In Vitro and *In Vivo* Release of Microencapsulated Chlorothiazide

J. R. NIXON

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Abstract □ Microcapsules of chlorothiazide were prepared by the complex coacervate technique using gelatin and acacia as the wall-forming materials. The release of drug from the microcapsules and compressed tablets of microcapsules were studied *in vitro* and *in vivo*. *In vitro* dissolution was characterized by a rapid release of drug followed by a slower, more sustained release. The effects of pH and concentration are discussed. *In vivo* release of drug was determined from urine, and the volume of urine passed was studied.

Keyphrases □ Chlorothiazide—*in vitro* and *in vivo* release rates evaluated from plain tablets, compressed tablets of microcapsules, and microcapsules □ Gelatin–acacia coacervates—chlorothiazide, *in vitro* and *in vivo* release rates evaluated from compressed tablets of microcapsules, plain tablets, and microcapsules □ Microencapsulation—symposium, chlorothiazide release rates evaluated from plain tablets, compressed tablets of microcapsules, and microcapsules *in vitro* and *in vivo*

The preparation of gelatin–acacia microcapsules containing water-insoluble drugs is well known (1), although the number of modifications to the basic technique (2), in particular to the extraction of a fine powder, still precludes setting up a simple general method. The dosage forms in which microcapsules are used usually are not specified, although their use as suspensions, powders, and tablets all have been suggested (3, 4). Most published data regarding dissolution is from *in vitro* work (5–8), but some *in vivo* studies have been reported (9). Few attempts have been made to compare the performance of different formulations of the same microcapsules and to correlate *in vivo–in vitro* properties within and between preparations.

The present work attempts to investigate how chlorothiazide behaves when it is microencapsulated and formulated as powder and tableted dosage forms.

BACKGROUND

Chlorothiazide and related compounds are diuretics that reduce the reabsorption of electrolyte from the renal tubules and, therefore, increase the excretion of sodium and chloride ions and, as a consequence, water.

Unmicroencapsulated diuretics given orally may produce diuresis after 2 hr; this diuresis can be maintained for up to 12 hr, although a more normal time is from 5 to 9 hr. Tolerance does not develop, and this diuretic may be used over a long period. Because of the long continuous activity, continuous administration usually is not needed; after an initial dose of 1 or 2 g, it is sufficient to give maintenance doses, which rarely exceed 1 g every few days.

Mild symptoms of discomfort have been reported for chlorothiazide and occasionally there are more serious toxic effects, particularly with patients suffering from diabetes and hepatic disorders (10–12). As with many materials given as tablets, ulceration of the gut is possible, particularly when the drug is in the form of a layered tablet that contains potassium chloride as a potassium supplement. The use of supplementary potassium with chlorothiazide is recommended, and the preparation of potassium chloride microcapsules is reported elsewhere. Subsequently, it is hoped that both types of microcapsules can be combined into a single dosage form that will allow a more sustained chlorothiazide diuretic effect and prevent gut ulceration due to the potassium chloride.

As a reasonable reference compound to study *in vivo*, chlorothiazide has a number of advantages. It allows measurement of urinary excretion rates (metabolism is not an important factor in the elimination of thiazide from the body), the rate of flow of urine and changes in the total urine volume, and changes in the elimination of sodium and potassium.

EXPERIMENTAL

Preparation of Microcapsules—The microcapsules were prepared by gelatin coacervate techniques outlined previously (13, 14). With complex gelatin–acacia coacervates, it was immaterial whether the drug was dispersed in the gelatin or acacia prior to mixing (15, 16). Extraction was carried out by treating the mixture with formaldehyde solution (formalin), filtering, washing with isopropanol, and drying in a stream of air. The recovery was between 86 and 92%, and the mean size of the microcapsules was 73 μm with 80% of the sizes between 5 and 136 μm.

Preparation of Tablets of Chlorothiazide Microcapsules—These tablets were compressed in a single-punch hand compressor which had a 30-sec compression and a 10-sec rest under compression, followed by a 10-sec stage during which compression was released. In all cases, flat punches were used making 5-mm diameter tablets. The weight of the microcapsules was 250 mg for each tablet, and compression varied between 0.5 and 5 tonnes.

