STUDY ON MILK PROTEIN BASED COMPOSITE ANTACID TABLETS

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS

submitted in partial fulfillment of the requirements
for the award of the degree of

Master of Technology
in
Dairy Technology

Supervisor
Dr. Arvind

Submitted By
Ridhi Pandey

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Dear Sir,

I have great pleasure in forwarding the thesis entitled **"STUDY ON MILK PROTEIN BASED COMPOSITE ANTACID TABLETS"** submitted by **Miss Ridhi Pandey, I.D. No. 20412MDT010, Enrolment No. 433979,** in partial fulfilment of the requirement for the degree of **Master of Technology** in **Dairy Technology**, Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

This is to certify that the work has been carried out solely by **Miss Ridhi Pandey** under my supervision and guidance and her findings and data presented herein are genuine and original to the best of my knowledge and belief and no part of the work has been submitted for any other degree or institution.

Thanking you,

Forwarded by Yours faithfully

(Dr. Arvind)
Supervisor

(Head of the Department)

BHU capital of knowledge

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Date:

Place: *Varanasi* ***(Ridhi Pandey)***

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
AE	Aqueous extract
α-La	Alpha Lactoalbumin
ANC	Acid Neutralizing Capacity
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CCP	Colloidal calcium phosphate
CDI	Chewing Difficulty Index
FTIR	Fourier Transform Infrared
HCl	Hydrochloric acid
KBr	Potassium Bromide
ME	Methanol extract
mEq	milli equivalent
mg	milli gram
ml	milli litre
mm	milli meter
MPa	Mega Pascal
PGE2	Prostaglandins E2
Rpm	Rotation Per Min
SEM	Standard Error of Mean
Sp.gr.	Specific Gravity
TGA	Thermogravimetric Analysis

Chapter I

INTRODUCTION

The stomach is a digestive tract organ responsible for digesting food and preparing it for intestine absorption. One of its most important roles is the secretion of gastric juice, which produces 1.2 to 1.5 liters each day**.** Hydrochloric acid is one of the essential components of gastric juice in the digestive process. It also stops infections from multiplying and transforms pepsinogen into pepsin, a necessary enzyme for protein digestion (Wolfe *et al.*, 1988). Acidity is usually caused by extrinsic variables such as dietary habits, worry and stress, smoking and alcohol intake, lack of physical activity, irregular feeding schedule, and prolonged use of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs).

Erosions or ulcerations develop when aggressive stimuli overpower the gastrointestinal mucosa's defence factors. As a result, gastritis, peptic ulcers, and gastroesophageal reflux disease develop. Acid and pepsin are the two critical aggressive variables that have been widely established for decades. As a result, antacids, H_2 receptor antagonists, and proton pump inhibitors are commonly used to treat peptic ulcer disease. Hence peptic ulcer diseases are mostly treated with antacids, H_2 receptor antagonists, and proton pump inhibitors (Sandhya *et al.*, 2012).

Men are more likely than women to develop peptic ulcers due to their increased acid production, ranging from 2:1 to 5:1 in youngsters (Celebi *et al.,* 2017). A meta-analysis of 96 papers from 37 countries published in 2020 found that the global pooled prevalence of GERD was 13.98 %, with substantial regional and country heterogeneity. Asia had a 12.92 % estimated rate, compared to 19.55 % in North America and 14.12 % in Europe (Nirwan *et al.,* 2020). Similarly, a prior study found that Asian countries have lower GERD prevalence rates than Western countries (10 % vs. 14.1 - 21.3 %) (Eusebi *et al.,* 2018). Between 2006 and 2016, the number of younger patients with GERD increased significantly, especially among those aged 30 to 39 years in the following manner 15–19 years (0.2 %); 20–29 years (2.4 %); 30–39 years (3.2 %); 40–49 years (2.8 %); 50–59 years (2.5 %) and 60–69 years (0.8 %), all having a significant difference with P 0.001. Obesity and bad eating habits may contribute to the rising prevalence of GERD in the younger generation (Yamasaki *et al.*, 2018).

Generally, Histaminic H_2 Receptor antagonists, proton pump inhibitors, and pharmaceutical antacids are used to control it. Among these, the first two suppress the acid secretion while pharmaceutical antacids neutralize the acid in the stomach (Rang *et al.,* 1997). Gastric acid is produced by the gastric parietal cells when histamine, acetylcholine, and gastrin bind with H_2 receptors. H_2 receptor antagonists arrest acid production by reversibly competing with histamine for binding to H_2 receptors in the gastric parietal cells. For this binding to occur, H_2 receptors must be taken in the bloodstream, and for this to happen, it takes more than 30 minutes or more for H_2 receptors to work. Some typical H_2 receptors are ranitidine and cimetidine. Hepatic and renal impairment are two of the most common adverse effects. Cimetidine is most commonly associated with headache, dizziness, diarrhea, and muscle soreness (Vestergaard *et al.,* 2006).

Proton pump inhibitors are derivatives of benzimidazole that bind covalently at the secretory surface of the gastric parietal cells and irreversibly inhibit the H+ /K+ ATPase enzyme system blocking the final step and hence suppressing acid production. Like H_2 antagonists, PPI is a slow-acting drug but provides a long-term effect. Some common proton pump inhibitors omeprazole, lansoprazole, rabeprazole, etc. Nausea, abdominal pain, constipation, flatulence, diarrhea, subacute myopathy, arthralgias, headaches, and other adverse effects associated with PPI (Vestergaard *et al.*, 2006).

Antacids are one of the most commonly prescribed pharmacological types for self-medication. Antacids differ from acid-reducing medicines like H_2-receptor antagonists or proton-pump inhibitors. They do not destroy the bacteria Helicobacter pylori, which is responsible for most ulcers. Pharmaceutical antacids are the most often used acid-controlling agents because of their fast action (less than 5 minutes). They are alkaline medications that neutralize gastric acidity and act as a buffer to keep the pH of the stomach stable. They do not prevent excessive acid production, but they neutralize the excess acid and raise the pH of gastric contents in the stomach (optimum activity between pH 2-4). However, stomach acid will still splash into the esophagus, and it will be neutralized, resulting in reduced or eliminated heartburn symptoms. For decades, antacids have been proven to be useful in treating gastric and duodenal ulcers, peptic ulcers, heartburn, dyspepsia, and GERD. Calcium carbonate, aluminum hydroxide, magnesium carbonate, sodium bicarbonate, and other antacids are common.

Pharmaceutical antacids can have negative side effects and are frequently severe in action (Mankoo *et al.,* 2021)**.**

Systemic or absorbable antacids and non-systemic or non-absorbable antacids are the two types of antacids. Absorbable antacids are easily absorbed into the systemic circulation and can cause alkalosis and systemic electrolytic changes (e.g., sodium bicarbonate). Aluminum hydroxide, aluminum phosphate, calcium carbonate, and magnesium hydroxide are non-absorbable antacids that do not absorb well (Garg *et al.,* 2022). Systemic alkalosis is the most serious side effect of sodium bicarbonate. Other side effects of systemic antacids include nausea, belching, flatulence, fullness, and peptic ulcer rupture. Non-systemic antacids, on the other hand, are insoluble and poorly absorbed medicines. These medications react in the stomach after absorption, forming chloride salt. Constipation is the most common side effect of aluminum hydroxide. Osteomalacia, encephalopathy, and osteodystrophy are the other side effects. The most common negative effect of magnesium hydroxide is diarrhoea (Neuvonen *et al.*, 1991).

Several features should be present in the ideal antacid: (i) it must respond quickly, (ii) It should be enjoyable to eat, (iii) It should not be absorbed or cause diarrhoea or constipation, (iv) It should be inexpensive, (v) It should be active in all three forms: liquid, powder, and tablet and (vi) There should be no other medications in the same formulation, such as anticholinergic drugs or sedatives, because their activity may decide the maximum dosage achievable, preventing an effective dose of the antacid component (Piper *et al.,* 1964). Altschuler reported that aluminum, particularly aluminum-containing antacids, may play a role in the aetiology of idiopathic Parkinson's disease (Altschuler *et al.,* 1999). Aluminum accumulation has also been detected in serum, bone, and the central nervous system and impaired vitamin A and D absorption, thiamine inactivation, and phosphate depletion (Cooke *et al.,* 1978). Constipation/diarrhoea, reduced gastrointestinal absorption of other drugs such as tetracycline, iron, and cimetidine, and mineral metabolic disturbances such as hypomagnesaemia, hyperaluminumaemia, or phosphate depletion syndrome with osteomalacia are all well-known side effects of antacids in high doses (Berstad *et al.*, 1986).

Aloe vera is well-known for its anta-acid and H_2 receptor antagonist capabilities. It's probably due to the presence of lectins in aloe vera, which inhibit acid release (Jimmy *et al.,* 2015). Papaya is utilised in the production of antacids and for the treatment of ulcers (Ali *et al.*, 2011). Antacid property of Aloe vera, Basil leaves, Cinnamon, Cumin, Cucumber, Garlic, Ginger, Honey, Papaya and turmeric was evaluated and it showed that papaya and turmeric showed the highest antacid property (Divya *et al.*, 2021).

Malik *et al.* (2014) investigated the buffering capability of milk and other milk products and advised their use in hyperacidity/non-ulcer dyspepsia (NUD) circumstances. When compared to dried and coagulated milk products, the per unit protein level in liquid milks has a better buffering potential against HCI.

According to Al-Dabbas *et al.* (2011), the high protein content of dairy products ensures a relatively high buffering capacity due to the buffering effect of amino groups present and the creation of the casein network, which lowers dry matter loss during draining. Purified caseins have the greatest buffering ability at pH 5–5.5 due to phosphoserine and histidine residues (Srilaorkul *et al.*, 1989). Whey proteins have the greatest buffering capability between pH 3 and pH 4 (Srilaorkul *et al.*, 1989). The presence of acidic amino acids is related to this buffering area. Mann *et al.* (1996), on the other hand, described another buffering area at pH levels greater than 8 due to the presence of basic amino acids.

Considering the above facts, the present study **"STUDY ON MILK PROTEIN BASED COMPOSITE ANTACID TABLETS"** was undertaken with the following objectives:

1. To optimize the ingredients for milk protein based composite antacid tablets.
2. To study the proximate and phytochemical composition of optimized tablets.

Chapter II

REVIEW OF LITERATURE

2.1 ALOEVERA

Aloe vera has a thick and fleshy structure that thrives in dry to wet regions (16-33 °C) and belongs to the lily (Lillaceae) family. Aloe vera is known for its antacid properties and H_2 receptor antagonist properties. It is most likely because aloe vera contains lectins, restricting acid secretion (Jimmy *et al.,* 2015). Aloe vera also helps to prevent ulcers. Its anti-ulcer efficacy could be related to anti-oxidant, anti-inflammatory, mucus-secreting, cytoprotective, or healing properties. The leaves of Aloe vera contain a transparent gel that is commonly used as medication. Phenolic compounds (flavonoids), anthraquinone glycosides, saponins, tannins, auxin, gibberellins, amino acids, sitosterol, lignin, and lectins are all anti-inflammatory substances found in Aloe vera. This phytochemical property could be helpful in the treatment of stomach ulcers (Oktaviani *et al.*, 2021).

A stomach ulcer is a lesion that affects the mucosa, submucosa, and muscularis line. Gastric ulcers are caused by an imbalance of aggressive factors such as gastric acid secretion, pepsin, and bacterial infection with Helicobacter pylori and defensive factors such as prostaglandin production, gastric mucus, bicarbonate, and mucosal blood flow (Oktaviani *et al.*, 2021).

Sai *et al.* (2011) studied the anti-ulcer activity of Aloe vera in rats in which ulcer was induced using non-steroidal anti-inflammatory drug which showed Aloe vera to have statistically significant anti-ulcer activity comparable to standard drug omeprazole. Similarly, Oktaviani *et al.* (2021) studied the anti-ulcer activity of aloe vera compared to standard drug ranitidine which showed a potential antiulcer effects of aloe vera. Antiulcer activity of aloe vera was observed by a decrease in the number and severity of ulcers which was induced by using aspirin solution per orally.

Aloe vera extract inhibits gastric acid secretion and has no gastroprotective activity against HCl-induced damage in rats at higher doses. However, the current study found that the extract has gastric acid inhibitory properties and gastroprotective activity at lower concentrations, which could explain its use in the treatment of peptic ulcers. However, the biological processes underlying these effects have yet to be discovered (Yusuf *et al.*, 2004).

2.2 Papaya

Papaya is utilized in the production of antacids, the treatment of ulcers, and the prevention of constipation (Ali *et al.*, 2011). Papaya tablets are advertised for usage as natural antacids, ulcer relief, and constipation alleviation (Lim *et al.*, 2012).

Ezike *et al.* (2009) studied the anti-ulcer potentials of aqueous (AE) and methanol (ME) extracts of whole unripe *Carica papaya* fruit, using ethanol- and indomethacin-induced stomach ulcer models in rats. The extracts' influence on small intestine propulsion was also studied. When compared to the control group, the extracts considerably reduced the ulcer index in both experimental models.

2.3 Main components contributing to buffering capacity in dairy products-

Many components of milk is responsible for buffering capacity in dairy products. These are proteins with many acid base groups and other compounds with acid base groups. Total buffering capacity is the compound effect of buffering capacity of all the compounds which is often effected by interactions between them.

2.3.1 Salts and organic acids

Milk salts account for approximately 37% and 58% of the total buffering capacity of cow milk (Shugailo *et al.*, 1983) and cow skim milk (Srilaorkul *et al.*, 1989), respectively. Ions with buffering capacity include phosphate, citrate, lactate, carbonate, acetate, and propionate. These ions are formed from phosphoric, citric, lactic, carbonic, acetic, and propionic acids, and their degree of dissociation is affected by pH. Their pKa values change depending on physicochemical parameters including ionic strength and mineral surroundings. As a result, as ionic strength increases, the pK value decreases; thus, pK is referred to as apparent pK (pKa). It is worth mentioning that the term pKa is most accurate in most circumstances because the ionic strength is never zero. Cations (calcium, magnesium) on the other hand, can change the acid–base balance of anions (phosphate, citrate, carbonate) and hence their pKa values. Furthermore, insolubility events involving phosphate, citrate, and calcium can occur, particularly at neutral or basic pH (Salaün *et al.*, 2005).

