BUFFER CAPACITY AND VISCOSITY OF BUFFALO MILK AS INFLUENCED BY VARIOUS ADDITIVES

Dissertation
Submitted in partial fulfilment of the requirements for the degree of Master of Science in Dairying (Dairy Chemistry) to the Kurukshetra University, Kurukshetra

By
JEET RAM DHAKA
Division of Dairy Chemistry
National Dairy Research Institute (Indian Council of Agricultural Research)
Karnal (Haryana) India
1982

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DEDICATED TO MY PARENTS
This is to certify that Shri Jaat Ram of National Dairy Research Institute, Karnal participated in the planning of this study, carried out the experimental work involved, analysed the data and prepared this report on "Buffer capacity and viscosity of buffalo milk as influenced by various additives". He did this in part fulfilment of the requirements for the degree of MASTER OF SCIENCE in Dairying (Dairy Chemistry) of the Kurukshetra University, Kurukshetra, under my personal supervision. Help and assistance given by the individuals as well as the Institute in the preparation of the work has been duly acknowledged.

(R.C. Malik)
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( JEEV RAm DHAkA )
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INTRODUCTION
Physical properties of milk have been extensively investigated by a number of chemists and dairy technologists for more than a century. The pH, titratable acidity, buffering capacity and viscosity measurements are being routinely made on milk and its concentrates.

The buffer capacity, pH, acidity and viscosity are all factors to assess the aggregation or dispersion of casein micelles, whey proteins and fat globules. The acid-base equilibrium in milk arises from the presence of substances that liberate protons and those that combine with them and is among the most important factors in controlling the stability of milk suspensions. This equilibrium in milk is influenced by a delicate physical balance existing between colloidal and soluble protein, salts and minerals. Such treatments which influence the state of dispersion of proteins and salts, as heating and cooling or changes in milk composition by addition of salts, are reflected in variations of the above mentioned physical properties.

It is observed from the literature that most of the work reported so far deals with an understanding of these physical properties of cow milk for its processing for the production of sterilised and evaporated milk. It has been shown that the addition of salts like sodium citrate, phosphates, calcium chloride, etc. has different effects on different types of milk. However, it is observed that citrates and
Phosphates have a beneficial effect on the heat stability while calcium has the opposite effect.

The established variation in composition of cow and buffalo milk and their heat stability test demanded critical investigations on titratable acidity, buffer capacity and viscosity of buffalo milk. In the present investigation, an attempt has been made to understand the effect of fat, salts like phosphates, citrates, calcium, EDTA and temperature on titratable acidity, buffer capacity and viscosity of buffalo milk.
CHAPTER II

REVIEW OF LITERATURE
REVIEW OF LITERATURE

Studies on buffer capacity, pH and viscosity of milk have been extensively reported by many workers in Western countries. Such studies, however, were concerned with multifarious objectives. The effect of various factors as fat, temperature and additives (sodium citrate, sodium orthophosphate, sodium phosphate dibasic, ethylene diamine tetra acetic acid, calcium chloride, etc) were studied on milk. The following paragraphs give brief resume of such work on pH, buffer capacity and viscosity of milk.

1. **Effect of fat on pH and buffer capacity**

The effect of fat on pH of milk was reported by Dastur and Rao as early as in 1956 (Dastur and Rao, 1956). They reported that there was no correlation between pH and fat or SNF content of the samples. They also reported that buffer values of milk were highest in acid range, the peak being obtained in the range of pH 4.8 to 5.2 and either side of this range, there was a steep fall. In acid range buffalo milk showed highest buffer value than cow milk while at the reverse was the case in alkaline range. At the peak the buffer value of cow milk was 0.0359 and that of buffalo 0.0417. They observed also that skimming in most cases increased the pH of milk slightly. Dilution of milk increased the pH, the increase was 0.01 unit with 5%, 0.04% with 15%, and 0.08% with 25% water.
Parkash and Puri (1960) have determined the electrometric titration curves for cows' and buffaloes' milk, buffaloes' skim milk and casein. The average initial pH of cows' and buffaloes' milk was 5.51 and 6.63, respectively, while the pH values at the points of inflection were 8.3 - 8.4. The amount of alkali required to reach this point ranged from 12 to 16 ml 0.1N NaOH/100 ml. The quantity of alkali required for neutralization was not affected by the butter fat and ash contents of the milk, but it was related to the protein content. They also observed a decrease in buffer indices with increase in pH.

Minieri, Franciscis and Intrieri (1965) from their study established that there was significant correlation between pH and SNF (r = 0.3083) and between pH and lactose (r = 0.2921), but not between pH and fat or protein in buffaloes' milk.

Abd El-Salam and El-Shibiny (1966) found the average acidity of 100 samples of buffaloes' milk to be 0.21% lactic acid, and the pH value to be 6.45 - 6.50.

