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DETERMINATION OF *p*-AMINOPHENOL IN THE PRESENCE OF PARACETAMOL IN PHARMACEUTICAL PREPARATIONS

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ÖZET

FARMASOTİK PREPARATLARDA, PARASETAMOL YANINDA *p*-AMİNOFENOL TAYİNİ

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Yüksek Lisans Tezi, Kimya Anabilim Dalı
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Bu tezde *p*-aminofenol (PAP) ve parasetamolün tayini spektrofotometrik bir yöntemden yararlanılarak gerçekleştirilmiştir. Yöntem, oda sıcaklığında PAP'ün bir yükseltgen varlığında sodyum sülfür (Na_2S) ile tepkimesi sonucu oluşan metilen mavisi benzeri boyar maddenin 550 nm deki soğurmasının ölçümüne dayanmaktadır.

İlk bölümde, oda sıcaklığında, sulu çözeltideki ve etanol çözeltisindeki PAP'ün aydınlık/karanlık ve azotlu/azotsuz (O_2'li/O_2'siz) ortamlardaki kararlılığı spektrofotometrik davranışı gözlemlenerek incelendi. Çeşitli koşullarda, PAP'ün ethanol çözeltisinin sulu çözeltisinden daha kararlı olduğu ve PAP'ün 70 saatin üzerinde kararlı kalmasından dolayı, en kararlı durumun karanlıkta azotlu (O_2'siz) ortamda elde edildiği görüldü.

Çalışmalarda yükseltgen olarak Ce(IV) ve Fe(III) kullanıldı. Reaktif katım sırasının her iki yükseltgen için de önemli olduğu görüldü. Optimum koşullarının saptanması için renkli bileşiğin soğurma şiddetine, H_2SO_4, Na_2S ve yükseltgen derişimlerinin ve zamanın etkileri incelendi. Optimum koşullar ve renkli bileşiğin kararlı olduğu zaman aralığı, Ce(IV) ve Fe(III) için belirlendi. Optimum koşullar Ce(IV) için; $4{,}2\times10^{-4}$ M Ce(IV), $6{,}4\times10^{-5}$ M Na_2S, ~0,03 M H_2SO_4 ve Fe(III) için; $1{,}5\times10^{-3}$ M Fe(III), $2{,}57\times10^{-4}$ M Na_2S, 0,01 M H_2SO_4 olarak bulundu. Tepkime ürününün Ce(IV) için 20. dakikadan sonra kısa süreliğine Fe(III) için ~25. dakikadan sonra en az 2 saat kararlı kaldığı görüldü. Optimum koşullarda hazırlanan standart PAP çözeltileri ile kalibrasyon grafikleri oluşturuldu. PAP

tayini için uygun doğrusal aralıklar, kalibrasyon grafiklerinden Ce(IV) için 2-14 ppm ve Fe(III) için 2-22 ppm olarak bulundu. Sonuç olarak, renkli bileşiğin oluşturulduğu ve yükseltgen olarak Fe(III) ve Ce(IV)'ün kullanıldığı optimum koşullardaki denemelere göre, Fe(III) ile daha yüksek absorbans değerlerinin elde edildiği görüldü. Ayrıca, Fe(III) çalışmalarındaki PAP tayin aralığının daha geniş olduğu görüldü. Bu nedenle daha sonraki çalışmalarda yükseltgen olarak Ce(IV) yerine Fe(III) kullanıldı.

Na_2S yerine CH_3CSNH_2(tiyoasetamid) kullanılarak çeşitli denemeler gerçekleştirildi. Renkli bileşiğe ait düşük absorbans değerleri elde edildi. Bu nedenle, Na_2S yerine tiyoasetamid kullanılmasının uygun olmadığı görüldü.

Deneme, parasetamol hidrolizlenmeden, ilaç tabletinin PAP içeriğini tayin etmek için gerçekleştirildi. Fakat tepkime ürününün oluşumu gözlenmedi. Aynı miktarda PAP içeren örneklere artan miktarlarda parasetamol eklenerek denemeler yapıldı. Renkli tepkime bileşiğinin absorbans değerlerinin değişmediği saptandı. Bu nedenle belirlenen konsantrasyon aralığında parasetamol yanında PAP tayininin yapılabileceği görüldü. Dolaylı parasetamol tayini için kalibrasyon grafiği hazırlandı. Yöntem, 500 mg parasetamol/tablet içeren ilaç tabletlerine uygulandı ve ilacın 575.50±15.00 mg parasetamol/tablet içerdiği bulundu.

Anahtar Sözcükler: *p*-Aminofenol, parasetamol, farmasotik analiz, Spektrofotometri

ABSTRACT

DETERMINATION OF *p*-AMINOPHENOL IN THE PRESENCE OF PARACETAMOL IN PHARMACEUTICAL PREPARATIONS

Burcu TANRIKULU DEMİRCİ

Msc Thesis in Chemistry
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In this thesis, the determination of *p*-aminophenol (PAP) and paracetamol have been carried out by the help of a spectrophotometric method. The method is based on the measurement of the absorbance of methylene blue-like dye at 550 nm which produces by the reaction of PAP at room temperature with sodium sulphide (Na_2S) in the presence of an oxidant.

In the first phase, the stability of PAP was studied by observing the spectrophotometric behaviour of PAP at room temperature in the aqueous and ethanol solution of it in the light/dark and in the medium with/without nitrogen (with O_2/without O_2). It was seen that the ethanol solution of PAP is more stable than the aqueous solution of PAP in various conditions and the most stable condition was obtained in the medium with nitrogen (without O_2) in the dark because of the fact that PAP remained stable over 70 h.

Ce(IV) and Fe(III) were used as the oxidants in the studies. It was seen that the order of reagent addition was important for both oxidants. The effect of H_2SO_4, sodium sulfide and oxidant concentration and the effect of time on the absorption intensity of the coloured product were investigated in order to determine optimum conditions. The optimum conditions and the time range that the coloured product is stable were determined for Ce(IV) and Fe(III). The optimum conditions for Ce(IV) were found as follows: $4{,}2 \times 10^{-4}$ M Ce(IV), $6{,}4 \times 10^{-5}$ M Na_2S, ~0,03 M H_2SO_4. The optimum conditions for Fe(III) were found as follows: $1{,}5 \times 10^{-3}$ M Fe(III), $2{,}57 \times 10^{-4}$ M Na_2S, 0,01 M H_2SO_4. The reaction

product was seen to remain stable after 20 min for a short time and after ~25 min for at least 2 h for Ce(IV) and Fe(III), respectively. The calibration graphs were prepared with the standart PAP solutions which were prepared in optimum conditions. The appropriate linear ranges for the determination of PAP were found from the calibration graphs as 2-14 ppm and 2-22 ppm for Ce(IV) and Fe(III), respectively. As a result, according to the studies which the coloured product was formed and Ce(IV) and Fe(III) were used as the oxidants, it was seen that the higher absorption values were obtained with Fe(III). Besides, it was seen that the determination range of PAP in Fe(III) studies was more wide. For this reason, Fe(III) was used instead of Ce(IV) as an oxidant in the following studies.

Various studies were carried out by using CH_3CSNH_2(thioasetamid) instead of Na_2S. The low absorption values which belong to coloured product were obtained. For this reason, it was seen that using thioacetamid instead of Na_2S was not available.

The study was carried out for determining the tablet's PAP content without hydrolysing paracetamol. But the formation of the reaction product wasn't observed. The studies were carried out by adding increasing amount of paracetamol to the samples including same amount of PAP. It was determined that the absorbance values of the coloured reaction product didn't change. For this reason, it was seen that the determination of PAP in the presence of paracetamol could be done in the determined concentration range. The calibration graph was prepared for the indirect determination of paracetamol. The method was applied to the drug tablets containing 500 mg paracetamol/tablet and it was found that the drug included 575.50±15.00 mg paracetamol/tablet.

Keywords: *p*-Aminophenol, paracetamol, pharmaceutical analysis, spectrophotometry

TEŞEKKÜR

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1. INTRODUCTION

1.1 *p*-Aminophenol

p-Aminophenol is 4-aminophenol and may be represented by the following formula:

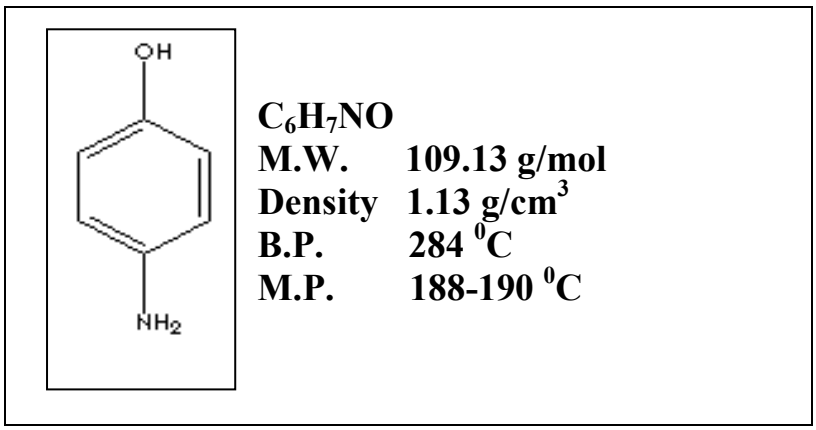

Figure 1.1 The structure of *p*-aminophenol.

In some publications, it is described as 4-hydroxyaniline or 4-amino-1-hydroxybenzene.

p-Aminophenol is the hydrolytic product of paracetamol.

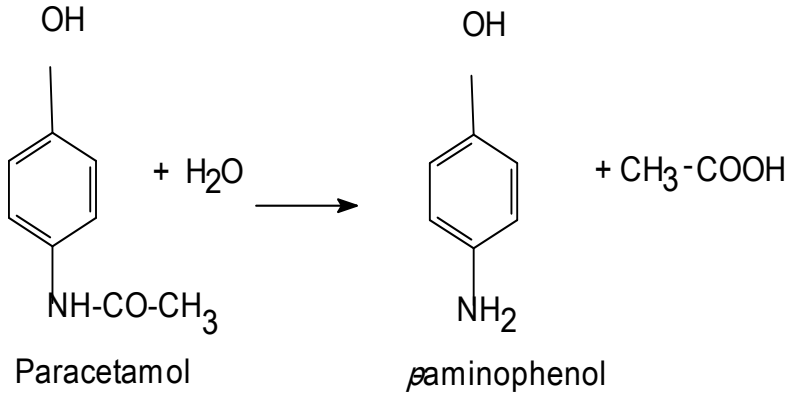

Figure 1.2 The hydrolysis reaction of paracetamol.

Solubility in water; 0.39% at 13^0C, 0.65% at 24^0C, 0.80% at 30^0C. Solubility in ethyl methyl ketone; 9.3% at 58.5^0C and solubility in absolute ethanol; 4.5% at 0^0C. It is very slightly soluble in benzene and chloroform. It may discolor on exposure to light and air. Because of that, it must be kept in dark and at 4^0C.

p-Aminophenol is the organic compound. Typically available as a white powder, it is commonly used as a developer in black and white film, marketed under the name Rodinal. Reflecting its slight hydrophilic character, the white powder is moderately soluble in alcohols and can be recrystallised from hot water. In the presence of base, it oxidizes readily. N-methyl and N,N-dimethyl derivatives are of commercial value. The compound is one of three isomeric aminophenols, the other two being 3-aminophenol and 2-aminophenol. It is produced from phenol by nitration followed by reduction with iron. Alternatively, the partial hydrogenation of nitrobenzene affords phenylhydroxylamine, which rearranges primarily to p-aminophenol:

$$C_6H_5NO_2 + 2H_2 \longrightarrow C_6H_5NHOH + H_2O$$
$$C_6H_5NHOH \longrightarrow HOC_6H_4NH_2$$

p-Aminophenol may be reacted with acetic anhydride to give paracetamol.

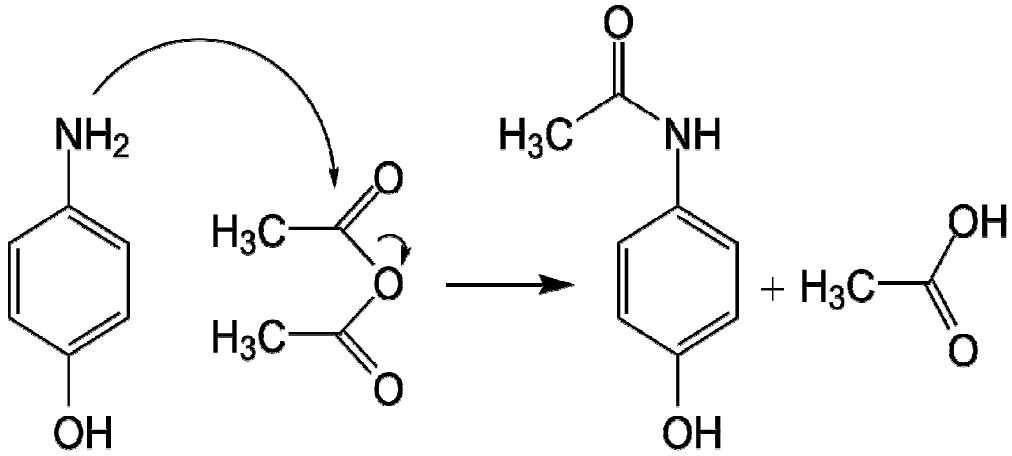

Figure 1.3 The reaction of PAP with acetic anhydride to give paracetamol.

(Wikipedia The Free Encyclopedia, 2009)

1.2 Facts About *p*-Aminophenol

p-Aminophenol has been widely used as raw chemical material and important intermediate in various fields, such as medicine, sulfur and azo dyes, rubber, feeding-stuff, petroluem and photoraphy, etc. As a result, large amounts of PAP may enter the environment as a pollutant. Moreover, it is a parent material for the production of paracetamol as one of the most produced pharmaceuticals worldwide. In addition, it is a harmful substance, because it increases the body temperature in humans, and its biological half-life (i.e. the time required for half of PAP to be removed from the organism by biophysical and biochemical processes) is long. It appears in environment mainly from the chemical and pharmaceutical industrial wastes (water and powdered pollutant emission). That is, PAP shows both biochemical and environmental hazards.

p-Aminophenol is also a degradation product of paracetamol; it is reported to have significant nephrotoxic and teretogenic effects, therefore its amount should be strictly controlled. It is limited to a low level of 0.005% in the drug substance by the European Pharmacopoeia (Ph. Eur.). The limits for PAP may vary in different products depending on the dosage form and formulation; the monograph of paracetamol tablets in BP (British Pharmacopoeia) allows 0.1%.

1.3 Paracetamol

Paracetamol (acetaminophen or N-acetyl-para-aminophenol) is an acylated aromatic amide, which was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893 and has been in use as an analgesic for home medication for over 30 years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin. It is a major ingredient in numerous cold and flu medications and many prescription analgesics. It is remarkably safe in standart doses, but, because of its wide availability, deliberate or accidental overdoses are not uncommon. Paracetamol, unlike other common analgesics such as aspirin and ibuprofen, has no anti-inflammatory properties, and so it is not a member of the class of drugs

known as non-steroidal anti-inflammatory drugs or NSAIDs. In normal doses, paracetamol does not irritate the lining of the stomach or affect blood coagulation, the kidneys, or the fetal ductus arterious. Like NSAIDs and unlike opioid analgesics, paracetamol has not been found to cause euphoria or alter mood in any way. Paracetamol ve NSAIDs have the benefit of bearing no risk of addiction, dependence, tolerance and withdrawal. Data about chemical, physical and biopharmaceutical properties are easily available.

Recent studies have shown that paracetamol is associated to hepatic toxicity and renal failure despite of its apparent innocuous character. Hepatic toxicity begins with plasma levels of paracetamol in the 120 μg mL^{-1} range 4 h after the ingestion and an acute damage is presented with plasmatic levels up to 200 μg mL^{-1} 4 h after the ingestion.

At normal therapeutic doses, paracetamol is metabolised very fast and completely by undergoing glucuronidation and sulphation to inactive metabolites that are eliminated in the urine. However, paracetamol higher doses produce toxic metabolite accumulation that causes hepatocyte death. Paracetamol overdose is a frequent cause of fulminating hepatic failure in Europe and US. (Bosch et al., 2006)

1.4 Determination Methods of *p*-Aminophenol and Paracetamol

Because of hazardous effects of *p*-aminophenol, establishment of a simple, economical, and accurate analytical method for the simultaneous determination of *p*-aminophenol and paracetamol would be useful for investigation of the stability of paracetamol, for pharmaceutical analysis, and for quality control of paracetamol containing products. Thus, a variety of methods are described in the literature. Spectrophotometry, liquid chromatography, conventional capillary electrophoresis(CE), enzyme-based assays methods, flow injection analysis (FIA), and electrochemistry, have been widely employed for the determination of paracetamol and *p*-aminophenol in body fluids and pharmaceutical preparations. Many of these prosedures suffer from interferences from other active ingredients

or additives especially those carrying phenolic and amine functional groups, and chromatographic seperation is usually required.

1.4.1 Optical methods

1.4.1.1 Spectrophotometric methods

A specific spectrophotometric method was developed for the determination of *p*-aminophenol and acetaminophen. The method is based on the reaction of *p*-aminophenol at ambient temperature with sodium sulphide in presence of an oxidant to produce a methylene blue-like dye. Different oxidizing agents were tried, e.g. Ce(IV) and Fe(III). The colour developed within 10 min and remained stable for at least 3 h. The method was applied successfully to the determination of *p*-aminophenol in the presence of acetaminophen without prior separation. The method was also applied to the analysis of various commercially available acetaminophen dosage forms and excellent recoveries were obtained comparable to those obtained by official procedures. The reaction product was isolated and a possible reaction mechanism was suggested.

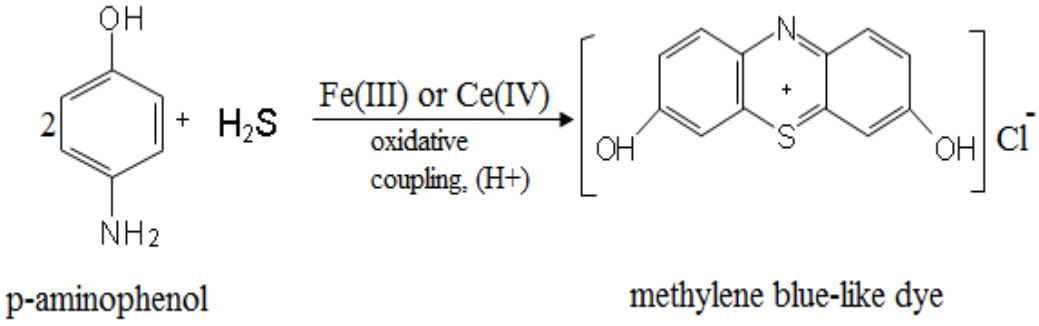

Figure 1.4 Suggested mechanism for the reaction *p*-aminophenol with Na_2S and Fe(III) or Ce(IV) to form a methylene blue-like dye.

As a result of this investigation, a rapid, sensitive and selective spectrophotometric method for the determination of paracetamol and *p*-aminophenol was developed. Advantages are that the reaction occurs at room temperature and using distilled water as a solvent. The absorption spectra of the

coloured reaction product (λ_{max}=550 nm) and pure *p*-aminophenol are shown in Figure 1.5.

