predicts that the reaction is essentially (~99%) complete after 30 min at room temperature.

As shown in Fig. 7, 100:1 dilution of this reaction mixture with 10^{-4} N H_2SO_4 allowed direct plastic electrode determination of the alkylated amine without separation from the excess reactants since no interference from benzyl bromide or the piperidine base was seen.

CONCLUSION

Previous reports reviewed the many difficulties in handling and analyzing hydrophobic amines in biological samples, including glass adsorption, protein binding, and poor detector sensitivity (11–15). Coupled with ion-pair extraction, the plastic ion-selective electrode detection method described here offers potential advantages for efficient isolation and quantitation of hydrophobic drugs in whole blood at low levels. Furthermore, the derivatization procedure affords a means of enhancing plastic electrode detector sensitivity up to two orders of magnitude and may, therefore, extend the applicability of the method to other, less hydrophobic primary and secondary amines.

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Colorimetric Acetaminophen Determination in Pharmaceutical Formulations

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Abstract □ Different approaches for the colorimetric determination of acetaminophen, based on its coupling with diazotized *o*-nitroaniline, are described. Copper(II) chelation with the coupled compound makes the method highly selective. Sensitivity is increased when the acetaminophen assay is carried out indirectly through the determination of the chelate's copper content. Optimum conditions for performing the different approaches are described. The stoichiometric balance for the reactants in the coupled compound and chelate is determined. The degree of dissociation and the instability constant are computed. The rectilinear relationship between the absorbance of the different products and the concentration of acetaminophen allows for its determination in different pharmaceutical formulations. Compared with the official method, the proposed methods give more accurate results.

Keyphrases □ Acetaminophen—analysis, colorimetry, various pharmaceutical formulations □ Colorimetry—analysis, acetaminophen in various pharmaceutical formulations □ Analgesics—acetaminophen, analysis, colorimetry, various pharmaceutical formulations □ Antipyretics—acetaminophen, analysis, colorimetry, various pharmaceutical formulations

Acetaminophen hydrolysis with subsequent condensation with anisaldehyde (1), *p*-dimethylaminobenzaldehyde (2), *p*-dimethylaminocinnamaldehyde (3), or vanillin (4) or reaction with alkaline 2-naphthol (5) has been used for colorimetric acetaminophen determination in unit-dose formulations. Other methods were based on the reaction with phenol together with hypobromite (6), hypochlorite in the presence of metaarsenite (7), or ferricyanide (8). Nitrosatin (9) and nitration (10, 11) were utilized also for colorimetric determination. Most of these methods are not selective.

The fluorometric method, based on acetaminophen oxidation with alkali ferricyanide (12, 13) or on its hydrolysis followed by reaction with benzylamine (14), is sensitive to many interfering substances present in pharmaceutical formulations.

Acetaminophen was also determined by cerimetric titration (15, 16), gravimetry (17), titration of the hydrolytic product with nitrite (18), and polarography (19). These methods are not sufficiently sensitive.

Acetaminophen, containing the acetamido group $NHCOCH_3$, was determined by hydrolysis, diazotization

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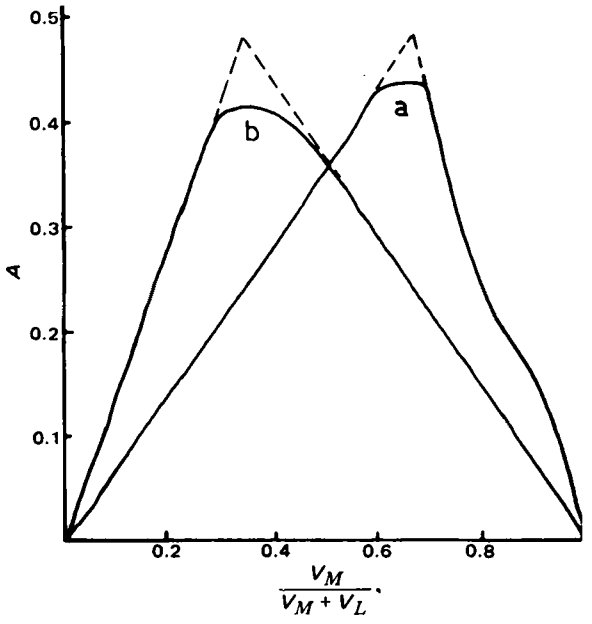


