Investigation of the Degradation Mechanism of 5-Aminosalicylic Acid in Aqueous Solution

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The solution degradation of the antiinflammatory agent 5-aminosalicylic acid (5-ASA) was investigated in order to elucidate a mechanism for degradation. Two degradation pathways were considered: decarboxylation by analogy to 4-aminosalicyclic acid (4-ASA) decomposition and oxidation from consideration of 5-ASA's aromatic ring substitution pattern (i.e., relation to *p*-aminophenol). The oxidation of 5-ASA was investigated using cyclic voltammetry and flow electrolysis. These studies showed that 5-ASA is more easily oxidized than is 4-ASA and that 5-ASA undergoes a two-electron, two-proton oxidation consistent with formation of 5-ASA-quinoneimine (5-ASA-QI). This oxidation is followed by subsequent complex chemistry. The decomposition of 5-ASA in solution was examined under a variety of conditions. 5-ASA decomposes most rapidly under conditions promoting oxidation and is most stable under conditions tending to inhibit oxidation. Decarboxylation was not found to be a significant degradation pathway.

KEY WORDS: 5-aminosalicylic acid; oxidative decomposition; solution stability; electrochemical studies.

INTRODUCTION

5-Aminosalicylic acid (5-ASA, mesalamine, mesalazine) is an antiinflammatory drug primarily employed in the treatment of inflammatory bowel disease, Crohn's disease and ulcerative colitis (1,2). Previous work on 5-ASA stability has focused on preventing decomposition in drug formulations and biological samples (3–6) rather than on determining a decomposition pathway. Degradation of 5-ASA through decarboxylation has been suggested by analogy to mechanisms for decomposition of the more widely studied 4-aminosalicyclic acid (4-ASA), which has been shown to decarboxylate (7,8). Decarboxylation of 5-ASA has been studied and has been found to occur in nonaqueous solution at elevated temperatures (9) but has not been investigated in aqueous solution.

Another possible pathway for degradation of 5-ASA is via oxidation. 5-ASA is structurally analogous to *p*-aminophenol (PAP), which is easily oxidized to the quinoneimine (Scheme I) (10). The oxidation of 5-ASA has been employed indirectly for the trace determination of Fe^{3+} (11). 4-ASA, on the other hand, is an analogue of *m*-aminophenol, which is much more difficult to oxidize and cannot form a quinoneimine structure. Therefore, oxidative processes are expected to be of much greater importance in the chemistry of 5-ASA relative to that of 4-ASA. This is further suggested by 5-ASA's biological activity as a free radical scavenger and antioxidant (12–15).

The purpose of this study was to elucidate the degradation pathways of 5-ASA in aqueous solution, to evaluate the relative importance of oxidation and decarboxylation, and to demonstrate the utility of various electroanalytical techniques for these studies.

MATERIALS AND METHODS

Materials

5-Aminosalicylic acid (>98%) was supplied by Marion Merrell–Dow (Kansas City, MO). Resorcinol (RS), hydroquinone (HQ), and gentisic acid (GA) were purchased from Sigma Chemical Company (St. Louis, MO); 4-ASA, *m*-aminophenol (MAP), *p*-aminophenol (PAP), and 2,4-dihydroxybenzoic acid (2,4DHBA) from Aldrich Chemical Company (Milwaukee, WI); and salicylic acid (SA) from Mallinckrodt Chemical Works (St. Louis, MO).

N-Acetyl-5-aminosalicylic acid (Ac-5-ASA) was synthesized by reaction of 5-ASA with acetic anhydride (EM Science, Gibbstown, NJ). Liquid chromatographic analysis determined a single product with no remaining 5-ASA. Identification of the product as Ac-5-ASA was confirmed by mass spectrometry and melting-point determinations [product m.p. 219°C; literature m.p. 218°C (16)].

HPLC-grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ). HPLC-grade water was obtained using a Barnstead Nanopure II system (Fisher Scientific). All other chemicals were reagent grade or better and used as received.

