amperometric detector electrode probably accounts for the poor performance of this detector when the injected thimerosal amount exceeded approximately 100 ng.

We also examined the influence of electrode potential on analytical performance using the coulometric detector. Decreasing the applied potential to + 0.55 or + 0.45 V contributed to significant deviations from observed recovery and response linearity relative to the case of the coulometric detector at + 0.75 V. In principle, a linear current-concentration relationship holds for every point along the hydrodynamic current-potential curve in HPLC analysis with electrochemical detection. In practice however, small variations in potential yield relatively large current deviations when the applied potential resides on the upslope of the hydrodynamic curve. Thus possible advantages in the improved selectivity inherent with lower operating potentials (17) must be evaluated versus attendant losses in sensitivity and linearity before selecting an applied potential below the plateau region of a hydrodynamic voltammogram.

Finally, the spectrophotometric and amperometric detectors were tested for precision of response with 10 spiked placebo samples, and both detectors gave statistically identical results (paired t-test at the 95 % confidence level: UV = 24.1 ± 0.84 μg/ml, EC = 23.9 ± 0.53 μg/ml).

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Studies on the Dissolution of Drugs from Tablets using Perturbed Angular Correlation

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Abstract: The perturbed angular correlation (P.A.C.) technique was used to assess the dissolution of drugs coprecipitated with [^{111}In]indium chloride in tablets *in vitro*. The amount of drug in solution was monitored simultaneously with the amount of radioactivity in solution and these were correlated to the anisotropy values obtained by P.A.C. measurements. The results indicated that the rate of drug dissolution paralleled the change in measured anisotropy of the system. Thus, the measurement of anisotropic changes in drug-indium complexes by P.A.C. is a reliable indicator of drug dissolution and can provide meaningful dissolution data for noninvasive *in vivo* studies.

Imaging techniques, in particular gamma scintigraphy, are now well established as tools in pharmaceutical research (1). When studying solid dosage forms, one drawback of this technique is that it is impossible to distinguish between the radionuclide in solid form or in solution. This problem can, in part, be overcome by the combined use of gamma scintigraphy with perturbed angular correlation measurements (P.A.C.) (2). Indium-111 decays by emitting two gamma rays in cascade that exhibit a certain angular correlation between their direction of propagation. This correlation can be perturbed if the intermediate state of the nucleus, during decay, is perturbed, for example, by dissolution of material containing the radiolabel. Hence, Beihn and Digenis were able to monitor the dissolution of ^{111}indium chloride from tablets by P.A.C. and showed that changes in the angular correlation paralleled the rate of dissolution of ^{111}indium chloride as measured by sampling and counting (2). The technique could be used as a non-invasive assessment of dissolution *in vivo* and has since been successfully applied to the *in vivo* release from suppositories (3). As pointed out by Beihn and Digenis, the P.A.C. technique measures the dissolution of the radionuclide, and not the drug, and this may be considered as a limitation of its usefulness.

One approach to overcoming this problem would be to radiolabel the drug itself. In certain specific cases, this may be possible. This paper examines a more

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general approach, that is, precipitating the drug in the presence of the radioactive indium chloride, with a view to forming a "coprecipitate" of the two materials. On subsequent dissolution, the rate of dissolution of the indium chloride may then reflect the rate of dissolution of the drug.

Materials and Methods

Materials

The major materials used, together with their standards, were as follows: salicylic acid, U.S.P., Paracetamol, U.S.P., phenacetin, U.S.P. (all Mallinckrodt, St. Louis, U.S.A.), anhydrous lactose (Sheffield Products, Memphis, U.S.A.), Sugartab (directly compressible sugar, E. Mendell, New York, U.S.A.), and Primogel (Sodium carboxy methyl starch, Generichem, Little Falls, N.J., U.S.A.). [^{111}In]Indium chloride ($^{111}InCl_3$) was obtained from Medi-Physics (Emoryville, CA, U.S.A.).

Coprecipitation

An appropriate quantity of the drug to prepare 4 tablets was dissolved in sufficient acetone to effect dissolution. 200 μCi of $^{111}InCl_3$ was added and the solution rapidly evaporated to dryness under vacuum using a rotary evaporator (Büchi, R110, Switzerland), with a water bath temperature of 65°C. The resultant material was powdered, and then further dried at 60°C for 4 hours.