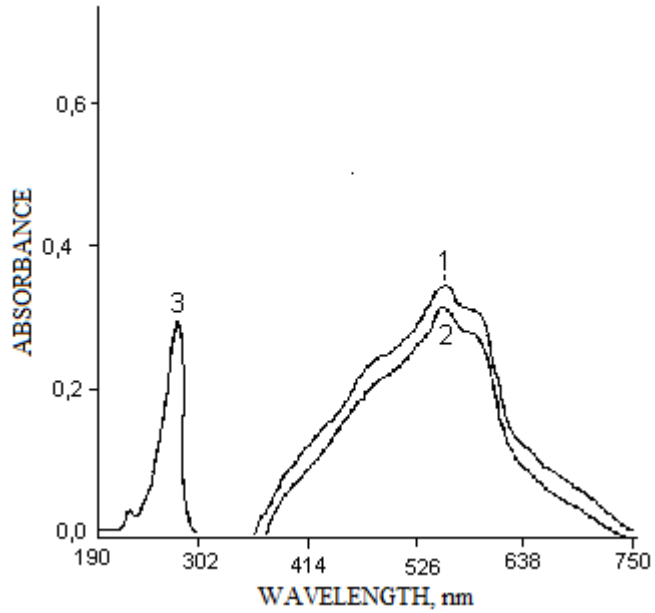

Figure 1.5 Absorption spectra for the reaction product of 10µg mL^{-1} *p*-aminophenol with (1) Fe(III) and (2) Ce(IV) and Na_2S reagents and (3) for the pure compound.

The effect of changing the concentrations of the reagents on the absorbances of solutions containing 10 µg mL^{-1} *p*-aminophenol was studied. The optimum conditions were obtained for the sodium sulphide (0.025% (w/v)), sulphuric acid (0.03 M) and oxidant (0.5% (w/v) Fe(III) or 0.08% (w/v) Ce(IV) dissolved in 0.03 M sulphuric acid) concentrations. And the order of reagent addition was determined. It was seen that the sulphide solution has to be added to the sample and finally the oxidant. Changing the order produced low results. After dilution of the coloured solutions with distilled water, a gradual increase in absorption intensity was observed throughout the first 10 min and it then remained constant for more than 3 h. Therefore, absorption measurements were carried out 10 min after dilution with distilled water. (Mohamed et al., 1997)

A rapid spectrophotometric method for the determination of paracetamol is proposed in the study. The proposed method is based on the microwave assisted alkaline hydrolysis of paracetamol to *p*-aminophenol that reacts with S^{-2} in the presence of Fe^{3+} as oxidant to produce a methylene blue-like dye having an

absorptivity maximum at 540 nm. The experiment showed that paracetamol could be hydrolysed quantitatively to *p*-aminophenol in only 1.5 min under radiation power 640W using a microwave in NaOH medium. The system obeys Beer's law in the range of 0-3.0x10^{-4} mol L^{-1} paracetamol. The method has been applied successfully to analysis of paracetamol in pharmaceutical preparation. (Xu et al., 2004)

A method to determine paracetamol and *p*-aminophenol in pharmaceutical products is presented. The purpose of the study is to introduce 2,2'-(1,4-phenylenedivinylene) bis-8-hydroxyquinoline (PBHQ) as a novel coupling agent. This developed method is based on the microwave assisted alkaline hydrolysis of paracetamol to *p*-aminophenol, which reacts in mildly alkaline medium with 2,2-(1,4-phenylenedivinylene)bis-8-hydroxyquinoline (PBHQ) as a novel coupling reagent. The formed product shows maximum absorbance at 650 nm with molar absorptivity= 3.4×10^{4} L mol^{-1} cm^{-1} and the method was linear in the 0.44–5.5 mg L^{-1} concentration range. The proposed method is successfully adapted for the determination of paracetamol and *p*-aminophenol in various pharmaceutical preparations. (Filik et al., 2005)

2,2'-(1,4-Phenylenedivinylene)bis-8-hydroxyquinoline (PBHQ), a highly sensitive reagent used for the colorimetric determination of *p*-aminophenol (PAP), was successfully immobilised on XAD-7 and coupled with optical fibres to investigate a sensor-based approach for determining *p*-aminophenol. The solid-state sensor is based on the reaction of PAP with PBHQ in the presence of an oxidant to produce an indophenol dye.The reflectance measurements were carried out at a wavelength of 647 nm since it yielded the largest divergence different in reflectance spectra before and after reaction with the analyte. As this PAP sensor is irreversible, a fresh sensor has to be used for each measurement. Using the optical sensing probe, PAP in pharmaceutical wastewater and paracetamol was determined. (Filik et al., 2008)

A fibre-optic sensor for *p*-aminophenol (PAP) based on the use of 25,26,27,28-tetrahydroxycalix[4]arene (CAL4) immobilised onto Amberlite XAD-16 and reflectance spectrometry has been developed. The sensor is based on

the reaction of PAP with CAL4 in presence of an oxidant to produce an indophenol dye. The reflectance measurements were carried out at a wavelength of 620nm since it yielded the largest divergence different in reflectance spectra before and after reaction with the analyte. Using the optical sensing probe, PAP in urine, pharmaceutical and pharmaceutical wastewater was determined. Although aminophenols showed serious interference, the method was not affected from common ions and from various important drug active constituents present in urine and pharmaceutical effluents such as acetaminophen, acetylsalicylic acid, sorbitol, and caffeine. (Filik et al.,2009)

The method presents a highly sensitive, precise and automated method employing the technique of Flow Injection (FI) analysis to quantitatively assay low levels of *p*-aminophenol. A solution of the drug substance, or an extract of the tablets, containing *p*-aminophenol and paracetamol is injected into a solvent carrier stream and merged on-line with alkaline sodium nitroprusside reagent, to form a specific blue derivative which is detected spectrophotometrically at 710 nm. Standard HPLC equipment is used throughout. The procedure is fully quantitative and has been optimised for sensitivity and robustness using a multivariate experimental design (multi-level 'Central Composite' response surface)model. The method has been fully validated and is linear down to 0.01 μg mL^{-1}. The approach should be applicable to arange of paracetamol products. (Bloomfield, 2002)

A simple method for the rapid determination of paracetamol in pharmaceutical formulations is described. The method involves oxidation of paracetamol by potassium hexacynoferrate(III) and a subsequent reaction with phenol in the presence of ammonia. The blue complex formed is measured at 630 nm. The system has a sample frequency of 27 samples per h with a detection limit of 0.2 mg l^{-1}. (Staden et al., 2002)

A method for the determination of 4-aminophenol as an impurity in paracetamol (N-(4-hydroxyphenyl)-acetamide) by proton nuclear magnetic resonance (1H-NMR) spectroscopy has been developed. The ^{13}C-satellite from the protons in the ortho position from the hydroxyl group in paracetamol was used as

an internal standard, although these peaks interfered with the peaks from the protons in 4-aminophenol. Because of interference in the spectra and non-linearity over a wide calibration range, a Bayesian regularized neural network model was used for calibration. Various kinds of data preprocessing were examined: zero filling, multiplication by a negative exponential function (line broadening), followed by Fourier transformation of the free induction decay (FID). The NMR spectral data were automatically phased and shift-adjusted by means of a genetic algorithm. Multiplicative scatter correction and data compression by wavelets and sequential zeroing of weights variable selection were performed to obtain an optimal calibration model. Neither zero filling of the FID nor line broadening improved the calibration models with regard to error of prediction, so these processes were excluded in the final model. The generated Bayesian regularized network model was evaluated with an independent test set. Four different models with different test sets were constructed to explore the quality of the calibration. The mean error of the optimal calibration model was 25.3×10^{-6} weight of 4-aminophenol per weight paracetamol. The method is characterized by being relative fast, simple and sufficient sensitive for typical pharmaceutical impurity determinations. (Forshed et al., 2002)

A spectrophotometric method for rapid quantification of acetaminophen in serum is described. Free unconjugated acetaminophen is seperated from other endogenous interferents by extracting the drug into ethyl acetate and hydrolysis to *p*-aminophenol by treatment with heat and acid. The latter compound is capable of undergoing an oxidative coupling reaction with *p*-xylenol (2,5-dimethylphenol) catalyzed by sodium periodate. The resultant indophenol derivative formed is measured spectrophotometrically at 635 nm. The proposed method for acetaminophen determination is simple and rapid and is especially suitable for screening for drug ovedose in an emergency situation. (Afshari et al., 2001)

1.4.1.2 Spectrofluorimetric methods

A method paper describes a fluorimetric method to quantify the low amount of 4-aminophenol (50 ppm) in a pharmaceutical preparation, i.e. in paracetamol tablets. The fluorimetric method was validated and the linearity, precision, trueness, range, limit of detection and limit of quantification were determined. They were found acceptable to assay the low amounts of 4-aminophenol in paracetamol tablets. (Dejaegher et al., 2008)

A spectrofluorimetrical method was designed for determination of paracetamol. The employed methodology involves coumarinic compound formation obtained by reaction between paracetamol and ethylacetoacetate in the presence of sulphuric acid as catalyst. The reaction product is highly fluorescent at 478 nm, being excited at 446 nm. (Oliva et al., 2005)

The native fluorescence of paracetamol in the solid state is demonstrated, allowing the development of a rapid, simple and rugged method for direct analysis of pharmaceutical formulations. It is easily adaptable to any spectrofluorimeter, and no chemical treatment of the sample is needed. The fluorescence measurements (λ_{ex} = 333 nm; λ_{em} = 382 nm) are performed directly on the powdered sample, the active substance being diluted in lactose, maize starch, poly(vinylpyrrolidone), talc and stearic acid. (Moreira et al., 2005)

A stopped-flow method with fluorescence detection for the determination of paracetamol based on its oxidation with hexacyanoferrate(III) is described. A kinetic study of the reaction is developed measuring the initial rate of change of the fluorescence intensity of the oxidised product formed at 241 and 426 nm excitation and emission wavelengths, respectively. (Pulgarin et al., 1996)

1.4.1.3 Chemiluminescence methods

A flow injection procedure was developed for the determination of *p*-aminophenol (PAP) based on the inhibition by PAP of the chemiluminescence from luminol–dimethylsulfoxide (DMSO)–NaOH–EDTA system. The method

has merits of higher sensitivity, wider linear range, simpler procedure, and a more rapid analyzing speed. It is applicable for the determination of PAP in the range of $2.5x10^{-10}$–$5.0x10^{-8}$ gmL^{-1} with a detection limit of $1.9x10^{-10}$ gmL^{-1}. The relative standard deviation (RSD) for $5.0x10^{-9}$ gmL^{-1} PAP is 0.78% (n=15). The method has been successfully used to determine PAP in industrial wastewaters and environmental waters. (Xu et al., 2005)

At a large drug scanning, the system Luminol-H_2O_2-$Fe(CN)_6^{3-}$ is proposed for first time for the indirect determination of paracetamol. The method is based on the oxidation of paracetamol by hexacyanoferrate (III) and the subsequent inhibitory effect on the reaction between luminol and hydrogen peroxide. The prosedure resulted in a linear calibration graph over the range 2.5-12.5 $\mu g\ mL^{-1}$ of paracetamol with a sample throughput of 87 samples h^{-1}. The influence of foreign compounds was studied and, the method was applied to determination of the drug in three different pharmaceutical formulations. (Alapont et al., 1999)

1.4.1.4 IR spectrophotometric methods

Near infrared transmittance spectroscopy was used to determine the analgesic paracetamol in a pharmaceutical preparation commercially available as tablets. Spectra were recorded on a dedicated instrument that measures the transmission of intact tablets over the wavelength range 600-1900 nm. Spectral data were processed by using two multivariate calibration methods, viz. stepwise multiple linear regression (SMLR) and partial least-squares regression (PLSR). The analgesic contents provided by the two calibration methods were comparable and differed by less than 1% from the reference (UV spectrophotometric) value. The calibration graphs constructed to determine the mean paracetamol content were used to analyse content uniformity in the tablets. (Eustaquio et al.,1999)

A new FI/FTIR method for the determination of acetaminophen (paracetamol, N-acetyl-p-aminophenol) involving on-line reaction is described. The proposed method is based on the alkaline hydrolysis of the analyte to produce *p*-aminophenol and its oxidation reaction with potassium ferricyanide to produce *p*-benzoquinone-monoimine which eventually oxidizes to form *p*-benzoquinone.

The chemistry of the reaction was studied both, in the visible and IR regions of the spectrum and the method has been developed by the application of flow-injection methodology. The reaction was carried out in aqueous media and at room temperature. The micro-flow version of the CIRCLE IR accessory, which is compatible with aqueous solutions, was used. Measurements were carried out at the OH-phenolic deformation (1274.1 cm^{-1}) and the aromatic ring mode (1498.2 cm^{-1}) infrared vibrations for the hydrolysis product, *p*-aminophenol. The method was applied to the determination of acetaminophen in commercial tablets, and mean detection values of 512 and 491 mg were found at 1274.1 and 1498.2 cm^{-1}, respectively. (Ramos et al., 1998)

1.4.2 Electroanalytical methods

Carboxylated multiwalled carbon nanotubes (MWCNT-COOH) were used to modify the working electrode surface of different screen-printed electrodes. The effect of this modification on the electrodic characteristics (double layer capacitance, electroactive area and heterogeneous rate constants for the electron transfer) was evaluated and optimized for the cyclic voltammetric determination of *p*-aminophenol. The enzymatic hydrolysis of *p*-aminophenylphosphate was employed for the quantification of alkaline phosphatase, one of the most important label enzymes in immunoassays. Finally, ELISA assays were carried out to quantify pneumolysin using this enzymatic system. (Lamas-Ardisana et al., 2008)

A sensitive electroanalytical methodology for the determination of paracetamol using adsorptive stripping voltammetry (AdsSV) at a multiwalled carbon nanotube modified basal plane pyrolytic graphite electrode (MWCNT-BPPGE) is presented. Both cyclic voltammetric and square wave adsorptive stripping voltammetric techniques are compared. The method was then successfully utilised for the determination of paracetamol in a real sample of "ANADIN EXTRA" tablets and a recovery of 95% was obtained without interference from aspirin or caffeine. The proposed electroanalytical method using MWCNT-BPPGE is the most sensitive method for determination of paracetamol with lowest limit of detection to date. It has also advantages such as easy

handling, resistance against surface fouling, and low cost. (Kachoosangi et al., 2008)

Thin film electro-coated poly[N-vinylcarbazole-co-vinylbenzene sulfonic acid] (p[NVCzVBSA]), poly[carbazole-co-methylthiophene] (p[CzMeTh]) and polycarbazole (p[Cz]) carbon fibre microelectrodes (CFMEs) were characterised by scanning electron microscopy (SEM) and FTIR-ATR spectroscopy. These modified carbon fibre electrodes were found to be effective systems for the determination of *para*-aminophenol. Thin film coated p[NVCzVBSA] was the most suitable modified electrode for the detection of *para*-aminophenol. (Jamal et al., 2004)

A single-wall carbon nanotubes (SWNT)-Nafion film coated glassy carbon electrode (GCE) was described for the determination of 4-aminophenol. In pH 3.0 sodium citrate-HCl buffer, the oxidation peak current of 4-aminophenol increases greatly at the SWNT-Nafion film coated GCE in contrast to that at both bare GCE and Nafion-film coated GCE. Moreover, the oxidation peak potential shifts to more negative potential. All the experimental parameters were optimized for the determination of 4-aminophenol. Using the proposed method, 4-aminophenol in water samples was determined. (Huang et al., 2003)

1.4.3 Chromatographic methods

A method for the determination of 4-aminophenol, the main impurity of paracetamol, by high-performance liquid chromatographic (HPLC) method with amperometric detection has been developed. The analysis was performed in an isocratic mode on a reversed phase Luna column 5 mm C-18 (100 x 4.6 mm). A mobile phase (0.05 mol L^{-1} LiCl solution containing 18% methanol adjusted to pH 4.0 with orthophosphoric acid) was suitable for the separation and determination of 4-aminophenol. Chromatograms were recorded for 250 s by means of an amperometric detector at a potential of +325 mV of the glassy carbon electrode versus the reference electrode Ag/AgCl. The proposed liquid chromatographic method was successfully applied to the analysis of commercially available multicomponent dosage forms. The method developed in this study is sensitive

and selective and can be applied for routine studies of pharmaceuticals in the form of tablets or capsules. (Wyszecka-Kaszuba et al., 2003)

A high-performance liquid chromatography (HPLC) method has been developed and validated for the simultaneous determination of n-propionyl-p-aminophenol, 3-chloro-4-hydroxyacetanilide, 4'-hydroxyacetophenone, 4-hydroxyacetophenone oxime, 4-acetoxyacetanilide and 4'-chloroacetanilide, the main impurities in acetaminophen drug substance. The chromatographic separation was achieved on an Eclipse XDB-18 reversed-phase column using a gradient elution, being solvent A: 0.01M phosphate buffer at pH 3.0 and solvent B: methanol. The proposed method was successfully applied to the analyses of different lots and different manufactures of acetaminophen drug substance. The proposed method can be used for the routine quality control of acetaminophen. (Kamberi et al., 2004)

A micellar electrokinetic chromatography (MEKC) method was established for determination of paracetamol and chlorpheniramine maleate (CPM) in cold tablets. Separation of both drugs, as well as other seven cold remedy ingredients, was achieved in 25.5 min using a sodium dihydrogenphosphate–sodium tetraborate buffer (10 mM, pH 9.0) containing sodium dodecyl sulfate (SDS) (50 mM) and acetonitrile (26% v/v). The effective capillary length of 50 cm, the separating voltage of 15 kV and the temperature of 30 °C was optimized. Detection was by a diode array detector at 214 nm. The developed method was applied to the determination of ingredients in cold tablets and was found to be simple, rapid and efficient. (Suntornsuk et al., 2003)

A method to simultaneously quantify paracetamol, paracetamol glucuronide and paracetamol sulphate in plasma and urine with superior sensitivity is therefore desired, especially if the volume of plasma available is low. A simple isocratic reverse phase high-performance liquid chromatography (HPLC) assay with spectrophotometric detection has been developed. The method, requiring only 100 μL of plasma and 50 μL of urine, utilizes a reversed-phase C18 column, a wavelength of 254 nm for detection and a mobile phase composed of potassium dihydrogen orthophosphate (0.1 M)–isopropanol–tetrahydrofuran (THF)

(100:1.5:0.1, v/v/v) adjusted to pH 3.7 with phosphoric acid. The assay has been used to measure concentrations of paracetamol and the two metabolites in plasma collected by finger-prick sampling and of the metabolites in urine from healthy volunteers administered a single oral dose of 1000 mg of paracetamol. (Jensen et al., 2004)

An isocratic and rapid HPLC method for the simultaneous determination of the three compounds, acetaminophen, phenylephrine and chlorpheniramine, in capsules as pharmaceutical formulations, including the separation of impurities (4-aminophenol and 4-chloracetanilide) and excipients, has been developed and validated. The final chromatographic conditions employed a Supelco Discovery HS PEG column poly(ethyleneglycol) 15 x 0.46 cm, 5 μm. The mobile phase was 20 m*M* phosphate buffer, pH 7.0–acetonitrile (90:10, v/v) at a flow-rate of 1mL/min. UV detection was performed at 215 nm for all the compounds except acetaminophen, which was measured at 310 nm. (Garcia et al., 2003)

