

Chemistry Extended Essay

The effect of storage relative humidity on the degradation of paracetamol
(over-the-counter acetaminophen) at 22 °C.

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1 Introduction

1.1 Background

Paracetamol (acetaminophen), one of the most widely used over-the-counter drugs, is commonly stored for extended periods in domestic settings. Its stability is not only important for maintaining its efficacy but also for preventing potential toxicity from degradation products such as p-aminophenol (PAP), which has been shown to cause liver and kidney damage (Karaarslan, Numan, 2021). Pharmaceutical stability studies show that degradation is caused by factors such as improper temperature, light, and most significantly, moisture. The control of these variables will be discussed later (Ahmad and Shaikh, 2023).

Paracetamol (N-(4-hydroxyphenyl)acetamide) contains a phenol group and an amide linkage (Figure 1), and undergoes amide hydrolysis under humid conditions to yield p-aminophenol and ethanoic acid (Figure 2).

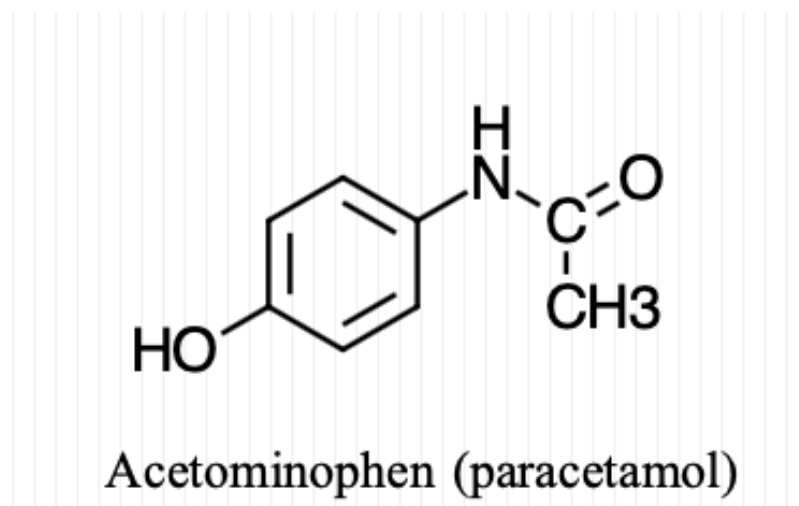


Figure 1. Acetaminophen, phenol group represented by ring structure bound to -OH ($\text{C}_6\text{O}_5\text{OH}$), amide group represented by $-\text{NHCOCH}_3$.

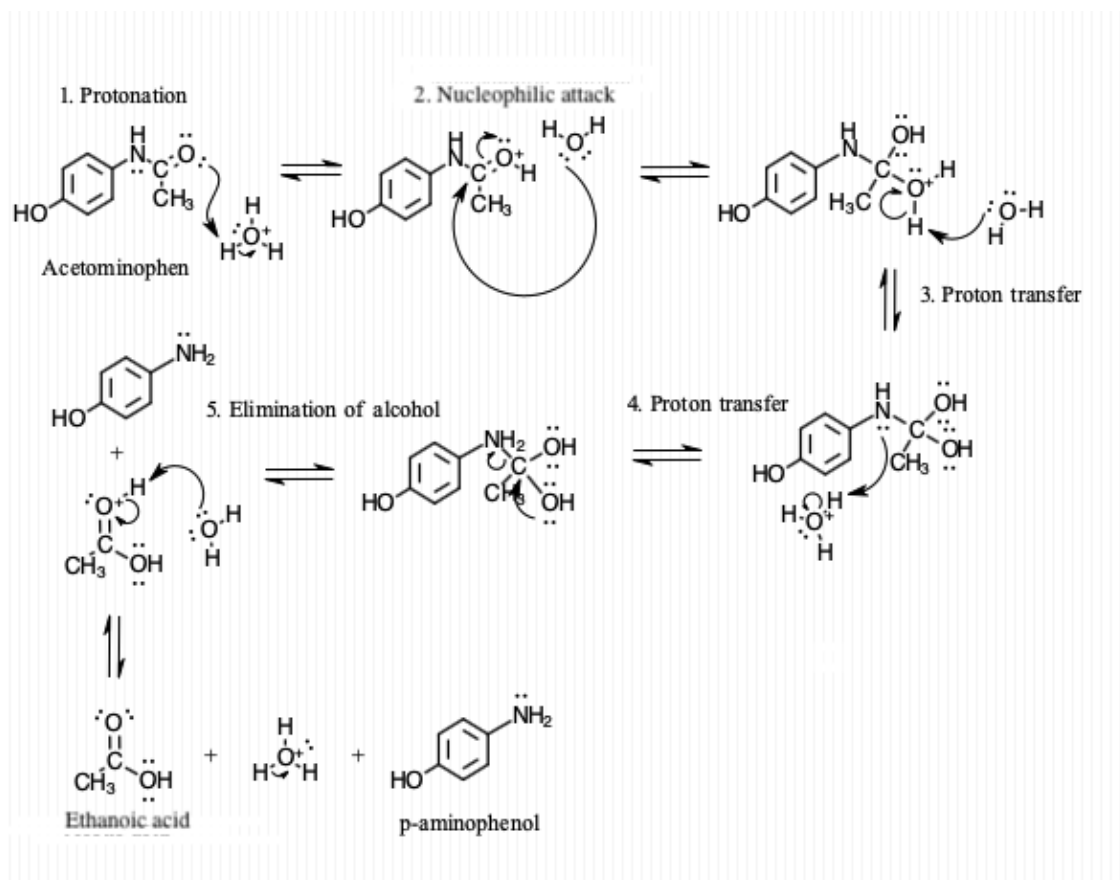


Figure 2. Acetaminophen undergoing slow acid-catalysed hydrolysis to form p-aminophenol and ethanoic acid in the presence of moisture, mechanism written by the author, based on Clark et al., 2015.