Liquid Chromatographic Analysis

A reverse-phase liquid chromatographic method was developed providing separation of 5-ASA and possible degradation products (PAP, GA, SA, HQ). The same method was employed for 4-ASA and its possible degradation products (MAP, 2,4-DHBA, SA, RS). The chromatographic system consisted of a reverse-phase column (Brownlee Spherisorb ODS, 5 μm, 4.6 × 100 mm, with a 4.6 × 20-mm guard column), a Shimadzu LC-6A pump, a Rheodyne 7010 injector with a 20-μl sample loop, and either an electrochemical (Bioanalytical Systems LC-4B) or a photodiode array detector (Groton Technology Inc. PF-1). A mobile phase of pH 4.8 ammonium phosphate (0.05 *M*) buffer filtered through a 0.2-μm filter was used at a flow rate of 1.0 ml/min. Sample solutions were diluted 1:100 with mobile phase prior to injection on the chromatographic system.

The photodiode array detector recorded UV spectra in the range from 200 to 400 nm for each chromatographic time point as well as providing single-wavelength detection. The electrochemical detector was operated in the dual-electrode parallel configuration, with electrodes operated at +600 mV and −100 mV versus Ag/AgCl in order to detect both oxi-

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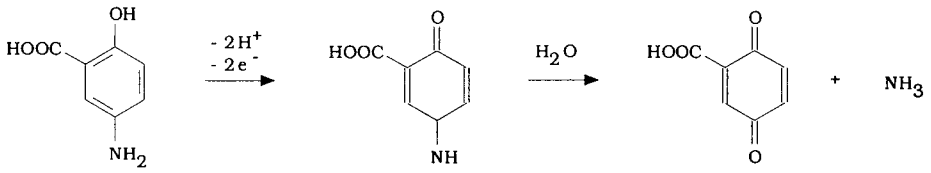


Scheme I

dizable and reducible species. Glassy carbon working electrodes and a stainless steel auxiliary electrode were used.

Cyclic Voltammetry

Cyclic voltammograms were recorded with a Bioanalytical Systems CV-47 scanning potentiostat using glassy carbon working, Ag/AgCl reference, and platinum auxiliary electrodes. Solutions were prepared to be 1 m*M* 5-ASA, 4-ASA, or Ac-5-ASA, 0.05 *M* phosphate buffer, and 0.1 *M* KNO_3. All solutions were deoxygenated with argon for 10 min prior to obtaining the voltammogram. Cyclic voltammetry of 5-ASA was performed in pH 2.0, 5.0, 7.0, and 10.0 phosphate buffers. Cyclic voltammetry of 4-ASA and Ac-5-ASA was performed at pH 7.0, and that of gentisic acid at pH 2.0. The potential was scanned from −200 to +1200 mV at scan rates of 10, 100, and 500 mV/sec.

Flow Electrolysis

Electrolysis experiments were performed using a flow cell built in-house, a Bioanalytical Systems CV-47 potentiostat, and a Sage Instruments Model 351 syringe pump. A flow rate of 1.3 ml/min was used. Solutions were prepared in pH 7.0 phosphate (0.05 *M*) buffer. Electrolysis was performed at an applied potential of +600 mV versus Ag/AgCl.

The electrolytic flow cell was constructed as described elsewhere (17). A flow-through, packed carbon bed working electrode (48-mesh carbon packed in porous Vycor tubing, 7-mm o.d., 4-mm i.d.) was employed, with a platinum auxiliary electrode and a Ag/AgCl reference electrode. The auxiliary and reference electrodes were placed in a saturated KNO_3 solution isolated from the working electrode by Vycor tubing. Electrical contact was maintained through the tubing. Under these conditions this cell achieved complete electrolysis.