The method presents a HPLC method for simultaneous determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets, using chromatographic system consisting a Bio Rad 18 01 solvent pump, Rheodine 71 25 injector and Bio Rad 18 01 UV–Vis Detector. Separation was achieved using Bio SiL HL C18, 5 μm, 250 × 4.6 mm column. Mixture of acetonitrile–water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid was used as a mobile phase at a flow rate of 2.0 mL min^{-1}. UV detection was at 207 nm range 0.01 AUFS. Under the same conditions it was possible to determine the level of salicylic acid. The chromatographic parameters such as retention times, capacity factor, peak asymmetry, selectivity factor and resolution factor was determined. The proposed HPLC method has been applied for the determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in Malophenum tablets. The developed method is rapid and sensitive and therefore suitable for routine control of these drugs in dosage form. (Franeta et al., 2002)

A simple, rapid and convenient HPLC method, which permits the simultaneous determination of paracetamol, 4-aminophenol and 4-chloracetanilide in pharmaceutical preparation has been developed. The chromatographic

separation was achieved on porous graphitized carbon (PGC) column using a isocratic mixture of 80/20 (v/v) acetonitrile/0.05 M potassium phosphate buffer (pH 5.5) and ultraviolet detection at 244 nm. The chromatographic behaviour of the three compounds was examined under variable mobile phase compositions and pH. The retention selectivity of these compounds on PGC was compared with those of octadecylsilica (ODS) packing materials in reversed phase liquid chromatography. It is suggested that the proposed method should be used for routine quality control and dosage form assay of paracetamol in pharmaceutical preparations. (Monser et al., 2002)

A micellar electrokinetic chromatographic (MEKC) method was developed for the quality control of paracetamol containing pharmaceutical preparations. The influence of several factors (surfactant concentration, buffer concentration, pH and applied voltage) was studied during development and optimisation of the method. The separation was performed in an uncoated fused silica capillary with a total length of 64.5 cm and effective length of 56 cm (50 μm i.d.) with a bubble cell (optical path length 150 μm). Separations were carried out using normal polarity at an applied voltage of 25 kV. Detection was carried out at 240 nm. The method was successfully applied for the quality control of paracetamol containing products. (Nemeth et al., 2008)

A rapid and efficient oil-in-water microemulsion liquid chromatographic method has been optimised and validated for the analysis of paracetamol in a suppository formulation. Excellent linearity, accuracy, precision and assay results were obtained. Lengthy sample pre-treatment/extraction procedures were eliminated due to the solubilising power of the microemulsion and rapid analysis times were achieved. The method was optimised to achieve rapid analysis time and relatively high peak efficiencies. Astandard microemulsion composition of 33 g SDS, 66 g butan-1-ol, 8 g n-octane in 1 L of 0.05% TFA modified with acetonitrile has been shown to be suitable for the rapid analysis of paracetamol in highly hydrophobic preparations under isocratic conditions. Validated assay results and overall analysis time of the optimised method was compared to British Pharmacopoeia reference methods. Sample preparation and analysis times for the MELC analysis of paracetamol in a suppository were extremely rapid compared

to the reference method and similar assay results were achieved. A gradient MELC method using the same microemulsion has been optimised for the resolution of paracetamol and five of its related substances in approximately 7 min. (McEvoy et al., 2007)

1.4.4 Capillary electrophoretic methods

Poly(dimethylsiloxane) microchip capillary electrophoresis with amperometric detection has been used for rapid separation and determination of acetaminophen and its hydrolysate, i.e. *p*-aminophenol. A Pt ultramicroelectrode with a diameter of 10 μm positioned at the outlet of the separation channel was used as a working electrode for amperometric detection. Factors influencing separation and detection were investigated and optimized. This method has been successfully applied to the detection of traces of *p*-aminophenol in paracetamol tablets. (He et al., 2004)

Capability of fast analysis of a novel miniaturized capillary electrophoresis with carbon disk electrode amperometric detection (mini-CE-AD) system was demonstrated by determining acetaminophen and *p*-aminophenol in dosage forms. Factors influencing the separation and detection processes were examined and optimized. Under the optimum conditions, the end-capillary 300μm carbon disc electrode amperometric detector offered favorable signal-to-noise characteristics at a relatively low potential (+600mV versus Ag/AgCl) for detecting acetaminophen and *p*-aminophenol. The proposed mini-CE-AD system should find a wide range of analytical applications in pharmaceutical formulations as an alternative to conventional CE and μ-CE. (Chu et al., 2008)

The migration behaviour of acetaminophen and *p*-aminophenol was investigated by capillary electrophoresis. The influence of different parameters (pH, nature and concentration of the running buffer and applied voltage) on the migration time, peak symmetry, efficiency and resolution was systematically investigated. The two analytes can be well separated within 4 min in a 57 cm fused-silica capillary at a separation voltage of 18 kV in a 50 mM borate buffer adjusted to pH 9.5. The method was applied to the analysis of various

commercially available acetaminophen dosage forms with recoveries of 98.4–100.7%. (Perez-Ruiz et al., 2005)

A method based on capillary electrophoresis with electrochemical detection (CE-ED) was developed for the simultaneous determination of *p*-aminophenol and acetaminophen in the hydrolysates of acetaminophen. Effects of several important factors such as the acidity and concentration of running buffer, separation voltage, injection time, and working potential were investigated to acquire the optimum conditions. The detection electrode was a 300 μm carbon disc electrode at a working potential of +0.80 V (versus SCE). The two analytes can be well separated within 6 min in a 50 cm length fused silica capillary at a separation voltage of 18 kV in a 25 mM phosphate buffer (pH 6.5). The rate constants of acetaminophen hydrolysis in 0.5 M HCl at different temperatures were determined by monitoring the concentration changes of acetaminophen. The activation energy for acetaminophen hydrolysis was calculated to be 68.13 kJ mol^{-1}, which is in good agreement with the value in the literature. (Chen et al., 2002)

The method describes capillary electrophoresis (CE) for the determination of paracetamol and its main metabolites in urine and serum. Due to its high efficacy, CE enables the analysis of drugs directly in complex matrices. Thus, simple, rapid and reliable assays could be developed that made use of some of the main advantages of this analytical technique. In order to prevent the peaks from tailing, a water zone was injected behind the sample. Occasionally occurring peak splittings of paracetamol were investigated and methods to suppress these splittings were developed. Paracetamol, its main metabolites, paracetamol glucuronide, paracetamol sulfate as well as paracetamol cysteinate and paracetamol mercapturate, as metabolites of the oxidative pathway were identified in urine using diode-array detection and coupling of the CE instruments to electrospray–mass spectrometry. The assays were validated. Their usefulness was demonstrated by applying them to the analysis of urine and serum samples of healthy volunteers as well as to urine samples from children under anticancer therapy. (Heitmeier et al., 1999)

A capillary electrophoresis (CE) method was developed using paracetamol glucuronide as a novel probe for human β-glucuronidase activity. Using UV detection without prior sample clean-up procedures, fast and reliable quantitation of the released paracetamol was possible. The suitability of the method has been shown for enzyme kinetic studies using different liver and kidney homogenates, respectively. The data clearly demonstrate that paracetamol glucuronide is cleaved by human β-glucuronidase thereby releasing paracetamol. The CE method presented is not only a valuable tool for measuring human β-glucuronidase activity, but also allows investigation of the contribution of deglucuronidation of paracetamol glucuronide to the disposition of paracetamol. (Bohnenstengel et al., 1999)

1.4.5 The other methods

A sensitive, accurate and precise thermometric titration is described for the direct determination of ≥ 75 mg of acetaminophen in pure form or in pharmaceutical formulations. Titrations are with aqueous sodium hydroxide solution. Excipients tested (starch, talc, lactose) had no effect on the accuracy and precision. (Burgot et al., 1997)

A simple and accurate titrimetric method has been developed for the assay of paracetamol in pure form and in dosage forms, using *N,N*-dibromo dimethylhydantoin (DBH). Though a number of N-halo compounds are available for the determination of pharmaceuticals. DBH has greater advantages over such reagents. (Kumar et al., 1997)

1.5 The Aim of The Thesis

Paracetamol is a commonly used analgesic and antipyretic drug that is used for the relief of fever, headaches and other minor aches and pains. *p*-aminophenol is the hydrolytic product of paracetamol and is reported to have significant nephrotoxicity and teratogenic effects and has been detected in paracetamol as an impurity or synthetic intermediate. Therefore, its amount should be strictly controlled. So it will be useful that a rapid, sensitive, selective, accurate, simple,

economic analytical method concerning determination of *p*-aminophenol in the presence of paracetamol is developed for the studies about stability of paracetamol, quality control process in drug production and pharmaceutical analysis. The aim of the present study is to investigate determination conditions of *p*-aminophenol with a spectrophotometric method (Mohamed et al., 1997) in the presence of paracetamol in pharmaceutical preparations.

1.6 UV/Visible Moleculer Absorption Spectrometry

The information about UV/Visible Moleculer Absorption Spectrometry was provided from the book named Principles of Instrumental Analysis.(Skoog et al., 1998)

Absorption measurements based upon ultraviolet and visible radiation find widespread application for the quantitative determination of a large variety of inorganic and organic species.

Molecular absorption spectroscopy is based on the measurement of the transmittance *T* or the absorbance A of solutions contained in transparent cells having a path length of b cm. Ordinarily, the concentration c of an absorbing analyte is linearly related to absorbance as represented by the equation

$$A = - \log T = \log \frac{P_0}{P} = \varepsilon bc \qquad (1)$$

All of the variables in this equation are defined in Table 1-1. This equation is a mathematical represented of Beer's law.

Measurement of Transmittance and Absorbance

Ordinarily, transmittance and absorbance, as defined in Table 1-1, cannot be measured in the laboratory because the analyte solution must be held in some sort of a transparent container, or cell. As shown in Figure 1-6, reflection occurs at the

two air/wall interfaces as well as at the two wall/solution interfaces. The resulting beam attenuation is substantial. In addition, attenuation of a beam may occur as a result of scattering by large molecules and sometimes from absorption by the container walls. To compensate for these effects, the power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an idential cell containing only solvent.

Table 1-1 Important Terms and Symbols for Measurement of Absorption.

Term and Symbol	**Definition**	**Alternative Name and Symbol**
Radiant power P,P_o	Energy of radiation (in ergs) impinging on a 1-cm^2 area of a dedector per second	Radiation intensity I,I_o
Absorbance A	log P_o/ P	Optical density D; extinction E
Transmittance T	P/P_o	Transmission T
Path length of radiation b	_	I, d
Absorptivity a	A/bc	Extiction coefficient k
Molar absorptivity ε	A/bc	Molar extinction coefficient

An experimental transmittance and absorbance that closely approximate the true transmittance and absorbance are then obtained with the equations

$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0} \qquad (2)$$

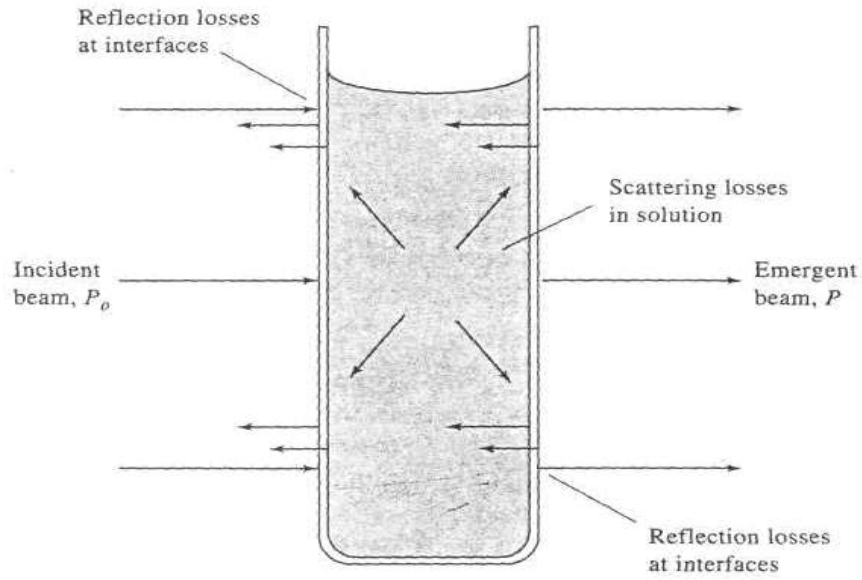

Figure 1-6 Reflection and scattering losses.

$$A = \log \frac{P_{\text{solvent}}}{P_{\text{solution}}} \approx \log \frac{P_o}{P} \qquad (3)$$

The terms P_o and P refer to the power of radiation after it has passed through cells containing the solvent and the analyte, respectively.

Beer's Law

Equation 1 represents Beer's law. This relationship can be rationalized as follows. Consider the block of absorbing matter (solid, liquid, or gas) shown in Figure 1-7. A beam of paralel monochromatic radiation with power P_o strikes the block perpendicular to a surface; after passing through a length b of the material, which contains n absorbing atoms, ions, or molecules, its power is decreased to P as a result of absorption. As a result, Beer's law was derived from this approach.

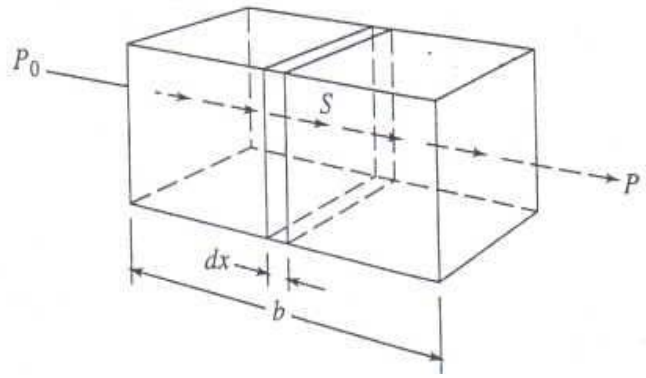

Figure 1-7 Attenuation of radiation with initial power P_o by a solution containing c moles per liter of absorbing solute and with a path length of b cm. $P < P_o$.

Limitations to Beer's Law

Few exceptions are found to the generalization that absorbance is linearly related to path length. On the other hand, deviations from the direct proportionality between the measured absorbance and concentration when b is constant are frequently encountered. Some of these deviations are fundamental and represent real limitations of the law. Others ocur as a consequence of the manner in which the absorbance measurements are made or as a result of chemical changes associated with concentration changes; the latter two are sometimes known, respectively, as instrumental deviations and chemical deviations.

Real Limitations to Beer's Law

Beer's law is succesful in describing the absorption behavior of media containing relatively low analyte concentrations; in this sense, it is a limiting law. At high concentrations (usually > 0.01M), the average distance between the molecules responsible for absorption is diminished to the point where each molecule affects the charge distribution of its neighbors. This interaction, in turn, can alter the ability of the molecules to absorb a given wavelength of radiation. Because the extent of interaction depends upon concentration, the occurence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. A similar effect is sometimes encountered in media containing low absorber concentrations but high concentrations of other species,

particularly electrolytes. The close proximity of ions to the absorber alters the molar absorptivity of the latter by electrostatic interactions; the effect is lessened by dilution.

While the effect of molecular interactions is ordinarily not significant at concentrations below 0.01 M, some exceptions occur among certain large organic ions or molecules. For example, the molar absorptivity at 436 nm for the cation of methylene blue in aqueous solutions is reported to increase by 88% as the dye concentration is increased from 10^{-5} to 10^{-2} M; even below 10^{-6} M, strict adherence to Beer's law is not observed.

Deviations from Beer's law also arise because ε is dependent upon the refractive index of the medium. Thus, if concentration changes cause significant alterations in the refractive index n of a solution, departures from Beer's law are observed. A correction for this effect can be made by substitution of the quantity $\varepsilon n/(n^2 + 2)^2$ for ε in equation 1. In general, this correction is never very large and is rarely significant at concentrations less than 0.01 M.

Apparent Chemical Deviations

Apparent deviations from Beer's law arise when an analyte dissociates, associates, or reacts with a solvent to produce a product having a different absorption spectrum from the analyte. A common example of this behaviour is found with aqueous solutions of acid/base indicators. For example, the color change associated with a typical indicator HIn arises from shifts in the equilibrium

$$\underset{\text{color 1}}{HIn} \rightleftharpoons H^+ + \underset{\text{color 2}}{In^-}$$

Data in Table 1-2 demonstrate how the shift in this equilibrium with dilution results in deviation from Beer's law.

Table 1-2 Concentration and Absorbance Data for the HIn equilibrium.

C_{HIn}, M	[HIn]	$[In^-]$	A_{430}	A_{570}
2.00×10^{-5}	0.88×10^{-5}	1.12×10^{-5}	0.236	0.073
4.00×10^{-5}	2.22×10^{-5}	1.78×10^{-5}	0.381	0.175
8.00×10^{-5}	5.27×10^{-5}	2.73×10^{-5}	0.596	0.401
12.00×10^{-5}	8.52×10^{-5}	3.48×10^{-5}	0.771	0.640
16.00×10^{-5}	11.9×10^{-5}	4.11×10^{-5}	0.922	0.887

Figure 1-8 is a plot of the data shown in Table 1-2, which illustrates the kinds of departures from Beer's law that arise when the absorbing system is capable of undergoing dissociation or association. The direction of curvature is opposite at the two wavelengths.

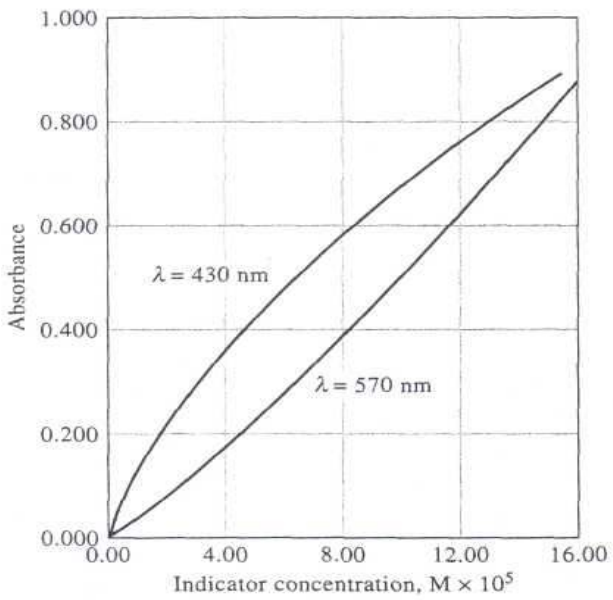

Figure 1-8 Chemical deviations from Beer's law for unbuffered solutions of the indicator HIn. For data, see Table 1-2.

Apparent Instrumental Deviations with Polychromatic Radiation

Strict adherence to Beer's law is observed only with truly monochromatic radiation; this observation is yet another manifestation of the limiting character of the law. Unfortunately, the use of radiation that is restricted to a single

wavelength is seldom practical because devices that isolate portions of the output from a continium source produce a more or less symmetric band of wavelengths around the desired one.

The following derivation shows the effect of polychromatic radiation on Beer's law.