Paracetamol contains an amide group ($-\text{NHCOCH}_3$) that slowly hydrolyses in the presence of moisture via acid catalysis: H_3O^+ protonates the carbonyl oxygen, enabling nucleophilic attack by water to form a tetrahedral intermediate, which collapses to release p-aminophenol and ethanoic acid while regenerating H_3O^+ . Because $[\text{H}_3\text{O}^+]$ is very low in neutral water, this reaction is slow, consistent with gradual degradation during long-term humid storage.

Hydrolysis rates increase in the presence of water, particularly at elevated temperatures or under improper pH storage conditions (Touw et al., 2023). PAP can subsequently undergo further oxidation to quinone-imine-type species that are associated with tablet discoloration and toxicity (Ahmad, Shaikh, 1993).

1.2 Use of FeCl₃ to detect the extent of degradation to PAP

Fe(III) forms intensely coloured complexes with phenols (the ferric chloride test); colour varies with the phenol and intensity increases with concentration. Both paracetamol and PAP, the key degradation product of paracetamol, contain a phenolic hydroxyl group (-OH) and therefore react in this test, both initially producing a violet/purple color. Typically used to verify paracetamol synthesis (Hasegawa, Usui, 1992; Parveen et al., 2023), here the same reagent instead quantifies the extent of degradation through a process described in Al-Abadleh, Hind A et al., 2022, and in Ahmad, Shaikh, 1993.

The intense colour of the iron(III)-phenolate complex arises from d-d electronic transitions within the split d-orbitals of Fe(III); the energy gap determines the absorbed wavelength and therefore the transmitted colour. This was the basis of choosing a suitable wavelength to measure absorbance, specifically 580 nm, as it corresponds closest to the yellow wavelength of light that would be absorbed for violet colored complexes formed by FeCl₃ and PAP/acetaminophen (Gore and Newman, 1964); therefore, it was decided that this was also the best wavelength to detect change by oxidation of PAP, as explained below.

As PAP accumulates, it becomes the key species that reacts with the FeCl₃ solution used in this investigation, this is because PAP is significantly more redox-active than paracetamol: in acidic solution, Fe(III) can oxidise *p*-aminophenols spontaneously (thermodynamically favourable), whereas analogous Fe(III) redox reactions with simple phenols are reported as non-spontaneous under the same acidic conditions (Al-Abadleh, Hind A et al., 2022). The increased redox activity of *p*-aminophenol compared to paracetamol likely arises from the presence of a free amine group (-NH₂), which is known to donate electron density into the aromatic ring, and thereby the phenolic hydroxyl group (-OH). In contrast, the amide group in paracetamol (-NHCO-) withdraws electron density from the ring toward the carbonyl group, creating the opposite effect and making oxidation by Fe(III) less favourable (Trout, 2022). Oxidation of PAP produces quinone-imine intermediates that are reactive and then undergo oxidative coupling to form larger oligomeric products, as described by Al-Abadleh, Hind A, et al. Eventually, literature suggests that coupling continues, with the product of coupling being poly(*p*-aminophenol), see Figure 3. As extended conjugation happens, π -electron delocalization increases over increasingly larger structures. More delocalization lowers the energy required to excite electrons, shifting absorption into the visible region (Royal Society of Chemistry, 2025). Al-Abadleh, Hind A et al., 2022 report that the Fe(III) system and PAP produces dark colored oligomers, which is consistent with strong visible

absorption across a wide range of wavelengths, also including at 580 nm.

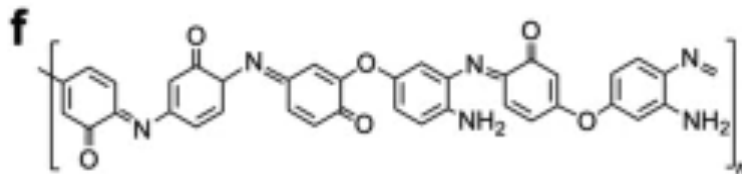


Figure 3. Al-Abadleh, Hind A et al., 2022; suggested structure for poly(*p*-aminophenol).

1.3 Preparation of Iron(III) Chloride solution

In aqueous solution, Fe(III) hydrolyses readily, producing the yellow colour of the solution; the reagent was therefore prepared in 0.1 mol/dm³ HCl to suppress hydration and maintain Fe(III) stability.



Adding HCl provides extra H⁺ ions once fully dissociated, which move the equilibrium to the left due to Le Chatelier's principle, creating a more stable solution that is a better fit for color development, as the Fe (III) ions are responsible for forming colored complexes and for oxidising PAP. Preparing Iron Chloride solutions in dilute HCl is a standard procedure for detecting phenols, described in Hasegawa and Usui 1992; moreover, it is necessary for the conditions to form oligomeric products as described previously, requiring Fe(III) ions.

