

## Molecular Integrity Of Aspirin In Relation

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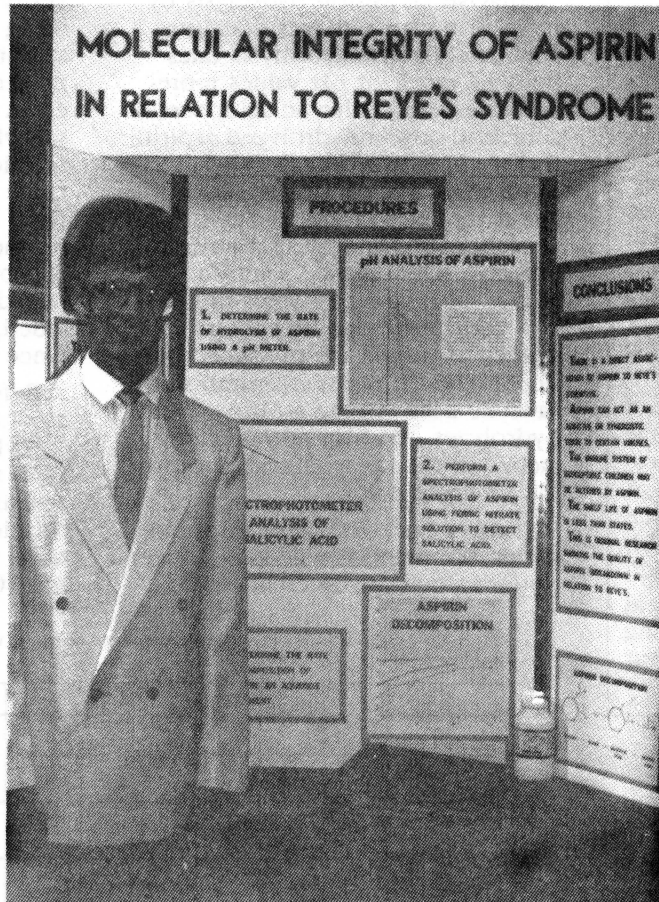
*Aspirin decomposes into salicylic acid and acetic acid, neither of which has any pain-relieving or temperature-reducing characteristics. Preliminary analysis of pure aspirin using a pH meter suggested a breakdown much faster than published values and the stated shelf life would indicate. The resulting data also suggested a rate order conflicting with the published zero order rate. Further analysis of aspirin with a spectrophotometer, utilizing a range of temperature and time conditions, reinforced the hydrolysis data.*

*Reye's syndrome is an acute, life-threatening disease affecting children from infancy to late teen years, and is one of the 10 major causes of death in children. It is characterized by vomiting and delirium following the apparent recovery from a prodromal illness, primarily influenza or chickenpox. The illness progresses to cerebral edema and coma.*

*Case/control studies showed that between 96 per cent and 100 per cent of the cases and between 44 per cent and 71 per cent of controls received salicylates during a viral respiratory illness or bout with chickenpox; however, nowhere in the literature is the quality of the ingested aspirin discussed. The immune system of susceptible children may be altered by aspirin which may act as an additive or synergistic toxin in relation to certain viruses.*

### Introduction

Acetylsalicylic acid, commonly known as aspirin, was introduced in



Scott Caveny of Wheeling, now a West Virginia University student, in front of his research exhibit which won first place in the West Virginia Science Talent Search (Westinghouse Research Award) and four other first-place awards and a grand prize in other science fair competition (date of photo April, 1987, age 17).

1899 and is used worldwide to relieve pain, fever, and inflammation. Aspirin hydrolyzes into salicylate and acetate in an aqueous environment (1). In light of the widespread use of this drug in such areas as arthritis, heart disease, dental discomfort, and headache relief, knowledge of the hydrolysis rate may provide valuable information as to the stability of aspirin in relation to the shelf life of this pharmaceutical product.

Data presented in this investigation showed a breakdown of aspirin

faster than previously reported (2). As a result, people dependent on aspirin may be taking into their systems a large influx of salicylic acid and acetic acid. The full implications of this influx are not presently known; however, a correlation between Reye's syndrome cases and the ingestion of aspirin during prodromal disease has been reported (3).

