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Original Research Article

In vitro evaluation of raft-forming and non-raft forming antacids marketed in India

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ABSTRACT

Background: Raft properties of alginate-antacids may vary based on their formulation. This study compared the physicochemical characteristics such as raft properties of Digeraft Plus® versus four other raft-forming (brands A to D) and acid neutralizing properties of raft-forming antacids versus two non-raft forming (brands E, F) antacids marketed in India.

Methods: The study assessed acid neutralizing capacity (ANC), and acid neutralizing potential (ANP) of the raft and non-raft forming antacids and the effect of raft structure on neutralization profile, raft strength, and raft resilience of the raft-forming antacids. Sodium alginate content of the raft-forming antacids was measured by a validated high-performance liquid chromatography (HPLC) assay.

Results: The raft-forming formulations were able to neutralize acid better than non-raft forming formulations. Total duration of acid neutralization of Digeraft Plus[®] was found to be 72 ± 5.33 mins while for the other raft as well as non-raft forming antacid products, it was in the range of 40 to 60 mins. Additionally, it also had raft strength of around 20.5 gm which was significantly higher than other raft-forming brands (p<0.05) indicating enhanced raft integrity and retention. This performance correlates with its sodium alginate content, which was greater than 90%.

Conclusions: Results indicate that minor differences in formulation can affect the raft properties, and consequently, its in-vivo clinical efficacy. The strength and resilience of the rafts varied between the different alginate-based antacids available in the Indian market. Compared to other antacids, Digeraft Plus® had greater ANP, raft strength, and raft resilience.

Keywords: Acid neutralization, Antacids, Digeraft Plus®, Gastroesophageal reflux disease, Rafts, Sodium alginate

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic upper gastrointestinal disease in which stomach content persistently and regularly flows up into the esophagus, resulting in symptoms such as dysphagia, heartburn, regurgitation, and non-cardiac chest pain.^{1,2}

Antacids, including alginate-based raft-forming formulations are commonly used for symptom relief of gastroesophageal reflux disease.^{3,4} Alginate-based raft-

forming formulations work through a unique non-systemic mechanism by forming a protective gel or raft in the stomach.^{5,6} In the presence of gastric acid, the alginate precipitates to form an insoluble alginic acid gel, while the bicarbonate salt gets converted to carbon dioxide, which gets entrapped within the alginate gel matrix, giving it the buoyancy to float over the gastric contents, thus forming a raft.⁷ The divalent calcium ions discharged from calcium carbonate react with alginic acid to increase raft strength.⁶ Rafts formed by alginate/antacid anti-reflux preparations are expected to be cohesive, buoyant, voluminous,

resistant to reflux into the oesophagus, and not easily broken up by movement in the stomach.^{8,9} Calcium carbonate-based antacids have been shown to create stronger and more porous rafts compared to aluminium or magnesium-based antacids. The amount of sodium alginate as well as the other ingredients such as gasforming or gel-forming agents found in the formulation exert a significant effect on raft formation and its mechanical properties, and consequently the in vivo performance.^{6,10}

This study aimed to evaluate the physicochemical characteristics of Digeraft Plus® (Abbott India Ltd.) as an indicator of its in-vivo performance and compare the raft properties with four other raft-forming formulations having similar sodium alginate content and the acid neutralization properties with two non-raft forming formulations marketed in India.

METHODS

This in-vitro study was conducted in accordance with lab standard operating procedures and in compliance with protocol at the Pharmaceutics Department of the Bombay College of Pharmacy-Autonomous, Mumbai, India (6 months).

The raft-forming products were Digeraft Plus® and other four products were labelled as brand A, brand B, brand C and brand D. The two non-raft forming products were labelled as Brand E and Brand F. They were procured from local medical stores and stored as per recommended storage conditions. The composition of each product is depicted in Table 1. Three bottles of same manufacturing date and manufacturing batch number were procured for each product. All evaluations were carried out in triplicates.

Table 1: Products with the composition.