Determination of Chlorothiazide—*Microcapsules*—To determine the amount of chlorothiazide in the microcapsules, they were extracted with either water at pH 10 or isopropanol. In both cases, a 500-mg charge of the microcapsules was refluxed for 10–15 min at 100° with water or at 80° with isopropanol. The normal spectrophotometric assay at 513 nm was performed. The system was refluxed for an additional 15 min with fresh solvent to be certain that all chlorothiazide had been extracted.

Urine—Protein-free urine samples were obtained by diluting 1 ml with 4 ml of 20% *p*-toluenesulfonic acid and diluting to 20 ml with water. Five milliliters of this solution was heated in a water bath for 30 min with 1 ml of 3.75 *N* NaOH, during which chlorothiazide was hydrolyzed to a 6-aminodisulfonamide along with any conjugates present.

After this hydrolysis step, the assay followed the Bratton–Marshall method for sulfonamides. Five milliliters of the cooled solution was acidified with hydrochloric acid, sodium nitrite was added to diazotize the amine, and the tube was left in ice for 3 min. The excess nitrous acid was destroyed by ammonium sulfamate, and the solution was allowed to stand at room temperature for 5 min. The diazonium salt was coupled with *N*-(1-naphthyl)ethylenediamine hydrochloride, and the color developed after 10 min was read spectrophotometrically at 513 nm. The standard solutions for comparison were made from chlorothiazide in 4% *p*-toluenesulfonic acid.

***In Vitro* Dissolution**—The *in vitro* release from chlorothiazide microcapsules was studied using 250 mg of microcapsules containing either 163.7 or 95.6 mg of drug. This quantity was dispersed in 2 liters of water at 37° and adjusted to pH 2, 5, 7, or 9 with hydrochloric acid or sodium hydroxide. The stirring rate was 100 rpm.

***In Vivo* Dissolution**—The *in vivo* dissolution of the drug from the microcapsules was studied by giving 27 healthy volunteers on three separate occasions either plain chlorothiazide tablets, microcapsules, or tableted microcapsules. All dosages contained 500 mg of chlorothiazide, and 1 week elapsed between a repeat dose of drug to any subject. The normal volume of urine over 3 days was monitored for the subjects prior to taking the preparation, and all urines were assayed to ensure that a positive assay for chlorothiazide did not exist. No special diet was used, and sodium and potassium intake was not monitored or supplemented.

RESULTS AND DISCUSSION

The results of the *in vitro* dissolutions are shown in Figs. 1 and 2. All samples at different pH values showed the familiar pattern of a rapid initial release followed by a slower sustained release. With all samples other than those at pH 2, ~50–60% of the drug was released within 20 min for the higher chlorothiazide concentration. However, with the lower

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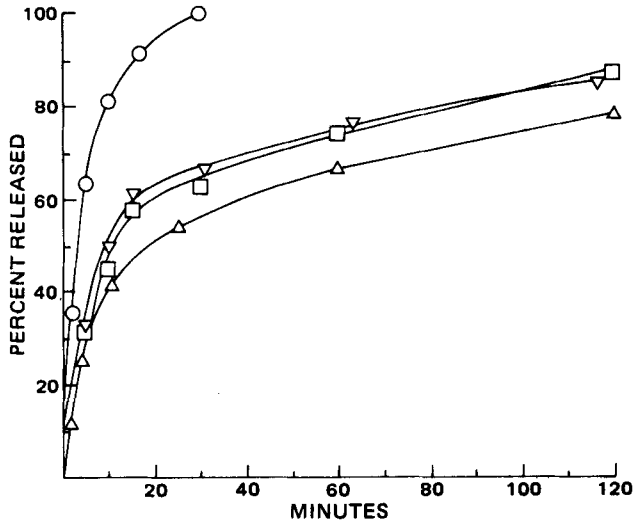

Figure 1—*Effect of pH on the* in vitro *release of chlorothiazide from formaldehyde solution-treated gelatin–acacia microcapsules. The concentration of chlorothiazide was 65.48% in a charge of 250 mg in 2 liters of water at 37°. Key:* ○, *pH 2;* ▽, *pH 5;* □, *pH 7; and* △, *pH 9.*

concentration, the change in the release rate was more gradual, and it was difficult to discern any break that could be regarded as the start of a sustained, or slower release, stage.

