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An Improved Procedure for the Iodination of Phenols Using Sodium Iodide and *tert*-Butyl Hypochlorite

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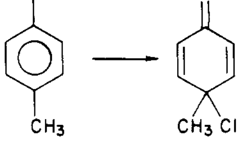
Electrophilic aromatic iodination constitutes a singularly important procedure for the synthesis of radiopharmaceuticals.[1] In connection with several projects involving the synthesis of radioiodinated photoprobes,[2] we required an efficient iodination reaction of substituted phenols **1** that would proceed under neutral conditions to furnish monoiodinated products **2** or diiodinated products **3**. A survey of the literature revealed that most common iodinating agents such as iodine,[3] iodine monochloride,[4] *N*-iodosuccinimide,[5] *N*,*N*′-diiodo-5,5-dimethylhydantoin,[6] and metal iodides[7] were used in conjunction with various acids, bases, or oxidizing agents that were incompatible with other sensitive functional groups to be incorporated in the photoprobes. Moreover, the scope of the literature procedures with regard to other aromatic substituents was often unclear.

Our initial foray in this area included a survey of various iodination conditions that would proceed in the presence of pH 7.4 buffer and that would accommodate a minimal amount of a polar organic cosolvent. In this way, we anticipated that we would be able to iodinate our photoprobe reagents either before or after linking to a protein receptor. Ultimately, we found that the combination of 1.2 equiv each of sodium iodide and chloramine T in DMF, Me_2SO, or acetonitrile with or without an aqueous buffer would monoiodinate various phenols in good yield.[8] Previous investigators[9] who utilized this same reagent combination in acetic acid were not concerned with iodinations of phenols bearing chemically sensitive functionality, and our modification using pH 7.4 buffered solvent system accommodated reactive carboxylate derivatives such as the *N*-hydroxysuccinimidyl (NHS) esters. However, the chloramine T/sodium iodide combination produced *p*-toluenesulfonamide as a byproduct which invariably required chromatographic separation. The use of polymer-bound chloramine T circumvents this difficulty but is best suited only for small-scale iodinations. The chromatography of the crude sodium iodide/chloramine T reactions precluded the direct isolation of certain NHS esters such as **2n** in Table I and is particularly unwelcome in the case of radioiodination. The synthesis of a labeled, NHS-containing photoprobe would require chromatography of a radioiodinated intermediate and subsequent activation as an NHS ester. Clearly, it would be preferable to radioiodinate as the penultimate step.

We have developed sodium iodide/*tert*-butyl hypochlorite as a superior reagent for the iodination of phenols. As shown in Table I, the combination of sodium iodide and *tert*-butyl hypochlorite was particularly effective for the diiodination of various phenols including the NHS ester **1m** which was converted to the diiodinated product **3m** in 96% isolated yield without requiring chromatography. The *tert*-butyl alcohol byproduct was readily removed during the aqueous workup procedure. The identification of the iodinated products was facilitated by the ^{13}C NMR spectra which displayed the iodine-bearing carbon at δ 80–88 (except for those cases which also possessed an ortho azide group).

The observation that the use of 1.2 equiv each of sodium iodide and *tert*-butyl hypochlorite led to ca. 1:2 monoiodination–diiodination ratios (e.g., **1e** gave 23% **2e** and 45% **3e**) whereas 1.2 equiv each of sodium iodide and chloramine T led to 3:1 monoiodination–diiodination ratios (e.g., **1e** gave 75% **2e** and 23% **3e**) led us to suspect that the true iodination species in each reagent pair may be different. Similarly, the halogenation of 4-methylanisole using the former reagent pair led to the dienone[10] **4** whereas 4-methylanisole was inert to the latter reagent pair. Although we regarded iodine monochloride as a potential iodinating agent,[11] we found that the treatment of 4-cresol (**1c**) with 1.2 equiv of iodine monochloride in 1:5 solution of pH 7.4 buffer–acetonitrile led to only 2% of 2-iodo-4-cresol (**2c**). The addition of *tert*-butyl alcohol to the sodium iodide/chloramine T reagent or the addition of *p*-toluenesulfonamide to the sodium iodide/*tert*-butyl hypochlorite reagent failed to alter the monoiodination–diiodination ratios discussed above. Additional experiments involving iodine itself in combination with *tert*-butyl



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(9) Sodium iodide and chloramine T in acetic acid was reported previously to effect the iodination of certain phenols (Jones, B.; Richardson, E. N. J. *Chem. Soc.* **1953**, 713), but under these conditions, a variety of phenols gave intractable products which under the conditions developed by us, gave useful, iodinated products.

