——□, 50 mg tablets; ■——■, 100 mg tablets.

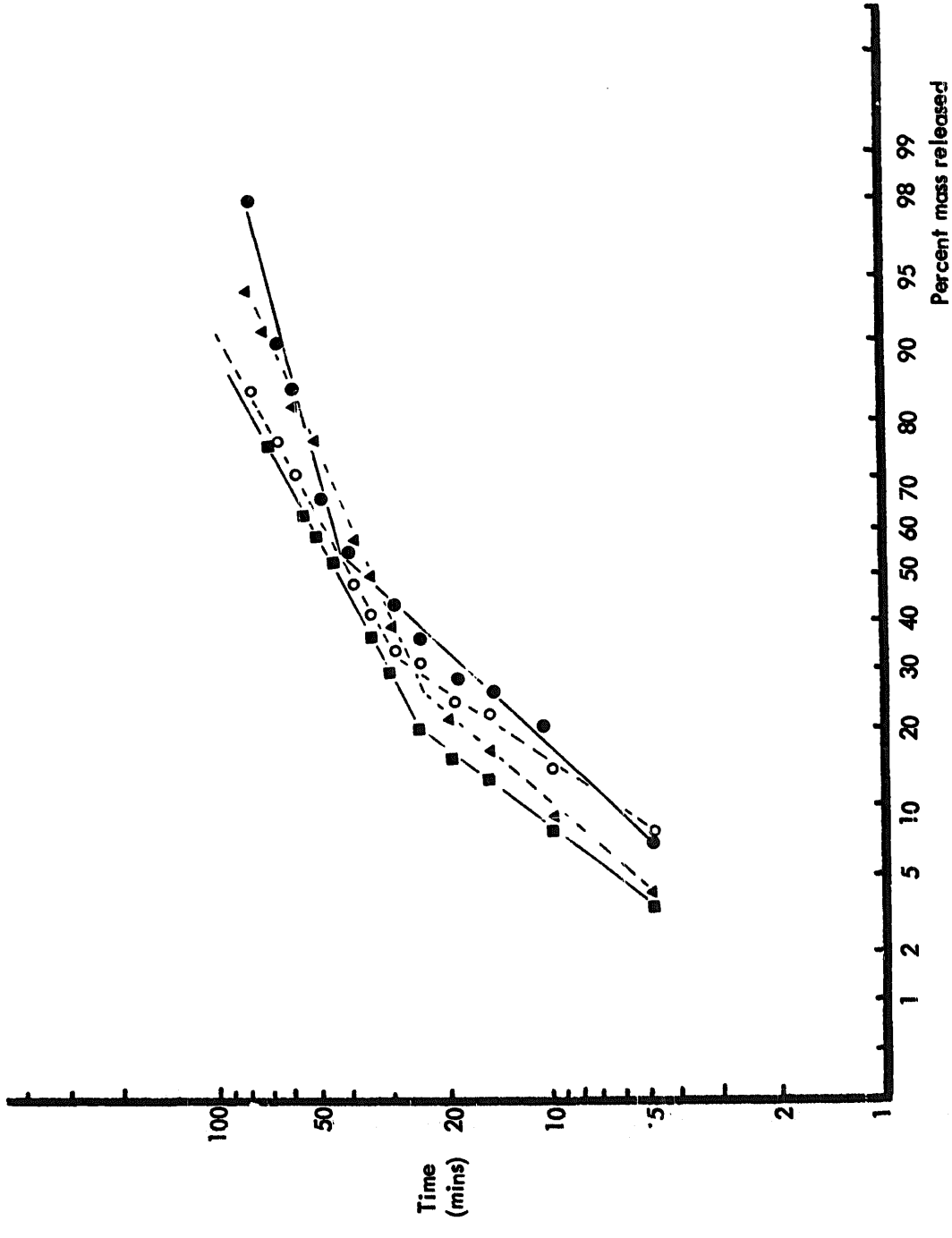

Fig. 2. Release of phenytoin from commercial sodium phenytoin capsules (mean of 5) at 37°C. o-----o, 50 mg in water; ●——●, 100 mg in water; ■——■, 100 mg in buffer pH 9.0; ▲- - - -▲, 100 mg in buffer pH 10.0. No results obtained at pH 1.0.