With both high and low concentrations of drug in the microcapsules, the fastest release was at pH 2; and since the microcapsules had been formaldehyde solution treated, it appears that this treatment did not produce any enteric coating effect. This observation suggests that *in vivo* the bulk of the drug would be released in and absorbed from the stomach. Also shown in Fig. 2 is the rate of solubility of the unencapsulated drug in water. Although the dissolution rate from the microcapsules at pH 2 was rapid, there was a slight delay in release when compared with the dissolution rate of raw drug.

Figure 3 shows the percentage released as a function of both pH and the percentage of drug in the microcapsules. A linear relationship existed between the percentage of drug present and the quantity released. In the first 30 min, irrespective of the pH other than pH 2, a higher percentage of drug was released from those microcapsules containing the larger percentages. This result was not unexpected because, with the method of manufacture employed, these microcapsules have the thinnest protective wall. However, after 60 min, the core content appeared to have little effect on the total percentage of drug released, except at pH 9 where the microcapsules with the highest content of chlorothiazide were releasing a higher total percentage of drug than the thicker walled, lower drug content ones.

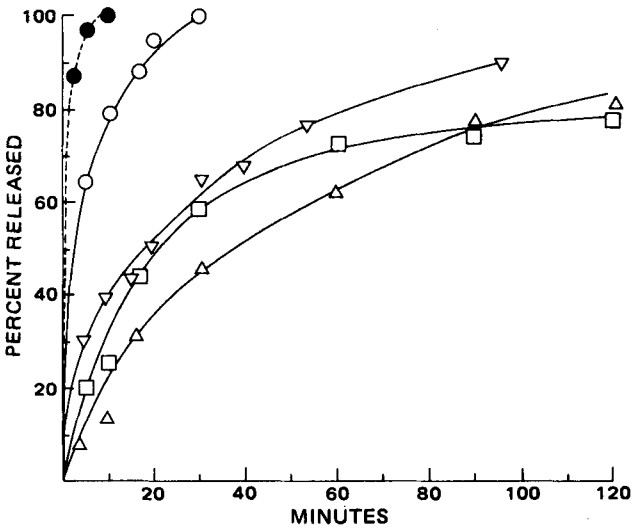

Figure 2—*Effect of pH on the* in vitro *release of chlorothiazide from formaldehyde solution-treated gelatin–acacia microcapsules. The concentration of chlorothiazide was 38.24% in a charge of 250 mg in 2 liters of water at 37°. Key:* ○, ●, *pH 2;* ▽, *pH 5;* □, *pH 7;* △, *pH 9;* ○, ▽, □, △, *microcapsules; and* ●, *raw drug.*

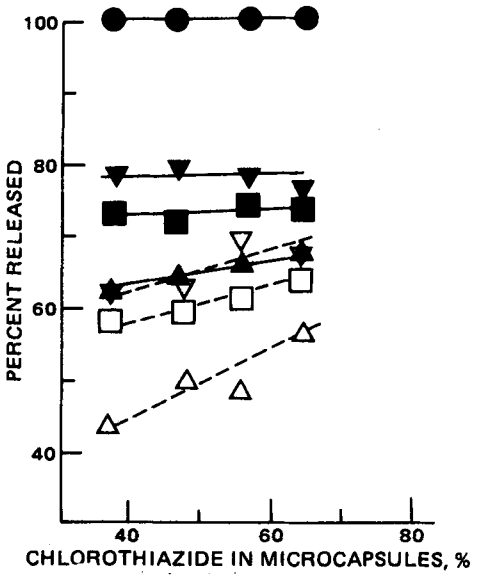

Figure 3—*Effect of pH and drug concentration on the* in vitro *release of chlorothiazide from a charge of 250 mg of microcapsules at 37°. Key:* ●, *pH 2;* ▽, ▼, *pH 5;* □, ■, *pH 7;* △, ▲, *pH 9;* ●, ▲, ■, ▼, *60 min; and* △, □, ▽, *30 min.*