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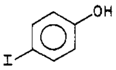
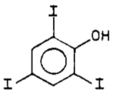
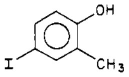
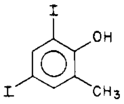
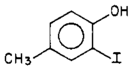
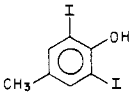
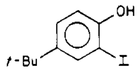
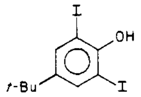
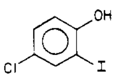
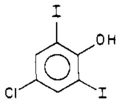
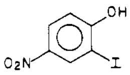
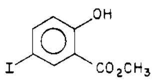
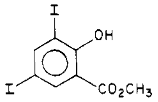
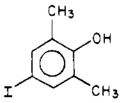
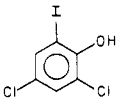
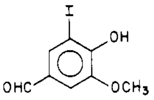
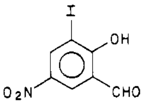
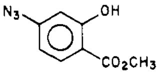
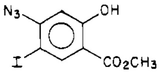
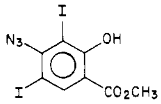
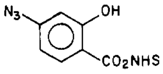
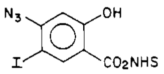
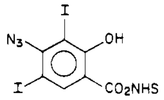
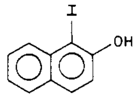
Table I. A Comparison of Phenol Iodinations Mediated by Sodium Iodide and either Chloramine T or *tert*-Butyl Hypochlorite

	substrate 1	conditions^d	products (% isolated yield)	
			monoiodination 2	polyiodination 3
a	phenol	A E	 (97)	 (90)
b	2-cresol	A D	 (76)	 (12) (95)
c	4-cresol	A B C^c D	 (60) (21)	 (21) (71) (35) (85)
d	4-*tert*-butylphenol	A	 (69)	 (25)
e	4-chlorophenol	A C^c	 (75) (23)	 (23) (45)
f	4-nitrophenol	A	 (69)	
g	methyl salicylate	A D	 (78)	 (91)
h	2,6-dimethylphenol	A C	 (64) (70)	
i	2,4-dichlorophenol	C	 (97)	
j	vanillin	A C	 (94) (97)	
k	5-nitrosalicylaldehyde	C	 (88)	
l		A C D	 (88) (66)	 (94)
m^b		A D	 (42)^a	 (96)
n	2-naphthol	A C	 (66) (95)	
o	17β-estradiol	A	2-iodo (27) + 4-iodo (20)	2,4-diiodo (15)

[a] Yield determined by conversion to *N*-*n*-butylamine derivative. [b] Ji, T. H.; Ji, I. *Anal. Chem.* **1982**, *121*, 286. [c] Balance of the product mixture in these cases was unreacted starting material. [d] Conditions: A, 1.2 equiv NaI, 1.2 equiv chloramine T; B, 2.2 equiv NaI, 2.2 equiv chloramine T; C, 1.1 equiv NaI, 1.1 equiv *t*-BuOCl; D, 2.2 equiv NaI, 2.2 equiv *t*-BuOCl; E, 3.3 equiv NaI, 3.3 equiv *t*-BuOCl.

hypochlorite or chloramine T were equally unrewarding. Although the mechanism for these iodinations is uncertain, the practical utility of these methods is clear.

Experimental Section

Infrared spectra were determined on a Beckman Microlab 600 spectrometer. The abbreviation TF denotes thin film. NMR spectra were determined on a JEOL 270-MHz NMR spectrometer. Mass spectra were determined on a VG ZAB-1F mass spectrometer. Melting points were determined by using a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

4-Azido-2-hydroxybenzoic Acid. To a stirred suspension of 10.00 g of 4-amino-2-hydroxybenzoic acid (Sigma) in 250 mL of water at 0 °C was added 325 mL of cold 6 M hydrochloric acid dropwise over 1 h. After the mixture was stirred for 20 min, 4.96 g of sodium nitrite in 35 mL of water was added dropwise over 15 min, and the resulting bright yellow-colored solution was stirred for 60 min at 0 °C. The insoluble crude product was filtered and recrystallized twice from acetone–water to afford 3.37 g (29%) of azide: mp 175 °C dec; IR (KBr) 3200–2400 (br), 2109, 1646, 1268 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.64–7.82 (m, Ar H); ^{13}C NMR (Me_2SO-d_6) δ 106.7, 109.8, 110.4, 132.1, 146.4, 162.4, 171.4; mass spectrum (70 eV), *m/e* (relative intensity) 179 (14), 151 (7), 133 (19), 105 (19), 77 (21), 32 (25), 28 (100).

