

TABLE II.—ANALYSIS OF MIXTURES OF GLUTETHIMIDE AND 4-ETHYL-4-PHENYLGLUTARAMIC ACID BY DIFFERENTIAL TITRATION

Glutethimide		4-Ethyl-4-phenylglutaramic Acid	
Weighed Amt., mg.	Recovery, %	Weighed Amt., mg.	Recovery, %
147.5	99.72	141.5	103.67
103.4	102.80	149.3	98.19
147.4	100.75	130.6	101.45
163.7	99.42	45.75	101.68
170.0	100.65	52.15	97.34
160.2	100.75	52.55	99.26

The proposed assay permits the differential titration of glutethimide and its breakdown product. Synthetic mixtures of glutethimide and 4-ethyl-4-phenylglutaramic acid in varying ratios of the two components were analyzed by the proposed method. A typical differential titration curve is shown in Fig. 1, curve B. The data for the analysis of the synthetic mixtures are recorded in Table II. The significant difference in pKa values (glutethimide, 9.2; 4-ethyl-4-phenylglutaramic acid, 4.6) accounts for the two inflections in the titration curve. The

data in Table II indicate that excellent resolution of the mixtures was achieved.

The proposed nonaqueous titration was applied to aminoglutethimide powder and tablets and bemegride powder and injection. The data are shown in Table I.

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## Notes

### Solubility of Pentaerythritol Tetranitrate-1,2-<sup>14</sup>C in Water and Saline

By EDWARD J. MERRILL

The solubility of clinically useful pentaerythritol tetranitrate has been determined in water and saline utilizing the radioactive labeled compound. The solubility agrees with one of the two previously reported values. The synthetic work has been included.

Two vastly differing values for the solubility of pentaerythritol tetranitrate (PETN) have been reported. Desvergues (1) found the solubility to be 100 mcg./ml., while Leslie (2) reported a value of 1.5 mcg./ml. When PETN labeled with <sup>14</sup>C was prepared for an extensive biological evaluation, it became possible to re-evaluate this solubility problem and resolve this discrepancy. The older methods for the determination of the solubility of compounds having a limited solubility suffer from the inherent error of weighing a small amount of solid, or in this case, of an analytical method designed to detect inorganic nitrate ions and not intact PETN. If hydrolysis of the PETN had occurred, the results

would be high due to the ready solubility of the nitrate ion.

#### DISCUSSION

Essentially, the method was to equilibrate 5 mg. of PETN-1,2-<sup>14</sup>C with 5 or 15 ml. of water or saline, filter to remove colloidal material,<sup>1</sup> then count<sup>2</sup> an aliquot in a scintillation solution composed of 0.7% PPO, 0.03% dimethyl POPOP, and 100 Gm. of naphthalene adjusted to 1 L. with freshly distilled 1,4-dioxane. The counting efficiency was 69%, and each sample was corrected for quenching by the addition of benzoic acid-<sup>14</sup>C as an internal standard.

It was found necessary to use a 0.22- $\mu$  filter (Millipore)<sup>3</sup> to remove the colloidal material completely. However, successive passes through similar filters removed material from solution. At these low concentrations, this phenomena is not unexpected (3-5). These results are shown in Table I.

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<sup>1</sup> This was brought to the author's attention by Mr. M. Geller, Applied Analytical Research Department, Warner-Lambert Research Institute, Morris Plains, N. J.

<sup>2</sup> Tri-Carb scintillation spectrometer, model 314 X, Packard Instrument Co.

<sup>3</sup> Millipore Filter Corp., Bedford, Mass.

TABLE I.—EFFECT OF SUCCESSIVE FILTRATIONS THROUGH A 0.22- $\mu$  FILTER (MILLIPORE)

Sample <sup>a</sup>	First Filtration		Second Filtration		Third Filtration	
	c.p.m.	Solubility <sup>b</sup>	c.p.m.	Solubility <sup>b</sup>	c.p.m.	Solubility <sup>b</sup>
15 ml. H <sub>2</sub> O A	2820	1.9 (av.)	2540	1.8 (av.)	2420	1.7 (av.)
15 ml. H <sub>2</sub> O B	2620		2600		2450	

<sup>a</sup> Five milligrams of PETN-1,2-<sup>14</sup>C in indicated volume of solvent. <sup>b</sup> Expressed as mcg./ml.