1.4 Humidity control via saturated salts

In this investigation, relative humidity is controlled using saturated aqueous solutions of different soluble salts placed at the bottom of sealed containers, with the tablet samples held on trays above the liquid. At a given temperature, saturated salts and solutions of such salts have a fixed water activity, so the equilibrium vapour pressure of water above is lower than that of pure water because non-volatile salt ions dilute the liquid phase and hinder the escape of water molecules into the gas phase. The ratio between this reduced vapor pressure and the vapor pressure of pure water at the same temperature defines a characteristic equilibrium relative humidity, so each salt-temperature pair establishes a reproducible RH point. According to Greenspan and Lewis (1977), saturated MgCl₂ produces 33 % RH, NaBr ≈ 58 % RH, NaCl ≈ 75 % RH, and KNO₃ ≈ 93 % RH (at 25 °C to 30 °C). Moreover, silica

gel was used to produce very low relative humidity conditions for a control group to measure changes in absorbance without high humidity conditions. Salt was added until undissolved solid remained visible at the bottom, confirming saturation.

All chambers were verified as saturated prior to use, with relative humidity tested using a digital humidity meter ($\pm 1\%$ RH). Samples were placed after 24 hours (to allow development of equilibrium), confirming target conditions were established.

1.5 The Role of Ethanoic Acid in the Detection Signal

In the acidic medium used here, excess H^+ suppresses ethanoic acid dissociation (Le Chatelier's principle), making pH measurement unreliable, therefore it was not considered. Ethanoic acid is also not considered further as its absorption maximum falls below the visible range (NIST, 2025).

1.6 Research question

How does storage relative humidity, controlled by saturated salt solutions, influence the extent of paracetamol degradation in crushed tablet samples after 70, 85, and 100 days at $22^\circ C$, as quantified by visible colorimetry following reaction with $FeCl_3$?

1.7 Hypothesis

Higher storage humidity (RH) will produce greater $FeCl_3$ colorimetric absorbance at 580 nm, indicating more phenolic degradation products such as p-aminophenol. After 70, 85, and 100 days at $22^\circ C$, if RH increases from 8% to 98% then absorbance will increase steadily across time points.

1.8 Preliminary investigation

Before commencing the main degradation study, a short preliminary investigation was carried out to confirm that the saturated salt solutions used produced relative humidities that were

appropriate for the experiment. With limited access to salts, for instance KNO_3 (a controlled substance), the following available salts were used for testing: NaCl , CaCl_2 , MgCl_2 , with the addition of a small container filled with distilled water, and 10 g of silica gel. Over the course of a week, conditions were changed such that relative humidities of $< 10\%$, 40% , 50% , 70% , 80% , and $> 95\%$ would be achieved. Saturated solutions were placed in a small open dish within each sealed container; in each case, salt was added to the solution until undissolved solid visibly remained at the bottom, confirming saturation. Containers were sealed and left for 24 h with a digital RH meter ($\pm 1\%$ RH) until equilibrium was reached.

- 8% (10.0 g of silica gel)
- 40% (3.40 g of aqueous MgCl_2 in saturated solution with 6 cm^3 of water)
- 50% (5.50 g of aqueous CaCl_2 in 6 cm^3 of water)
- 70% (5.50 g of aqueous NaCl in saturated solution with 6 cm^3 of water)
- 80% (5.50 g of aqueous NaCl in saturated solution with 8 cm^3 of water)
- 98% (10 cm^3 of distilled water)

Preliminary results showed that different relative humidity conditions were possible to maintain distinct and reproducible humidity environments, justifying their use in a longer acetaminophen experiment.

2 Methodology

2.1 Variables

- **Independent variable:** Relative humidity (RH): 8% (10.0 g silica gel), 40% (3.40 g MgCl_2 in 6 cm^3 water), 50% (5.50 g CaCl_2 in 6 cm^3 water), 70% (5.50 g NaCl in 6 cm^3 water), 80% (5.50 g NaCl in 8 cm^3 water), and 98% (10 cm^3 distilled water).
- **Dependent variable:** Extent of degradation at 70, 85, and 100 days, quantified as visible absorbance at 580 nm after addition of FeCl_3 to tablet extracts. Higher absorbance indicates greater formation of conjugated oligomeric degradation products. Measurements were performed using 1.00 cm path-length cuvettes.

- **Controlled variables:**

- **Temperature:** 22.0 °C (± 0.1 °C) (thermostatically controlled laboratory) important to note temperature was set at 22 °C, however, this was assumed to be constant as measurement could not be attend at all times.
- **Light exposure:** dark storage (aluminium foil wrapping) to prevent photodegradation (Dao et al., 2018).
- **Sample form and mass:** 500 mg Tesco Health paracetamol tablets (single batch), crushed for homogeneity. Two tablets ($\approx 1.000 \text{ g} \pm 0.001 \text{ g}$) were placed in each chamber to maintain a consistent surface area.
- **Container geometry and headspace:** identical airtight plastic containers used for all humidity chambers.
- **FeCl₃ reagent:** single 5 % w/v (0.308 mol/dm^3) FeCl₃ batch prepared in 0.1 mol/dm^3 HCl ($\pm 0.01 \text{ mol/dm}^3$); fixed volume of 2.00 cm^3 added to 10.0 cm^3 sample.
- **Reaction time:** 40 min development period before absorbance measurement.
- **Colorimeter conditions:** same instrument used throughout, calibrated with distilled water before each session; 1.00 cm plastic cuvettes cleaned before use.
- **Replicates:** triplicate concordant readings required ($\Delta A \leq 0.02 \text{ a.u.}$).
- **Control condition:** desiccated control maintained at 8 % RH.

2.2 Materials

- Tesco Health 500 mg paracetamol tablets (same batch).
- Salts for saturated RH chambers: MgCl₂ · 6 H₂O, NaCl (anhydrous), CaCl₂ · 6 H₂O.
- Silica gel (amorphous SiO₂).
- Airtight plastic containers.
- Digital thermostat (laboratory): controlled room temperature at 22.0 °C with ± 0.1 °C uncertainty.
- Digital relative humidity (RH) measurement device (uncertainty: ± 1 % RH).

