RESEARCH ARTICLE

Optimization of UV Spectrophotometric Conditions for Aspirin Analysis Using Experimental Design Methodology

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Abstract

Introduction: Forced degradation studies are important for drug research because they give important information about how stable drug substances are under different types of stress. These studies help find ways that chemicals break down, possible breakdown products, and things that affect chemical stability during storage, formulation, manufacturing, and packaging. Acetylsalicylic acid (Aspirin) is a non-steroidal anti-inflammatory drug (NSAID) that has been around since the late 1890s. It is often used to treat minor aches and pains, lower fevers, control inflammation, and lower the risk of heart disease. Because it is used so much and can be found in wastewater effluents, it is important to know how it breaks down for both pharmaceutical stability and environmental risk assessment.

Method: The current study examined the oxidative degradation characteristics of aspirin through a Design of Experiments (DoE) methodology. A structured experimental design was used to test three independent variables—oxidant concentration, temperature, and time—at three different levels. The oxidizing agent was hydrogen peroxide. Absorbance, measured by UV spectrophotometry, was chosen as the response parameter to demonstrate the level of degradation under diverse stress conditions.

Result: The results demonstrated that all three independent variables oxidant concentration, temperature, and time significantly influenced the degradation of aspirin. An increase in temperature, oxidant concentration, and exposure time resulted in increased degradation, as reflected by corresponding changes in absorbance values. Interaction effects between the variables were also observed, indicating a combined influence on the degradation process.

Discussion: The findings confirm that oxidative degradation of aspirin is strongly dependent on environmental and stress-related factors. The application of the DoE approach enabled systematic evaluation of factor interactions and provided a predictive understanding of degradation behavior. These results are valuable for optimizing formulation strategies, improving storage conditions, and assessing the environmental fate and risk associated with aspirin.

Keywords: Aspirin, Design of experiments, Non-steroidal anti-inflammatory drug, Mortality, Forced degradation.
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Introduction

Aspirin is the most commonly used medicine in the world. A daily aspirin doubles the chances of living a long life. Studies have demonstrated that an aspirin regimen for people over 50 can lengthen life since aspirin lowers the risk of numerous aging-related illnesses. The first applications of decoctions or preparations of plants containing salicylate date back thousands of years in the history of aspirin.[1] Acetylsalicylic acid (ASA), also known as aspirin, is a popular non-steroidal anti-inflammatory medication (NSAID) for treating minor aches and pains. Moreover, it is used as an antipyretic to reduce fever, as an anti-inflammatory drug, and to reduce the risk of mortality from a heart attack. Particularly when taken in larger doses, gastrointestinal ulcers, stomach bleeding, and tinnitus are the main side effects of aspirin.[2,3]

Aspirin is a non-steroidal anti-inflammatory drug (NSAID) and a member of the salicylate family, which all have salicylic acid as their active ingredient. Salicylic acid is made up of a benzene ring, one hydroxyl radical, and one carboxyl radical. Salicylate's hydroxyl group is esterified into an acetyl group in the acetylsalicylic acid found in aspirin. Aspirin's pharmacological qualities are identical to those of salicylates as well as to the biological effects assigned to salicylate itself, and because of its reactive acetate group, it also possesses additional independent effects.[4] Salicylate and acetate groups, which are both physiologically active, function separately from one another at various places. Aspirin inhibits the activity of the enzyme now called cyclooxygenase(COX). There are at least two different cyclooxygenase isozymes: COX-1 (PTGS1) and COX-2 (PTGS2). Both forms are permanently

and non-selectively inhibited by aspirin.[5] It accomplishes this by acetylating a serine residue's hydroxyl group.[6] Prostaglandins, the majority of which are pro-inflammatory, and thromboxane, which encourage clotting, are normally produced by COX. The majority of the lipoxins produced by aspirin-modified COX-2 are anti-inflammatory.

In an aqueous environment, aspirin breaks down into several hazardous intermediates that harm the environment and people's health. It is difficult and expensive to remove these chemical molecules using conventional methods (adsorption, ionization, etc.). These techniques typically result in gaseous emissions and concentrated effluent streams, which are bad for the environment and need to be treated further before being dumped into a landfill or adjacent river.[7,8] When applying the standard electrochemical approach to study aspirin degradation, hydrogen peroxide was found to be efficient. Additionally, a degrading process based on the production of a hydroxyl group in hydrogen peroxide was suggested.[9]

Forced Degradation Studies

Forced degradation (or stress testing) typically involves exposure of drug substances to heat, humidity, and light for solid-state studies. For solution-state studies, the drug substance is exposed to a range of pH values. The experimental samples produced are then used to demonstrate that a proposed analytical method is "Stability Indicating," i.e., the method is capable of detecting the loss in content of the active component and subsequent increase in degradation products. Ideally, loss in content of the active component and increase in degradation products should be monitored by a single analytical method.