Figure 1—*Continuous variation plot for:* (a) *diazotized* o-*nitroaniline* (V_M)-*acetaminophen* (V_L) *coupled compound, and* (b) *2,6-di-(o-nitrophenylazo)-4-acetamidophenol* (V_L)-*copper(II) ion* (V_M).

of the resulting amine, and subsequent coupling with *N*-1-naphthylethylenediamine (20).

Acetaminophen as a phenolic compound can couple with a chromogenic reagent, diazotized *o*-nitroaniline. The coupled compound can be used as a chelating agent to copper(II) ion. These approaches constitute the bases for the acetaminophen assay described in this article.

EXPERIMENTAL

Apparatus, Reagents, and Materials—All reagents were analytical grade.

A photoelectric spectrophotometer[1] with 1-cm cells was used.

Acetaminophen[2] (BP 1973), acetaminophen tablets[3], acetaminophen syrup[4], and acetaminophen drops[5] were used.

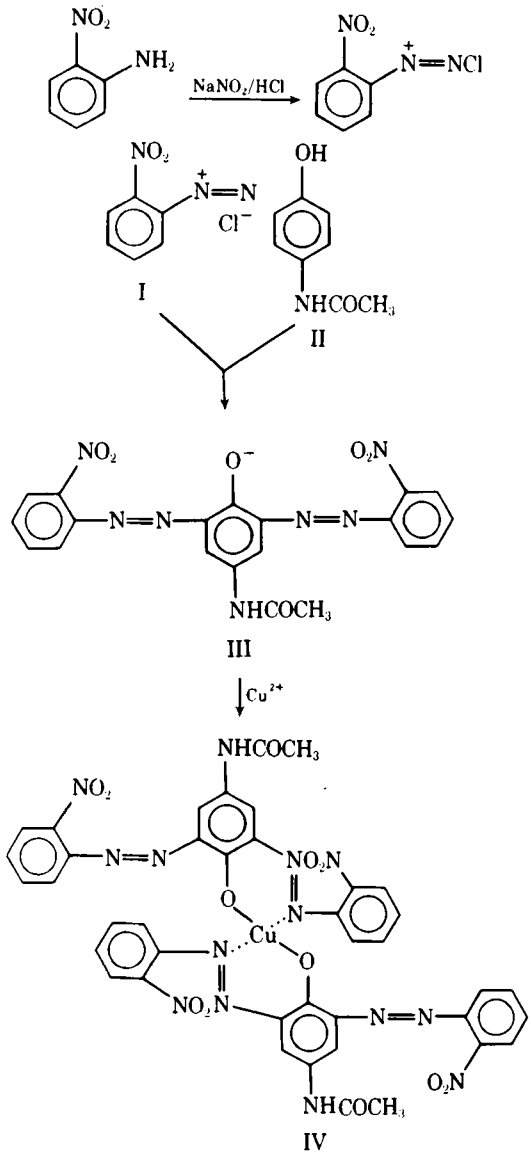
Preparation of Assay Solution—*Tablets*—Twenty tablets were powdered and mixed; an accurately weighed amount, equivalent to one tablet, was transferred to a 100-ml graduated flask. The sample was dissolved as completely as possible in water, diluted to volume, and filtered. An aliquot of filtrate was removed, after the first portion was discarded, measured, suitably diluted, and treated as described for Procedure A, B, or C.

Syrups or Drops—An aliquot was diluted with water to attain a concentration suitable for Procedure A, B, or C.

