Preparation of Artificial Saliva Formulation

Andi Sri Suriati Amal[1]*, Samsinah Hussain[1], Mohd. Amin Jalaluddin[1]
[1]*Department of Pharmacy, Faculty of Health Sciences, University of Darussalam Gontor Ponorogo, East Jawa, Indonesia*
*E-mail: andiinci73@gmail.com

***Abstract*—Dry mouth or throat (xerostomia) is a clinical condition characterized by desiccation of the intraoral tissues. Patients with chronic or temporary sensation of dry mouth need some kind of treatment to relieve the symptoms. Causes of dry mouth include medications, autoimmune disease (Sjogren's syndrome), radiotherapy or chemotherapy for cancer, hormone disorders and infections. The project is important not only because saliva substitutes are not manufactured locally, but also because most saliva substitutes use mucin (porcin in origin). Therefore there is a need to produce one with other source which has properties to mucin itself. The objective of this project is to produce saliva substitutes that can serve as mouth and throat lubricants. The first step was pre-formulation studies that involved characterization of active ingredients (physical, chemical, and mechanical properties) in order to choose what other ingredients (excipients) should be used in the preparation. Formulation studies also considered such factors as solubility, viscosity, and pH. The last step was assessment of safety and stability of the final product. The new artificial saliva formulations containing various ratios of SCMC (Sodium carboxymethyl cellulose), MC (methyl cellulose) and HPMC (hydroxypropyl methycellulose) have been developed. Combination of cellulose derivatives and albumin in these formulations resulted in the physical properties of these new artificial saliva substitutes closely resembling human saliva and mucin-based saliva substitutes. Formula we choose were the most suitable formulae due to their viscosity and pH properties which closely resemble human saliva and mucin based saliva substitutes.**

Keywords—Artificial Saliva; Saliva Substitute; Mouth and Throat Lubricant; Mouth and Throat Moisturizer

I. INTRODUCTION

The decreased in salivary production may be caused by pharmacological agentspathological conditions as in Sjogren's Syndrome and physiological changes in the body. Dry mouth or throat (xerostomia) is a clinical condition that is characterized by a dessication of the intraoral tissues. The most frequent cause seems to be side effects from drugs (Regelink *et al*. 1998, Philip 1997). Patients with chronic or temporary sensation of dry mouth need some kind of treatment to relieve the symptoms. These patients are often advised to drink water *ad libitum,* using chewing gum, candies and to try either a saliva stimulant or a saliva substitute (Grotz 2003; Allal, Dulquerov & Bieri 2000; Tenovuo 1981).

A decreased in the flow of saliva is often associated with a number of secondary effects, namely hampered movements of the lips and tongue, matiscatory difficulties and disturbances in swallowing, speech and taste (Vissink *et al*. 1983). With regards to the mouth, it is important to keep it well lubricated. This can be achieved naturally with the use of artificial saliva preparations or salivary stimulants. This should help to reduce the risk of problems such as secondary infection of the mouth and throat. When this occurs, it usually takes the form of thrush, which is a fungal infection causing painful redness over the tongue and throat (Kelly 2001). Saliva is dilute, colourless and opalescent fluid with specific gravity ranging between 1002 to 1010 (average 1003). It consists of albumin, amino acids, ammonia, enzymes, proteins, electrolytes, and vitamins. Depending on its exposure to air and bacterial action, the pH of saliva varies from 5.75 to 7.05. Saliva is normally hypotonic, its osmolarity being half to three-fourths that of plasma (Srivastava 2004).

The composition of saliva varies with the rate of secretion and the type of stimulus initiating it. Sodium concentration in saliva may vary from 5 to 100 mEq/L depending on its flow rate. The potassium concentration is relatively higher than in plasma, mixed saliva usually containing 8 to 20 mEq/L. Salivary calcium concentration may reach up to 3 to 4 mEq/L and like sodium increase with the flow rate. The salivary chloride concentration is always below that of plasma and may vary from 5 to 7 mEq/L. The carbonate concentration is directly influenced by the partial pressure of CO_2 in arterial blood. The phosphate concentration is

about twice that of plasma, and appears to be independent of the flow rate (Srivastava 2004).