Consider a beam consisting of just two wavelengths λ' and λ". Assuming that Beer's law applies strictly for each of these individual wavelengths, w emay write for radiation λ'

$$A' = \log P'_o/P' = \varepsilon' bc$$

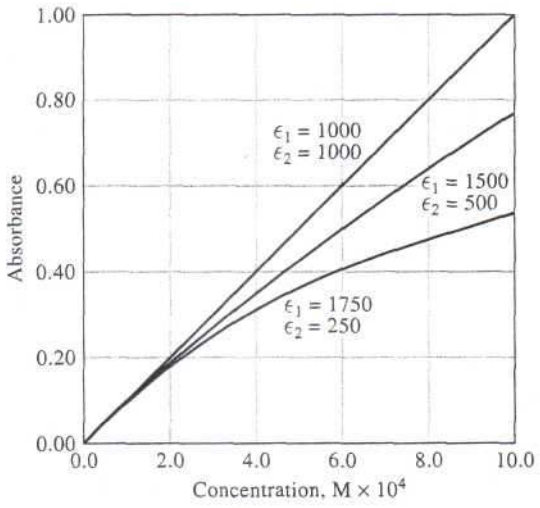

Figure 1-9 Deviations from Beer's law with polychromatic light. The absorber has the indicated molar absorptivities at the two wavelengths λ' and λ".

or

$$P'_o/P' = 10^{\varepsilon' bc}$$

and

$$P' = P'_o 10^{-\varepsilon' bc}$$

Similarly, for λ"

$$P'' = P''_o 10^{-\varepsilon'' bc}$$

When an absorbance measurement is made with radiation composed of both wavelengths, the power of the beam emerging from the solution is given by $P'+P''$ and that of the beam from the solvent by $P'_o+P''_o$. Therefore, the measured absorbance A_m is

$$A_m = \log \frac{\left(P_0' + P_0''\right)}{\left(P' + P''\right)}$$

Substituting for P' and P'' yields

$$A_m = \log \frac{\left(P_0' + P_0''\right)}{\left(P_0'.10^{-\varepsilon' bc} + P_0''.10^{-\varepsilon'' bc}\right)}$$

When ε' = ε'', this equation simplifies to

$$A_m = \varepsilon' bc$$

and Beer's law is followed. As shown in Figure 1-9, however, the relationship between A_m and concentration is no longer linear when the molar absorptivities differ; moreover, greater departures from linearity can be expected with increasing differences between ε' and ε''. This derivation can be expanded to include additional wavelengths; the effect remains the same.

It is an experimental fact that deviations from Beer's law resulting from the use of a polychromatic beam are not appreciable, provided the radiation used does not enpcompass a spectral region in which the absorber exhibits large changes in absorption as a function of wavelength. This observation is illustrated in Figure 1-10.

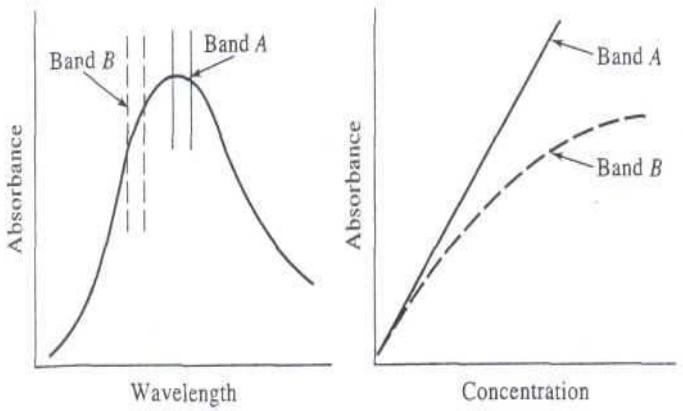

Figure 1-10 The effect of polychromatic radiation upon Beer's law. Band *A* shows little deviation because ε does not change greatly throughout the band. Band *B* shows marked deviation because ε undergoes significant changes in this region.

It is also found experimentally that for absorbance measurements at the maximum of narrow peaks, departures from Beer's law are not significant if the effective bandwidth of the monochromator or filter $\Delta\lambda_{eff}$ is less than 1/10 of the half width of the absorption peak at half height.

Instrumental Deviations in the Presence of Stray Radiation

The radiation exiting from a monochromator is ordinarily contamined with small amounts of scattered or stray radiation, which reaches the exit slit as a result of scattering and reflections from various internal surfaces. Stray radiation often differs greatly greatly in wavelengths from that of the principal radiation and, in addition, may not have passed through the sample.

When measurements are made in the presence of stray radiation, the observed absorbance is given by

$$A' = \log \frac{(P_0 + P_S)}{(P + P_S)}$$

where P_S is the power of nonabsorbed stray radiation. Figure 1-11 shows a plot of A' versus concentration for various between P_S and P_0. It is noteworthy that at high concentrations and at longer path lengths, stray radiation can also cause

significant deviations from the linear relationship between absorbance and path length.

The instrumental deviations illustrated in Figure 1-10 and 1-11 result in absorbances that are smaller than theoretical. It can be shown that instrumental deviations always lead to negative absorbance errors.

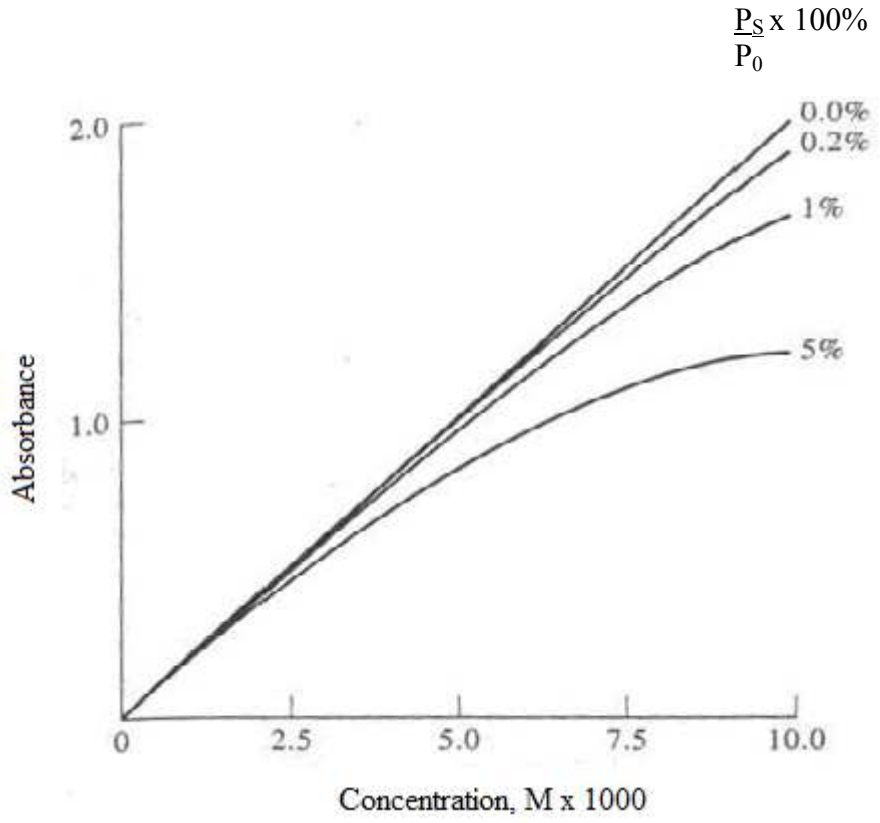

Figure 1-11 Apparent deviation from Beer's law brought about by various amounts of stray radiation.

2. EXPERIMENTAL

2.1 Apparatus

Spectrophotometric measurements were carried out using a Schimadzu UV-160A Spectrophotometer and Hellma quartz cuvettes.

Tacussel Electronique Potentiometer (Ag electrode as a working electrode, calomel electrode as a reference electrode, $AgNO_3$ as a titrant) with a system including KNO_3 salt bridge was used to standardize of sodium sulpfide.

Tacussel Electronique Potentiometer (Pt electrode as a working electrode, calomel electrode as a reference electrode, $Ce(SO_4)_2$ as a titrant) was used to standardize of ammonium iron (III) sulphate ($NH_4Fe(SO_4)_2$).

A Şimşek Laboteknik Model SS 200 instrument was used to produce distilled water.

A Gec Avery Model analytical balance was used.

2.2 Chemicals and Reagents

All reagents were of analytical grade. *p*-Aminophenol and paracetamol was provided from Sigma Aldrich. $Ce(SO_4)_2.4H_2O$, concentrated H_2SO_4, $AgNO_3$, $NH_4Fe(SO_4)_2.12H_2O$, C_2H_5OH, CH_3CSNH_2 were provided from Merck. $Na_2S.3H_20$ was provided from Atabay Kimya. Na_2CO_3 (to standardize H_2SO_4 solutions) was provided from Horasan Kimya. Distilled water was used throughout.

2.3 Reagent Solutions

0.025% (w/v) sodium sulphide aqueous solution was daily prepared from $Na_2S.3H_20$. 0.5% (w/v) Fe(III) solution was prepared from $NH_4Fe(SO_4)_2.12H_2O$ in 0.03 M H_2SO_4 and 0.08% (w/v) Ce(IV) solution was daily prepared from $Ce(SO_4)_2.4H_2O$ in 0.03 M H_2SO_4. H_2SO_4 solutions were prepared from concentrated H_2SO_4 (18.4 M) solution. $3{,}21x10^{-3}$ M Thioacetamid solution was prepared from CH_3CSNH_2 in distilled water.

2.4 Preparation of Standart Solution

p-aminophenol: prepare a 50 µg mL^{-1} *p*-aminophenol aqueous solution. Stock solution must be daily prepared and kept in a dark.

Intact paracetamol: prepare a 1 mg mL^{-1} paracetamol aqueous solution.

Hydrolysed paracetamol: transfer 0.100 g of paracetamol into a beaker, add 20 mL of 5 M H_2SO_4, heat in a boiling water-bath for 30 min, cool and dilute to volume with distilled water in a 100 mL volumetric flask. Dilute in order to obtain a concentration of 50 µg mL^{-1}. Stock solution must be daily prepared and kept in a dark.

2.5 Preparation of Sample

Tablets: Weigh and finely powder 10 tablets. Transfer an accurately weighed amount of powder equivalent to 100 mg of paracetamol into a beaker, add 20 mL of 5 M H_2SO_4, mix well and place in a boiling water-bath for 30 min, then cool and dilute to volume with distilled water in a 100 mL volumetric flask. Filter through a fitler-paper. Discard the first portion of the filtrate. Dilute in order to obtain a concentration of 50 µg mL^{-1}. Stock solution must be daily prepared and kept in a dark.

2.6 Procedure

Transfer 10,0 mL of either *p*-aminophenol or hydrolysed paracetamol standart or sample solution into a 50 mL volumetric flask. Add 5,0 mL of sodium sulphide reagent followed by 5,0 mL of Fe(III) or Ce(IV) solution. Stoper the flask and shake for about 30 s, then allow it to stand for 15 min in the case of Fe(III) and 10 min in the case of Ce(IV). Dilute to volume with 0.03 M H_2SO_4, allow to stand for a further 10 min, then measure the absorbance at 550 nm against the corresponding reagent blank.

3. RESULTS AND DISCUSSION

3.1 The Investigation of The Stability of *p*-Aminophenol

The spectrophotometric behaviour of 10^{-3} M aqueous and ethanol solution of PAP was investigated at room temperature in the light and dark. Besides, the influence of nitrogenising these solutions kept in the light and dark on the stability of PAP was evaluated. The aim of nitrogenising is to remove the oxygen in the medium.

The spectra similar to the spectra in Figure 3.1 were observed for the aqueous solution of PAP in the medium with nitrogen in the light and in the medium with/without nitrogen in the dark. The spectra similar to the spectra in Figure 3.2 were observed for the ethanol solution of PAP in the medium with nitrogen in the light and in the medium with/without nitrogen in the dark.

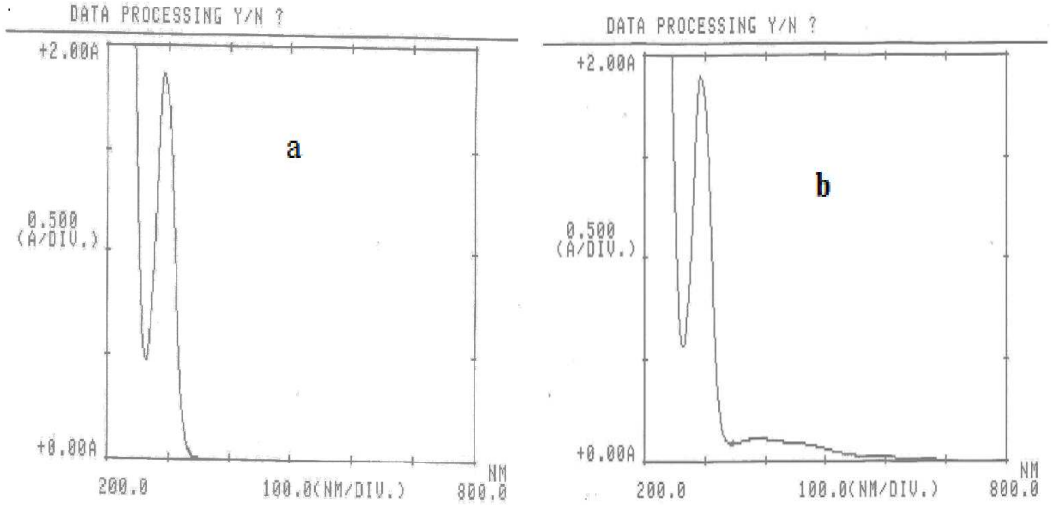

Figure 3.1 The initial (a) and degradation (b) spectra of 10^{-3} M aqueous solution of PAP kept in the medium without nitrogen(with O_2) in the light at room temperature.

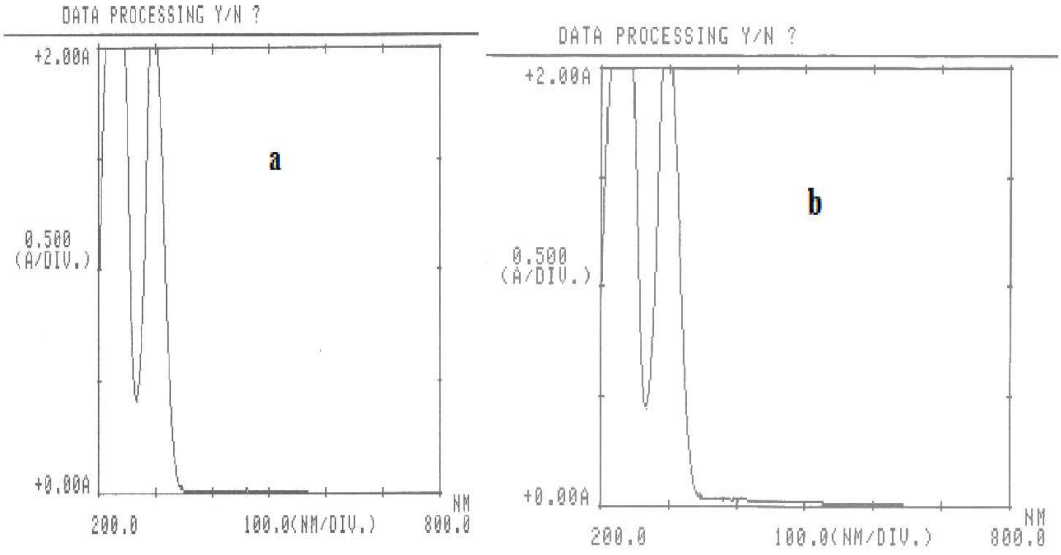

Figure 3.2 The initial (a) and degradation (b) spectra of 10^{-3} M ethanol solution of PAP kept in the medium without nitrogen(with O_2) in the light at room temperature.

As a result, it was seen that a new peak (degradation peak) at ~390 nm started to form with age in the aqueous solution of PAP and a slight difference in the absorption intensity between 300 and 700 nm occurred in the ethanol solution of PAP.

3.1.1 The investigation of the stability of aqueous solution of PAP

The stability tests of 10^{-3} M aqueous solution of PAP kept in the medium with/without nitrogen(without/with O_2) in the light/dark were carried out in this study.

3.1.1.1 <u>The stability of aqueous solution of PAP in the medium without nitrogen(with O_2) in the light</u>

The data obtained from the stability tests of 10^{-3} M aqueous solution of PAP kept in the medium without nitrogen(with O_2) in the light are given Table 3.1 ; Figure 3.3.

Table 3.1 The absorption values of 10^{-3} M aqueous solution of PAP against time in the medium without nitrogen(with O_2) in the light.

t(h)	A(230nm)	A(294nm)	A(390nm)
0,0	2,499	1,839	
0,5	2,498	1,844	
1,0	2,499	1,849	
1,5	2,499	1,854	
2,0	2,499	1,868	
2,5	2,500	1,862	
3,0	2,500	1,861	
3,5	2,500	1,869	
4,0	2,499	1,879	0,059
4,5	2,499	1,893	0,085
5,0	2,500	1,892	0,112
5,5	2,499	1,906	0,137
24,0	2,493	1,759	0,492
25,0	2,490	1,748	0,480
26,0	2,488	1,706	0,444
27,0	2,480	1,683	0,405
28,0	2,491	1,615	0,353

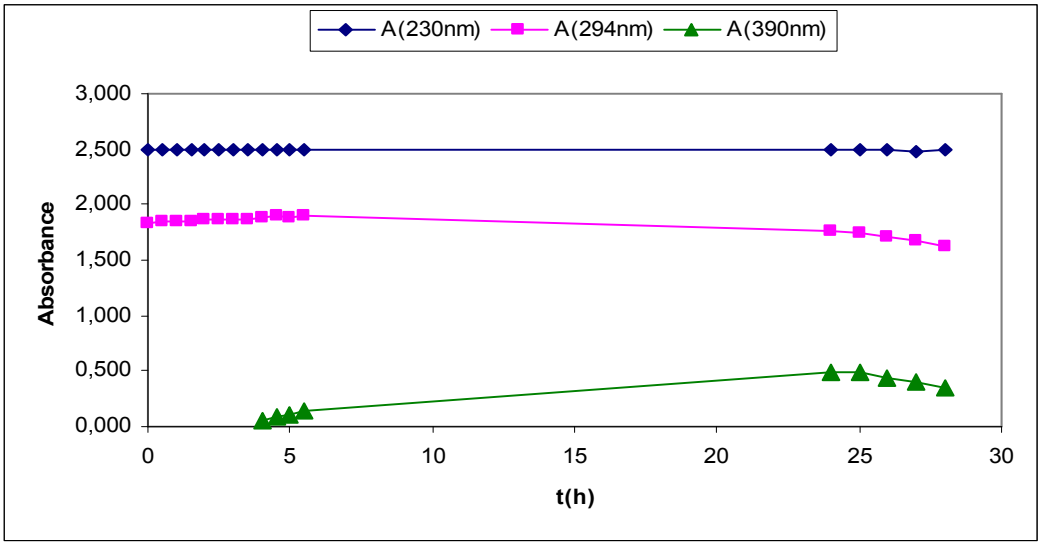

Figure 3.3 The variation of the absorption values of 10^{-3} M aqueous solution of PAP depending on time in the medium without nitrogen(with O_2) in the light.

From Table 3.1 and Figure 3.3, it was seen that the aqueous solution of PAP remained stable ~4 hours.

3.1.1.2 The stability of aqueous solution of PAP in the medium with nitrogen(without O_2) in the light

The data obtained from the stability tests of 10^{-3} M aqueous solution of PAP kept in the medium with nitrogen(without O_2) in the light are given Table 3.2 ; Figure 3.4.

Table 3.2 The absorption values of 10^{-3} M aqueous solution of PAP against time in the medium with nitrogen(without O_2) in the light.

t(h)	A(230nm)	A(294nm)	A(374nm)
0,0	2,498	1,918	
1,0	2,495	1,945	
2,0	2,496	1,999	
3,0	2,493	2,023	
4,0	2,493	2,028	
5,0	2,493	2,024	
6,0	2,493	2,018	
7,0	2,493	2,027	
8,0	2,494	2,009	
24,0	2,496	2,010	
25,0	2,496	2,014	
26,0	2,494	2,015	
27,0	2,494	2,011	
28,0	2,492	2,014	
29,0	2,494	2,026	
30,0	2,491	2,030	
31,0	2,493	2,026	
32,0	2,492	2,029	
•49,0	2,491	2,042	0,081
50,0	2,491	2,042	0,095
51,0	2,491	2,059	0,113
52,0	2,489	2,060	0,127
53,0	2,490	2,064	0,133

• The colour change was observed with the naked eye after 36 hours.