The number of electrons (n) transferred in the electrochemical oxidation was determined using the derivative form of the Faraday equation

$$i = nF(dN/dt) = nFCf_r$$

where i is the current (amp), F is the Faraday constant (96,500 C/mol electrons), N is the number of moles of analyte, t is time (sec), C is the analyte concentration (M), and f_r is the flow rate through the cell (L/sec). In addition, the oxidation products were collected and analyzed by liquid chromatography.

Decomposition Studies

The stability of 5-ASA in aqueous solution was examined under a variety of conditions intended to promote decarboxylation [i.e., low pH, as reported for 4-ASA (8)], or oxidation (i.e., high pH, solution oxygenation, hydrogen peroxide). Conditions known to inhibit oxidation (i.e., solution deoxygenation, added antioxidant) were also examined. Degradation was studied as a function of pH in buffered solution. The percentage decomposition was determined by liquid chromatographic analysis with comparison to standard 5-ASA solutions.

The decomposition of 5-ASA in aqueous solution was followed under a variety of conditions as outlined in Table I. All solutions were prepared to be 1 m*M* in 5-ASA, 4-ASA, or Ac-5-ASA. Buffers used were sodium phosphate (0.05 *M*) adjusted to constant ionic strength (μ = 0.5) with sodium chloride. Deoxygenation was maintained by continuous bubbling with argon. Oxygenated solutions were saturated with O_2 at 20°C. The concentration of ascorbic acid in the applicable solutions was 1.4 mg/ml.

RESULTS

Electrochemical Studies

Cyclic voltammograms obtained for 5-ASA, 4-ASA, and Ac-5-ASA are shown in Fig. 1. 4-ASA (E_p = 850 mV) is much more difficult to oxidize than is 5-ASA (E_p = 300 mV), as predicted from comparison of their aminophenol analogs. Ac-5-ASA, with the amino group blocked, is also more difficult to oxidize than is 5-ASA but less so than 4-ASA.

The effect of the potential scan rate on the electrochemistry of 5-ASA is shown in Fig. 2. At slow scan rates an irreversible oxidation wave is observed. A single reduction

Table I. Stability of 5-ASA in Solution

Conditions[a]	Time	% decomposition
Water	48 hr	2
	7 days	42
Water, heated[b]	2 hr	2
Water, UV light	48 hr	7
0.1 *N* HCl	180 days	1
0.1 *N* HCl, heated[b]	2 hr	1
0.1 *N* NaOH		
Deoxygenated	2 hr	2
Oxygenated	2 hr	72
Oxygenated, ascorbic acid added	2 hr	1
4% H_2O_2	24 hr	33
4% H_2O_2, EDTA	24 hr	32
4% H_2O_2, trace Fe^{3+}	1 min	86
Trace Fe^{3+}, oxygenated	1 hr	20
4-ASA (1 m*M*), 0.1 *N* HCl	48 hr	25
Ac-5-ASA (1 m*M*), 0.1 *N* NaOH	24 hr	2

[a] Except where otherwise noted, all solutions were prepared 1 m*M* in 5-ASA.

[b] Solutions were heated to boiling.

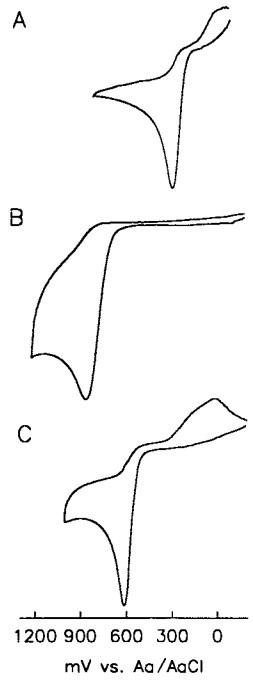


Fig. 1. Cyclic voltammograms of (A) 5-ASA, (B) 4-ASA, and (C) Ac-5-ASA taken at 100 mV/sec and pH 7.

wave is seen at a much more negative potential than would be observed for the reversible reduction. This indicates that the initial product of the oxidation undergoes a chemical reaction to yield a second product which can be reduced at this more negative potential.

