# THE KINETIC BEHAVIOUR OF PHENYLBUTAZONE

# IN VARIOUS SOLVENT SYSTEMS

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## ABSTRACT

The kinetic behaviour of the degradation of phenylbutazone in four solvent systems suitable for injection was investigated. The rate constants for the degradation reactions were determined and shelf-lives for the various products were predicted.

Chromatography was used to give tentative identifications of degradation products and one such product was positively identified using various analytical techniques.

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The kinetic behaviour of phenylbutazone in four solvent systems suitable for injection was investigated. The rate constants for the degradation reactions were determined and shelf-lives for the various products were predicted.

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# 1. INTRODUCTION

## 1.1 History

Phenylbutazone, 1,2-diphenyl-3,5-diketo-4-n-butyl-pyrazolidine, was first synthesized by H. Stenzl in 1946[1]. Initially, it was used to increase the solubility of aminopyrine in an injectable form. Investigators noticed that patients with rheumatoid arthritis receiving aminopyrine injection showed a better response than those receiving oral dosage forms. This observation led to the discovery that phenylbutazone was useful in the treatment of rheumatic diseases. The drug was introduced into therapy in 1948 by Wilhelmi and Domenjoz, Pulver, and Gsell and Müller[2].

## 1.2 Pharmacology[1,3,4]

Phenylbutazone is used most effectively in therapy of rheumatic and acute inflammatory diseases. Many authors have reported excellent response in rheumatic fever treated with phenylbutazone. Acute attacks of gout have also been successfully treated. Perhaps the most common use of the drug is in rheumatoid arthritis. Here treatment is most effective in the early stages of the disease. Dramatic effects have also been obtained from use in ankylosing spondylitis.

Oral dosage in humans is generally 300-600 mg initially, with a maintenance dose of 100-400 mg. Oral veterinary doses are 2-4 g/1000 lb for horses and 100 mg/5 lb for dogs. In humans, doses above 800 mg are seldom justified since the blood level tends to reach a maximum and then

level off despite increases in dosage[1]. This may be due to increased metabolism caused by the increase in concentration of free drug in the plasma when protein binding capacity is exceeded.

The drug is rapidly and completely absorbed, probably from the small intestine, causing the concentration in the plasma to reach a maximum in approximately two hours. Phenylbutazone has a relatively long biological half-life, approximately 72 hours. The drug exists in the enol form in the blood and is highly protein bound.

Phenylbutazone is metabolized in the liver (Figure 1.5) and excreted in the urine with only traces remaining unchanged.