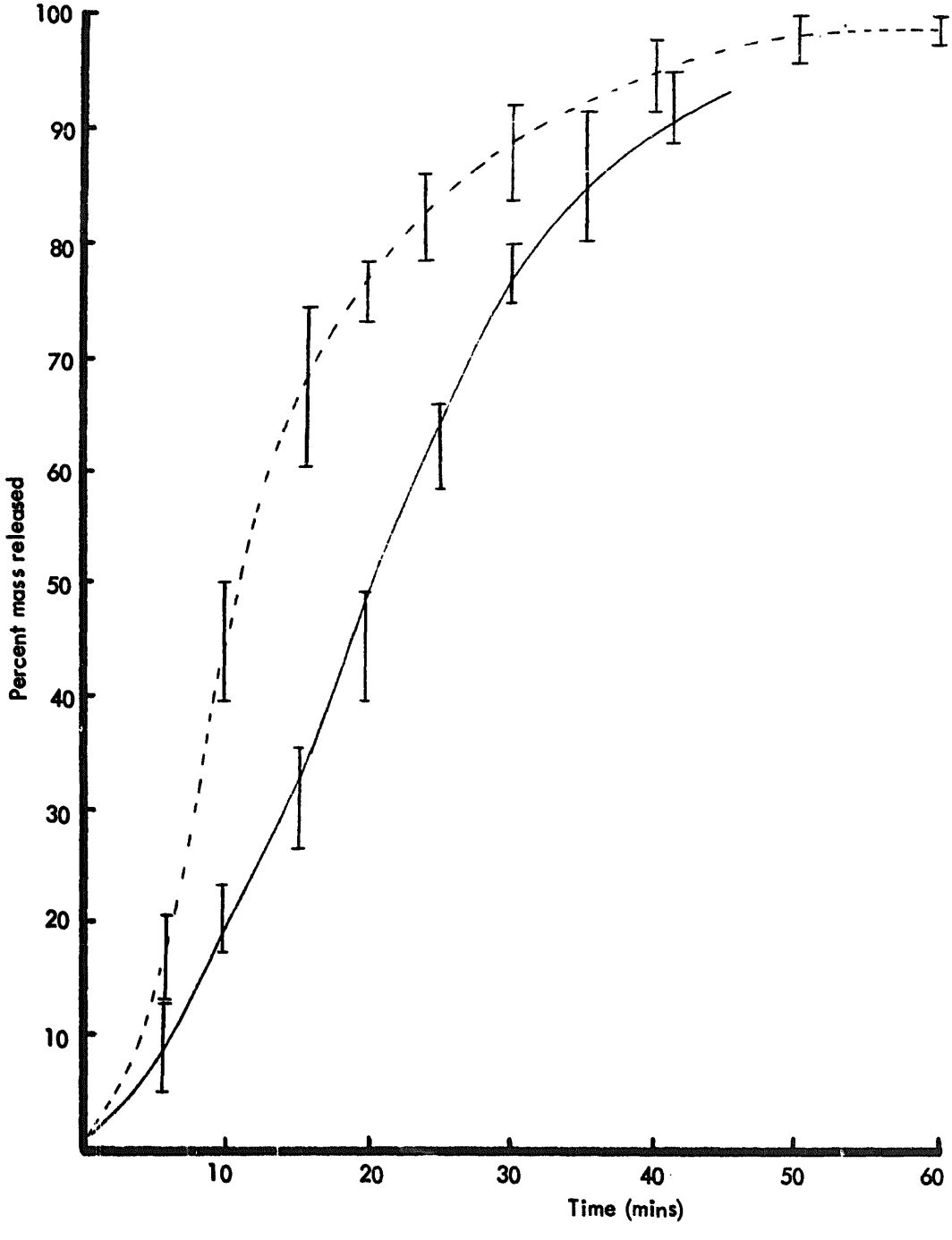

Fig. 3. Dissolution profiles of capsules made from tablets (———) and tablets made from capsules (- - - - - -) (see text). Bar lines indicate scatter from 5 replicates.