Procedure A—*Coupling with Diazotized Nitroaniline*—One milliliter of *o*-nitroaniline solution (1 mg/ml of 1.5 *N* HCl) was transferred to a 25-ml graduated flask to which 2 ml of sodium nitrite solution (3% aqueous) had been added. The contents were mixed and left for 10 min. An aliquot of acetaminophen solution (0.4 mg in water) was added, followed by 3 ml of 1 *N* NaOH. The contents were mixed, left for 5 min, and diluted to volume with water. The absorbance was measured at 426 nm against a blank.

Stoichiometric Reactant Balance—Using Procedure A, 0.5–4.5 ml of 2×10^{-3} *M* (V_M) samples of *o*-nitroaniline were diazotized and coupled with the corresponding complementary volume of 2×10^{-3} *M* acetaminophen (V_L) to make a total 5-ml volume for $V_M + V_L$.

Procedure B—*Subsequent Chelation with Copper(II) Ion*—*o*-Nitroaniline (1 ml) and sodium nitrite solution (2 ml) were transferred to a 100-ml separator. The contents were mixed and left to stand for 10 min.



Scheme I

Acetaminophen solution (0.3 mg/5.0 ml of water) was added followed by 3 ml of 1 *N* NaOH. The contents were mixed and left to stand for 5 min. A copper(II) sulfate solution (5 ml, 0.125 *M*) was added followed by 6 ml of 1 *N* H_2SO_4. The mixture was extracted three times with a total of 25 ml of chloroform. The absorbance was measured at 400 nm against a blank.

Stoichiometric Balance—Procedure B was used on 0.5–4.5 ml of 2×10^{-3} *M* acetaminophen (V_L) previously coupled with diazotized *o*-nitroaniline and treated with the corresponding complementary volume of copper(II) sulfate (V_M) to give a total volume of 5 ml for V_M and V_L in each case.

Procedure C: Indirect Determination—The copper(II) chelate chloroform extract (5.0 ml) obtained by applying Procedure B was transferred to a 10-ml graduated flask. The volume was completed with 5.0 ml of sodium diethyldithiocarbamate solution (0.5 g % in ethanol). The contents were mixed, and the absorbance was measured at 442 nm against a blank.

RESULTS

Coupling with Diazotized *o*-Nitroaniline—The phenolic hydroxyl group *para* to the acetamido group ($NHCOCH_3$) in acetaminophen causes coupling with diazotized *o*-nitroaniline to occur *ortho* to the hydroxyl group. To investigate the molecular reactant ratio, Job's method of continuous variation (21) was employed (Fig. 1*a*). The maximum absorbance was attained when $V_M/(V_L + V_M)$ equaled 0.66. This finding indicated that the diazonium salt-acetaminophen molecular ratio was

[1] Aprolabo.
[2] Alexandria Co. for Pharmaceutical and Chemical Industries, Egypt.
[3] Paracetamol, Misr Co. for Pharmaceutical Industries, Cairo, Egypt.
[4] Paracetamol, Misr Co. for Pharmaceutical Industries; and pyral, Elkahira Pharmaceutical and Chemical Industries Co., Cairo, Egypt.
[5] Pyral, Elkahira Pharmaceutical and Chemical Industries Co., Cairo, Egypt.

Table I—Acetaminophen in Different Pharmaceutical Formulations

Pharmaceutical Formulation	Mean Percentage ± *SD*			
	Official Method (n = 12)[a]	Procedure A (n = 14)	Procedure B (n = 16)	Procedure C (n = 14)
Laboratory-made tablet	—	100.24 ± 0.28	100.35 ± 0.51	100.37 ± 0.67
Acetaminophen tablets	101.02 ± 0.8	99.56 ± 0.41 (5.99)[b]	99.60 ± 0.60 (5.38)	99.54 ± 0.45 (5.93)
Acetaminophen syrup	101.75 ± 1.09	103.71 ± 0.29 (3.44)	103.77 ± 51.0 (3.18)	103.46 ± 0.39 (4.14)
Acetaminophen syrup	107.53 ± 0.73	105.38 ± 0.24 (10.41)	105.25 ± 0.44 (10.28)	105.57 ± 0.38 (8.77)
Acetaminophen drops	102.32 ± 0.20	101.36 ± 0.32 (8.98)	101.72 ± 0.37 (5.07)	101.57 ± 0.31 (7.19)

