



FORMULATION AND EVALUATION OF N-ACETYLCYSTEINE LOZENGES

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ABSTRACT

N-Acetylcysteine is used as a beneficial drug in the management of Asthma. The objective of the study is to enhance bioavailability of N-Acetylcysteine using modification in dosage form. N-Acetylcysteine is a mucolytic agent which has 10% bioavailability. The N-Acetylcysteine's absolute bioavailability is very low as it undergoes an extensive hepatic metabolism in the gut wall and liver. To overcome this problem, lozenges of N-Acetylcysteine were prepared to avoid first pass metabolism by heating and congealing method to enhance bioavailability. The different natural polymers like xanthan gum, tragacanth and gelatin were used in the formulation. The lozenges were assessed by their physical characteristics, weight uniformity, thickness, diameter, hardness, friability, drug content, moisture levels, in vitro drug release, and stability. Total 09 batches were prepared, and from the results, Batch A6 was identified as the optimized formulation as it gives 99.45 % drug release in 75 mins. Stability study showed no significant changes. Present study suggested that lozenges of N-Acetylcysteine containing tragacanth polymer provided rapid drug release 99.54 % within a short period of time.

KEYWORDS: N-Acetylcysteine, lozenges, Heating and Congealing method.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are both chronic inflammatory diseases of the airways that induce airflow limitation. Asthma often starts in childhood, in such cases being commonly associated with allergies. It may remit and recur in adulthood.^[1] There are many treatments available in the market used in the management of Asthma and COPD. The World Health Organization (WHO) estimates that around 500 million people globally have either asthma or COPD, and these conditions together cause nearly 4 million deaths each year, with over 1 million being premature deaths. Approximately 90% of premature deaths from these diseases occur in low- and middle-income countries. Asthma has a global prevalence of about 3.6% and COPD around 3.9% in adults over 30. To overcome this problem, there is need to incorporate new remedies for the treatment of Asthma and COPD. For the present study, N-Acetylcysteine (BCS Class I drug) was selected and lozenges were formulated as the desired dosage form for the treatment of Asthma. N-Acetylcysteine is a mucolytic agent and used in the treatment of chronic bronchitis, ulcerative colitis, liver cancer, muscle performance, hemodialysis, asthma, Alzheimer and Parkinson.^[2]

Lozenges are flavored therapeutic dosage forms that contain one or more medications, usually in a sweetened base, and are meant to dissolve or disintegrate in the mouth or pharynx. Patients who are unable to swallow solid oral dosage forms and those taking medications that release gradually to maintain a steady level of drug in the oral cavity and coat the tissues in the throat with a drug solution are both treated using lozenges. Lozenges have a local effect at a specific location in the mouth and a systemic effect where the medication circulates in the blood and demonstrates its pharmacological action.^[3-4]

MATERIALS AND METHOD

MATERIALS: Ahem Lifecare LLP Ahmedabad, India provided N-Acetylcysteine as a gift sample. Sucrose, Glucose and Xanthan gum were obtained from Chemdyes Corporation, Ahmedabad (India). Rest all polymer such as Gum tragacanth and Gelatin, Methyl Paraben, Color and Flavor were obtained from Astron Chemicals, Ahmedabad (India).

METHOD: Lozenges of N-acetylcysteine were made using the heating and congealing method. In a beaker, the necessary amounts of sucrose were dissolved in water, heated to 140 °C, and stirred for two hours to create the syrup basis. A tiny amount of water was mixed with glucose, and the mixture was heated to 110°C until the glucose entirely dissolved and formed a clear, viscous syrup. The sucrose syrup was then mixed with the glucose solution. The temperature was raised to 160 °C for two hours while stirring and heating until the color turned golden yellow. A plastic mass was achieved by lowering the temperature to 90 °C. After 30 minutes of mixing, the drug, polymer, color, and flavor were introduced.^[5] The Lozenges were prepared using various ingredients with concentrations as mentioned in table 1.