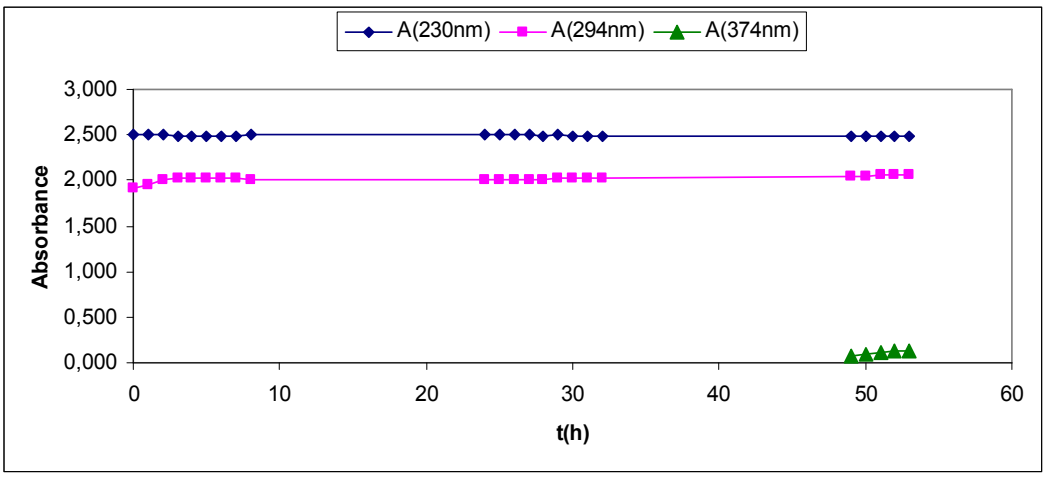

Figure 3.4 The variation of the absorption values of 10^{-3} M aqueous solution of PAP depending on time in the medium with nitrogen(without O_2) in the light.

In the stability tests, it was observed with the naked eye from the colour of the solution that the aqueous solution of PAP started to degrade after 36 hours.

3.1.1.3 The stability of aqueous solution of PAP in the medium without nitrogen(with O_2) in the dark

The data obtained from the stability tests of 10^{-3} M aqueous solution of PAP kept in the medium without nitrogen(with O_2) in the dark are given Table 3.3; Figure 3.5.

Table 3.3 The absorption values of 10^{-3} M aqueous solution of PAP against time in the medium without nitrogen(with O_2) in the dark.

t(h)	A(230nm)	A(294nm)	A(390nm)
0,0	2,499	1,847	
1,0	2,496	1,862	
2,0	2,499	1,857	
3,0	2,499	1,866	
4,0	2,496	1,876	
5,0	2,499	1,866	
6,0	2,499	1,875	
7,0	2,499	1,864	
•23,0	2,498	1,873	0,075
24,0	2,498	1,886	0,134
25,0	2,496	1,916	0,204
26,0	2,496	1,917	0,256
27,0	2,496	1,934	0,313
28,0	2,496	1,932	0,365
29,0	2,494	1,938	0,398
30,0	2,493	1,928	0,399
31,0	2,490	1,902	0,375

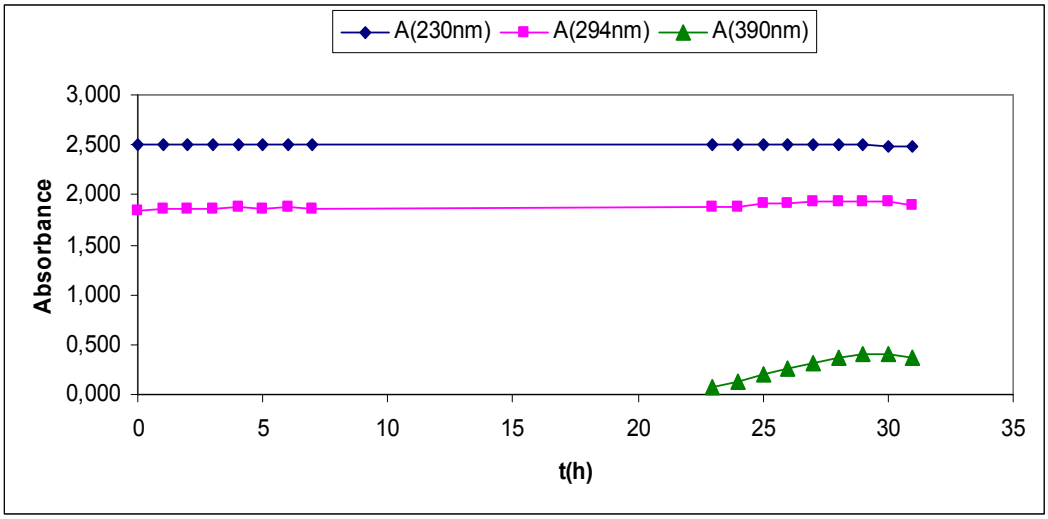

Figure 3.5 The variation of the absorption values of 10^{-3} M aqueous solution of PAP depending on time in the medium without nitrogen(with O_2) in the dark.

In the stability tests, it was observed with the naked eye from the colour of the solution that the aqueous solution of PAP started to degrade after 11 hours.

• The colour change was observed with the naked eye after 11 hours.

3.1.1.4 The stability of aqueous solution of PAP in the medium with nitrogen(without O_2) in the dark

The data obtained from the stability tests of 10^{-3} M aqueous solution of PAP kept in the medium with nitrogen(without O_2) in the dark are given Table 3.4; Figure 3.6.

Table 3.4 The absorption values of 10^{-3} M aqueous solution of PAP against time in the medium with nitrogen(without O_2) in the dark.

t(h)	A(230nm)	A(294nm)	A(372nm)
0,0	2,498	1,755	
1,0	2,496	1,832	
2,0	2,496	1,952	
3,0	2,498	1,941	
4,0	2,498	1,935	
5,0	2,496	1,941	
6,0	2,496	1,958	
7,0	2,495	1,962	
24,0	2,496	1,934	
25,0	2,496	1,953	
26,0	2,493	1,958	
27,0	2,496	1,949	
28,0	2,496	1,943	
29,0	2,496	1,962	
30,0	2,493	1,971	
31,0	2,493	1,967	
32,0	2,496	1,969	
49,0	2,496	1,952	
50,0	2,493	1,981	
51,0	2,494	1,979	
•52,0	2,496	1,984	0,089
53,0	2,496	1,985	0,094
54,0	2,496	2,000	0,107

• The colour change was observed with the naked eye after 50 hours.

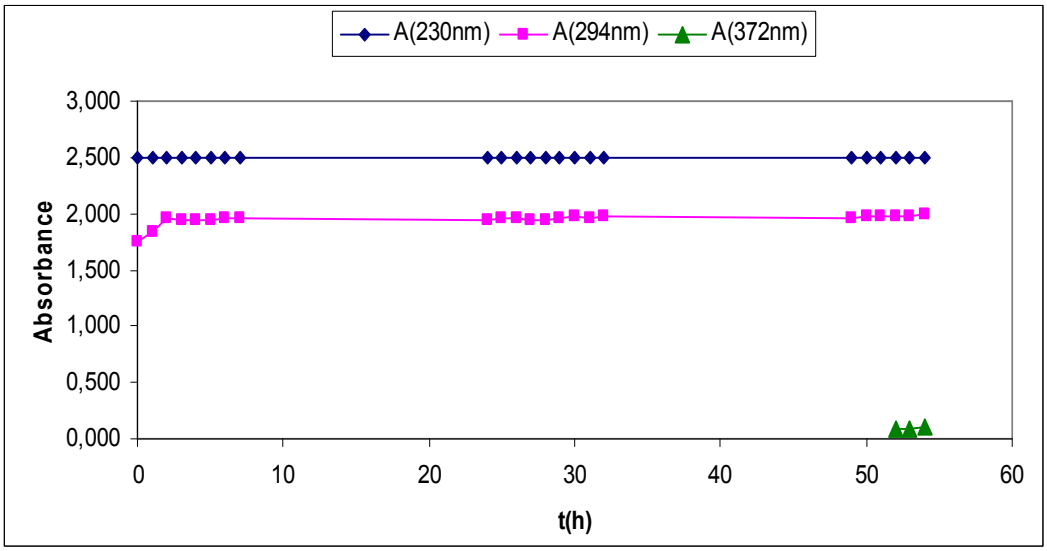

Figure 3.6 The variation of the absorption values of 10^{-3} M aqueous solution of PAP depending on time in the medium with nitrogen(without O_2) in the dark.

In the stability tests, it was observed with the naked eye from the colour of the solution and the formation of the peak at 372 nm that the aqueous solution of PAP started to degrade after 50 hours.

3.1.2 The investigation of the stability of ethanol solution of PAP

The stability tests of 10^{-3} M ethanol solution of PAP kept in the medium with/without nitrogen(without/with O_2) in the light/dark were carried out in this study.

3.1.2.1 <u>The stability of ethanol solution of PAP in the medium without nitrogen(with O_2) in the light</u>

The data obtained from the stability tests of 10^{-3} M ethanol solution of PAP kept in the medium without nitrogen(with O_2) in the light are given Table 3.5 ; Figure 3.7.

Table 3.5 The absorption values of 10^{-3} M ethanol solution of PAP against time in the medium without nitrogen(with O_2) in the light.

t(h)	A(233nm)	A(296nm)
0,0	2,250	2,129
2,0	2,253	2,157
4,0	2,253	2,164
6,0	2,252	2,172
•22,0	2,259	2,165
24,0	2,256	2,168
26,0	2,258	2,170
28,0	2,259	2,178
30,0	2,253	2,172
47,0	2,263	2,189
49,0	2,259	2,190
51,0	2,267	2,193

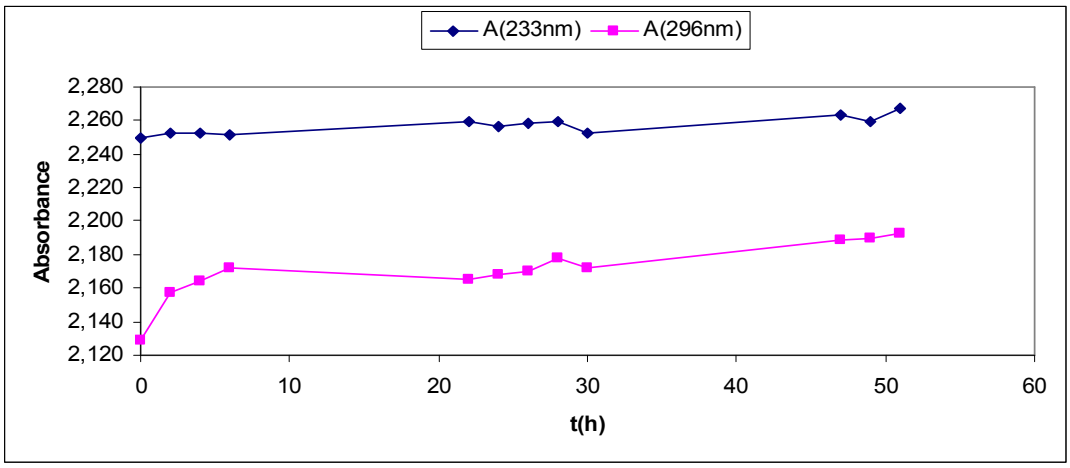

Figure 3.7 The variation of the absorption values of 10^{-3} M ethanol solution of PAP depending on time in the medium without nitrogen(with O_2) in the light.

In the stability tests, it was observed with the naked eye from the colour of the solution that the ethanol solution of PAP started to degrade after 14 hours.

• The colour change was observed with the naked eye after 14 hours.

3.1.2.2 The stability of ethanol solution of PAP in the medium with nitrogen(without O_2) in the light

The data obtained from the stability tests of 10^{-3} M ethanol solution of PAP kept in the medium with nitrogen(without O_2) in the light are given Table 3.6 ; Figure 3.8.

Table 3.6 The absorption values of 10^{-3} M ethanol solution of PAP against time in the medium with nitrogen(without O_2) in the light.

t(h)	A(240nm)	A(296nm)
0,0	1,998	2,096
2,0	1,997	2,106
4,0	1,995	2,122
6,0	2,002	2,124
24,0	2,001	2,125
26,0	1,998	2,127
28,0	2,002	2,125
30,0	2,014	2,133
•48,0	2,065	2,139
50,0	2,072	2,140
52,0	2,070	2,152

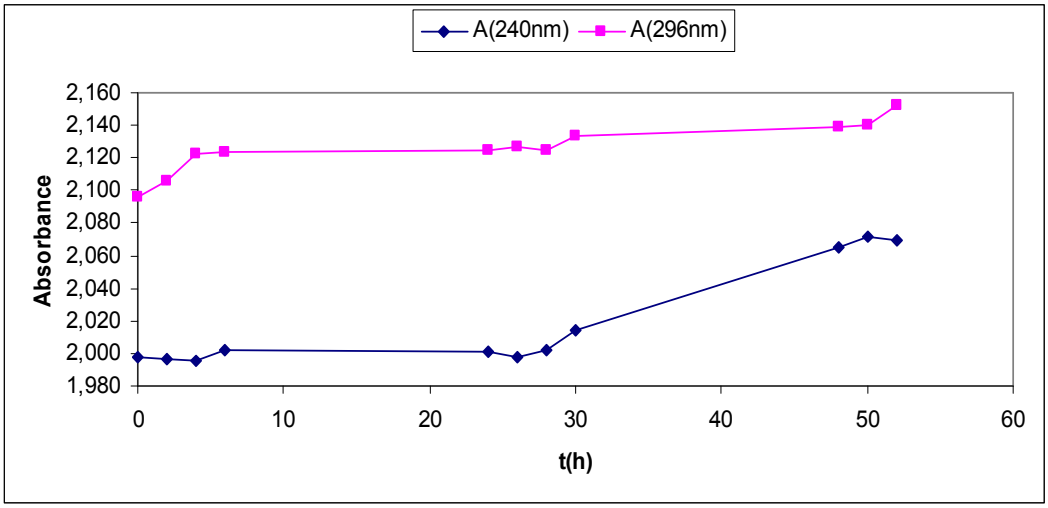

Figure 3.8 The variation of the absorption values of 10^{-3} M ethanol solution of PAP depending on time in the medium with nitrogen(without O_2) in the light.

In the stability tests, it was observed with the naked eye from the colour of the solution that the ethanol solution of PAP started to degrade after 48 hours.

• The colour change was observed with the naked eye after 48 hours.

3.1.2.3 The stability of ethanol solution of PAP in the medium without nitrogen(with O_2) in the dark

The data obtained from the stability tests of 10^{-3} M ethanol solution of PAP kept in the medium without nitrogen(with O_2) in the dark are given Table 3.7 ; Figure 3.9.

Table 3.7 The absorption values of 10^{-3} M ethanol solution of PAP against time in the medium without nitrogen(with O_2) in the dark.

t(h)	A(233nm)	A(296nm)
0,0	2,246	2,132
2,0	2,251	2,150
4,0	2,251	2,148
6,0	2,257	2,149
•22,0	2,259	2,151
24,0	2,251	2,153
26,0	2,259	2,157
28,0	2,257	2,161
30,0	2,254	2,156
47,0	2,265	2,169
49,0	2,266	2,168
51,0	2,261	2,175

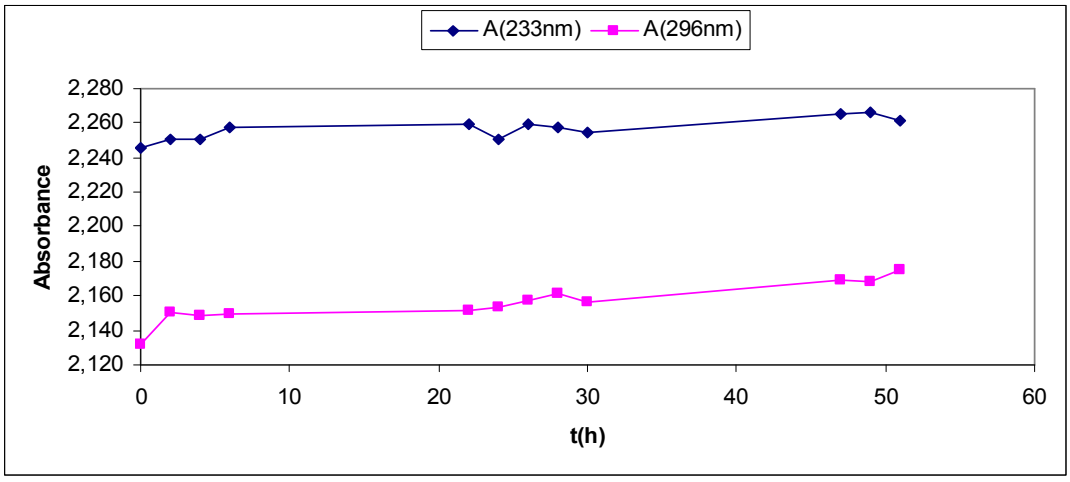

Figure 3.9 The variation of the absorption values of 10^{-3} M ethanol solution of PAP depending on time in the medium without nitrogen(with O_2) in the dark.

In the stability tests, it was observed with the naked eye from the colour of the solution that the ethanol solution of PAP started to degrade after 20 hours.

• The colour change was observed with the naked eye after 20 hours.

3.1.2.4 The stability of ethanol solution of PAP in the medium with nitrogen(without O_2) in the dark

The data obtained from the stability tests of 10^{-3} M ethanol solution of PAP kept in the medium with nitrogen(without O_2) in the dark are given Table 3.8 ; Figure 3.10.

Table 3.8 The absorption values of 10^{-3} M ethanol solution of PAP against time in the medium with nitrogen(without O_2) in the dark.

t(h)	A(240nm)	A(296nm)
0,0	2,000	2,082
2,0	2,008	2,098
4,0	2,003	2,086
6,0	2,000	2,099
24,0	2,014	2,096
26,0	2,013	2,102
28,0	2,016	2,093
30,0	2,021	2,098
48,0	2,080	2,096
50,0	2,077	2,108
52,0	2,080	2,117
•71,0	2,076	2,119
73,0	2,080	2,136

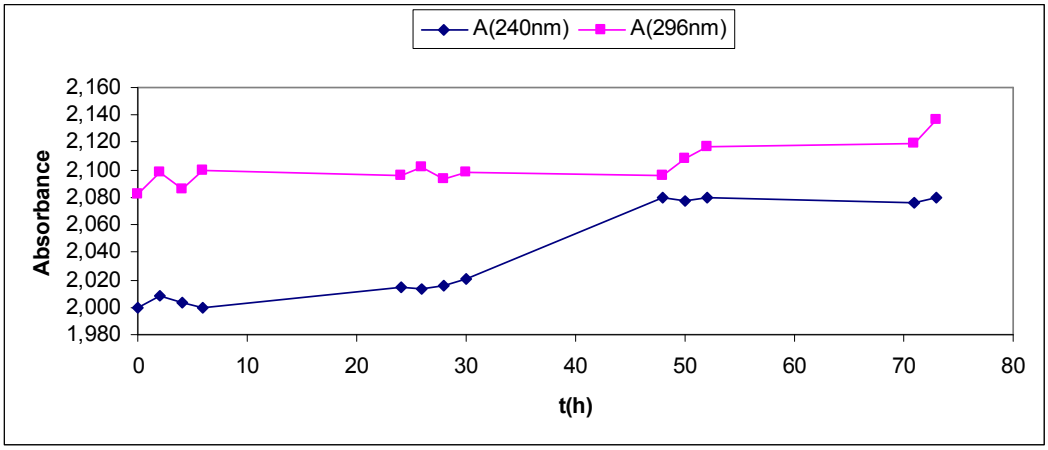

Figure 3.10 The variation of the absorption values of 10^{-3} M ethanol solution of PAP depending on time in the medium with nitrogen(without O_2) in the dark.