2.3.2 Milk proteins

Whey proteins have the greatest buffering capability between pH 3 and pH 4 (Srilaorkul *et al.*, 1989). The presence of acidic amino acids is related to this buffering area. Mann *et al.* (1996), on the other hand, described another buffering area at pH levels greater than 8 due to the presence of basic amino acids. Purified caseins have the greatest buffering ability at pH 5–5.5 due to phosphoserine and histidine residues (Srilaorkul *et al.*, 1989).

Protein pH titration is a complicated function since it is affected by the protein's sequence, spatial structure, and environment. Some ionizable groups are inaccessible within a protein, but they can become accessible following a pH change or denaturation. (Singh *et al.*, 1997). Thus, the pKa value of phosphoserine residues (2nd ionisation) is determined by (i) protein structure; the pKa of phosphoserine amino acid is approximately 5.9, whereas that of ß-casein is between 6.34 and 6.34; and (ii) their localizations in casein; for ß-casein, the pKa values of phosphoserine residues in positions 15, 17, 18, 19, and 35 are 6.55 (Baumy *et al.*, 1989).

On the other hand, the interactions of cations with acid–base groups of proteins affect their buffering capacities. According to Barraquio *et al.* (1990) and Szpendowski *et al.* (1997), sodium and calcium caseinate have the same maximum buffering capacity (pH=5.6). Calcium caseinate, on the other hand, has a greater buffering capacity than sodium caseinate. The electrostatic interaction of calcium with phosphoserine most likely alters the acid–base equilibrium of this residue.

2.3.3 Anti ulcerative properties of bovine α-lactoalbumin

It is generally known that peptic ulcerogenesis (gastric and duodenal) is caused by an imbalance between protective substances such as mucus, bicarbonate, prostaglandins (PGE2 and PGI2), sulfhydryl compounds such as proteins, glutathione, and others, as well as blood flux to the mucosa cells, and infectious agent like the bacteria Helicobacter pylori or aggressive chemical agent (Abdel-Salam *et al.*, 2001).

Mezzaroba *et al.* (2006) investigated ability of α-lactoalbumin to protect the rat stomach mucosa from ulcerative lesions caused by indomethacin or ethanol. The protective action of α-lactoalbumin was observed with increased prostaglandin E2 (PGE2) and mucus production. Protective action of whey is attributed to a combination of enhanced prostaglandin and mucus production and due to the presence of naturally occurring sulfhydryl compounds, as well as sulfhydryl groups in the a-La. The major whey protein contributing toward protection against ulcerative lesions caused by indomethacin or ethanol is α-lactoalbumin. According to the findings of this study, whey protein and whey protein hydrolysates protect the rat stomach mucosa against ulcerative lesions induced by indomethacin or ethanol.

Similar study done by Ushida *et al.* (2003) reported α-lactoalbumin showed increased protective activity in naïve rats for both PG-dependent and PG-independent gastric defence mechanisms. He reported the following to be defence mechanism of α-lactoalbumin. Increased endogenous PGE2 levels in gastric tissue; accelerated mucus secretion with thickening of the mucus gel layer; increased gastric luminal pH; increased gastric fluid volume; and delayed gastric emptying.

2.4 Variation in Buffering Capacity of milk

2.4.1 Cow milk

Imam *et al.* (1974) reported that cow milk has a higher buffering capacity at acid than alkaline pH and its pKa value was 4.9. The maximum buffering capacity of cow milk has been reported at a pH in the range of 5.0-5.6, more frequently pH 5.2-5.3. Lucey *et al.* (1993) reported that the maximum buffering occurring at approximately pH 5.1 was due to the solubilization of CCP resulting in the formation of phosphate ions which combine with H+ causing increased action.

2.4.2 Buffalo milk

Buffalo milk has a higher buffering capacity than cow milk. In comparison to cow milk, buffalo milk includes more total solids, casein, and minerals such as calcium, magnesium, and inorganic phosphate. It has a higher buffering capacity than goat milk. Buffalo milk has a better buffering capacity at acidic pH than at alkaline pH. Buffalo milk has the greatest buffering capacity at pH 5.3-5.4 and a pKa of 5.32 (Imam *et al.*, 1974).

Rao *et al.* (1956) reported that, Murrah buffalo milk has the highest buffer intensity value of 0.0417. On the alkaline side, the buffering capacity of buffalo milk decreased steadily, and the average buffer values at pH 7.2, 8.0, and 9.0 were 0.01102, 0.0069, and 0.0058, respectively.

2.4.3 Goat milk

Buffering capacity of goat milk is higher than cow or human milk because of higher SNF content (Lan *et al.*, 2000; Joshi *et al.*, 1967). Higher capacity is observed at acid pH than alkaline pH (Imam *et al.*, 1974). At pH 5.3, the greatest buffering capacity was observed, with an average value of 0.0396, it showed a similar trend as colostrum, where there is decrease in buffering capacity in the alkaline region up to pH8 and then increase in buffering capacity was observed (Joshi *et al.*, 1967).

Park *et al.* (1992) reported that the buffering capacity of Nubian goat's milk is higher than that of Alpine goat's milk. This higher buffering capacity of Nubian goat's milk is related to the higher levels of total nitrogen and phosphate. Park studied the relative buffering capacities of goat milk (Alpine, Nubian), cow milk (Holstein, Jersey), soy-based infant formulas, and non-prescription antacid drugs.

2.4.4 Human milk

Lower protein and phosphate content of human milk compared to cow milk results in poor buffering capacity of human milk. (Bullen *et al.*, 1977) A significant contribution to buffering capacity of human milk is because of minerals, followed by casein and whey protein, 57%, 31%, and 12%, respectively (Shugailo *et al.*, 1983). Variation in the buffering capacity of human milk has been observed during lactation with buffering capacity of 0.57 ml during 1st week, 0.45 ml at the end of the first week, 0.68 ml after 3rd week, and 0.88 ml after 6 months.

2.4.5 Colostrum

Colostrum has a higher buffering capacity than normal milk, and it was maximum at pH 5.0 (McIntyre *et al.,* 1952; Rao *et al.,* 1956). However, Rao observed two peaks at pH 6.2 and pH 6.8. These three areas peaks correlate to the highest levels of buffering provided by calcium phosphate, bicarbonate, and sodium and potassium phosphates, respectively. The buffer value of buffalo milk colostrum has a higher maximum value than cow milk colostrum (Rao *et al.,* 1956). In the alkaline region, buffering value showed a decrease up to pH 8; after that it increased. Mclntyre *et al.* (1952) observed no marked reduction in buffering capacity during the first four milking but then, with an increasing number of milking decrease in buffering capacity was followed.

2.4.6 Mastitic milk

Buffering peak of mastitic milk observed at pH 5.1 was slightly higher than normal milk. Higher concentration of colloidal calcium phosphate in mastitic milk was stated to be the reason for this (Lane *et al.,* 1970; Singh *et al.,* 1997). Mastitic cow milk has maximum buffering value of 0.032 (Lane *et al.,* 1971). Gajdusek *et al.* (1996) reported that, there is a statistically significant decline in buffering capacity with increasing somatic cell counts (more than 100,000).

2.5 Influence of physico-chemical conditions on the buffering capacity of milk

2.5.1 Anion addition

The milk buffering capacity can be modified differently depending on the nature of the added anion. The shifts are caused by the anion's acid-base characteristics and its interaction with minerals, particularly calcium. As a result, adding the exact molar amounts of different anions results in qualitative and quantitative alterations. Qualitative changes include a change in buffering capacity, and quantitative changes involve changes in buffering intensity corresponding to the number of anions added.

2.5.2 Carbonate (carbonic acid)

Carbonate has a buffering capacity and alters salt balance by forming calcium carbonate salt. The addition of CO_2 to milk causes qualitative and quantitative alterations in buffering capacity: carbonated milk has two maximal buffering peaks at pH 4.95 and 5.4, whereas control milk has only one at pH 5.1 (De La Fuente *et al.,* 1998; Gevaudan *et al*., 1996). Changing the pressure from 500kPa to 1500kPa, changes the peak at pH from 5.4 to 5.1 (Gevaudan *et al.,* 1996). A decrease in buffering capacity happens in the latter case. These alterations in buffering capacity for milk acidified at pH 5.5 are reversible when the pH is raised to 6.6, as opposed to those generated by acidification at pH 4.6–5.0 (Lucey *et al.,* 1996).

2.5.3 Citrate and Phosphate

When citrate or phosphate salts are added to milk, the buffering capacity changes qualitatively and quantitatively. These changes are caused by the anions' buffering characteristics and the interaction of anions with calcium.

Citrate salts are calcium chelators, and their addition to milk causes colloidal calcium phosphate to be solubilized; the buffering capacity is pushed towards high pH because some colloidal inorganic phosphate is solubilized, and it is also enhanced because citrate salts have buffering capacity. The effects of phosphate salts on buffering capacity vary. When orthophosphate is given to milk (which is already saturated in calcium phosphate), the soluble phosphate concentration rises (the buffering capacity rises) while insoluble calcium phosphate salts develop (buffering capacity is shifted towards low pH). When pyro or polyphosphate (both chelators) are administered to milk, a portion of the colloidal calcium phosphate is solubilized, same as when citrate salts are added, and the effects on the buffering capacity are the same.

Ramadan *et al.* (1997) studied that 5 mM disodium phosphate or trisodium citrate increased buffering capacity in the pH ranges 6–6.9 or 5.8–6.5, respectively. Ibrahim *et al.* (1989) also proved the effectiveness of adding a citric acid and disodium phosphate mixture to boost the buffering capacity of cheese making milk. Increase in buffering capacity of milk at neutral pH was observed by addition of sodium citrate and sodium pyrophosphate. These additives prevent milk instability caused by intense heat treatments (Ismail *et al.,* 1973).

2.5.4 Addition of calcium

When $CaCl_2$ is added to milk, which is already saturated in calcium phosphate, a decrease in the concentration of phosphate and citrate in the aqueous phase is observed. This decrease is associated with calcium phosphate and calcium citrate salts in the micellar phase (Philippe *et al.*, 2003; Udabage *et al.*, 2000). These associations are responsible for shifts in buffering capacity towards low pH.

2.5.5 Presence of alcohol

Study conducted by O'Connell *et al.* (2001), regarding the mechanism of ethanol-dependent heat-induced dissociation of casein micelles and he reported that alcohol causes a shift in buffering capacity towards alkaline pH by reducing the dielectric constant. This change is connected to the pKa values of the phosphoserine residues' second acidity, which are more alkaline in the presence of alcohol.

2.6 Influence of different treatment on buffering capacity of milk

2.6.1 Heat treatment

Buffering capacity can be modified to some degrees by changing the intensity of the heat treatment and composition if milk. Heat treatment lower than 100 °C increases buffering capacity around pH 5 but more intense heat treatment like 120 °C for 10 min changes the maximum buffering capacity from pH 5.0–5.2 for untreated milk to pH 4.3–4.5 for heated milk. The major mechanism behind these shifts is complex due to various changes occurring biochemical changes occurring these treatment. Changes occurring during heat treatment are calcium phosphate precipitation, changes in the structure and composition of micellar calcium phosphate, lactose degradation in formate, casein dephosphorylation with release of inorganic phosphate, and casein degradation (Lucey *et al.*, 1993).

The urea content in milk may also influence the products of its thermal degradation, particularly carbonic acid, which may increase the buffering capacity (Gaucheron *et al.*, 2000). Metwalli *et al.* (1996) reported that in the presence of urea supplied at doses of 5 and 10 mM, the heat-induced decrease in pH is minimised. The authors suggests that the combination of caseins and ß-lactoglobulin is to reason for this reduction (Metwally *et al.*, 2001).

The buffering capacities of the low and high heat milk powders were the same as those of the corresponding milk (Lutchman *et al.*, 2006). It was observed that the intensity of buffering capacity of skim milk powder decreased with increasing heat treatment done during spray drying. Buffering intensity in the range of pH 5.2-6 for low heat reconstituted SMP and high heat reconstituted SMP was 1 and 0.5 in the range of pH 4.2-6.0.

2.6.2 Milk concentration by membrane separation technology

Milk ultrafiltration allows for the partial removal of mineral contained in the aqueous phase without altering the micellar minerals. Its use causes whey protein and micellar phase concentration. This causes increase in buffering capacity in proportion to protein content between pH 5.0 and pH 5.3 (Mistry *et al.*, 1985; Srilaorkul *et al.*, 1989).

When milk is concentrated via microfiltration, the outcomes are similar. However, the contribution of whey proteins to buffering capacity is diminished in this circumstance. Data from our laboratory reveal that the buffering capacity increases with micellar casein content. Furthermore, as the buffering capacity increases in accordance with the concentration factor during ultrafiltration, the buffering capacity shifts towards acid pH: there is a 0.4 pH unit shift between a milk and its ultrafiltration retentate (Srilaorkul *et al.*, 1989). Because the aqueous phase becomes more concentrated and saturated in minerals during acidification of retentate, the pH must be reduced to a lower value to induce colloidal mineral solubilisation (Le Grae¨t *et al.*, 1999).

The contribution of the various elements to the buffering capacity of milk, on the other hand, differs from that of its comparable ultrafiltration retentate (volumetric concentration factor=5) (36 vs. 53.8% for caseins, 5.4 vs. 9.7% for whey proteins and 58.6 vs. 36.5% for milk salts) (Srilaorkul *et al.*, 1989). Retentate dilution with water, on the other hand, reduces the retentate buffering capability more efficiently than dilution with permeate, which contains some buffering minerals (Abd El-Salam *et al.*, 1982).