As the scan rate is increased the oxidation of 5-ASA becomes quasi-reversible. In addition, a third reduction wave is seen between the reversible reduction and that observed at slow scan rates. This indicates a much more complex mechanism where the initial oxidation is followed by multiple chemical reactions to yield several electroactive species. Whether these reactions are sequential or simultaneous is not easily ascertained. The reversible reduction wave corresponds to the immediate oxidation product, 5-ASA-quinoneimine. The potential of the intermediate reduction wave (E = 300 mV) is identical to that for the reduction of oxidized gentisic acid, showing probable hydrolysis of the 5-ASA-quinoneimine to gentisic acid (Scheme I).

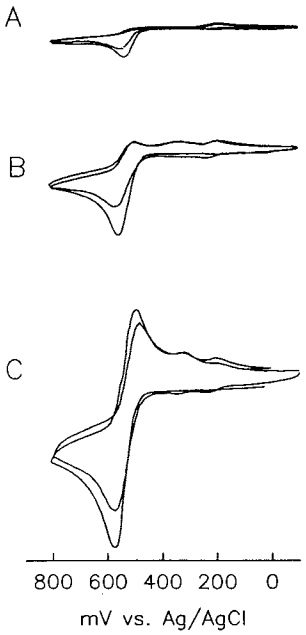


Fig. 2. Cyclic voltammograms of 5-ASA (pH 2) taken at scan rates of (A) 10, (B) 100, and (C) 500 mV/sec.

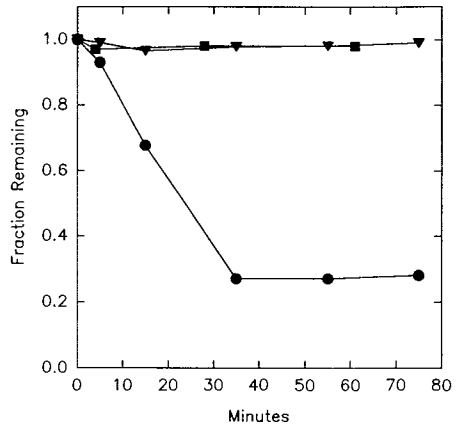


Fig. 3. Stability of 5-ASA (1 mM) in 0.1 N NaOH in oxygenated solution (●), deoxygenated solution (■), and oxygenated solution with added ascorbic acid (▼).

The oxidation peak potential (E_p) of 5-ASA was plotted as a function of pH. Through the Nernst equation (18) the slope of this plot yields the ratio of protons to electrons transferred in the reaction.

$$E = \underbrace{\left(E^{\circ\prime} - \frac{0.059}{n} \log \frac{[R]}{[O]}\right)}_{y\ \text{Intercept}} - \underbrace{\frac{0.059a}{n} pH}_{\text{Slope}}$$

Using $E = E_p$ the ratio [R]/[O] is a constant. The slope of the E_p versus pH plot, when divided by -0.059, yields the pro-

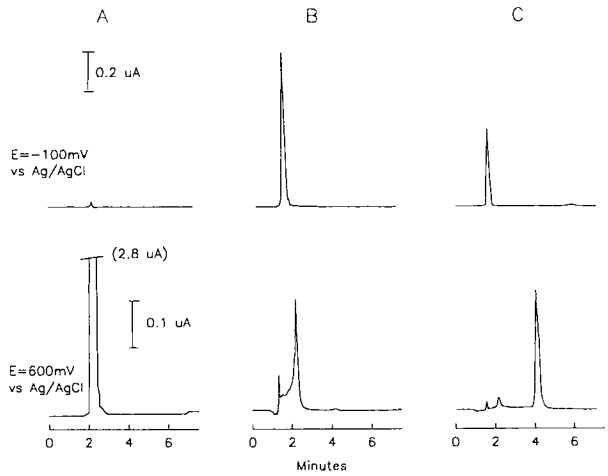


Fig. 4. Dual electrochemical detection chromatograms of (A) 5-ASA standard and flow-cell oxidation products (B) immediately following oxidation and (C) 55 min following oxidation.