- 5 % FeCl_3 reagent solution (by mass; 0.308 mol/dm^3), prepared by dissolving 8.330 g of $\text{FeCl}_3 \cdot 6 \text{ H}_2\text{O}$ in 0.1 mol/dm^3 HCl and making up to 100 cm^3 in a volumetric flask (single batch used throughout, stored in an airtight container). Uncertainty: $\pm 0.001 \text{ g}$ (mass), $\pm 0.1 \text{ cm}^3$ (volume).
- Deionized water.
- Mortar and pestle.
- Analytical balance (uncertainty $\pm 0.001 \text{ g}$).
- Volumetric flasks, pipettes, beakers, test tubes, filter paper and/or syringe filters.
- Colorimeter (visible range; absorbance at 580 nm); matched 1 cm cuvettes (uncertainty $\pm 0.001 \text{ a.u.}$).
- PPE: lab coat, goggles, nitrile gloves, and chemical waste containers.

2.3 Procedure

Six sealed humidity chambers were prepared using identical airtight plastic containers containing saturated aqueous solutions of $\text{MgCl}_2 \cdot 6 \text{ H}_2\text{O}$, NaCl , $\text{CaCl}_2 \cdot 6 \text{ H}_2\text{O}$, distilled water (98 % RH), or 10.0 g silica gel (8 % RH control). Approximately 10 cm^3 of each solution was placed in a weighing boat, physically separated from the tablet samples to prevent direct contact. Chambers were left for 24 h to reach target relative humidities (8 %, 40 %, 50 %, 70 %, 80 % and 98 % RH), verified using a digital hygrometer ($\pm 1 \text{ % RH}$). All chambers were stored at $22.0^\circ\text{C} \pm 0.1^\circ\text{C}$ in darkness.

Tesco Health 500 mg paracetamol tablets (single batch) were crushed to a homogeneous powder. Portions of 1.000 g ($\pm 0.001 \text{ g}$) were placed into each chamber and stored for 70, 85, or 100 days.

At each time point, $0.250 \text{ g} \pm 0.001 \text{ g}$ of sample was transferred to a 100.0 cm^3 volumetric flask ($\pm 0.02 \text{ cm}^3$), dissolved in deionised water, made up to volume, homogenised, and filtered to remove insoluble excipients. A 10.0 cm^3 aliquot ($\pm 0.1 \text{ cm}^3$) was transferred to a test tube, and $2.00 \text{ cm}^3 \pm 0.1 \text{ cm}^3$ of 5 % w/v (0.308 mol/dm^3) FeCl_3 in 0.1 mol/dm^3 HCl was added. After mixing, solutions were left for 40 min at 22°C to allow development of oligomeric products (Al-Abadleh et al., 2022).

Absorbance was measured at 580 nm using a visible-light colorimeter with 1.00 cm path-length cuvettes. The instrument was calibrated with distilled water before each session. Measurements were recorded to three decimal places, and triplicate concordant readings ($\Delta A \leq 0.02$ a.u.) were obtained for each RH–time condition.

2.4 Statistical analysis methods

Triplicate absorbance values were summarised as mean $\pm$ SD for each RH–time combination, representing mean response and experimental scatter respectively.

Trends were explored visually by plotting absorbance vs RH (per time point) and vs time (per RH), with SD error bars to judge patterns against measurement variability. To test whether absorbance increases monotonically with RH (RH is ordered, but the relationship may not be strictly linear, and the sample size is small), Spearman’s rank correlation (ρ) was used at each time point.

To quantify how quickly absorbance changes over time within each humidity condition, a linear regression was fitted over the 70–100 day interval; the slope provides an interpretable rate of change (in arbitrary absorbance units per day) that allows comparison between different RH conditions.

2.5 Uncertainty propagation

Error bars in all graphical representations correspond to ± 1 standard deviation (SD) of triplicate absorbance measurements for each RH–time condition. SD was chosen over instrumental uncertainty propagation because absorbance is read directly from the colorimeter.

SD (typically 0.005–0.010 a.u.) substantially exceeded the colorimeter’s stated instrumental uncertainty (0.001 a.u.); replicate SD therefore captures dominant experimental variability from sample preparation, filtration, cuvette positioning, and reaction development. Systematic uncertainty in the absolute absorbance reading could not be accurately estimated, as no certified reference standard was available; its potential contribution is considered in the Evaluation.

2.6 Safety considerations

All methodology procedures followed CLEAPSS HazCards; specific sections are cited below.

Table 1: Risk assessment and safety precautions for chemicals and equipment used.

Safety consideration	Risk assessment	Precautions taken
0.1 mol/dm ³ HCl solution (HazCard 20)	Irritant to eyes/skin, low hazard at 0.1 mol/dm ³ dilution.	Wear eye protection; avoid skin contact; rinse spills with water.
5 % w/v FeCl ₃ solution (0.308 mol/dm ³) (HazCard 38)	Harmful if swallowed or inhaled (in powdered form); corrosive to skin/eyes.	Eye protection mandatory; handle in a fume hood if necessary, gloves recommended.
Salts (NaCl, CaCl ₂ , MgCl ₂) and silica gel (HazCards 34–36, 97)	Dust inhalation irritates the respiratory system; silica gel dehydrates skin.	Avoid dry transfer, use eye protection, and a respirator if necessary.
Glassware (student safety sheet 71)	Cuts/lacerations from breakage.	Inspect before use, wear gloves, and sweep with a brush if broken.
Crushed tablets (HazCard 97)	Fine dust inhalation causes respiratory irritation/allergy.	Wet-wipe surfaces; wear a respirator if necessary.