2.6.3 High-pressure trea K tment

Physico-chemical changes in casein are seen when milk is subjected to high-pressure treatment. Slight demineralization of casein micelle is reported (Famelart *et al.,* 1997; Gaucheron *et al.,* 1997). The effect of high-pressure treatment on the buffering capacity of milk is observed despite the only slight change in mineral distribution. Buffering peak for untreated milk is at a pH of 4.8-5.0, which changes to a pH of 5.2-5.4 after treatment at 250, 450, or 600 MPa. At pH 5.0, buffering capacity of untreated milk is higher, but between pH 5.2 and 6.0, it is higher in high pressure treated milk (Famelart *et al.,* 1997).

Solubilisation of micellar casein during acidification occur at high value of pH when milk is high pressure treated. As a result, at pH 5.2, there are 30% more calcium ions in the aqueous phase of a milk compressed at 600 MPa for 30 minutes than in untreated milk. The differential in buffering capacity is most likely related to changes in micellar calcium phosphate composition during the pressurization–depressurization cycle. Studies show destruction of micellar structure under pressure. When the caseins are restored to atmospheric pressure, they are differently structured, and the micellar calcium phosphate is not in its native state (Huppertz *et al.*, 2002).

2.6.4 Carbonation

Carbonate changes the salt balance by forming calcium carbonate salt. The amount of CO_2 dissolved, hydrated, and protonated in the aqueous phase of a food determines the extent of pH reduction, which is dependent on the intrinsic qualities of the aqueous phase, such as buffering capacity and initial pH (Gill, 1988).

Upon CO_2 addition, a reduction in milk pH is accompanied by a progressive solubilisation of CCP and other colloidal salts solubilized into the serum phase from casein. Carbonated milk had two maximal buffering peaks at pH 4.95 and 5.4, whereas control milk had only one at pH 5.1. Furthermore, at a pressure of 500 kPa, a peak at pH 5.4 was detected, which changes to pH 5.05 at a pressure of 1500 kPa. (Gevaudan *et al.*, 1996). A decrease in buffering capacity happens in the latter case. In contrast to those generated by acidification at pH 4.6–5.0, these alterations in buffering capacity for milk acidified to pH 5.5 are reversible when the pH is raised to 6.6 (Lucey *et al.,* 1996).

Raouche *et al.* (2007) reported that milk buffering capacity in the pH range of 4.5-5.5 reduced after neutralisation of carbonated milk, but rose during refrigerated storage. The physicochemical properties of casein micelles were found to be unaffected by holding time of carbonated milk at low pH**.**

2.6.5 Extrusion

Extrusion of para-caseinate and acid casein increased their buffering capacity due to formation of aggregates stabilized by hydrophobic bonds with no reduction in their biological value. But with neutralized casein, buffering capacity and biological value is decreased by extrusion (Szpendowski *et al.,* 1991).

2.6.6 Enzymatic hydrolysis

Hassan *et al.* (2002) reported that enzymatic hydrolysis of buffalo milk protein into products showed increased buffering capacity with increased hydrolysis time by Maxrien enzyme. Similar study done in Emmental cheese showed increased buffering capacity with the reason may be hydrolysis of protein into smaller peptides (Blanc *et al.,* 1979). However, Korchik *et al.* (1988) reported that partial hydrolysis of cow milk proteins impaired buffering capacity.

2.7 Buffering capacity of dairy products

2.7.1 Yoghurts

Yoghurts are high in lactic acid, caseins, and inorganic phosphate, and have the greatest buffering capacity at pH 3.6 and between pH 5 and 6.The presence of urea in milk, which is degraded by urease into CO_2 and NH_3, causes a slowing of acidification during their production. As observed during heat treatment, the generation of CO_2 (in acid–base equilibrium with carbonate and bicarbonate) increases buffering capacity at roughly pH 6.5 and hence slows the rate of acidification. However, replacing some of the skim milk powder with whey proteins increases buffering capacity at pH 4 while decreasing buffering capacity between pH 5 and pH 6 (Kailasapathy *et al.,* 1996). These changes are due to differences in buffering capacity between whey protein concentrate and skim milk powder, which have maximum buffering capacities at pH 4 and pH 5 and pH 6, respectively.

2.7.2 Milk powder

The buffering capacity of cow milk powder was much larger than that of goat milk powder or soya milk powder, with values of 0.3196, 0.0221, and 0.0192, respectively. It's because cow's milk includes casein, a protein with the highest buffering capacity, whereas goat's milk and soy milk powder have none or very little casein. The buffering capacities of the low and high heat milk powders were the same as those of the corresponding milk. (Lutchman *et al.,* 2006). It was observed that the intensity of buffering capacity of skim milk powder decreased with increasing heat treatment done during spray drying. Buffering intensity in the range of pH 5.2- pH 6 for low heat reconstituted SMP and high heat reconstituted SMP was 1 and 0.5 in the range of pH 4.2-6.0 (Metwally *et al.,* 2001). Mistry *et al.* (2002) used ultrafiltration and diafiltration with no pH adjustment to create a high-protein powder rich in milk proteins, casein and whey proteins, and lactose-free. The powder, which included around 84 % total protein, had a strong buffering capacity when combined with non-fat dry milk, allowing the development of an active bulk lactic starter.

2.7.3 Infant formula

Alekseev *et al.* (1982) compared milk based infant formulas from six different countries for their buffering capacity. Their buffering capacity ranged from 0.76 to 2.93. The buffering capacity content of infant food made from cow milk was reported to be 4-5 times higher than that of human milk (Shugailo *et al*, 1983). Dried infant food manufactured from buffalo milk showed good buffering capacity comparable to cow milk but lower than human milk (Kuchroo *et al.,* 1982).

Modifying buffering capacity of cow milk was done by Korchik *et al.* (1988) to produce baby food. Increased whey protein and lower total protein concentration were found to diminish buffering capacity. Optimum buffering capacity obtained when whey used was ultrafiltered or demineralized by electro dialysis.

The amount of casein in infant formula was found to be a significant factor in its buffering capacity. No linear relationship was found between the buffering capacities of milk-based infant formula and the amount of casein (Ahmari *et al.,* 2000)

2.7.4 Conventional cheese

The curd's buffering capacity during mould removal and ripening is an important element in enzymatic activity and microbial development. Variations in this component cause varying rates of alkalinisation and ripening, which might impact the ultimate cheese quality. Caseins and whey proteins, as well as their degradation products, inorganic phosphate, and organic acids are the key ingredients that contribute to cheese buffering ability (Lawrence *et al.,* 1982).

Amount of these elements in cheese varies depending on milk composition, technical treatments of milk prior to transformation into curd, the cheese making process, salting and ripening conditions. And so buffering capacity of cheese depends on these many elements (Salaün *et al.,* 2005).

2.8 Milk in the form of tablets

Milk has a variety of components that have medicinal purposes. There have been found components with applications in fields such as enteric infections, skin therapies, tissue repair, medication delivery, and tablet formulations. Furthermore, genetic engineering techniques can be utilised to produce milk components with specialised applications, making milk a valuable source of medications (Dionysius *et al.,* 1991).

The tablet can also be utilised in mass catering, military requirements, and as a survival ration to replace milk and sugar in tea/coffee. One milk tablet every hour could help to maintain regular stomach acidity. As a result, it has the potential to be used to treat peptic ulcers and other forms of elevated stomach acidity (Konzelmann *et al.,* 1968). The inclusion of dried milk in aspirin tablets may reduce the stomach irritating impact of aspirin (Borchers *et al.,* 1999).

Many researchers developed tablets by using milk or milk component for one or more functions. Most of the formulations are patented. Many researchers created tablets that used milk or milk components to perform one or more functions. The majority of the formulations are protected by patent.

Metadier *et al.* (1974) created a revolutionary natural milk-based food product in the shape of a solid tablet by compressing dry milk extract or dried milk with ingredients such as sugar, chocolate, coffee, vanilla, caramel, fruit, and so on. This product can be used as a snack, survival food, low-calorie diet component, and so on. Compressing a mixture of dried whole milk, instant skim milk, and dextrose resulted in solid shaped goods such as cubic, cylindrical, and tablets. The final composition was 20-60% dextrose, 5-10% dried whole milk, 1-3% moisture, and the rest was instant milk (Legrand *et al.*, 1972).

The procedure for producing milk pills is described in a German patent. By adding water to dried milk, a plastic product having a moisture percentage of less than 60%, preferably 15- 20%, is created. After adding additives and sweeteners, it is formed into a tablet and dried to a moisture content of less than 4%, ideally under vacuum, to allow for expansion. Because it does not lose its crispiness when soaked in milk, the final product was crisp and could be used as a breakfast cereal, etc. (Morgan *et al.*, 1969).

Milk tablets based on milk protein also have been reported. Natvaratat *et al.* (2007) developed milk protein tablets with a high nutritional value were produced to provide adequate protein supplements to rural schoolchildren and to improve the nutritional status and health of rural schoolchildren. The optimal formula contained 25% dried egg yolk powder, 55% milk powder, 15% sugar, 0.4 % cab-o-sil, 1% talcum, 3.5 % comprecel, and 0.1 % strawberry flavour. The protein content was approximately 15.8 %, the fat content was 22.9 %, and the energy content was 457.85 kcal.

2.9 Market status of milk tablets

Lac-nutrients Inc. introduced pure milk pills in 1968. Westfield, New Jersey It may eventually be blended with soup, eggs, meat, fruit, and other things under the label 'Milk to Eat.' (Verma *et al.*, 2001). Milk tablets, goat milk tablets, and colostrum tablets are currently available in China, New Zealand, Thailand, Australia, Switzerland, France, India, and other nations. Milk tablets are manufactured in China by companies such as Chaozhou Anbu Liqiang Food Co., Ltd., Rainia Import and Export Co., Ltd., Golden Coast Industry and Trade Co., Ltd., Shantou Honeycandy Food Factory, Shen Zhen Wan Hao Da Industrial Co., Ltd., Chaozhou Anbu Liqiang Food Co., Ltd., Shanghai Zhonghe Packing Machinery Co., Ltd., and others. Bee Products Industry Co., Ltd. manufactures She-Cow Milk Tablet, which is popular in Thailand. In Hong Kong, Befy Development Co., Ltd. also manufactures milk tablets. In France, the tablets are branded as Bio RestEzy Milk Tablet and are produced from Lactium®, a patented milk protein hydrolysate (MPH) from Ingredia. Swiss milk tablet is a Scottish medium-hard, sweet dessert. It is created by boiling sugar, condensed milk (originally made in Switzerland, hence the name swiss milk), and butter to a soft-ball stage and then allowing it to crystallise. It is frequently flavoured with vanilla and may contain nut pieces. Guangzhou Cinjep Biotechnology Co., Ltd. manufactures calcium milk tablets. Pine pollen calcium milk tablets are available in the Chinese market. This item contains pore-smashed pine pollen powder, full cream milk powder, powdered whey, xylitol, edible calcium carbonate, zinc gluconate, oligosaccharides, and mint essence. It contains a lot of amino acids, vitamins, and microelements. In New Zealand, goat milk strawberry chewable tablets are available. KiwiCorp Products Ltd., Deep Blue Health Ltd., Laniazs Enterprise, NZ Green Health Ltd., and other companies in New Zealand are also involved in the production of goat milk tablets.New essentials pure goat milk tablets are now available in the Netherlands. Goat milk tablets are manufactured in Singapore by Superbee Network Singapore Pte Ltd, Akid Enterprise, and others, and in Australia by Life Time Health Products Pty Ltd. Goat milk tablets have recently entered the Indian market and are now accessible in Ahmedabad, Gujarat. Green Health Limited in New Zealand produces chewable colostrum tablets that are also high in calcium. Healtheries Colostrum Milk Tablets are also seen in New Zealand market with strawberry flavour. Nutrientsnz, New Zealand also manufactures colostrum milk tablets. Horlicks Malted Milk Tablets used to come in a glass bottle resembling a Bayer Asprin bottle.

Chapter III

MATERIALS AND METHODS

The manufacturing and analysis of the tablets was carried out in Department of Pharmaceutical Engineering and Technology, IIT BHU and Department of Dairy Science and Food Technology, IAS, BHU. This chapter deals with the materials used and the methods employed in present investigation. Methodologies related to the technological aspects as well as the physical, chemical, and statistical analyses are delineated hereunder.

3.1 RAW MATERIAL/INGREDIENTS

Casein- Casein was procured from Barbell Training Private Limited, New Zealand.

Whey protein- Whey protein was procured from AS-IT-IS Nutrition, Medizen Labs private Limited, Bangalore, India.

Aloe vera powder - Aloe vera powder was obtained from Heilen Biopharm Pvt ltd, India.

Papaya powder – Papaya powder was obtained from Annapurna Agro Export, India.

Starch- Starch was procured from Mahaveer Marketing, India.

Mannitol - Mannitol was obtained from Profoods nutrition, India.

Magnesium stearate- Magnesium stearate was procured from Purenso Global, India.

3.2 EQUIPMENTS

pH meter	Thermo Scientific, Sn B21889, Singapore
Magnetic stirrer	Spinot, India
Electronic Weighing balance	Labtech LCB 1021 v Daihan Lab Tech India Pvt. Ltd
Hot Air oven	992 Perfit India
Muffle Furnace	SNOL 82/1100-1LZ Pagaminta Lietuvoje, Lithuania
Hot Plate	Lapro 131, Moglix Pvt. Ltd., India

Hot water bath	Borosil WBC 250 W 2 Flask Position Mini Water Bath, Moglix Pvt. Ltd, Delhi
Kjeldahl Apparatus	KEL PLUS Protein Estimation System, Pelican Equipments Ltd., India
Friability tester	Erweka Apparatebau, Germany
Disintegration tester	Veego Pharma, LLC is located in Somerset, NJ, United States
Hardness tester	A Monsanto hardness tester (Monsanto Chemical Corp, USA)
TGA	TA Instruments Q500 analyser
Tablet compression machine	Rimek Tablet Compression Machine

3.3 METHOD OF MANUFACTURE OF MILK PROTEIN BASED COMPOSITE ANTACID

3.3.1 Precaution taken during tablet manufacture

- All the equipment used in the manufacturing, i.e., sieve, a tray for granules drying, S.S. vessel, etc., were cleaned thoroughly and dried before use. The manufacturing area was cleaned.
- Production personnel wore a clean apron. Their nails were ensured to be trimmed adequately during the handling of materials.
- The unit premises were maintained clean and free from dust.
- Before manufacturing, equipment was cleaned so that there was no chance of contamination of the product.
- Any semi-finished material/tablets were stored in airtight and labeled polythene bags.