Table 1: Formulation design for N-Acetylcysteine Lozenges

Ingredients (mg)	Formulation (Batch) code								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
N-Acetylcysteine	600	600	600	600	600	600	600	600	600
Sucrose	2000	2000	2000	2000	2000	2000	2000	2000	2000
Glucose	750	750	750	750	750	750	750	750	750
Xanthan gum	400	600	800	-	-	-	-	-	-
Tragacanth	-	-	-	400	600	800	-	-	-
Gelatine	-	-	-	-	-	-	400	600	800
Methylparaben	100	100	100	100	100	100	100	100	100
Orange flavour	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Colour	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Total weight	4000	4000	4000	4000	4000	4000	4000	4000	4000

CHARACTERIZATION OF N-ACETYL CYSTEINE BY UV-VISIBLE SPECTROPHOTOMETRY

Identification by UV Spectrophotometric Method:

To obtain 60 µg/ml of N-acetylcysteine, pipette 6 ml of N-acetylcysteine solution (100 µg/ml) into separate 100 ml volumetric flask and dilute with 90 % methanol to the appropriate level. Solution was scanned between 200 and 400 nm.

Methodology for Derivatization of N-acetylcysteine:

Blank Preparation: A solution of 10% Schiff Base Reagent (1 ml Schiff Base Reagent in 10 ml of 90 % methanol as mark-up solvent) was placed into 25 mL comparison tube and is diluted to graduation and mixed well, this was considered as reagent blank solution.

Sample Preparation: Another 25 mL comparison tube was filled with a 10% Schiff Base Reagent solution (1 ml Schiff Base Reagent in 10 ml of 90 % methanol as the solvent) and 1 ml reagent was added to each sample solution of N-Acetylcysteine. The mixture was thoroughly mixed and diluted to graduation. This solution is referred to as the sample solution. After allowing the sample and

reagent blank solutions to react for a while at room temperature, the absorbance was measured at 214 nm using a 1.0 cm cuvette in comparison to the reagent blank.

Determination of Melting point of N-Acetylcysteine: Melting point of N-Acetylcysteine was determined by open capillary method in which the API was filled in the capillary tubes and melting point was measured thrice.

Determination of N-Acetylcysteine by FTIR: FTIR spectroscopy of pure drug and physical mixture of drug and excipients was carried out to check the compatibility of drug and excipients.

EVALUATION OF N-ACETYLCYSTEINE LOZENGES

Physical characteristics: The consistency, texture, and clarity of the medicated lozenges were evaluated. Visual examination of the product was used to assess the stickiness of the lozenges' texture.^[7]

Weight Variation Test: A Digital balance was used to weigh ten lozenges in grams from each batch. The standard deviations and average weight are computed. Each lozenge's individual weight is likewise determined using the same method and contrasted with the average weight.

$$\% \text{ Deviation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100$$

Thickness and diameter Test: Thickness and diameter of six lozenges was measured individually by Vernier Calipers in millimeters. The average thickness with the standard deviation are reported.

Hardness Test: Six lozenges with known weight and thickness of each batch were taken and their hardness were recorded in kg/cm² and the average hardness with the standard deviation is reported.

Friability test: Lozenges were weighed and placed in a Roche friability tester, and all parameters were set. The device was rotated at 25 rpm (100 revolutions) for 4 minutes. After rotation, the lozenges were removed and reweighed. The maximum average weight loss of the sample did not exceed 1.0%. % Friability was determined using following equation.

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

% Drug Content: Lozenges were powdered and weight equivalent to 600 mg was weighed, then the powder was taken in 100 ml volumetric flask prefilled with 50 ml pH 6.8 phosphate buffer solution and allowed 30 mins to stand. The volume was made upto 100 ml with pH 6.8 buffer solution. The stock solution was then diluted and absorption was recorded at 214 nm. The official potency range for most of the larger dose drugs in lozenge form, permitted is not < 90% not > 110% of the labelled amount.^[8]

Moisture Content: 1 lozenge is weighed, placed in a desiccator for 24 hrs and after 24 hrs the final weight was calculated using below formula:

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

In vitro Drug Release study: The modified dissolution test apparatus USP type II (paddle) was used, 900 ml of the phosphate buffer pH 6.8 was placed in the beaker dissolution medium maintained at temperature 37.5°C ± 0.5°C. 1 lozenge was added and stirred at 100 rpm. At 15 mins time interval 5 ml aliquot samples were withdrawn and replaced with an equal volume of phosphate buffer pH 6.8 immediately. By UV-Visible spectrophotometer, each aliquot was diluted and analyzed using phosphate buffer at pH 6.8 as blank. The drug release was determined from the standard calibration curve of pure drug and the samples were analyzed at 214 nm.^[9-13]

Stability study of optimized formulations: Stability study was performed for 3 months at 40°C /75% RH for the optimized formulation. The data were collected at the end of 3 months and compared with initial data to check stability of the formulation.