In the stability tests, it was observed with the naked eye from the colour of the solution that the ethanol solution of PAP started to degrade after 70 hours.

• The colour change was observed with the naked eye after 70 hours

3.1.3 The results of the stability studies of PAP

According to the results of the above-mentioned studies, Table 3.9 demonstrate that how the stability time (at which the colour change in solution started to be observed or the peak at ~390 nm started to form) changed depending on various conditions.

Table 3.9 The stability time of 10^{-3} M PAP solutions in various conditions.

	time for the stability of aqueous solution of PAP (h)		**time for the stability of ethanol solution of PAP (h)**	
	light	**dark**	**light**	**dark**
with nitrogen (without O_2)	36	50	48	>70
without nitrogen (with O_2)	4	11	14	20

As a consequence of the degradation in the aqueous and ethanol solutions of PAP, the colour of the solutions changed from colourless to dark brown with time. A new peak was observed at ~390 nm in the aqueous solution of PAP. A new peak didn't form and only a slight difference in the absorption intensity between 300 and 700 nm occurred in the ethanol solution of PAP. Because the degradation in the colour of ethanol solution was very slow according to the degradation in the aqueous solution, it was hard to determine stability time in the ethanol solution of PAP.

From Table 3.9, It was seen that the ethanol solution of PAP is more stable than the aqueous solution of PAP in various conditions and the most stable condition was obtained in the medium with nitrogen (without O_2) in the dark.

3.2 The Spectrophotometric Determination of *p*-Aminophenol Oxidized by Ce(IV) or Fe(III)

According to the procedure which is described in the article that we benefit from, the method is based on the reaction of *p*-aminophenol at ambient temperature with sodium sulphide in presence of an oxidant (Ce(IV) or Fe(III)) to produce a methylene blue-like dye. Then the absorbance is measured at 550 nm.

In the studies carried out with Ce(IV) and Fe(III) as oxidants, the effect of variation of sodium sulfide, sulphuric acid and oxidant concentration and the effect of time on the absorption intensity of the reaction product were investigated in order to determine optimum conditions and the calibration graphs were prepared for the determination of PAP.

3.2.1 The spectrophotometric determination of *p*-aminophenol oxidized by Ce(IV)

The effect of variation of sodium sulfide, sulphuric acid and oxidant concentration and the effect of time on the absorption intensity of the reaction product were investigated in order to determine optimum conditions by using Ce(IV) as an oxidant. The calibration graph was prepared for the determination of PAP.

3.2.1.1 The effect of sulphuric acid concentration

In order to study the influence of sulphuric acid concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution, 2,50 mL of 0,0032 M Na_2S, 2,50 mL of 0,0024 M Ce(IV). Then 1,0 M H_2SO_4 and distilled water in different volumes were added into each flask to give total volume of 15,00 mL. The flask was shaked for about 30 s, allowed to stand for 20 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.10 and the graph obtained is shown in Figure 3.11.

Table 3.10 The absorption values obtained from the reaction product with Ce(IV) as an oxidant by using different concentrations of H_2SO_4.

Distilled Water (mL)	1,0 M H_2SO_4 (mL)	C (mol/L) H_2SO_4	A(550nm)
15,00	0,00	0,003	0,154
14,75	0,25	0,010	0,149
14,50	0,50	0,020	0,156
14,25	0,75	0,030	0,154
14,00	1,00	0,040	0,154
13,75	1,25	0,050	0,153
13,50	1,50	0,060	0,155

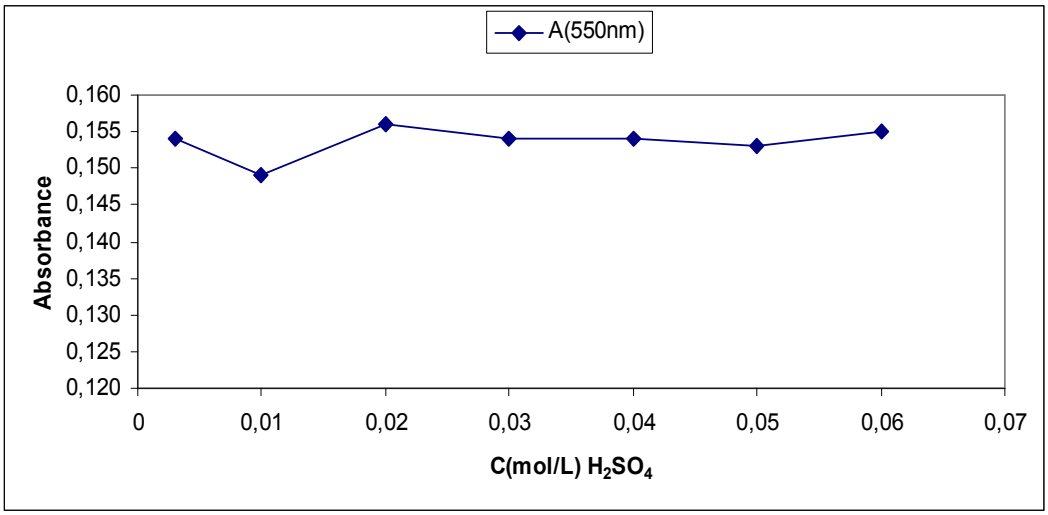

Figure 3.11 Effect of variation of sulphuric acid concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Ce(IV) and Na_2S reagents.

From Table 3.10 and Figure 3.11, it is seen that the absorbance reachs a maximum on using >0,02 M H_2SO_4. Therefore, it was seen that 0,03 M H_2SO_4 was appropriate for the studies. 0,03 M H_2SO_4 was determined as an optimum value.

3.2.1.2 The effect of sodium sulphide concentration

In order to study the influence of sodium sulphide concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution. 0,0032 M Na_2S and distilled water in different volumes were added into each flask to give total volume of 5,00 mL. Then 2,50 mL of 0,0024 M Ce(IV) was added and the flask was shaked for about 30 s, allowed to stand for 10 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.11 and the graph obtained is shown in Figure 3.12.

Table 3.11 The absorption values obtained from the reaction product with Ce(IV) as an oxidant by using different concentrations of Na_2S.

0,0032 M Na_2S (mL)	**Distilled water (mL)**	**C(mol/L) Na_2S**	**A (550nm)**
0,00	5,00	0,00 $x10^{-4}$	0,000
0,10	4,90	0,13 $x10^{-4}$	0,073
0,20	4,80	0,26 $x10^{-4}$	0,142
0,30	4,70	0,39 $x10^{-4}$	0,194
0,40	4,60	0,51 $x10^{-4}$	0,200
0,50	4,50	0,64 $x10^{-4}$	0,272
1,00	4,00	1,28 $x10^{-4}$	0,242
1,50	3,50	1,93 $x10^{-4}$	0,227
2,00	3,00	2,57 $x10^{-4}$	0,201
2,50	2,50	3,21 $x10^{-4}$	0,185
3,00	2,00	3,85 $x10^{-4}$	0,176
3,50	1,50	4,49 $x10^{-4}$	0,131
4,00	1,00	5,14 $x10^{-4}$	0,088

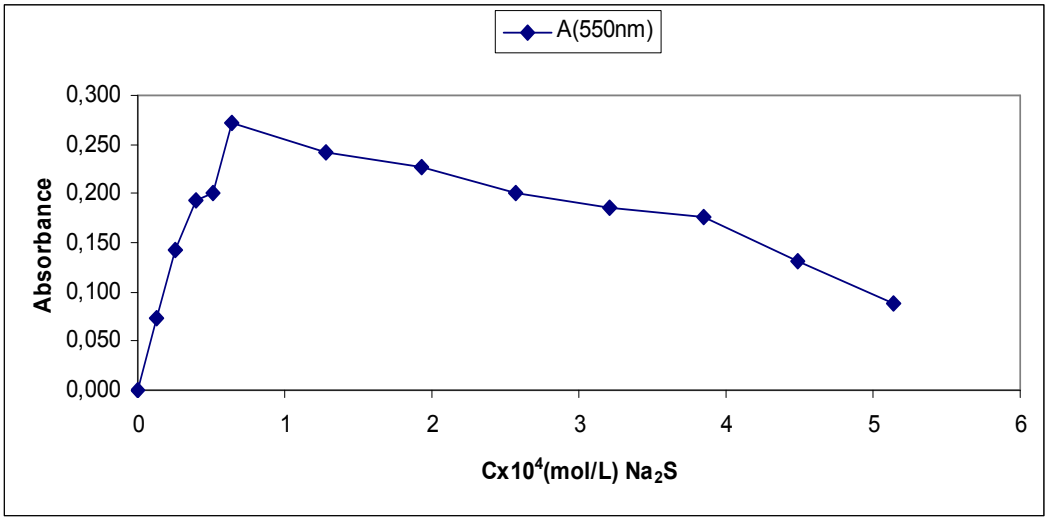

Figure 3.12 Effect of variation of sodium sulphide concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Ce(IV) and Na_2S reagents.

From Table 3.11 and Figure 3.12, it is evident that the absorbance reachs a maximum on using $0{,}64x10^{-4}$ M Na_2S. Therefore, $0{,}64x10^{-4}$ M Na_2S was determined as an optimum value.

3.2.1.3 The effect of Ce(IV) concentration

In order to study the influence of Ce(IV) concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution, 2,50 mL of 0,0032 M Na_2S. Then 2,50 mL of Ce(IV) stock solution in different concentrations and 2,50 mL of distilled water were added into each flask. The flask was shaked for about 30 s, allowed to stand for 10 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.12 and the graph obtained is shown in Figure 3.13.

Table 3.12 The absorption values obtained from the reaction product with Ce(IV) as an oxidant by using different concentrations of Ce(IV).

Ce(IV) Stock Solution (mol/L)	Ce(IV) (mL)	Distilled Water (mL)	C (mol/L) Ce(IV)	A (550nm)
0,00 x 10^{-3}	0,00	2,50	0,00 x 10^{-4}	0,000
1,20 x 10^{-3}	2,50	2,50	1,20 x 10^{-4}	0,042
1,80 x 10^{-3}	2,50	2,50	1,80 x 10^{-4}	0,060
2,40 x 10^{-3}	2,50	2,50	2,40 x 10^{-4}	0,134
3,00 x 10^{-3}	2,50	2,50	3,00 x 10^{-4}	0,166
3,60 x 10^{-3}	2,50	2,50	3,60 x 10^{-4}	0,210
4,20 x 10^{-3}	2,50	2,50	4,20 x 10^{-4}	0,246
5,40 x 10^{-3}	2,50	2,50	5,40 x 10^{-4}	0,212
6,60 x 10^{-3}	2,50	2,50	6,60 x 10^{-4}	0,242
7,80 x 10^{-3}	2,50	2,50	7,80 x 10^{-4}	0,230
8,82 x 10^{-3}	2,50	2,50	8,82 x 10^{-4}	0,235

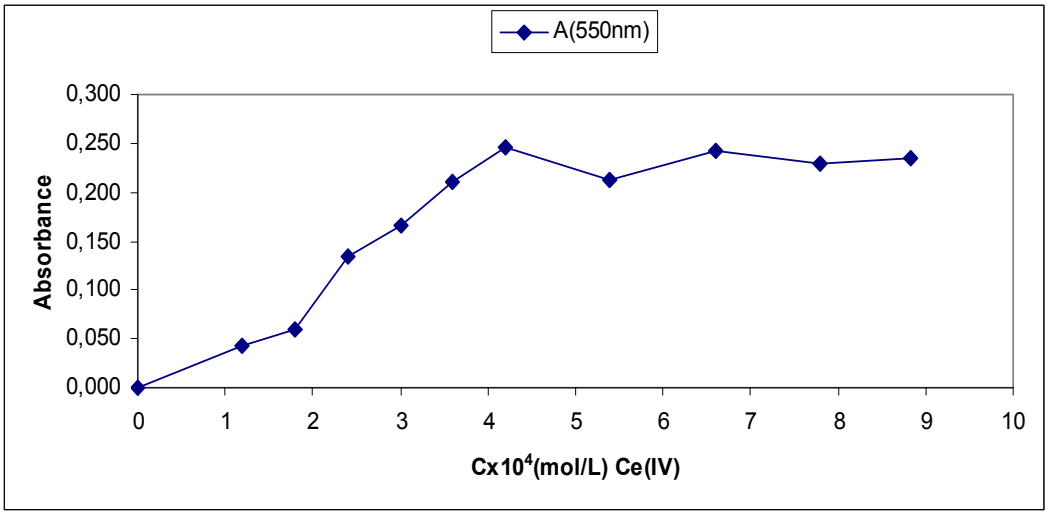

Figure 3.13 Effect of variation of Ce(IV) concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Ce(IV) and Na_2S reagents.

From Table 3.12 and Figure 3.13, it is evident that the absorbance reachs a maximum on using 4,2x10^{-4} M Ce(IV). Therefore, 4,2x10^{-4} M Ce(IV) was determined as an optimum value.

3.2.1.4 The investigation of the stability of methylene blue-like dye with Ce(IV)

In order to obtain the reaction product in optimum conditions, 5,00 mL of 50 ppm PAP solution, 0,50 mL of 0,0032 M Na_2S, 4,40 mL of 0,0024 M Ce(IV) were transferred into a 25 mL volumetric flask. The flask was shaked for about 30 s, allowed to stand for 10 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. The absorption spectra of the reaction product is shown in Figure 3.14.

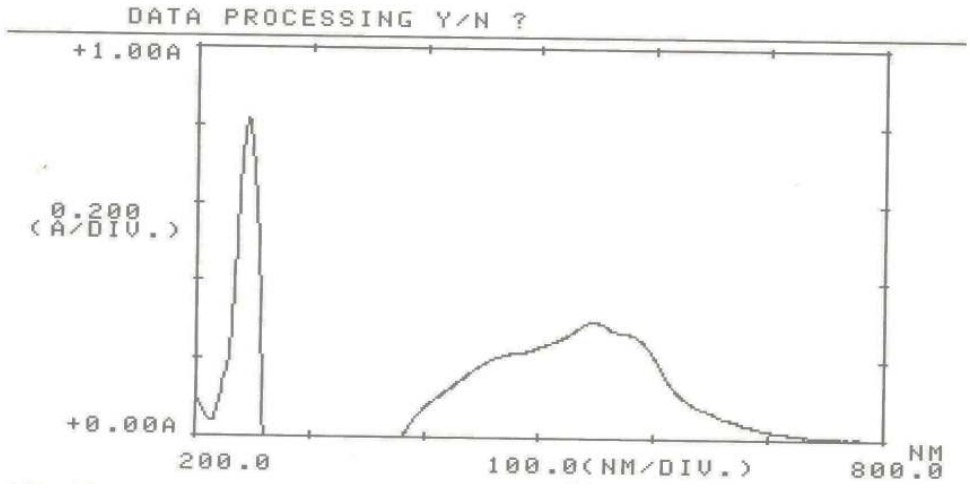

Figure 3.14 The absorption spectra of the reaction product which is obtained by using Ce(IV) as an oxidant in optimum conditions.

Maximum absorbance which belongs to methylene blue-like dye was observed at 550 nm. The measured absorption values of methylene blue-like dye at 550 nm against time are given in Table 3.13 and the graph obtained is shown in Figure 3.15.

Table 3.13 The absorption values of methylene blue-like dye obtained by using optimum values of the reagents against time.

t(min)	A(550nm)
2,0	0,334
4,0	0,336
6,0	0,337
8,0	0,339
10,0	0,339
12,0	0,339
14,0	0,338
16,0	0,338
18,0	0,337
20,0	0,337
22,0	0,337
24,0	0,337
26,0	0,337
28,0	0,337
30,0	0,336
60,0	0,334
90,0	0,333
120,0	0,332
150,0	0,332
180,0	0,331
210,0	0,329
240,0	0,329
270,0	0,329

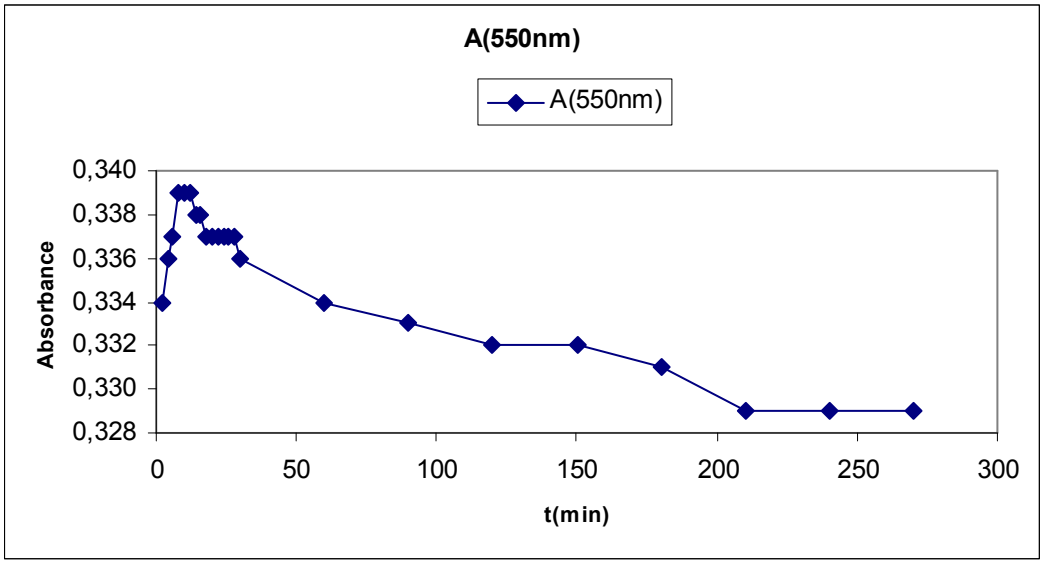

Figure 3.15 Effect of time on absorption intensity of methylene blue-like dye in optimum conditions.

From Table 3.13 and Figure 3.15, the absorption values of the reaction product were seen to remain stable after 20 min for a short time. For this reason, the measurements were done after 20 min.

3.2.1.5 Preparation of the calibration graph with Ce(IV) for the determination of *p*-aminophenol

In order to prepare calibration graph in optimum conditions, the reaction was carried out in a series of 25 mL calibrated flasks containing different volumes of 50 ppm PAP solution. Then distilled water was added into each flask to give total volume of 15,00 mL. 0,50 mL of 0,0032 M Na_2S, 8,30 mL of 0,00126 M Ce(IV) were transferred into the flasks. The flask was shaked for about 30 s, allowed to stand for 10 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.14 and the graph obtained is shown in Figure 3.16.

Table 3.14 The absorption values of the samples (which were prepared in optimum conditions) containing different concentrations of PAP.