Several international guidelines outline forced degradation research. The International Committee for Harmonization of Technical Standards for Registration of Pharmaceuticals for Human Use (ICH)[9] is responsible for these standards. The ICH guidelines that apply to forced degradation studies are:

- ICH Q1A – Stability Testing of New Drug Substances and Products.[10]
- ICH Q1B – Photostability Testing of New Drug Substances and Products.[11]
- ICH Q2B – Validation of Analytical Procedures: Methodology.[12]

Materials And Method

Chemicals and Reagents

Analytically pure aspirin was obtained from Central Drug House (P) Ltd. All chemicals and reagents used were of analytical grade. Hydrogen peroxide was obtained from Central Drug House (P) Ltd.

Instrumentation

UV-visible spectrophotometer (Shimadzu 1800), Hot Plate (HICON), water bath (HICON), micropipette (CWS Series), weighing balance (Scale-Tec), Distilled water (In-house distillery), Refrigerator (Godrej).

Methodology

A precise amount of 0.1 mg of aspirin was placed in a 100 mL volumetric flask, and hydrogen peroxide was added to the mark to make the stock solution. Five milliliters of this stock solution were pipetted into three different 100 mL volumetric flasks. Each flask was then filled with a different amount of hydrogen peroxide: 1, 3, and 5%, respectively. These were then used as working solutions for oxidative degradation studies. To make hydrogen peroxide solutions (1, 3, and 5% v/v), concentrated hydrogen peroxide was mixed with distilled water in the right amounts.

The prepared aspirin solutions were moved into different volumetric flasks in 10 mL amounts and put in different temperature settings

- 5°C (in the fridge)
- 30°C (the temperature in the room)
- 55°C (hot plate condition)

A UV-Visible spectrophotometer was used to measure the absorbance of each solution at the beginning (0 min) at the predetermined λmax of aspirin. Then, absorbance readings were taken at 120 and 240 minutes to find out how much oxidative degradation had happened.

A central composite design (CCD) within response surface methodology (RSM) was utilized to examine the impact of essential process variables on the oxidative degradation of aspirin. The chosen independent variables for the study were

- Concentration of oxidizing agent (% H_2O_2)
- Temperature (°C)
- Time (min)

The change in absorbance was the response variable, and it was used to figure out how much aspirin had broken down. The CCD model made it possible to assess

- Main effects of individual factors
- Interaction effects between variables
- Quadratic effects for optimization

Statistical analysis and model generation were utilized to ascertain the significance of variables and to identify the optimal conditions for oxidative degradation.