Preparation of Tablets

Salicylic acid tablets contained 150 mg salicylic acid and 250 mg anhydrous lactose. Paracetamol and phenacetin tablets contained 50 mg of the drug with 350 mg sugar tab. When a disintegrant was required, 2% w/w primogel was added. Tablets were prepared using a one-half inch flat faced punch and die set, lubricated with a 1% suspension of magnesium stearate in acetone, at a compression pressure of 105 MN/m^2 using a hydraulic press (F. Carver Lab. WI, U.S.A.).

Dissolution and P.A.C. Measurements

These were carried out in an identical manner to that described by Beihn and Digenis (2). The dissolution fluid was 350 ml of either distilled water or 0.1 N HCl contained in a 500 ml jacketed beaker and maintained at 37°C. One ml samples were taken at suitable time intervals, the radioactive counts determined and the samples analyzed for drug content by U.V. spectroscopy at the appropriate wavelength of maximum absorption. The excipients used did not interfere with the assay.

Results and Discussion

The previous work of Beihn and Digenis (2) and Jay et al. (3) clearly established the close correlation between the change in anisotropy as measured by P.A.C. and the percentage of indium-111 in solution. Hence, it was unnecessary to monitor P.A.C. on each occasion, but merely to establish the correlation for this system. Figure 1 shows the relationships between the percentage change in anisotropy, the percentage of indium-111 in solution, and the percentage of salicylic acid dissolved with time.

There is a good agreement between all three determinations showing that the P.A.C. measurements reflect the dissolution of the indium and that this, in turn, reflects the dissolution of the drug.

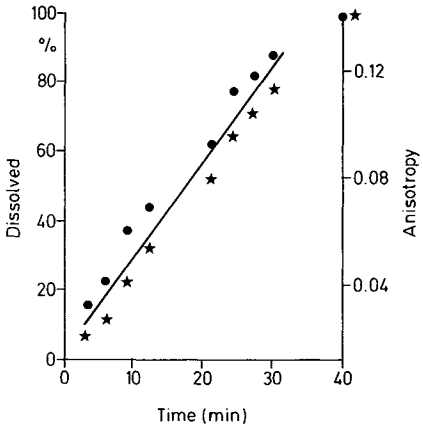

Fig. 1 *In vitro* dissolution in water of a tablet containing a salicylic acid – $^{111}InCl_3$ coprecipitate. The stars represent dissolved salicylic acid, the circles represent the amount of indium-111 in solution, and the solid line reflects continous anisotropy observations as measured by perturbed angular correlation.

This finding indicates that the coprecipitation associates the indium chloride with the salicylic acid in some way, as the aqueous solubilities of the two materials are very different. It is known that small quantities of impurities can be incorporated into a crystal structure during crystallization (4) and this is a possible mechanism. It should be noted that for a tablet containing 150 mg of drug and 50 μCi of indium-111, the ratio of drug to indium is 1.27×10^9. There is an early rise in the radioactive counts which is not mirrored by the salicylic acid, but over the major part of the experiment the two curves are parallel. This phenomenon was noted in most of the experiments and is possibly due to the presence of a small amount of "free" indium chloride.

Because the relationships are linear over the majority of the observation period, the slopes of the plots can be used as a basis for comparison, and these are shown for replicate experiments in Table 1. No attempt was made to achieve reproducible conditions between experiments, as only correlations within a single experiment were important. The results show good agreement between the rate of dissolution of the indium chloride and that of the salicylic acid. The change of solvent from water to 0.1 N HCl reduces the solubility of salicylic acid while that of indium chloride is virtually unaffected. Results from a disintegrating tablet are shown in Figure 2.

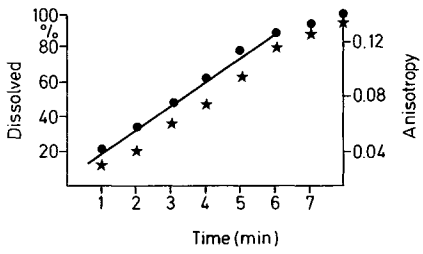

Fig. 2 *In vitro* dissolution in 0.1 N HCl of a disintegrating tablet containing a salicylic acid – $^{111}InCl_3$ coprecipitate. See Fig. 1 for explanation of symbols.

Table I. Comparison of the Slopes of % Dissolved Versus Time Plots for Salicylic Acid and Indium Chloride

Tablet Number	Dissolution Media	Salicylic Acid		Indium Chloride	
		Slope	c.c.	Slope	c.c.
1	Water	2.82	0.997	2.67	0.991
2	Water	1.38	0.996	1.76	0.996
3	Water	1.99	0.996	2.16	0.993
4	Water	1.82	0.999	1.98	0.999
5	0.1 N HCl	1.38	0.994	1.42	0.985
6	0.1 N HCl	1.39	0.998	1.44	0.999

c.c. = correlation coefficient

The technique was extended to include two further materials differing widely in solubility, paracetamol and phenacetin. The results from experiments using these materials are shown in Figs. 3 and 4. Again, good agreement between these measurements can be seen.