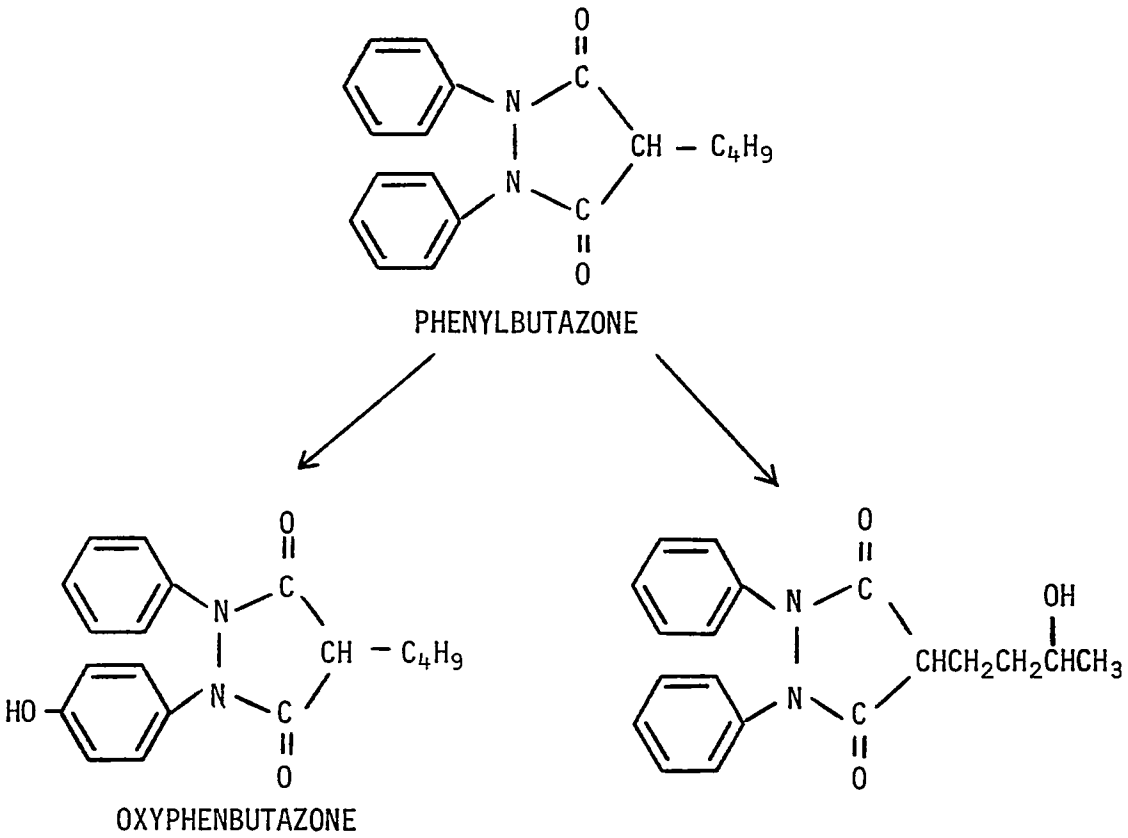



Figure 1.1: Metabolic Transformations of Phenylbutazone

Side effects most often reported are salt and water retention resulting in edema, nausea, rash and irritation of gastric mucosa sometimes resulting in ulceration. There have also been reports of gastric hemmorrhage, cardiac failure, thrombocytopenia and agranulocytosis. Fortunately these more serious complications are rare. The incidence of side effects as reported from several authors is estimated at approximately 15% of the patients treated. Treatment had to be discontinued in approximately one-half of these cases.

## 1.3 Chemical and Physical Properties[1]

The structure of phenylbutazone is shown in Figure 1.2.

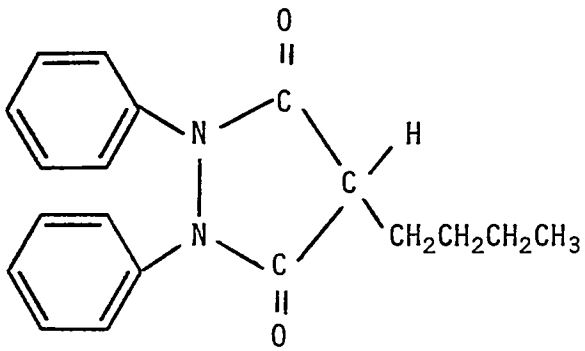



Figure 1.2: Structure of 1,2-diphenyl-3,5-diketo-4-n-butyl pyrazolidine

It is manufactured by means of a condensation reaction between hydrazobenzene and the diethyl ester of n-butylmalonic acid (Figure 1.3).

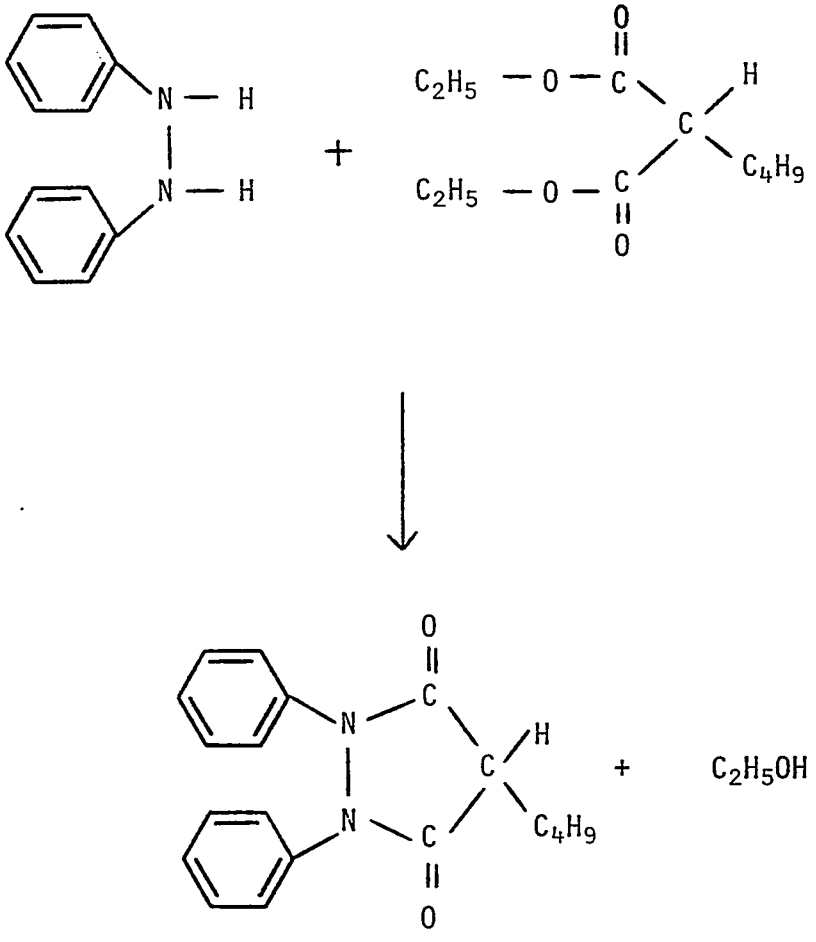


Figure 1.3: Condensation of Hydrazobenzene and the Diethyl Ester of n-butylmalonic acid

Phenylbutazone exhibits distinctly acidic properties, forming water-soluble salts with sodium, potassium, lithium, calcium, magnesium, strontium, dimethylaminoethanol, and diethylaminoethanol by virtue of the -CO-CH-CO- grouping. It exists as colourless crystals (mp 104.5° - 106.5°) which are soluble in acetone, ethanol and ethyl acetate and sparingly soluble in water.