No marked difference in the pH of buffaloes' and cows' milk was noticed by Hofi et al. (1965), the mean values varying between 6.53 and 6.50. The acidity of milk varied from 0.175 to 0.185% lactic acid.

Pasteurized and boiled milk showed the same pH and acidity as raw milk. Sterilization increased the acidity without affecting the pH.

From the analysis of 50 goat milk samples, Joshi and Vadanayakam (1967) concluded the average SNF content was found to be 9.37% and the average peak value in acid range was 0.0396 at pH 5.3. The average keeping quality at the atmospheric temperature was 10.31 hrs.
The correlation coefficient between SNF content and keeping quality, and buffer index and keeping quality was 0.665 and 0.624, respectively which was highly significant. It was observed that higher the SNF content, higher is the buffer value and greater is the keeping quality.

Ismail, Beest and Eldifrawi (1973) reported the effect of fat on buffer capacity while studying values for skim milk. They observed that maximum buffering capacity of cows' and buffaloes' milk occurred at pH 5.3-5.4 and values for skim milk, standardized milk and whole milk were 0.0290, 0.0283 and 0.0270 for buffaloes' milk and 0.0333, 0.0283 and 0.0277 for cows' milk.

While studying buffer value, pKa and buffer intensity curves of buffaloes', cows', ewes' and goats' milks, Imam et al. (1974) observed TS, fat, protein and ash contents and also pH values for ewe's milk were higher than those of buffaloes, and those of latter were higher than those of goats. They further reported that all examined types of milks were found to have the higher buffer values at the alkaline side. The pKa of buffaloes', cows', ewes' and goats' milk were 5.32, 4.90, 5.05 and 5.25, respectively. Ewe's milk had higher buffering capacity than both cows' and goats' milk which had approximately the same buffering capacity.

Chandra and Roy (1977) reported the highly significant negative correlation between milk fat content and pH, other correlation between pH and TS or protein were not significant.

2. Effect of temperature on pH and buffer capacity

The pH of cows' milk is commonly stated as falling between pH 6.5 and 6.7 with 6.6 the most usual value applies only at temperatures of
measurement near 25°C. The pH of milk exhibits a greater dependence on temperature than that of such buffers as phosphate, which is the principal buffer component of milk at pH 6.6. Miller and Sommer (1940) reported a specimen with pH of 6.64 at 20°C decreasing to 6.23 at 60°C. Over the same temperature range a phosphate buffer decreases only from pH 6.88 to 6.84. Likewise, Dixton (1953) observed that the pH of milk decreases by about 0.01 unit/°C between 10 and 30°C, and emphasised the importance of careful temperature control in pH measurements. The marked temperature dependence of the pH of milk probably is attributed to insolubilization of calcium phosphate as the temperature is raised and its solution as temperature is lowered.

Rao and Dastur (1956a) reported that pasteurisation and boiling increased the pH by 0.04 unit. Sterilized milk showed an average decrease of 0.18 units.

While studying changes in pH of milk during freezing and frozen storage, Berg Vanden (1961) concluded that when raw or pasteurised milk and milk ultrafiltersates were frozen slowly (0.1°C/hr) or less, a decrease in pH to a minimum of 5.8 at -10°C occurred followed by an increase, but rapid freezing (1°C/hr or more) had little effect. Marked decreases (0.5 - 1.0 pH unit below the prefreezing value) for the first two-three weeks of storage at -7°C or -12°C were observed. They also reported that pasteurisation and the addition of potassium citrate, potassium phosphate or calcium chloride to raw milk tended to lower the pH minimum during storage, the subsequent increase being less rapid.

While studying the pH of buffaloes' milk in different seasons,
Francisca et al. (1963) observed that pH values ranging from 6.73 in August, 6.85 in December and January.

Hofi et al. (1966) reported that significant decreases in pH were obtained by sterilizing both cows' and buffaloes' milk and by boiling buffaloes' milk.

Chandra and Rao (1977) concluded that decrease in pH with increasing temperature (t) was described by the linear regression equation: \( \text{pH} = 6.913 - 0.0093 \ t \).