RESULTS AND DISCUSSION

Estimation of drug by UV spectrophotometry

The absorbance of N-Acetylcysteine in a pH 6.8 phosphate buffer solution was scanned using UV Visible Spectrophotometer between the ranges of 200-400 nm wavelength. The resulting spectrum of N-Acetylcysteine exhibited a maximum absorbance at 214 nm (λ_{max}). To construct the calibration curve of N-Acetylcysteine, solutions of 20, 40, 60, 80, and 100 $\mu\text{g/ml}$ of drug in pH 6.8 Phosphate Buffer was prepared and the overlay UV spectra of N-Acetylcysteine were generated by scanning those different concentration at 214 nm. The overlay spectra for N-Acetylcysteine is represented in Fig. 1. The absorbance of different concentration of N-Acetylcysteine in phosphate buffer at pH 6.8 showed in Table 2. The regression analysis of N-Acetylcysteine showed in Table 3.

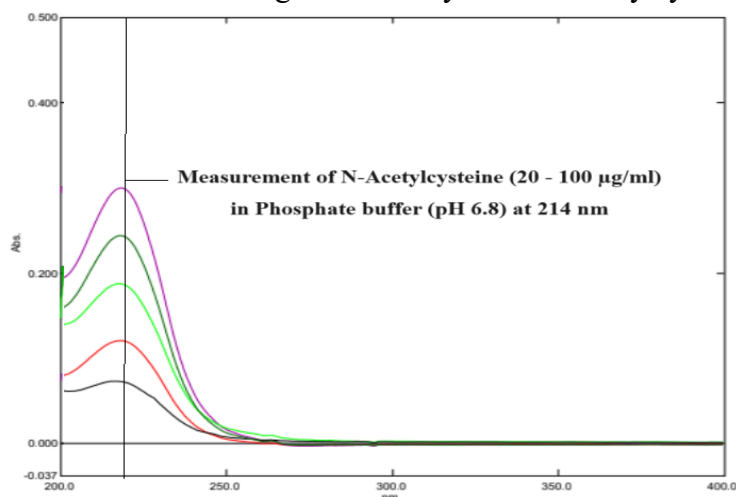


Fig. 1: Overlay UV Spectra of N-Acetylcysteine (20 - 100 $\mu\text{g/ml}$) at 214 nm

Table 2: Absorbance of different concentration of N-Acetylcysteine in phosphate buffer at pH 6.8

Sr. No.	Concentration ($\mu\text{g/ml}$)	Absorbance			Mean Absorbance $\pm$ SD
		I	II	III	
1	20	0.075	0.079	0.078	0.077 ± 0.002
2	40	0.125	0.122	0.126	0.124 ± 0.002
3	60	0.189	0.185	0.186	0.186 ± 0.002
4	80	0.245	0.251	0.248	0.248 ± 0.003
5	100	0.302	0.303	0.304	0.303 ± 0.001

The calibration curve for the prepared working solutions of N-acetylcysteine (20-100 $\mu\text{g/ml}$) was constructed by plotting the calibration curve of concentration versus mean absorbance (Fig. 2).

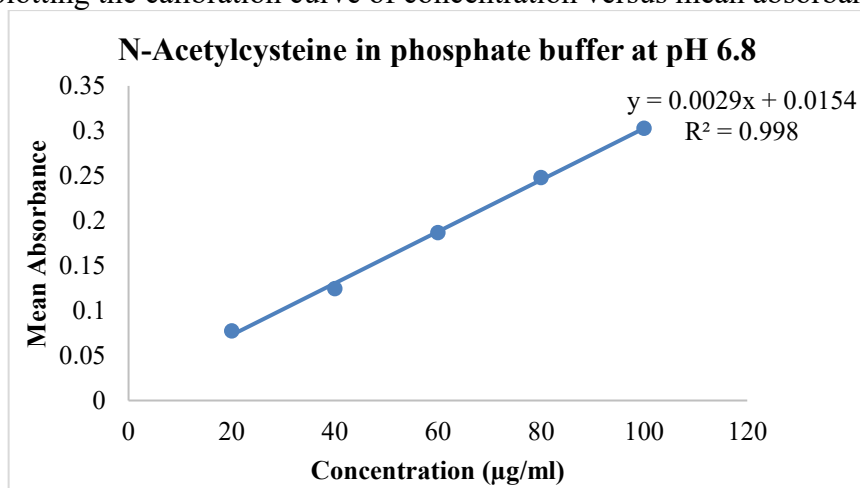


Fig. 2: Calibration curve of N-Acetylcysteine in pH 6.8 phosphate buffer solution