Infra-red spectra as a Nujol mull and chloroform solution reveal a doublet at 5.70 and 5.82 μ indicating the presence of two >C=O groups. Thus in the absence of bases the compound exists predominantly in the diketone form. However, various authors have published work which indicates that it exists as a monovalent anion in the blood.

Ultraviolet spectra of phenylbutazone are documented for solutions in slightly acidic methanol (λ max = 240 mμ, log ε = 4.19) and dilute alkali (λ max = 264 mμ, log ε = 4.32). In this work spectra were run from 200 to 450 mμ in 95% ethanol using a Unicam SP 800 spectrophotometer (λ max = 240 mμ, log ε = 4.14). A sample spectrum for a solution of 0.01 mg/ml in 95% ethanol is given in Figure 1.4.

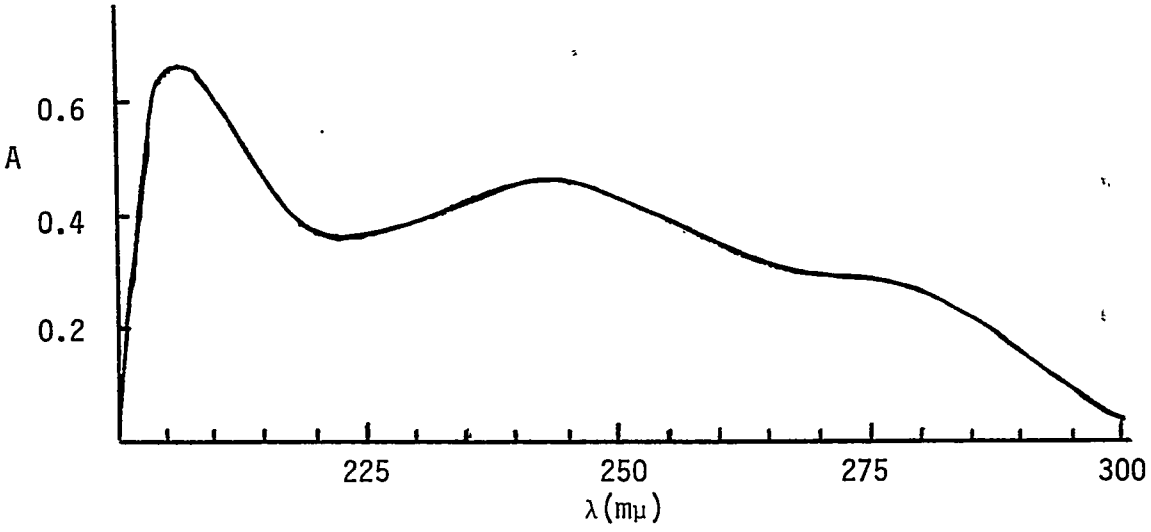



Figure 1.4: Absorption Spectrum of Phenylbutazone in Ethanol (0.01 mg/ml)

## 1.4 Assay

Various procedures have been suggested for the assay of phenylbutazone in the presence of decomposition products. The official method given in the B.P. 1963[5] and N.F. XII[6] employs titration by NaOH. This method suffers from the disadvantage that several of the decomposition products identified in dosage forms are also acidic and would therefore lead to higher than actual assay results (Figure 1.5). Spectrophotometric analysis is similarly non-specific since degradation products such as the carboxylic acid (II), α-hydroxy carboxylic acid (III), 4-hydroxyphenylbutazone (IV), and n-caproyl hydrazobenzene (V), can interfere with the U.V. assay[7].

