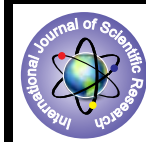


Analysis Of pH, Titratable Acidity and Total Soluble Solid Content of Mouthrinses with Different Active Ingredients and Concentration Commercially Available in India: An in Vitro Study



Medical Science

KEYWORDS : Mouthrinses; pH; titratable acidity; total soluble solid content; tooth erosion.

Ankita Goyal	P.G. Student, Department of Public Health Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
Manjunath BC	Senior Professor and Head, Department of Public Health Dentistry, Postgraduate Institute of Dental Sciences, Pt. BD Sharma University of Health Sciences, PGIMS Campus, Rohtak, Haryana, India
Anup N	Professor and HOD, Department of Public Health Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
Vikas Jeph	Senior Lecturer, Department of Public Health Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
Manohar Bhat	Professor and HOD, Department of Pedodontics and Preventive Dentistry, Jaipur Dental College, Jaipur, Rajasthan, India
Priyanka Agrawal	Assistant Professor, Department of Periodontics, Bharati Vidyapeeth Dental College and Hospital, Pune- 43, India

ABSTRACT

Objectives: To analyze in vitro pH, titratable acidity (TA) and total soluble solid content (TSSC) of mouthrinses with different active ingredients and concentration available in India. Material and methods: 14 commercial brands of mouthrinses containing different active ingredients and concentration available in India were included for the in vitro study. The endogenous pH was evaluated by using pH meter, TA was evaluated by the addition of 0.05N NaOH increments to the mouthrinses, and TSSC readings by Gravimetric methods. Results: pH values ranged from 2.57 + 0.06 to 6.77 + 0.33. The TA values ranged from 0.1 + 0.00 to 16.67 + 0.12. Original Listerine & Eludril presented the lowest and the highest TSSC, respectively. Significant correlation ($p < 0.05$) was found between mouthrinses and endogenous pH, TA, TSSC. Conclusion: Some of the mouthrinses presented low pH ($pH < 5.5$), high TA and high TSSC which may be potentially erosive to the dental tissues.

INTRODUCTION:

Dental erosion is a complex process with interaction between chemical, biological and behavioral factors. It is commonly associated with consumption of beverages and other acidic agents. The erosive potential of products like beverages depend on chemical factors such as endogenous pH, titratable acidity, total soluble solid content (TSSC), mineral content, clearance on tooth surface and calcium-chelation properties.^{1,2} The incidence of dental erosion is increasing throughout the world due to multitude of factors, and hence strategies have to be undertaken to prevent it.^{3,4} It is essential for the dentists to have a thorough knowledge of the possible signs, clinical appearance, progression of erosive lesions and their causes so as to initiate adequate preventive and curative measures.^{1,5}

The presence of potentially erosive ingredients in many therapeutic products used for maintenance of good oral health is also a matter of concern. The control of dental biofilm (dental plaque) is the corner stone in prevention of various oral diseases and to achieve this, many mechanical and chemical methods or a combination of both may be used.^{6,7} Chemical methods include the use of mouthrinses which contain anti plaque / antiseptic agents along with acids as one of their ingredients and hence indiscriminate use may increase the risk of erosion of the teeth. A pH of 5.5 or less is critical for the enamel dissolution.⁸ Acids or EDTA are added along with the therapeutic agents as low endogenous pH of the oral care products improve chemical stability of fluoride compounds by incorporation of fluoride ions into voids in hydroxyapatite crystals and their by stabilizing the crystal lattice. Even though, enamel surface becomes stronger after incorporation of fluoride ions and has a protective effect against bacterial dissolution, the protective effects are lost when the pH is too low which may result in dental erosion and further research is necessary to reveal benefits of fluoride in prevention of dental erosion.^{9,10}

The erosive potential of mouthrinses, salivary substitutes, and chewable tablets is due to the low pH and high titratable acids and hence both the practitioner and the consumer should

be aware of it.¹¹ There has been a spurt in the consumption of mouthrinses by the general public throughout the world, as it is available over the counter and also endorsed by health care practitioners.¹² So it is necessary to identify erosive agents present in the mouthrinses responsible for dental erosion. Hence, an in vitro study was carried out with the objective to analyze endogenous pH, titratable acidity (TA) and total soluble solid content (TSSC) of mouthrinses with different active ingredients and concentration commercially available in India.