3.3.2 Formulation of different types of antacid tablets based on milk protein, papaya and aloe vera.

Previous studies on the buffering capacity of milk proteins and antacid capacity of milk protein tablets has been reported by Thesiya *et al.* (2018). Antacid activity of papaya and aloe vera was also reported by Divya *et al.* (2021). Based on the above findings, formulation of the antacid tablets was designed.

Table 3.1: Formulation of different type of antacid tablets

	Aloe vera	**Papaya**	**PA**	**Control**
Casein %	50	50	50	50
Whey protein%	20	20	20	50
Aloe vera%	30	-	15	-
Papaya %	-	30	15	-

3.3.3 Binder preparation

Starch 5% w/w was mixed with boiling distilled water to prepare starch slurry. Sucralose was also added to this Starch was constantly mixed slowly to prepare a homogenous slurry. Starch slurry thus obtained was cooled to room temperature.

3.3.4 Weighing

All the ingredients, casein, whey protein, papaya powder and aloe vera powder were weighed according to the formulation. The weighed ingredients were transferred to vessel and it was dry mixed to produce uniform mixture.

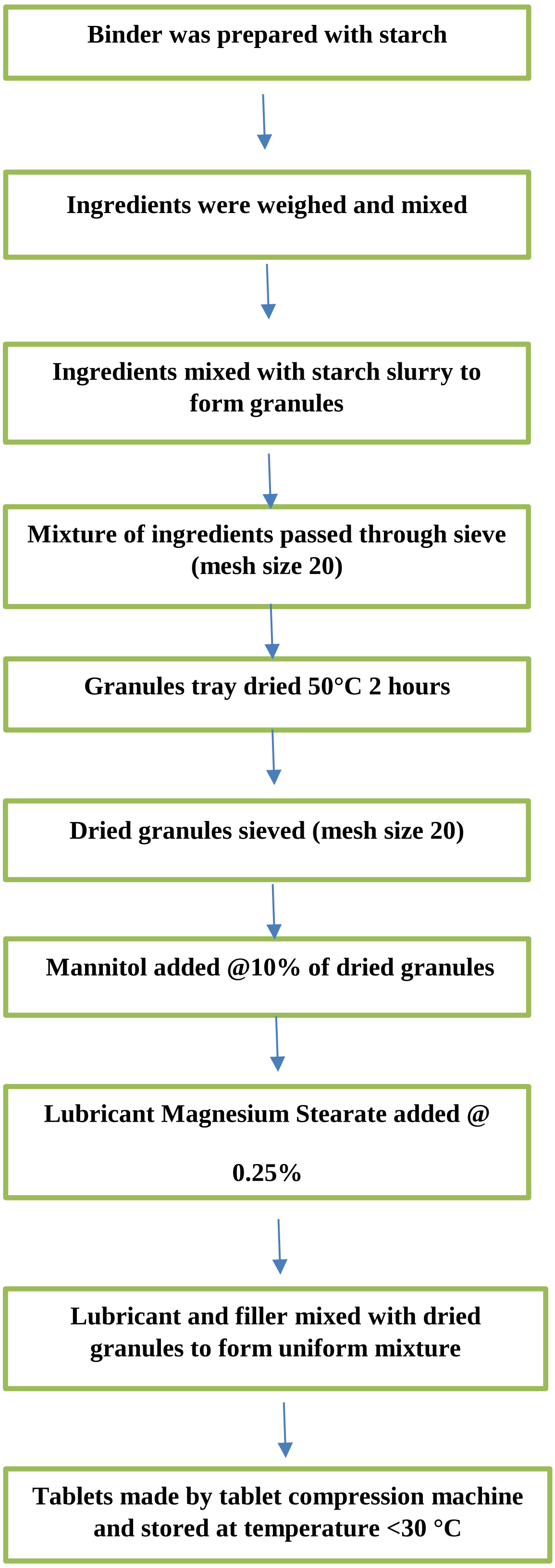

Figure 3.1: Flow diagram of preparation of tablets through wet granulation method

3.3.5 Granulation

Wet Granulation method was used for manufacturing of tablets. The weighed ingredients were mixed with starch slurry previously prepared. These were mixed thoroughly to prepare small granules.

3.3.6 Sieving

The mixed mass of ingredients and binder starch was passed through SS sieve of mess size 20 and the granules obtained was dried.

3.3.7 Tray drying

The granules obtained were tray dried at 50 °C for 2 hours so that the moisture content was reduced to 3-5%.

3.3.8 Sieving

The dried granules were passed again through the sieve 20.

3.3.9 Mannitol addition

Mannitol was added as filler @ 10 % of the dried weight of granules.

3.3.10 Lubrication

Magnesium stearate was added @ 0.25% of dried weight of granules as lubricating material for smooth flow through the tablet compression machine.

3.3.11 Tablet compression

Tablets were made by passing dried granules in tablet compression machine.

Figure 3.2: Granules for Aloe vera tablets

Figure: 3.3 Granules for Papaya tablets

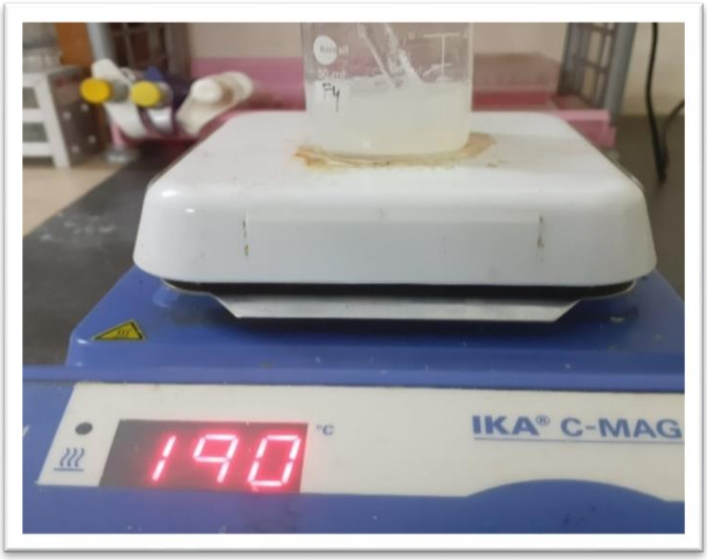

Figure 3.4: Starch slurry

Figure 3.5: Granules for control tablets

Figure 3.6: Granules for PA tablets

Figure3.7: Friabilator

Figure 3.8: Hardness tester

Figure 3.9: Tablet compression machine

Figure 3.10: Disintegration test apparatus

Figure 3.11(a): Aloe vera based composite antacid tablets

Figure 3.11(b): Papaya based composite antacid tablets

Figure3.11 (c): Papaya and Aloe vera based composite antacid tablets

Figure 3.11(d): Control antacid tablets

3.4 ANALYSES OF MILK BASED COMPOSITE ANTACID TABLETS

3.4.1 Acid Neutralizing Capacity (USP 23)

Tablet equivalent to minimum dosage was ground to fine powder and transferred to a beaker of 250ml. 70 ml distilled water is added to this and this solution was mixed on magnetic stirrer for 1 minute. 30 ml of 1 N HCl was then added and the sample stirred for a further 15 minutes at a rate of 300 rpm. pH meter was connected to the beaker to monitor the change in pH. Titration was carried out immediately to determine excess HCl using 0.5N NaOH to attain a stable pH of 3.5. The experiment was carried out at a temperature of 37 °C ± 3 (Yafout *et al.*, 2022).

The number of mill equivalents (mEq) of acid consumed was calculated by the formula:

$$ANC\ (mEq) = \ (30 \times N\ HCl) - (\ V\ NaOH\ \ \times N\ NaOH)$$

Where N HCl and N NaOH are the normalities of HCl and NaOH respectively and V NaOH is the volume of NaOH added to obtain a stable pH of 3.5.

3.4.2 Bulk Density

Bulk density of the composite antacid tablets were measured by the method as described by Thesiya *et al.* (2018). Glycerol solution was used to measure the bulk density of the tablets. At first 35ml of glycerol was takin in 50ml measuring cylinder. 10 tablets were weighed and then were immersed in the glycerol solution. Increase in the level of glycerol obtained were noted. Bulk density was measured by the ratio of weight of the sample to increase in volume of the glycerol solution.

3.4.3 Weight variation test

Twenty tablets from each batch were collected randomly and weight of individual tablet was determined using an electric balance. The average weight of the 20 tablets and percentage deviation in weight of each tablet from the average weight were calculated and tabulated (Tarkase *et al.*, 2015).

The weight variation of the tablets was calculated using the following formula:

$$Weight\ Variation = \frac{(\ intial\ weight - average\ weight)}{average\ weight} \times 100$$

3.4.4 Tablet dimension

Tablet dimensions were measured by using Venire-callipers. Diameter and thickness were measured in millimetres.

3.4.5 Friability

During manufacture, packing, and transportation, tablets were constantly subjected to mechanical shocks and abrasion. Such forces can cause tablet chipping, abrasion, or even fracture. As a result, it is critical that the tablet be designed to bear such forces without damage. Friability is defined as the percentage of weight lost by the tablets during the test due to mechanical action.

Friability testing was performed using Roche friabilator. It is a rotating transparent plastic drum with a cover was used fitted on the drum wall was a radial curved blade which fitted the tablets along with it up to the central height and let them fall off while drum was in rotation. Thus the tablets rubbed against each other. The drum rotated at a fixed speed of 30 rpm for 4 minutes (Tarkase *et al.*, 2015). The tablets were weighed before and after testing.

Percentage weight loss was calculated using following formula-

$$\%Friability = \frac{(intial\ weight - final\ weight)}{intial\ weight} \times 100$$

3.4.6 Disintegration time test

Disintegration testing apparatus consist of rack with six test tube type structure with stainless steel wire-gauze bottom on their base, on which tablets are kept. It consist of two beakers of one litre capacity in which 0.1 N HCl was kept and the temperature was maintained at 37°C. These basket rack moved in vertical direction in beaker containing HCl. At random, six tablets were selected from each formulation and each tablet was placed in each of the tubes of the basket rack system of disintegration.

Disintegration time was noted by visual observations and was calculated as till no granule of any tablet was left on the mesh. It was observed that the antacid products tended to undergo erosion rather than disintegration. (Nyamweya *et al.,* 2020)

3.4.7 Sensory evaluation

The milk-protein based antacid tablet samples were subjected to sensory evaluation on a 9-point hedonic scale by a panel of judges. These products were subjected to sensory evaluation of colour and appearance, body and texture/ chewability, flavour and overall acceptability. The score card used is given in Appendix – I.

3.4.8 Hardness test

The resistance of tablets to capping, abrasion, or breakage under conditions of storage, transportation, and handling before usage depends on its hardness. Tablet hardness is defined as the load required crushing or fracture a tablet placed on its edge. Sometimes, it is also termed as tablet crushing strength.

A Monsanto hardness tester was used at room temperature to determine the load required to diametrically break the tablet. Five tablets were taken from each formulation and were placed between the spindles of the hardness tester instrument diametrically and pressure was applied. The pressure was then increased as slowly and the pressure at which the tablets broke, was recorded (Gandhi *et al.,* 2011).

3.4.9 Chewing Difficulty Index (CDI)

The Chewing Difficulty Index (CDI) is a parameter that has been recently proposed as a measure of the ease or difficulty of chewing a tablet. (Nyamweya *et al.,* 2020). It is calculated according to the following equation-

$$CDI = F \times H$$

F is the breaking force, and H is the tablet thickness.

3.4.10 Thermo gravimetric analysis

Thermogravimetric analysis was done to study the thermal stability of the developed antacid tablets. It was done at the Department of Chemistry, Institute of Science, BHU**.** It was performed by using TA Instruments Q500 analyser. The average sample size was 5.3 mg and was placed in a platinum container. The analysis was done in the range of 30 °C –700 °C with heating rate of 10 °C/min, in nitrogen atmosphere. The flow rate of nitrogen was 20.0 ml/min. Sample was prepared by weighing 10 mg of tablet fines and was spread evenly on the bottom of crucible and it was covered with aluminum lid and with a hole already drilled in it to cover the crucible in order to prevent the material from spilling out of the crucible. (Dolatowska-Żebrowska *et al.*, 2019)

3.4.11 FTIR of milk protein based composite tablets

FTIR analysis was done at the Department of Chemistry, Institute of Science, BHU. FTIR analysis of tablets were done using FTIR spectrophotometer model Perkin Elmrt Spectrum Version 10.4.3 (Perkin Elmer Co., MA, USA) using the KBr disc method.