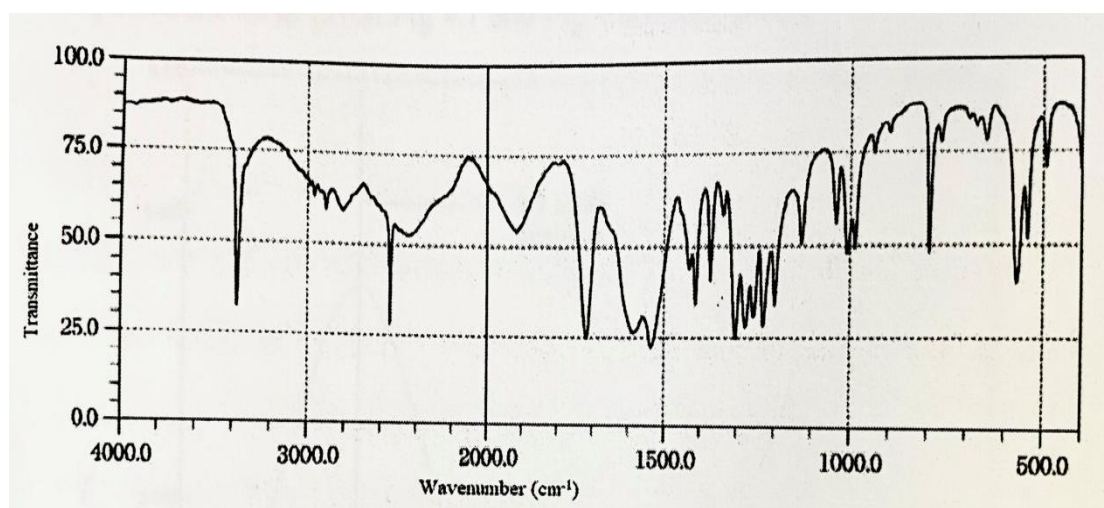
Table 3: Regression Analysis of N-Acetylcysteine in pH 6.8 phosphate buffer solution

Sr. No.	Parameters	Result
1	Regression equation	$y = 0.0029x + 0.0154$
2	Correlation coefficient	$R^2 = 0.998$
3	Linearity range	20 - 100 $\mu\text{g/ml}$

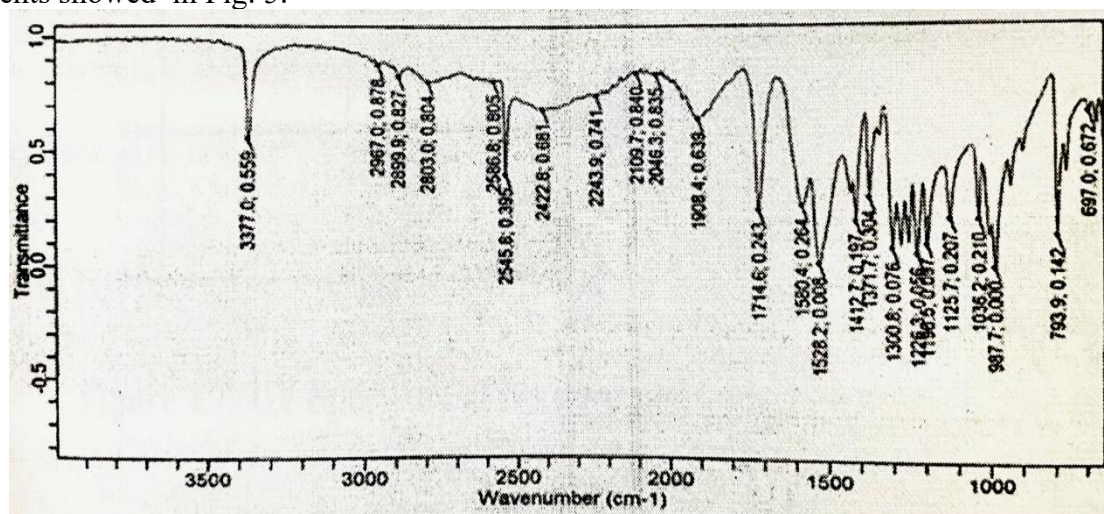
Determination of Melting Point of N-Acetylcysteine: Melting point of N-Acetylcysteine was found in the range of 110°C - 112°C. by using open capillary method. Reported melting point of N-Acetylcysteine is 109°C - 111°C. [27] Thus, the measured melting point was in the range of reported melting point of N-Acetylcysteine, so it was concluded that received API was N-Acetylcysteine.