PAP (ppm)	A (550nm)
2,0	0,044
4,0	0,072
6,0	0,116
8,0	0,171
10,0	0,283
12,0	0,297
14,0	0,334
16,0	0,344
18,0	0,295
20,0	0,298
22,0	0,340
24,0	0,324
26,0	0,338
28,0	0,295
30,0	0,290

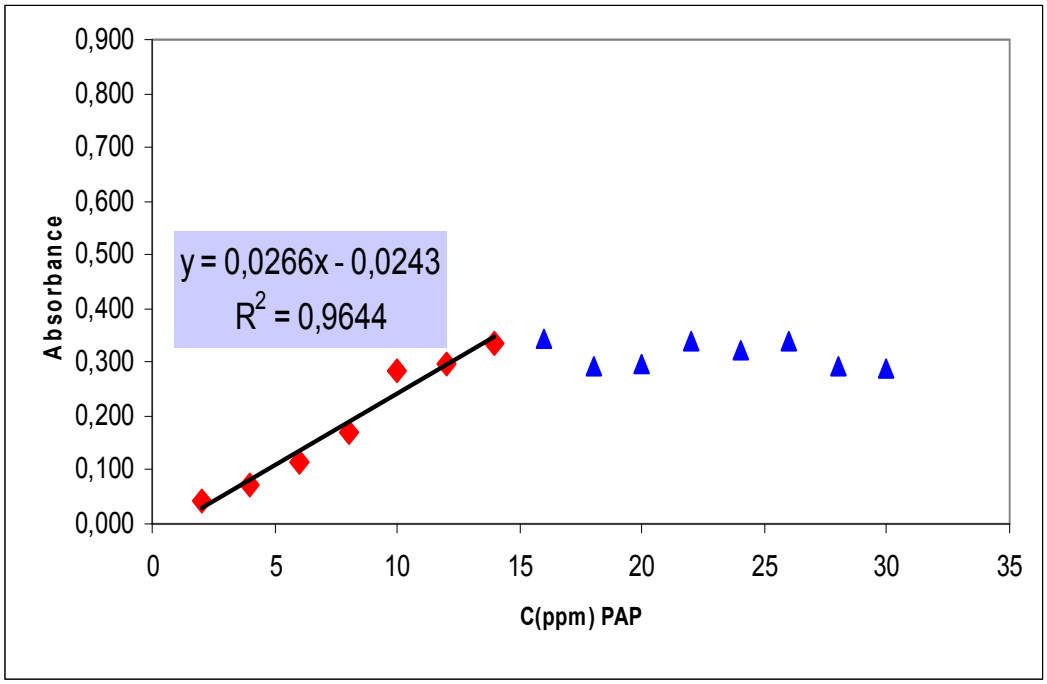

Figure 3.16 The calibration graph prepared with Ce(IV) as an oxidant for the determination of PAP.

The calibration graph shown in Figure 3.16 is linear between 2-14 ppm.

3.2.2 The spectrophotometric determination of *p*-aminophenol oxidized by Fe(III)

The order of reagent addition in the studies carried out with Fe(III) according to the method in question was changed. Because, when we added Fe(III) solution before 0,03 M H_2SO_4, it was observed that the colour of the solution turned brown instead of methylene blue due to the fact that the acidity of the medium was not enough and it caused formation of $Fe(OH)_3$. Because of this, Na_2S solution, 0,03 M H_2SO_4 and Fe(III) solution have to be added to the sample respectively.

3.2.2.1 The effect of sulphuric acid concentration

In order to study the influence of sulphuric acid concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution, 2,50 mL 0,0032 M Na_2S. Then 0,5 M H_2SO_4 or 1,0 M H_2SO_4 and distilled water in different volumes were added into each flask to give total volume of 15,00 mL. Finally 2,50 mL of 0,019 M Fe(III) was transferred into the flask. The flask was shaked for about 30 s, allowed to stand for 25 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.15 and the graph obtained is shown in Figure 3.17.

Table 3.15 The absorption values obtained from the reaction product with Fe(III) as an oxidant by using different concentrations of H_2SO_4.

Distilled Water (mL)	0,5 M H_2SO_4 (mL)	1,0 M H_2SO_4 (mL)	C (mol/L) H_2SO_4	A(550nm)
15,00	-	-	0,003	0,240
14,90	0,10	-	0,005	0,322
14,80	0,20	-	0,007	0,343
14,75	0,25	-	0,008	0,359
14,70	0,30	-	0,009	0,365
14,75	-	0,25	0,010	0,371
14,50	-	0,50	0,020	0,359
14,25	-	0,75	0,030	0,322
14,00	-	1,00	0,040	0,296
13,75	-	1,25	0,050	0,283

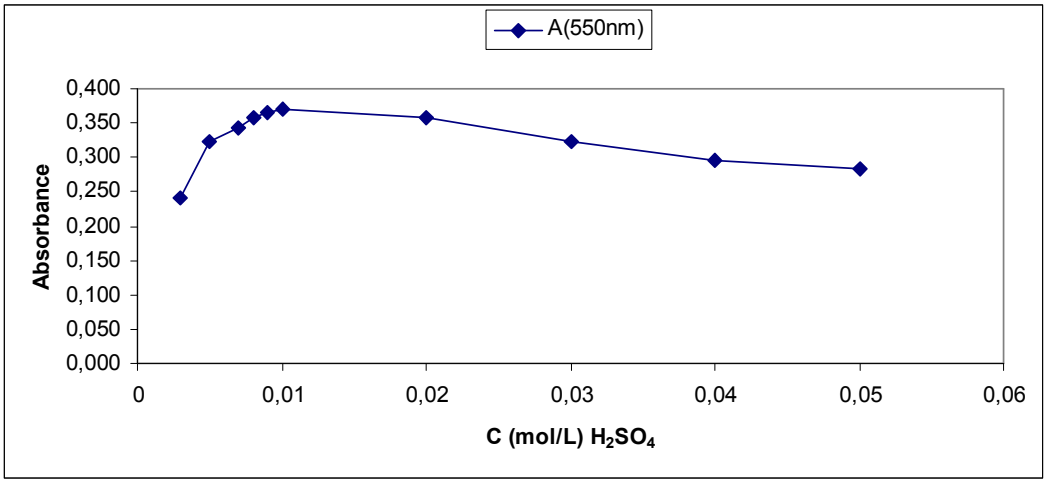

Figure 3.17 Effect of variation of sulphuric acid concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Fe(III) and Na_2S reagents.

From Table 3.15 and Figure 3.17, it is seen that the absorbance reachs a maximum on using 0,01 M H_2SO_4. Therefore, 0,01 M H_2SO_4 was determined as an optimum value.

3.2.2.2 The effect of sodium sulphide concentration

In order to study the influence of sodium sulphide concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution. 0,0032 M Na_2S and distilled water in different volumes were added into each flask to give total volume of 5,00 mL. Then 10,00 mL of 0,03 M H_2SO_4 and 2,50 mL of 0,019 M Fe(III) were transferred into the flasks. The flask was shaked for about 30 s, allowed to stand for 15 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.16 and the graph obtained is shown in Figure 3.18.

Table 3.16 The absorption values obtained from the reaction product with Fe(III) as an oxidant by using different concentrations of Na_2S.

0,0032 M Na_2S (mL)	**Distilled Water(mL)**	**C (mol/L)Na_2S**	**A (550nm)**
0,00	5,00	0,00 x10^{-4}	0,000
0,10	4,90	0,13 x10^{-4}	0,021
0,20	4,80	0,26 x10^{-4}	0,092
0,30	4,70	0,39 x10^{-4}	0,148
0,40	4,60	0,51 x10^{-4}	0,192
0,50	4,50	0,64 x10^{-4}	0,234
1,00	4,00	1,28 x10^{-4}	0,350
1,50	3,50	1,93 x10^{-4}	0,403
2,00	3,00	2,57 x10^{-4}	0,412
2,50	2,50	3,21 x10^{-4}	0,409
3,00	2,00	3,85 x10^{-4}	0,400
3,50	1,50	4,49 x10^{-4}	0,385
4,00	1,00	5,14 x10^{-4}	0,367

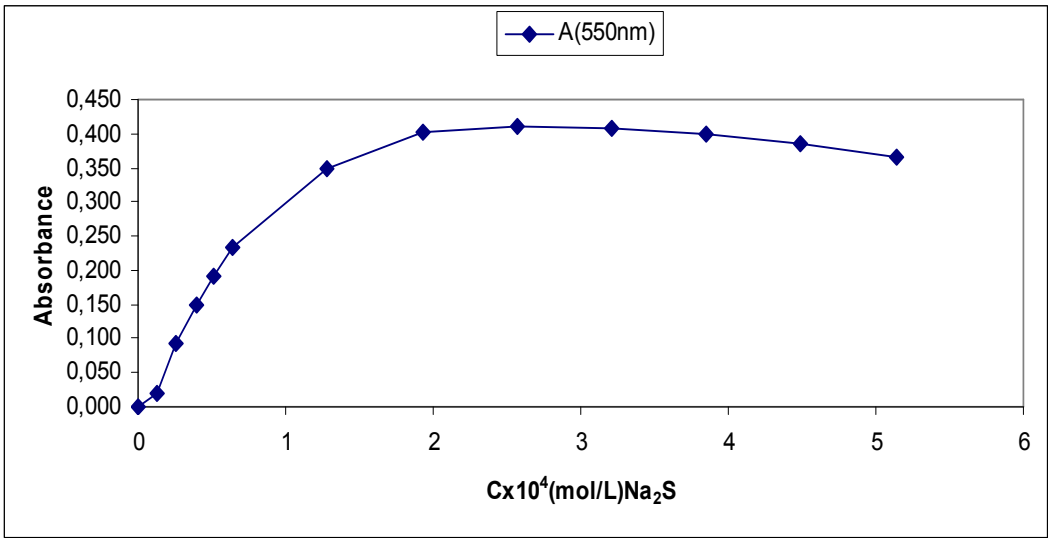

Figure 3.18 Effect of variation of sodium sulphide concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Fe(III) and Na_2S reagents.

From Table 3.16 and Figure 3.18, it is seen that the absorbance reachs a maximum on using $2,57x10^{-4}$ M Na_2S. Therefore, $2,57x10^{-4}$ M Na_2S was determined as an optimum value.

3.2.2.3 <u>The effect of Fe(III) concentration</u>

In order to study the influence of Fe(III) concentration, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution, 2,50 mL of 0,0032 M Na_2S and 10,00 mL of 0,03 M H_2SO_4. 0,019 M Fe(III) and distilled water in different volumes were added into each flask to give total volume of 5,00 mL. The flask was shaked for about 30 s, allowed to stand for 15 min. 0,03 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.17 and the graph obtained is shown in Figure 3.19.

Table 3.17 The absorption values obtained from the reaction product with Fe(III) as an oxidant by using different concentrations of Fe(III).

0,019 M Fe(III) (mL)	Distilled Water (mL)	C (mol/L) Fe(III)	A (550nm)
0,00	5,00	$0,00 \times 10^{-3}$	0,000
0,66	4,34	$0,50 \times 10^{-3}$	0,166
1,45	3,55	$1,10 \times 10^{-3}$	0,306
1,71	3,29	$1,30 \times 10^{-3}$	0,313
1,97	3,03	$1,50 \times 10^{-3}$	0,321
2,50	2,50	$1,90 \times 10^{-3}$	0,311
3,03	1,97	$2,30 \times 10^{-3}$	0,309
3,42	1,58	$2,60 \times 10^{-3}$	0,293
3,95	1,05	$3,00 \times 10^{-3}$	0,289

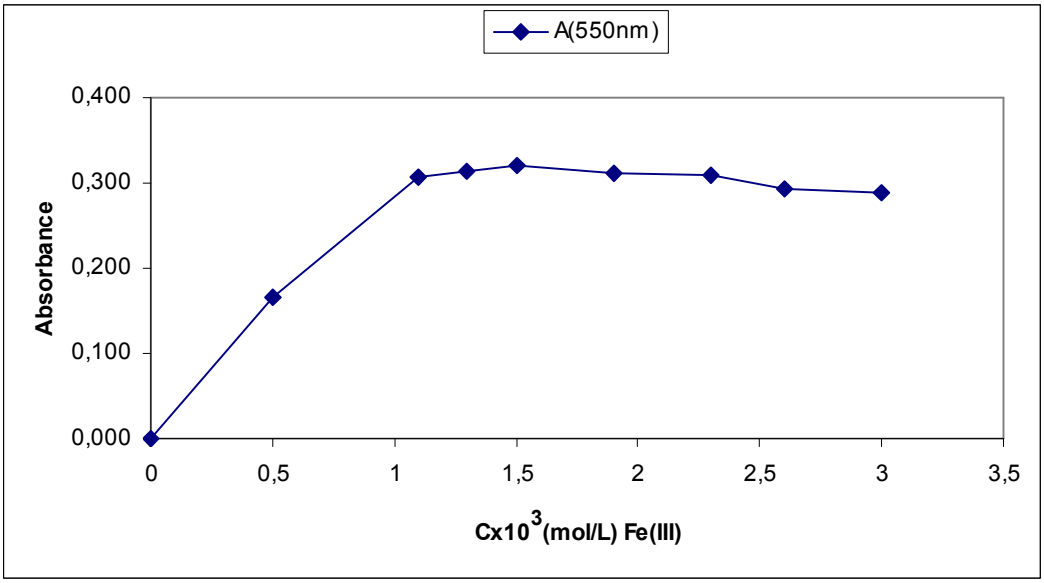

Figure 3.19 Effect of variation of Fe(III) concentration on the absorption intensity of the reaction products of 10 µg mL^{-1} *p*-aminophenol with Fe(III) and Na_2S reagents.

From Table 3.17 and Figure 3.19, it is seen that the absorbance reachs a maximum on using $1,5x10^{-3}$ M Fe(III). Therefore, $1,5x10^{-3}$ M Fe(III) was determined as an optimum value.

3.2.2.4 The investigation of the stability of methylene blue-like dye with Fe(III)

In order to obtain the reaction product in optimum conditions, 5,00 mL of 50 ppm PAP solution, 2,00 mL of 0,0032 M Na_2S, 10,00 mL of 0,01 M H_2SO_4, 1,97 mL of 0,019 M Fe(III) were transferred into a 25 mL volumetric flask. The flask was shaked for about 30 s, allowed to stand for 15 min. 0,01 M H_2SO_4 was added to dilute to volume and it was allowed to stand for a further 10 min. The absorption spectra of the reaction product is shown in Figure 3.20.

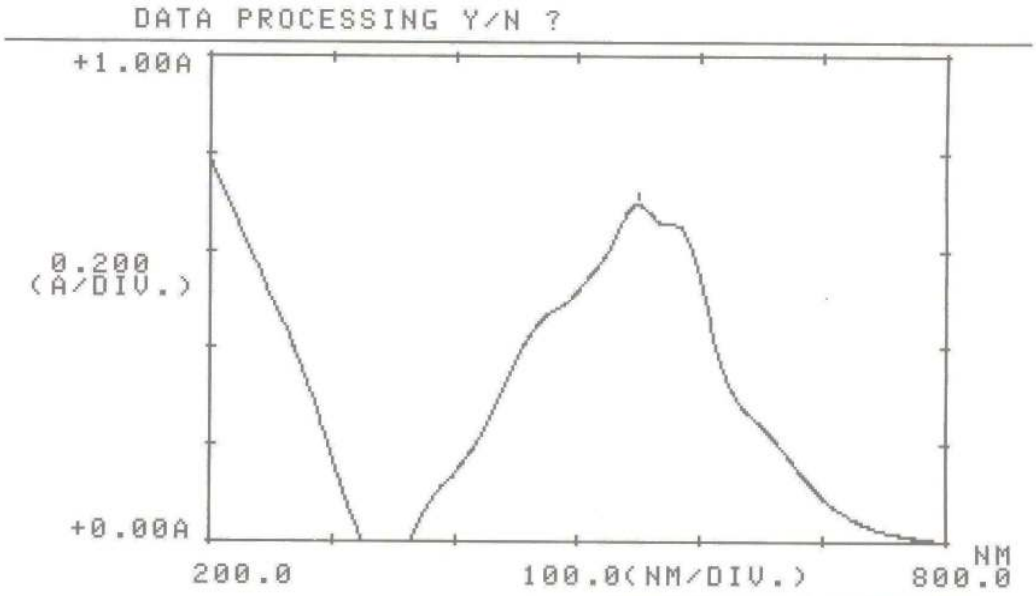

Figure 3.20 The absorption spectra of the reaction product which is obtained by using 28 ppm PAP and Fe(III) as an oxidant in optimum conditions.

Maximum absorbance which belongs to methylene blue-like dye was observed at 550 nm. The measured absorption values of methylene blue-like dye at 550 nm against time are given in Table 3.18 and the graph obtained is shown in Figure 3.21.

Table 3.18 The absorption values of methylene blue-like dye obtained by using optimum values of the reagents against time.

t(min)	A(550nm)
2,0	0,273
4,0	0,294
6,0	0,312
8,0	0,321
10,0	0,324
12,0	0,327
14,0	0,328
16,0	0,330
18,0	0,330
20,0	0,331
22,0	0,330
24,0	0,331
26,0	0,330
28,0	0,330
30,0	0,331
60,0	0,330
90,0	0,329
120,0	0,329
150,0	0,328
180,0	0,327
210,0	0,327
240,0	0,326

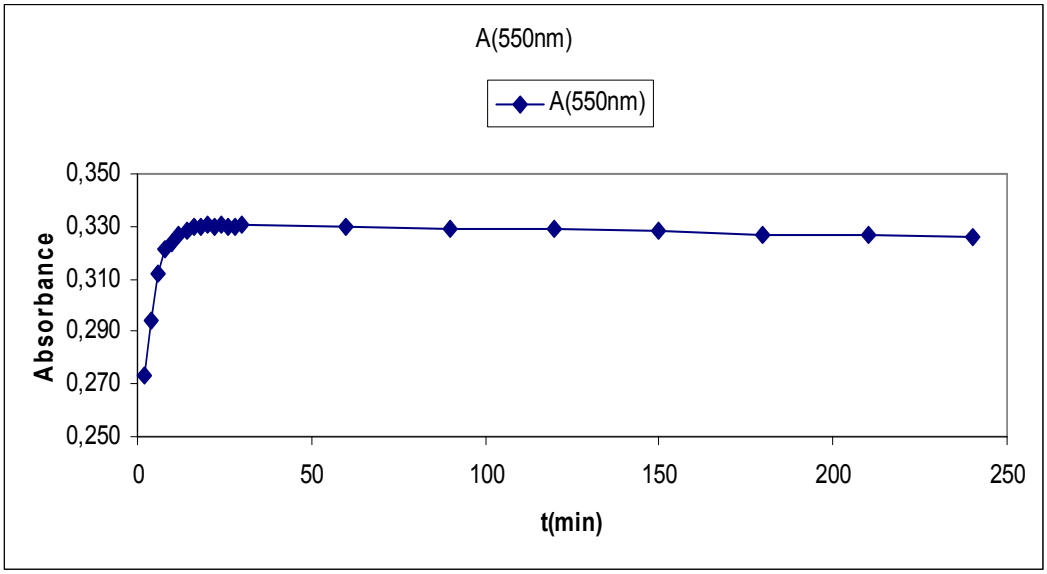

Figure 3.21 Effect of time on absorption intensity of methylene blue-like dye in optimum conditions.

From Table 3.18 and Figure 3.21, the absorption values of the reaction product were seen to remain stable after ~25 min for at least 2 h. For this reason, the measurements were done after 25 min.

