Microbial spoilage, instability risk of antacid suspension in the presence of commonly used preservative system

Jamshaid Ali Khan[1]*, Imran Ullah Khan[1], Zafar Iqbal[1], Fazli Nasir[1], Salar Muhammad[2], Peer Abdul Hannan[1] and Irfan Ullah[1]

[1]Department of Pharmacy, University of Peshawar, Peshawar, KPK, Pakistan
[2]Department of pharmacy, Abdul Wali Khan University, Mardan, KPK, Pakistan

Abstract: Manifestation of microbial spoilage of any product by bacteria and to assess the effectiveness of the anti-microbial preservatives (parabens) used for the prevention and stability purpose. The aim of the present work is to study the effectiveness of preservatives used in the antacid suspensions and to analyze the effect of microbial growth on the quality of respective antacid suspensions. Samples of various antacid suspensions were randomly collected from local market and Government hospital pharmacies. Three different antacid formulations were prepared in the laboratory. All the formulations were preliminarily evaluated on the basis of organoleptic characteristics, pH, viscosity and assay. Efficacy of the preservative system in suspension formulation was determined by inoculating the samples in its final container, with specific strains of bacteria i.e. *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538, taking samples from the inoculated preparation at specified intervals of time i.e. 0 time, 07 days, 14 days and 28 days, growing it on nutrient agar medium and colony forming units (CFUs) were scored by plate count. At the same time the samples were also subjected to qualitative and quantitative testing. The decrease in CFU and alteration in assay, pH and viscosity was observed in all the formulations except formulation M2 and F3 that showed stability throughout the study period.

Keywords: Antacid Suspension, microbial contamination, stability, microbial assay, pasteurization, parabens, colony forming units (CFU).

INTRODUCTION

The presence of high concentration of free water in pharmaceutical product may lead to their contamination by microorganisms which not only make them hazardous from the infections standpoint, but may also change the physical, chemical and organoleptic properties of the drugs, alter the contents of active ingredients, or convert them to toxic products (Judge *et al.,* 2008; Denyer and Baird, 1990). It has been shown under laboratory conditions that various microorganisms can metabolize a wide range of drugs, resulting in loss of activity and potency. Many preservatives and disinfectants can be metabolized by various Gram-negative bacteria, most commonly at concentration below their effective use levels. Pseudomonas species can metabolize 4-hydroxy-benzoate esters (preservatives) contained in eye drops, oral solutions and suspension (Baird *et al.,* 2004).

Antacids are the drugs that neutralize stomach acidity, containing at least one of the active acid neutralizing agent e.g. aluminium, magnesium or calcium salts. Although the alkaline pH of the antacid suspension is a critical factor in their acid neutralizing capacity and controlling microbial growth within the suspension, these formulations can still be susceptible to microbial contamination (Beveridge, 1998; Bloomfield, 1996). As the antacid suspension is available as multidose medicament there is possibility of further contamination during its usage period.

Pharmaceutical preparations are protected by adding antimicrobial preservatives that prevent or inhibit the growth of microorganisms (Beveridge, 1999). Sodium benzoate, potassium sorbate, and methyl paraben in the concentratins range of 0.1-0.2%, 0.1-0.2% and 0.1-0.25% (w/w) respectively, are among the most commonly used preservatives in liquid pharmaceutical preparations (Beringer and Felton, 2006). The alkyl esters of parahydroxybezoic acid particularly methyl and propyl esters are most commonly used preservatives in oral preparations as they give the most effective options such as broad spectrum of activity, low toxicity and good stability (Soni *et al*., 2002), but they undergo hydrolysis at alkaline pH (Shija *et al.*,1992).

Methyl and propyl parabens are frequently used in combination due to the observed synergestic effect. Poor preservation may lead to the microorganisms getting exposed to the sub-lethal concentration of the preservatives and develop resistant variants (Baird and Pertris, 1991; Khan *et al*., 2008). However, high concentration may prove to be toxic for consumer's health. Therefore, it is essential to determine the appropriate preservation conditions for efficacy and usefulness of the product during its storage period

**Corresponding author:* e-mail: jamshaidkhan@upesh.edu.pk

Table 1: Laboratory prepared antacid suspensions

S. No.	Materials	Formulation1	Formulation2	Formulation3
		Quantity	Quantity	Quantity
1	Aluminium hydroxide	57.15 gm	57.15gm	57.15 gm
2	Magnesium hydroxide	17.37 gm	17.37gm	17.37 gm
3	Simethicone	5.688 gm	5.688gm	5.688 gm
4	Sorbitol	60 gm	60gm	60 gm
5	Xanthan gum	4 gm	4gm	4 gm
6	Saccharin sodium	1.1 gm	1.1gm	1.1 gm
7	Methyl paraben	1.150 gm	1.150gm	1.150 gm
8	Propyl paraben	0.2 gm	0.2 gm	0.2 gm
9	Aerosil	0.584 gm	0.584gm	0.584 gm
10	Benzyl alcohol	10ml	------	-------
11	Citric acid	2 gm	2 gm	2 gm
12	Flavourpeppermint	0.725 ml	0.725ml	0.725 ml
13	Distilled water	Q.S. 1000 ml	Q.S. 1000ml	Q.S. 1000 ml