The *in vitro* release rate of tablets of microcapsules is shown in Fig. 4. *In vitro* dissolution tests, carried out as described previously, showed a much slower release from the compressed microcapsules. Contrary to expectation, the tablets did not disintegrate and were still present as definite entities at the end of the 2-hr *in vitro* experiments. The effect of pressure followed the expected norm; as with the uncompressed material, release into acidic pH was far more rapid than in pH 9. As with the uncompressed microcapsules, higher drug concentrations seemed to lead to a more rapid total release, although the difference was not so noticeable as in the early release from uncompressed material. Separate, detailed studies into the compression characteristics of microcapsules are being conducted.

Figure 5 shows the mean urine excretion found with all subjects up to 28 hr. The subjects measured the volume of urine passed and retained a sample for subsequent analysis. The normal, nondiuretic-induced condition for the subjects showed a fairly steady increase in total urine passed throughout the day. As expected, this volume was lower at all times than after diuretic dosage. A standard tablet of nonmicroencap-

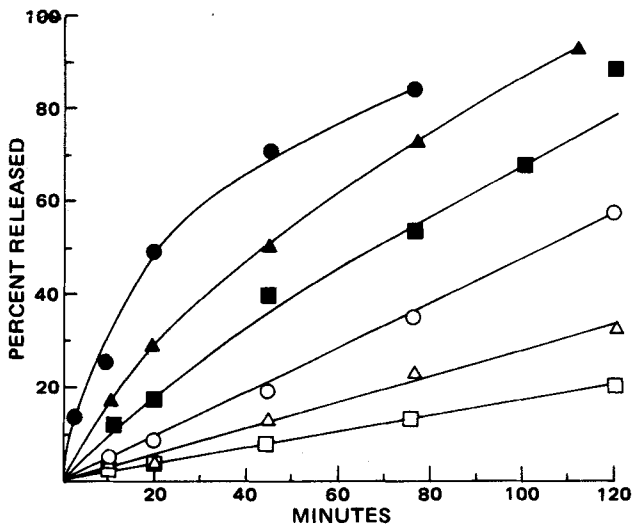

Figure 4—*Effect of pH and compression pressure on the* in vitro *release of chlorothiazide from tableted microcapsules. The concentration of chlorothiazide was 65.48% in a charge of one 250-mg tablet in 2 liters of water at 37°. Key:* ●, ▲, ■, *pH 2;* ○, △, □, *pH 9;* ○, ●, *0.5 tonne of pressure;* △, ▲, *3 tonnes of pressure; and* □, ■, *5 tonnes of pressure.*

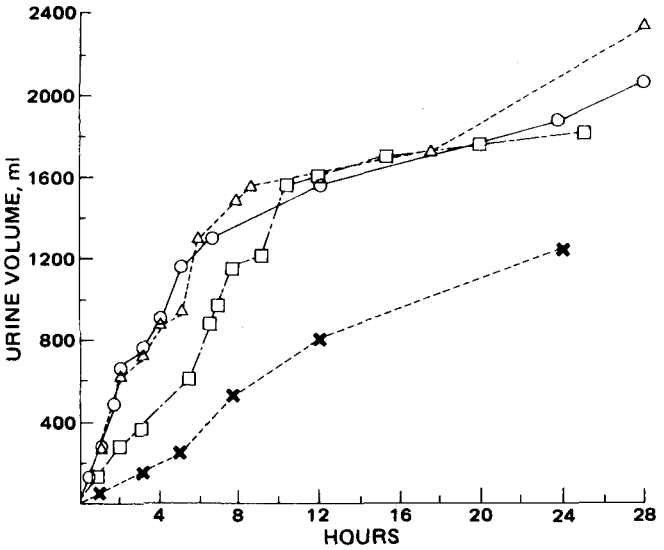

Figure 5—*Mean volume of urine passed after ingestion of 500 mg of chlorothiazide in various dosage forms. Key: ×, control; ○, standard tablet; △, microcapsules; and □, tableted microcapsules. Tablet compression pressure was 5 tonnes.*

sulated chlorothiazide produced a steady rapid release of urine over the first 2 hr when compared with the nondosed control state. This release fell slightly but rose again after the midday meal. About 10 hr after ingestion of the tablet, the rate of urine production followed approximately the normal condition.