Reye's syndrome, first diagnosed in 1963 (4), is an acute, life-threatening disease affecting children from infancy to late teen years, and is one of the 10 major causes of death in children. Although Reye's syndrome primarily attacks children, it is not confined to any age group. In fact, cases have been reported in people over 50 (5). It is characterized by vomiting, delirium, aggressiveness, and irrational behavior following apparent recovery from a febrile illness, primarily influenza or chickenpox (6). The peak season for Reye's syndrome is in January, February, and March, coinciding with increases in influenza and chickenpox cases.

Unfortunately, most parents are not fully aware of the possible association of salicylates and Reye's syndrome (7). The purpose of this project is to investigate the relationship between aspirin purity and the incidence of Reye's syndrome.

### Materials and Methods

Preliminary determination of the rate of aspirin hydrolysis was done using an Orion® pH meter, a strip chart recorder, pharmaceutical-grade aspirin, and triply distilled water. Each test was recorded on the strip

# To Reye's Syndrome

## Editor's Note:

"Originality is simply a fresh pair of eyes."

—Woodrow Wilson

It is unusual for the *Journal* to publish a paper from such a source. Scott Caveney did this research on aspirin as a senior in high school at Linsly in Wheeling. This paper won first place in the West Virginia Science Talent Search sponsored by Westinghouse and the West Virginia Academy of Science.

Dr. Otis Bowen, Secretary of Health and Human Services, gave Scott an award of merit for "creativity, concern and commitment in developing a science project which may have direct benefit in health programs affecting young people."

Scott is currently a freshman at West Virginia University studying pre-medicine on a full four-year WVU Foundation scholarship.

Analysis of aspirin hydrolysis by monitoring pH changes does not adequately show hydrolysis rates because resultant pH values represent the pH of salicylic acid, acetic acid, and any unhydrolyzed aspirin; therefore, it was necessary to determine what per cent of the hydrolyzed aspirin was salicylic acid. This was accomplished by using a four-per cent ferric nitrate solution.

When salicylic acid comes in contact with ferric nitrate, various shades of purple are produced depending on the concentration of salicylic acid. As the aspirin hydrolyzes, the iron complex attaches to the salicylic acid, producing a purple color.

Using a Spec-20<sup>®</sup> spectrophotometer, accurate salicylic acid concentrations could be ascertained. To

verify spectrophotometer analysis using the ferric nitrate solution, the stability of ferric nitrate in the presence of salicylic acid was checked. A color was developed with the salicylic acid and the ferric nitrate solution, and the per cent transmittance was monitored for several days. The test showed that the color reached a maximum intensity after approximately five minutes, maintained it for several hours, and then faded, making the per cent transmittance rise.

Before tests were done on aspirin, a concentration curve of pure salicylic acid was established (Figure 1). A stock solution of 1.0 g salicylic acid in one liter of ethyl alcohol solution was prepared. Serial dilutions were made by taking 10 ml, 15 ml, 20 ml, 25 ml, and 30 ml of the

Method: Dilutions made using a 1.0g/l Salicylic Acid solution measured to 10, 15, 20, 25, and 30 ml quantities and then each diluted to 100 ml