Sample preparation – 300 mg of dried IR- Grade KBr was weighed and placed in a marble mortar and pestle and was ground to completely powder it. 3 mg of tablets was placed in the marble mortar and pestle with the KBr and ground for 60 seconds to thoroughly mix it. The KBr- sample mixture was placed into an evaluable die on a hydraulic press and was press in vacuum for more than 5 KN pressure. The pressure was released, the die was removed from the press and disassembled and the KBr pellet was removed. The KBr pellet was placed in a pellet holder and put it into sample beam of the IR. (Markoska *et al.*, 2019)

3.5 Proximate Analysis

3.5.1 Moisture

For the measurement of moisture content, five grams of sample was crushed to powder and taken in petri dish. The petri dish was previously dried and weighed. Accurately weighed 5g sample was taken in petri dish, which was previously dried and weighed. The moisture cup along with sample was placed in the oven maintained at 105 ±2 °C for 3- 4 hours, by repeating the process of drying, cooling and weighing at 30 min intervals, until the difference between consecutive weights was less than 1 mg then it was transferred to desiccator, cooled and weighed. Moisture content calculated from the following formula

$$Moisture\ content\ \% = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

W1= weight of empty petri dish

W2 weight of petri dish +sample before drying

W3 = weight of petri dish + sample after drying

3.5.2 Fat AOAC

The fat contents of the developed antacid tablets were determined by Mojonnier extraction method (IS: 18, Part-XI, 1981) as follow:

A 3 g sample (previously grinded) was taken in to a Mojonnier fat extraction tube. 1 ml concentrated ammonia solution (Sp.gr. 0.88) was added in each tube and mixed properly. Then 10 ml ethyl alcohol (95-96% w/w) was added. For sample, 1 g of antacid tablet powder were taken in 50ml beakers. 9 ml of 0.5 % (w/v) sodium chloride solution were added and swirled gently to disperse the sample. The mixture was transferred into Mojonnier fat extraction tube with 10 ml ethyl alcohol (95-96% w/w) and mixed well. 25 ml diethyl ether (Sp.gr. 0.72) was added through the beaker used for weighing sample and the tube was tightly closed with a bark cork and was vigorously shaken for 1 min 25 ml of light petroleum ether (boiling point, 40-600 C) was then added in the tube and the contents mixed vigorously for 1 min. The tube was allowed to stand for not less than 30 min. The ether layer was carefully decanted into a previously dried, cooled and weighed conical flask. The extraction and decantation step was repeated twice by using 15 ml each of diethyl ether and petroleum ether. The solvent was first evaporated on a hot plate and the residual fat was dried in the hot air oven at 102± 2ºC for 1 h. The flasks were cooled in a desiccator. Drying, cooling and weighing were repeated until successive weight did not vary by more than 1 mg. A blank was run simultaneously using distilled water in place of milk. The fat content was calculated by deducting the blank value.

3.5.3 Protein (IS: 18, Part-XI, 1981)

Protein content of antacid tablets was determined by Kjeldahl method-

Digestion:

Tablets weighing 1.5 g was digested in 20 ml of concentrated H_2SO_4 and 1 g digestion mixture which contained potassium sulphate and mercuric oxide in 10:0.5 ratio. Digestion was done for 2-3 h at 400°C till a clear solution was obtained.

Distillation:

The digested sample was transferred to volumetric flask and the volume was made up to 100ml with distilled water. 10 ml from the diluted flask was taken into 500 ml Kjeldahl flask and was assembled in distillation unit. Through an opening on the top of distillation assembly, 8 ml of sodium hydroxide-sodium thiosulphate solution was added slowly into the Kjeldahl flask. Steam was started after closing the opening. 50 ml conical flask containing 10ml of saturated boric acid with 2-3 drops of mixed indicator, was placed for collection of distillate. About 25 ml of distillate was collected.

Titration:

Titration of the distillate collected was done with 0.02 N HCL till appearance of violet colour. The titre volume for distillate and blank was recorded.

The protein content was calculated as follows:

$$\text{Protein (\%)} = \frac{X \times 0.14 \times V \times 6.25 \times 100}{1000 \times V1 \times W}$$

Where,

X= Volume of sulphuric acid consumed for titration after nullyfying with the titre value of blank sample

V= Total volume digest

V1= Volume of digest for distillation

W= Weight of sample for digestion

3.5.4 Carbohydrate

Carbohydrate content of the sample was determined by subtracting protein, fat and ash from total solids.

Carbohydrate = % Total solid - (% Protein + % Fat + % Ash)

3.5.5 Ash

The ash content of the milk-protein based antacid tablets was determined by using the BIS method (IS: SP: 18 -Part XI – 1981) as follows:

Tablets weighing four to five gram were taken and were finely ground. Silica disc which was previously ignited, was kept in desiccator for cooling. Tablet fines were transferred to this silica disc and weighed. The crucible was kept in muffle furnace at 550±10°C for 3-4 hours until the ash was free from carbon. The silica disc was kept in a desiccator and was weighed. Ash content was calculated from the following-

$$\% \text{ Ash} = \frac{Weight\ of\ residue}{Weight\ of\ sample} \times 100$$

3.6 Statistical Analysis

All the analysis were done in triplicate. The data of the analysis were pooled and averaged and the mean and the standard error were calculated using MS Excel Software. Experiments were laid out in a completely randomized block design with three replications. Data on ANC, friability, disintegration time, bulk density, and hardness were subjected to Analysis of Variance (one way ANOVA). Difference of $p<0.05$ were considered significant. For computation of data, software application programmes like Microsoft Excel was used.

Chapter IV

RESULTS AND DISCUSSION

The present study entitled "**STUDY ON MILK PROTEIN BASED COMPOSITE ANTACID TABLETS**" was carried out at the Department of Dairy Science and Food Technology, IAS, BHU. The milk based composite antacid tablets were prepared using milk protein namely casein, whey, aloe vera and papaya. The manufacturing of the tablets was carried out at Department of Pharmaceutical Engineering and Technology, IIT BHU. Composite antacid based on milk protein were developed by wet granulation method. Four different formulation with different amount of papaya, aloe vera, and milk protein was produced, with mannitol as filler material, starch as granulation aid and magnesium stearate as lubricating agent. Acid neutralising capacity of the developed antacid were considered for optimization of tablets.

4.1 Nutritional labelling of the ingredients used for composite antacid tablets-

Table 4.1 (a): Nutritional Labelling of Casein

Values based on	Per 100 g
Calories (Kcal)	372
Total Fat(g)	1.4
Cholesterol (mg)	28.4
Sodium (mg)	156
Protein (g)	85
Carbohydrates(g)	4
Calcium(mg)	2600

Table 4.1 (b): Nutritional Labelling of Whey

Value based	Per 100 g
Calories (Cal)	396
Protein (g)	80
Total fat (g)	4.9

Table 4.1 (c): Nutritional Labelling of Papaya powder

Value based on	Per 100 g
Calories	43
Total fat (g)	0.3
Saturated fat (g)	0.1
Sodium (mg)	8
Total Carbohydrate (g)	11
Dietary Fibre (g)	1.7
Sugars (g)	7.8
Protein (g)	0.5

Table 4.1 (d): Nutritional Labelling of Aloe vera powder

Value based	Per 100g
Calories (Cal)	166.5
Sodium (mg)	999
Carbohydrate(mg)	33.3
Sugar	16.65

4.1 Acid Neutralizing Capacity (USP 23)

The acid neutralizing capacity of milk protein based composite antacid tablets was measured for the all four formulation. Aloe vera based antacid tablets were found to have maximum acid neutralizing capacity with 5.23±0.05 mEq, followed by control with 4.63±0.050 mEq, PA with 2.33±0.05 and papaya based antacid tablet with lowest acid neutralizing capacity with 2.10±0.05 mEq. ANC results were analysed by ANOVA and according to the ANOVA results there is significant difference between acid neutralizing capacities of all the four formulations. Gandhi *et al.* (2011) formulated and developed orodispersible antacid tablets for geriatric patient, whose acid neutralising capacity ranged from 5.33-10.47. Prajapati *et al.* (2012) formulated raft forming chewable tablets of H_2 antagonist (Famotidine) using a raft-forming agent along with an antacid- and gas-generating agent. The acid neutralizing capacity of these tablets ranged from 6.7-9.2 mEq. Yafout *et al.* (2022) evaluated the acid neutralizing capacity of twelve antacid tablets, effervescent and oral suspensions, marketed in Morocco and the ANC ranged from 6.50-49.85 mEq of acid consumed. On the basis of acid neutralizing capacity, aloe vera based antacid was selected as optimized tablets since it has the highest acid neutralizing capacity.

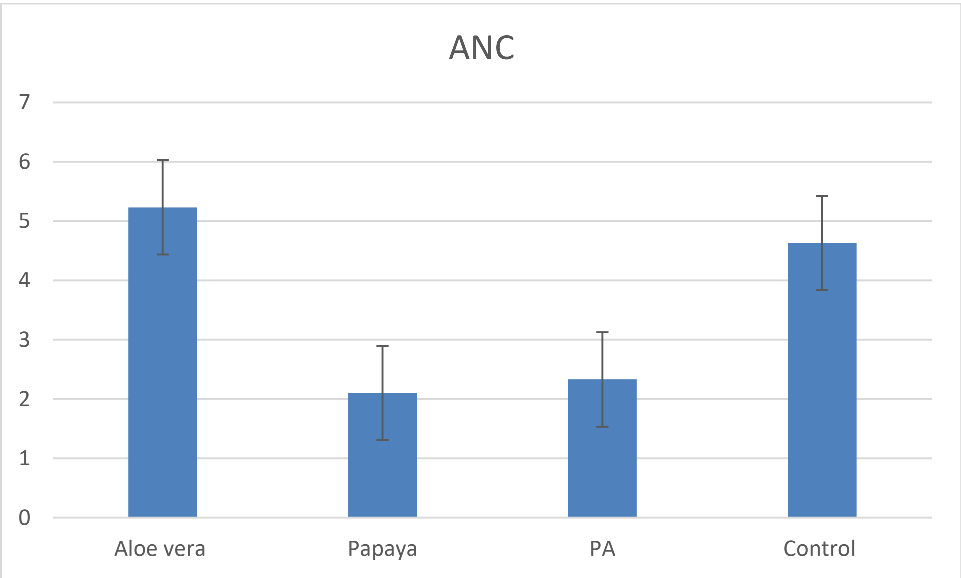

Figure 4.1: ANC of different tablets

4.2 Bulk density

Bulk density of the developed antacid varied in between 1.25±0.00 to 1.25±0.00g/cm³. Aloe vera based tablets had the highest the highest bulk density 1.46±0.00 g/cm³, followed by Papaya based tablets 1.30±0.00 g/cm³ and control tablets 1.30±0.00 g/cm³. PA tablets had the lowest bulk density, 1.25±0.00 g/cm³. Bulk density of all the four formulation was analysed by ANOVA, significant difference was observed. Thesiya *et al.* (2018) analysed the bulk density of milk protein based antacid tablets, the tablet density ranged from 0.92 to 1 g/cm³ with a mean value of 1.06 g/cm³. Soni *et al.* (2016) reported that the raft-forming chewable tablet density ranged from 0.39 to 0.46 g/cm³.

4.3 Weight variation test

Tablets of all the formulation were measured and the weight ranged between 0.32±0.00 g to 0.38±0.0 g. Tablet weight ranges of raft-forming chewable tablet from 0.996 to 0.999 g were reported by Soni *et al.* (2016). Thesiya *et al.* (2018) developed milk protein based antacid tablets and the weight of the antacid tablets ranged from 0.88 g to 1.1 g with a mean value of 1.05 g.

4.4 Tablet dimension

Tablet dimension of the tablets developed are measured in mm with Vernier calliper. Tablet diameter varied from 10.07±0.01mm to 10.20±0.01 mm and thickness from 3.59±0.06mm to 4.47±0.06mm. The variation is due to the changes in pressure of the compression machine. The variation among twenty tablets of all the four formulation, was less than 7%.

4.5 Friability

Friability of the pharmaceutical product is done to check the physical stability of the tablets during packaging, handling and transportation. Friability of the aloe vera, papaya and PA tablets was found to be 2% and the control tablet had a friability of 9%. Friability of the control tablets were highest indicating that aloe vera and papaya contributed significantly to the friability of the composite tablets.

Table 4.2: Result of analysis of different milk protein based composite antacid tablets

	ANC (mEq)	Bulk Density (g/cm³)	Weight (g)	Diameter (mm)	Thickness (mm)	Friability (%)	Disintegration time (minute)	Hardness (kg/cm²)	CDI
Aloe vera	5.23±0.05	1.46±0.00	0.36±0.00	10.07±0.01	4.00±0.06	0.02± 0.00	54.33±1.35	2.5±0.37	11.93±1.4
Papaya	2.10±0.05	1.39±0.00	0.33±0.00	10.14±0.01	3.59±0.06	0.02± 0.00	46.66±1.35	4.0±0.37	14.29±1.4
PA	2.33±0.05	1.25±0.00	0.32±0.00	10.18±0.01	3.64±0.06	0.02± 0.00	48.00±1.35	3.0±0.37	10.91±1.4
Control	4.63±0.05	1.30±0.00	0.38±0.00	10.20±0.01	4.47±0.06	0.09± 0.00	47.66±1.35	2.0±0.37	8.93±1.4

Data as presented as mean±standard error

Friability of orodispersible antacid tablets for geriatric patient ranged from 0.55%-1.14% (Gandhi *et al.*, 2011). Aloe vera herbal suppositories formulated by Tarkase *et al*. 2015, reported friability within the range of 37-.43%.

4.6 Disintegration time test

Disintegration time of the developed antacid ranged between 46.66±1.35 minutes to 54.33±1.35 minutes, which was comparable to commercially available antacids. The minimum disintegration time was for papaya based tablets with 46.66±1.35 minutes and maximum disintegration time was for Aloe vera based antacid, 54.33±1.35 minutes. Disintegration time for PA and control tablets were 48.00±1.35 minutes and 47.66±1.35 minutes, respectively. Disintegration time of all the four formulation was subjected to analysis by ANOVA. Significant difference was seen between all the four formulations. Disintegration time of five commercially available antacid was evaluated by Nyamweya *et al.* (2020), which ranged from 9 to over 60 min.