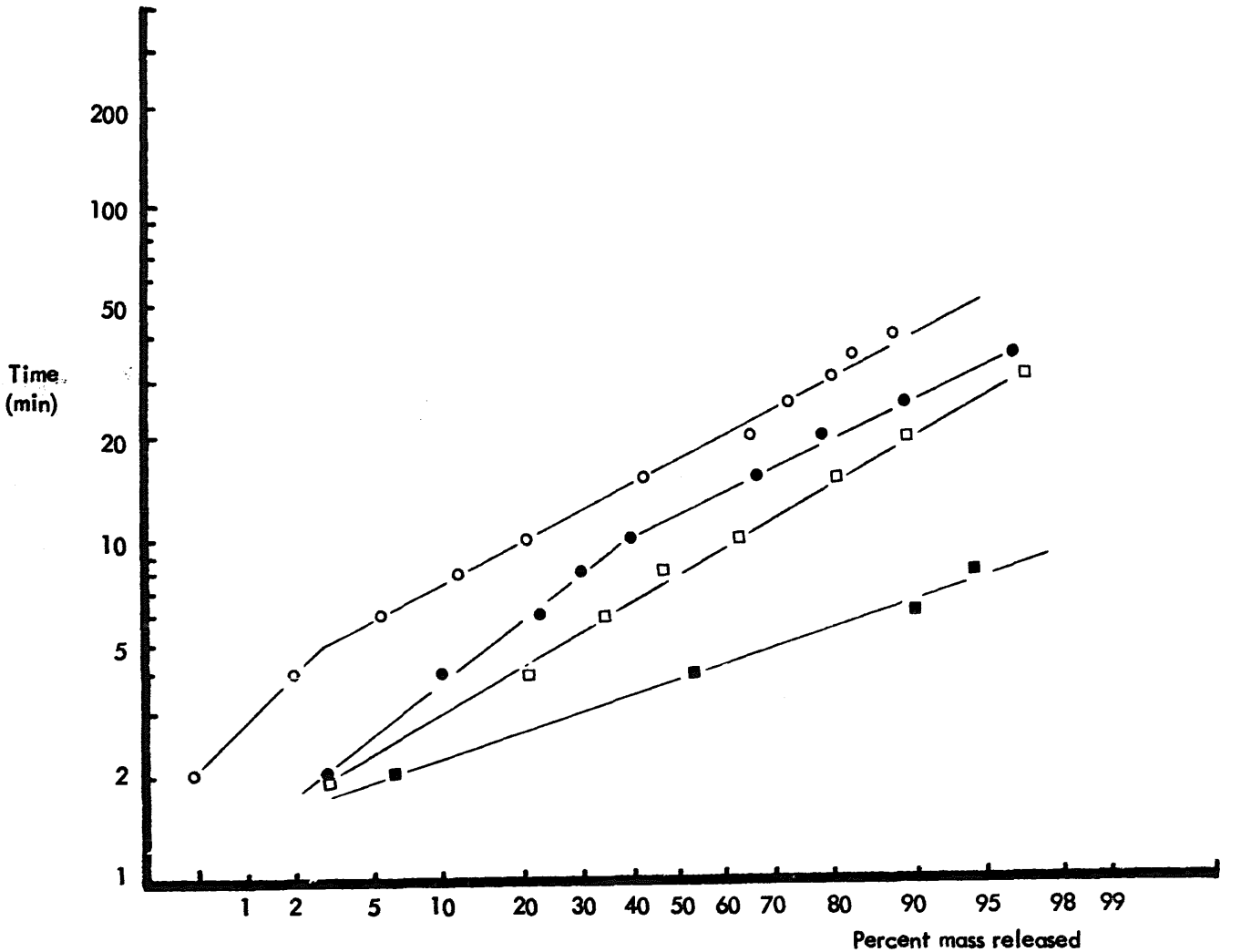

Fig. 4. The effect of packing 100 mg sodium phenytoin (d_{vs} ~ 100 μm) with varying amounts of lactose into a size 2 capsule. ■———■, 0; □———□, 40 mg lactose; ●———●, 70 mg lactose; ○———○, 130 mg lactose.