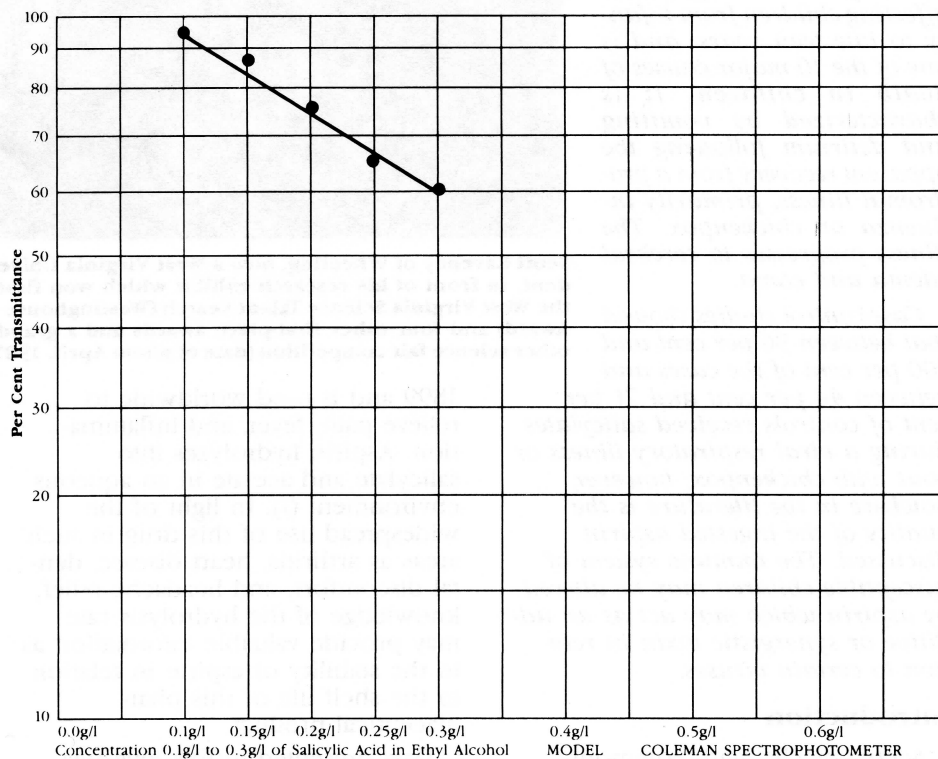


Figure 1. Spectrophotometer analysis of salicylic acid.

chart recorder, which also acted as a time base for each test.

One tenth gram of powdered aspirin was dissolved in 100 ml of distilled water. Simultaneously, the electrode from the pH meter was placed into the solution and the strip chart recorder and magnetic stirrer were turned on. The pH readings were recorded every 24 seconds, and data collection was terminated following a consistently low pH level. Data from the plotted graph were analyzed using linear regression to obtain a mathematical model of the event. A second similar investigation into the hydrolysis of aspirin involved the addition of 1.0 g of aspirin in 100 ml water.

To evaluate the accuracy of the hydrolysis rate, a 0.1 g sample of aspirin dissolved in 100 ml of water was boiled for five minutes. Boiling the sample assured driving the reaction to completion.

stock solution and diluting to a final volume of 100 ml. The spectrophotometer was blanked with the ferric nitrate solution. Five ml of a sample and 45 ml of the ferric nitrate solution were put in a volumetric flask, shaken thoroughly, and put in the spectrophotometer. The per cent transmittance was read five minutes from the time the color solution was added with results recorded on the graph. Based on the result of this graph, the following procedure was used to detect the amount of salicylic acid in a sample of aspirin:

Using the molecular masses of aspirin and salicylic acid, 180 g/mole and 134 g/mole, respectively, it was stoichiometrically determined that 0.040 g aspirin in 100 ml of distilled water would produce the best results for the graph. The spectrophotometer was blanked with the ferric nitrate solution. Five ml of the aspirin sample and 45 ml of ferric nitrate solution were combined, shaken thoroughly, and put in the spectrophotometer. The per cent transmittance was read and recorded exactly five minutes from the time the color solution was added. Using the salicylic acid concentration curve, the weight of salicylic acid in the solution was determined. The per cent decomposition was determined using the following equation:

$$\text{Per cent decomposition} = \frac{\text{X g/L Salicylic acid} \times 100}{0.298 \text{ g/L Salicylic acid}}$$

Using this procedure, several conditions could be altered to determine more accurately the breakdown of aspirin in an aqueous environment. Three samples were used.

Sample #1: 0.040 g aspirin in 100 ml water, per cent transmittance was measured five minutes after it was put in water.

Sample #2: 0.040 g aspirin in 100 ml water, boiled for 15 minutes, cooled, refilled to 100 ml, then per cent transmittance measured.

Sample #3: 0.040 g aspirin in 100 ml water, boiled for 15 minutes, covered with a watch glass, boiled for an additional 15 minutes, cooled, refilled to 100 ml, then per cent transmittance measured.

Each sample was monitored for a period of four days and the results recorded (Figure 2).