3. Effect of additives on pH and buffer capacity

Heat treatment is known to cause reduction of both the total soluble and ionic calcium. Under pasteurizing conditions, the reduction is slight but significant losses of soluble calcium and phosphorus occur above 76°C (Bell, 1925; Hilgeman et al., 1951; Ter Horst, 1950) values reported for changes in salt distribution in heated milk show large discrepancies, which may be caused by inadequate precautions against subsequent shifts in equilibrium and by variations in the time lapses between heat treatment and analysis. It shows the initial loss of about 25% of the soluble calcium in milk heated to 76°C for 30 min (Hilgeman et al., 1951). When the milk is aged at 5°C, gradual reversion toward the original soluble calcium level occurs over a period of 24 to 48 hr. Soluble phosphorus undergoes a similar change. This decrease in soluble calcium and phosphorus upon heat treatment and its reversion on cool aging has also been confirmed by analysis of calcium and phosphorus in centrifuged milk (Edmonson et al., 1955) as well as in ultrafiltrate (Ross et al., 1959).
The influence of titratable acidity or pH upon heat coagulation has long been a recognized correlation. The general relationship between adjusted pH and spontaneous coagulation temperatures of raw skim milk, as defined by Miller and Sommer (1940), shows a high order of sensitivity to pH for values between 6.2 and 6.4. At pH values below 6.4, added phosphate tends to displace the curve toward higher stability, while calcium has the reverse effect. Such heat stability curve has been found to exhibit a maximum at a pH that appears to be a specific characteristic for each milk.

The acidity of milk increases with temperature, partially as a result of changes in the buffer capacity of the milk salts and the expulsion of CO₂ on heating. Miller and Sommer (1940) observed that the pH of skim milk decreases approximately 0.1 pH for each 10°C temperature rise.

Rao and Dastur (1956b) observed that the main buffering constituents of milk are casein and acid phosphate, and other minor contributing factors being albumin, salts of citric acid, carbonate and lactate. Rice and Markley (1924) showed that milk having high apparent acidity contained greater amount of all nutrients and was particularly rich in phosphate. High acid milk contained more casein also, but difference was not enough to account for more than a small part of the rise in acidity in the high acid sample milk with apparent acidity on account of its high buffer value had long keeping quality.

Rose et al. (1959) reported that the hydrogen ion concentration of a 94°C milk ultrafilterate is at least twice that of a comparable 25°C
ultrafiltrate. Concentration of milk is accompanied by a significant
decrease in pH, which may contribute to the greater heat-susceptibility
of condensed milks. Under prolonged heat treatment at elevated tempera-
tures, additional acidity is developed as a result of further changes
in the milk. This acidity may be derived from thermal decomposition of
the lactose to organic acids, interaction of lactose with the milk
proteins, hydrolytic dephosphorylation of casein and displacement of the
calcium-phosphate equilibrium.

In 1961, Rose also reported that the heat stability of milk
passes through a maximum and minimum between pH 6.4 and 7.0. The pH of
maximum lowered by the addition of phosphate and raised by calcium. The
difference between the maximum and minimum heat stabilities of casein
phosphate complex in milk ultrafiltrate is progressively increased by
the addition of $\beta$-lactoglobulin. It is suggested that the denatured
$\beta$-lactoglobulin casein complex is the pH sensitive component.

Jenness and Parkash (1967) have noted that part of the differen-
tces in heat stability and pH stability curves between individual milks
could be eliminated by dialysis against bulk milk. This dialysis did
not equalize the concentration of ultrafilterable calcium, magnesium or
phosphorus of the test samples and the bulk milk. However, removal of
colloidal calcium phosphate by acidification and then neutralization
usually resulted in increased heat stability of both unconcentrated and
concentrated (2:1) skim milk, but the pH stability curve was of the same
type as that of the original skim milk.

While studying the effects of pyrophosphate and citrate ions
upon colloidal caseinate phosphate micelles and ultrafilterate, Morr (1967) reported that (a) apparent size and chemical composition of the colloidal caseinate phosphate micelles, (b) chemical composition and Sephadex gel filtration properties of the resulting ultrafilterate, and (c) ultracentrifugal sedimentation characteristics of caseinate-phosphate micelles dispersed in phosphate buffer, treated with oxalate or dialysed against tetra sodium pyrophosphate (TSPP).

Heating skim milk, containing TSPP and citrate ions, resulted in substantial disaggregation of the colloidal caseinate-phosphate micelles. The resulting micelles in heated TSPP-skim milk contained greater proportions of calcium phosphate than those sedimented from control skim milk.

Added TSPP ions resulted in lower levels of ultrafilterable calcium, but higher levels of ultrafilterable inorganic phosphate, whereas added citrate ions caused an increase in both the ultrafilterable calcium and inorganic phosphate over those of the control. Heating TSPP and citrate skim milk resulted in minor additional changes in the amount of ultrafilterable calcium phosphate.

Sedimentation experiments showed that TSPP ions disaggregated the colloidal caseinate phosphate micelles from heated TSPP-skim milk more effectively than those from heated control skim milk and to a much greater extent than was produced by removal of calcium with oxalate.