Microencapsulated drug was given in both tableted and powder forms. The microencapsulated tablet showed a urine production intermediate between that produced by the nondosed and normal tablet-dosed form. The urine production increased after 5 hr, probably corresponding to increased release of chlorothiazide from the tablet because of either diffusion or disintegration. After 5 hr, the tablet would be expected to progress to the more alkaline region of the small intestine, where the formaldehyde solution-treated microcapsules should disintegrate more than in the acidic conditions of the stomach. However, the *in vitro* dissolution data showed a more rapid release of drug under acidic conditions. Therefore, under *in vivo* conditions, it may take time for tableted microcapsules to build up a high diuretic concentration in the body and to exert a marked diuretic effect, even though drug is released from the moment of ingestion. After 10 hr, the amount of urine produced was approximately the same as that resulting from the standard tablet.

Nontableted microcapsules showed no significant difference in urine production over a normal tablet, indicating that the drug dose had been released rapidly in the acidic pH conditions of the stomach and was available almost immediately. This result was expected from the *in vitro* dissolution data, which showed 100% release of the drug within a period far shorter than the normal stomach-emptying time.

All doses of the drug were small when compared with the normal therapeutic dose, but the same pattern probably is obtained with initial doses of up to 2 g of chlorothiazide.

To determine how much drug was excreted, urine samples were assayed by the modified Bratton–Marshall method. The urinary concentration of drug followed the same pattern as the urine production from a given formulation (Fig. 6). Thus, with microcapsules of chlorothiazide and ordinary tablets, there was a rapid buildup of drug excreted in the urine; after 10 hr, there was little further increase. With the tableted microcapsules, only slightly less chlorothiazide was excreted over the first 2 hr, but the mean urine production from this dosage form was smaller. However, after 2 hr, there was significantly less drug in the urine from the tableted microcapsules. This was still the case after 10 hr, although

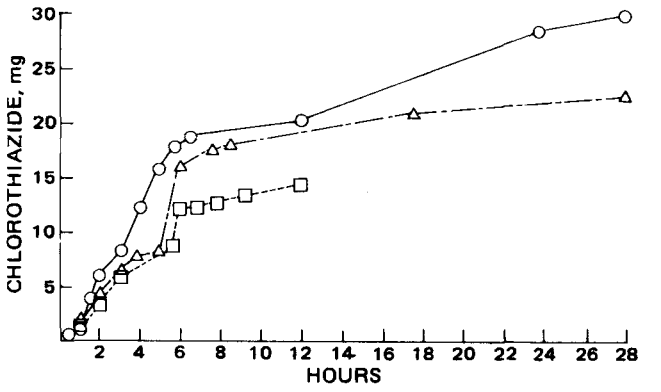

Figure 6—*Release of chlorothiazide in urine after a 500-mg dose. Key: ○, standard tablet; △, microcapsules; and □, tableted microcapsules. Tablet compression pressure was 5 tonnes.*

the urine production curves had shown that there was no significant difference in urine production with any formulation after this time.

Only a fraction of the drug (<10%) was found in the urine, indicating either significant body retention of the drug or very incomplete absorption from the GI tract.

There is a potassium loss from the body during the use of chlorothiazide, and studies on the use of microencapsulated potassium chloride incorporated with microencapsulated chlorothiazide are underway. This work is being extended to a study of the physical characteristics involved in tableting microcapsules. So that there is only one microcapsule formulation to investigate, the water-soluble potassium chloride is encapsulated in a gelatin–acacia coacervate, which normally is restricted to use with water-insoluble drugs.

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