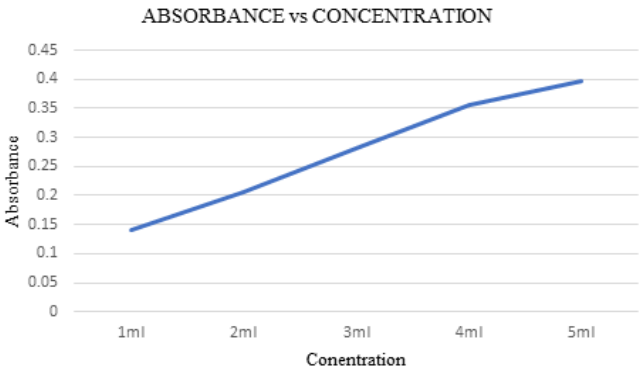

Figure 1: Calibration plot of aspirin

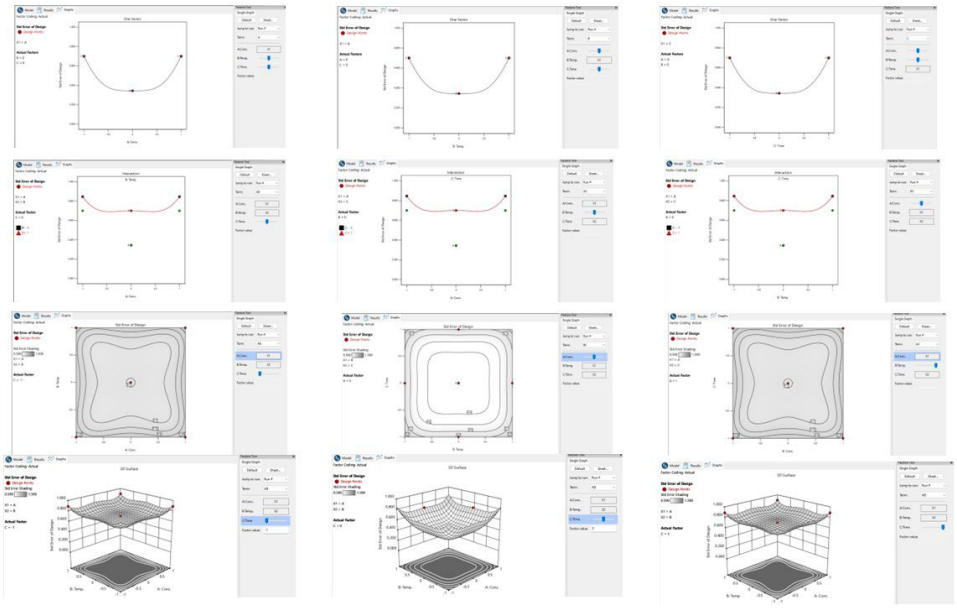

Figure 2: a) Plot showing showing effect of single factor i.e., concentration of oxidizing agent b) plot showing showing effect of single factor i.e., temperature c) plot showing showing effect of single factor i.e., time d) plot showing effect of two factors i.e concentration and temperature e) plot showing effect of two factors i.e concentration and time f) plot showing effect of two factors i.e., temperature and time g) plot showing simultaneous effect of two factors i.e. concentration and temperature h) plot showing simultaneous effect of two factors i.e. temperature and time i) plot showing simultaneous effect of two factors i.e. concentration and time j) contour plot between temperature and time when h2o2 is 1% k) contour plot between temperature and time when h2o2 is 3% l) contour plot between temperature and time when h2o2 is 5%

Result and Discussion

The samples were taken according to Table 1 and were analyzed using the spectrum mode of a UV spectrometer at a wavelength of 275 nm. Absorbance was taken as the response, and the data were analyzed using Design Expert 13.

A calibration plot was made using different concentrations of aspirin (10, 20, 30, 40 & 50 ppm) to check linearity Fig 1 & Table 2. Fig 2 shows that with an increase in concentration (1–5%), an increase in absorbance is seen, which means the effect of concentration on the degradation of aspirin is high as compared to temperature & time. With an increase in temperature, there is a significant increase in the absorbance. Similarly, with an increase in time, a decrease in absorbance was seen. When two factors (a) concentration of oxidant and (b) temperature (time is kept constant as 0 min) are studied simultaneously, it is observed that degradation is minimum at low concentration and at a low time point. The degradation increases with increased concentration of oxidant and time. When two factors (a) temperature and (b) time (concentration of oxidant is kept constant as 3%) are studied simultaneously, it is observed that degradation is minimum at low temperature and at a low time point. The degradation increases with increased temperature and for a longer period of time. When two factors (a) concentration of oxidant and (b) time (temperature is kept constant at 55°) are studied simultaneously, it is observed that degradation is minimum at low concentration and at a low time point. The degradation increases with increased concentration of oxidant and for a longer period of time. Contour plots of Fig

Table 1: Design with factors as Concentration of Oxidant, Time & Temperature

Run	*Concentration of oxidant*	*Time*	*Temperature*
1.	-1	0	0
2.	0	0	0
3.	0	0	1
4.	0	-1	0
5.	0	0	0
6.	1	1	-1
7.	0	1	0
8.	-1	1	1
9.	-1	-1	1
10.	-1	-1	-1
11.	0	0	-1
12.	0	0	0
13.	1	-1	-1
14.	1	1	1
15.	0	0	0
16.	0	0	0
17.	0	0	0
18.	1	0	0
19.	-1	1	-1
20.	1	-1	1

Table 2: Absorbance of aspirin at different concentrations

S.NO	*Concentration (ppm)*	*Absorbance*
1.	10	0.140
2.	20	0.205
3.	30	0.282
4.	40	0.356
5.	50	0.397

2 show the interaction between the factors. Absorbance is less when all three factors are at their lowest levels. When the concentration of H_2O_2 has increased to 3%, the absorbance decreases (time and temperature at their lowest level), but the absorbance increases to some extent when all three factors are at their maximum levels.

Conclusion

The degradation studies of aspirin conclude that the three parameters responsible for degradation were time, temperature, and oxidant concentration. A greater understanding of the environmental fate of aspirin, which is frequently discovered as a substantial wastewater discharge, comes from research on the mechanisms influencing its degradation. The degradation is mostly impacted by temperature and oxidant concentration, according to the single-factor response. It was found that when different parameters were used in degradation studies, all factors have some relative effects. At low oxidant concentrations, temperatures, and times, degradation is less, but it increases if any of the parameters are increased. Contour plots demonstrated that, even at low temperatures and times, there is a considerable interaction between the variables and degradation that increases with an increase in oxidant content. Additionally, when all three components are operating at maximum capacity, there is a noticeable rise in absorbance. This is an interesting discovering, and the rise in reaction could be brought about by the breakdown product becoming more absorbent than the parent chemical. However, advanced equipment like an LC-MS is needed to validate it. In conclusion, this research will help manage or regulate aspirin's degradation to lower its risk. This will provide a deeper understanding of the variables influencing the aspirin degradation rate.

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Conflict of Interest

None

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