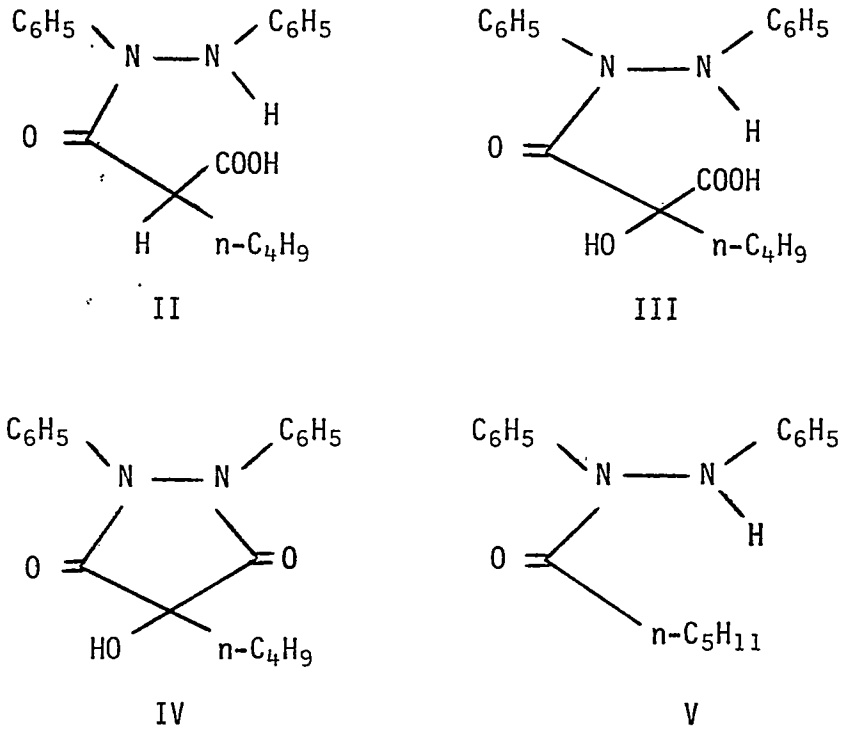



Figure 1.5: Degradation Products of Phenylbutazone

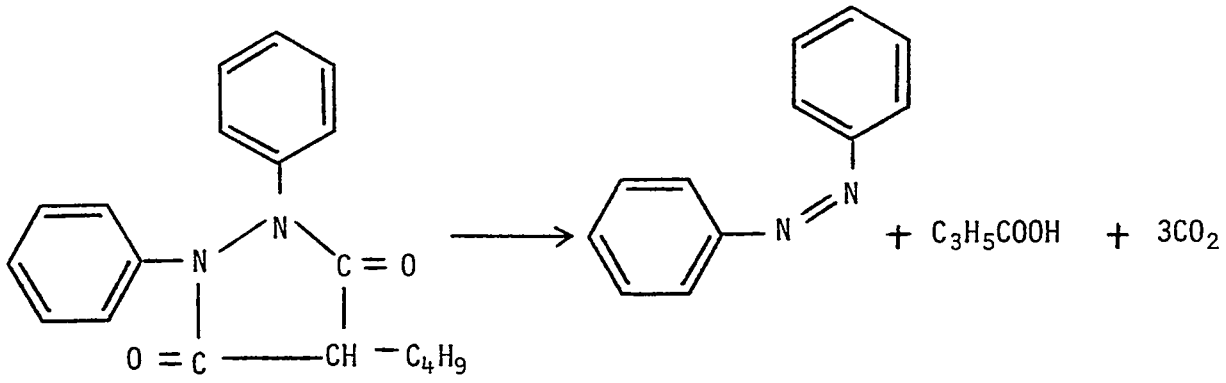
Other assay methods tried have been polarography by Kalvoda and Zyka, hydrolysis followed by colour formation of the products by Pulver, Pemberton, Hochlein and Magiorelli et al, colourimetry, halometry and gravimetry[7].

Qualitative determination of the drug and its decomposition products was carried out using thin-layer chromatography by Pawelczyk, Wachowiak and Romanowski. The plates were developed for 45 min in a solvent of cyclohexane-trichloroethylene-acetone (9:8:3) and spots were visualized by spraying with 0.1 M $Ce_2SO_4$ in 2 N $H_2SO_4$[8].

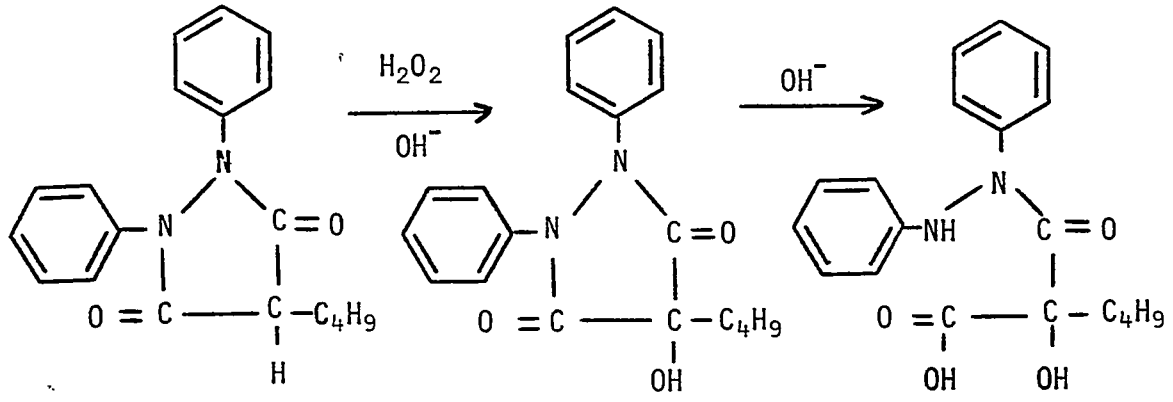
Beckstead, Kaistha and Smith employed an ultraviolet assay using simultaneous equations to calculate the amount of drug present in a sample containing degradation products. This method is complicated by the fact that more than one degradation product is usually present. Thus an estimation of the absorptivity of the combined degradation products must be decided upon and this introduces an additional error. These authors also refer to a method of developing thin-layer chromatograms using a solvent of cyclohexane-chloroform-methanol-glacial acetic acid (60:30:5:5) and a chlorine / o-toluidine spray reagent[7].