The titration of aspirin was another procedure to be utilized. Using a pH meter, 0.452 g aspirin was titrated against 0.1 Molar NaOH.

Once an accelerated breakdown in pure aspirin was noted, brand-name aspirin samples were compared. Four samples of aspirin with varying dosages and expiration dates were used:

Sample #1: Schein® aspirin, no expiration date provided, 227 mg aspirin/tablet, four tablets used.

Sample #2: Anacin® aspirin, expiration date 9/84, 400 mg aspirin/tablet, three tablets used, packaged individually.

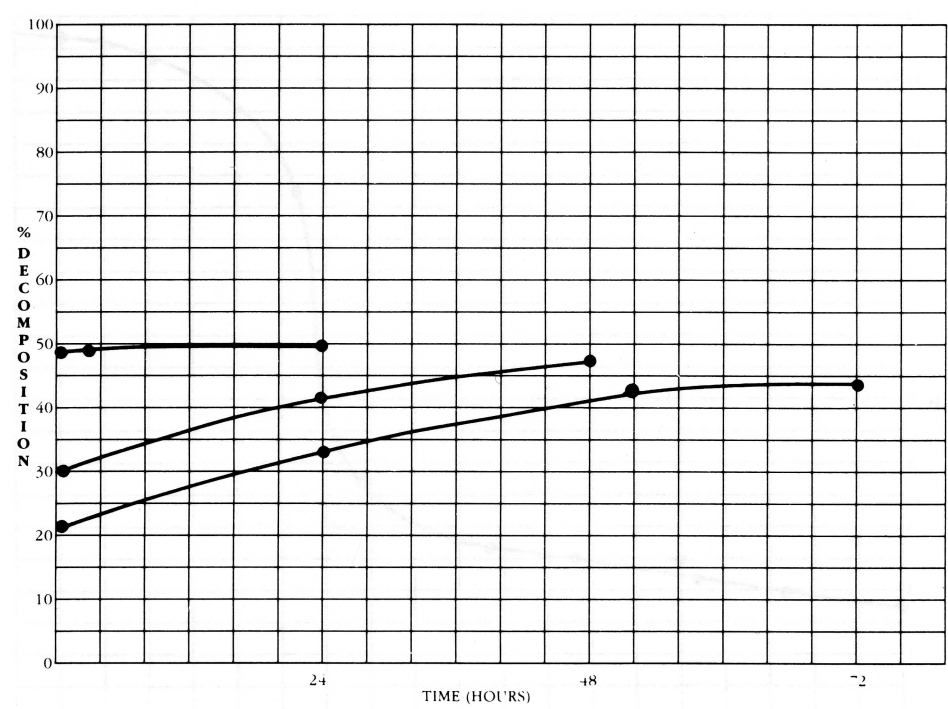
Sample #3: Anacin® aspirin, expiration date 12/88, 400 mg aspirin/tablet, three tablets used.

Sample #4: Empirin® compound #3, expiration date 6/26/81, 325 mg aspirin/tablet, three tablets used, also contained 30 mg codeine.

Due to variability in aspirin dosages, a variable number of tablets was used to reach the weight of 1.0 g instead of crushing and weighing the tablets individually. Each sample was crushed, put in 250 ml of distilled water and monitored for four days.

### Results

The graph of the pH analysis (Figure 3) suggested an order rate other than zero as proposed by many pharmaceutical houses. The zero order rate reaction would mean that concentration does not