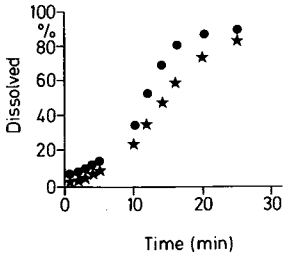

Fig. 3 *In vitro* dissolution of a tablet containing a paracetamol – $^{111}InCl_3$ coprecipitate. See Fig. 1 for explanation of symbols.

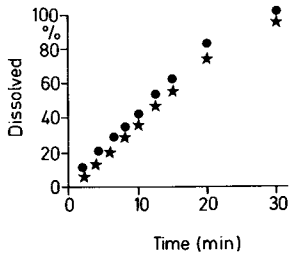

Fig. 4 *In vitro* dissolution of a tablet containing a phenacetin – $^{111}InCl_3$ coprecipitate. See Fig. 1 for explanation of symbols.

Conclusion

The ability to monitor the dissolution rate of a drug *in vivo* is important in pharmaceutical research and development. A combination of P.A.C. studies and gamma scintigraphy appears to offer one approach to achieving this goal. This paper has examined the potential of monitoring drug dissolution by P.A.C. studies. By precipitating the drug in the presence of the radionuclide, the radionuclide is associated with the drug crystals in a way that its release rate reflects that of the drug. It may be argued that the process involved changes the original physical properties of the drug, but this can be easily ascertained, and the process altered accordingly. Current novel approaches to the administration of drugs given as solids often require precise release rates or almost immediate dissolution followed by controlled release of solution. A combination of P.A.C. studies with suitably labelled drug material and gamma scintigraphy give the possibility of following these processes *in vivo*.

Dissolution studies using P.A.C. measurements are not without certain limitations. The *in vivo* P.A.C. dissolution studies performed to date were limited to dosage forms that dissolved within a confined anatomical region, i.e., the stomach or rectum. Dosage forms that spread throughout the gastrointestinal tract while undergoing dissolution may be technically difficult to measure by P.A.C. because of geometric problems with the detectors. In addition, free ^{111}In may bind to specific components of mucous secretions resulting in alterations in the observed anisotropy value. It has been shown that this type of binding does not occur in the stomach (2), but does occur in the rectum (3). Further experiments are needed to determine if such binding can occur throughout the remainder of the gastrointestinal tract. Newer approaches currently under study that employ a single-detector system may circumvent some of the shortcomings of the present detection system.

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The Interaction of Cyanonaphthyridinomycin with DNA

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Abstract: The characteristics of the *in vitro* interaction of cyanonaphthyridinomycin (CYANO) with DNA are described. Unlike naphthyridinomycin (NAP), CYANO is extremely dependent on reductive activation with dithiothreitol (DTT) to bind DNA. The reaction of CYANO with DNA is kinetically slower than that observed for NAP and is still linear after six hours incubation at room temperature. The extent of binding is pH dependent with acidic pH being inhibitory. CYANO, as with NAP, appears to bind to dG:dC base pairs in the minor groove of double stranded DNA. Studies using [C^3H_3:^{14}CN] CYANO demonstrated that the cyanide group is lost when the drug binds to DNA. In the absence of DNA but in the presence of DTT, cyanide is still released from CYANO and the extent of release is also inhibited by acid pH conditions. These results suggest that the cyanide group comes off prior to binding of the antibiotic to DNA. The rate limiting step in the reaction of CYANO with DNA would appear to be the release of cyanide from the drug molecule.

Naphthyridinomycin (NAP) is an antitumor antibiotic produced by *Streptomyces lusitanus* (1). We have previously demonstrated that this compound inhibits DNA and RNA synthesis in susceptible organisms by apparently binding to dG:dC base pairs in the minor groove of DNA (2–4). The rate of the *in vitro* interaction is enhanced by the presence of reducing reagents suggesting that NAP is a bio-reductively activated antitumor agent (4). The drug-DNA adduct is presumably formed through the α-carbinolamine of the antibiotic and the N-2 nitrogen of guanine in the minor groove of DNA (4) as has also been proposed for the pyrrolo[1,4]benzodiazepine antibiotics (5) and saframycin A and S (6, 7).

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