FTIR Study of N-Acetylcysteine and physical mixture

FTIR Spectra of Drug standard was obtained by FTIR Spectrophotometer. Small quantities of standard was kept directly in the sample compartment of FT-IR and scanning was done in the range of 400-4000 cm^{-1} . FT-IR Spectra of N-Acetylcysteine was interpreted. FTIR Spectrum of Standard N-Acetylcysteine showed in Fig. 3.

**Fig. 3: IR Spectrum of Standard N-Acetylcysteine**

FTIR Spectrum of N-Acetylcysteine API is given in Fig. 4 and FTIR spectrum of API with excipients showed in Fig. 5.

**Fig. 4: FTIR Spectrum of N-Acetylcysteine API**

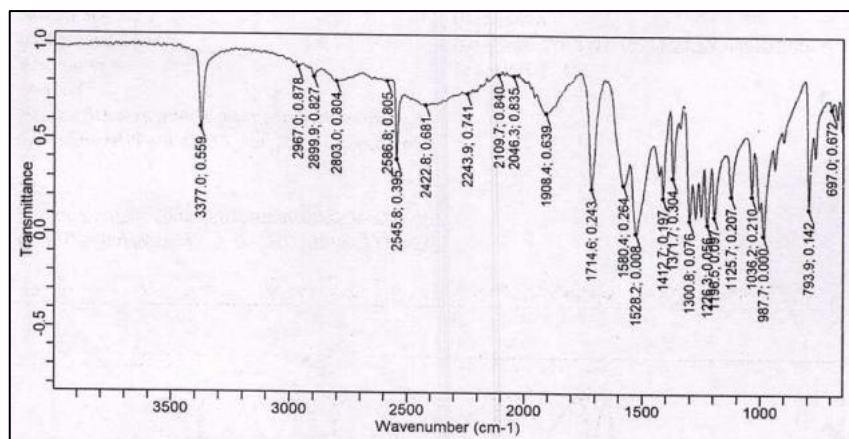


Fig. 5: FTIR of N-Acetylcysteine with Excipients

From the above graph, interpretation was found that major functional groups are present in the reported structure of N-Acetylcysteine and are present in the FTIR of N-Acetylcysteine and excipients with no significant change in band width.

Physical appearance: Observations regarding the physical appearance parameters revealed that batch A1 exhibited a sticky texture, while all other batches were non-sticky. Additionally, batches A3, A8 and A9 displayed slightly rough appearance, whereas all other batches had a smooth texture. However, all batches remained within acceptable limits. Furthermore, all batches were translucent in appearance (Table 4).

The lozenges prepared using Gelatine, Xanthan Gum and Tragacanth showed in Fig. 6.

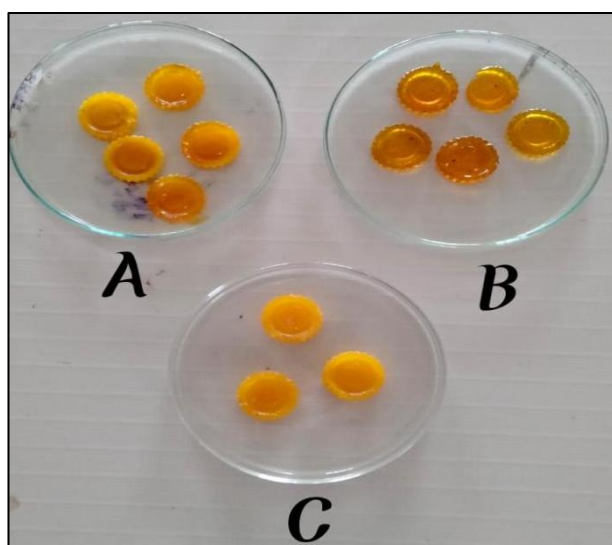


Fig. 6: Image of lozenge (A) Gelatin, (B) Xanthan Gum and (C) Tragacanth

Table 4: Physical appearance of Lozenges

Batch code	Clarity	Stickiness	Texture
A1	Yellowish translucent	Sticky	Smooth
A2	Yellowish translucent	Non-Sticky	Smooth
A3	Yellowish translucent	Non-Sticky	slightly rough
A4	Yellowish translucent	Non-Sticky	Smooth
A5	Yellowish translucent	Non-Sticky	Smooth
A6	Yellowish translucent	Non-Sticky	Smooth
A7	Yellowish translucent	Non-Sticky	Smooth
A8	Yellowish translucent	Non-Sticky	slightly rough
A9	Yellowish translucent	Non-Sticky	slightly rough

Diameter (mm): Diameter was calculated for the formulated batches and was found in the range of 13.85 ± 0.06 mm to 14.10 ± 0.01 mm.