Table 2: Preliminary evaluation of the collected samples

Formulation	pH	Viscosity	% assay	
		Maps	$Al(OH)_3$	$Mg(OH)_2$
M 2	7.79±0.01	1061±1	100.30±0.42	100.30±0.42
DP	8.8±0.01	1052±2	106.15±0.46	110.50±0.85
Gd	8.83±0.01	2685±2	106.78±0.44	101.44±0.88
Tx	7.78±0.01	1665±1	106.15±0.46	110.55±0.88
Md	8.43±0.01	1395±4	94.72±0.46	100.82±0.88
Sl	8.15±0.01	2713±4	108.63±0.44	109.93±0.82
F 1	8.4±0.01	1168.67±0.47	91.66±0.78	95.38±0.86
F 2	8.6±0.01	1135.67±1.25	106.46±0.78	112.38±0.86
F 3	8.3±0.01	1139.33±1.25	100.92±0.76	101.44±0.88

(Hossain *et al*., 2004). Several factors affect the activity of the preservatives, which include temperature, pH, chemical composition, conditions of microorganism, concentration of preservatives, the presence or absence of interfering factors and the possible interaction of the preservatives with containers. These factors must be considered when selecting proper antimicrobial preservative (Beveridge, 1998; Russell, 2003).

MATERIAL AND METHOD

Collection of sample

Samples of antacid suspensions were collected from Government hospital pharmacies and local market pharmacies.

Sample preparation

Three different formulations were developed in the laboratory for the testing procedures shown in table 1. In formulation 2 the slurry of aluminum hydroxide and magnesium hydroxide was tyndallized while for the formulation 3 pasteurization was done.

Qualitative and quantitative analysis of the samples

Preliminary test i.e. Physical appearance, pH, assay and viscosity were conducted in accordance to the protocol mentioned in the United States Pharmacopoeia (2009) for all the samples including laboratory prepared samples.

Analysis of the effective preservatives

The preparations were challenged in its final container according to the procedure mentioned in United States Pharmacopoeia, (2009), with a prescribed inocula of suitable microorganisms i.e. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739.The inoculated preparations were stored at room temperature, samples were withdraw from the container at specified intervals of time i.e. 0, 07 days, 14 days and 28 days for microbial counts and analysis.

Data interpretation

Data was expressed as mean ± standard deviation, one way ANOVA and appropriate statistical procedures were applied for analysis of the data.

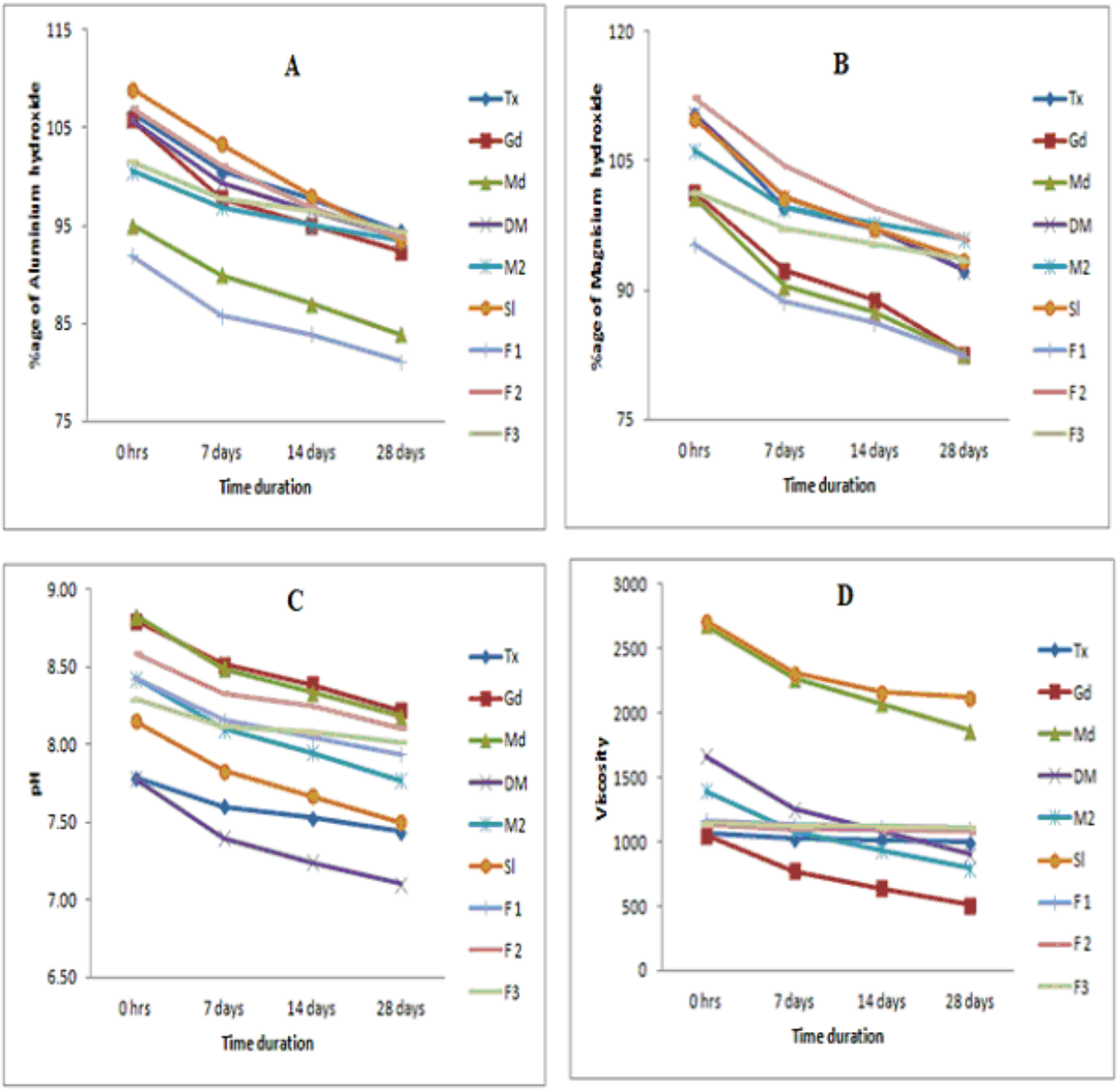

Fig. 1: Effect of *Staphylococcus aureus* ATCC 6538 on the assay (A, B), pH (C) and viscosity (D) of antacid suspensions.