[a] n = number of experiments. [b] Figures in parentheses are the calculated values for which theoretical value $t_{0.975}$ equals 2.06.

2:1, as was anticipated due to the two free positions *ortho* to the acetaminophen hydroxyl when the diazonium salt coupling was assumed to occur (Compound III, Scheme I).

The coupled product stability was attained by: (*a*) keeping the *o*-nitroaniline with nitrous acid under the conditions previously mentioned for 10 min; (*b*) making the medium alkaline after addition of acetaminophen since the alkaline medium was essential for the coupling reaction, and (*c*) leaving the coupled product for 5 min before dilution with water. For alkalinization, 3 ml of 1 *N* NaOH was the optimum; a lower concentration was not sufficient for the full color development, and a higher concentration caused azo dye precipitation.

The colored azo dye (λ_{max} 426 nm) was stable for 6 hr. The dissociation degree, α, and the instability constant, K_i (22), equaled 0.0952 and 6.1105 × 10^{-6}, respectively.

Coupling and Subsequent Chelation—The investigation was extended to utilize the coupled compound as a chelating agent for copper(II) ion since the former has a free hydroxyl group with an easily replaceable proton and a diazo group (—N=N—) that offers a lone electron pair. The copper (II) chelate was hardly soluble in water but was easily extracted with chloroform to give a yellow solution (λ_{max} 400 nm).

The stoichiometric balance (Fig. 1*b*) determined by Job's method of continuous variation equaled 2:1 [2,6-di-(*o*-nitrophenylazo)-4-acetamidophenol–copper(II) ion]. The copper(II) ion satisfied its four coordination valency by combination with two free acetaminophen hydroxyl groups and with two diazo groups (Compound IV, Scheme I). The chelate was stable in chloroform for at least 4 hr after extraction. The dissociation degree, α, and the instability constant, K_i, for the chelate equaled 0.1375 and 19.2899 × 10^{-6}, respectively.

An acidic medium favored copper(II) chelate formation. Addition of 6 ml of 1 *N* H_2SO_4 ensured maximum quantitative chelate extraction. More important, the order of reagent addition (copper sulfate followed by sulfuric acid) was essential since addition of acid before copper(II) sulfate destroyed the azo dye.

Linearity of Beer's Plots—Standard curves for the azo dye and its copper(II) chelate were constructed by plotting observed absorbance readings *versus* the acetaminophen concentration treated similarly as described for Procedures A and B. Such a relationship followed Beer's law within a concentration range of 0.4–2.8 mg % (Procedure A) or 0.4–4 mg (Procedure B) of acetaminophen calculated in the final dilution. The regression equations, derived by the least-squares method (23), for the calibration curves were:

$$A_{426} = -0.0020 + 0.2503C \quad \text{(Procedure A)} \qquad \text{(Eq. 1)}$$

$$A_{400} = -0.0025 + 0.1216C \quad \text{(Procedure B)} \qquad \text{(Eq. 2)}$$

for which the percentage fits (24) were 99.97 and 99.91, respectively.

The validity of these equations was tested by analyzing known acetaminophen amounts mixed with common tablet excipients or additives in a ratio approximating sample commercial tablet weights. The results (Table I) were highly accurate, indicating no interference from tablet base ingredients.

