1039. *The Determination and Reaction of 2,2-Diphenyl-1-picryl-hydrazyl with Thiosalicylic Acid* [1]

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An accurate method for analysis of 2,2-diphenyl-1-picrylhydrazyl (DPPH) has been developed, using a simple titration procedure with thiosalicylic acid. The products of the reaction were 1,1-diphenyl-2-picrylhydrazine and 2,2′-dithiodibenzoic acid (dithiosalicylic acid). It was also observed that several forms of solvent-free DPPH exist.

THE stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was first prepared by Goldschmidt and Renn,[2] who also reported a method of determining it by reaction with hydroquinone. In electron paramagnetic resonance (e.p.r.) work, it can serve as a standard for spin concentration, since it saturates negligibly for usual microwave or RF powers, as well as a standard for line position (g), for field scale and for resolution. Because of this and its other extensive uses, we have evolved an easier and more accurate method of analysis for DPPH than those hitherto available, making use of its ability to abstract hydrogen atoms from mercaptans.[3]

RESULTS AND DISCUSSION

Determination of DPPH.—DPPH reacts cleanly and rapidly with solutions of thiosalicylic acid (TSA). Since TSA and the reaction products are not highly coloured, it is convenient to use the intense purple colour of the DPPH as indicator. In titrating DPPH with TSA, the colour changes from purple to brown-orange to bright yellow (in concentrated solutions, finally to bright orange). The change to yellow (orange) was taken as the end-point, confirmed in one run by monitoring the disappearance of the free-radical content by e.p.r.

TSA was selected as reducing agent partly because it was expected that its solutions might be easily standardised acidometrically, and for solutions in ethanol or acetone this was found to be so. The end-point is easily found by potentiometric titration; even more conveniently, use of the indicator curcumin gives a sharp colour change (yellow to orange) which is not adversely shifted or weakened by organic solvents.[4]

The accuracy of the analysis was tested by titrating very pure samples of DPPH, [DPPH (I)], prepared by the chromatographic technique of Laederich and Traynard;[5] these samples were found by elemental analysis to be solvent-free. The ability of DPPH to crystallise in fairly well-defined complexes with solvent molecules has been investigated by several authors,[6] and appears to depend somewhat on the exact experimental conditions. In Table 1, we present some detailed analytical results obtained in one set of titrations. Similar results were obtained in numerous other runs.

TABLE 1

Titration of DPPH with TSA

Solvent	DPPH* (gm.)	Concn. TSA (M) (in ethanol)	Vol. TSA soln. used (ml.)	M, DPPH (calc.)
Acetone	0·01014	$5{\cdot}82_7 \times 10^{-4}$	$44{\cdot}0_0$	$397{\cdot}_3$
Acetone	0·01063	$5{\cdot}85_1 \times 10^{-4}$	$45{\cdot}7_0$	$397{\cdot}_7$
Ethanol	0·01032	$5{\cdot}82_7 \times 10^{-4}$	$44{\cdot}8_0$	$398{\cdot}_0$
Ethanol	0·01011	$5{\cdot}82_7 \times 10^{-4}$	$43{\cdot}8_0$	$398{\cdot}_0$
			M (calc. for $C_{18}H_{12}O_6N_5$):	394·32

* Dissolved in *ca.* 10 ml. to yield $2{\cdot}5 \times 10^{-3}$ M-solutions.

[1] Based on work performed under the auspices of the U.S. Atomic Energy Commission.

[2] (*a*) S. Goldschmidt and K. Renn, *Ber.*, 1922, **55**, 628; (*b*) R. H. Poirier, E. J. Kahler, and F. Benington, *J. Org. Chem.*, 1952, **17**, 1437.

[3] (*a*) K. E. Russell, *J. Phys. Chem.*, 1954, **58**, 437; (*b*) A. G. Brook, R. J. Anderson, and J. Tissot Van Patot, *Canad. J. Chem.*, 1958, **36**, 159; (*c*) A. H. Ewald, *Trans. Faraday Soc.*, 1959, **55**, 792.

[4] I. M. Kolthoff and C. Rosenblum, "Acid–Base Indicators," MacMillan, 1937, Ch. 6, pp. 197—215.

[5] T. Laederich and P. Traynard, *Compt. rend.*, 1962, **254**, 1826.