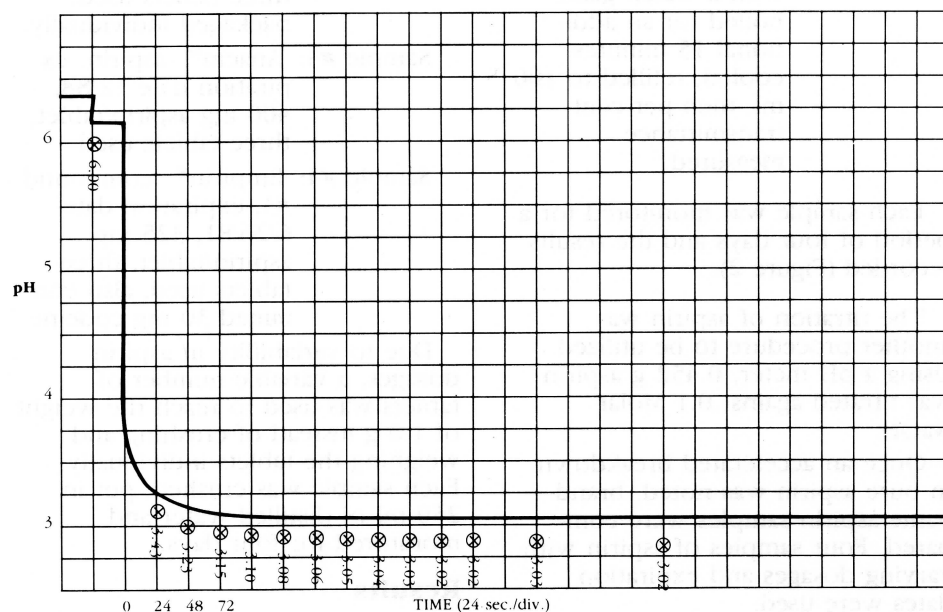


Top Line—0.040g aspirin in 100ml water, boiled for 15 minutes, covered with a looking dish, boiled for an additional 15 minutes, cooled slightly, refilled to 100ml, and measured  
 Center Line—0.040g aspirin in 100ml water, boiled for 15 minutes, cooled, refilled to 100ml, and measured  
 Bottom Line—0.040g aspirin in 100ml water, % transmittance measured 5 minutes after put in water  
 Each sample was measured on a daily basis.

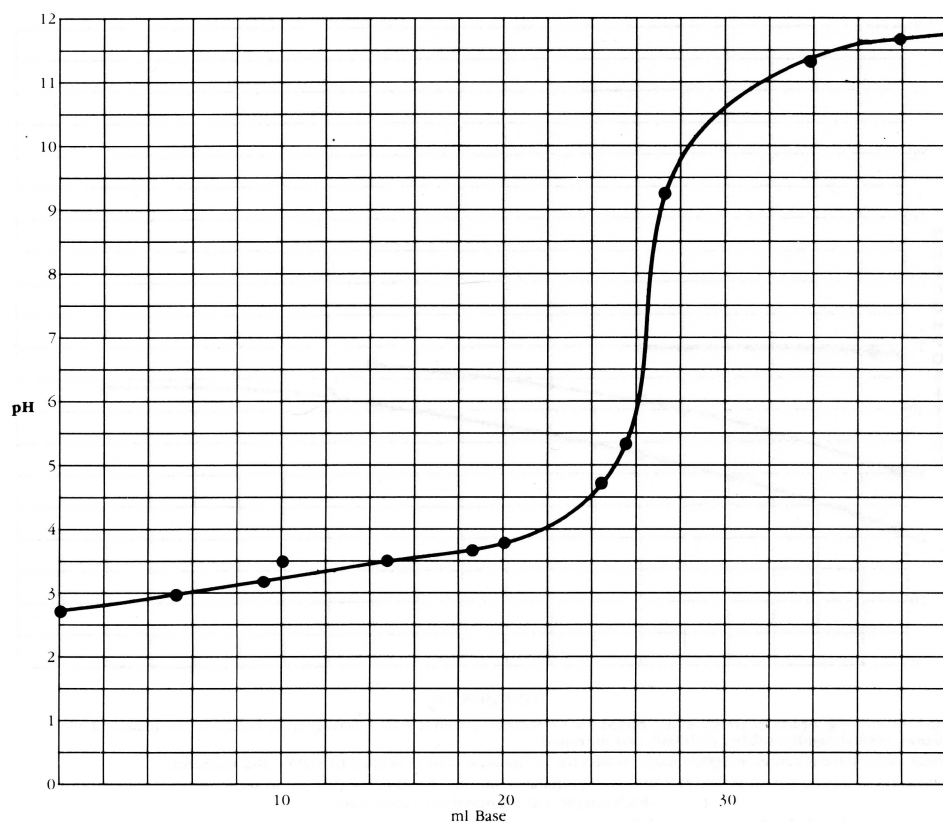
**Figure 2. Aspirin Decomposition.**

affect the curve. Linear regression analysis gave an equation for the line to be  $y = 4.690X - 0.082$  with a

correlation coefficient of 0.97236. with maximum hydrolysis within 200 seconds. The test using 1.0 g