Indirect Method—In spite of the high copper(II) chelate stability in chloroform, addition of 5 ml of diethyldithiocarbamate (0.5 g % ethanolic solution) displaced 2,6-di-(*o*-nitrophenylazo)-4-acetamidophenol to give copper(II) diethyldithiocarbamate, a complex of λ_{max} 442 nm. The absorbance was proportional to the acetaminophen concentration in the 0.2–2 mg % range. The calibration curve was described by the following regression equation:

$$A_{442} = -0.0047 + 0.3081C \quad \text{(Procedure C)} \qquad \text{(Eq. 3)}$$

for which the percentage fits equaled 99.59.

DISCUSSION

Acetaminophen coupling with diazotized *o*-nitroaniline had an advantage over the method involving acetaminophen hydrolysis with subsequent diazotization and coupling (20, 21). In the latter technique, any hydrolytic product present in pharmaceutical formulations during unsuitable storage interfered during the analysis. Any phenolic compound could interfere with Procedure A. To increase the method selectivity, coupling with subsequent chelation was proposed. The common analgesic–antipyretics likely to be present with acetaminophen exhibited no interference. Aspirin, codeine, caffeine, and salicylamide did not interfere[6].

The slope of the calibration curve for Procedure C was 1.23- or 2.53-fold higher than that for Procedure A or B, respectively. This finding indicated the high indirect method sensitivity.

BP 1973 (25) described a UV assay for acetaminophen in which diluents and binders in tablets or sweetening agents, preservatives, and coloring matter in syrups interfere during analysis. Therefore, it is not surprising that the proposed procedures give more accurate results than the official method since t-calculated exceeds t-theoretical (Table I).

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Pharmacokinetics of Morphine and Its Surrogates III: Morphine and Morphine 3-Monoglucuronide Pharmacokinetics in the Dog as a Function of Dose

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Abstract □ The pharmacokinetics of morphine and its derived metabolite, morphine 3-monoglucuronide, were studied in normal and bile-cannulated dogs. High doses (7.2–7.7 mg/kg iv) caused renal and biliary shutdowns and time lags in urinary drug and metabolite excretion and in biliary secretion of the hepatically formed conjugate. Intermediate doses (0.41–0.47 mg/kg iv) inhibited urine flow but not renal clearance. Low doses (0.019–0.07 mg/kg iv) had no apparent effect. Dose-related effects on the total, metabolic, and biliary clearances imply saturable enzymes and/or dose-inhibited hepatic flows, accounting for the major elimination half-lives of 83 ± 8 and 37 ± 13 min at the high and low doses, respectively. The slow terminal phase in plasma morphine and metabolite elimination and urinary accumulation is due apparently to the enterohepatic metabolite recirculation after biliary excretion, gastrointestinal hydrolysis, and hepatic first-pass reconjugation. Bile-cannulated dogs showed no fecal drug and no slow terminal plasma and urine elimination phases. Intravenous morphine 3-monoglucuronide was eliminated only renally and showed neither biliary excretion nor prolonged hepatically formed glucuronide elimination. Hepatic morphine clearances at normal therapeutic doses parallel hepatic blood flow and explain the lack of oral morphine bioavailability by anticipating complete first-pass liver metabolism. Renal morphine and morphine conjugate clearances were 85 (± 9 *SEM*) and 41 (± 4 *SEM*) ml/min, respectively, indicating glomerular filtration for the latter and glomerular filtration plus tubular secretion for the former. Urinary morphine and morphine conjugate excretion accounted for ~83% of the dose. Biliary secretion accounted for 11–14% of the dose. Morphine showed dose-independent plasma protein binding of 36 (± 1 *SEM*) % and a red cell–plasma water partition coefficient of 1.11 ± 0.04 *SD*. New equations were developed to model the discontinuous morphine and morphine metabolite pharmacokinetics.