RESULTS

Preliminary evaluation of formulations

Samples of six different pharmaceutical manufacturing companies were investigated. Results of the various tests such as pH, organoleptic properties, viscosity and weight per ml are summarized in table 2. The effect of *Staphylococcus aureus* ATCC 6538 on the assay, pH and viscosity of antacid suspensions is summarized in table 3 and fig. 1. The effect of *Escherichia coli* ATCC 8739 on the assay, pH and viscosity of antacid suspensions is summarized in table 4 and fig. 2. The effect of *Pseudomonas aeruginosa* ATCC 9027on the assay, pH and viscosity of antacid suspensions is summarized in table 5 and fig. 3.

Preservatives efficacy testing of suspension formulations

Preservative efficacy testing was based on a sample inoculation using a microbial suspension with determined CFU count. In the current study, preservative system of nine samples was challenged by inoculating *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538 bacterial types into the preparations in their final containers.

DISCUSSION

Qualitative and quantitative aspects of antacid suspensions in the presence of microorganisms were studied during the preservatives efficacy testing. The contamination of microbes deteriorated the antacid suspensions as the peppermint flavour of antacid suspension was replaced by alcoholic odour, which indicated the fermentation process in the preparation. Assay of the active ingredients was decreased gradually as shown in fig. 1, pH of the suspension changed towards acidic range and viscosity was also decreased. Two samples i.e. M2 and F3 showed stability to the deterioration due to microbial contamination. The effects of microbial contamination on quality of antacid suspensions are shown in table 3, 4 and 5.

A significant decrease in percentage of aluminum hydroxide has been shown for all the samples except samples M2 and F3, which showed a lesser/slight decrease. Fig. 1 and graph B show % fall of magnesium hydroxide. Again the samples Tx, Gd, Md, DM, Sl, F1 and F2 have shown significant decrease in the percentage of magnesium hydroxide. Fig. 1 and graph C show a decrease in the pH of antacid suspensions. The graph C shows a gradual decrease in the pH for all the samples due to microbial contamination but samples M2 and F3 show a slight decrease in the pH compared to other samples. Changes can occur in pH of the product, depending on whether acidic or basic metabolites are produced and such alteration can inflict secondary attack by microbes previously inhibited by the pH (Hoq, *et al.*, 1991). The parabens used in the formulations may be

