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# Development of spectroscopic method for quantification of sodium hydroxide in milk

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**Abstract**
A spectroscopic technique was utilized to develop method for quantification of sodium hydroxide in milk. Colour was developed by reaction between sodium hydroxide and rosolic acid and absorption was measured at 554 nm. Standard curve was constructed for concentration of sodium hydroxide versus absorbance. Recovery factor was developed from amount of spiked sodium hydroxide and amount of sodium hydroxide obtained from standard curve. After developing the method, it was validated for performance by true (known) sample approach. The concentration of sodium hydroxide obtained by application of the developed method was compared with the concentration of the sodium hydroxide actually added for preparation of the sample. For statistical analysis t-test: two sample assuming equal variances was applied. The validation of method developed for quantification of sodium hydroxide suggested that the difference between the estimated amount of sodium hydroxide and amount of sodium hydroxide actually added to milk was statistically non-significant.

**Keywords:** Milk preservation, sodium hydroxide, quantification, spectroscopy

## Introduction
Though it is not legally permitted, addition of preservatives in milk is a common practice. The preservative, when applied to milk, may be defined as any chemical compound which retards alterations caused by the growth of microorganisms or enables physical properties, chemical composition and original nutritional value to remain unaffected by microbial spoilage [1].
Sodium hydroxide is added in the milk to avoid spoilage of milk but their addition to milk is not permitted under legal provision [2]. The addition of sodium hydroxide to milk and cream intended for consumption as such is prohibited and constitutes adulteration; the neutralization of cream for butter making, on the other hand, is a recognized practice of general occurrence [3].
Many qualitative methods have been developed for the detection of sodium hydroxide in milk such as rosolic acid test [4, 5, 6, 7, 8, 9], pH determination [3, 9], indicator method [3] and methyl alcohol method [3]. However, the addition of sodium hydroxide in small quantities and neutralization of added sodium hydroxide by developed acidity hinder their detection using these methods [10].
It appears from the above that numerous work for quantitative determination of sodium hydroxide in milk has been reported. However, these methods involved sophisticated instruments, cumbersome with lengthy procedures for sample preparation, expensive reagents, trained manpower for sample analysis, etc. Dairy industry requires simple, rapid and cost effective tests for routine analysis of the milk. Therefore, there is a need for the development of simple test for quantitative estimation of sodium hydroxide in milk.

## Materials and Methods
### Chemicals and reagents
Sodium Hydroxide (LOBA Chemie Pvt. Ltd.), Rectified spirit **(**Shree Madhi Vibhag Khand Udhyog Sahakari Mandi Ltd. Madhi), Rosolic Acid Solution (Spectrochem Pvt. LTD., Vadodara).

**Preparation of rosolic acid solution (1% w/v):** The reagent was prepared by dissolving 1 g of rosolic acid in 95 per cent ethyl alcohol and made up to 100 ml with ethyl alcohol.

**Instrument**

Spectrophotometer (Micro controller based UV-Vis spectrophotometer CL- 1320).

**Milk Samples**

The ratio of cow milk to buffalo milk was taken as 40:60 to prepare mixed milk sample. The genuine cow milk was brought from Livestock Research Station and the genuine buffalo milk was brought from Reproductive Biology Research Unit, Anand Agricultural University, Anand, Gujarat.

**Test procedures used for determination of sodium hydroxide in milk**

**A. Preparation of standard curve:**

1. 100 ml milk was taken in a glass beaker and 0.075 g of NaOH was added to it and mixed well.
2. An aliquot of 10 ml was taken from the mixture.
3. 40 ml Rectified spirit was added to the above mixture.
4. The content was mixed thoroughly and filtered through Whatman filter paper No.1.
5. The same procedure was followed for pure milk (Control) simultaneously.
6. Filtrates of both sample (with NaOH) and control (without NaOH) were collected in clean and dry conical flasks.
7. For preparation of standard curve different volume of filtrate (0, 1, 2, 3, 4, 5 ml) containing NaOH were taken so as to get concentration of 0, 0.15, 0.30, 0.45, 0.60, 0.75 mg NaOH per test tube. Total volume was made up to 5 ml in each test tube with control filtrate.
8. 20 µl of rosolic acid (1 % w/v) was added to each test tube with the help of micropipette and mixed well (Development of rose red colour was observed).
9. Absorbance was measured at 554 nm against blank as filtrate obtained from pure milk with rosolic acid solution using spectrophotometer.
10. A standard curve was plotted using absorbance and NaOH concentration (in mg).