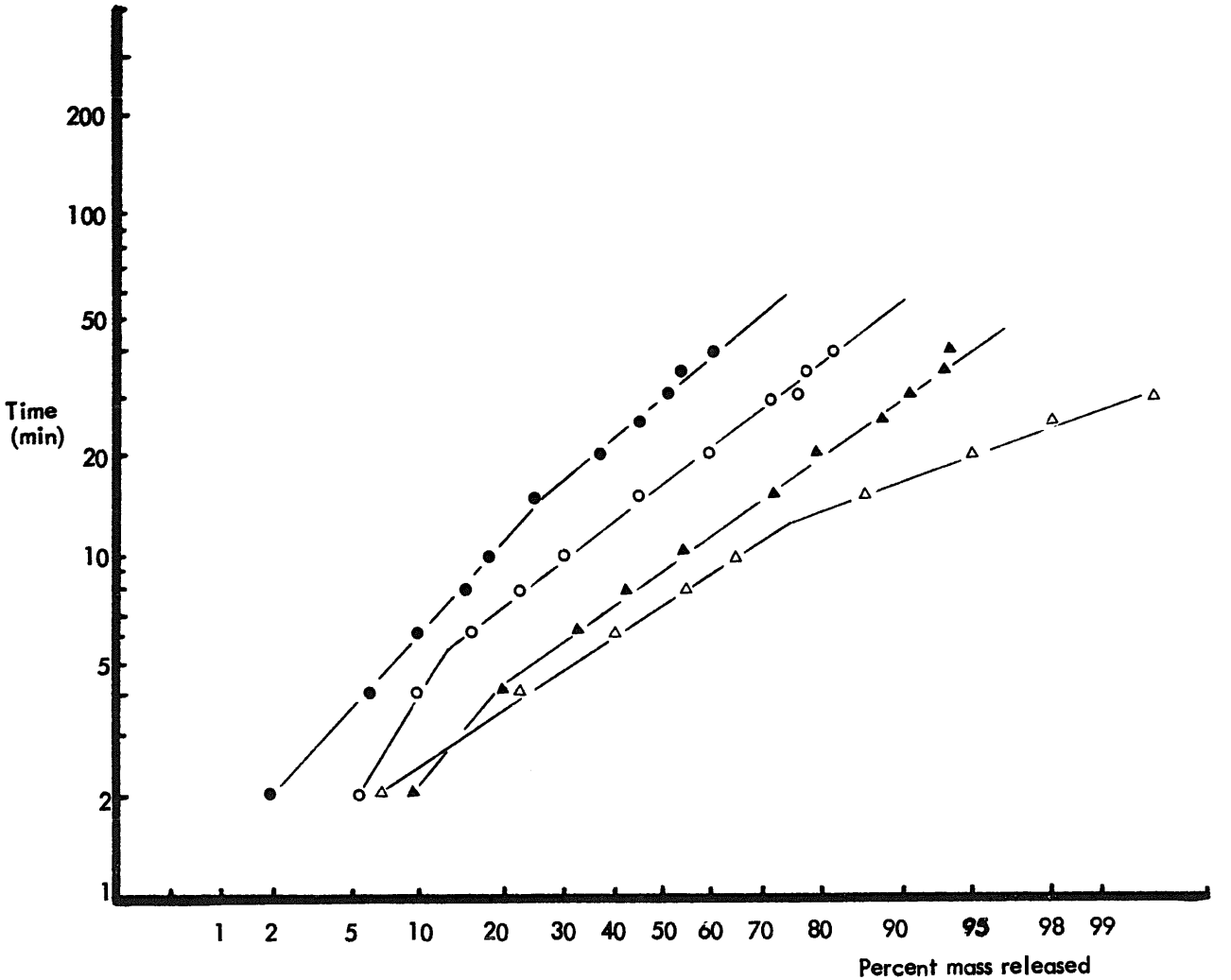

Fig. 5. Dissolution of phenytoin from capsules containing 100 mg sodium phenytoin of different particle sizes and 150 mg lactose. ●———●, mean particle size (d_{vs}) 150 μm; ○———○, mean particle size (d_{vs}) 80 μm; ▲———▲, mean particle size (d_{vs}) 47 μm; △———△, mean particle size (d_{vs}) 22 μm.