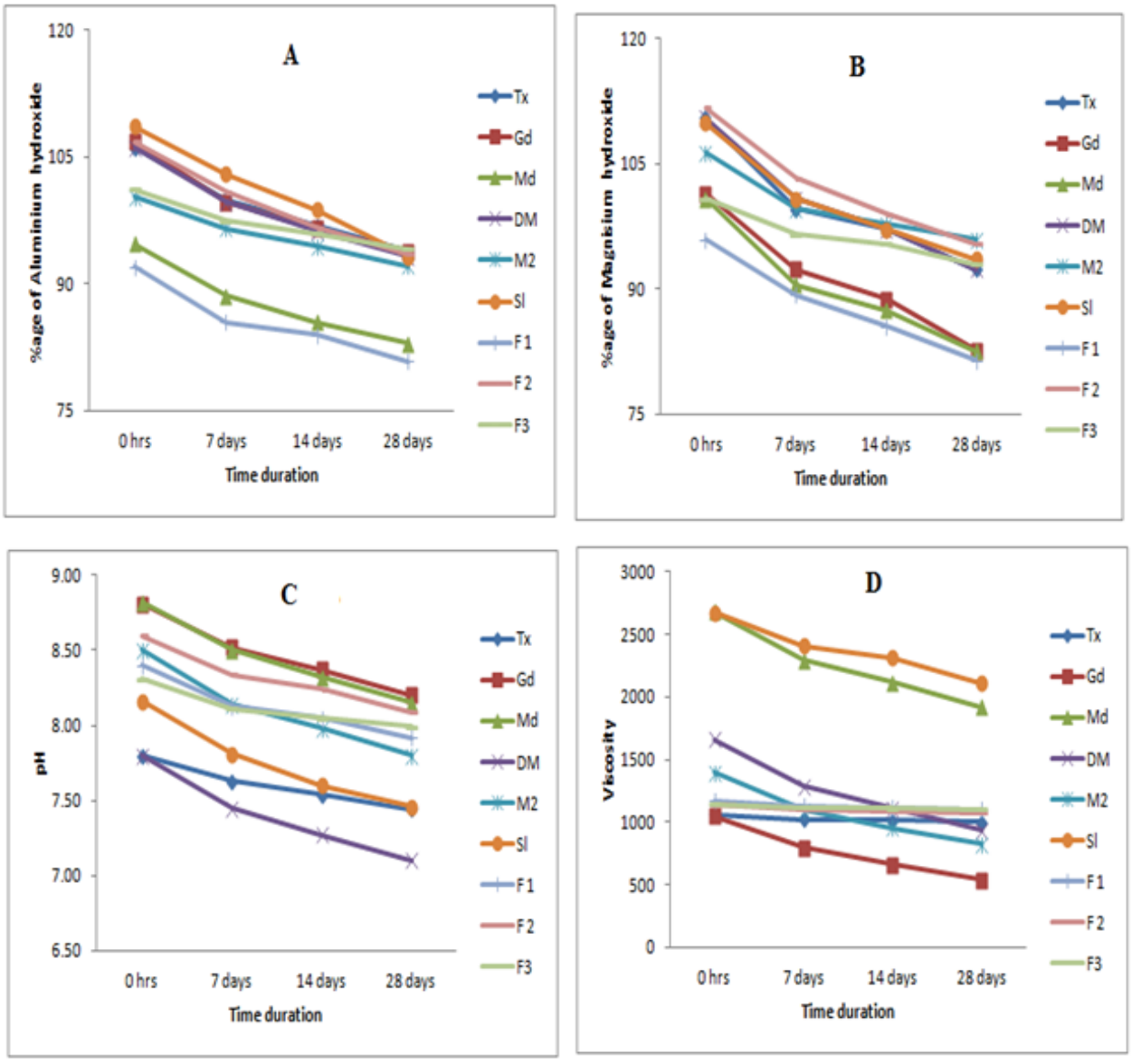

Fig. 2: Effect of *Escherichia coli* ATCC 8739on the assay (A, B), pH (C) and viscosity (D) of antacid suspensions.

hydrolyzed to benzoic acid, which may be the cause of reduction pH of the formulation (Ali and Amini, 2007). Benzoic acid is also a good preservatives but it is effective at pH 2.5 to 4.5. It activity is approximately lost at pH exceeding to 5 (Burlini *et al.*, 1993). Fig. 1 graph D showed decrease in the viscosity of the samples due to microbial contamination. All sample showed a decrease in the viscosity. The decrease in the viscosity may be due the microbial activity on the viscosity building agent in the formulation followed by degradation of these agents.

and graph A. A significant decrease in percentage of aluminum hydroxide has been shown for all the samples except samples M2 and F3, which showed a slight decrease. Fig. 2 graph B shows a decrease in the percentage of magnesium hydroxide. Again the samples TX, Gd, Md, DM, Sl, F1 and F2 have shown significant decrease in the percentage of magnesium hydroxide. In fig. 2 graph C shows decrease in the pH of antacid suspensions. Fig. 2 graph D shows a decrease in the viscosity of the samples due to microbial contamination. All the samples have shown decrease in the viscosity.