**B. Estimation of mixed NaOH in milk samples:**

1. Adulterated milk samples mixed with 0.012, 0.029, 0.035, 0.055 and 0.067 g sodium hydroxide in 100 ml milk respectively.
2. To obtain the filtrate, steps 1 to 4 of the procedure used in Section (A) was followed.
3. Aliquots of 5 ml filtrate were collected test tubes to which 20µl of 1% rosolic acid solution was added.
4. The absorbance was measured at 554 nm against blank using spectrophotometer.

**C. Calculation**

Read from the graph the concentration of NaOH (mg) corresponding to absorbance of the sample.

Say the absorbance for the sample be X and the corresponding concentration from the standard curve for NaOH = Y mg.

Therefore, 5 ml filtrate from sample has = Y mg NaOH

50 ml (10 milk+40 ml rectified spirit) filtrate has= (Y/5) * 50 mg NaOH

But, 50 ml filtrate is obtained from 10 ml milk,

Therefore, 10 ml milk has =(Y/5) * 50 mg NaOH

*i.e.* 100 ml milk has = (Y/5*10) * 50 * 100 mg NaOH = Y * 100 mg NaOH

= Y * 0.1 g NaOH

**Results and Discussion**

For quantitative determination of added sodium hydroxide in milk, use of qualitative tests known as rosolic acid test. A rose-red colour indicates the presence of sodium hydroxide in milk. Rosolic acid is a pH indicator, which changes its colour from yellow (pH 6.0) to red (pH 7.6). Neutralization of milk leads to increase in pH, changing the colour of milk to red when rosolic acid solution is added. Preferably, the rosolic acid solution is prepared in ethanol as its solubility is higher in alcohol as compared to water.

In the present study, attempt was made to use this reaction for developing a spectrophotometric method to estimate amount of sodium hydroxide mixed in the milk. This study was divided into 4 parts: to develop colour and find out the maxima of the coloured complex formed, construction of standard curve, estimation of the recovery in method and validation of the method.

**Spectral Characteristics**

In order to get spectra of sodium hydroxide mixed milk, the neutralizer was added to 100 ml milk to make adulterated sample. It was coagulated by rectified spirit followed by filtration through Whatman filter paper grade 1. To the filtrate, 20µl of 1per cent (w/v) rosolic acid was added to develop the colour and scanned for absorption maxima in visible region (400-800 nm) in Micro Controller Based UV-Vis Spectrophotometer CL- 1320). The spectrum for the same is presented in Fig. 1.

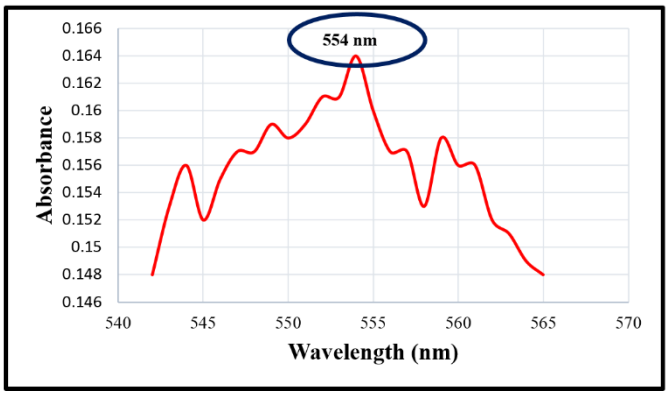


**Fig 1:** Absorption maxima ($\lambda_{max}$) for coloured complex

Different peaks were observed during scanning, *i.e.* 544, 551, 554 and 559 nm, among which the most prominent and highest peak was obtained at 554 nm. Therefore, this maxima was selected for further study.

**Construction of standard curve**

In the second part, standard curve was prepared for sodium hydroxide. For preparation of the standard curve, 0.075 g sodium hydroxide was mixed in 100 ml milk and filtrate was prepared according to the procedure described in Section 1 Different aliquots (0, 1, 2, 3, 4, 5 ml of filtrate from sample mixed with sodium hydroxide) were taken in test tubes so as to get concentration of 0, 0.15, 0.30, 0.45, 0.60, 0.75 mg per test tube and total volume was made to 5 ml with the filtrate of control milk sample, wherein, no sodium hydroxide was mixed. Colour was developed by using the procedure as described above in Section 1. The intensity of the colour obtained with varying amount of sodium hydroxide is presented in Fig 2.