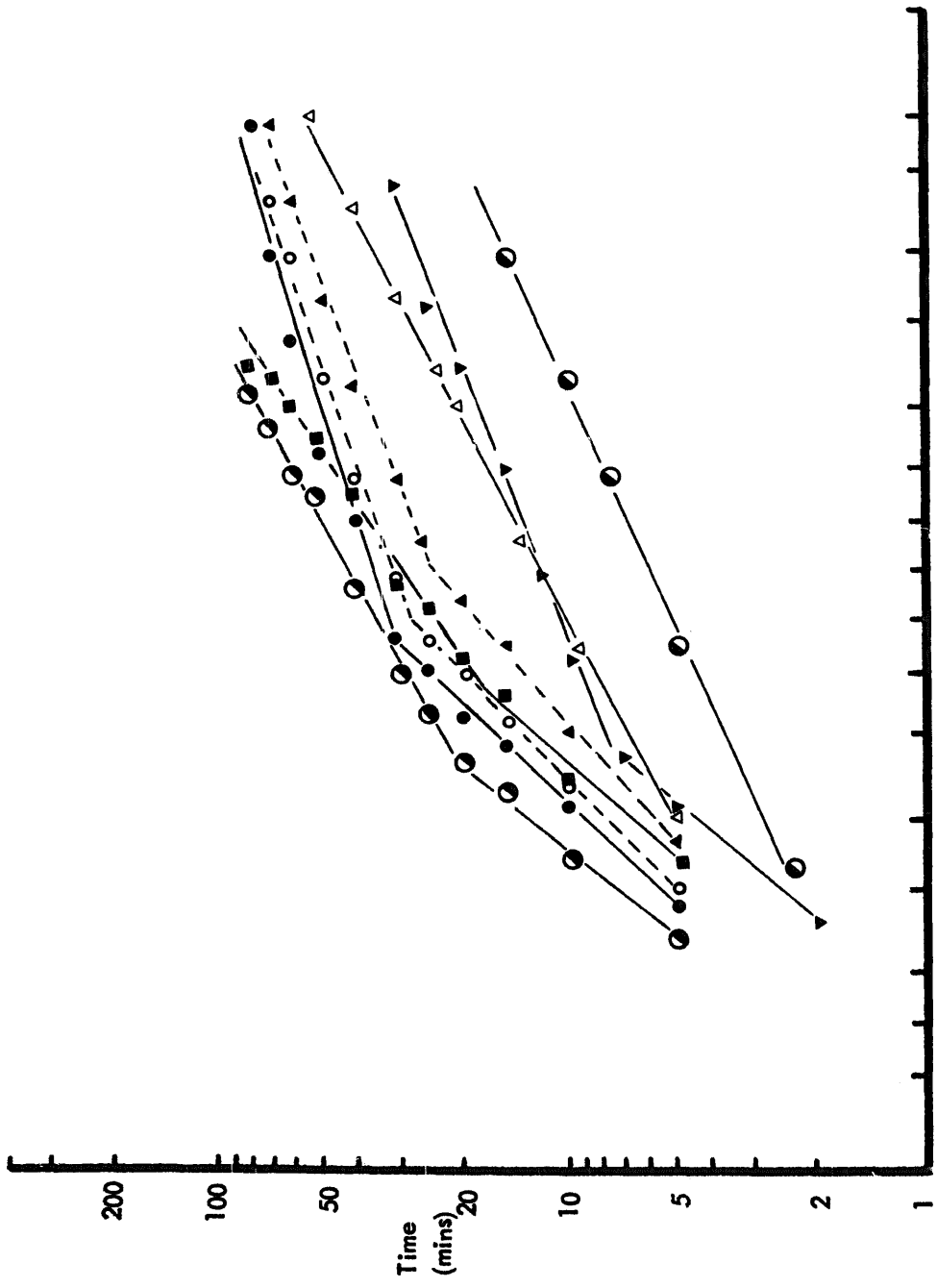

Fig. 6. The effect of formulation variables on the release of phenytoin and sodium phenytoin from capsules into water at 37°C. △——△, sodium phenytoin 100 mg lactose 130 mg; ▲- - - - -▲, sodium phenytoin 100 mg lactose 80 mg corn starch 50 mg; ○- - - - -○, sodium phenytoin 100 mg lactose 30 mg corn starch 100 mg; ●——●, sodium phenytoin 100 mg corn starch 130 mg; ◐——◐, sodium phenytoin 100 mg lactose 130 mg + 1% sodium lauryl sulphate; ▼——▼, sodium phenytoin 100 mg corn starch 130 mg + 1% sodium lauryl sulphate; ◕——◕, phenytoin acid 100 mg lactose 130 mg + 1% sodium lauryl sulphate; ■——■, phenytoin acid 100 mg corn starch 130 mg + 1% sodium lauryl sulphate.