3 Data Collection and Processing

3.1 Raw data

Tables 2–4 show the raw absorbance measurements (three trials) and the calculated mean and standard deviation for each storage condition.

Table 2: Raw absorbance data after 70 d at 22 °C. Absorbance is reported in arbitrary units (a.u.).

Condition	RH / %	Time (d)	Absorbance 1 (a.u.)	Absorbance 2 (a.u.)	Absorbance 3 (a.u.)	Mean (a.u.)	SD (a.u.)
10 mL of water	98	70	0.694	0.684	0.682	0.687	0.006
NaCl + 8 mL water	80	70	0.529	0.523	0.517	0.523	0.006
NaCl + 6 mL water	70	70	0.577	0.582	0.574	0.578	0.004
CaCl salt	50	70	0.520	0.524	0.535	0.526	0.008
MgCl salt	40	70	0.569	0.572	0.564	0.568	0.004
10 grams of silica gel (control)	8	70	0.463	0.476	0.474	0.471	0.007

Table 3: Raw absorbance data after 85 d at 22 °C. Absorbance is reported in arbitrary units (a.u.).

Condition	RH / %	Time (d)	Absorbance 1 (a.u.)	Absorbance 2 (a.u.)	Absorbance 3 (a.u.)	Mean (a.u.)	SD (a.u.)
10 mL of water	98	85	0.755	0.763	0.746	0.755	0.009
NaCl + 8 mL water	80	85	0.518	0.507	0.513	0.513	0.006
NaCl + 6 mL water	70	85	0.491	0.482	0.477	0.483	0.007
CaCl salt	50	85	0.497	0.502	0.501	0.500	0.002
MgCl salt	40	85	0.504	0.509	0.511	0.508	0.004
10 grams of silica gel (control)	8	85	0.468	0.465	0.472	0.468	0.004

Table 4: Raw absorbance data after 100 d at 22 °C. Absorbance is reported in arbitrary units (a.u.).

Condition	RH / %	Time (d)	Absorbance 1 (a.u.)	Absorbance 2 (a.u.)	Absorbance 3 (a.u.)	Mean (a.u.)	SD (a.u.)
10 mL of water	98	100	0.765	0.768	0.770	0.768	0.003
NaCl + 8 mL water	80	100	0.605	0.607	0.598	0.603	0.005
NaCl + 6 mL water	70	100	0.586	0.599	0.582	0.589	0.009
CaCl salt	50	100	0.503	0.490	0.500	0.498	0.007
MgCl salt	40	100	0.554	0.566	0.573	0.564	0.010
10 grams of silica gel (control)	8	100	0.461	0.473	0.471	0.468	0.006

3.2 Sample calculations

The following is a worked example of the first row of table 2:

Raw absorbance readings: 0.694, 0.684, and 0.682 a.u.

Mean absorbance

$$\bar{x} = \frac{x_1 + x_2 + x_3}{3} = \frac{0.694 + 0.684 + 0.682}{3} = 0.6867 \approx 0.687 \text{ a.u.}$$

Standard deviation (sample SD)

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}, \quad n = 3$$

$$s = \sqrt{\frac{(0.694 - 0.6867)^2 + (0.684 - 0.6867)^2 + (0.682 - 0.6867)^2}{2}}$$

$$s = 0.00643 \approx 0.006 \text{ a.u.}$$

3.3 Processed data

Figure 4 shows that at each time point (70, 85, 100 days) the mean absorbance generally increases with storage RH, with the control at 8% RH giving the lowest values and 98% RH the highest. The spread between curves widens from 70 to 100 days, suggesting that humidity-driven degradation becomes more pronounced over time.