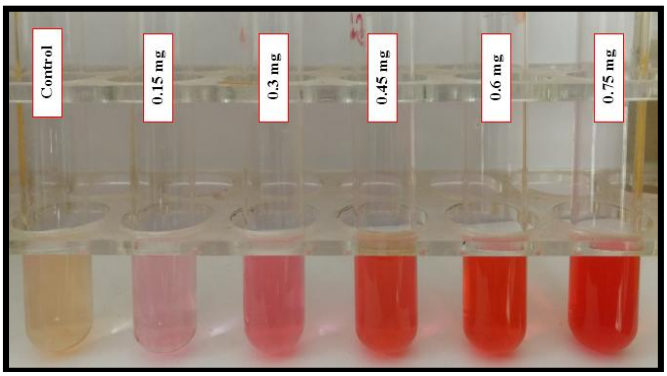


**Fig 2:** Intensity of the colour obtained with varying amounts of sodium hydroxide

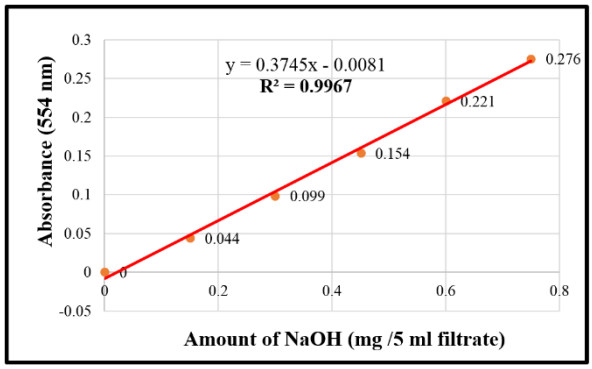


**Fig 3:** Standard curve for Sodium hydroxide

It was evident from the Fig 2 that intensity of the colour formed was linearly increasing with the increase in concentration of the sodium hydroxide mixed in the milk.
The resulted intensity of the colour was determined by measuring the absorbance at 554 nm. Three replications were conducted and average values of this exercise are given in the Table 1. The graph (standard curve) of absorbance versus concentration of sodium hydroxide was plotted and presented in Fig. 3.

**Table 1:** Observations taken for preparation of standard curve

| S. No | Sodium hydroxide (mg/5 ml filtrate) | Absorbance (554 nm) |
|---|---|---|
| 1 | 0.00 | 0.000 |
| 2 | 0.15 | 0.044 |
| 3 | 0.30 | 0.099 |
| 4 | 0.45 | 0.154 |
| 5 | 0.60 | 0.221 |
| 6 | 0.75 | 0.276 |
| The value are average of three replications | | |

It was evident from data given in Table 4.10 and Fig. 4.10 that absorbance values of the reaction mixture were linearly increasing with the increase in concentration of the Sodium hydroxide mixed in the milk and coinciding very well with increasing intensity of the colour. The slope ($R^2$ = 0.9967) indicated that the regression line was very well fitted in the data.

**Estimation of Recovery**

After developing the standard curve, experiments were conducted to estimate amount of sodium hydroxide obtained from standard curves after mixing known amount of sodium hydroxide in the milk. The sodium hydroxide was mixed in the milk in the range of 0.012 to 0.067 g per 100 ml of milk. Different amounts of sodium hydroxide were selected in such a way that entire range of standard curve get covered. The amount of sodium hydroxide in each case was estimated from the standard curve. The per cent recovery of sodium hydroxide obtained in the experiment was derived from amount of sodium hydroxide expected in the 5 ml of filtrate and the amount of sodium hydroxide estimated in 5 ml of filtrate from standard curve. Three replications were conducted. The data obtained for the recovery of sodium hydroxide are presented in Table 2.

**Table 2:** Estimation for recovery of sodium hydroxide in the experiment

| Replication | Amount of sodium hydroxide mixed (mg/5 ml filtrate) | Amount of sodium hydroxide estimated (mg/5 ml filtrate) | Recovery (percent) |
|---|---|---|---|
| R1 | 0.12 | 0.110 | 91.66 |
| | 0.29 | 0.260 | 89.65 |
| | 0.35 | 0.302 | 86.25 |
| | 0.55 | 0.500 | 90.90 |
| | 0.67 | 0.601 | 90.00 |
| R2 | 0.12 | 0.110 | 91.66 |
| | 0.29 | 0.286 | 98.62 |
| | 0.35 | 0.330 | 94.28 |
| | 0.55 | 0.521 | 94.72 |
| | 0.67 | 0.640 | 95.52 |
| R3 | 0.12 | 0.123 | 102.5 |
| | 0.29 | 0.293 | 101.0 |
| | 0.35 | 0.352 | 100.5 |
| | 0.55 | 0.547 | 99.45 |
| | 0.67 | 0.691 | 103.1 |
| Average | | | 95.32 |

It appeared from the results that the recovery of sodium hydroxide in the experiments varied from 86.25 to 103.1 per cent, with an average of 95.32 per cent. Therefore, to estimate the actual amount of sodium hydroxide mixed in the milk, results obtained from the experiment to be multiplied by the recovery factor. This recovery factor was obtained through dividing 100 by average per cent recovery observed in the experiment. Hence, the recovery factor for the sodium hydroxide in the present study was 1.049 (*i.e.* 100/95.32).
The amount of sodium hydroxide recovered in the filtrate was lower than the amount of sodium hydroxide mixed in the milk. This might be attributed to some losses of sodium hydroxide during preparation of the filtrate. The losses may occur due to retention of sodium hydroxide in the coagulum.