Product		Content (as per label)	
Raft-	Digeraft Plus®	Each 10 ml contains Sodium alginate- 500 mg Sodium bicarbonate- 213 mg Calcium carbonate- 325 mg	
forming	A	Each 5 ml contains	
formulation	В	Sodium alginate- 250 mg	
	С	Sodium bicarbonate- 133.5	
	D	mg Calcium carbonate- 80 mg	
Non-raft- forming	E	Each 5 ml contains Aluminium hydroxide- 291 mg Magnesium hydroxide- 98 mg Oxetacaine- 10 mg	
formulation	F	Each 5 ml contains Magaldrate- 540 mg Simethicone- 50 mg Oxetacaine- 10 mg	

Simulated gastric fluid (SGF) made up of 1 mg/ml pepsin (HiMedia Laboratories Pvt. Ltd, Mumbai) and 2 mg/ml sodium chloride dissolved in 0.1 M hydrochloric acid (HCl, pH 1.0±0.02) was used for the assessments. To form a raft,10 ml of the raft-forming products, which is equivalent to 500 mg of sodium alginate, was added using a syringe (without attached needle) in 5-second periods to 150 ml of SGF (equilibrated to 37°C on a water bath for 20 minutes). The formed rafts were allowed to mature by maintaining them at 37°C for 30 minutes in the water bath. These matured rafts were used for assessing ANC and ANP.

When the rafts were formed within seconds of the addition of the suspension to SGF, the speed of raft formation was considered to be immediate and their floatation was complete. While the formation speed was slow when the products required 8-10 minutes to form rafts. In the case of products where some part of the raft floated on the surface of SGF while the remaining part sank to the bottom of the beaker throughout the maturation period, the floatation time was considered moderate. When the raft forming antacid remained at the bottom of the beaker throughout the 30-minute raft maturation time, the floatation was considered to be incomplete. The six raft-forming products were classified into three groups average, good, and excellent for coherence based on the intactness of the rafts. ¹⁰

Neutralization studies

The capacity of antacids to neutralize acid and the duration of neutralization were assessed.

Raft-forming formulations

Acid neutralization capacity (ANC) of the rafts was measured to understand their ability to act as a reservoir of antacid within its structure.6 This method was adapted from previously published methods. 10,11 Mature rafts were given a series of washing: thrice with 40 ml deionized water, thrice with 40 ml deionized water (4°C) and twice with 40 ml ethanol (4°C). Each time, the raft was suspended in the washing solvent for 3 minutes followed by mild vacuum filtration. The rafts were then oven-dried at 40°C to a constant weight and powdered. The powder was weighed and transferred to a 250 ml beaker to which 70 ml deionized water (37°C) was added. The beaker was placed on a water bath shaker (37°C and 100 rpm), 30 ml of 1 M HCl (37°C) was added after 1 minute, and the beaker remained on the shaker for another 15 minutes at 100 rpm. Then the contents were titrated against 0.5 M sodium hydroxide (NaOH) at a constant increment of 0.7 ml/min until pH 3.5 was reached. ANC was calculated using the following formula:

ANC (mEq) =
$$\frac{[V(ml) - T(ml)] \times 0.5 \times total \ mass \ of \ raft \ (mg)}{sample \ weight \ (mg)}$$

Where, V = volume of HCl added to the sample, T = volume of titer consumed by the sample, total mass of raft

= weight of dried raft, sample weight = weight of powder obtained after crushing the raft.

Non-raft forming formulations

The method used was adapted from a previously published method. ¹² The minimum labelled dose of the antacid was transferred to a 250 ml beaker to which deionized water was added up to a total volume of 70 ml. The solution was stirred on the magnetic stirrer for 1 minute, 30 ml of 1 M HCl was added, and it was further stirred for 15 minutes at 300 rpm. The contents were titrated against 0.5 M NaOH at a constant increment of 0.7-1 ml/min until a stable pH of 3.5 was reached.

ANC was calculated using the formula:

ANC (mEq) = $(30 \times N_{HCl}) - (V_{NaOH} \times N_{NaOH})$

Where N_{HCI} and N_{NaOH} are the normalities of HCl and NaOH respectively, and V_{NaOH} is the volume of NaOH added.

Neutralization profile

The method used was adapted from previously published methods for both raft-forming formulations, and non-raft forming formulations. ^{10,11,13}

Raft-forming formulations

The matured rafts were transferred into a Buchner funnel, mild vacuum filtration was applied to remove any excess SGF and pH was recorded. Thereafter, 3 ml of 0.04 M HCl (pH 1.4±0.05) was added to the raft, allowed to settle for 5 min, and then removed using mild vacuum filtration for over 3 minutes. The pH of filtrate was recorded. This step was repeated until the HCl was no longer neutralized by the raft, i.e. the pH of the filtrate was ≤ 4 or \leq the previous reading. The remaining raft was transferred into a 100 ml beaker and broken using a spatula. To this, 3 ml of 0.04 M HCl was added and allowed to settle. The raft was transferred back to the Buchner funnel and filtered using a mild vacuum. The pH of the filtrate was recorded. During filtration, observations were made on how the acid was filtering- through or from the sides of the raft, and the speed of filtration.