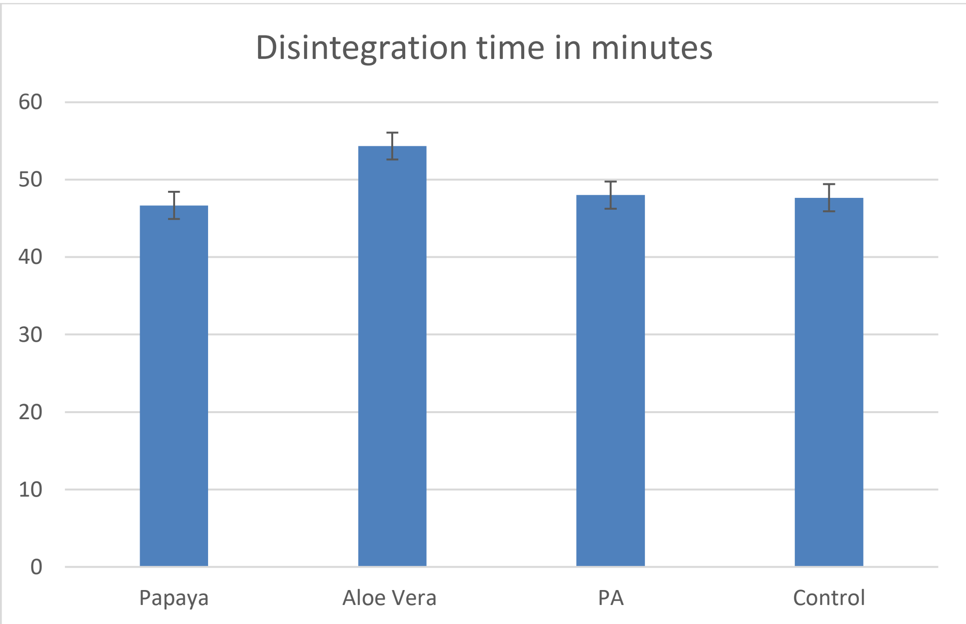

Figure 4.2: Disintegration time of milk protein based composite tablets

4.7 Hardness test

Hardness of the developed tablets ranged from 2.5±0.37 kg/cm² to 4.0±0.37 kg/cm². Papaya based hardest with hardness value of 4.0±0.37 kg/cm², followed by PA, 3.0±0.37 kg/cm² and aloe vera based tablets2.5±0.37 kg/cm². Lowest hardness was found in control tablets 2.5±0.37 kg/cm². ANOVA was used to analyse the hardness of all the four formulation and significant difference between them was observed. Gandhi *et al*. (2011) formulated and developed orodispersible antacid tablets for geriatric patient, whose hardness ranged from 4.3-5 kg/cm². Natvaratat *et al.* (2007) developed high nutritive milk tablets with 52% DEY, 55% milk powder, 15% sugar, 0.4% cab-o-sil, 1% talcum, 3.5% comprecel and 0.1% strawberry flavour whose hardness ranged from 1.86- 3.89 kg/cm². Tarkase *et al.* (2015) formulated herbal suppositories by heat molding from Aloe vera with hardness value ranging from 2.42- 3.01 kg/cm².

4.8 Chewing Difficulty Index (CDI)

Chewing Difficulty Index was measured for all the four formulation to check the ease of chewing of the tablets. . The CDI of the developed tablets were found to be optimal in the range of 8.93±1.4 to 14.29±1.4. Lower CDI indicates ease of chewing. Control tablets had the lowest CDI of 8.93±1.4 and hence the easiest to chew among all the four formulation. . It can be inferred from CDI that tablets with more hardness will be difficult to chew. ANOVA of Chewing difficult index showed no significant difference. Similarly, Nyamweya *et al.* (2020) also evaluated that the chewing difficulty index of commercially available chewable antacid tablets.The CDI of the tablets varied between 8.2-23.9.

4.9 Sensory evaluation

The sensory analysis of milk protein based composite antacid tablets was done using a 9-point hedonic scale. Tablets shows the sensory score of all the four formulation with different ration of aloe vera, papaya and milk protein.

A semi-trained panel of judges assessed the sensory quality of milk protein based composite antacid tablets. Teachers and students of the department of Dairy Science and Food Technology, IAS, BHU evaluated the composite antacid tablets.

Body and Texture, Flavour, Colour and appearance and Overall acceptability were assessed as sensory criteria.

Table 4.3: Sensory score of the developed tablets

	Body and texture	**Flavour**	**Colour and appearance**	**Overall Acceptability**
Aloe vera	7.5±0.40	**7**±0.40	7.6±0.47	7.3±0.62
Papaya	7.5±0.40	7.3±0.2	8.16±0.05	7.6±0.22
PA	7.06±0.09	7.06±0.09	7.3±0.22	7.06±0.09
Control	7.5±0.40	7.5±0.40	7.3±0.47	7.8±0.23

Data as presented as mean ± SD (n=10)

Body and texture/ Chewability – From the Table 4.3, it can be observed that the minimum score was for Body and texture/ Chewability was recorded for PA tablet 7.06±0.09. All other three formulations had similar score which indicates the use of similar amount of filler and lubricating agents with use of similar pressure for preparation of the tablets.

Flavour – From the sensory table, the flavour score was highest for control tablets with a sensory score of 7.06±0.09 with lowest score for aloe vera based antacid tablets **7**±0.40. The reason for high score of flavour of control was may be due to plain flavour of milk protein and lower score of aloe vera tablets was due to bitter flavour of aloe vera.

Colour and appearance- Sensory score for colour and appearance was highest for papaya based antacid 8.16±0.05 due to the colour imparted to the tablets by papaya and lowest for

Overall Acceptability- Overall acceptability of all the tablets was in the range of 'Like moderately' to 'Like Very Much'.

4.10 Thermo gravimetric analysis-

For the analysis of thermal stability of tablets, change in weight was measured as a function of increasing temperature.

TGA curve for the aloe vera bases antacid tablets in the figure 4.3(a), showed two region of weight loss in temperature region of 30° C- 200° C with 10.637 %weight loss, due to loss of physically adsorbed and hydrogen bonded water molecules. The second region in the temperature range of 200° C- 694° C with around additional 64.042 % of weight loss due to thermal degradation of aloe vera and carbonization of material. TGA curve of the papaya based antacid tablets in figure 4.3(b), showed four regions of weight loss in the 30° C - 150° C with weight loss of 8.284 %, this drop of mass can be assumed as water loss during the temperature rise. Second region being in the 150° C - 250° C with 17.174 %, third region in the range of 250° C -370 ° C with additional weight loss 32.561 % and final temperature range of 370 °C- 690° C with further weight loss of 15.557%. TGA curve for PA tablets showed in figure 4.3 (c), three region of weight loss the 30° C - 150° C, 150° C -250° C, and 250°- 690° C with following % weight loss 7.902 %, 12.549 %, and 51.875% was observed respectively.

Control tablets made up of only milk protein showed a TGA curve in the figure 4.3 (d), with three regions of weight loss with the range of temperature being same but the weight loss different from the TGA curve of PA tablets. The weight loss was 6.083 %, 5.925 %, and 65.310 %respectively. The first stage, observed up to 30° C - 150° C, was related to the loss of adsorbed and bound water. The degradation of pure system occur at 250° C, suggesting different primary and secondary structure of milk protein do not significantly affect the thermal degradation process in nitrogen atmosphere.