3.2.2.5 Preparation of the calibration graph with Fe(III) for the determination of *p*-aminophenol

In order to prepare calibration graph in optimum conditions, the reaction was carried out in a series of 25 mL calibrated flasks containing different volumes of 50 ppm PAP solution. 2,00 mL of 0,0032 M Na_2S, 6,03 mL of 0,01 M H_2SO_4, 1,97 mL of 0,019 M Fe(III) were transferred into the flasks. Then distilled water was added into each flask to give total volume of 15,00 mL with PAP solution (PAP solution +distilled water= 15,00 mL). The flask was shaked for about 30 s, allowed to stand for 25 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.19 and the graph obtained is shown in Figure 3.22.

Table 3.19 The absorption values of the samples (which were prepared in optimum conditions) containing different concentrations of PAP**.**

C(ppm)PAP	A(550nm)
2,0	0,030
4,0	0,080
6,0	0,124
8,0	0,189
10,0	0,254
12,0	0,315
14,0	0,374
16,0	0,436
18,0	0,494
20,0	0,549
22,0	0,612
24,0	0,655
26,0	0,696
28,0	0,736
30,0	0,783

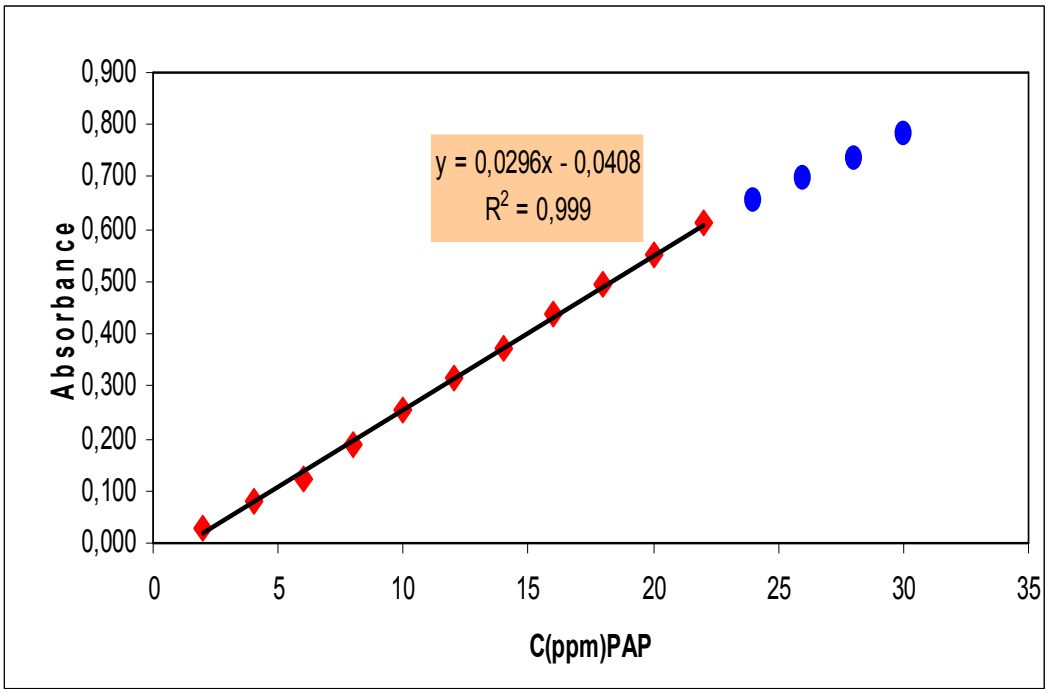

Figure 3.22 The calibration graph prepared with Fe(III) as an oxidant for the determination of PAP.

The calibration graph shown in Figure 3.22 is linear between 2-22 ppm.

3.3 The Studies with Thioacetamid Instead of Sodium Sulfide

In order to determine the effect of thioacetamid instead of sodium sulphide on the absorption intensity of the coloured product, the reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution. $3{,}21 \times 10^{-3}$ M thioasetamid in different volumes and $2{,}7 \times 10^{-2}$ M Fe(III) in different volumes were transferred into the flasks. The flask was shaked for about 30 s, allowed to stand for 15 min. Then distilled water or 0,01 M H_2SO_4 were added into each flask to give total volume of 25 mL and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption spectra of the reaction product is shown in Figure 3.23.

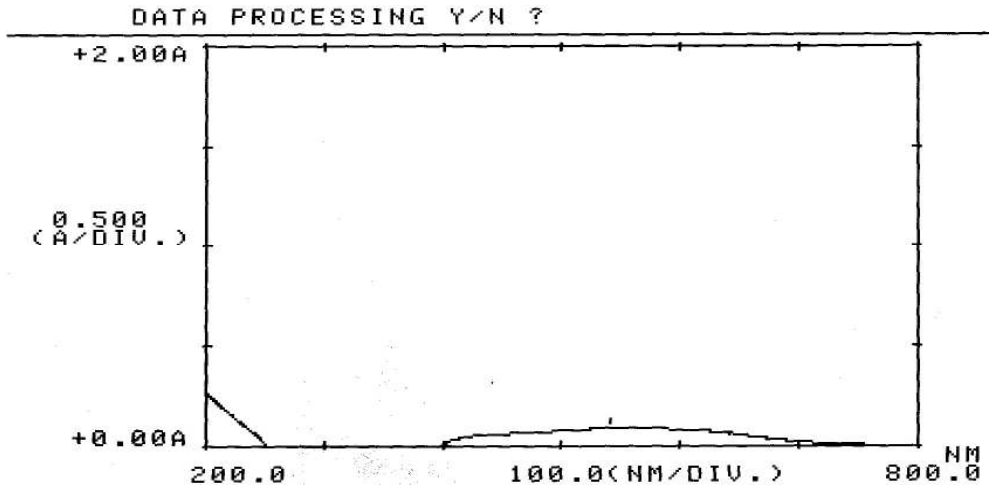

Figure 3.23 The absorption spectra of the reaction product which is obtained by using thioacetamid instead of Na_2S.

From Figure 3.23, it is seen that the absorbance of the reaction product at ~550 nm is very low. The absorption values are given in Table 3.20.

Table 3.20 The absorption values of coloured reaction product which is obtained by using thioacetamid instead of Na_2S.

3,21x 10^{-3} M Thioacetamid (mL)	**2,7x 10^{-2} M Fe(III) (mL)**	**Distilled Water (mL)**	**0,01 M H_2SO_4**	**C (mol/L) Thioacetamid**	**A (550nm)**
1,00	2,50	16,5	-	1,28 x 10^{-4}	0,085
2,00	2,50	15,5	-	2,57 x 10^{-4}	0,080
3,00	2,50	14,5	-	3,85 x 10^{-4}	0,072
4,00	2,50	13,5	-	5,14 x 10^{-4}	0,070
5,00	2,50	12,5	-	6,42 x 10^{-4}	0,067
2,50	1,00	16,5	-	3,21 x 10^{-4}	0,082
2,50	2,00	15,5	-	3,21 x 10^{-4}	0,079
2,50	3,00	14,5	-	3,21 x 10^{-4}	0,082
2,50	4,00	13,5	-	3,21 x 10^{-4}	0,081
2,50	5,00	12,5	-	3,21 x 10^{-4}	0,082
1,00	2,50	-	16,5	1,28 x 10^{-4}	0,088
2,00	2,50	-	15,5	2,57 x 10^{-4}	0,085
3,00	2,50	-	14,5	3,85 x 10^{-4}	0,073
4,00	2,50	-	13,5	5,14 x 10^{-4}	0,070
5,00	2,50	-	12,5	6,42 x 10^{-4}	0,066

Because of the fact that low absorption intensities were obtained, it was decided that the studies related to thioacetamid must be developed.

3.4 The Determination of *p*-Aminophenol in The Presence of Paracetamol

The reaction was carried out in a series of 25 mL calibrated flasks containing 5,00 mL of 50 ppm PAP solution. 50 ppm paracetamol in different volumes, 2,00 mL of $3{,}21 \times 10^{-3}$ M Na_2S, 10,00 mL of 0,01 M H_2SO_4 and 1,40 mL of $2{,}7 \times 10^{-2}$ M Fe(III) were transferred into the flasks. The flask was shaked for about 30 s, allowed to stand for 15 min. Then 0,01 M H_2SO_4 was added into each flask to give total volume of 25 mL and it was allowed to stand for a further 10 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.21 and the graph obtained is shown in Figure 3.23.

Table 3.21 The absorption values of coloured reaction product of 10 µg mL^{-1} *p*-aminophenol which are obtained by adding increasing amount of paracetamol to the samples.

50 ppm Paracetamol (mL)	C(ppm) Parasetamol	A(550nm)
0,00	0,0	0,190
1,00	2,0	0,191
2,00	4,0	0,197
3,00	6,0	0,200
4,00	8,0	0,192
5,00	10,0	0,201
6,00	12,0	0,198

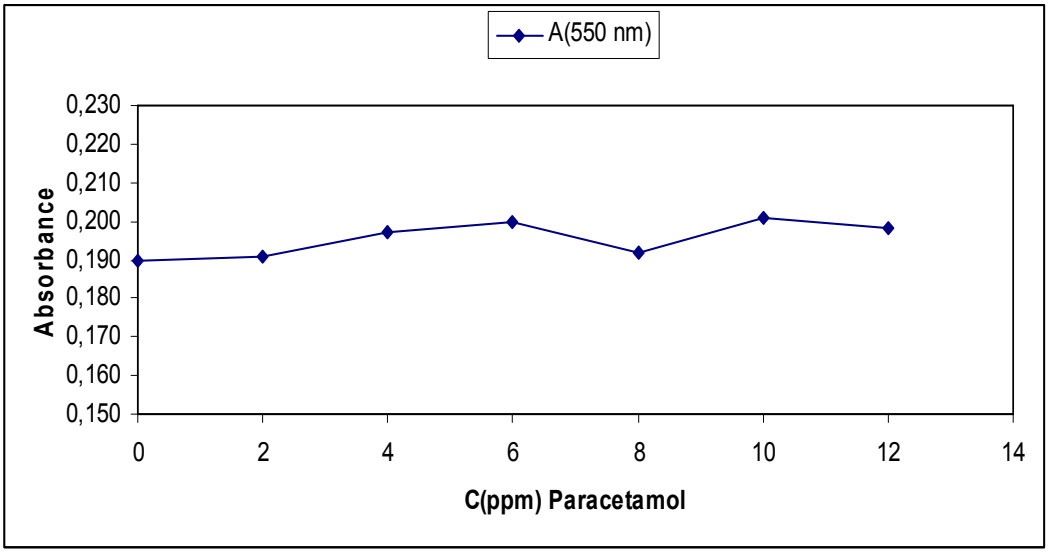

Figure 3.24 The effect of increasing paracetamol on the absorption intensity of the coloured reaction product.

The proposed method was applied in order to evaluate the applicability to the determination of *p*-aminophenol in the presence of paracetamol. The method allowed the determination of *p*-aminophenol in the presence of paracetamol without prior seperation. Even though the amount of paracetamol increased, the absorbance value of the coloured reaction product didn't change.

3.5 Determination of PAP in The Presence of Paracetamol in Drugs

In order to determine PAP content in drugs in the presence of paracetamol, PAP content of a tablet containing 500 mg paracetamol/tablet was tried to be found without hydrolysing paracetamol.

For this purpose, 6 tablets were weighed (3,5812 g) and powdered. The amount of powder equivalent to 100 mg of paracetamol was weighed (0,1194 g) and transferred into a 100 mL volumetric flask, diluted to volume with distilled water and filtered though black band fitler-paper.

The reaction was carried out in a 25 mL calibrated flask containing 5,00 mL of the above-mentioned sample. 2,00 mL of 0,0032 M Na_2S, 10,00 mL of 0,01 M H_2SO_4, 1,40 mL of 0,027 M Fe(III) were transferred into the flask. The flask was shaked for about 30 s, allowed to stand for 15 min. Then 0,01 M H_2SO_4 was added into the flask to give total volume of 25 mL and it was allowed to stand for a further 10 min. Then the absorbance was tried to measure at 550 nm against the reagent blank. But any absorbance at 550 nm could be measured.

Because of the fact that the coloured reaction product didn't form, it was understood that the tablet didn't contain PAP in the level that we could observe in the presence of paracetamol.

3.6 Determination of Paracetamol in Drugs

The method was applied to the drug tablets containing 500 mg paracetamol /tablet.

3.6.1 Preparation of the calibration graph for the paracetamol analysis in drugs

100 mg of pure paracetamol was weighed and transferred into a beaker. 20,00 mL of 5 M H_2SO_4 was added in it and it was placed in a boiling water-bath for 30 min, then cooled, diluted to volume with distilled water in a 100 mL volumetric flask. (Thus, paracetamol was hydrolysed to *p*-aminophenol.) Then the solution was diluted with distilled water in order to obtain a concentration of 50 ppm.

The reaction was carried out in a series of 25 mL calibrated flasks containing different volumes of 50 ppm PAP solution. 2,00 mL of 0,0032 M Na_2S, 6,60 mL of 0,01 M H_2SO_4, 1,40 mL of 0,027 M Fe(III) were transferred into the flasks. Then distilled water was added into each flask to give total volume of 15,00 mL with PAP solution (PAP solution +distilled water= 15,00 mL). The flask was shaked for about 30 s, allowed to stand for 25 min. Then the absorbance was measured at 550 nm against the reagent blank. The absorption values are given in Table 3.22 and the graph obtained is shown in Figure 3.25.

Table 3.22 The absorption values of the samples (which were prepared by the hydrolysis of paracetamol to *p*-aminophenol) containing different concentrations of PAP.

C(ppm)PAP	A(550nm)
1,44	0,035
2,89	0,064
4,33	0,104
5,78	0,148
7,22	0,191
8,66	0,224
10,11	0,255
11,55	0,274
13,00	0,330
14,44	0,350
15,88	0,374
17,33	0,400
18,77	0,402
20,21	0,435
21,66	0,455

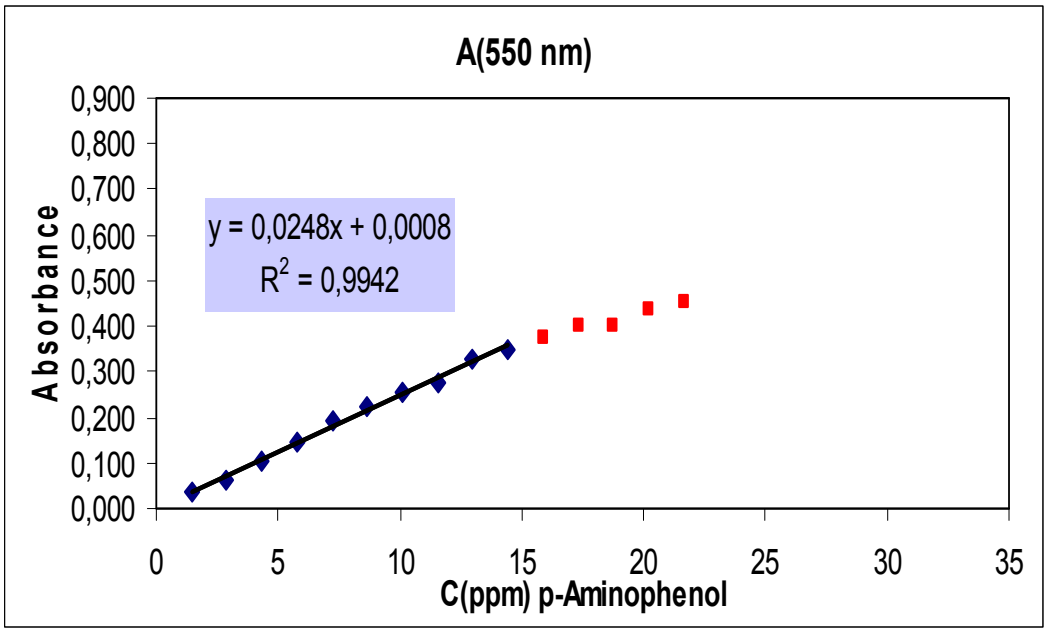

Figure 3.25 The calibration graph for the determination of paracetamol in drugs

The calibration graph shown in Figure 3.25 is linear between 1,44-14,44 ppm.

3.6.2 Determination of paracetamol content in the tablet

In order to determine the amount of paracetamol in tablets, 6 tablets were weighed (3,5822 g) and powdered. The amount of powder equivalent to 100 mg of paracetamol was weighed (0,1194 g) and transferred into a beaker. 20,00 mL of 5 M H_2SO_4 was added in it and it was placed in a boiling water-bath for 30 min, then cooled, diluted to volume with distilled water in a 100 mL volumetric flask and filtered through blue-band filter- paper. (Thus, paracetamol was hydrolysed to *p*-aminophenol.) Then the solution was diluted with distilled water in order to obtain a concentration of 50 ppm.

The reaction was carried out in 25 mL of calibrated flask containing 5,00 mL of 50 ppm the above-mentioned solution. 2,00 mL of 0,0032 M Na_2S, 10,00 mL of 0,01 M H_2SO_4 and 1,40 mL of 0,027 M Fe(III) were transferred into the flask. The flask was shaked for about 30 s, allowed to stand for 15 min. Then 0,01 M H_2SO_4 was added into the flask to give total volume of 25 mL and it was

allowed to stand for a further 10 min. Then the absorbance of the sample was measured at 550 nm against the reagent blank. The tests were repeated 5 times by using the same powdered sample. The absorption values were found as follows; 0,200, 0,203, 0,215, 0,208, 0,208.

From the calibration graphs (Figure 3.22 and Figure 3.25), 388.60± 0.4 and 575.50±15 mg paracetamol/ tablet were found in the tablet by using the above-mentioned absorption values which belong to 5 samples.

The different results show that the hydrolysis of paracetamol didn't complete.

4. CONCLUSION

According to the stability tests of PAP aqueous and ethanol solution, it was shown that the ethanol solution of PAP is more stable than the aqueous solution of PAP and the most stable condition was obtained in the medium with nitrogen(without O_2) in the dark. Because of the fact that the aqueous solution of PAP is suitable for study conditions (From Table 3.9, it is seen that PAP can remain stable ~4 h in the medium with O_2 in the light), the aqueous solution of PAP was used through the study.

This study demonstrated that PAP could be determined in the presence of paracetamol by using the proposed spectrophotometric method (Mohamed et al., 1997). For this purpose, Ce(IV) and Fe(III) were used as oxidants. The optimum conditions for Ce(IV) were found as follows: $4{,}2x10^{-4}$ M Ce(IV), $6{,}4x10^{-5}$ M Na_2S, ~0,03 M H_2SO_4. The optimum conditions for Fe(III) were found as follows: $1{,}5x10^{-3}$ M Fe(III), $2{,}57x10^{-4}$ M Na_2S, 0,01 M H_2SO_4. The highest absorption intensities were obtained by using Fe(III). The calibration graphs were obtained linear between 2-14 ppm and 2-22 ppm using Ce(IV) and Fe(III), respectively. It was seen that PAP could be determined in these linear ranges.

It was understood that PAP could be determined with the proposed method in the presence of paracetamol because of the fact that paracetamol didn’t give a reaction similar to PAP and the determination of paracetamol could be carried out indirectly with the so-called method after the hydrolysis of paracetamol to PAP.

The proposed method is rapid, simple,sensitive and cheap according to many other methods but has some disadvantages. The reproducibility of the method is low and the working concentration range is narrow. All the reagent solutions except Fe(III) solution must be must be daily prepared and kept in the dark.

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