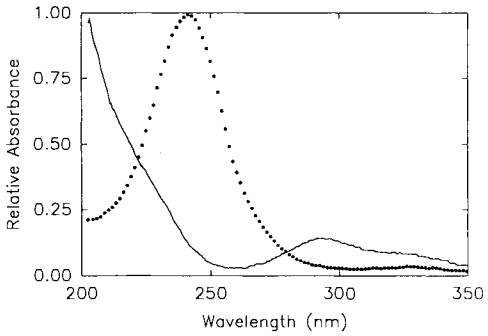


Fig. 5. UV spectra of 5-ASA (——) and 5-ASA-quinoneimine (---) (flow-cell oxidation product).

ton-to-electron ratio (a/n) for the reaction. This ratio for the oxidation of 5-ASA is 1.16 ± 0.26.

The number of electrons transferred in the oxidation was determined using controlled electrolysis. A 5-ASA solution of known concentration (1.0 m*M*) was pumped through an electrolytic flow cell as described above. The oxidation current was measured and related through the Faraday equation with the flow rate and 5-ASA concentration to yield n = 1.9 ± 0.1 electrons transferred in the oxidation. This indicates an overall two-electron, two-proton oxidation of 5-ASA, which is expected for the formation of the 5-ASA-quinoneimine (5-ASA-QI).