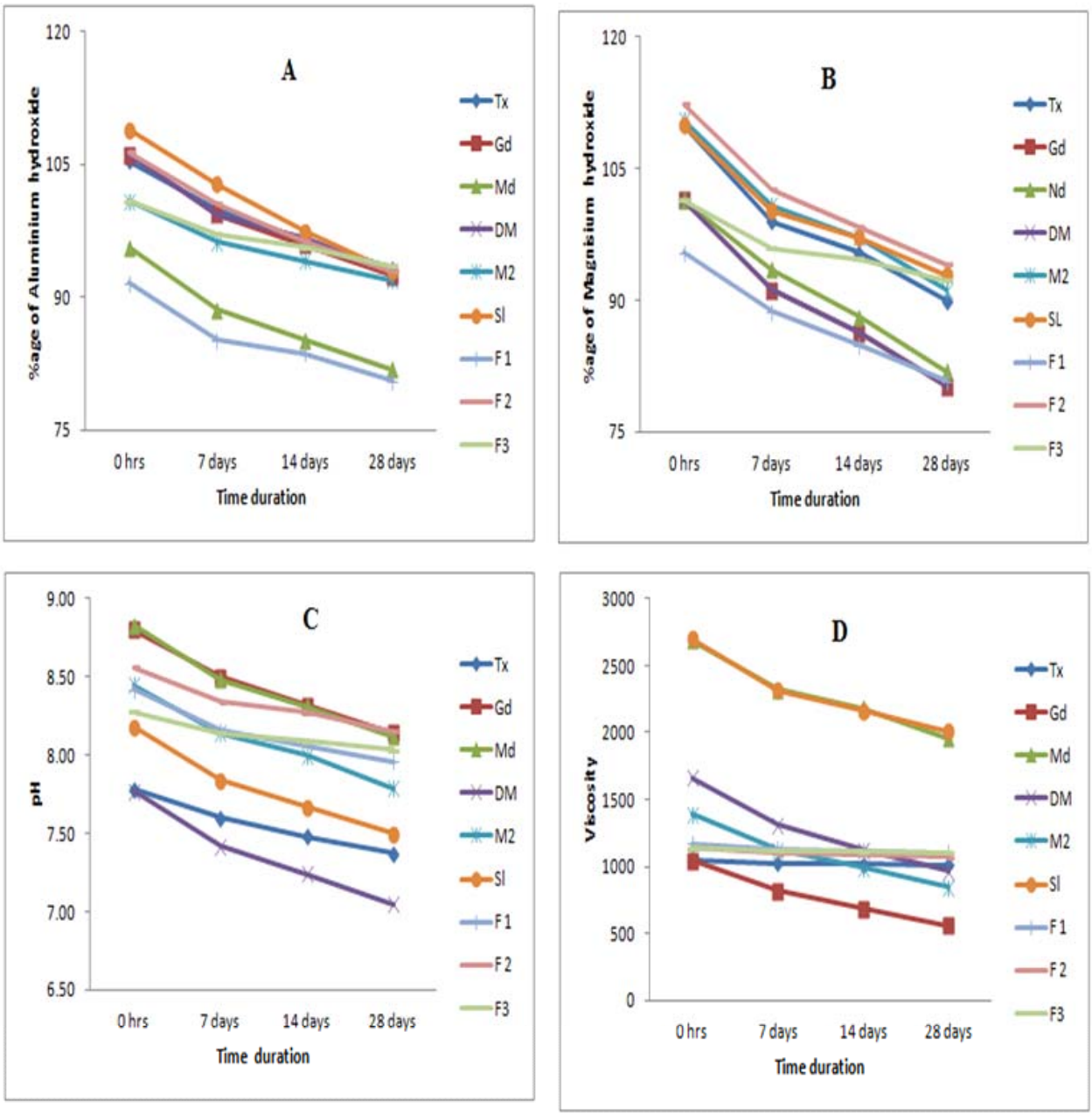

Fig. 3: Effect of *Pseudomonas aeruginosa* ATCC 9027on the assay (A, B), pH (C) and viscosity (D) of antacid suspensions.

Fig. 3 graph A is showing a gradual decrease in the percentage of aluminum hydroxide. A significant decrease in percentage of aluminum hydroxide has been shown for all the samples except M2 and F3 samples which showed a slight decrease. In fig. 3a and 3b graph B a decrease in the percentage of magnesium hydroxide has been shown. Again the samples Tx, Gd, Md, DM, Sl, F1 and F2 have shown a significant decrease in the percentage of magnesium hydroxide. In fig. 3 graph C a decrease in the pH of antacid suspensions has been indicated. fig. 3a and 3bgraph D show a decrease in the viscosity of the samples due to microbial contamination. All the samples showed a decrease in the viscosity.

The CFU of *Escherichia coli* ATCC 2739 in the samples DM, GD, Tx, MD, Sl, F1 and F2 remained >400 CFU and did not show decrease in the count from zero time till 28 days. While the samples M2 and F3 showed a significant reduction i.e. from >400 to 28 in sample M2 and from >400 to 40 in sample F3, the sample F3 which was prepared in laboratory pasteurization was applied. For *Pseudomonas aeruginosa* ATCC 9027 all the remaining

Table 3: Effect of *Staphylococcus aureus* ATCC 6538 on the quality of antacid suspensions

Product	Tests		0 time	07 days	14 Days	28 days
Tx	Assay	Aluminum hydroxide	106.46±0.78	100.60±0.42	97.80±0.78	94.42±0.76
		Magnesium hydroxide	111.14±1.45	99±0.85	95.35±0.85	94.42±0.76
	pH		7.78±0.01	7.40±0.01	7.24±0.02	7.1±0.01
	Viscosity		1665±1	1252±2	1083±2	904±2
GD	Assay	Aluminum hydroxide	105.82±0.46	97.80±0.42	95.05±0.46	92.41±0.98
		Magnesium hydroxide	100.82±0.88	89.91±0.86	86.26±0.86	82.60±0.85
	pH		8.83±0.01	8.49±0.02	8.34±0.01	8.18±0.01
	Viscosity		2685±2	2372±2	2194±2	1962±2
MD	Assay	Aluminum hydroxide	95.05±0.46	89.97±0.52	87.02±0.76	83.96±0.43
		Magnesium hydroxide	100.82±0.88	91.12±1.49	86.32±0.94	80.79±0.85
	pH		8.43±0.01	8.10±0.00	7.95±0.01	7.77±0.01
	Viscosity		1395±4	1075±3	934±3	793±2
DM	Assay	Aluminum hydroxide	105.82±0.46	99.38±0.44	96.60±0.42	93.80±0.42
		Magnesium hydroxide	112.32±0.82	99.00±0.85	95.38±0.86	91.73±0.86
	pH		8.8±0.01	8.52±0.02	8.39±0.01	8.22±0.00
	Viscosity		1052±2	825±3	690±3	550±5
M2	Assay	Aluminum hydroxide	100.60±0.64	96.90±0.00	95.05±0.69	93.50±0.64
		Magnesium hydroxide	105.09±0.86	99.60±0.85	99.00±0.85	97.19±0.85
	pH		7.79±0.01	7.6±0.00	7.53±0.01	7.44±0.01
	Viscosity		1061±1	1021±1	1015±3	999±2
S1	Assay	Aluminum hydroxide	108.94±0.44	103.37±0.47	98.12±0.76	93.50±0.73
		Magnesium hydroxide	109.96±0.86	100.84±0.86	99.01±0.86	94.77±1.49
	pH		8.15±0.01	7.83±0.01	7.67±0.01	7.5±0.01
	Viscosity		2713±4	2312±3	2162±2	2123±3
F 1		Aluminum hydroxide	91.98±0.44	85.80±0.42	83.96±0.43	81.17±0.47
	Assay	Magnesium hydroxide	95.38±0.86	88.69±0.86	86.26±0.86	82.44±0.61
	pH		8.42±0.01	8.16±0.01	8.06±0.01	7.96±0.01
	Viscosity		1165.67±1.25	1131.67±1.25	1120.00±1.63	1107.1.25
F 2	Assay	Aluminum hydroxide	107.09±0.44	101.22±0.45	96.90±0.42	93.80±0.42
		Magnesium hydroxide	112.38±0.86	104.42±0.76	99.60±0.85	95.98±0.86
	pH		8.56±0.00	8.34±0.001	8.27±0.001	8.15±0.001
	Viscosity		1130.00±0.82	1099.33±0.47	1090.00±0.82	1076.67±1.25
F 3	Assay	Aluminum hydroxide	101.53±0.45	97.80±0.42	96.60±0.42	94.42±0.76
		Magnesium hydroxide	101.44±0.8	97.19±0.85	95.38±0.86	93.55±0.86
	pH		8.28±0.00	8.14±0.00	8.09±0.01	8.03±0.00
	Viscosity		1142.33±1.25	1121.67±1.25	1115.3±0.47	1108.6±1.25