The identity of the material in solution was established by repeatedly spotting the aqueous filtrate on a TLC plate with Silica Gel G as the adsorbent. This gave, after irrigation, a single radioactive spot at  $R_f = 0.71$  using the system of DiCarlo *et al.* (6) and was identical to that of authentic PETN.<sup>4</sup>

Using a 1-ml. aliquot, the solubility was determined from the following formula (Table II):

$$\text{solubility}^5 = \text{c.p.m.} \times \text{dil. factor} \times 1/\text{eff.} \times 1/\text{conversion factor}^6 \times 1/4.72 \mu\text{c./mg.}$$

### EXPERIMENTAL<sup>7</sup>

Although the synthesis of pentaerythritol-1-<sup>14</sup>C (PE) has been described by Trevoy and Myers (7) the author used essentially the procedure of Schurink (8), modified by bubbling 25 mmoles acetaldehyde-1,2-<sup>14</sup>C,<sup>8</sup> into a suspension of 15 mmoles Ca(OH)<sub>2</sub>, 250 mmoles formaldehyde as a 37% aqueous solution, and 15.1 ml. of water. After removal of the calcium as its sulfate, the filtrate was stripped and the oily residue dissolved in water and steam distilled until the distillate gave a negative test with chromotropic acid (9). The solution containing the crude PE-<sup>14</sup>C was stripped on an oil pump, and the resulting solid was triturated with 3 × 5 ml. absolute EtOH, to give 2.28 Gm. of crude PE-1,2-<sup>14</sup>C melting at 195–225°. This was converted to the tetraacetate (PETA) according to the method of Wiersma *et al.* (10). After removal of the first crop of PETA-1,2-<sup>14</sup>C, saturation of the aqueous HOAc solution with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and extraction with ether gave an additional crop of the tetraacetate. The combined yield of 3.66 Gm. of PETA-1,2-<sup>14</sup>C, m.p. 76–77°, was dissolved in 55 ml. of MeOH containing 3.66 ml. of 8 N ethanolic HCl (11) and left at room temperature overnight. The precipitated PE-1,2-<sup>14</sup>C was filtered and an additional crop obtained by concentration of the filtrate. A 1.42-Gm. (42%) quantity of PE-1,2-<sup>14</sup>C was obtained, melting at 260–263°.

The nitration was performed as follows using a combination of the various methods described by Berlow *et al.* (12).

<sup>4</sup> Scanning performed using a model C100A Actigraph II with TLC adapter, Nuclear-Chicago Co.

<sup>5</sup> Expressed as mg./ml.

<sup>6</sup> Equivalent to 2.2 × 10<sup>6</sup> dpm/1  $\mu$ c.

<sup>7</sup> All melting points were taken on a Mel-Temp apparatus and are corrected.

<sup>8</sup> Purchased from New England Nuclear Corp. having a specific activity of 1.6 mc./mmoles.

TABLE II.—SOLUBILITY OF PETN-1,2-<sup>14</sup>C IN WATER AND SALINE

Sample	c.p.m.	Solubility <sup>a</sup>
5 ml. H <sub>2</sub> O A	3120	2.1 (av.)
5 ml. H <sub>2</sub> O B	2790	
15 ml. H <sub>2</sub> O A	2820	1.9 (av.)
15 ml. H <sub>2</sub> O B	2620	
5 ml. saline A	2650	1.9 (av.)
5 ml. saline B	2620	

<sup>a</sup> See Table I.

To 4.7 ml. of 98% HNO<sub>3</sub> cooled in an ice bath was added 1.42 Gm. (10.4 mmoles) of PE-1,2-<sup>14</sup>C portionwise over 20 min. The suspension was stirred for an additional 30 min. and 51 ml. of cold water added. After 30 min., the precipitate was filtered, and the crude PETN-1,2-<sup>14</sup>C was washed with 1 N NH<sub>4</sub>OH, water, and absolute EtOH. Recrystallization from aqueous acetone containing a trace of Na<sub>2</sub>CO<sub>3</sub> gave 3.10 Gm. of PETN-1,2-<sup>14</sup>C, melting at 140–142°. This material gave a single radioactive spot on TLC with  $R_f = 0.7$ . Aliquots were counted in a scintillation solution of 0.4% PPO and 0.01% diMePOPOP in toluene and found to have a specific activity of 4.72 mc./Gm.

The pure PETN-1,2-<sup>14</sup>C was dissolved in acetone and diluted (1:7) with lactose (previously passed through a 200-mesh sieve) and the stable powder again passed through a 200-mesh sieve. The specific activity of this powder was 0.59 mc./Gm. The PETN-1,2-<sup>14</sup>C can be extracted quantitatively from this lactose powder by extraction in a Soxhlet with anhydrous ether for about 1 hr.

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