Addition of polyphosphate and citrate ions to milk brings substantial disaggregation of the colloidal caseinate-phosphate micelles (Wilson et al., 1963) either by being incorporated into the micelles
(Herraid et al., 1963; Leviton, 1964; Odagiri et al., 1965) or by chelation of divalent calcium ions, thereby, causing a shift in the distribution of the colloidal and soluble salts (Man, 1966; Odagiri et al., 1965). As a result, a number of interrelated chemical and physical properties of the milk system are altered: (a) reduced sedimentation of the micelles by high speed centrifugation (Morr and Kenkare, 1964); (b) increased viscosity; (c) reduced turbidity and increased translucency (Odagiri et al., 1964); and (d) stabilization of the protein component against rennet coagulation (Satra and GisMann, 1964; Man, 1966; Odagiri et al., 1964), heat aggregation (Leviton, 1963), gelatin in HTST sterile concentrates (Leviton, 1963; Wilson et al., 1963) and flocculation of frozen milk (Leviton et al., 1966).

While studying the effect of additives on buffer capacity, Ismail, Aeb and Eldifrawi (1973) concluded that addition of calcium lactate, EDTA, sodium pyrophosphate, potassium chloride and sodium citrate (20 or 40 ml; 0.2M solution) to standardized milk changes the pH of maximum buffer capacity to 5.3 - 5.4, 5.2, 6.55, 5.6 and 6.4, respectively and increased the maximum buffer capacity. These effects were greater in cows' than in buffaloes' milk. Thus, sodium citrate and sodium pyrophosphate were the most favourable additives for milk stabilization as they shift the pH of maximum buffering capacity towards that of milk and also cause the greatest decrease in milk reflectance due to calcium and magnesium precipitation.

Kirchmaier (1980) reported that the contribution of milk proteins to the total buffer capacity is about 35%. The colloidal phosphate bound to casein is contributing about 13%. The colloidal phosphate
has an apparent pK value of 5.5 due to its binding to casein versus 7.2 in its soluble state. When milk is acidified with lactic acid, a pronounced buffer effect can be stated at a pH below 5.2 by the increased lactate ion concentration reducing the dissociation of lactic acid.

4. Effect of additives on viscosity

Spettel and Gneist (1942) studied the viscosity of milk. They reported that the salt content does not affect the viscosity of milk.

Puri and Gupta (1955) reported that addition of ammonia solution to milk increases its viscosity to an appreciable extent. The viscosity of the mixture changes with time, but ultimately a constant value which is independent within limits of the concentration of the ammonia solution. The viscosity of the mixture varies appreciably with change in its protein content and an empirical formula connecting the two quantities enables the evaluation of proteins in milk.

Puri, Prakash and Totaja (1963) published that addition of 10% cane-sugar or starch produced no change while additives of same amount of gelatine caused a noticeable increase. Addition of rennin caused first a decrease and then an increase. Addition of ethyl alcohol caused a linear increase till the approach of visible coagulation.

Puri et al. (1972) investigated that viscosity of 1.0% cows' and buffaloes' milk casein in NaOH and Ca (OH)₂ increased with rise in pH of the solution to pH 11 (the point maximum viscosity and complete neutralization) than decreased. This observation gave a convenient method for
total base binding capacity of caseins. Maximum viscosity was greater in NaOH than in Ca(OH)$_2$. Viscosity in NH$_3$ also increased with pH, and was higher in this liquid at any given pH than in NaOH or Ca(OH)$_2$.

While studying the viscosity of buffalo and cow casein, and casein co-precipitates, Hagraas et al. (1976) concluded that the viscosities of casein coprecipitates increased with increasing calcium content. Addition of sodium hexametaphosphate increased the viscosity of low calcium coprecipitation with medium and high calcium coprecipitates. Its effects varied according to pH and temperature with gel formation occurring at higher pH and temperature values.

Yadav and Roy (1977) studied the role of mineral cation on the viscosity of model milk system. They prepared model milk systems containing critical concentration of caseins from buffaloes’ milk (8.64%) and cows’ milk (8.20%) and lactose mineral and other dialysable constituents of the milks in normal proportions. These systems do not contain milk lipids or whey proteins. Incorporation of calcium and magnesium as their chlorides cause considerable and significant reduction in viscosity of the most model milk systems, but did not noticeably affect their pH. Influence of calcium was more pronounced than that of magnesium. Role of calcium in decreasing viscosity was more marked in buffalo model milk systems as compared with the cow system. It was also observed that the accompanying chloride ions caused only a slight rise in viscosity of the model milk system without appreciably changing the role of cations.