Table 4: Effect of *Escherichia coli* ATCC 8739 contamination on the quality of antacid suspensions

Product	Tests		0 time	07 days	14 Days	28 days
Tx	Assay	Aluminum hydroxide	106.15±0.46	99.69±0.75	96.90±0.42	93.80±0.42
		Magnesium hydroxide	110.55±0.85	99.60±0.85	97.19±0.85	92.33±0.86
	pH		7.8±0.00	7.45±0.01	7.27±0.01	7.1±0.00
	Viscosity		1663±2	1285±2	1107±2	936±3
GD	Assay	Aluminum hydroxide	106.78±0.44	99.69±0.44	96.90±0.42	93.80±0.42
		Magnesium hydroxide	101.44±0.88	92.33±0.86	88.83±0.66	82.63±0.89
	pH		8.82±0.01	8.5±0.02	8.32±0.01	8.2±0.01
	Viscosity		2684±3	2295±2	2115±1	1918±2
MD	Assay	Aluminum hydroxide	94.72±0.46	88.59±0.44	85.50±0.42	83.01±0.41
		Magnesium hydroxide	100.82±0.88	90.51±0.86	87.48±01.49	82.60±0.85
	pH		8.46±0.01	8.14±0.01	7.98±0.02	7.8±0.01
	Viscosity		1396±2	1096±2	954± 2	825±3
DM	Assay	Aluminum hydroxide	106.15±0.44	99.69±0.44	96.29±0.75	93.20±0.42
		Magnesium hydroxide	110.50±0.85	100.82±0.88	97.19±0.85	92.33±0.86
	pH		8.81±0.01	8.52±0.01	8.37±0.01	8.2±0.01
	Viscosity		1050±2	795±3	662±2	538±2
M2	Assay	Aluminum hydroxide	100.30±0.42	96.60±0.42	94.42±0.76	92.11±0.70
		Magnesium hydroxide	106.30±0.85	99.60±0.85	97.80±0.85	95.98±0.86
	pH		7.8±0.01	7.63±0.01	7.54±0.01	7.44±0.00
	Viscosity		1060±2	1024±2	1016±3	1004±2
S1	Assay	Aluminum hydroxide	108.63±0.44	103.03±0.47	98.75±0.46	93.20±0.42
		Magnesium hydroxide	109.93±0.82	100.82±0.88	97.19±0.85	93.55±0.86
	pH		8.16±0.01	7.81±0.01	7.62±0.01	7.46±0.01
	Viscosity		2672±2	2412±2	2313±2	2113±4
F 1	Assay	Aluminum hydroxide	91.98±0.44	85.50±0.42	83.96±0.43	80.83±0.47
		Magnesium hydroxide	95.98±0.86	89.30±1.49	85.48±1.71	81.40±0.86
	pH		8.43±0.01	8.16±0.01	8.05±0.01	7.94±0.01
	Viscosity		1167.6±1.25	1130.6±1.25	1119±0.82	1104.3±1.25
F 2	Assay	Aluminum hydroxide	106.78±0.44	100.92±0.76	96.63±0.47	93.50±0.73
		Magnesium hydroxide	111.78±0.86	103.27±0.86	99.00±0.85	95.38±0.86
	pH		8.59±0.01	8.33±0.01	8.25±0.01	8.11±0.01
	Viscosity		1133.6±1.25	1099.6±1.25	1088±1.63	1072.6±1.25
F 3	Assay	Aluminum hydroxide	101.22±0.45	97.50±0.42	95.99±0.44	94.10±0.42
		Magnesium hydroxide	100.82±0.88	96.59±1.48	95.38±0.86	92.94±1.49
	pH		8.29±0.01	8.12±0.01	8.08±0.01	8.02±0.01
	Viscosity		1141.6±1.25	1118.6±1.25	1112.3±0.47	1103.6±1.25

samples except followed by M2 and F3, failed to decrease the CFU count but M2 and F3 showed a significant decrease in the CFU count as in case of M2 from >400 to 25 and F3 from >400 to 35 after zero time till 28 days. In case of *Staphylococcus aureus* ATCC 6538only the samples M2 and F3 showed a decrease in the CFU count as in case of M2 from >400 to 20 and F3 from >400 to 30 and the remaining samples were unable to show a clear cut decrease in the CFU count.