Anal. Calcd. for $C_7H_5O_3N_3$: C, 46.93; H, 2.81; N, 23.46. Found: C, 47.04; H, 2.86; N, 23.44.

Methyl 4-Azido-2-hydroxybenzoate (1l). A solution of 1.00 g of 4-azido-2-hydroxybenzoic acid and 1 mL of concentrated sulfuric acid in 150 mL of methanol was refluxed for 49 h. The methanol was removed under reduced pressure at 20 °C and replaced with 70 mL of ethyl acetate. The ethyl acetate solution was washed successively with three 25-mL portions of a saturated sodium bicarbonate solution and two 25-mL portions of brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent afforded 830 mg (77%) of methyl ester (1l) as a pale yellow solid: R_f 0.56 (1:5 ethyl acetate–hexane); IR (TF) 2130, 1675, 1616, 1576 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95 (s, 3, CO_2CH_3), 6.52–7.83 (m, 3, Ar H); ^{13}C NMR ($CDCl_3$) δ 52.3, 107.2, 110.5, 131.6, 147.3, 162.9, 170.0; mass spectrum (70 eV), *m/e* (relative intensity) 193 (M^+, 45), 165 (40), 133 (100), 105 (75), 77 (60).

Typical Experimental Procedure for Iodination Using Sodium Iodide and Chloramine T. See ref 8.

Typical Experimental Procedure for Iodination Using Sodium Iodide and *tert*-Butyl Hypochlorite. 2,6-Dichloro-4-iodophenol (2h). To a solution of 100 mg (0.816 mmol) of 2,6-dimethylphenol (1h) and 135 mg (0.897 mmol, 1.1 equiv) of sodium iodide in 0.5 mL of water and 2.5 mL of acetonitrile at 0 °C was added 101 μL (0.897 mmol, 1.1 equiv) of *tert*-butyl hypochlorite (Fisher) dropwise. The solution was stirred for 10 min at 0 °C and diluted with 50 mL of ethyl acetate. The organic solution was washed successively with 5% aqueous sodium thiosulfate solution and brine and dried over anhydrous magnesium sulfate. The crude product was chromatographed on a 20 × 20 cm Macherey–Nagel silica gel F254 preparative layer plate to afford 142 mg (70%) of 2,6-dichloro-4-iodophenol (2h): mp 101–102 °C (lit.[12] mp 102–102.5 °C); ^1H NMR ($CDCl_3$) δ 2.17 (s, 6, CH_3), 4.74 (br s, 1, OH), 7.26 (s, 2, Ar H); ^{13}C NMR ($CDCl_3$) δ 15.5, 82.3 (CI), 125.6, 136.9, 152.1.

Physical Data/Spectral Data for Monoiodination Products 2 and Polyiodination Products 3 in Table I. The following products were identical with commercial samples or had data in agreement with literature values: **2a**, **2b**,[13] **2c**,[14] **2d**,[15] **2e**,[16] **2f**,[17] **2g**,[18] **2i**,[19] **2j**,[20] **2k**,[21] **2n**,[22] **2o**,[23] **3a**,[22] **3b**,[22] **3c**,[22] **3d**,[24] **3e**,[16] **3g**,[25] and **3o**.[23] NMR spectral data is given below since it is unavailable elsewhere.

2,4-Dichloro-6-iodophenol (2i): ^1H NMR ($CDCl_3$) δ 5.94 (br s, 1, OH), 7.31 (d, *J* = 2.0 Hz, 1, Ar H), 7.58 (d, *J* = 2.0 Hz, 1, Ar H); ^{13}C NMR ($CDCl_3$) δ 83.2 (CI), 119.1, 126.2, 129.2, 136.9, 149.8.

