essential to convert the penicillamine in plasma to the hydrochloride before freeze-drying since penicillamine base is only slightly soluble in ethanol. It was also necessary to use EDTA as anticoagulant, since penicillamine could not be detected in ethanol extracts if either heparin or citrate was present as anticoagulant or if penicillamine was added to serum. Although Saetre & Rabenstein (1978) used EDTA they did not comment on its necessity. The requirement for EDTA implies that penicillamine circulates in plasma complexed with a cation such as copper. Penicillamine-copper complex has been reported to possess activity as a superoxide dismutator (Lengfelder & Elstner 1978), and a deficiency of superoxide dismutase activity in rheumatoid arthritis has been observed (Rister et al 1978). The therapeutic activity of penicillamine may arise from the ability of its copper complex to protect cells from superoxide ions generated in phagocytosis.

The plasma concentrations of penicillamine administered by four different routes to rabbits are shown in Fig. 1. Oral absorption was rapid (Fig. 1a) and the plasma concentration fell to a steady value 3 h after administration. When penicillamine was given intravenously removal from plasma followed a biphasic pattern (Fig. 1b). An initial rapid phase occurred with a $T_{1/2}$ of 21 min followed by a slower phase with a $T_{1/2}$ of 55 min. Penicillamine was rapidly absorbed from the peritoneal cavity (Fig. 1c). Absorption was slowest following subcutaneous administration; peak plasma concentrations had not been reached by 4 h.

When rabbits were given 20 mg kg^{-1} i.v. daily doses of penicillamine for 21 days, no detectable penicillamine was present in the plasma 25 h after the final dose.

The plasma from six rheumatoid arthritis patients receiving chronic penicillamine therapy was also analysed by the method. Two h after they had taken 125 mg penicillamine the penicillamine plasma concentrations were less than 2 $\mu g\ ml^{-1}$. Saetre & Rabenstein (1978) reported values of $1{\cdot}67 \pm 0{\cdot}90\ \mu g\ ml^{-1}$ in a group of six rheumatoid patients receiving 250 mg penicillamine three times daily. Only one other report of plasma penicillamine concentrations in man has been recorded. Assem (1974), using a radioimmune assay, found 8–10 $\mu g\ ml^{-1}$ in a single subject 2–3 h after a single oral dose of 250 mg penicillamine. This higher value for penicillamine may be a consequence of the radioimmune assay detecting metabolites, such as the disulphide of penicillamine. Alternatively, the chronic administration of penicillamine to patients may induce a more rapid metabolic destruction of the drug.

The co-operation of Dr A. J. Swannell and his patients at Nottingham City Hospital is gratefully acknowledged. Mrs P. A. Fisher and Mr A. Holland assisted with some experiments.

February 20, 1979

REFERENCES

Assem, E. S. K. (1974) Curr. Med. Res. Opin. 2: 568–572

Assem, E. S. K., Vickers, M. R. (1974) Postgrad. Med. J. (Aug. Suppl.) 10–13

Borner, K. (1965) Hoppe-Seyler's Z. Physiol. Chem. 341: 264–267

Ellman, G. L. (1959) Arch. Biochem. Biophys. 82: 70–77

Lengfelder, E., Elstner, E. F. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359: 751–757

Pal, P. R. (1959) J. Biol. Chem. 234: 618–619

Rister, M., Bauermeister, K., Gravert, U., Gladtke, E. (1978) Lancet 1: 1094

Saetre, R., Rabenstein, D. L. (1978) Anal. Chem. 50: 276–280

Polarographic assay of glyceryl trinitrate by an internal standardization method

W. J. PUGH, *Welsh School of Pharmacy, UWIST, Cathays Park, Cardiff, U.K.*

Studies by Flann (1969) and Thoma & Groening (1975) have amply demonstrated the application of a polarographic method which is well suited to single dosage form assays. Although Flann (1969) reported good reproducibility of the (glyceryl trinitrate concentration/diffusion current) ratio over a period of 7 weeks in a thermostatted system, it is common practice to make use of an internal standard to overcome the effect of variation in instrumental factors, and it was decided to test the reproducibility of a non-thermostatted system over a long period during which the instrument would be used intermittently by other workers, mainly students. Because the polarographic reduction of glyceryl trinitrate is an irreversible process (Flann 1969) it was considered desirable to use a standard chemically similar to it.

Polarographic solvent: 800 ml n-propanol, 100 ml 1·0 M tetramethylammonium chloride, 50 ml 0·2 M ammonium chloride, 50 ml 0·2 M ammonium hydroxide. Methyl nitrate (BDH Poole U.K.), 0·2 mg ml^{-1} in acetone, was used as the internal standard. The glyceryl trinitrate standard was extracted from mannitol-based tablets (A.P.S., Cleckheaton, U.K.) by dry ether. Evaporation under vacuum of the ethereal extract yielded an oil which gave a single peak on g.l.c. analysis (Rosseel & Bogaert 1972) which was dissolved in acetone for the polarographic assays. Polarograph: Cambridge Instruments Recording Polarograph. 1 ml aliquots of glycerol trinitrate in acetone and methyl nitrate internal standard solutions were added to 3 ml of polarographic solvent in the polarograph cell and de-oxygenated by bubbling nitrogen for 5 min. The polarograms were recorded between 0 and —2·2 V using a dropping mercury cathode and mercury anode, and the diffusion