[6] (*a*) J. A. Lyons and W. F. Watson, *J. Polymer Sci.*, 1955, **18**, 141; (*b*) J. J. Lothe and G. Eia, *Acta Chem. Scand.*, 1958, **12**, 1535.

In terms of the molecular weight of DPPH, the precision of the method is better than 1·0 (0·2%) and the accuracy is better than 4·0 (1·0%) with results consistently slightly higher than the expected value (see below).

The DPPH concentrations over which the titration appears useful lie between *ca.* 1×10^{-6}M and 1×10^{-2}M, limited at both extremes by the difficulty in judging the end-point. The optimal concentration range is $1—5 \times 10^{-3}$M. DPPH in ethanol or acetone can be titrated with TSA in either ethanol or acetone, whereas DPPH in benzene or chloroform is best analysed using TSA in acetone.

The major contaminants of DPPH are likely to be solvents of crystallisation [6] and 1,1-diphenyl-2-picrylhydrazine (present because of incomplete oxidation during the preparation of the DPPH, or because of reaction of DPPH with any of numerous hydrogen-donating reactants). The initial presence of small amounts of the hydrazine was found not to interfere with the titration, although the presence of large amounts ($>10^{-2}$M) did make the end-point difficult to detect. Because of the possible presence of these impurities, the absorption peak of DPPH near 525 mμ can be used only with caution for quantitative analysis.[2b,3c,6a]

Polymorphs, Solvates and Derivatives of DPPH.—It was found that DPPH could be prepared, solvent-free, in several forms. Published m. p.s for DPPH include 127—129° (decomp.), from 2 : 1 benzene–light petroleum;[2b] 137°, from benzene;[6a] 127—129°, from carbon tetrachloride;[7a] and 143—144°, from benzene–light petroleum.[7b]

DPPH (I), above, had m. p. 106°. Material which had been oxidised with active lead dioxide, recrystallised from benzene, and then heated *in vacuo* [DPPH (II)] had m. p. 137°; *M*, by titration 396·7, 396·8, 397·4. Material recrystallised from carbon disulphide gave black, acicular crystals [DPPH (III)] of m. p. 128—129°; *M*, 396·4, 396·7.

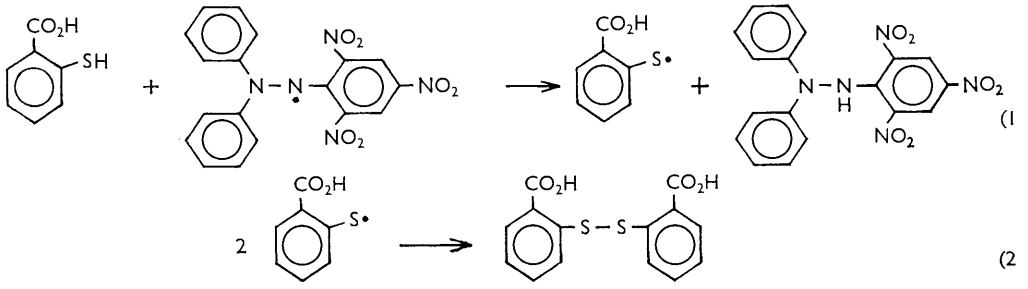
It thus appears that more than one crystal form of solvent-free DPPH exists, in addition to the various complexes with solvent molecules. The e.p.r. powder line-widths (between points of maximum slope) at 9500 Mc./sec. (room temp.) were $3{\cdot}1_3$oe with broad wings [DPPH (I)], $2{\cdot}5_7$oe [DPPH (II)], and $1{\cdot}0_6$oe [DPPH (III)]; all three samples gave $g = 2{\cdot}0037 \pm 0{\cdot}0002$. The *X*-ray powder patterns of the three samples confirm the presence of three distinct polymorphic forms. More complete *X*-ray investigations of the crystal forms and complete structure are being undertaken elsewhere.[8] It is to be noted that polymorphism is relatively common in nitro-aromatic compounds. For hydrazyl, only one partial *X*-ray structural analysis, for the DPPH–benzene complex, has been reported.[9]