**Figure 3. pH analysis of aspirin.** This is a record of a pH test done with an Orion® pH meter with the graph recorded using a strip chart recorder. 0.1 grams of aspirin were placed in 100 ml. of distilled water and the graph was then started. The solution was agitated with a magnetic stirrer. pH points were recorded at time intervals determined on the graph paper. The reaction was run to completion with equilibrium occurring in 200 seconds. This procedure was repeated with the same results being obtained.



**Figure 4. Titration curve of aspirin against NaOH using pH meter.**

aspirin in 100 ml of water resulted in a hydrolysis rate that dropped much more quickly than the previous test, demonstrating that concentration had a significant effect on the graph, also suggesting an order rate other than zero. The sample boiled for five minutes had a resulting pH = 3.17, compared to the original sample of pH = 3.02.

The spectrophotometric analysis of salicylic acid showed a linear relationship between per cent transmittance and concentration. From this, the grams per liter of salicylic acid in the aspirin samples were read and converted into per cent decomposition. The results were recorded on a daily basis (Figure 2).

Results from the aspirin titration showed that aspirin acted like a monoprotic acid, which produces a large break in the pH (Figure 4).

Results from the analysis on the various brands of aspirin showed that older aspirin had lower transmittance, thus indicating lower purity. Several important points were noted from this test. Older aspirin, when crushed and placed in solution, had large fragments of undissolved aspirin or "binder" on the top and bottom, making the solution cloudy. When crushing the tablets, the exterior of older aspirin was much softer than the new tablets. The spectrophotometer test also revealed that individually wrapped aspirin were not well protected, thus indicating hydrolysis had occurred inside the package, which is not vacuum sealed.

## Discussion and Conclusions

The relationship between Reye's syndrome and prodromal illness has long been recognized, yet the exact cause of Reye's syndrome remains unknown despite many years of study (3).

Due to the high statistical association of salicylate ingestion with Reye's syndrome, it appears that there is a direct relationship between aspirin and Reye's syndrome. Symptoms of salicylate toxicity usually include nausea, disorientation, vomiting, dehydration, fever, oliguria, coma, and convulsions.



Unusual symptoms include bleeding, respiratory depression and pulmonary edema. Because of the similarities between salicylate toxicity and Reye's syndrome, it appears that, of the two aspirin components, salicylic acid seems to be the trigger mechanism for Reye's syndrome. The severity of the illness depends on the quantity ingested and the weight of the patient, yet the condition can be potentially lethal (8).

Research on aspirin presented in this study shows that the shelf life is less than stated on the bottle. Aspirin cannot remain in a pure state for the stated two to five years when exposed to fluctuating levels of humidity.

Aspirin in a pure state, or impure state, can act as an additive or synergistic toxin to certain viruses such as influenza and chickenpox. The immune system of susceptible, unsensitized children may be altered by aspirin. This research showed the quality of aspirin in relation to Reye's syndrome.

Case studies of Reye's syndrome have failed to look at the quality of the aspirin ingested by the patient. Continuing needs exist for parental and public education and awareness regarding the risk of giving aspirin to children with influenza, chickenpox and other respiratory diseases. Increased awareness of the purity of drugs such as aspirin would also lessen the threat of diseases such as Reye's syndrome.

A cure for Reye's syndrome may be a long time in coming; however, an understanding of the causes of this disease is vital to lives in the future. Studies of Reye's syndrome in laboratory animals such as mice and puppies have proved relatively unsuccessful. This fact, unfortunately, confines studying Reye's syndrome to human patients, probably prolonging the amount of time until a cure can be found. The number of Reye's syndrome victims remains fairly constant, but, thanks to improved diagnostic and treatment procedures, the death rate has fallen to approximately 25 per cent in the last few years (9).

### Acknowledgement

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