Yadav and Roy (1978) also reported that model milk system prepared from buffaloes’ milk (BMS) and from cows’ milk (CMS) contain 8.64
and 3.20 g of casein and 4.74 and 4.35 g lactose/100 g, respectively. Incorporation of citrate (as iso pH solution) increased the viscosity (P = 0.01) of both BMS and CMS, phosphate also increased viscosity but significantly only in BMS and chloride produced a non-significant increase in viscosity. Addition of lactose in conjunction with citrate and phosphate enhanced the effect of the anions. Preheating at 96-97°C for 5 min or storage at 4-6°C for 7 days reduced (P = 0.01) the viscosity of phosphate and chloride, but not citrate treated samples. Phosphate reached an early saturation effect with BMS and citrate with CMS. BMS had the higher initial phosphate and CMS the higher initial citrate content. Citrate had a greater influence than phosphate on viscosity in both BMS and CMS.
CHAPTER III

MATERIALS AND METHODS
MATERIALS AND METHODS

Materials

The milk samples used for the experimental study were collected from buffaloes belonging to the Institute herd. Individual sample was collected during morning and evening milkings. Samples were taken just after milking. Each sample was taken in a stoppered sterilised corning glass conical flask. It was stored in a refrigerator at temperature 5-10°C for about 1 hr till it was used for experimental work. This sample was uniformly mixed by gentle agitation and aliquots were taken for determination of pH, buffer capacity and viscosity. Skimming was done using laboratory cream separator. The amount of the fat in skin milk was less than 0.1%.

Following additives were used in the present study:

i) Tri sodium citrate (Na₃C₆H₅O₇·2H₂O) Analytical grade, BDH

ii) Sodium dihydrogen orthophosphate (Na₂HPO₄·2H₂O) Analytical grade, BDH

iii) Sodium phosphate dibasic (Na₂HPO₄·2H₂O) GR, Sarabhai M.Chemicals

iv) Ethylene Diamine Tetra Acetic Acid (EDTA) Analytical grade, BDH

v) Calcium chloride 2-hydrate cryst (CaCl₂·2H₂O) GR Merck
To understand the effect of fat on the physical properties of milk, a series of mixtures were prepared by mixing skim milk and fat from the same sample of milk. The composition of the mixture is as:

i) Skim milk
ii) 75 ml skim milk + 25 ml cream
iii) 50 ml skim milk + 50 ml cream
iv) 25 ml skim milk + 75 ml cream
v) Whole milk

Methods

Determination of fat

The fat content of the mixture in each case was determined by the Gerber method as follows:

10 ml of Gerber sulphuric acid acid (density 1.807 - 1.812 gm/ml at 27°C) was taken in a butyrometer. 10.75 ml of the milk sample (prepared as above) was layered gently on sulphuric acid. After that 1 ml of amyl alcohol (density 0.803 - 0.805 gm/ml at 27°C) was added to the same butyrometer. A lock stopper was put in the mouth of the butyrometer. The contents of the butyrometer were well mixed and kept in a water bath at 65 ± 3°C for about 5 minutes. The butyrometer was now centrifuged in a Gerber centrifuge (1200-1400 rpm) for 4-5 minutes. After this butyrometer was kept in a water bath at 65°C for 3 minutes before taking the reading. The fat percentage was directly recorded as per the reading of the fat column.

Determination of pH

In the course of present investigation, pH determination of all...
the samples have been carried out electrometrically using a digital pH meter (Electronic Corporation of India Ltd.) throughout the work. The pH meter was calibrated using a suitable buffer solution of pH 9.2. Necessary arrangement was made to maintain the samples during pH measurement at respective temperatures of 10, 20, 30, 40, 50, 60 and 70°C ± 0.1°C.

**Determination of buffer capacity**

The variation in buffer capacity over the titration range can be expressed more exactly in terms of 'Buffer Index' \( \frac{dB}{dpH} \) which is defined as the number of equivalents of acid or base required to shift the pH of a litre of solution by one unit.

\[
\frac{dB}{dpH} = \frac{\Delta E}{\Delta pH}
\]

The buffer index was determined in acid and alkali range covering a pH range of 4.0 to 10.0. For the analysis, 50 ml of the sample was taken in a beaker placed in water bath maintained at the desired temperature. To this was added a small increment (1 ml) of 0.1N NaOH each time and reading of pH taken after thorough mixing. This process was continued till a pH around 10.0 was attained. In the same way the experiment was repeated with another 50 ml of the sample and 0.1N HCl till a pH of 4.0 was obtained.

From the pH change and the volume of acid or alkali added, the buffer value \( \frac{dB}{dpH} \) was calculated as follows:

\[
\frac{dB}{dpH} = \frac{(\text{ml of acid or alkali added}) \times (\text{normality of acid or alkali})}{(\text{volume of sample in ml}) \times (\text{pH change produced})}
\]
Viscosity determination

Viscosity was determined by capillary flow method using Ostwald viscometer under an atmospheric pressure and fixed experimental temperatures by adding different additives (sodium citrate, sodium dihydrogen orthophosphate, sodium phosphate dibasic, calcium chloride, EDTA).