CONCLUSION

Antacid suspensions are used for neutralization of gastric acidity. They are commonly preferred over tablets due to their rapid activity and greater ability of suspension to neutralize gastric acid. These suspensions have aluminum hydroxide and magnesium hydroxide as active drugs which are acid neutralizing agents. Sometimes antacid suspension gives bad odour on opening the sealed bottle, which may be due to the microbial contamination. The active drugs of antacid suspension are of natural origin i.e. from soil source, which are highly contaminated with microorganisms. The use of such contaminated raw materials in the formulation of pharmaceuticals will lead to contamination in the final preparation. Operating personnel and poor manufacturing environment constitute the other sources of microbial contamination in the final preparation. The occurrence of microorganisms in

Table 5: Effect of *Pseudomonas aeruginosa* ATCC 9027 on the quality of antacid suspensions

Product	Tests		0 time	07 days	14 Days	28 days
Tx	Assay	Aluminum hydroxide	105.85±0.46	99.99±0.75	96.60±0.42	93.20±0.42
		Magnesium hydroxide	109.96±0.86	99±0.85	95.38±0.86	89.91±0.86
	pH		7.77±0.00	7.42±0.00	7.24±0.01	7.05±0.00
	Viscosity		1666±1	1314±2	1124±3	965±4
GD	Assay	Aluminum hydroxide	106.11±0.43	99.38±0.44	95.97±0.47	92.41± 0.98
		Magnesium hydroxide	101.44±0.88	93.55±0.86	88.09±0.86	81.96±0.86
	pH		8.83±0.01	8.28±0.02	8.31±0.01	8.12±0.01
	Viscosity		2685±2	2325±3	2185±5	1955±3
MD	Assay	Aluminum hydroxide	95.67±0.45	88.59±0.44	85.17±0.78	81.81±0.43
		Magnesium hydroxide	101.44±0.88	91.12±1.49	86.26±0.86	80.18±1.49
	pH		8.45±0.01	8.14±0.01	8.0±0.02	7.79±0.01
	Viscosity		1392±2	1124±2	994±2	849±4
DM	Assay	Aluminum hydroxide	106.11±0.43	99.38±0.44	96.66±0.50	92.90±0.42
		Magnesium hydroxide	110.56±0.86	100.82±0.85	97.19±0.85	91.12±1.49
	pH		8.9±0.01	8.5±0.01	8.3±0.01	8.2±0.01
	Viscosity		1045±2	817±2	683±2	563±1
M2	Assay	Aluminum hydroxide	100.20±0.76	96.29±0.75	94.10±0.42	91.95±0.62
		Magnesium hydroxide	105.09±0.86	99.60±0.85	97.19±0.85	95.38±0.86
	pH		7.78±0.01	7.60±0.01	7.48±0.01	7.37±0.00
	Viscosity		1055±2	1026±1	1016±2	1009±3
S1	Assay	Aluminum hydroxide	108.94±0.44	102.75±0.76	97.50±0.42	92.90±0.42
		Magnesium hydroxide	109.93±0.82	100.22±1.49	97.19±0.85	92.94±1.49
	pH		8.18±0.01	7.84±0.01	7.67±0.01	7.5±0.01
	Viscosity		2699±1	2313±3	2163±2	2012±3
F 1		Aluminum hydroxide	91.66±0.78	85.19±0.75	83.66±0.43	80.55±0.76
	Assay	Magnesium hydroxide	95.38±0.86	88.69±0.86	84.87±1.11	80.80±0.86
	pH		8.4±0.01	8.13±0.01	8.05±0.01	7.92±0.01
	Viscosity		1168.6±0.47	1129.6±1.70	1118.3±1.25	1102.3±1.25
F 2	Assay	Aluminum hydroxide	106.46±0.78	100.6±0.42	96.32±0.80	92.90±0.42
		Magnesium hydroxide	112.38±0.86	102.67±0.86	98.40±1.147	94.16±0.86
	pH		8.6 ± 0.01	8.34±0.01	8.24±0.01	8.09±0.01
	Viscosity		1135.6±1.25	1100.6±1.25	1088.6±1.25	1071.3±1.25
F 3	Assay	Aluminum hydroxide	100.92±0.76	97.20±0.73	95.68±0.44	93.50±0.73
		Magnesium hydroxide	101.44±0.88	95.98±0.86	94.77±1.49	92.33±0.86
	pH		8.31±0.01	8.11±0.01	8.05±0.01	7.99±0.01
	Viscosity		1139.3±1.25	1118.6±1.25	1111.6±1.25	1103±1.63

pharmaceuticals not only makes them hazardous from the infection point of view, but can also alter the chemical, physical and organoleptic characteristics of the preparation, change in the contents of active constituents and/or change them to harmful products. To overcome the problem of microbial contamination, antimicrobial preservatives are used in the formulation. Methyl paraben, propyl paraben, sodium benzoate and potassium sorbate are the most commonly used preservatives. All these preservatives are effective over acidic pH range and are least effective over alkaline pH. Furthermore, these preservatives are poorly water-soluble. In order to achieve adequate preservative level during the shelf life of a product with alkaline pH like antacid suspension is practically not possible. Lowering the pH of antacid suspension by the addition of organic acids for effectiveness to preservatives, will adversely affect the acid neutralizing capacity of the antacid. To combat this situation, pasteurization of antacid suspension improves its effectiveness as this process destroys the initial bioburden. So the product could be microbiologically safe for rest of the product shelf life. Raw materials used in the formulation should be of minimal bioburden and such bioburden should be properly checked at raw material analysis stage. Development of a preservatives system, which may be effective atacidic range that will also be helpful in addressing the problem of microbial contamination in antacid suspension.