Non-raft forming formulation

The minimum labelled dose of well-mixed antacid was taken in a 250-ml beaker and volume was made up to 30 ml using deionized water. The beaker was stirred on a magnetic stirrer at 300±30 rpm for 1 minute. Then 70 ml of 0.1 N HCl was added, and allowed to stand for 10 minutes after which the content was stirred at 300±30 rpm for 10 minutes. The contents were titrated against 0.1 N HCl at a constant rate of 2.0 ml/minute. The pH-time profile was recorded continuously, using a pH meter

placed into the beaker contents, until the pH of the mixture fell below 3.0.¹³

Raft strength

Raft strength was estimated using an in-house fabricated modified pan balance, and is based on a previously published method.⁸ Rafts were allowed to develop around an L-shaped wire probe (diameter: 1 mm, length of vertical arm: 9 cm, length of horizontal arm: 2 cm), held in a 250-ml glass beaker, by adding the maximum recommended dose of the product to 150 ml of SGF at 37°C. The formed rafts were allowed to mature at 37°C for 30 minutes while holding the wire probe upright in the central axis of the beaker throughout the maturation period. The beaker and the raft on the wire probe were then carefully attached to one scale of the modified pan balance. After maturation, water was added drop-wise to a paper cup placed on the opposite pan of the balance and the weight of water in grams required to break the raft was recorded.

Raft resilience

The method used was adapted from previously published methodology. ^{10,14} The maximum recommended dose of the products was added to 150 ml of SGF at 37°C in 250 ml glass beakers, and the rafts formed were allowed to mature at 37°C for 30 minutes. Then the SGF was replaced with same quantity of fresh SGF, and placed in water bath shaker (37°C and 50 rpm). At hourly intervals, the rafts were examined for integrity, number of pieces and time required for rafts to completely disappear.

Sodium alginate content

A high performance liquid chromatography (HPLC) assay was used which is based on a validated method. ¹⁵ The HPLC system consisted of Dionex P100 series with quaternary pump, ultraviolet detector, and autosampler. Chromatographic peak separation was performed on Hi-Qsil (250 mm \times 4.6 mm), 5 μ m column. Data was analysed using Chromeleon software (version 6.80) (Thermo Fisher Scientific Inc., Waltham, MA).

The mobile phase was prepared by adding 0.05% of orthophosphoric acid in 1000 ml of deionized water. The pH was adjusted to 7.0 using 0.1 N NaOH and the solution was filtered through 0.45 μ membrane filter (PALL). The working standard was made up of 50 mg alginate standard dissolved by sonication in 30 ml deionized water in a volumetric flask, and volume made up to 50 ml with deionized water. The blank mobile phase and the working standard solution were injected into the HPLC system, and the chromatograms recorded. Isocratic elution was carried out using 100% buffer solution at a flow rate of 0.7 ml/minute. The injection volume was 20 μ l and detection wavelength was 200 nm.

System suitability was validated by injecting 6 replicates of alginate standard solution (500 parts per million in

diluent). The relative standard deviation of the six replicate injections was to be not more than 2%. For validation of linearity, six different concentrations of alginate standard were analysed in triplicate, and a linearity graph of area versus concentration was plotted. The correlation coefficient was to be not less than 0.995.

Assay of the products for sodium alginate content was performed in triplicate. 5 ml of the raft-forming formulation was dispersed in 200 ml deionized water in a 250 ml volumetric flask and sonicated for 30 minutes. After cooling, the volume was made up to 250 ml with deionized water, and centrifuged at 4000 rpm for 5 minutes. The supernatant was filtered through a 0.22 μm syringe filter and analysed by the HPLC method described above. The sodium alginate concentration in each sample was determined using the linear equation (R²>0.995) obtained from the standard graph. The percent assay of sodium alginate for each product was determined using the following equation:

% assay of sodium alginate = $\frac{[X (mg/ml) \times 250 \ ml \times Dilution \ factor \times 100]}{LC}$

where, X (mg/ml) = sodium alginate concentration in each sample, LC = Label claim (mg of sodium alginate per 5 ml of suspension).

Statistical analysis

Analysis was performed using GraphPad Prism 8 (GraphPad Software, La Jolla, California). ANC data, neutralization time, raft strength and alginate content of

Digeraft Plus® versus brands A to D were analysed using one-way analysis of variance (ANOVA) with Bonferroni post-test for multiple comparisons. The p values of <0.05 were considered statistically significant.