The sodium hydroxide may retain in the coagulum due to two possible reasons. One is interaction of the sodium hydroxide with casein. Another reason may be retention of sodium hydroxide in the serum portion remained in the coagulum as moisture.

**Validation of Method**

After developing the recovery factor for different sodium hydroxide, the amount (g) of sodium hydroxide derived from standard curve was multiplied by recovery factor to get amount of sodium hydroxide mixed in 100 ml of milk by this experiment. The data obtained for amount of sodium hydroxide actually added in the milk and corresponding amount of sodium hydroxide practically estimated in the experiment, along with their statistical analysis are presented in Table 3.

**Table 3:** Amount of sodium hydroxide mixed and estimated in 100 ml of milk

| S. No | NaOH from std. curve (A) (g/100 ml milk) | NaOH estimated (A x 1.049*) (g/100 ml milk) | NaOH mixed (g/100 ml milk) |
|---|---|---|---|
| 1 | 0.0110 | 0.0115 | 0.012 |
| 2 | 0.0260 | 0.0272 | 0.029 |
| 3 | 0.0302 | 0.0316 | 0.035 |
| 4 | 0.0500 | 0.0524 | 0.055 |
| 5 | 0.0601 | 0.0630 | 0.067 |
| 6 | 0.0110 | 0.0115 | 0.012 |
| 7 | 0.0286 | 0.030 | 0.029 |
| 8 | 0.0330 | 0.0346 | 0.035 |
| 9 | 0.0521 | 0.0546 | 0.055 |
| 10 | 0.0640 | 0.0671 | 0.067 |
| 11 | 0.0120 | 0.0125 | 0.012 |
| 12 | 0.0290 | 0.0304 | 0.029 |
| 13 | 0.0352 | 0.0369 | 0.035 |
| 14 | 0.0547 | 0.0573 | 0.055 |
| 15 | 0.0691 | 0.0724 | 0.067 |
| Average | 0.0377 | 0.0395 | 0.0396 |
| t Stat | | 0.0090 | |
| P(T<=t) two-tail | | 0.9928 | |
| Test | | NS | |

* Recovery factor
NS = Non significant

The sodium hydroxide was mixed in milk in range of 0.012 to 0.067 g per 100 ml of milk, with an average of 0.0396 g per 100 ml of milk. The amount of sodium hydroxide estimated in the milk by the method developed in this study ranged from 0.0115 to 0.0724 g per 100 ml of milk, with an average of 0.0395 g per 100 ml of milk. The comparison between actual amount of sodium hydroxide mixed per 100 ml milk and amount of sodium hydroxide estimated per 100 ml milk by the method of the study revealed that the difference between the two was statistically non- significant. The results suggested that method worked out in the study was suitable for practical purpose.

**Conclusion**

For quantification of sodium hydroxide, spectroscopic method based on rosolic acid test used to detect sodium hydroxide in milk was used. After developing the rose red colour by reaction between sodium hydroxide and rosolic acid in milk serum, the maxima of colour absorption was obtained in visible region and it was found to be 554 nm. Then standard curve was constructed by developing the colour with sodium hydroxide at the rate of 0.00 0.15, 0.30, 0.45, 0.60 and 0.75 mg per 5 ml milk serum. After constructing standard curve, work was carried out to know actual recovery obtained from the standard curve. From the average per cent recovery obtained in the experiment (95.32%), the recovery factor was derived (1.049). The amount of sodium hydroxide obtained from the standard curve were converted into amount of sodium hydroxide in 100 ml of milk by back calculation. The amount of sodium hydroxide thus obtained per 100 ml of milk was multiplied by recovery factor to compensate the losses of sodium hydroxide occurring during the procedure followed in the experiment. Finally, performance of the method was tested by comparison of amount of sodium hydroxide obtained in milk by the developed method with actual amount of sodium hydroxide mixed in milk. The difference between two was statistically non-significant. The results suggested that method worked out in the study was simple, relatively faster and suitable for practical purpose.

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