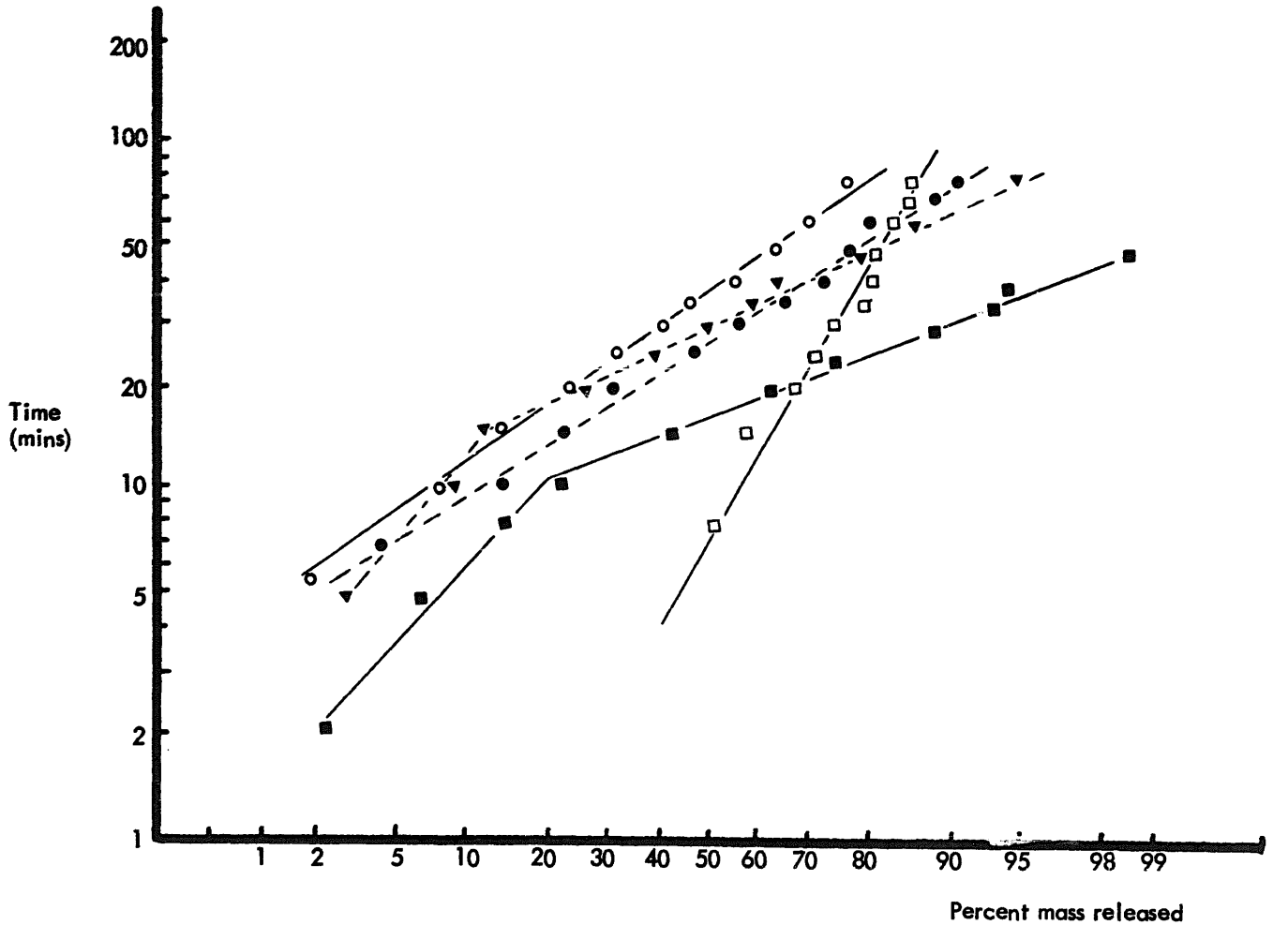

Fig. 7. Release of phenytoin from capsules containing various adjuvants. ■———■, sodium phenytoin 100 mg lactose 130 mg; ▼- - - - - -▼, sodium phenytoin 100 mg $CaSO_4$, $2H_2O$ 130 mg*; ●- - - - - -●, sodium phenytoin 100 mg lactose 130 mg + 5% magnesium stearate; ○———○, sodium phenytoin 100 mg $CaSO_4$, $2H_2O$ 130 mg + 5% magnesium stearate; □———□, sodium phenytoin 100 mg $CaSO_4$, $2H_2O$ 130 mg + 1% sodium lauryl sulphate*. No release was obtained from a capsule containing 100 mg sodium phenytoin and 130 mg magnesium sulphate.

* Not all the drug was released in these samples and the curves are estimated based on the amount filled into the capsule.