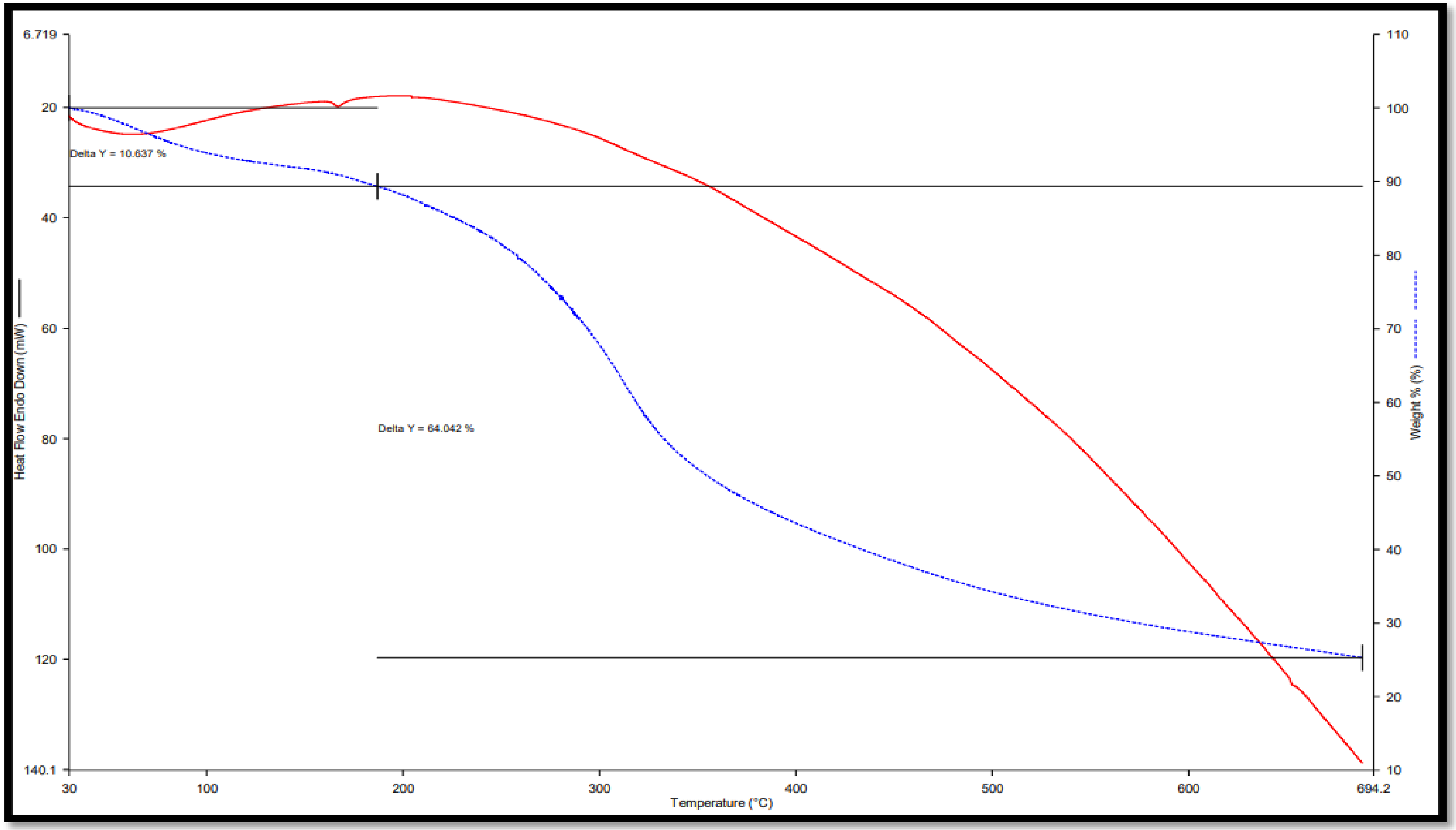

Figure 4.3 (a): TGA graph of aloe vera based antacid tablet

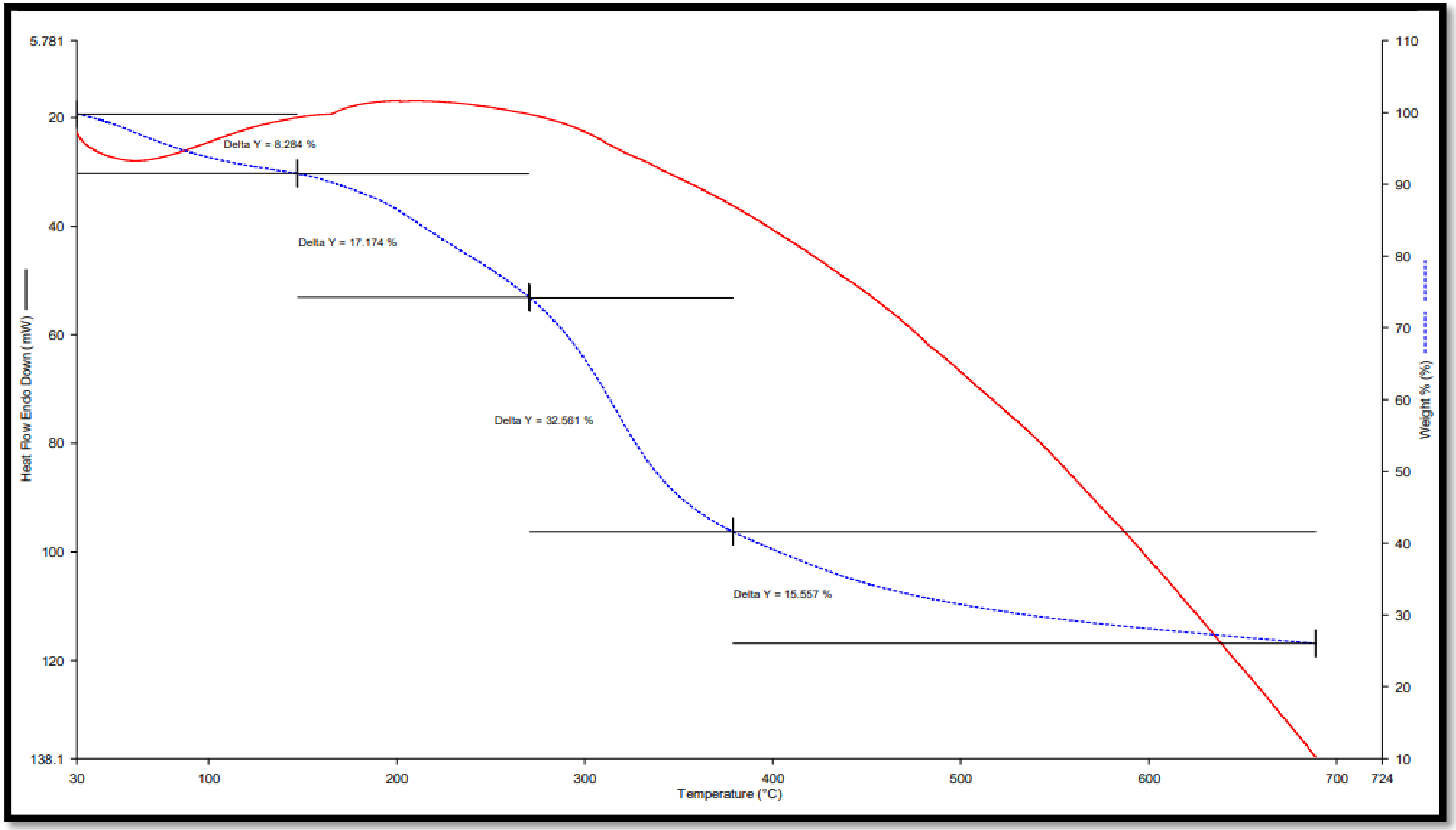

Figure 4.3 (b): TGA graph of Papaya based antacid tablets

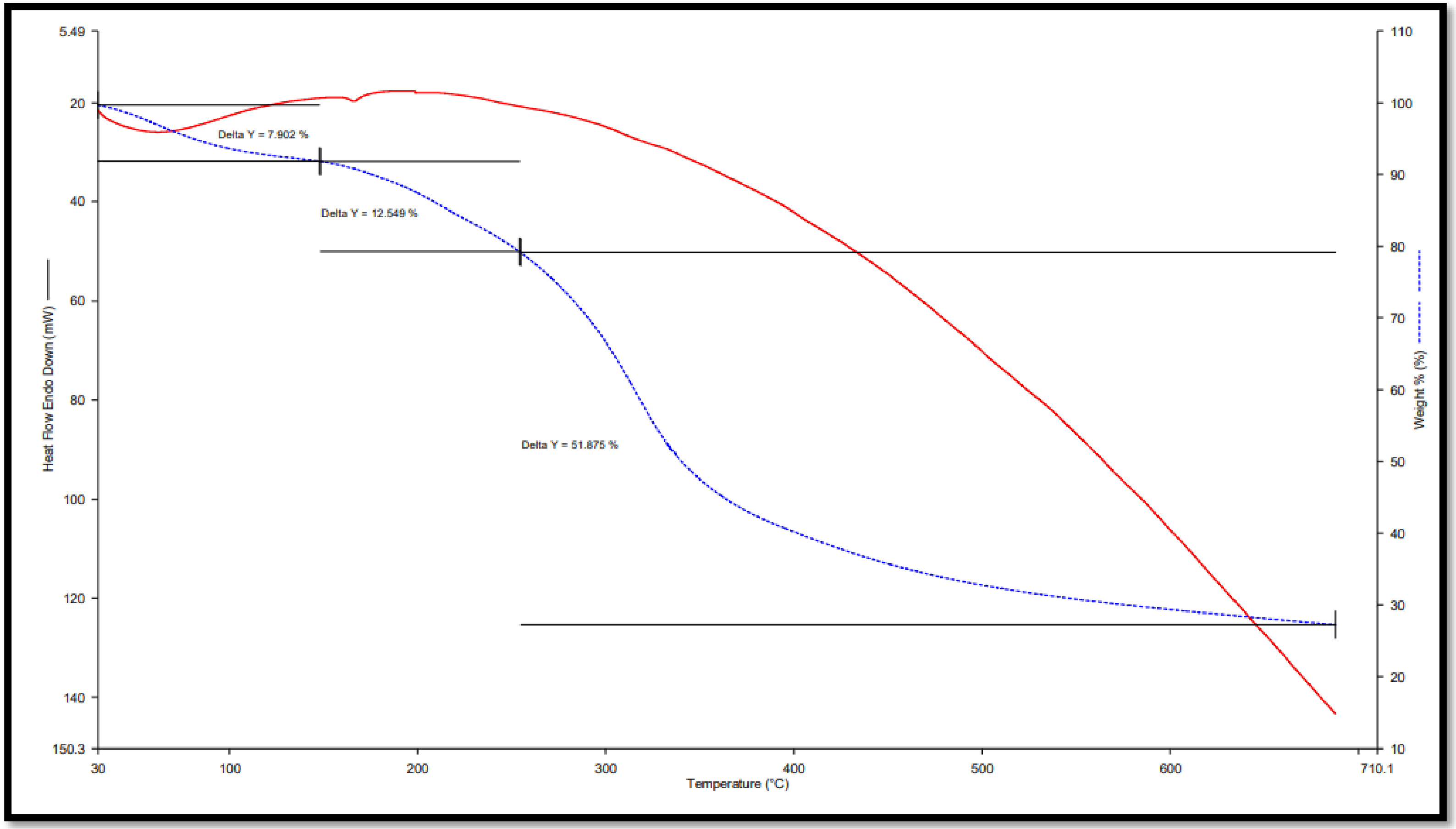

Figure 4.3 (c) : TGA graph for PA composite antacid tablets

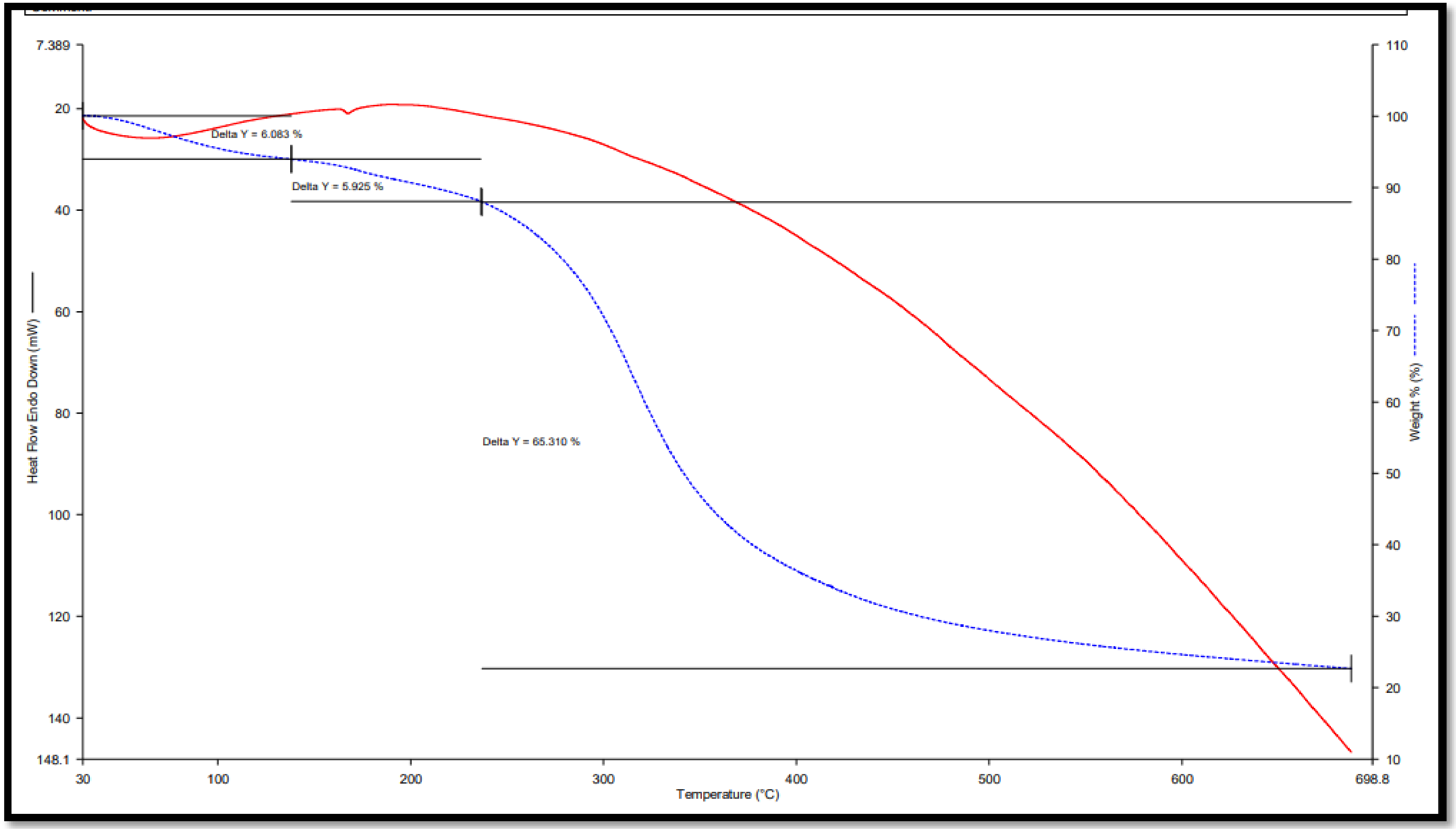

Figure 4.3 (d): TGA curve of control tablets

4.11 FTIR of milk protein based composite antacid tablets

The characterization of milk protein based composite antacid tablets was done using FTIR. All the four formulation were analysed by FTIR. From the FTIR graph for aloe vera given in figure 4.4 (a), it can be inferred that peaks in the range of 995-985 cm^{-1}showed C=C bending with alkene being the principle functional group. Peaks in the range of 2830-2695 cm^{-1}, represents C-H stretching, indicating aldehyde group. C-H stretching in the peak range of 2840-3000, represents presence of alkane, suggesting scission of the main protein chain The transmittance peak in the range of 3100-3000 cm^{-1}representing presence of alkene, showing stretching C=C in vinyl ether and aloin components.

The FTIR of papaya based antacid tablets in the figure 4.4 (b), showed a similar peaks as in Aloe vera based, in the region of 985-995 cm^{-1}, 2695-2830 cm^{-1}, 2840-3000 cm^{-1}, 3000-3100 cm^{-1}, representing alkene, aldehyde, alkane and alkene compound, respectively. Composite tablets containing papaya, aloe vera and milk protein in the figure 4.4 (c), showed a slight difference peaks in the range 2695-2830 cm^{-1} indicating aldehyde group with H–C=O: C–H stretch, =C–H stretch in the range of 3000-3100 cm^{-1} and a O–H stretch in the range of 500-3300 cm^{-1} indicating presence of carboxylic. The FTIR graph of control tablets in the figure 4.4 (d), showed peak at 2349 cm^{-1}, representing O=C=O stretching, indicating presence of carbon dioxide.

Table 4.4(a): Functional Group present in Aloe vera tablets

Range cm^{-1}	Wavelength cm^{-1}	Appearance	Bond	Functional Compound
995-985	991	Strong	C=C bending	Alkene
2830-2695	2811	Medium	C-H stretching	Aldehyde
3000-2840	2919	Medium	C-H stretching	Alkane
3100-3000	3096	Medium	C-H stretching	Alkene

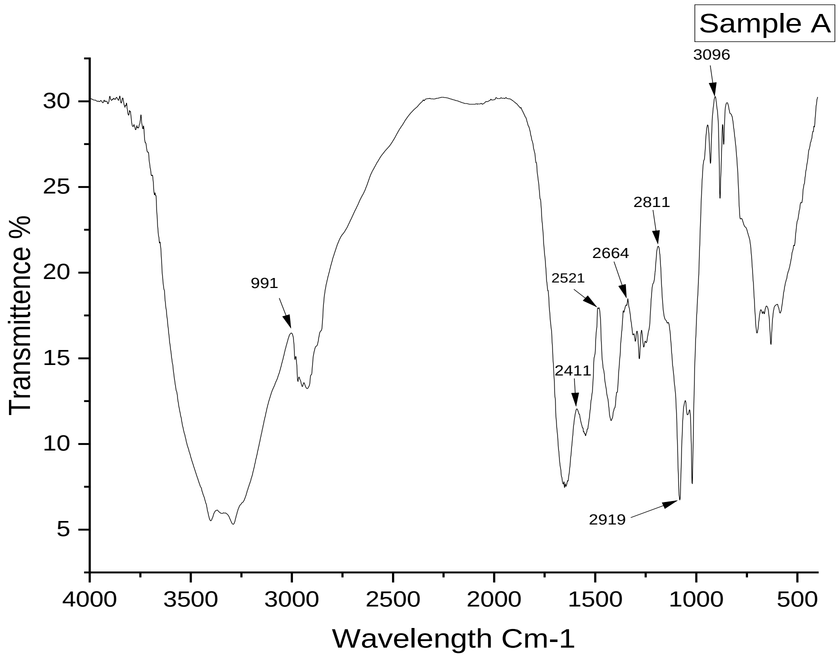

Figure 4.4 (a): FTIR curve of Aloe vera based antacid tablets

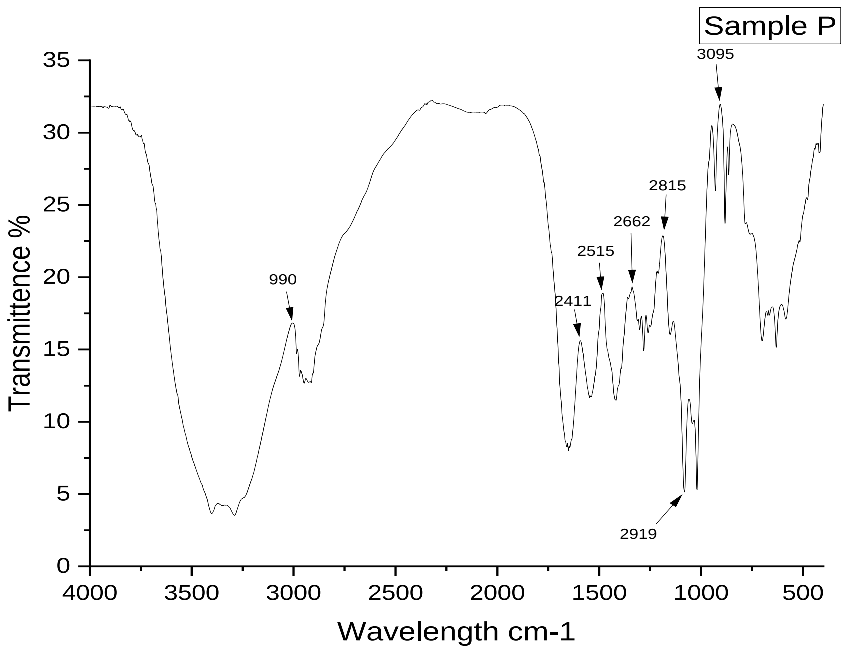

Figure 4.4 (b): FTIR curve of Papaya based antacid tablets

Table 4.4(b): Functional group present on Papaya Tablets

Range cm^{-1}	Wavelength cm^{-1}	Appearance	Bond	Functional Compound
995-985	990	Strong	C=C bending	Alkene
2830-2695	2815	Medium	C-H stretch	Aldehyde
3000-2840	2919	Medium	C-H stretch	Alkane
3100-3000	3095	Medium	C-H stretch	Alkene