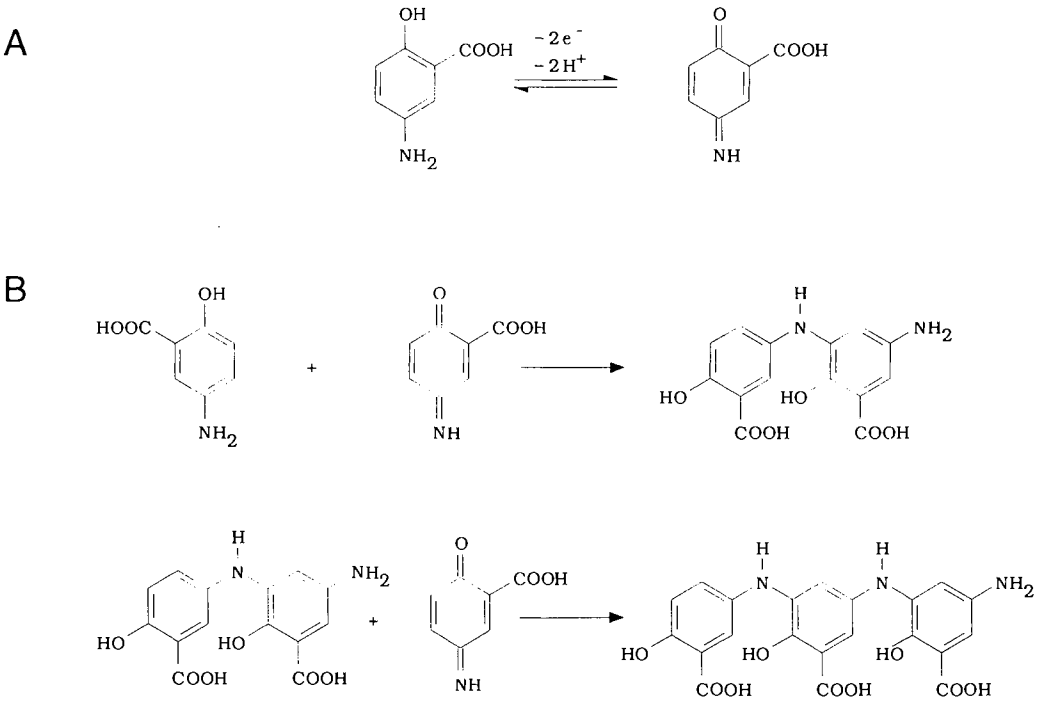
Decomposition Studies

The stability of 5-ASA was initially examined under conditions previously used for decomposition of 4-ASA, which has been reported to decarboxylate most rapidly at low pH (7,8). In 0.1 *N* HCl, 4-ASA was found to decarboxylate slowly to *m*-aminophenol. 5-ASA, however, was stable for over seven days under these conditions. 5-ASA slowly decomposes in water, with slight discoloration, and rapidly decomposes in 0.1 *N* NaOH. No degradation products were detected following separation by reverse-phase liquid chromatography during decomposition of 5-ASA in either water or basic solution.

Figure 3 shows the dependence of the alkaline degradation of 5-ASA on the presence of oxygen. In an oxygenated basic solution 5-ASA is rapidly decomposed. Deoxygenation stabilizes 5-ASA in alkaline solution, as does addition of the antioxidant ascorbic acid. The decomposition of 5-ASA in solution was found to be highly pH dependent, as shown in Table II. At low pH values 5-ASA is stable. As the solution pH is increased, the rate of decomposition of 5-ASA increases.

5-ASA was found to degrade slowly in dilute hydrogen peroxide solution. LC determination indicated the presence of small amounts of gentisic acid which appeared transiently early in the degradation. No other products were detected. EDTA was added to both basic and peroxide solutions of 5-ASA to mask by complexation any metal ions which might be present catalyzing degradation. No change in the rates of decomposition was noted. The addition of a catalytic amount of Fe^{3+} (i.e., approximately 0.5 mg $Fe(NO_3)_3$/100 ml) to the peroxide solution in the absence of EDTA caused the reaction to proceed much more rapidly, with only 15% of the 5-ASA initially present remaining after 1 min.

The products of the oxidation by flow electrolysis were collected and analyzed, both immediately following oxidation and upon standing, by LC with electrochemical and photodiode array detection. A reducible product was detected immediately following oxidation (Fig. 4B). The UV spectra of 5-ASA and the oxidation product are shown in



Scheme II

Fig. 5. The λ_{max} of the product is shifted to longer wavelengths as is generally observed for quinones and quinoneimines (19). This reducible product was therefore identified as the 5-ASA-quinoneimine. Upon standing, this peak diminished and a new chromatographic peak grew in, corresponding to gentisic acid (Fig. 4C). The peak's identity was confirmed by comparison of retention time and UV spectrum with an authentic sample of gentisic acid. The solution was colorless immediately following oxidation but darkened quickly on standing. The products detected immediately following oxidation correspond to only 30% of the material originally present. At 2 hr following oxidation, no products were detected in the oxidized solution using this chromatographic method.

DISCUSSION

The initial step in the degradation of 5-ASA in solution requires oxidation of 5-ASA to the 5-ASA-quinoneimine (Scheme IIA) and does not involve decarboxylation. This is supported by the pH dependence of the reaction, the effect of oxygen on reaction rate, the iron catalysis of the peroxide degradation, and the observed electrochemistry. The solution stability of 5-ASA under a variety of conditions is summarized in Tables I and II. 5-ASA was found to decompose most rapidly under conditions promoting oxidation (i.e., high pH, oxygenated solutions, hydrogen peroxide) and to be most stable under conditions inhibiting oxidation (i.e., low pH, deoxygenated solutions, added antioxidant). The cyclic voltammetric and electrolytic studies indicate a mechanism involving the initial two-electron, two-proton oxidation of 5-ASA to the quinoneimine, followed by one or more chemical reactions yielding additional electroactive species. This is also supported by analysis of the flow cell oxidation products.