MATERIALS AND METHODS

Fourteen commercial brands of mouthrinses commercially available in Indian market comprising of various active ingredients and concentrations were selected for this study (Table 1). The study was carried out at Department of Biotechnology, Birla Institute of Scientific Research Jaipur. Ethical clearance was obtained from the Ethical research review board of Jaipur Dental College, Jaipur. The study was double blinded (investigator analyzing the properties of mouthrinses and statistician). Coding using alphabetical order was done for the mouthrinses (Fig.1). The products were evaluated in a randomized experiment, with 3 repetitions for each sample, with calculation of mean value. The pH of each mouthrinse was measured immediately after package was opened using a Control dynamics APX 175 E/C Model pH meter (Fig.2) with a relative accuracy of ± 0.01 pH unit⁸. The pH meter was first calibrated according to the manufacturer's instructions, using buffer standards of pH 7 and pH 4. Titratable acidity was measured by the amount of 0.05 N Sodium hydroxide (NaOH) solution needed for the product i.e. 10ml of each mouthrinse sample to reach pH equal to neutral pH i.e. titration method¹³ (Fig.3). Total Soluble Solid Content was measured by Gravimetric method¹⁴ (Fig.4). The Total Soluble Solid Content were reported as percentage (%) by converting mg/l to gm/100ml. Data were analyzed using Medcalc 14 version and ANOVA test was used to find the significance with alpha value fixed at .05.

RESULTS

The mean values of endogenous pH, Titratable Acidity and

Total Soluble Solids Content are shown in table 2, 3, 4 respectively. It was found that ten of fourteen mouthrinses were acidic i.e. pH <5.5. The pH value ranged from 2.57 to 6.77. Betadine mouthrinse (Povidine Iodine) was found to be highly acidic with pH value 2.57 in spite of not containing fluoride according to label of mouthrinse. It has been labeled on the mouthrinse, that it should be used after diluting with an equal volume of water. This may increase the pH of the mouthrinse.

Among the tested mouthrinses, Colgate Phos-Flur (Sodium fluoride) (16.67) exhibited high titratable acidity and Colgate Plax (Cetylpyridine) (0.1) and Eludril (Chlorhexidine Gluconate) (0.1) exhibited low titratable acidity which indicate that some of the ingredients present in its composition react with base used to neutralize the mouthrinse (0.05N NaOH) making it less erosive. Eludril (33.50%) presented the highest and Original Listerine (Eucalyptol, Methyl Salicylate, Thymol, Menthol) (0.14%) lowest total soluble solids. Significant correlation ($p < 0.05$) was found between mouthrinses and pH, TA, TSSC using One Way Analysis of Variance.

DISCUSSION

Dental erosion is slowly becoming a health problem which can be attributed to the use of various therapeutic products and beverages which are acidic in nature.^{3,8,11} Low pH value of any solution (i.e. pH < 5.5) may cause erosion and it manifests, when there is contact of such solution with the teeth for prolonged duration, and repeated over time as well.^{1,6,8,15} There will be initial softening of the enamel surface followed by loss of protective hard tissue of the tooth while a softened layer persists at the surface of the remaining tissue. As the critical pH of dental enamel is approximately 5.5, dissolution of the enamel takes place due to exposure to acids combined with an insufficient salivary flow rate.

The effects of various interactions result in a permanent ion / substance exchange and reorganization within the tooth material or at its interface, thus altering its strength and structure.^{6,8,11,15,16} The severity of erosion is determined by the susceptibility of the dental tissues towards dissolution. Because the mineral content of enamel is less soluble than dentine, it tends to erode more slowly.^{6,8,11,16,17} It has been established that long term usage of mouthrinse with a pH value of 5.5 or less is considered to be critical for the destruction of the enamel.^{8,11} Mouthrinses have been more commonly prescribed by the dentists and also are readily available over the counter increasing the risk of dental erosion.^{8,18-20}

Hence, analysis of endogenous pH, titratable acidity and the TSSC of these mouthrinses to assess the erosive potential of the mouthrinses become necessary. The present investigation evaluated physicochemical properties of fourteen important mouthrinses commercially available in Jaipur, India.