DISCUSSION

Phenytoin is a weak organic acid which is substantially insoluble in water but dissolves in alkali. It was therefore surprising to note that the sodium salt and the free acid differed considerably in solubility when dissolved in aqueous media of different pH. The sodium salt may have a higher solubility than the acid because it always has a higher degree of ionization, irrespective of the pH. In vivo it might be anticipated that the sodium salt would be precipitated as the acid at the pH of the stomach conditions, but the state of the precipitate, especially the particle size and the presence of other materials acting as precipitation nuclei, could exercise a considerable influence on the subsequent rate of solution during passage down the intestine. This consideration must also be made when attempting to correlate in vitro and in vivo performance and it is clear that the pH of the dissolution medium requires to be specified with considerable precision. The reduction of the t_{50} times obtained by increasing the pH of the dissolution medium from 9 to 10 was not in relation to the apparent increase in solubility at room temperature and would not appear to be likely to be significant if tested under clinical conditions. This would suggest that the overall dissolution behaviour of the drug, especially if presented in hard gelatin capsules, may be an altogether more complex phenomenon.

The complexity of behaviour is also suggested by the apparent bimodal character of the release pattern from capsules when plotted on log-probability paper. Experience using this type of presentation suggests that it is a useful way of linearizing dissolution data, at least for tablets which disintegrate progressively into smaller fragments. The main advantage of log-probability linearization is that it is easier to interpolate release figures for given times or the times corresponding to give percentages. However, the slope or standard deviation of the line is a measure of the release rate (probit of the mass change as a function of the logarithm of the time). The fact that the release from capsules appears to take place at different rates may be due partially to the influence of the gelatin shell itself, and later, to penetration of water and subsequent release from the power mass contained within the shell. Although much faster in release than the formulations they were derived from, the capsules made from the powdered tablets became bimodal in behaviour. This suggests that, under the right conditions of powder packing, the gelatin may protect the powder bed from deaggegating under the conditions of the test. The capsule formulation is clearly not ideal when tabletted and disintegrates very rapidly (Fig. 3).

The effect of progressively increasing the amount of diluent in a fixed volume of capsule is shown in Fig. 4 as a significant reduction in the release rates and the mean times, t_{50}, are increased markedly. Replacing the lactose by corn starch (Fig. 6) had the effect of increasing the capsule disintegration rate, but reduced the drug dissolution. The replacement of the lactose by calcium sulphate dihydrate reduced the release rate (Fig. 7), although no effect on the disintegration was noted. The addition of magnesium stearate retarded release and sodium lauryl sulphate accelerated it, as might be anticipated when considering changes brought about in the packing of the powder bed or by changes likely to be induced in the hydrophobic character of the constituent particles in the bed (Newton, 1972).

The effect of particle size reduction (Fig. 5) was also as anticipated from the work of Johansen (1972), Johansen and Weise (1970) and Neuvonen et al. (1977). Examination

of the contents of the commercial capsules in polarized light suggested that the mean size of the sodium phenytoin was around 70 μm, with a wide spread of size. The same examination could not be undertaken for the tablet owing to the presence of interfering birefringent materials. However, it is clear that the particle size of the drug is one of the critical biopharmaceutical properties which requires careful quality control.

Use of the free acid produced a reduction in the dissolution rate which is rather less marked than might have been anticipated. This might well support the contention of Tammisto et al. (1976) that the free acid is clinically not significantly different from the sodium salt.

Returning to the effect of various diluents, it was noted that the release in the presence of calcium sulphate dihydrate was apparently not complete. This is shown in Fig. 7 since the amount filled into each capsule was known, and suggested that between 10 and 15% of the drug did not go into solution under the conditions of the test. When the readily soluble magnesium sulphate was used as a diluent interference was complete and no drug could be detected in the dissolution medium. These observations may be compared with the suggestions of Tyrer et al. (1970) that not all the drug is absorbed in the formulations containing calcium sulphate. The fact that the faecal level of phenytoin was also increased would indicate that there is some formation of an insoluble calcium salt in vivo. This may well be variable in extent but could be complete if a soluble calcium or magnesium salt could be formed in vivo from otherwise insoluble adjuvants which could then precipitate a proportion of the drug. This inadvertant formation of insoluble drug salts from adjuvants used in preparing a drug delivery system obviously requires further investigation since, on present evidence, it would appear to be a possible factor in the biopharmaceutical variability of this particular drug.

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