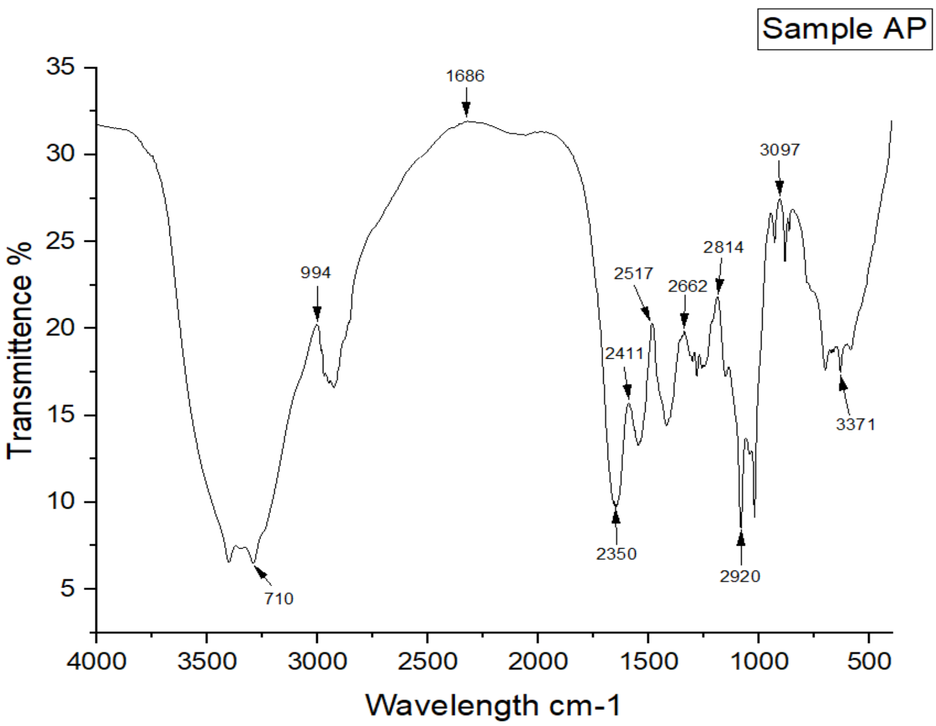

Figure 4.4(c): FTIR curve of PA composite antacid tablets

Table 4.4(c): Functional group present in PA tablets

Range cm^{-1}	Wavelength cm^{-1}	Appearance	Bond	Functional Compound
2830–2695	2814	Medium	H–C=O: C–H stretch	Aldehydes
3000–2850	2920	Medium	C–H stretch	Alkanes
3100–3000	3097	Medium	=C–H stretch	Alkenes
3300–2500	3371	Medium	O–H stretch	carboxylic acids

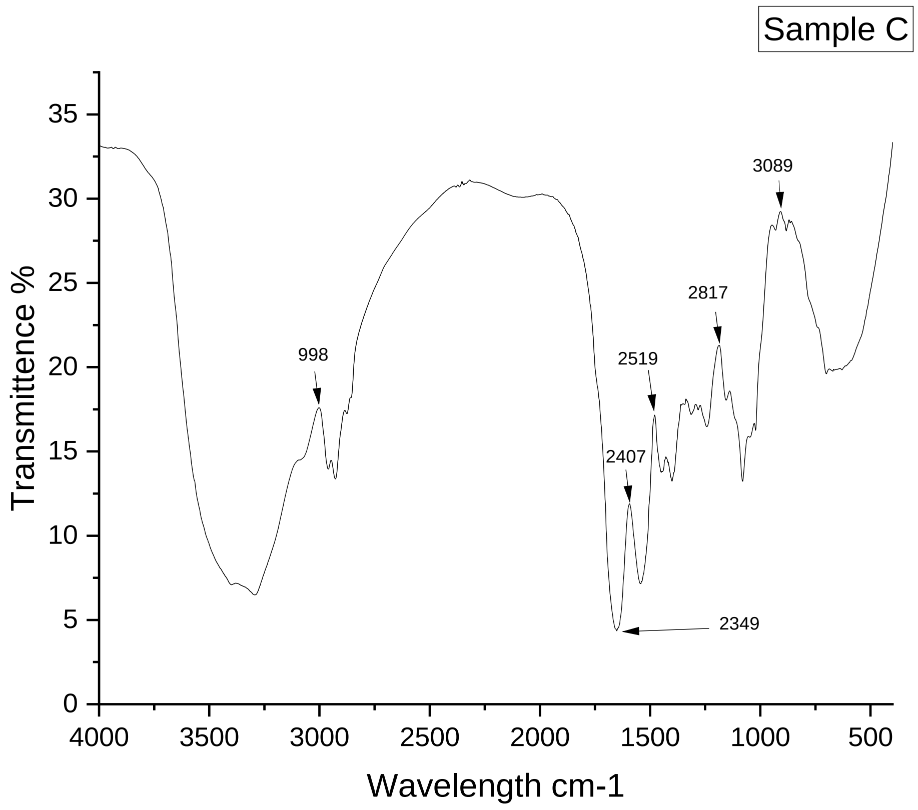

Figure 4.4(d): FTIR curve of control tablets

Table 4.4(d): Functional group in Control tablets

Range cm^{-1}	Wavelength cm^{-1}	Appearance	Bond	Functional Compound
995-985	998	Strong	C=C bending	Alkene
2349	2349	Strong	O=C=O stretching	Carbon dioxide
2830-2695	2817	Medium	C-H stretching	Aldehyde
3100-3000	3089	Medium	C-H stretching	Alkene

4.12 Proximate Analysis of Optimized antacid tablets

Proximate analysis of the optimized tablet and control tablet was done. Moisture, total solids, fat, protein and ash contents were determined using standard methods. Proximate analysis of milk protein based antacid by Thesiya *et al.* (2018) reported antacid tablet with Moisture (%w/w) 4.76±0.16, Fat (%w/w) 2.26±0.02, Protein (%w/w), 61.33±0.42, Ash (%w/w) 12.51±0.10, and Carbohydrate (% w/w) 18.85. As milk protein is used as ingredient, along with aloe vera, protein and carbohydrate from this would provide some amount of energy. Energy provided by the optimized and control tablets per 100 g were 289.46 Kcal and 308.24 Kcal respectively. Energy value per gram for optimized and control tablets are 2.89Kcal and 3.08 Kcal. The nutritional contribution of these tablets are apparently non-significant and the major reason for use of protein lies in partial substitution of the buffering salts and thus the developed tablets are more system friendly when compared with the commercially available antacid. Thesiya *et al.* (2018) reported the energy value of the milk protein antacid tablets 4.72 Kcal per unit of antacid tablet. The result of proximate analysis of aloe vera based and control tablets are given in table 4.5

Table 4.5: Proximate of Milk protein based Aloe vera and Control Tablets per 100 g

	Aloe vera	Control
Total fat	0.9 g	1.28 g
Protein	23.09 g	29.65 g
Carbohydrate	47.25 g	44.68 g
Moisture	0.19%	0.22%
Ash	3.25%	3.20%
Energy value(per 100 g)	289.46	308.24

4.13 Comparative analysis of Acid Neutralizing capacity of the optimized and control antacid tablets with marketed sample of antacid.

Optimized antacid tablet and control antacid tablets were compared with antacids available commercially. It can be seen from Table 4.6 that the commercially available antacid have better acid neutralizing capacity when compared to the developed composite antacid tablets but less chemical content of the developed antacid masks up for lower acid neutralizing capacity with decreased side effects on the body.

Table 4.6: ANC of the optimized and control antacid tablets with marketed sample of antacid

	Market sample-1	Market sample-2	Market sample-3	Market sample-4	Optimized tablet	Control tablet
ANC (mEq)	9.46	9.26	11.70	6.16	5.23	4.63

Malik *et al.*, 2014 compared milk and milk product with Antacid Pharmacological Preparations for buffering ability and milk product were found to have comparable buffering ability. Yafout *et al.* (2022) compared ANC antacids commercially available in Morocco and those were found to meet the minimum FDA requirement for acid neutralizing capacity.

Chapter VI

SUMMARY AND CONCLUSION

The present study entitled **STUDY ON MILK PROTEIN BASED COMPOSITE ANTACID TABLETS** has been prepared at the department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India and Department of Pharmaceutical Engineering and Technology, IIT BHU. The study was conducted in two phases namely optimization of the ingredients for milk protein based composite antacid tablets and study of the proximate and phytochemical composition of optimized tablets.

The buffering capacity of milk protein namely casein and whey protein, has been utilized in making of the tablets. Along with milk protein, papaya and aloe vera was used as ingredients because of their known antacid property. The result obtained are summarised below:

5.1 Manufacturing of milk protein based composite antacid tablets

Composite tablets were made by wet granulation method, using mannitol as filler and magnesium stearate as lubricating agent. Four different formulation were made with milk protein, papaya and aloe vera in different proportion.

5.2 Analysis of the developed antacid tablets

- Acid neutralizing capacity of all the four formulation was evaluated, out of which tablet based on aloe vera was found to have highest acid neutralizing capacity of 5.23±0.05 mEq of acid.
- Bulk density of all the tablets was between 1.25±0.00 to 1.46±0.00.
- Weight of the tablets was between 0.32±0.00g to 0.38±0.00 g.
- Tablet diameter varied from 10.07±0.01mm to 10.20±0.01mm and thickness from 3.59±0.06 to 4.47±0.06mm.
- Friability of the aloe vera based antacid tablet was 2% though the control tablet was highly friable with 9% friability.
- Disintegration time of tablets ranged from 46.66±1.35minutes to 54.33±1.35minutes.

- Hardness of the tablets ranged in between 2.0±0.37 kg/cm² to 4.0±0.37 kg/cm².
- Sensory evaluation of the tablets using 9 point hedonic scale was done which showed overall acceptability in the range of 7.06±0.09-7.8±.23 indicating like moderately.
- FTIR analysis of all four formulation was done for characterization of tablets.
- Thermogravimetric analysis was done to evaluate the thermal stability of the developed antacid tablets.

5.3 Optimization of milk protein based composite antacid

The principal factor for optimization of antacid tablets was acid neutralizing capacity and aloe vera based antacid was found to have highest acid neutralizing capacity of 5.23±0.05 mEq of acid.

5.4 Proximate analysis and energy value of developed antacid

Proximate analysis of optimized aloe vera based antacid tablet and control tablet was done and energy value of both the tablets per 100 gram were calculated. Aloe vera based tablet had 289.46 Kcal/100 g and for control tablet it was 308.24Kcal /100 g.

5.5 Comparative analysis of developed antacid with commercial sample

Acid neutralizing capacity of commercially available antacid tablets in market was compared with developed milk protein based composite antacid tablets. Acid neutralizing capacity of market sample were found to be superior to the developed antacids.

Conclusion-

From the above, it can be concluded that antacid tablets developed from milk protein and aloe vera can be used as antacid, with less chemical content and hence possess less side effects on the body as compared to commercially available antacid. Along with the antacid effect, it can act as source of protein and hence a potential nutraceutical. It has a potential anti ulcerative property due to use of ingredients such as aloe vera and whey protein. The developed antacid meets the FDA criteria for acid neutralizing capacity and hence can be used as antacid. Recommendation for further studies in the development of antacid can include the following-

- Study of Shelf life of tablets with accelerated shelf life study and activation energy.
- In-vivo study of the tablets for further investigation.
- Study on use of some GRAS preservative to extend the shelf life of tablets.

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Appendix- I

Score Card for Sensory Evaluation of Milk protein based composite Antacid Tablets

Date: _________

Kindly evaluate the given sample of Antacid Tablets using the following 9-point hedonic scale and enter the score for given samples in the space provided in the table below-

Rating	**Score**
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Sensory Attributes	**Sample No**			
	1	**2**	**3**	**4**
Body & Texture/ Chewability				
Flavour				
Colour & Appearance				
Overall acceptability				

Remarks (if any):

Signature: ____________________

Name: ______________________

Appendix-II

Friability ANOVA

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups		3	0.003025	639.7159	7.3E-10	4.066181
Within Groups	3.78E-05	8	4.73E-06			
Total	0.009114	11				

Disintegration time ANOVA

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	109.6667	3	36.55556	0.937322	0.466468	4.066181
Within Groups	312	8	39			
Total	421.6667	11				

Hardness ANOVA

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.3125	3	2.104167	6.636005	0.006827	3.490295
Within Groups	3.805	12	0.317083			
Total	10.1175	15				

Bulk Density ANOVA

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.079425	3	0.026475	64.83673	5.9E-06	4.066181
Within Groups	0.003267	8	0.000408			
Total	0.082692	11				

Acid Neutralizing Capacity ANOVA

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22.7625	3	7.5875	758.75	3.7E-10	4.066181
Within Groups	0.08	8	0.01			
Total	22.8425	11				

Chewing Difficulty Index

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	42.1742	3	14.05807	3.007539	0.072335	3.490295
Within Groups	56.0913	12	4.674275			
Total	98.2655	15				