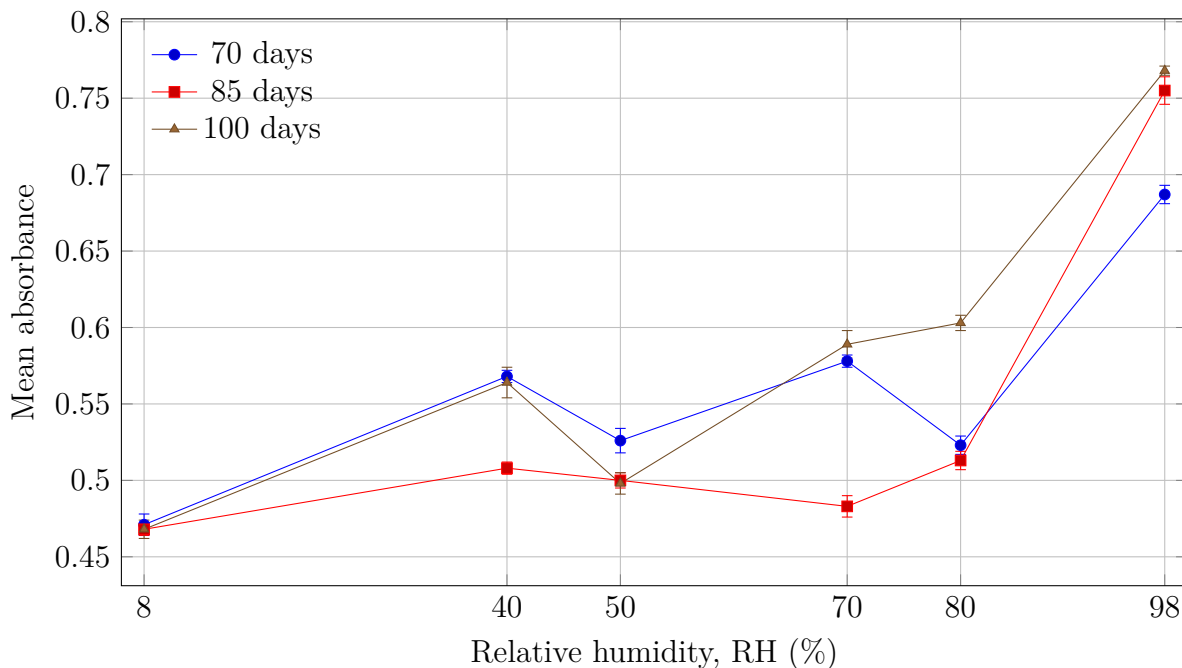


Figure 4. Absorbance vs relative humidity (RH) at 22 °C for 70, 85, and 100 days. Points show mean $\pm$ SD ($n = 3$).

Figure 5 presents the change in mean absorbance over time (70–100 days) for each storage RH condition. At 98% and 80% RH, absorbance increases noticeably with time, whereas at

8 % (control), 40 % and 50 % RH the slopes are close to zero or slightly negative within the experimental uncertainty.

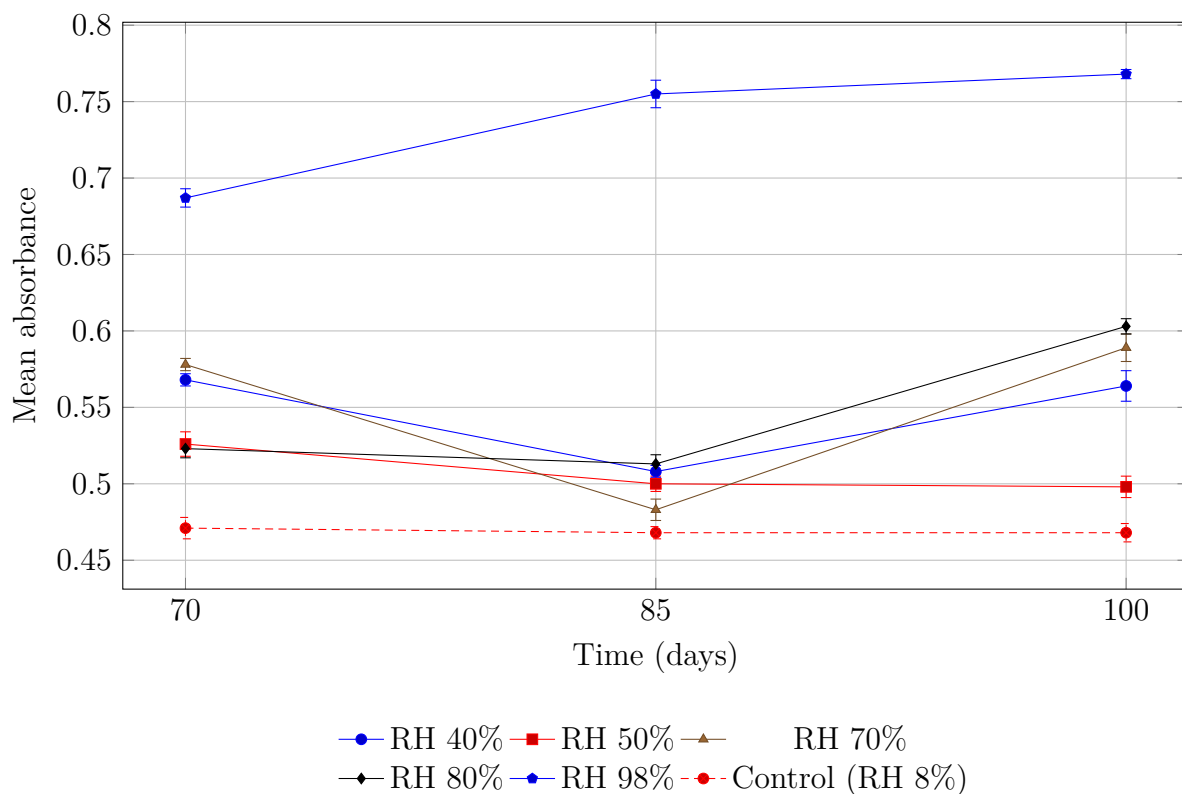


Figure 5. Absorbance vs time at 22 °C for each relative humidity condition (RH 40 %, 50 %, 70 %, 80 % and 98 %). Points show mean $\pm$ SD ($n = 3$).

Figure 6 summarizes the 100-day results by comparing mean absorbance across RH conditions. At 100 days, absorbance at 98% RH (0.768 a.u.) was 64% higher than the desiccated control (0.468 a.u.), illustrating the cumulative impact of humidity on degradation.