## 1.5 Degradation and Stability

Several authors have studied the products formed from decomposition of phenylbutazone. Awe and Kienert reported the products formed by oxidation processes[9]. In acidic permanganate they found the reaction to be:




In alkaline hydrogen peroxide solution they report the following reaction:




Pawelczyk, Wachowiak and Romanowski have reported a method

of qualitative analysis of decomposition products of phenylbutazone preparations[8]. They employed a method of thin-layer chromatography on a silica gel G layer impregnated with acidic $Bi(NO_3)_3$ solution. Chromatograms were developed in cyclohexane-trichloroethylene-acetone (9:8:3) and visualized by spraying with cerium sulfate in 2 N $H_2SO_4$.

In a recent paper Schmid identified phenylbutazone breakdown products as 4-hydroxyphenylbutazone (IV) and the carboxylic acid (II) formed by reversible hydrolysis[10]. He stated that IV is the main breakdown product in suppositories and II in injectables. In ampoules the hydroxy acid (III) is also formed by oxidation to 4-hydroxyphenylbutazone and subsequent hydrolysis. After heating ampoules for one hour at 120°C a further product V is formed, the decarboxylation product of II.

As mentioned earlier, Beckstead et al identified these same degradation products by use of a chromatographic method on silica gel GF[7].

Schmid has dealt with the kinetics of phenylbutazone degradation in a recent paper[11]. Here he postulated that the reaction mechanism of the degradation reaction is of the type:

$$I \underset{k_2}{\overset{k_1}{\rightleftarrows}} II$$

at low temperatures and:

$$I \underset{k_2}{\overset{k_1}{\rightleftarrows}} II \xrightarrow{k_3} V$$

at high temperatures. He reported that reaction rate and the position of equilibrium depend on the solvent but not on the pH. The temperature dependence and rate constants are given for a number of different media. In addition he has determined the effects of substitution in the 4-position and the 4'- and 4"-positions on the reaction rates.

## 2. DEFINITION OF THE PROBLEM

Initially, the problem was to develop a stable injectable form of phenylbutazone suitable for use in veterinary medicine. In the first phase of the work an aqueous vehicle, employing propylene glycol and sodium bicarbonate, was investigated. Because of the difficulty of dissolving the drug in such a vehicle, solubilization was attempted; and because it was felt that the aqueous system might be contributing to the instability of the product, several non-aqueous systems were tried. The three non-aqueous vehicles that were finally tested were diethyl carbonate, N,N-dimethyl acetamide and N,N-dimethyl formamide. The problem therefore became one of testing the stability of the drug in these four systems to determine the best vehicle for the formulation of an injectable solution.

A major portion of the work was involved in selecting a suitable assay technique which could be used with confidence in the presence of degradation products.

## 3. PRELIMINARY INVESTIGATION

Initial studies were carried out on an injectable solution with a concentration of 100 mg phenylbutazone per ml. The vehicle was propylene glycol (60%) in water with 3% (w/v) $NaHCO_3$ added.* A straight UV assay was employed with no adjustment made to account for interference due to degradation products.

It was found that on boiling ampoules for various lengths of time, full-strength solutions exhibited what appeared to be zero-order kinetics while dilute solutions showed first-order kinetics. It is now known that this "change" in kinetics is due to the increased interference of the degradation products with the assay technique.

The effect of pH was also investigated and it appeared that there was little change in stability as pH was increased from 7.6 to 9.5.

On storing at various temperatures the full-strength product again exhibited zero-order kinetics due to the inadequacy of the assay technique.

Since the solution developed a yellow colour on storage, various antioxidants were tried in an effort to eliminate colour development. The antioxidants tried were maleic acid, propyl gallate, sodium bisulfite, sodium metabisulfite, thiourea and monothioglycerol. Although none of these substances appeared to solve the problem, monothioglycerol did inhibit colour development somewhat.

* pH 7.6

## 4. FORMULATION OF SEVERAL INJECTIONS

### 4.1 Solubilizing with N-methyl Glucamine

Since several authors have reported an increase in water solubility of a number of compounds on addition of N-methyl glucamine (1-deoxy-1-methylamino glucitol), it was felt that perhaps the solubility of phenylbutazone might be increased in this way.

Nakanishi and Suzuki reported an increase in solubility of N-(p-hydroxyphenyl) salicylamide used as an injectable choleretic[12].

Lim, Thompson and Dosi have noted an increase in solubility of phenobarbital which they attribute to salt formation or complexation[13].