5-Iodovanillin (2j): ^1H NMR ($CDCl_3$) δ 3.98 (s, 3, OCH_3), 6.69 (s, 1, OH), 7.38 (d, *J* = 1.6 Hz, 1, C-2H), 7.82 (d, *J* = 1.6 Hz, 1, C-6H), 9.77 (s, 1, CHO); ^{13}C NMR (Me_2SO-d_6) δ 54.2, 82.0 (CI), 108.1, 128.1, 132.8, 145.3, 150.3, 188.0.

3-Iodo-5-nitrosalicylaldehyde (2k): ^1H NMR (Me_2SO-d_6) δ 8.67 (d, *J* = 2.6 Hz, 1, Ar H), 8.74 (d, *J* = 2.6 Hz, 1, Ar H), 10.10 (s, 1, CHO), 11.34 (br s, 1, OH); ^{13}C NMR (Me_2SO-d_6) δ 88.6 (CI), 120.3, 127.9, 138.9, 140.2, 164.2, 194.0.

Methyl 5-iodo-4-azidosalicylate (2l): mp 142–143 °C (recrystallized from ethyl acetate–hexane); IR (KBr) 2095, 1662 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (s, 3, OCH_3), 6.75 (s, 1, C-3H), 8.19 (s, 1, C-6H), 10.88 (s, 1, OH); ^{13}C NMR (Me_2SO-d_6) δ 50.9, 73.4 (CI), 105.5, 109.8, 138.9, 146.2, 160.1, 166.2.

Anal. Calcd for $C_8H_6O_3N_3I$: C, 30.12; H, 1.90. Found: C, 30.21; H, 1.95.

1-Iodo-2-naphthol (2n): ^1H NMR ($CDCl_3$) δ 5.82 (s, 1, OH), 7.20 (d, *J* = 8.8 Hz, 1, Ar H), 7.33 (t, *J* = 7.3 Hz, 1, Ar H), 7.49 (t, *J* = 7.3 Hz, 1, Ar H), 7.61 (m, 2, Ar H), 7.88 (d, *J* = 8.8 Hz, 1, Ar H); ^{13}C NMR ($CDCl_3$) δ 86.2 (CI), 116.3, 124.1, 128.1, 128.2, 129.5, 130.1, 130.5, 134.6, 153.6.

4,6-Diiodo-2-cresol (3b): ^1H NMR $CDCl_3$) δ 2.25 (s, 3, CH_3), 5.30 (s, 1, OH), 7.37 (d, *J* = 1.3 Hz, 1, C-3H), 7.75 (d, *J* = 1.3 Hz, 1, C-5H); ^{13}C NMR ($CDCl_3$) δ 16.9, 82.8 (CI), 86.9 (CI), 127.1, 139.7, 142.5, 152.8.

2,6-Diiodo-4-cresol (3c): ^1H NMR ($CDCl_3$) δ 2.22 (s, 3, CH_3), 5.57 (s, 1, OH), 7.48 (s, 2, Ar H); ^{13}C NMR ($CDCl_3$) δ 19.4, 82.0 (CI), 133.5, 139.3, 151.0.

Methyl 3,5-diiodosalicylate (3g): ^1H NMR ($CDCl_3$) δ 3.97 (s, 3, OCH_3), 8.08 (d, *J* = 1.9 Hz, 1, C-4H), 8.16 (d, *J* = 1.9 Hz, 1, C-6H), 11.56 (s, 1, OH); ^{13}C NMR ($CDCl_3$) δ 53.1, 80.7 (CI), 86.8 (CI), 114.0, 138.3, 151.9, 161.1, 168.7.

Methyl 4-azido-3,5-diiodosalicylate (3l): mp 99–100 °C (recrystallized from ethyl acetate/hexane) dec; IR (KBr) 2101, 1661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.99 (s, 3, OCH_3), 8.21 (s, 1, C-6H), 11.76 (s, 1, OH); ^{13}C NMR ($CDCl_3$) δ 53.2, 77.8 (CI), 83.7 (CI), 111.3, 140.3, 147.6, 161.5, 168.2.

Anal. Calcd. for $C_8H_5O_3N_3I_2$: C, 21.60; H, 1.13. Found: C, 21.70; H, 1.14.

***N*-Hydroxysuccinimidyl 4-azido-3,5-diiodosalicylate (3m):** mp 180–181 °C (recrystallized from ethyl acetate) dec; IR (KBr) 3296, 2106, 1728 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.93 (s, 4, succinimidyl H), 8.32 (s, 1, C-6H); ^{13}C NMR (Me_2SO-d_6) δ 25.5, 79.7 (CI), 88.8 (CI), 109.2, 139.4, 148.7, 160.5, 170.0, 172.7.