In the present study, ten mouthrinses were classified as potentially erosive (pH < 5.5) corroborating the findings of previous investigations.¹⁸⁻²⁰ Fluoride containing mouthrinses usually have a low pH as it makes the mouthrinse chemically more stable and also result in increased fluoride uptake by the enamel.^{8,21} The product labels were examined to identify mouthrinses containing fluoride which revealed that among the ten mouthrinses with pH less than 5.5, Amflor (Amine Fluoride, Proylene Glycol), Colgate Phos-Flur, S-Flo (Sodium fluoride) and Shy-OR (Potassium Nitrate, Triclosan) has fluoride (480ppm, 4.4mg in 10ml, 904ppm, 904ppm respectively) in its formulation.

The label of the other six mouthrinses with pH below the critical value for enamel dissolution did not contain fluoride such as HiOra Regular (Pilu, Bibhitaka, Nagavalli), Apollo pharmacy Fresh breath (Thymol, Eucalyptol, Menthol), Betadine, Clohex (Chlorhexidine Gluconate), Tantum Oral Rinse (Benzylamine hydrochloride) and Original Listerine.

All the mouthrinses below the critical pH should be used judiciously to prevent dental erosion. Mechanical toothbrushing with use of fluoride toothpaste along with flossing is the best

method for maintenance of good oral hygiene.²² Daily rinsing with therapeutic mouthrinses is advised as an adjunct only for those individuals who are at the risk of dental caries and those who are unable to perform mechanical toothbrushing adequately.^{23,24} Even though, mouthrinses are available as over the counter drug, it is better to consult a dentist before starting daily mouth rinsing routine. Chlorhexidine mouthrinses should be used for only for a short duration of time to prevent many adverse effects.^{22,24} Mouthrinses which contain alcohol should not be advised for individuals with xerostomia.^{22,24} All the mouthrinses should be used only after toothbrushing and not before as it has a potential to cause dental erosion.²⁵

The potential erosive effect of a substance does not merely depend on its pH. Titratable acidity too is considered to have an erosive effect on the tooth. Products with a higher titratable acidity cause a prolonged drop in the pH and hence result in relatively more demineralization of the dental tissues.⁸ In the present study, Colgate Phos-Flur exhibited high titratable acidity with also pH less than 5.5. A possible explanation for this result is that some ingredients present in its composition did not react with the base used to neutralize the mouthrinse. Colgate Plax and Eludril exhibited low titratable acidity with pH more than 5.5 which was similar when compared with the findings of previous investigations.^{8,11,17-20,26}

Total Soluble Solid Content (TSSC) is a measure of the combined content of all inorganic and organic substances contained in a liquid in: molecular, ionized or micro-granular (colloidal sol) suspended form.²⁷ Almost all the medical formulations have their share of side effects. These may be local or systemic. The benefit – to – risk has to be determined prior to the use of any of the formulations. The risk is influenced by the incidence and the severity of the side effects. Four of the mouthrinses Colgate Phos-Flur, Disoral (Diclofenac), Eludril, Kidodent (Sodium fluoride, Triclosan) exhibited total soluble solids greater than 20%, that is, 20 g of solids dissolved in 100 ml of mouthrinse. Among the tested mouthrinses, Eludril presented the highest total soluble solids. Finding was consistent with the findings of an investigation on mouthrinses available in Brazilian market.⁸

Use of fluoride to reduce and prevent dental erosion: The use of toothpastes which contain sodium fluoride has been reported to be beneficial in preventing dental erosion when compared to other fluoride formulate ions.²⁸ In another in vitro study, stabilized stannous fluoride (SnF₂) toothpaste demonstrated a highly significant reduction in enamel surface loss when compared to sodium fluoride (NaF), or sodium monofluorophosphate (SMFP).²⁹ Messias DC et al, in their in vitro study have negated these findings and have reported that fluoridated toothpastes do not have the capacity to prevent dental erosion.³⁰ It has been reported that titanium tetrafluoride (TiF₄) is effective in prevention of both carious and erosive lesions. The mechanism of action of TiF₄ is due to the increased fluoride uptake which result in the formation of an acid resistant surface layer, which might reduce demineralization of dental hard tissues.^{31,32} Even though, in vitro studies involving TiF₄ reports to be more effective than other forms, there is lack of sufficient evidence through clinical trials.³¹⁻³⁴ In spite of having inconclusive evidence, formulations containing fluorides can be still advised for prevention of dental caries to all the individuals using mouthrinses.