Thickness (mm): Thickness of the formulated batches was found in the range of 6.06 ± 0.02 to 6.20 ± 0.01 mm.

Weight variation (mg): Weight of the lozenges was found to be from 3.80 ± 0.10 gm to 4.13 ± 0.58 gm. Thus, as per the Pharmacopeia weight variation limit, all the batches prepared complied the test.

Hardness (kg/cm²): Hardness of lozenges prepared by heating and congealing method was found in the range of 8.50 ± 0.87 kg/cm² to 13.00 ± 0.50 kg/cm².

Friability (%): Friability was found in the range of 0.53 % to 0.78 % for the prepared Lozenges.

Moisture content (%): Moisture content influences manufacturing and packaging of lozenges. Moisture content should be in the range of 0.5 to 1.5 % as per the standard limit. From the evaluation of prepared Lozenges, moisture content was obtained in the range of 0.69 to 1.49 % and hence it was within the standard limits.

Drug content (%): Drug content of the prepared lozenges was calculated to be 97.15 % to 98.86 %, thus drug content indicated that lozenges had uniform distribution and proper dose of active ingredient.

Diameter, Thickness, Hardness, Weight variation, Friability, Moisture Content and Drug Content

The Diameter, Thickness, Weight variation, Hardness data of lozenges were mentioned in Table 5.

Table 5: Diameter, Thickness, Weight variation and Hardness data

Batch	Diameter (mm±S.D.)	Thickness (mm±S.D.)	Weight Variation (gm±S.D.)	Hardness (kg/cm ² ± S.D.)
A1	14.06±0.02	6.19±0.02	4.13±0.58	8.50±0.87
A2	13.85±0.06	6.20±0.01	3.97±0.37	9.67±0.76
A3	14.10±0.01	6.16±0.01	4.07±0.15	8.67±0.76
A4	13.98±0.01	6.06±0.02	4.13±0.20	9.00±0.50
A5	14.01±0.01	6.14±0.03	4.00±0.26	12.50±0.87
A6	13.98±0.04	6.07±0.01	3.80±0.10	13.00±0.50
A7	14.08±0.02	6.06±0.02	3.90±0.11	11.67±0.76
A8	13.85±0.04	6.08±0.01	4.07±0.15	11.17±0.58
A9	13.92±0.08	6.16±0.01	3.93±0.15	10.83±0.76

The Friability, Moisture content and Drug Content data of lozenges were mentioned in Table 6.

Table 6: Friability, Moisture content and Drug Content data

Batch	Friability (%)	Moisture content (%)	Drug content (%)
A1	0.78	1.49	98.86
A2	0.71	1.19	97.82
A3	0.74	1.44	98.27
A4	0.69	1.15	98.02
A5	0.57	0.84	97.15
A6	0.53	0.69	98.43
A7	0.58	0.93	97.60
A8	0.66	1.02	98.83
A9	0.63	0.94	98.62

***In Vitro* Drug Release Profile of lozenges**

Dissolution test apparatus type II (paddle) was used for this study. 900 ml of the pH 6.8 phosphate buffer solution was taken as a dissolution medium and maintained at $37^\circ \pm 0.5^\circ \text{C}$ and 50 rpm. Results in the Table 7 showed, there is increase in the drug release as the concentration of polymer

increases. Also more than 50% of drug released in less than 30 mins. Formulations A1 to A3 shows drug release 98.63 %, 98.49 %, and 99.76 %, at 120, 105 and 120 mins, respectively. Formulation A4 to A6 shows drug release 99.21 %, 98.65 % and 99.54 % at the end of 105, 75 and 75 minutes, respectively. Formulations A7 to A9 shows drug release 99.32 %, 99.58 % and 98.99 % at the end of 90, 105 and 90 minutes, respectively. Drug release profile indicates that as concentration of Polymer increases from 0.4% to 0.8% (Fig. 7).

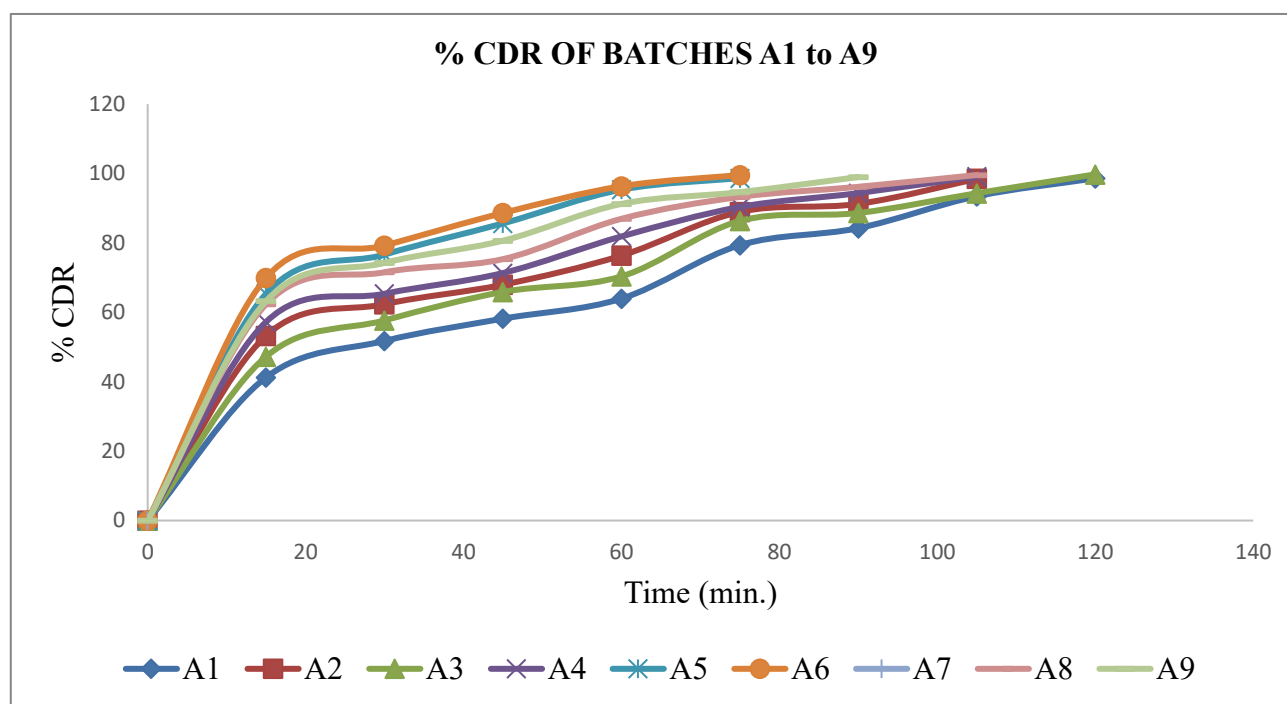


Fig. 7: % Drug release of Batches A1 to A9

Table 7: Percentage drug release of batches A1 to A9

Time (min)	% Drug release								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
0	0	0	0	0	0	0	0	0	0
15	41.26	53.26	47.26	57.26	65.36	69.96	65.96	62.36	63.26
30	51.76	62.36	57.68	65.36	76.65	79.25	76.59	71.56	74.26
45	58.18	67.96	65.89	71.34	85.63	88.63	85.66	75.34	80.66
60	63.96	76.36	70.36	81.88	95.36	96.34	91.65	86.96	91.25
75	79.36	88.96	86.36	90.36	98.65	99.54	95.39	93.26	94.63
90	84.26	91.25	88.63	94.36	-	-	99.32	96.17	98.99
105	93.45	98.49	94.32	99.21	-	-	-	99.58	-
120	98.63	-	99.76	-	-	-	-	-	-

Stability study: On the basis of all above parameters, batch A6 showed good physical characteristics, and Drug Content of N-Acetylcysteine was 98.43 %. Moreover, it showed % drug release of N-Acetylcysteine 99.54 % in just 75 minutes. Thus, batch A6 was selected as an optimized batch.

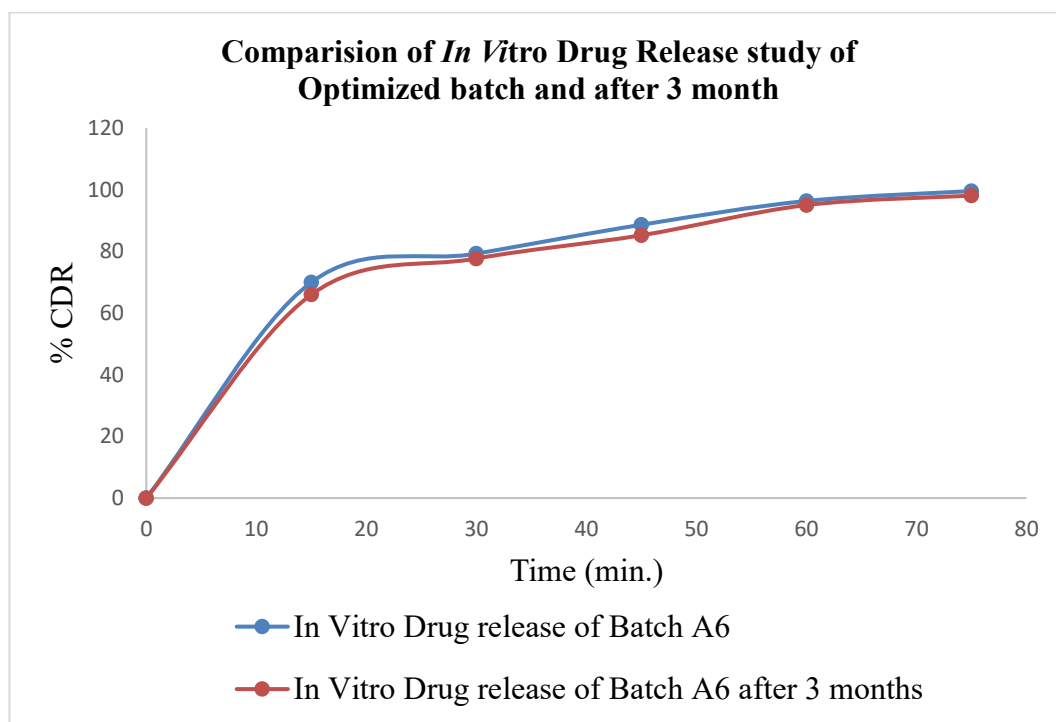
Table 8 and Table 9 shows the comparison of different parameters and % Drug Release of the Optimized batch A6 initially and after 3 months, respectively.

Table 8: Comparison of different parameters of the Stability study

Sr. No.	Evaluation Parameter	Results of optimized batch A6	Result after 3 months at 40° ± 2 °C and 75 ± 5 % RH
1	Hardness	13.00±0.50 Kg/cm ²	15.34±0.76 Kg/cm ²
2	Moisture content	0.69 %	0.62 %
3	Drug content	98.43 %	97.98 %

Table 9: Comparison of % Drug Release Study of the Stability Batch

Time (Min.)	% Drug Release of Optimized Batch A6 (%)	% Drug Release of batch A6 After Time Period of 3 Months (%)
0	0	0
15	69.96	68.92
30	79.25	78.66
45	88.63	87.93
60	96.34	95.48
75	99.54	99.03

**Fig. 8: Comparison of % Drug Release study of Optimized batch initial and after 3 months**

Comparison of % Drug Release of Optimized batch and after 3 months is presented in Fig. 8. Since the findings showed only a slight change, the data indicated that all the parameters were within reasonable limits. As a result, it was discovered that the optimized batch A6 remained stable for three months.

CONCLUSION

N-acetylcysteine lozenges were designed to boost bioavailability by absorbing via the oral mucosa, avoiding first-pass metabolism. It was discovered that the chosen lozenge optimized formula A6 (including Tragacanth) had a 99.54% in vitro release of N-acetylcysteine in just 75 minutes. Every formulation had good mechanical strength, as evidenced by the average weight, friability, and hardness being within compendial limits. The homogeneity of the drug content was within permissible bounds, indicating that the drug was distributed uniformly throughout the lozenges.

The result of stability study of the batch A6 showed that there was no significant change in Hardness, moisture content, drug content, and *In Vitro* dissolution profile when stored at room temperature for period of three months. Hence, it was concluded that N-Acetylcysteine Lozenges can be prepared effectively using Natural Polymers by heating and congealing method, which can provide rapid drug release within a short period time which can be used for the treatment of Asthma.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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