Table 6: Preservatives efficacy assessment of all samples

S. No.	Product	Testing Intervals				
		0 Hours	7th Day	14th Day	28th Day	Result
Escherichia coli ATCC 2739						
1	M 2	≥400	240±1	125±2	48±1	Effective
2	DM	≥400	≥400	≥400	≥400	Ineffective
3	Gd	≥400	≥400	≥400	≥400	Ineffective
4	Tx	≥400	≥400	≥400	≥400	Ineffective
5	Md	≥400	≥400	≥400	≥400	Ineffective
6	Sl	≥400	≥400	≥400	≥400	Ineffective
7	F1	≥400	≥400	≥400	≥400	Ineffective
8	F2	≥400	≥400	≥400	≥400	Ineffective
9	F3	≥400	195±2	110±3	40±1	Effective
Pseudomonas aeruginosa ATCC (9027)						
1	M2	≥400	125±1	62±1	25±1	Effective
2	DM	≥400	≥400	≥400	≥400	Ineffective
3	Gd	≥400	≥400	≥400	≥400	Ineffective
4	Tx	≥400	≥400	≥400	≥400	Ineffective
5	Md	≥400	≥400	≥400	≥400	Ineffective
6	Sl	≥400	≥400	≥400	≥400	Ineffective
7	F1	≥400	≥400	≥400	≥400	Ineffective
8	F2	≥400	≥400	≥400	≥400	Ineffective
9	F3	≥400	180±1	100±2	35±1	Effective
Staphylococcus aureus ATCC 6538						
1	M2	≥ 400	165±1	95±1	20±1	Effective
2	DM	≥ 400	≥ 400	≥ 400	≥ 400	Ineffective
3	Gd	≥ 400	≥ 400	≥ 400	≥ 400	Ineffective
4	Tx	≥ 400	≥ 400	≥ 400	≥ 400	Ineffective
5	Md	≥ 400	≥ 400	≥ 400	≥ 400	Ineffective
6	Sl	≥ 400	≥ 400	≥ 400	≥ 400	Ineffective
7	F1	≥400	≥400	≥400	≥400	Ineffective
8	F2	≥400	≥400	≥400	≥400	Ineffective
9	F3	≥400	170±2	95±1	30±1	Effective

REFERENCES

Ali RS and Amini M (2007). Deterioration of parabens in preserved magnesium hydroxide oral suspensions. *J.Appl. Sci*. **7**(21): 3322-3325.

Baird R (2004). Microbial spoilage, infection risk and contamination control, in hugo and russell's Pharmaceutical Microbiology. *In*: Denyer SP, Hodges NA and Gorman SP editors. Blackwell Science: Massachusetts U.S.A. pp.263-284.

Baird R and Petris P (1981). A study of microbiological contamination of oral medicaments. *Pharm. J*., **266**: 10-11.

Beringer P and Felton DMA (2006). Remington: The science and practice of pharmacy 21st Edition, Lippincott, Williams and Wilkins, United State Of America, pp.1633,1736.

Beveridge EG (1998). Microbial spoilage and preservation of pharmaceutical products, in Pharmaceutical microbiology, *In*: Hugo WD and Russell AD editor. Blackwell: London, pp.355-373.

Beveridge EG (1999). Preservation of medicines and cosmetics. Principles and Practice of Disinfection, Preservation and Sterilization, 3rd ed. Blackwell Science, Oxford, pp.457-484.

Bloomfield S (1996). Control of microbial contamination in cosmetics, toiletries and non-sterile pharmaceuticals. Microbial quality assurance in cosmetics, toiletries and non-sterile pharmaceuticals, 2nd ed. Taylor and Frances, London. pp.3-8.

Burlini N, Pellegrini R, fracheris P, Tortora P and Guerritore A (1993). Metabolic effects of benzoate and sorbate in the yeast Saccharomyces cerevisiae at neutal pH. *Arch. Microbiol*. **159**(3): 220-224.

Denyer SP and Baird RM (1990). Guide to microbiological control in pharmaceuticals, Ellis Horwood: New York, pp.157-181.

Hoq M, Morsheda S and Gomes D (1991). Development of appropriate preservative system for liquid antacid: Bacterial contaminants in antacid samples. *Banglad. J. Microbiol.,* **8**(1): 5-10.

Hossain M, Ara S and Rahman MZ (2004). Quantitative examination of aerobic bacteria and fungi in locally available antacid suspension and possible contamination by specified bacteria. *Pak. J. Biol. Sci.,* **7**(11): 2014-2017.

Judge V, Narasimhan B, Narang R and Ohlan R (2008). Preservative evaluation of novel 2, 4-hexadienoic acid derivatives in aluminium hydroxide gel-USP. *Scientia. Pharmaceutica.,* **76**(2): 269.

Khan JA, Iqbal Z, Rehman SU and Farzana K (2008). Prevalence and resistance pattern of pseudomonas aeruginosa against various antibiotics. *Pak. J. Pharm. Sci.*, **21**(3): 311-315.

Russell A (2003). Challenge testing: Principles and practice. *Int. J. Cosmet. Sci.,* **25**(3): 147-153.

Shija R, Bruce Sunderland V and McDonald C (1992). Alkaline hydrolysis of methyl, ethyl and n-propyl 4-hydroxybenzoate esters in the liquid and frozen states. *Int. J. Pharm*., **80**(1-3): 203-211.

Soni MG, Taylor SL and Greenberg NA (2002). Evaluation of the health aspects of methyl paraben: A review of the published literature. *Food Chem., Toxicol* **40**(10): 1335-1373.

ZaniF, Minutello A, Maggi L and Santi P (1997). Evaluation of preservative effectiveness in pharmaceutical products: The use of a wild strain of Pseudomonas cepacia. *J. Appl. Microbiol.,* **83**(3): 322-326.