Limitations: Since it was an in vitro study, further studies need to be carried out in order to determine the clinical implication of these mouthrinses.

SUMMARY AND CONCLUSION:

The present study demonstrated that 10 mouthrinses out of 14 were classified as potentially erosive (pH < 5.5), few with low endogenous pH, high titratable acidity, and high total soluble solid content which may make these products potentially erosive to dental tissue if not properly used. The manufacturers of these mouthrinses should take necessary steps to reduce the pH level of the mouthrinses.

RECOMMENDATIONS:

Children and parents or caretakers need to be educated regarding the importance of brushing prior to the use of mouthrinses and must be discouraged from using it before brushing. Taking in to consideration the present results, which have demonstrated the erosive potential of common mouthrinses, we recommend the following steps to mitigate any problems associated with their use:

1. The pH of the mouthrinse should be maintained above 6 as reports suggests that pH less than 5.5 is associated with dental erosion. Strong base producing salts including Ca₂⁺, Na⁺, and K⁺ - containing salts have the tendency to increase the pH of the mouthrinses.

2. Dental – care education should be instilled to minimize the possibility of developing dental erosion and also to promote dental health.
3. The manufacturers should formulate the mouthrinses so as to make them less erosive to the teeth.

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Table 1: List of mouthrinses according to the commercial brand, active ingredients, fluoride content and manufacturer’s name

Sr. no.	Brand name	Active Ingredients	Available fluoride content when packed given on label of mouthrinses	Manufacturer
1	Tantum Oral Rinse	Benzydamine hydrochloride BP 0.15% (Composition)	-	Elder Pharmaceuticals LTD
2	Clohex mouthrinse	Chlorhexidine Gluconate Solution IP equivalent to Chlorhexidine Gluconate – 0.20%	-	Group pharmaceuticals LTD
3	Eludril	Chlorhexidine Gluconate Solution IP equivalent to Chlorhexidine Gluconate – 0.10%	-	G.S.Pharmbutor Pvt. Ltd.
4	HiOra Mouthrinse Regular	Pilu- 5mg, Bibhitaka- 10mg, Nagavalli- 10mg	-	Himalaya drug company
5	Original Listerine mouthrinse	Eucalyptol, Methyl Salicylate, Thymol, Menthol	-	IDS Manufacturing LTD
6	Disoral	Diclofenac 0.074%	-	Elder Health Care LTD
7	Apollo pharmacy Fresh breath mouthrinse	Thymol IP 0.06%, Eucalyptol PC _x 0.09%, Menthol IP 0.04%	-	Vita Biopharma Pvt. Ltd.
8	Betadine mouthrinse	Povidine Iodine IP 2% (0.2% w/v available iodine	-	G.S.Pharmbutor Pvt. Ltd.
9	Shy-OR with xylitol	Potassium Nitrate BP – 3% Triclosan – 0.3%	904ppm	Group pharmaceuticals LTD
10	Amflor Oral Rinse	Amine Fluoride, Proylene Glycol	480 ppm	Group pharmaceuticals LTD
11	Colgate Phos- Flur	Sodium fluoride 4.4mg	4.4 mg in each 10ml	Vita Biopharma Pvt. Ltd.
12	S-Flo mouthrinse	NaF IP 0.2%	904ppm	Group pharmaceuticals LTD
13	Kidodent mouthrinse	Sodium fluoride- 0.05% Triclosan USP- 0.03%	226ppm	INDOCO REMEDIES LTD
14	Colgate Plax	Cetylpyridine, Sodium Fluoride	225 ppm	Vita Biopharma Pvt. Ltd.