It is significant to note that the N-acetyl derivative of 5-ASA, which has its oxidation potential shifted +300 mV relative to 5-ASA, is stable under conditions (alkaline solution) causing rapid decomposition of 5-ASA (Table I). This indicates that the amine function plays an essential role in 5-ASA degradation.

The initial oxidation of 5-ASA leads to substantial followup chemistry. Multiple pathways are expected for the succeeding reactions. One of these involves hydrolysis of the quinoneimine to produce gentisic acid as suggested by the electrolysis and cyclic voltammetry experiments. One possible pathway is 1,4 Michael addition of excess 5-ASA to the 5-ASA quinoneimine, resulting in a reduced dimer which can react further in the presence of additional quinoneimine (Scheme IIB). Such a polymerization has been reported for the ethyl ester of 5-ASA (20). Since the decomposition of 5-ASA results in a mixture of products, isolation and purification of those products will be necessary prior to identification.

Table II. pH Dependence of 5-ASA Stability

pH[a]	% decomposition[b]
2.0	<1
4.5	3
6.5	5
8.5	6
10	44
13[c]	95

[a] All solutions except pH 13 contained 1 m*M* 5-ASA in 0.05 *M* sodium phosphate buffer with ionic strength adjusted to 0.5 with NaCl.

[b] Decomposition after 72 hr in solution determined relative to known standard.

[c] Solution of 1 m*M* 5-ASA in 0.1 *N* NaOH with ionic strength adjusted to 0.5 with NaCl.

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REFERENCES

1. U. Klotz. Pharmacokinetic properties of 5-aminosalicylic acid (mesalazine). *Falk Symp.* 46:339–347 (1988).
2. S. Bondesen, S. N. Rasmussen, J. Rask-Madsen, O. H. Nielsen, K. Lauritsen, V. Binder, S. H. Hansen, and E. F. Hvidberg. 5-Aminosalicylic acid in the treatment of inflammatory bowel disease. *Acta Med. Scand.* 221:227–242 (1987).
3. E. Brendel, I. Meineke, E. Stüwe, and H. Osterwald. Stability of 5-aminosalicylic acid and 5-acetylaminosalicylic acid in plasma. *J. Chromatogr. Biomed. Appl.* 432:358–362 (1988).
4. C. Fischer, K. Maier, and U. Klotz. Simplified high-performance liquid chromatographic method for 5-aminosalicylic acid in plasma and urine. *J. Chromatogr.* 225:498–503 (1981).
5. H. A. Montgomery, F. M. Smith, B. E. Scott, S. J. White, and K. B. Gerald. Stability of 5-aminosalicylic acid in plasma. *Am. J. Hosp. Pharm.* 43:118–120 (1986).
6. I. Cendrowska, M. Drewnowska, A. Grzeszkiewicz, and K. Butkiewicz. Investigation of the stability of 5-aminosalicylic acid in tablets and suppositories by high-performance liquid chromatography. *J. Chromatogr.* 509:195–199 (1990).
7. R. F. Rekker and W. T. Nauta. The U.V. absorption spectra of p-amino-salicylic acid and some related compounds. III. The decomposition of p-amino-salicylic acid in aqueous solutions. *Pharm. Weekbl.* 91:693–704 (1956).
8. S. G. Jivani and V. J. Stella. Mechanism of decarboxylation of *p*-aminosalicylic acid. *J. Pharm. Sci.* 74:1274–1282 (1985).
9. G. E. Dunn, E. G. Janzen, and W. Rodewald. Mechanism of decarboxylation of substituted salicylic acids. I. Kinetics in quinoline solution. *Can. J. Chem.* 46:2905–2909 (1968).
10. W. K. Snead and A. E. Remick. Studies on oxidation-reduction mechanism. II. The anodic oxidation of *p*-aminophenol. *J. Am. Chem. Soc.* 79:6121–6127 (1957).
11. T. Nomura. Indirect polarographic determination of microgram amounts of iron by means of the catalytic oxidation of 5-aminosalicylic acid. *J. Electroanal. Chem.* 124:213–219 (1981).
12. B. J. Dull, K. Salata, A. v. Langenhove, and P. Goldman. 5-Aminosalicylate: Oxidation by activated leukocytes and protection of cultured cells from oxidative damage. *Biochem. Pharmacol.* 36:2467–2472 (1987).
13. O. I. Aruoma, M. Wasil, B. Halliwell, B. M. Hoey, and J. Butler. The scavenging of oxidants by sulphasalazine and its metabolites. *Biochem. Pharmacol.* 36:3739–3742 (1987).
14. I. Ahnfelt-Rønne and O. H. Nielsen. The antiinflammatory moiety of sulfasalazine, 5-aminosalicylic acid, is a radical scavenger. *Agents Actions* 21:191–194 (1987).
15. H. Strøm and I. Ahnfelt-Rønne. Second-line anti-rheumatic drugs and free radical scavenging. *Agents Actions* 26:235–237 (1989).
16. J. R. A. Pollock and R. Stevens (eds.). *Dictionary of Organic*