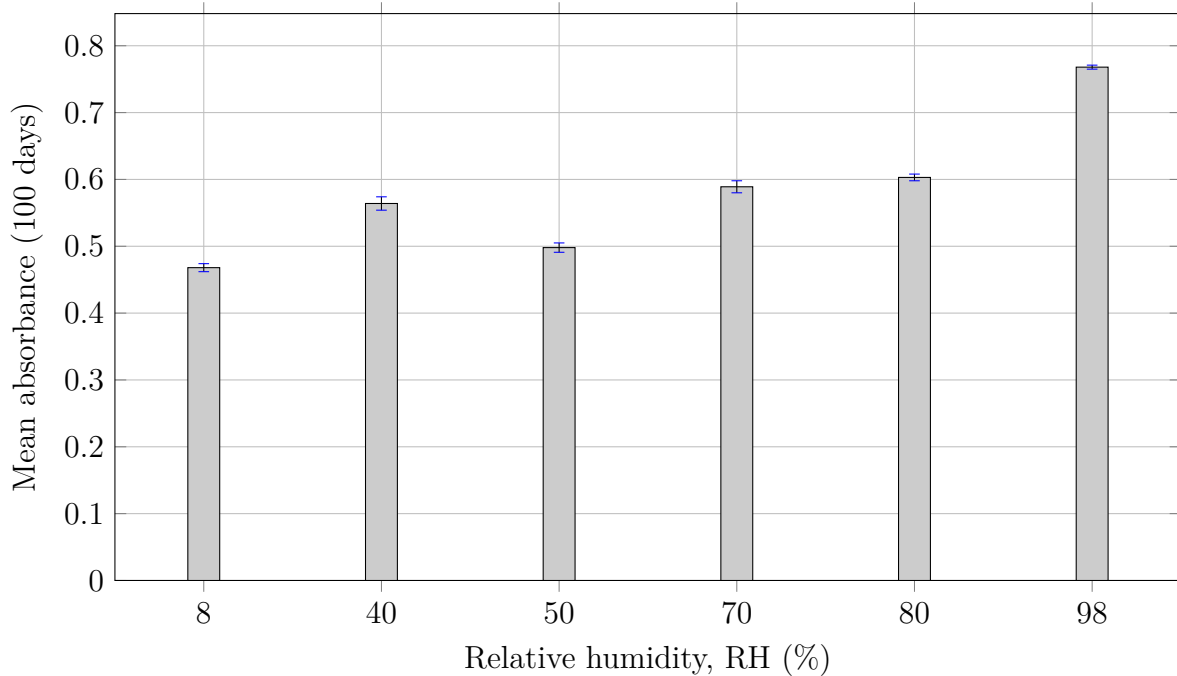


Figure 6. Mean absorbance at 100 days vs relative humidity (RH) at 22 °C. Error bars show $\pm$ SD ($n = 3$).

3.4 Linear regression: mean absorbance vs time

For each RH condition, a simple linear regression was performed using the three mean absorbance values at 70, 85, and 100 days, fitted as

$$\text{mean absorbance} = a + b(\text{time}).$$

Table 5 reports the slope b (rate of change in mean absorbance per day), its standard error, and R^2 .

The largest positive slopes were observed at 98 % and 80 % RH (0.002 70 and 0.002 67 a.u. day⁻¹, respectively), with relatively high R^2 values (0.867 and 0.658), indicating a consistent increase in absorbance over 70–100 days under very humid storage. By contrast, the slopes at 8 %, 40 % and 50 % RH are negative and small in magnitude ($-0.001\,50$ to $-0.000\,133$ a.u. day⁻¹), with low R^2 , suggesting no systematic increase and possibly only random variation within the measurement standard deviation.

3.5 Spearman rank correlation: RH vs mean absorbance (by time)

To test for a monotonic relationship between storage relative humidity (RH) and mean absorbance at each time point ($n = 6$ RH values per time, including the control group at RH 8%). The use of the control in analysis is justified because it is a distinct point on the experimental humidity gradient. Spearman’s rank correlation was calculated for 70, 85, and 100 days (Table 6). Spearman ρ was positive at all three time points (70, 85, and 100 days), indicating that higher RH generally corresponded to higher mean absorbance when the control group is included; the monotonic relationship appears strongest at 100 days (largest $|\rho|$). At 70 days, Spearman’s ρ is close to zero (0.143, $p = 0.787$), so a monotonic RH-absorbance trend cannot be distinguished from random scatter at this early time. By 100 days, ρ increases to 0.943 ($p = 0.00480$), which indicates a strong, statistically significant monotonic increase of mean absorbance with RH across the 8%–98% range.

Table 5: Ordinary least squares regression of mean absorbance vs time for each storage relative humidity (RH).

RH / %	slope / (AU day ⁻¹)	SE(slope) / (AU day ⁻¹)	R^2
8 (control)	-0.00150	0.00490	0.157
40	-0.000133	0.00223	0.003
50	-0.000933	0.00404	0.051
70	0.000367	0.00387	0.009
80	0.00267	0.00193	0.658
98	0.00270	0.00106	0.867

Table 6: Spearman rank correlation between relative humidity and mean absorbance at each storage time.

Time / days	Spearman ρ	p -value
70	0.143	0.787
85	0.429	0.397
100	0.943	0.00480

4 Analysis and Discussion

The data demonstrate that both relative humidity and storage time influence paracetamol degradation as measured by FeCl₃ colorimetry. The 8% RH control remained effectively constant across all time points (0.471, 0.468, 0.468 a.u.; SD $\approx$ 0.006 a.u.), confirming that

dry storage prevents amide hydrolysis, consistent with Haywood et al. (2006), who reported 95 % to 105 % label retention after 100 days below 60 % RH.

At each time point, absorbance increased with RH, with the divergence between low (8 % to 50 %) and high (80 % to 98 %) RH conditions widening progressively over time (Figure 4). Changes at 8 %, 40 %, and 50 % RH were negligible ($\Delta A < 0.005$ a.u.), whereas absorbance at 98 % and 80 % RH increased by 0.081 and 0.080 a.u. respectively between 70 and 100 days (Figure 5), consistent with moisture-driven amide hydrolysis producing PAP, which undergoes oxidative oligomerisation to strongly absorbing conjugated species (Al-Abadleh et al., 2022).