Table No. 2: Endogenous pH of different mouthrinses used in the study

Commercial brand	pH ₁	pH ₂	pH ₃	Mean ± SD	F Ratio	P value
Tantum Oral Rinse	4.35	5.28	4.79	4.81 ± 0.47	109.10	0.000
Clohex mouthrinse	4.87	5.3	5.07	5.08 ± 0.22		
Eludril	6.57	7.15	6.58	6.77 ± 0.33		
HiOra mouthrinse Regular	4.56	4.57	4.57	4.57 ± 0.01		
Original Listerine mouthrinse	3.78	4.14	3.98	3.97 ± 0.18		
Disoral	6.15	6.26	6.16	6.19 ± 0.06		
Apollo pharmacy Fresh breath mouthrinse	4.05	4.05	4.06	4.05 ± 0.01		
Betadine mouthrinse	2.51	2.58	2.62	2.57 ± 0.06		
Shy-OR with xylitol	5.17	5.18	5.18	5.18 ± 0.01		
Amflor Oral Rinse	4.56	4.55	4.56	4.56 ± 0.01		
Colgate Phos- Flur	4.2	4.22	4.24	4.22 ± 0.02		
S-Flo mouthrinse	5.26	5.45	5.43	5.38 ± 0.10		
Kidodent mouthrinse	5.85	6.14	5.82	5.94 ± 0.18		
Colgate Plax Peppermint	5.95	5.97	5.95	5.96 ± 0.01		

Table No. 3: Titratable Acidity (TA) of various mouthrinses used in the study

Commercial brand	TA ₁	TA ₂	TA ₃	Mean ± SD	F Ratio	P Value
Tantum Oral Rinse	0.25	0.25	0.25	0.25 ± 0.00	8471.19	0.000
Clohex mouthrinse	0.2	0.2	0.2	0.2 ± 0.00		
Eludril	0.1	0.1	0.1	0.1 ± 0.00		
HiOra Mouthrinse Regular	7	7.4	7.2	7.2 ± 0.20		
Original Listerine mouthrinse	2	2	2.0	2.00 ± 0.000		
Disoral	0.2	0.2	0.2	0.2 ± 0.000		
Apollo pharmacy Fresh breath mouthrinse	6.4	6.4	6.2	6.33 ± 0.12		
Betadine mouthrinse	2.6	2.4	2.6	2.53 ± 0.12		
Shy-OR with xylitol	6.2	6.2	6	6.13 ± 0.12		
Amflor Oral Rinse	4	4	3.8	3.93 ± 0.12		
Colgate Phos-Flur	16.6	16.6	16.8	16.67 ± 0.12		
S-Flo mouthrinse	0.6	0.6	0.6	0.6 ± 0.00		
Kidodent mouthrinse	0.2	0.2	0.2	0.2 ± 0.00		
Colgate Plax Peppermint	0.1	0.1	0.1	0.1 ± 0.00		



Figure 1: Coding using alphabetical order of different mouthrinses used in the study



Figure 2: Measuring Endogenous pH of different mouthrinses used in the study

Table No. 4: Total Soluble Solid Content (TSSC) of mouthrinses used in the study

Commercial brand	TSS ₁	TSS ₂	TSS ₃	Mean ± SD	F Ratio	P Value
Tantum Oral Rinse	2.28	1.44	2.14	1.95 ± 0.45	231.19	0.000
Clohex mouthrinse	13.41	13.32	13.68	13.47 ± 0.19		
Eludril	34.80	32.64	33.07	33.50 ± 1.14		
HiOra Mouthrinse Regular	11.02	9.59	9.9	10.17 ± .75		
Original Listerine mouthrinse	0.17	0.11	0.14	0.14 ± 0.03		
Disoral	26.17	24.46	25.75	25.46 ± 0.89		
Apollo pharmacy Fresh breath mouthrinse	9.87	10.02	11.1	10.33 ± 0.67		
Betadine mouthrinse	2.37	2.4	2.54	2.44 ± 0.09		
Shy-OR with xylitol	19.95	18.9	18.7	19.18 ± 0.67		
Amflor Oral Rinse	9.63	9.74	10.74	10.04 ± 0.61		
Colgate Phos-Flur	22.04	19.85	19.53	20.47 ± 1.37		
S-Flo mouthrinse	9.14	9.09	8.31	8.85 ± 0.47		
Kidodent mouthrinse	16.45	22.61	21.1	20.05 ± 3.21		
Colgate Plax Peppermint	7.70	7.64	7.33	7.56 ± 0.20		



Figure 3: Measuring Titratable Acidity of different mouthrinses used in the study



Figure 4: Measuring Total Soluble Solid Content (TSSC) of different mouthrinses used in the study

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