Other samples of DPPH, containing solvent of crystallisation (DPPH–benzene, DPPH–½ acetone) gave correspondingly higher molecular weights. The same degree of accuracy (better than 1%) was also obtained with other hydrazyls, *e.g.*, the mono- and di-*p*-nitrophenylpicrylhydrazyls prepared as described.[10]

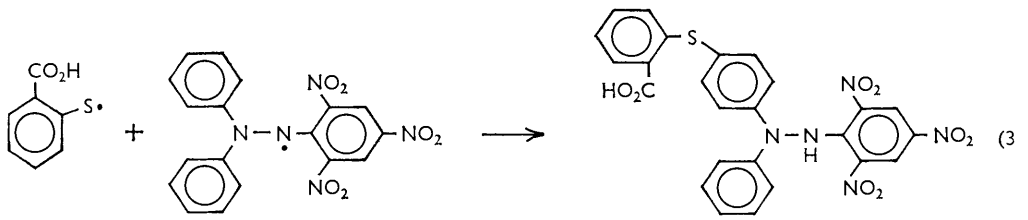
Identification of Products of Reaction between DPPH and TSA.—The nature of the reaction between DPPH and TSA was elucidated as follows. Large-scale (1—2 g.) reactions in acetone or ethanol were carried out by mixing equimolar amounts of DPPH and TSA, with stirring, until an orange-red colour (bright yellow on dilution) was obtained; the product was evaporated to dryness *in vacuo*. Addition of benzene yielded a red solution (A) and an insoluble light yellow powder (B). Evaporation of solution A, and recrystallisation from ethanol of the resulting red-brown powder, gave ~85% of 1,1-diphenyl-2-picrylhydrazine. Powder B was identified as 2,2′-dithiodibenzoic acid (dithiosalicylic acid) and was recovered in a yield of 86—88% by weight of the original TSA. Although no kinetic studies were attempted, one may conclude that the reaction between DPPH and TSA is reasonably uncomplicated and free from major side-reactions.

We believe that the primary reaction scheme, (1) and (2), could conceivably be com-

[7] (*a*) M. M. Chen, A. F. D'Adamo, jun., and R. I. Walter, *J. Org. Chem.*, 1961, **26**, 2721; (*b*) C. H. Bamford and A. D. Jenkins, *Proc. Roy. Soc.*, 1955, *A*, **228**, 220.
[8] D. E. Williams, *J.*, to be published.
[9] M. Sternberg, *Compt. rend.*, 1955, **240**, 990.
[10] J. A. Weil, K. V. Sane, and J. M. Kinkade, jun., *J. Phys. Chem.*, 1961, **65**, 710.



plicated by the side-reaction (3), in analogy to known reactions of DPPH with other free radicals.[3,10] We observed traces of a highly-coloured red material on chromatography of the products A and B and we note that the calculated molecular weights are all slightly higher than the theoretical value. Both observations are consistent with occurrence of a small contribution from reaction (3).



Experimental

2,2-Diphenyl-1-Picrylhydrazyl.—DPPH, obtained by oxidation with active lead dioxide [11] of commercial 1,1-diphenyl-2-picrylhydrazine (Eastman 7365) in benzene, was further treated as follows.

DPPH (I). The above material was subjected to chromatography,[5] using an acidic alumina column (Merck 71695) eluted with ether. The DPPH, after removal of the ether and pumping *in vacuo* for several days at room temperature, was not recrystallised further. It was solvent-free and appeared particularly fluffy and needle-like, m. p. 106°, lower on remelting a sample which had previously melted at 106°, indicating decomposition (Found: C, 54·3; H, 3·0; N, 17·7. Calc. for $C_{18}H_{12}N_5O_6$: C, 54·85; H, 3·05; N, 17·75%).

DPPH (*II*). The above material was recrystallised from benzene and heated *in vacuo* at 80° for 16 hr. giving black, amorphous particles with a bronzy sheen (Found: C, 55·0; H, 3·1; N, 17·35%); m. p., 137°, mixed m. p. with [DPPH (I)], 127—128°.

DPPH (*III*). The above material was recrystallised from carbon disulphide and subjected to high vacuum at room temperature for several days, yielding black, acicular sulphur-free crystals melting at 128—129° (Found: C, 54·95; H, 3·25; N, 17·65%).

DPPH is quite stable towards oxygen, but its solutions decompose slowly in light, and in ethanol. Generally, it is unwise to warm DPPH solutions to speed dissolution. Fresh solutions were used for all quantitative work described herein.