Linear regression and Spearman analysis (Tables 5 and 6) quantitatively support this picture. The largest slopes observed at 98 % and 80 % RH (≈ 0.0027 a.u. day⁻¹; $R^2 = 0.867$ and 0.658) are consistent with systematic degradation, while Spearman ρ strengthens from 0.143 ($p = 0.787$) at 70 days to 0.943 ($p = 0.0048$) at 100 days, indicating that the RH-absorbance relationship only becomes statistically significant with prolonged exposure.

These findings are broadly consistent with Ahmad and Shaikh (1993), who confirmed hydrolysis to 4-aminophenol via FeCl₃ and TLC after 180 days at 75 % RH, and with Haywood et al. (2006). The larger magnitude of change observed here—compared to packaged tablet studies—is attributable to direct humidity exposure of crushed powder with no packaging barrier, combined with increased surface area accelerating moisture ingress.

5 Evaluation

5.1 Evaluation and limitations

The method is different from established methods, hindering direct quantitative comparison with literature. For instance, three time points restrict regression analysis to local slopes rather than full first-order kinetics (as seen in Ahmad and Shaikh, 1993); this potentially could mask the extent of degradation, as measured through colorimetric assay. Moreover, FeCl₃ reaction measured change to oligomeric products, rather than p-aminophenol specifically, which produces values that are not in line with percent degradation. Saturated salt and other RH conditions achieved an 8 % to 98 % RH gradient; however, without consistent data logging, especially from day 1 to 70, minor changes could have weakened the correlations ob-

served. Despite such limitations, stable absorbance at RH 8 % (control), pronounced trends at RH $\geq$ 80 % (slopes $\approx$ 0.0027 a.u. day⁻¹), and strong Spearman $\rho = 0.943$ ($p = 0.00480$) at 100 days provide coherent evidence that elevated humidity at 22 °C substantially accelerates phenolic degradation in unprotected paracetamol powder, consistent with literature showing packaging as the key barrier to hydrolytic loss of active ingredient.

A source of systematic uncertainty concerns the reliability of humidity conditions over the full storage period. Saturated salt solutions maintain a fixed RH only while undissolved crystals remain present; once exhausted, the chamber RH drifts upward (Greenspan, 1977). Given that samples were stored over summer in a school laboratory, sustained slightly elevated temperatures, even within a thermostatically controlled building, could have accelerated crystal dissolution, pushing actual RH above nominal values. This could explain non-monotonic inversions between mid-RH conditions, such as 40 % RH recording higher absorbance than 50 % RH at day 100, without any error in measurement. Additionally, scatter within individual conditions is partly attributable to the nature of p-aminophenol oligomerisation. Fe(III)-catalysed oxidative coupling produces a distribution of oligomer chain lengths rather than a single defined product (Al-Abadleh et al., 2022), and longer chains absorb more strongly due to greater π -electron delocalisation; variation between triplicates could produce disproportionate absorbance scatter. For example, if shorter oligomers predominate, absorbance is lower even for the same initial PAP concentration and vice versa. Additionally, Colorimeter drift (can be attributable to instrument age) could not be quantified as no certified absorbance reference standard was available.

5.2 Improvements

Wavelength choice: UV–Vis spectrophotometry measuring paracetamol directly at its absorption maximum of 243 nm represents the standard analytical approach used across pharmaceutical stability literature (Patel et al., 2012), and would be a more appropriate method for this investigation. Unlike the indirect FeCl₃ colorimetric approach used here, direct UV–Vis measurement quantifies loss of paracetamol itself, yielding percentage degradation values that are directly comparable to published stability studies. This would both increase the accuracy of degradation quantification and allow meaningful comparison with existing literature values.

Calibration curve: Constructing a calibration curve using a standard solution of p-

aminophenol reacted with Fe(III) under identical experimental conditions could improve the quantitative reliability of the detection method, as plotting absorbance at 580 nm against known PAP concentrations would allow conversion of absorbance values into concentrations of products. However, this approach carries significant practical limitations that would require additional research before implementation: it would first need to be established whether the Fe(III)–PAP system produces a linear absorbance response across the relevant concentration range, and whether the coloured oligomeric species are sufficiently stable to yield reproducible readings from a single calibration. Furthermore, obtaining a pure sample of p-aminophenol or paracetamol at sufficient purity was not feasible in this school laboratory context, making a reliable calibration curve practically impossible without access to certified analytical-grade reagents.

Time points and larger sample size: Earlier time points (e.g., 30 or 50 days) were not feasible due to limited lab access and scheduling. The 70–100 day window was selected because significant hydrolysis is only expected within 1–3 months (Haywood et al., 2006; Ahmad Shaikh, 1993), and earlier measurement would have risked degradation falling below the school colorimeter’s detection capability meaning earlier time points and more advanced equipment would need to be implemented together.

6 Conclusion

Within the 70–100 day window studied, storing paracetamol at higher relative humidity and for longer time consistently produced higher absorbance with FeCl₃ reaction, indicating greater formation of oligomeric degradants under damp conditions. The data support the hypothesis that higher storage humidity (RH) produces greater FeCl₃ colorimetric absorbance at 580 nm.

Overall, absorbance was highest at 98% RH and lowest at the 8%RH control at all time points, with non-monotonic inversions observed among mid-RH conditions(40 %-70 %), likely attributable to RH drift in saturated salt chambers; absorbance rose consistently over time specifically at $\text{RH} \geq 80\%$, confirming that moisture accelerates degradation. Despite limitations, the trends align with the chemistry background and existing literature. Future UV–Vis measurements (e.g., at 243 nm) would enable more precise values for percent degradation, allowing better comparison and correlation of variables.

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