Ostwald viscometers of certified British Standard type B and C were used throughout the determinations. The viscometer was thoroughly cleaned, successively with dilute detergent solution, tap water, warm chromic acid, tap water and distilled water. It was then washed with acetone to remove water and finally dried by drawing dust free cold air. Measured quantity of sample (20 ml) was placed into the viscometer using a pipette. The viscometer filled with the sample was kept in a water bath for 30 minutes before the time of flow was recorded. The temperature of water bath was maintained at 30°C and was controlled thermostatically within ± 1°C. The time of flow of the sample from the upper to the lower marks in the viscometer limb was recorded by a stop watch calibrated to read 0.1 seconds. To record the time of flow for each sample, the averages of at least 6 readings within 0.1 seconds were taken. The relative viscosity of the sample was calculated from the times of flow for the sample and standard liquid (fresh, all glass, double distilled water).
CHAPTER IV

RESULTS AND DISCUSSION
RESULTS AND DISCUSSION

It is found from the literature that the pH of milk varies with milk temperature. It was thought to study the effect of temperature on the pH of buffalo milk. The data (mean of five samples) is presented in fig.1. It is observed from the graph that the pH of milk is highest around 25°C below and above this temperature, the pH shows a fall, however, the trend in the fall of pH around 25°C is not similar. The curve is more steeper on the higher temperature side, it is found that the fall is of the order of 0.45 pH units as we move from 25 to 80°C and this gives a decrease of about 0.008 units/°C. The corresponding decrease is about 0.007 unit/°C in the temperature range 25 to 0°C. The values reported in the case of cow milk is about 0.01 unit/°C between 20 to 30°C (Miller and Sommer, 1940; Dixton, 1963).

With a view to understand whether the change of pH with change of temperature was reversible or irreversible, the pH of milk was noted with fall of temperature from 80 to 25°C and rise of temperature from 0 to 25°C. The rate of fall or rise of temperature was kept constant. The data is reported in Fig.1. It is seen that the forward and backward curve do not overlap each other indicating irrevocability of the process. Between temperature 80 to 25°C, the backward curve remains below the forward curve and between 0 to 25°C it remains above the forward curve. The two curves intersect each other at 25°C.
FIG. 1. EFFECT OF TEMPERATURE ON THE pH OF MILK

- - 0 - 80 °C

- - 80 - 0 °C
It is well documented in the literature that during heating of milk soluble calcium phosphate changes to colloidal calcium phosphate, with the liberation of hydrogen ions and thus resulting in lowering of pH. It appears that the precipitated calcium phosphate produced during heating of milk from 25 to 80°C is resistant to dissolution on cooling. This observation also suggests that the colloidal calcium phosphate produced during rise of temperature is present as crystalline hydroxylapatite. The dissolution of colloidal calcium phosphate is appreciable around 45°C where the hysteresis loop is narrow.

Titration curves of a number of samples containing varying amounts of fat are shown in Fig. 2. It is seen that on the acid side there is a drop in the pH with increasing amount of fat. This may be expected as the fat will be acting as an inherent medium and decreasing the effective concentration of the proteins and minerals which are responsible for buffer capacity of milk. It is also reported that the presence of high concentration of fat promotes greater precipitation of calcium and magnesium ions through acting as an inert nucleus on which surface these cations could precipitate and so free phosphoric acid radical is liberated contributing to the pH drop (Ismail et al., 1959). On the alkaline side of the titration curve the pH increases with the same amount of alkalies used as the concentration of fat increases. The explanation for this is the same as in the case of acid range.

Effect of fat on the buffer intensity curves of milk is shown in Fig. 3. The height of the peak is proportional to the concentration of the buffer constituents of milk. It is highest with lowest amounts
FIG. 2. EFFECT OF FAT ON TITRATION CURVES OF MILK

- Milk (7.7% fat)
- Milk (15.7% fat)
- Milk (29.8% fat)
- Milk (44.5% fat)

0.1N HCl (ml)/20 ml milk ↔ 0.1N NaOH (ml)/20 ml milk
FIG. 3. EFFECT OF FAT ON BUFFER INTENSITY OF MILK

- Milk (7.7% fat)
- Milk (15.7% fat)
- Milk (29.8% fat)
- Milk (44.5% fat)
of fat and goes on decreasing with the concentration of fat. The maximum buffer indices are 0.0575, 0.0525, 0.0500 and 0.0475 at 7, 15.7, 29.8 and 44.5 percent fat, respectively.

The pka value of the buffer constituents is not influenced by the concentration of fat and is about 5.0. The slope of the buffer intensity curves shows a similar trend in all cases. There is steep fall of buffer intensity below pH 5.0. The pka value drops abruptly up to a pH of 4.6. On the other side of the peak it drops sharply up to a pH of 6.5, and then shows a steady fall up to pH 8.2, remains constant up to pH 9.0 and thereafter there is a slight increase.