Nakatani has suggested that the increased solubility of orotic acid may be explained by formation of a 1:1 complex[14].

Finally, coumarin has been formulated for oral or parenteral administration by addition of 2-25 moles N-methyl glucamine per mole of drug[15].

Unfortunately, when this procedure was tried with phenylbutazone, it was unsuccessful. Solubility appeared to be unaffected and 3% (w/v) $NaHCO_3$ still had to be added to the 60% propylene glycol-water vehicle to dissolve phenylbutazone to a concentration of 100 mg/ml. In addition the N-methyl glucamine used (practical grade) imparted a haziness to the solution which could only be removed by repeated filtration through a medium sintered glass funnel with a talc filter bed. This haziness persisted even after the N-methyl glucamine was recrystallized.

## 4.2 Solution in Several Organic Solvents

Since the solubility of phenylbutazone in water is so low it was felt that a more versatile injection might be formulated using organic solvents as vehicles. The dimethylated amides of formic and acetic acid are well known as almost universal solvents and so solution in N,N-dimethyl formamide and N,N-dimethyl acetamide as well as diethyl carbonate was attempted. Phenylbutazone dissolves readily in all three of these solvents thus forming injections which are very easy to prepare accurately at any concentration level.

## 5. TOXICITY OF NON-AQUEOUS SOLVENT SYSTEMS USED

Since organic solvents carry an inherent risk of toxicity, a literature search was conducted to assess the toxicity of dimethylformamide (DMF), dimethylacetamide (DMA), and diethyl carbonate (DEC).

Stasenkova has investigated the toxicity of dimethylformamide in rats and mice[16]. He found that the minimum lethal dose of DMF injected subcutaneously in rats and mice was 1 g/Kg and the $LD_{50}$ was 3.75 g/Kg. Orally in rats, the minimum lethal dose was 2 g/Kg and the $LD_{50}$ was 4 g/Kg.

McLaughlin, Marliac, Verrett, Mutchler, and Fitzhugh investigated fourteen volatile chemicals by the chick embryo method and found that DMF along with $CCl_4$ and acetone showed a low order of toxicity[17].

Kutzsche has reported that a single oral dose of 0.3 ml 30% DMF or local application of 0.2 ml 30% DMF induced necrosis of the liver in mice[18].

Auclair and Hameau investigated the toxicities of both DMF and DMA in mice and rats and the effects of various doses in rats, cats and dogs. They concluded that the compounds appear harmless if not given in doses greater than 0.5 ml/Kg to rats and greater than 0.1 ml/Kg to dogs and cats[19].

Tudisco tested the toxicity of DMF added to the diet of rats. He suggested that 0.3 g/Kg of diet was the mean between well tolerated and slightly toxic amounts as reported in the literature. He found no deaths or clinical toxicity after 90 days[20].

A study by Weiss and Orzel compared the acute oral and intraperitoneal toxicities of dimethylsulfoxide (DMSO), DMA, DMF, and propylene glycol. The compounds were compared in rats after 24 hours and 5 days. DMSO was found to be less toxic than DMA and DMF[21].

Thiersch has reported that DMA caused destruction of rat fetuses in the oviduct by causing abnormal mitosis with failure to implant in the uterus. He has pointed out that similar effects are possible in man since methylacetamides are widely used as solvents for pharmacologic agents, for example steroids, for intravenous injection[22].

Di Paolo and Elis reported that diethyl carbonate was teratogenic to hampsters. The teratogenic effects were subsequently correlated with skin tumor initiation and lung adenomas in mice[23].

From these reports it can be seen that the possibility of serious toxicity in man and animals still exists even though no really definitive studies have been done. Further toxicity data would, of course, be necessary before DMF, DMA, or DEC could be used in an injectable vehicle.

# 6. DEVELOPMENT OF AN ASSAY TECHNIQUE

## 6.1 Extraction into Organic Solvents

It was hoped that the degradation products would display some differences in solubility from phenylbutazone which could be utilized in removing them from degraded products.

Degraded solutions of phenylbutazone in DMF were diluted 1 ml to 10,000 ml with pH 10.8 buffer[24], and the UV spectrum determined from 200-450 mμ. Two peaks were observed -- one at 264 mμ corresponding to phenylbutazone and one at 234 mμ due to one or more of the degradation products. The sample was then shaken with an equal volume of chloroform and the spectrum determined again. No change in the spectrum was observed except one of intensity, presumably due to partitioning of all components into the chloroform layer.

This procedure was repeated using equilibration with cyclohexane and hexane but in both cases the results were similar.

## 6.2 Formation of the HCl Salt

The procedure adopted here was similar to that employed in alkaloidal assays where the alkaloids are extracted from ethereal or chloroformic solution by shaking with dilute acid[25]. Such a procedure is employed in the assay of Belladonna Leaf U.S.P.[26] where the alkaloids are extracted by maceration with ether or percolation with ether-chloroform (3:1) and the extract shaken with 0.5 N sulfuric acid forming the sulfate salt. The free alkaloids are then liberated by alkalinization of the

acid layer with ammonia water and determined by a titration method.