Keyphrases □ Morphine—pharmacokinetics, dose related, dogs □ Morphine monoglucuronide—pharmacokinetics, dose related, dogs □ Pharmacokinetics—morphine, morphine monoglucuronide, dose related, dogs

Early studies on the distribution and disposition of morphine were largely descriptive (1–6). Rigorous pharmacokinetic studies were limited and usually only related to the parent compound at high doses. Early time course studies (7–10) in the dog were conducted at large time intervals and at high 30-mg/kg levels (human therapeutic dose is 0.14 mg/kg) due to color complex assay sensitivity limitations (10). Early morphine studies (9) estimated that 78–97% of the administered morphine was recoverable in urinary and fecal excretions, 14% as free morphine and 55% as presumed glucuronide conjugate in the urine, and 6–26% in the feces with less than 2% of the total drug as the conjugate. The plasma [*N*-^{14}C-*methyl*]morphine half-life was estimated at ~1 hr (11).

BACKGROUND

A fluorometric method with a sensitivity of 1 μg/ml of plasma was used by Kupferberg *et al.* (12) in a single rabbit study of six time samples at 15 mg/kg to demonstrate apparent biphasic morphine decay in plasma with a terminal half-life that could be roughly estimated as 1 hr.

Only in the last decade has the morphine time course been monitored with sufficient attention to warrant any pharmacokinetic data analysis.

A highly sensitive GLC method was used to monitor the time course of morphine at 2.5 mg/kg iv in the rat (13). At least three exponentials were needed to fit the plasma level–time curve.

Radioimmunoassay was used to monitor morphine, 0.14 mg/kg iv, in human plasma (14, 15), and the results indicated an apparent triphasic loss that included a rapid initial decline during the first 5–10 min, a subsequent slower but precipitous decline with a half-life of 1.0–3.1 hr, and a terminal slow disappearance of 10–44 hr at plasma levels of <10 ng/ml. A separate study (16) at 1 mg/kg infused over 12 min could only demonstrate a two-compartment body model when conducted for 2 hr after the completion of the infusion. Total clearance was 378 ± 63 ml/min. The apparent half-lives were similar to those of the previous studies; mean values were 1 ± 1 and 137 ± 14 min, with the latter phase ranging from 1.3 to 3.5 hr. These half-lives were similar to those for the lower 0.14-mg/kg dose and suggested that the assumption of a saturable or capacity-limited process for morphine was not warranted. Since different subjects were used for the different doses, this concept cannot be rigorously concluded. Mean apparent volumes of distribution of the central compartment and the total apparent distribution space were 9 and 102% of body weight, respectively.

Radiolabeled morphine at 0.14 mg/kg in humans (17, 18) showed an initially fast distribution phase and an apparent terminal half-life of 2.1–2.6 hr. The sensitivity of 20–30 ng/ml of plasma may not have permitted the observation of a slow terminal phase. Conjugate quickly appeared in the plasma close to its maximum value at ~40 min. Cumulative urinary excretion of free morphine and of the conjugate was 8.5–9.3 and 66–70% of the dose, respectively. The renal drug and metabolite clearances were given as 109–171 and 50–115 ml/min, respectively. The qualitative formation of *N*-demethylated morphine was observed by following the time course of expired $^{14}CO_2$.

Catlin (19) investigated morphine pharmacokinetics in the rabbit by morphine radioimmunoassay methods with the intent of confirming the presence of multiphasic pharmacokinetics as indicated by previous studies that apparently demonstrated a terminal half-life of many hours or days. He clearly demonstrated that a two- or three-compartment body model in the rabbit could be observed, dependent on antiserum specificity and on possible interaction with metabolites. He stated that he would reject the third compartment for the rabbit and retain only two half-lives of 13 and 72 min. No pharmacokinetic dose dependency was observed between the 1- and 10-mg/kg doses. Catlin (19) issued the caveat for the previously cited radioimmunoassay studies in humans (14, 15), where an apparently long terminal half-life was observed, that "providing the antiserum used . . . did not seriously cross-react with morphine metabolites, it appears that in man the two compartment model is not sufficient

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