The effect of temperature on the titratable acidity was studied at two different temperatures of 20°C and 60°C. The results reported in Fig. 4 show that on the acid side milk requires smaller amounts of HCl to bring the same change in pH at 60°C than that required at 20°C confirming the fact that there is some release of hydrogen ion from the buffering constituents of milk on heating. This observation is confirmed on the alkaline.

Buffer intensity curves as shown in Fig. 5 indicate that the maxima of buffer intensity shifts towards acidic pH (around 4.6) at 60°C, while it is around 5.3 at 20°C. The two curves coincide with each other around pH of 6.2 and this continues further. This shift in pka value of buffer constituents of milk on heating has thus led us to deal with stronger acid. This may perhaps be due to the conversion of some of the HPO₄⁻ to PO₄³⁻.
FIG. 4. EFFECT OF TEMPERATURE ON TITRATION CURVES OF MILK

- Milk at 20°C
- Milk at 60°C

0.1 N HCl (ml)/20 ml milk → 0.1 N NaOH (ml)/20 ml milk
FIG. 5. EFFECT OF TEMPERATURE ON BUFFER INTENSITY OF MILK

- Milk at 20°C
- Milk at 60°C

dB/dpH

pH
The effect of additives like sodium citrate, sodium dihydrogen orthophosphate, sodium phosphate dibasic, EDTA and calcium chloride is shown in Figs. 6a, 6b, 6c, 6d and 6e, respectively. It is seen that the addition of sodium citrate and sodium phosphate dibasic increase the pH of milk, while the additives like sodium dihydrogen orthophosphate, EDTA and calcium chloride decrease the pH of milk.

The buffer intensity curve on addition of additives as sodium citrate, sodium dihydrogen orthophosphate, sodium phosphate dibasic, EDTA and calcium chloride are shown in Figs. 7a, 7b, 7c, 7d and 7e, respectively. On the addition of sodium citrate shifts the pH of maximum buffer index towards the alkaline side of the pH. This shift is not influenced by the amount of sodium citrate added. However, the peak in the buffer intensity curve increases with increase in the concentration of sodium citrate.

Sodium dihydrogen orthophosphate does not bring any shift in the change of maximum buffer intensity curve. The maxima is enhanced on the addition of the salt and this increase is proportional to the concentration of salt added.

Sodium phosphate dibasic shifts the maxima towards the alkaline side. EDTA brings the maxima towards acidic side. In this case also there is increase in the buffer intensity peak. Calcium chloride shifts the maxima towards right and there is decrease in the buffer intensity.

It is evident from the above results that the addition of stabilizing salts to milk before sterilization or evaporation shifts the pH of maximum buffer intensity towards the normal pH of milk and also
FIG. 6a. EFFECT OF SODIUM CITRATE ON TITRATION CURVES OF MILK

- Normal milk
- Normal milk + 50 mg Sodium citrate
- Normal milk + 90 mg Sodium citrate
FIG. 6b. EFFECT OF SODIUM DIHYDROGEN ORTHOPHOSPHATE ON TITRATION CURVES OF MILK

- Normal milk
- Normal milk + 50 mg Sod. dihydrogen orthophosphate
- Normal milk + 90 mg Sod. dihydrogen orthophosphate

0.1 N HCl (ml) / 50 ml milk | 0.1 N NaOH (ml) / 50 ml milk
Figure 6c. EFFECT OF SODIUM PHOSPHATE DIBASIC ON TITRATION CURVES OF MILK
FIG. 6d: EFFECT OF EDTA ON TITRATION CURVES OF MILK

- Normal milk
- Normal milk + 50 mg EDTA
- Normal milk + 100 mg EDTA

0.1 N HCl (ml)/50 ml milk vs. 0.1 N NaOH (ml)/50 ml milk

pH

4 5 6 7 8 9 10

40 32 24 16 8 0 8 16 24 32
FIG. 6e. EFFECT OF CALCIUM CHLORIDE ON TITRATION CURVES OF MILK

- Normal milk
- Normal milk + 78.0 mg CaCl₂
- Normal milk + 46.8 mg CaCl₂

pH

0.1 N HCl (ml)/50 ml milk ↔ 0.1 N NaOH (ml)/50 ml milk
enhances the buffering capacity of milk. The effect of sodium citrate and sodium phosphate dibasic is favourable. Sodium dihydrogen orthophosphate does not bring any shifts in pH while EDTA lowers the pH. This behaviour also arises from the fact that calcium phosphate is precipitated as pH is increased. Sodium citrate, sodium phosphate dibasic considerably raise the pH, while EDTA and calcium chloride lower the pH. The effect of sodium citrate and sodium phosphate dibasic in raising the pH of milk causes precipitation of calcium and magnesium ions. EDTA forms soluble chelate with calcium and magnesium ions and lowers the pH of milk. The effect of calcium and magnesium solubilization and change in pH on addition of additives may explain the variation in the behaviour of these compounds in stabilizing and destabilizing of milk suspensions.