Since the main degradation products mentioned in literature all differ from the parent compound by having an -N-H group it was felt that the hydrochloride salt might be formed which would remain in the aqueous phase while the phenylbutazone would be partitioned into the ether phase.

One ml of a degraded sample of phenylbutazone in DMF was added to 4 ml water and acidified with 50% v/v conc. HCL. This mixture was extracted with four 4 ml portions of ether. The ether layers were combined and evaporated to dryness at room temperature. The dry extract was dissolved in 100 ml ethanol and 1 ml of this solution was diluted to 100 ml with pH 10.8 buffer. The UV spectrum of this solution revealed peaks at 236 and 264 mμ; therefore, the degradation products had not been removed. Thin-layer chromatography on Bakerflex IB-F paper with a developing solvent of cyclohexane-chloroform-methanol-glacial acetic acid (60:30:5:5) also showed that all the original spots were still present after extraction.

This method was also repeated using chloroform, cyclohexane and hexane in place of ether, with similar results.

## 6.3 Quantitative TLC

Because of the difficulty of removing the degradation products from the samples to be assayed, it was felt that quantitative thin-layer chromatography might be a useful assay technique. This method suffers somewhat from a lack of precision due to the small amounts of substance

present and the number of steps involved. Bobbitt reports, however, that an accuracy of 3-5% is attainable using quantitative TLC combined with spectrophotometric determination after elution[27]. An integral part of the method finally employed is the statistical analysis which helps to minimize the experimental error and maximize the information obtainable.

Initially, chromatograms were run on Bakerflex IB-F thin-layer media. The adsorbent used in these layers was aluminum oxide. 2.00 µl of a 100 mg/ml solution of phenylbutazone in DMF was spotted on Bakerflex IB-F medium and run for 3 hours in a solvent of cyclohexane-chloroform-methanol-glacial acetic acid (60:30:5:5). The spot was scraped and extracted for 12 hours in 20 ml ethanol 95%. The sample was filtered through a medium sintered glass funnel and made up to 25 ml with ethanol 95%. The UV spectrum was determined and the recovery was found to be only about 50%. In addition the $\lambda_{max}$ moved from 240 mµ for phenylbutazone in ethanol to 269 mµ. Since complexation of phenylbutazone with the aluminum oxide adsorbent was suspected[28], a solution of 0.01 mg/ml phenylbutazone in ethanol was allowed to equilibrate 18 hours with excess $Al_2O_3$. This solution was then filtered through a medium sintered glass funnel until clear and the UV spectrum was taken. $\lambda_{max}$ was found to have shifted to 269 mµ and the intensity of absorption was increased (Figure 6.1). When approximately the same amount of $Al_2O_3$ was allowed to equilibrate for 18 hours in pure ethanol 95%, no change in UV absorption over the ethanol blank was observed.

It was, therefore, concluded that the phenylbutazone complexes with aluminum oxide or the aluminum ion causing the hyperchromic effect