currents measured at —1·5 V; drop time, 3 s; current density, 5×10^{-3} A mm^{-2}. The diffusion currents were expressed as pen response in mm. The difficulty of the methyl nitrate being reduced at approximately the same potential as the glyceryl trinitrate was overcome as follows. The response, i_1, of 1 ml standard methyl nitrate solution was measured; the response, i_2, for the mixture of 1 ml of internal standard and 1 ml unknown trinitrate solution was then corrected for i_1 giving

$$\text{Glyceryl trinitrate concentration} = \frac{(i_2 - i_1)c}{i_1 A}, \text{ mg ml}^{-1}$$

where c = concentration of internal standard,

$$A = \frac{\text{1 mg glyceryl trinitrate}}{\text{1 mg methyl nitrate response}} \text{ ratio.}$$

A series of three glyceryl trinitrate and three methyl nitrate solutions (0·2, 0·4 and 0·9 mg ml^{-1}) was assayed in a Latin square sequence and the response for 1 mg of both substances thus calculated from a mean of 18 readings. The results are given below with their fiducial limits ($P = 0{\cdot}05$).

1 mg glyceryl trinitrate response = 17·32 ± 0·53 mm.

1 mg methyl nitrate response = 46·12 ± 0·74 mm.

(1 mg glyceryl trinitrate/1 mg methyl nitrate) response ratio = A = 0·358—*0·376*—0·393

A re-determination 3 months later showed that the 1 mg glyceryl trinitrate response had changed significantly to 16·58 ± 0·67 mm, whereas the ratio, A was almost unchanged at 0·345—*0·365*—0·385, thus vindicating the internal standardization.

1 ml aliquots of methyl nitrate solution (approximately 0·3 mg $MeNO_3$) were mixed with 1 ml samples of a series of glyceryl trinitrate solutions of concentrations 0·1, 0·2, 0·4, 0·8, 1·6 mg ml^{-1}. The response was linear with respect to glyceryl trinitrate concentration and the gradient was 17·14 mm mg^{-1}, indicating no interference between the compounds.

An assay was performed on 6 samples taken from a powdered mass of 100 tablets. A weight of powder containing about 5 mg glyceryl trinitrate was mixed with 10 ml acetone on a 'Whirlimixer' for 3 min. The suspension was clarified by centrifugation and 1 ml supernatant liquid mixed with 1 ml methyl nitrate solution, 3 ml polarographic solvent and analysed. The glyceryl trinitrate content per mean tablet weight was 0·478 ± 0·004 mg ($P = 0{\cdot}05$).

Six samples analysed by the B.P. method gave a mean tablet content of 0·476 ± 0·005 mg ($P = 0{\cdot}05$). A variance ratio test showed that the proposed method and the B.P. method showed no significant difference in their precision (variance ratio = 1·80; F = 5·05 for $P = 0{\cdot}05$, 5 × 5 degrees of freedom), and a *t*-test showed that the means were not significantly different ($t = 1{\cdot}11$, tabulated value 2·23 at $P = 0{\cdot}05$, 10 degrees of freedom.

Single tablet assays were performed by placing one tablet in the polarographic cell, powdering with a glass rod, adding 2 ml of half strength methyl nitrate solution and 3 ml polarographic solvent. Results for 6 tablets of nominal strength 0·5 mg were 0·478, 0·492, 0·477, 0·465, 0·480, 0·480 mg; mean = 0·478 ± 0·009 mg ($P = 0{\cdot}05$). 6 tablets assayed by the B.P. method gave the results 0·476, 0·482, 0·481, 0·460, 0·487, 0·475, mean = 0·477 ± 0·010 mg ($P = 0{\cdot}05$), in good agreement with the results of the polarographic method.

October 2, 1978

REFERENCES

British Pharmacopoeia, H.M.S.O., London, 1973, p. 220

Flann, B. C. (1969) J. Pharm. Sci. 58: 122–124

Rosseel, M. T., Bogaert, M. G. (1972) J. Chromatogr. 64: 364–367

Thoma, K., Groening, R. (1975) Dtsch. Apoth. Ztg. 115: 1456–1460

Potentially biodegradable microcapsules with poly (alkyl 2-cyanoacrylate) membranes

A. T. FLORENCE*, T. L. WHATELEY†, D. A. WOOD†, *Department of Pharmaceutics and †Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow G1 1XW, U.K.*

The poly (alkyl 2-cyanoacrylates) are used as biodegradable tissue adhesives in surgery. The methyl ester in solid form degrades most rapidly yielding formaldehyde which is believed to be responsible for its histotoxicity. The butyl ester, with a slower degradation rate, is well-tolerated in vivo (Leonard 1970). In a previous paper, Florence et al (1976) showed that methyl 2-cyanoacrylate and butyl 2-cyanoacrylate dissolved in an oil phase formed polymeric films in contact with an aqueous phase and suggested that these monomers could be used in the preparation of microcapsules which would degrade in vivo. The present paper describes the preparation of microcapsules formed from butyl 2-cyanoacrylate monomer. The reaction involves a base catalysed anionic mechanism (Coover et al 1959) with the monomer dissolved in the oil phase of a water-in-oil emulsion and in situ polymerization occurring at the interface of the aqueous disperse phase. Aqueous protein solutions have been used as the microcapsule core materials; the protein is believed to act as an

* Correspondence.