The chief organic constituents of saliva are mucin, a glycoprotein responsible for the viscosity and lubricating properties of saliva, and an enzyme ptyalin, which is an α-amylase and catalyses the breakdown of starch to maltose. The other organic materials in saliva are made up of small amounts of other enzymes like carbonic anhydrase, free amino acids, urea, uric acid and creatinine. It contains a bacteriolyte enzyme called lysozyme, which is mucoprotein in nature. Saliva also contains soluble polysaccharides like ABO specific blood group (agglutinogens) substances, enzymes like kallikrein, and a protein component that acts as a nerve growth factor (NGF) (Srivastava 2004).

Artificial saliva preparations are designed to mimic natural saliva both chemically and physically. They are expected to have a visco-elastic pattern similar.to normal human saliva to provide similar viscosity and film forming properties (Alpöz *et al*. 2008). They do not stimulate natural salivary gland production and must be considered as replacement therapy and not as a cure for xerostomia (dry mouth). Artificial saliva closely resembles natural human saliva in the following characteristics: (i) viscosity (mucin, carboxymethylcellulose and glycerin are used to mimic natural saliva viscosity (Van der Reijden *et al*. 1997; Hatton *et al*. 1987; Vissink *et al*. 1984; 's-Gravenmade 1974); (ii) mineral content (all products contain calcium and phosphate ions, besides also containing fluoride) (Philip 1997; Leung & Darvell 1997; Joyston-Bechal & Kidd 1987); (iii) preservatives (all products except Salivart® contain preservatives such as methyl-or propyl paraben) (Sidney & James 1993; Sweetman 2007; Tenevuo 1981); (iv) palatability (the most common flavorings are mint, sorbitol, and xylitol).

It is important that the ionic composition of a substitute resembles that of natural saliva. Saliva substitute should not only alleviate discomfort but also help maintain the integrity of the teeth. This is relevant because of the increased susceptibility to dental caries is associated with a reduction in salivary secretion. To minimize dental caries, saliva substitutes should contain 0.4% stannous fluoride or 1.1% sodium fluoride (Myers & Ferris 2007). To produce artificial saliva with properties comparable to natural saliva, the salivary glycoproteins are replaced with carboxymethylcellulose (Glandosane®, Luborant®, Saliva substitute®, Salivart®), mucin (Saliva Orthana, Lozenges Orthana, AS Saliva Orthana), glycerol (Oracare D), linseed (Salinum) or sorbitol (Luborant). There have been many studies of synthetic saliva based on hydroxyethyl-, hydroxypropyl-, or carboxymethyl cellulose as the main component which may also be beneficial as palliative agents to relieve the discomfort of xerostomia by temporarily wetting the oral mucosa (Myers & Ferris 2007). Most researchers have indicated that mucin-containing artificial saliva preparations are considerably superior to carboxymethyl-cellulose formulations (Davies & Finlay 2005; Momm, Muller & Tsekos 2001; Duxbury, Thakker & Wastell 1989; Vissink *et al*. 1984).

The desired characteristics of saliva substitutes are excellent lubrication, surface wetting, inhibition of overgrowth of pathogenic microorganisms, maintenance of the hardness of dental structure, pleasant taste, long duration of effect, extended shelf life, and low cost (Burket *et al*. 2008). Vissink *et al*. (1984) reported that patients preferred a mucin-containing artificial saliva over a CMC-preparation because of the large improvement of oral functioning, large retention time and less amount needed daily.

II. MATERIALS AND METHODS

A. Materials

The materials used for preparing the artificial saliva are albumin (Sigma, Sigma-Aldrich CO, St Louis, MO USA), methyl cellulose (Sigma, Sigma-Aldrich CO, St Louis, MO USA), sodium carboxymethyl cellulose (Sigma, Sigma-Aldrich CO, St Louis, MO USA), hydroxypropylmethyl cellulose (Sigma, Sigma-Aldrich CO, St Louis, MO USA), potassium chloride (R&M chemicals, R&M Marketing, Essex, UK), di-potassium hydrogen phosphate (Merck, Merck KGaA, Darmstadt, Germany), sodium fluoride (Merck, Merck KGaA, Darmstadt, Germany), