International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 5, 2023

Original Article

IN VITRO MUCOLYTIC ACTIVITY DETERMINATION OF N-ACETYL CYSTEINE EFFERVESCENT TABLET USING SUSPENDED LEVEL VISCOMETER

NANGUDE SATISH[1*], RAVINDRA KAMBLE[1], VANHERE KAJAL[2]

[1]Faculty of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan-313001, India. [2]Department of Pharmaceutics, MVP Samaj's College of Pharmacy, Nashik, Maharashtra-422002, India

[*]Corresponding author: Nangude Satish; [*]Email: satishnangude4017@gmail.com

Received: 11 Apr 2023, Revised and Accepted: 19 Jul 2023

ABSTRACT

Objective: The objective of the present study was to determine the *in vitro* mucolytic activity of N-Acetyl Cysteine (NAC) effervescent tablet using egg white solution and suspended level viscometer.

Methods: Egg white has similar physicochemical characteristics with airway mucus, thus *in vitro* mucolytic activity of NAC effervescent tablets was assessed using an egg white solution and a suspended level viscometer. This was also compared with API and marketed effervescent tablets. Outcomes were statistically analysed using a single-factor ANOVA and a paired t-test.

Results: The results show, in all three cases, i.e., API, Test tablet, and Commercial tablet, viscosity reduced linearly as the concentration was raised from 10 to 60 mg/10 ml having R^2 values 0.9973, 0.9909, 0.9953 respectively. When compared to the negative control solution, viscosity rapidly decreased, which amounted to 71.10 %, 73.03 % and 84.63 % of API, Test tablet and Commercial tablet, respectively. The p values of single factor ANOVA and paired t-test were found to be very less than 0.05 in all cases.

Conclusion: NAC effervescent tablet's *in vitro* mucolytic activity was successfully assessed; results suggested that linearly decreases in viscosity as the concentration of NAC increases and statistical analysis shows significant differences in values.

Keywords: Mucolytic activity, NAC, Viscosity

DOI: https://dx.doi.org/10.22159/ijap.2023v15i5.48069. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Despite constant exposure to infections, particulates, and hazardous substances in inhaled air, the lungs are extremely resistant to environmental damage due to the tenacious nature of mucus [1]. Airway mucus, an extracellular gel with water and mucins, which are heavily glycosylated proteins as its main constituents, provides a highly efficient defence that is essential to their resistance. By ciliary beating and coughing, airway mucus captures inhaled poisons and transfers them out of the lungs with a clearance rate of 100 µm/sec [2–4].

Hypersecretion of the airway mucus, which can exacerbate chronic obstructive pulmonary disease, hasten the loss of forced expiratory volume, along with other consequences [5]. A healthy amount of mucus has 3% solids. Up to 15% more solids may be present due to hypersecretion of mucin or abnormal surface liquid volume, resulting in sticky and stretchy mucus that is difficult to expel [2].

By depolymerizing mucus, mucolytic substances can reduce the viscosity of airway mucous discharges and are thus helpful as adjuvant treatment agents in patients with chronic or acute bronchopulmonary illnesses including cystic fibrosis and emphysema [5, 6]. Expectorants and ion channel modifiers are examples of mucolytic drugs that provide water to the airway to reduce secretion viscosity. Classic mucolytics depolymerize the mucin network. Peptide mucolytics cleave aberrant polymers in a mucus-like DNA and filamentous actin [7]. Classic mucolytics, like N-Acetyl Cysteine, have free thiol groups that can break down mucus. Free thiol groups, which de-hydrolyse disulphide bonds, make it conceivable. *In vitro*, N-Acetyl Cysteine has the potential to reduce mucus viscosity [5, 6, 8–10].

The sulfhydryl-containing chemical N-acetylcysteine (NAC) having IUPAC name (2R)-2-acetamido-3-sulfanylpropanoic acid, molecular weight 163.2 g/mol, which was initially patented in 1960 and first reported for use in medicine in 1967. Since then, the usage of NAC has been extended to treat acetaminophen overdose and chronic obstructive pulmonary disease, and its therapeutic application is constantly growing. Over the past ten years, there have been numerous systematic reviews of NAC in the literature that have examined various clinical trials in psychiatry [11], neurology [12], metabolic disease [13], pulmonary disease [14], infectious diseases, including potential use in acute respiratory syndromes like coronavirus 2 (SARS-CoV-2) [15, 16], infertility [17], and as a metal chelator [18]. In addition to its anti-inflammatory [19], antioxidant [20] and mucolytic characteristics, NAC has the ability to increase the S-transferase activity of glutathione, replenish glutathione, scavenge free radicals, and by cross-linking cysteine disulphide bonds stabilise protein structures. NAC has a 6.25-h half-life, renal and nonrenal clearance, and diarrhoea, nausea, and vomiting as adverse effects [21–24].

Thiol reduction causes physical changes in the bronchial glycoprotein that are related to decreased sedimentation coefficient, molecular size, and viscosity [25, 26]. The disulphide bond (S-S) is converted by NAC to a sulfhydryl bond (-SH), which does not take part in crosslinking, lowering mucus viscosity and elasticity [8, 27]. NAC has equal effects on sputum that is purulent and non-purulent. Additional *in vitro* experiments showed that NAC's mucolytic activity increased in solutions with pH values greater than 5.5 to 8.0 [7].

To ascertain the airway mucus's *in vitro* mucolytic activity, an ideal model system should resemble cervical and respiratory mucus in terms of its biophysical (rheological) and biochemical characteristics [28]. In our search for such a system, we have considered a variety of possibilities, including saliva, egg white, and extracellular glycoprotein from yeasts. Egg white is heterogeneous in composition and contains 88% water, 10.6% proteins, 0.9% carbohydrates, and 0.5% minerals [29]. Normal bronchial mucus resembles egg whites in texture [2, 30].

Based on prior research [31–34] and the similar physicochemical characteristics of egg white with airway mucus, we made the decision to use egg white to test the *in vitro* mucolytic efficacy of NAC effervescent tablets. This can be done using a variety of viscometers, including Ostwald viscosimeter [34], Brookfield viscometer [31], stormer viscometer [32], semi-micro-Ostwald viscometer [8] etc.

This research aimed to determine the *in vitro* mucolytic activity of NAC effervescent tablets by using a suspended-level viscometer as specified in Indian Pharmacopoeia [35].

MATERIALS AND METHODS

Materials

N-Acetyl Cysteine was procured from Ningbo Honour Chem-Tech Co. Ltd., Ningbo, China, NAC effervescent tablets were manufactured by a novel technique at SciTech Specialities Pvt. Ltd. Sinnar, India, Egg white was obtained from Hen's eggs was brought from local market Sinnar, India. For comparison commercial tablets were brought from a local pharmacy store, Sinnar, India.

Method

Egg white and egg yolk were separated and stirred at 200 rpm to get a homogeneous solution of it. Test solution samples were prepared in water to get 10, 20, 30, 40, 50, and 60 mg/10 ml concentrations of API (Positive control sample), Test Effervescent Tablet, and Marketed Effervescent Tablet. Then, 25 ml of egg white solution was added to each 10 ml of the test solution and stirred at 300±20 rpm for 10 min. After that, the relative density was calculated using a specific gravity bottle, and the dynamic viscosity was calculated by determining the flow time of each sample using a suspended level viscometer as specified in the Indian Pharmacopoeia. The same procedure was repeated for the negative control sample containing egg white and only 10 ml water (without NAC).

For calculating dynamic viscosity (n) in millipascal seconds (mPa s) the following equation was used:

$n = KPt$,

Where t = time in seconds for the meniscus to fall from the upper mark to the lower mark,

P= mass/volume (g/cm^3) obtained by multiplying the relative density, of the liquid under examination by 0.998203.

K= The constant (K) of the instrument was determined on a liquid of known viscosity

Statistical analysis

Data were provided as mean±SD ($^*P<0.05$) for each experiment, which tested at least three samples. For statistical analysis, single factor analysis of variance (ANOVA) and paired t-test were carried out.

RESULTS AND DISCUSSION

The findings presented in this study hold significant importance in the field of mucolytic therapy for respiratory conditions. The results demonstrate the *in vitro* mucolytic activity of N-acetyl cysteine (NAC) effervescent tablets using an egg white model and a suspended-level viscometer. By investigating the relationship between NAC concentration and viscosity reduction, this study provides valuable insights into the effectiveness of NAC as a mucolytic agent.

The outcomes of each sample were displayed in table 1. These values were calculated by averaging the results of three tests (n=3) performed on each sample and adding their respective standard deviations. As the concentration was raised from 10 to 60 mg/10 ml in all three cases, i.e., API, Test tablet, and Commercial tablet, viscosity reduced linearly, having R^2 values 0.9973, 0.9909, and 0.9953, respectively (fig. 1). The observed linear decrease in viscosity as the concentration of NAC increases aligns with previous research on NAC's mucolytic action, [8] which involves the depolymerization of sputum to reduce its viscosity. These results further support the existing knowledge and understanding of NAC's therapeutic potential in treating respiratory conditions characterized by increased mucus viscosity.

The viscosities of each concentration of API (positive control sample) were found to be lower (2.5606 to 2.2149) than those of each concentration of Test tablet (2.7358 to 2.2751) as well as Commercial tablet (2.9540 to 2.6366). This could be due to excipient interference in effervescent tablets when compared to the positive control sample. Viscosities of Test tablets (2.7358 to 2.2751) were found to be lower than those of Commercial tablets (2.9540 to 2.6366) which shows greater mucolytic action of Test tablets when compared to commercial tablets. This suggests that the formulation or manufacturing technique employed for the Test tablet may contribute to its superior mucolytic activity. These findings have practical implications for the development and optimization of NAC effervescent tablets, highlighting the importance of formulation considerations in achieving enhanced therapeutic efficacy.

Table 1: Mean viscosity results with a standard deviation

S. No.	Concentration (mg/10 ml)	API (mPa s)	Test tablet (mPa s)	Commercial tablet (mPa s)
1	10	2.5606±0.010	2.7358±0.011	2.9540±0.010
2	20	2.4942±0.007	2.6353±0.009	2.9083±0.016
3	30	2.4179±0.011	2.5127±0.008	2.8443±0.007
4	40	2.3374±0.012	2.4267±0.008	2.7742±0.012
5	50	2.2856±0.015	2.3532±0.015	2.7152±0.012
6	60	2.2149±0.012	2.2751±0.008	2.6366±0.006

(All values are mean±SD; n=3)

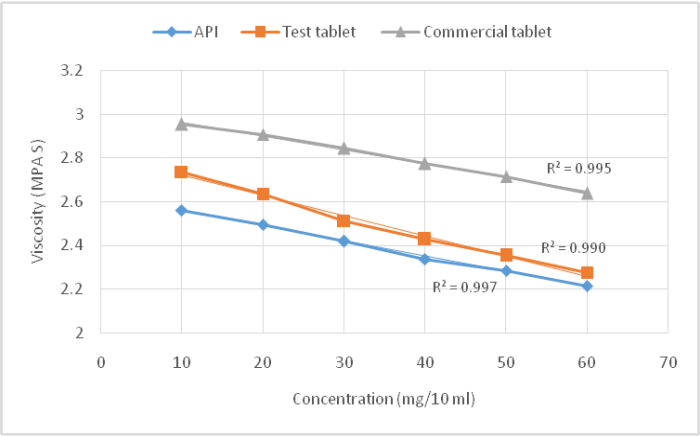

Fig. 1: Viscosity reduction with respect to concentration (mean; n=3)

The viscosity of the negative control sample (egg white+water) was found to be 3.1154±0.012 mPa s. which was further compared with all samples to determine the reduction of viscosity (fig. 2). Increasing the concentration of NAC to 6 mg/ml rapidly decreases the viscosity which was amount to 71.10 %, 73.03 % and 84.63 % of API, Test tablet and Commercial tablet respectively. These findings are consistent with previous research, which demonstrated *in vitro* viscosity reduction using an Ostwald viscometer at a concentration of 10 mmol NAC [34]. The comparable viscosity reduction percentages obtained in this study further validate the effectiveness of NAC in reducing viscosity and corroborate the previous research findings.

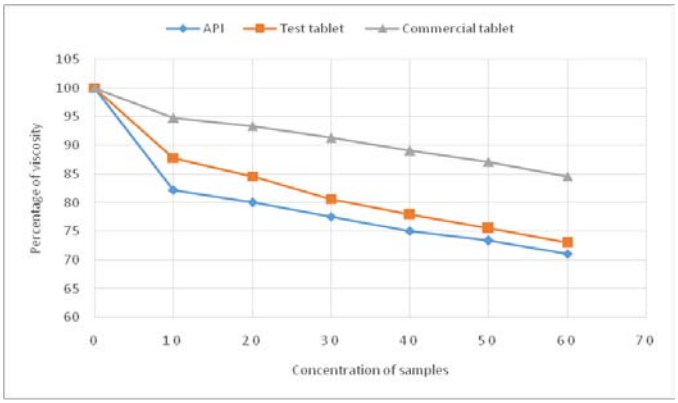

Fig. 2: % reduction in viscosity (mean; n=3)

The statistical analysis conducted in this study adds further robustness to the findings. Based on the viscosity measurement results of all samples, which were done in triplicate single factor analysis of variance (ANOVA) was carried out by taking a p-value less than 0.05(table 2). The P-value was found to be very less than 0.05 i.e., 2.34E-12, showing that the results obtained from viscosity measurement were significantly different, rejecting the null hypothesis and accepting the alternate hypothesis. F value was also greater than F critical value (47.39011>3.178799) also shows that obtained results were significantly different.

Table 2: Single-factor ANOVA

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F	P-value	F critical value
Between groups	1.723845	2	0.861922	47.39011	2.34E-12*	3.178799
Within groups	0.927578	51	0.018188			
Total	2.651423	53				

Paired t-test for further statistical analysis was also performed between all possible pairs of different samples i.e., API and Test tablet, API and Commercial tablet and Test tablet and Commercial tablet. Obtained p values of API and Test tablet, API and Commercial tablet and Test tablet and Commercial tablet were 9.2131E-09, 1.5745E-24, and 2.00054E-14, respectively (table 3) p values of all pairs were very less than 0.05 which shows a significant difference between all pairs. These findings hold paramount importance as they highlight the potential of the Test tablet as a more effective option for the intended purpose. The extremely low p-values obtained for all pairs (API and Test tablet, API and Commercial tablet, and Test tablet and Commercial tablet) indicate that there are significant distinctions in viscosity between each pair. This underscores the superior mucolytic activity of the Test tablet compared to both the API and the Commercial tablet.

The insights provided by this study into the comparative performance of the different formulations offer valuable guidance for further development and optimization. These results can serve as a foundation for future research and advancement in the field, aiding in the refinement of existing formulations and the development of novel approaches.

Table 3: Paired t-test

S. No.	Name of sample	P(T<=t) two-tail
1	API and Test tablet.	9.2131E-09*
2	API and Commercial tablet.	1.5745E-24*
3	Test and Commercial tablet	2.00054E-14*

CONCLUSION

NAC effervescent tablet's *in vitro* mucolytic activity was successfully assessed utilising an egg white solution and suspended level viscometer in accordance with Indian pharmacopoeia. Outcomes were evaluated with NAC API and with commercially available effervescent NAC tablets. Results suggested that linearly decrease in viscosity as the concentration of NAC increases. The obtained data is statistically analysed using the paired t-test and ANOVA, which reveal significant differences.

ACKNOWLEDGEMENT

The authors acknowledge SciTech Specialities Pvt. Ltd. Sinnar, India for providing financial assistance and research facilities to carry out this research work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Contribution of Mr. Satish Nangude: Preparation of study protocol, to get permission to carry out studies at Scitech Specialities Pvt. Ltd. to carry out actual research work as per protocol. Compilation and interpretation of results. Dr. Ravindra Kamble-Guidance for the study. Miss. Kajal Vanhere-Assistance in performing the experimental work. Statistical analysis of the data.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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