It is common practice in the industry to add various salts for enhancing the heat stability of milk. It was thought of interest to examine the effect of these salts on the viscosity of milk. Sodium citrate, sodium dihydrogen orthophosphate, sodium phosphate dibasic, EDTA and calcium chloride were added to milk so as to give a final concentration of 100 mmoles/litre of the system. The data, as relative viscosity with respect to that of water as one, is shown in Table 1. It is observed that these salts which are known to disperse the casein micelles increase the viscosity of milk. The increase in viscosity is in the order

Sodium phosphate dibasic > sodium citrate > sodium dihydrogen orthophosphate ~ EDTA
FIG. 7a. EFFECT OF SODIUM CITRATE ON BUFFER INTENSITY OF MILK

- Normal milk
- Normal milk + 50 mg Sod. citrate
- Normal milk + 90 mg Sod. citrate
FIG. 7b. EFFECT OF SODIUM DIHYDROGEN ORTHOPHOSPHATE ON BUFFER INTENSITY OF MILK

- Normal milk
- Normal milk + 50 mg Sod. dihydrogen orthophosphate
- Normal milk + 90 mg Sod. dihydrogen orthophosphate

$\text{dB/dpH}$ vs pH
FIG. 7c. EFFECT OF SODIUM PHOSPHATE DIBASIC ON BUFFER INTENSITY OF MILK

- Normal milk
- Normal milk + 50 mg Sod. phosphate dibasic
- Normal milk + 90 mg Sod. phosphate dibasic

P H

0.01

0.02

0.03

0.04

0.05

0.06

0

4.2 5.0 5.8 6.6 7.4 8.2 9.0 9.8

dB/dpH
FIG. 2d. EFFECT OF EDTA ON BUFFER INTENSITY OF MILK

- Normal milk
- Normal milk + 100 mg EDTA
- Normal milk + 50 mg EDTA
FIG. 7. EFFECT OF CALCIUM CHLORIDE ON BUFFER INTENSITY OF MILK

Normal milk
Normal milk + 46.8 mg CaCl₂
Normal milk + 78.0 mg CaCl₂
If we look at the pH of the samples, we find that this increase in viscosity is not due to change of pH. It is specific property of the ions of the salts.

Calcium chloride which is known to aggregate the micelles, decrease viscosity. The decrease in viscosity is, however, very small.
Table 1. Effect of salts on relative viscosity of milk at 30°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative viscosity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average value</td>
</tr>
<tr>
<td>Milk</td>
<td>1.57 - 1.89</td>
<td>1.71</td>
</tr>
<tr>
<td>Milk + sodium citrate</td>
<td>1.63 - 1.96</td>
<td>1.79</td>
</tr>
<tr>
<td>Milk + sodium dihydrogen orthophosphate</td>
<td>1.52 - 1.92</td>
<td>1.75</td>
</tr>
<tr>
<td>Milk + sodium phosphate dibasic</td>
<td>1.65 - 1.94</td>
<td>1.80</td>
</tr>
<tr>
<td>Milk + EDTA</td>
<td>1.55 - 1.86</td>
<td>1.75</td>
</tr>
<tr>
<td>Milk + calcium chloride</td>
<td>1.55 - 1.83</td>
<td>1.70</td>
</tr>
</tbody>
</table>

SUMMARY

In the present study the variations in the buffering capacities of milk with change in temperature, fat and on the addition of additives like sodium citrate, sodium dihydrogen orthophosphate, sodium phosphate dibasic, EDTA and calcium chloride on the individual sample of buffalo milk collected from the National Dairy Research Institute herd has been studied. The effect of above additives on the viscosity of milk is also studied.

It is found that pH of milk is highest around 25°C, below and above this temperature the pH shows a fall. It is also found that change of pH with temperature is not fully reversible.

It is seen that presence of high concentration of fat decreases the pH on acid side and increase on the alkaline side. The height of buffer intensity peak is biggest with lowest amounts of fat and goes on decreasing with the concentration of fat. The pKa value, however, is not influenced by concentration of fat.

It is found that for bringing the same change in pH the amount of HCl used at 60°C is less than the amount used at 20°C.

Sodium citrate, sodium phosphate dibasic increase the pH, while sodium dihydrogen orthophosphate, EDTA and calcium chloride decrease the pH. Sodium citrate, sodium phosphate dibasic and calcium chloride
change the maximum of buffer index towards alkaline side, EDTA brings the maxima towards acidic side and sodium dihydrogen orthophosphate does not bring any change.

Effect of additives on viscosity shows that sodium citrate, sodium phosphate dibasic, sodium dihydrogen orthophosphate and EDTA increase the viscosity, while calcium chloride decreases the viscosity.
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