Thiosalicylic Acid.—Powdered TSA, obtained commercially (*o*-mercaptobenzoic acid, Eastman P2805), was stable indefinitely after recrystallisation by dissolving in hot ethanol (4 c.c./gm. TSA), adding hot distilled water (8 c.c./gm. TSA) and boiling with decolorising carbon.[12] The stability of TSA towards oxygen was such that no special precautions were taken to exclude air from the solutions. Slight decomposition over 24-hr. periods was observed, even in stoppered bottles, and fresh solutions were made up every day.

Standardisation of TSA.—For most standardisations the indicator curcumin (Eastman 1179, used as received), dissolved in ethanol, was used. In a few check experiments, standardisations were also carried out potentiometrically, using a Beckman model G pH Meter with a glass

[11] (*a*) R. Kuhn and I. Hammer, *Chem. Ber.*, 1950, **83**, 413; (*b*) W. K. Wilmarth and N. Schwartz, *J. Amer. Chem. Soc.*, 1955, **77**, 4543.
[12] *Org. Synth.*, Coll. Vol. II, 1943, 580.

electrode (General Purpose) and a calomel electrode (Fibre Type). The results of two such standardisations with aqueous sodium hydroxide solutions are presented in Table 2.

TABLE 2

Standardisation of thiosalicylic acid solutions

TSA * (gm.)	Solvent for TSA	[NaOH] (M)	Volume of base added (ml.)		Concn. of TSA solution (M) Found		
			Indicator	Potentiometric	Indicator	Potentiometric	Calc. from Wt. TSA/l.
0·20125	Ethanol	$1·008 \times 10^{-2}$	$26·3_0$	$26·2_0$	$1·31_5 \times 10^{-3}$	$1·31_0 \times 10^{-3}$	$1·30_5 \times 10^{-3}$
0·20125	Ethanol	$1·008 \times 10^{-2}$	$26·0_0$	$25·9_5$	$1·30_0 \times 10^{-3}$	$1·29_8 \times 10^{-3}$	$1·30_5 \times 10^{-3}$
0·18271	Acetone	$1·008 \times 10^{-2}$	$23·7_5$	$23·6_5$	$1·18_8 \times 10^{-3}$	$1·18_3 \times 10^{-3}$	$1·18_5 \times 10^{-3}$
0·18271	Acetone	$1·008 \times 10^{-2}$	$23·6_5$	$23·6_5$	$1·18_3 \times 10^{-3}$	$1·18_3 \times 10^{-3}$	$1·18_5 \times 10^{-3}$

* Dissolved in a 1000·0 ± 0·3 ml. volumetric flask. Aliquot portions of 200·0 ± 0·1 ml. were taken for titration.

Products of Reaction Between DPPH and TSA.—Product A, 1,1-diphenyl-2-picrylhydrazine, was identified by its melting point (174—175°), elemental analysis (Found: C, 55·15; H, 3·3; N, 17·0. Calc. for $C_{18}H_{13}N_5O_6$: C, 54·7; H, 3·3; N, 17·7%), and p.m.r. spectrum,[10,13] as well as by the e.p.r. spectrum of the DPPH obtained by oxidation with lead dioxide. The yellow powder B was recrystallised from glacial acetic acid [14] as a light beige powder, m. p. 287—289°, insoluble in water and hydrochloric acid, slightly soluble in ethanol and ether, and soluble in 1M-sodium hydroxide. It was identified as 2,2′-dithiodibenzoic acid by m. p. (289°), mixed m. p., elemental analysis (Found: C, 54·0; H, 3·35; N, <0·3; S, 20·6. Calc. for $C_{14}H_{10}O_4S_2$: C, 54·95; H, 3·3; S, 20·9%), and p.m.r. spectrum using 1M-sodium hydroxide as solvent.

All melting points were taken on a Fisher–Johns Melting Point Apparatus, calibrated with suitable standards.

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[13] J. A. Weil and G. A. Janusonis, *J. Org. Chem.*, 1962, **27**, 1248; also see *J. Chem. Phys.*, 1964, **41**, 1033.

[14] E. Ziegler and E. Nolken, *Monatsh.*, 1960, **91**, 850.