RESULTS

The nature of formed rafts is depicted in Table 2. Raft-forming formulations except brand B, and the two non-raft forming formulations showed similar ANC (Figure 1). The raft-forming formulations had higher capacity to neutralize acid compared with non-raft forming formulations. The time of neutralization for all raft-forming formulation was similar (p>0.05) and was around 32 minutes, except for brand C which showed a sharp decrease in pH after 24 minutes (Figure 2). All raft-forming products were able to re-neutralize HCl, but to varying degrees, indicating that structure of the raft must have right level of porosity to allow the acid to be in contact with the antacid. The ANP of Digeraft Plus® was significantly higher (p<0.05) than all the other brands (Table 3).

Table 2: Nature of the formed rafts (n=3).

Product	Raft formation speed	Raft flotation	Raft coherence
Digeraft Plus®	Moderate	Complete	Excellent
Brand A	Moderate	Complete	Good
Brand B	Moderate	Complete	Excellent
Brand C	Immediate	Incomplete	Average
Brand D	Slow	Complete	Good

Table 3: Acid neutralization capacity, the duration of neutralization, and the speed of filtration.

Product		ANC ± SD (mEq)	Total duration of neutralization (minutes)	Speed of filtration on mild vacuum
Raft forming products	Digeraft Plus®	29.38±1.02	72±5.33	Slow
	Brand A	30.8±0.13	64±5.02	Moderate
	Brand B	26.8±3.82	56±7.32	Slow
	Brand C	29.8±1.02	61.33±3.77	Moderate
	Brand D	29.86±1.2	61.3±3.77	Moderate
Non-raft forming	Brand E	24.20±0.67	55.5±4.77	
products	Brand F	23.30±0.61	46.67±2.35	-

Table 4: Raft resilience.

Product	Observation
Digeraft Plus®	Major portion of the raft remained as a thick layer, intact after 24 hours
Brand A,	Raft broke into numerous smaller pieces in 1 hour and was settled at the bottom
Brand D	A small amount of raft remained after 24 hours
Brand B	Raft broke into numerous smaller pieces in 2 hours and was settled at the bottom
Di anu D	A major amount of raft as thin layer remained after 24 hours
Brand C	Raft broke into numerous smaller pieces in 2 hours and a major portion of raft was settled at the bottom
	A small portion of raft remained intact after 24 hours

Table 5: Percentage assay of sodium alginate.

Product	% Sodium alginate content
Digeraft Plus®	90.2±0.83
Brand A	71.4±0.64
Brand B	75.9±1.21
Brand C	81.7±0.85
Brand D	60.0±0.83

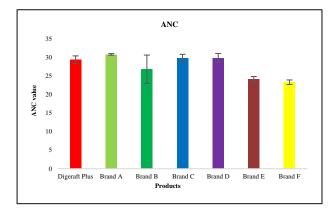


Figure 1: ANC of products.

Data represents a mean of three independent experiments; ANC-acid neutralization capacity; brand E and brand F are non-raft forming formulations.

The ANP was similar for brands A, C and D and lower for brand B (Table 3). The neutralization profile of brand E was greater than brand F (Table 3). The raft strength of Digeraft Plus® was significantly higher (p<0.05) than all the other brands (Figure 3). In line with this finding, the rafts of Digeraft Plus were found to be the most resilient (as a major amount of the raft was found intact even after 24 hours), and to retain their integrity for the longest time (>4 hours) compared with other products (Table 4). Only Digeraft Plus® (20.49±4 gm) and brand B (10.98±5.8 gm)

were within the BP specification of a raft strength of at least 7.5 grams for alginate raft-forming oral suspensions.¹⁶

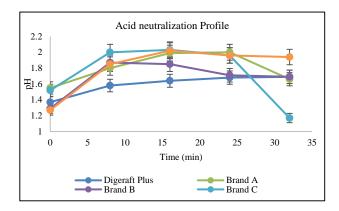


Figure 2: Neutralization profile of raft-forming formulations.

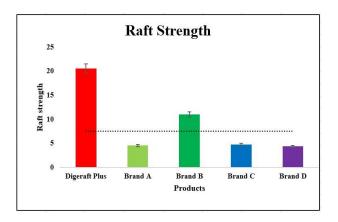


Figure 3: Raft strength of products.

Modified pan balance method *p<0.05 for Digeraft Plus® versus all other products. (Legend represents the correspondence to BP specification of raft strength more than 7.5 gm).

Table 6: Product classification depicting the statistical significance of the products evaluated.