Anal. Calcd for $C_{11}H_6O_5N_4I_2$: C, 25.02; H, 1.15. Found: C, 25.12; H, 1.18.

***N*-*n*-Butyl 5-Iodo-4-azidosalicylamide.** The iodination procedure described in ref 8 was repeated using 200 mg (0.724 mmol) of **1m** to afford a crude iodinated NHS ester. To this crude product in 3 mL of dimethyl sulfoxide was added 52.9 mg (0.724 mmol) of *n*-butylamine in 6 mL of dimethyl sulfoxide. The reaction mixture was stirred for 3 h at 25 °C. The product was diluted with ethyl acetate, and the organic layer was thoroughly washed with brine and dried over anhydrous magnesium sulfate. The residue was chromatographed on Macherey Nagel silica gel 60 with 1:5 ethyl acetate–hexane to afford 108.2 mg (42%) of the *N*-*n*-butylamide derivative of **2m**: mp 136–137 °C (recrystallized from ethyl acetate–hexane); IR (KBr) 3365, 2093, 1591 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, *J* = 7.6 Hz, 3, CH_3), 1.35–1.85, (m, 4, $CH_2(CH_2)_2CH_3$), 3.44 (m, 2, $NHCH_2$), 6.15 (br s, 1, NH), 6.77 (s, 1, C-3H), 7.68 (s, 1, C-6H), 12.70 (s, 1, OH).

Anal. Calcd for $C_{11}H_{13}N_4O_2I$: C, 36.68; H, 3.64. Found: C, 36.57; H, 3.67.

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Registry No. **1a**, 108-95-2; **1b**, 95-48-7; **1c**, 106-44-5; **1d**, 98-54-4; **1e**, 106-48-9; **1f**, 100-02-7; **1g**, 119-36-8; **1h**, 576-26-1; **1i**, 120-83-2; **1j**, 121-33-5; **1k**, 97-51-8; **1l**, 87877-90-5; **1m**, 96602-46-9; **1n**, 135-19-3; **1o**, 50-28-2; **2a**, 540-38-5; **2b**, 60577-30-2; **2c**, 16188-57-1; **2d**, 38941-98-9; **2e**, 71643-66-8; **2f**, 89487-91-2; **2g**, 4068-75-1; **2h**,

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10570-67-9; **2i**, 2040-83-7; **2j**, 5438-36-8; **2k**, 70331-81-6; **2l**, 99282-18-5; **2m**, 99298-06-3; **2o** (2-iodo), 24381-12-2; **2o** (4-iodo), 61748-87-6; 2n, 2033-42-3; **3a**, 609-23-4; **3b**, 4186-52-1; **3c**, 2432-18-0; **3d**, 75908-75-7; **3e**, 15459-50-4; **3g**, 18071-50-6; **3l**, 99282-19-6; **3m**, 99282-20-9; **3o**, 17756-86-4; 4-amino-2-hydroxybenzoic acid, 65-49-6; 4-azido-2-hydroxybenzoic acid, 66761-27-1.

FMN Reductase Catalyzed Regeneration of NAD(P) for Use in Enzymatic Synthesis

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The synthetic utility of horse liver alcohol dehydrogenase (HLADH) in the oxidation of alcohols to many potentially useful chiral synthons has been demonstrated.[1-3] These reactions and many other synthetically useful enzymatic oxidations require the cofactor nicotinamide adenine dinucleotide, NAD (or nicotinamide adenine dinucleotide phosphate, NADP), which is reduced to NADH (or NADPH) during the course of the reaction. While several practical procedures are available for the regeneration of reduced nicotinamide cofactors NAD(P)H, the problem of regeneration of *oxidized* nicotinamide cofactors NAD(P) is still not completely solved.[4] The high cost of NAD(P) requires the regeneration of NAD(P) from NAD(P)H to make the process economically feasible. The procedure for NAD(P) regeneration generally considered best is that using flavin mononucleotide (FMN) with dioxygen as the ultimate oxidizing agent.[5,6] This procedure is convenient, the reaction is energetically favorable and, FMN is innocuous to enzymes. However, this *noncatalytic* regeneration system is not efficient enough for routine use in large-scale synthesis because large quantities of FMN and NAD(P) are required to achieve useful rates, and separation of products from the cofactors is inconvenient. Many NAD(P)-dependent reactions are carried out at a substrate concentration of 0.1–0.5 M in order to be practical and to avoid inhibition by substrate and product. At these concentrations the NAD(P) concentration must be set at 0.1–0.5 mM to achieve a high turnover number (1000 or more) and to minimize the cost of the cofactor. Since the rate for the noncatalytic oxidation of NADH with FMN is governed by the bimolecular rate constant 0.2 M^{-1} s^{-1},[6] the number of moles of product produced per day by the synthesizing enzyme can be calculated and the results are shown in Table I. These data demonstrate the the productivity using this nonenzymatic regeneration system is quite low, especially at the desired low concentration of cofactor.