Compounds, 4th ed., Eyre and Spottiswoode, London, 1965, Vol. 1, p. 141.
17. D. J. Miner and P. T. Kissinger. Evidence for the involvement of *N*-acetyl-*p*-quinoneimine in acetaminophen metabolism. *Biochem. Pharmacol.* **28**:3285–3290 (1979).
18. P. T. Kissinger and W. R. Heineman. *Laboratory Techniques in Electroanalytical Chemistry,* Marcel Dekker, New York, 1984.
19. R. A. Friedel and M. Orchin. *Ultraviolet Spectra of Aromatic Compounds,* John Wiley and Sons, New York, 1951.
20. H. v. Euler, H. Hasselquist, and Uno Lööv. Oxydation des 5-aminosalicylsäure-äthylesters. *Arkiv Kemi Mineral. Geol.* **25A**:No. 14 (1948).
21. P. V. Khadikar and P. S. Deshmukh. Protonation constants of salicylic substituted salicylic acids. *J. Ind. Chem. Soc.* **54**:101 (1977).
22. H. Allgayer, J. Sonnenbichler, W. Kruis, and G. Paumgartner. Determination of the pK values of 5-aminosalicylic acid and N-acetylaminosalicylic acid and comparison of the pH dependent lipid-water partition coefficients of sulphasalazine and its metabolites. *Drug Res.* **35**(II):1457–1459 (1985).
23. M. De Vos, H. Verdievel, R. Schoonjans, R. Beke, G. A. De Weerdt, and F. Barbier. High-performance liquid chromatographic assay for the determination of 5-aminosalicylic acid and acetyl-5-aminosalicyclic acid concentrations in endoscopic intestinal biopsy in humans. *J. Chromatogr. Biomed. Appl.* **564**:296–302 (1991).
24. E. J. D. Lee and S. B. Ang. Simple and sensitive high performance liquid chromatographic assay for 5-aminosalicylic acid and acetyl-5-aminosalicylic acid in serum. *J. Chromatogr. Biomed. Appl.* **413**:300–304 (1987).
25. E. Brendel, I. Meineke, D. Witsch, and M. Zschunke. Simultaneous determination of 5-aminosalicylic acid and 5-acetylaminosalicylic acid by high-performance liquid chromatography. *J. Chromatogr.* **385**:299–304 (1987).
26. S. H. Hansen. Assay of 5-aminosalicylate and its acetylated metabolite in biological fluids by high-performance liquid chromatography on dynamically modified silica. *J. Chromatogr. Biomed. Appl.* **226**:504–509 (1981).
27. E. Nagy, I. Csipo, I. Degrell, and G. Szabo. High-performance liquid chromatographic assay of 5-aminosalicylic acid and its acetylated metabolite using electrochemical detection. *J. Chromatogr. Biomed. Appl.* **425**:214–219 (1988).