Parameters	Products	Statistical significance [Digeraft Plus® versus other raft-forming formulations]	Key findings	
	Brand A	P>0.9999		
	Brand B	P=0.0032	ANC of Digeraft Plus®, brand	
Acid neutralization	Brand C	P>0.9999	(A, C and D) was found to be	
capacity (ANC)	Brand D	p>0.9999	similarANC value of brand B, E and	
	Brand E	P<0.0001	 D was significantly different 	
	Brand F	P<0.0001	b was significantly different	
	Brand A	P<0.0001		
	Brand B	P<0.0001		
Acid neutralization	Brand C	P<0.0001	ANP of Digeraft Plus® was	
profile (ANP)	Brand D	P<0.0001	superior to other products	
	Brand E	P<0.0001		
	Brand F	P<0.0001		
Raft strength	Brand A	P<0.0001		
	Brand B	P<0.0001	Digeraft Plus® demonstrated	
	Brand C	P<0.0001	superior raft strength compare to other products	
	Brand D	P<0.0001		

Table 7: Ranking of products based on overall performance.

Products		Overall score	Rank
Raft-forming products	Digeraft Plus®	29	1
	Brand A	21	2
	Brand B	20	3
	Brand C	19	4
	Brand D	15	5
Non-raft forming	Brand E	6	6
products	Brand F	7	7

The sodium alginate content determined by HPLC was significantly higher in Digeraft Plus than all the other brands (p<0.05), and it was the only brand within the BP specification of the label claim (Table 4). Based on the overall study results, the products were ranked as summarized in Table 6. The scores from 1 to 10 have been assigned to the products based on their performance with the product showing best result getting the highest score and the score goes on reducing as the position of the product lowers. For ANC, higher scores were assigned for products having higher ANC values. The products having lower duration of neutralization were given lower scores and those forming rafts with higher raft strengths were given higher scores. Based on the results of the raft resilience study, the products were assigned scores such that higher the duration of time, the raft remains intact, the higher is the score. Similarly, for assay, products having percent assay close to 100% were assigned the highest score. The total score has been calculated by adding the scores for individual testing parameters and overall rank has been assigned based on the total score, the product with highest total score ranked first and so on (Table 7). 10,11 Based on this scoring, Digeraft Plus® was ranked the highest.

DISCUSSION

In alginate-based raft-forming formulations, relevant variables affecting raft formation include the species of alginate, the presence of stabilizing calcium ions, and the pH of the solution. 17,18 Successful raft formation requires a precise reaction between the ingredients in the formulation and even minor differences can have a significant effect on the interaction of alginates with antacids leading to significant differences in in vivo clinical efficacy of the products. 10 The extent to which the alginate content of the raft is present can affect how well the product preserves the integrity and strength of the raft and makes use of the other antacid components in the formulation. This is crucial because the alginate allows antacid trapping, which creates a pH-neutral barrier that refluxes preferentially ahead of any stomach acidity into the esophagus.9 However, there are very few publications that have studied the effect of alginate content on raft characteristics.¹⁴ Digeraft Plus® demonstrated significantly better ANC than brand B, and the two non-raft forming formulations.

Effective acid neutralization requires not only a high ANC but also a long duration of neutralization, which depends on raft structure/alginate content and its ability to retain a reservoir of antacid within its structure.9 Digeraft Plus® had absorbent and porous raft structures and exhibited a longer duration of neutralization, compared to all other products. In addition, Digeraft Plus® rafts had greater strength and resilience, which could be attributed to the higher calcium ion content and its raft strengthening properties, resulting in greater antacid trapping within the raft and more efficient acid neutralization. Also, the alginate content within the raft determined by HPLC method was within the BP specification only for Digeraft Plus® and the alginate content was significantly higher than that in other raft-forming products. Overall, study findings show better physicochemical properties such as neutralization profiles, raft strength and raft resilience in raft-forming antacid formulations with higher calcium carbonate content.

CONCLUSION

The in-vitro study assessed acid neutralizing capacity (ANC) and acid neutralizing potential (ANP) in various raft- and non-raft- forming antacids, and raft strength, raft resilience, and the sodium alginate content in various raft-forming antacids. Raft forming formulations demonstrated better acid neutralization than non-raft forming formulations. Furthermore, considerable differences were observed in the strength and resilience of the rafts formed by the different alginate-antacid products marketed in India that were tested. The rafts of Digeraft Plus® had high porosity and buoyancy, coupled with superior raft strength, raft resilience and acid neutralization profile.

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