We describe in this paper an improvement of this NAD regeneration system in which the enzyme FMN reductase (EC 1.6.8.1) from *Photobacterium fischerei* (ATCC 7744)[7] is added to the reactor (Scheme I). This enzyme catalyzes the oxidation of NAD(P)H by FMN, thereby increasing the rate of the reaction and making the process more efficient. The productivity depends on the amount of enzyme used. In a typical reaction condition of 0.1 mM NAD, 10 mM FMN, and 10 mg/L of FMN reductase, a productivity of 4×10^2 mmol/day/L can be accomplished. Without the enzyme the productivity would be only 17 mmol/day/L under otherwise identical conditions. This comparison and the comparison of productivities under other conditions is shown in Table I.

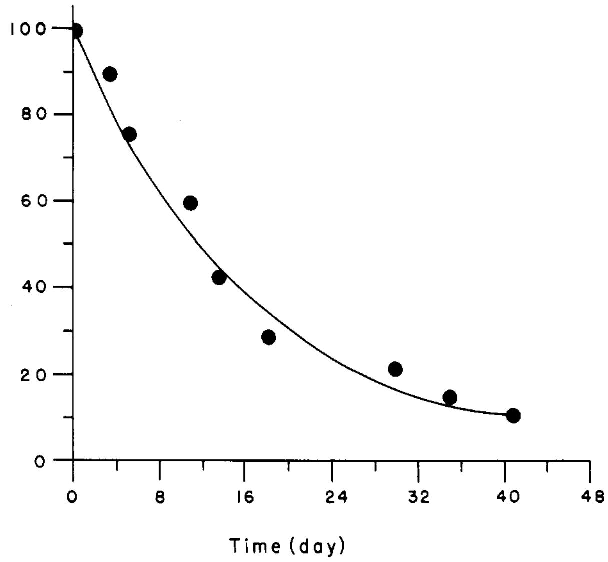
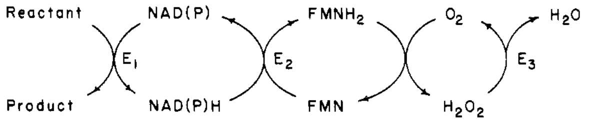
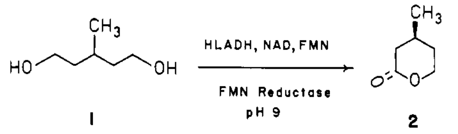


Figure 1. Stability of soluble FMN reductase in solution under air at pH 9.

Scheme I. FMN Reductase Catalyzed Regeneration of NAD(P): E_1, Horse Liver Alcohol Dehydrogenase; E_2, FMN Reductase; E_3, Catalase



Scheme II. HLADH-Catalyzed Oxidation Coupled with FMN Reductase Catalyzed Regeneration of NAD



Isolation of the enzyme FMN reductase used in this study was straightforward. Eight hundred grams of wet cells would produce about 260 units of the enzyme. The enzyme is also available commercially from Boehringer but is quite expensive.

The relative activity of the enzyme was determined at pHs 7, 8, and 9 with NADH and FMN as substrates. The enzyme had the greatest activity at pH 7, with 87% of this activity at pH 8 and 72% of this activity at pH 9. This indicates that the enzyme is sufficiently active to be useful synthetically at all of these pH regions. The high activity at alkaline region is particularly useful when the FMN reductase catalyzed reaction is coupled with the HLADH-catalyzed oxidations which have an optimal activity at pH 9.[5,6] The enzyme also shows comparable activity using NADPH as a substrate.[8] Stability studies

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