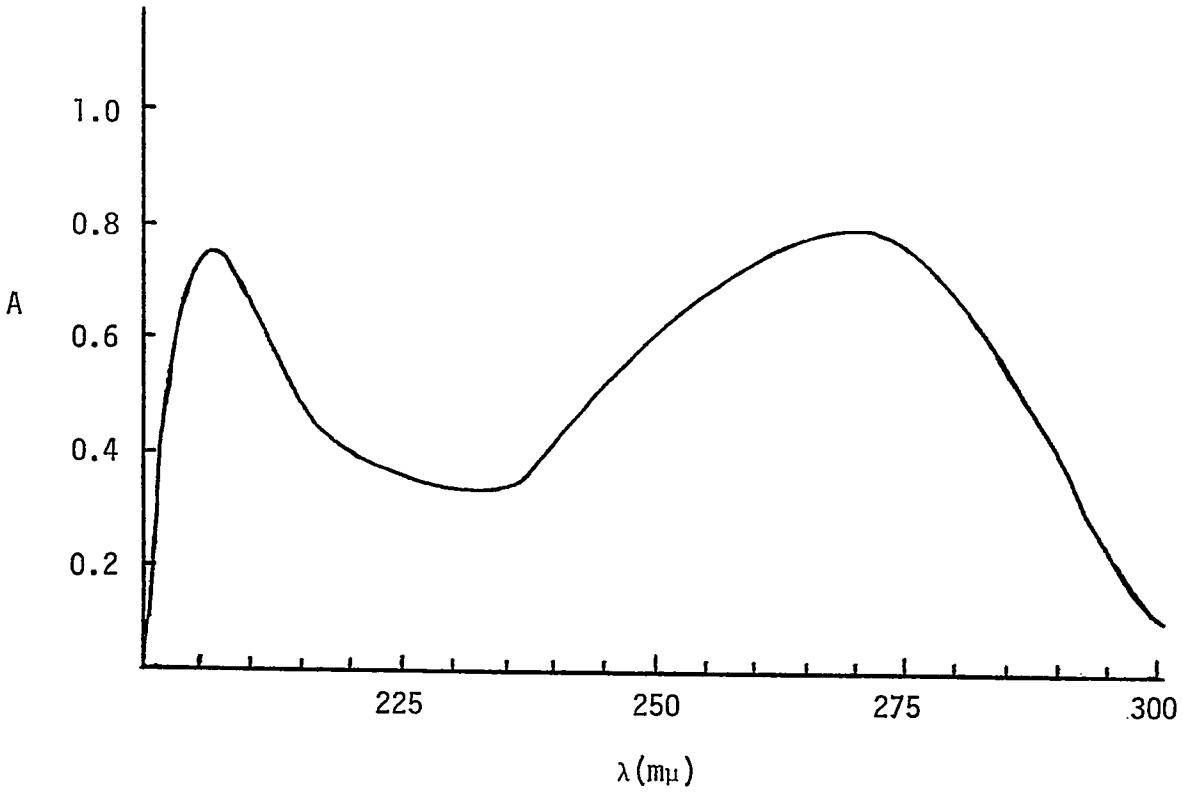



Figure 6.1: Change in Spectrum after Equilibration with $Al_2O_3$

and the bathochromic shift observed. No other report of in vitro complex formation is documented, but Wallenfels and Sund have reported the inhibition of alcohol dehydrogenase in yeast and liver and glutamic acid dehydrogenase in liver by phenylbutazone[29]. This was attributed to complexation with zinc attached to diphosphopyridine nucleotide-dependent systems.

Because of this complexation it was decided to use silica gel layers rather than the $Al_2O_3$. The assay technique finally adopted consisted of spotting 2.5 µl samples using a 10 µl Hamilton microsyringe on 250 µ (dry thickness) layers of silica gel HF. The layers were activated at 120°C for at least 3 hours. Before spotting, the plates were cooled for 15 minutes in an atmosphere with temperature controlled at 20°C and humidity at 45%. The spots were dried in a stream of cool air. The solvent was equilibrated for 20 minutes in the tank before running to allow complete saturation. Saturation was also ensured by lining the tank with absorbent filter paper. Chromatograms were run in cyclohexane-chloroform-methanol-glacial acetic acid (60:30:5:5) for 1 hour 15 minutes. This time was sufficient to allow the solvent front to reach the top of the plate, a technique which was employed to give more consistent $R_f$ values. Chromatograms were allowed to dry at room temperature before visualizing under short wavelength ultraviolet light. The phenylbutazone spot was marked and scraped off the plate. The silica gel with adsorbed phenylbutazone so obtained was eluted with 20 ml acetone for 15 minutes. The mixture was then filtered through a medium sintered glass funnel with the aid of vacuum. The acetone was allowed to evaporate at room temperature and the dry extract redissolved in 25 ml ethanol 95%. The UV spectrum of this ethanolic solution was then determined and the amount of phenylbutazone present estimated. Acetone was used as the original eluant because it readily dissolved phenylbutazone but not silicic acid which would have interfered with the UV assay; however, the acetone was not a suitable solvent for use in the UV region and was therefore removed before the spectrum was determined.

## 7. EXPERIMENTAL DESIGN[30]

The series of experiments to determine the kinetic degradation behaviour of phenylbutazone in the four solvent systems examined was designed to provide the maximum information from the data which could be obtained with the available resources.

Linear regression was used to obtain b, the slope of the straight line graph of log concentration vs. time. This b value could then be converted to a k value for the degradation reaction by multiplying by -2.303. At least three points had to be determined before this technique could be successfully used. The time intervals were equally spaced to increase the power of the statistical test. In addition to determining b, all data were used to test for linearity and linear regression.

The k values were determined for each solvent system at four different temperatures. Fairly low temperatures were used (30°C, 45°C, 60°C, 75°C) to ensure that the same kinetics as those at room temperature would be encountered. Analysis of covariance was employed here to test whether the Arrhenius plots (log k vs. $\frac{1}{T}$) for the four solvent systems were the same, thus implying that the stability of preparations was the same. In order to do the analysis of covariance calculations, at least two k values were needed at at least one value of $\frac{1}{T}$. To get this kind of data, four containers of each solution were stored at each temperature. The data for each container were analysed separately by linear regression to give a value of b. Thus four b values were obtained at four different

temperatures for each solvent system. The number of containers stored was limited by the number of spots which could be run on one chromatogram and was set at four because the length of time involved in analysing a larger number of samples would have been prohibitive.

Linear regression was also used on the log k vs. $\frac{1}{T}$ plots to determine the best straight lines for the data obtained.

Since the analysis of covariance is a general test of hypothesis, a specific test (the individual degree of freedom) was used to determine whether the slopes of the Arrhenius plots were the same.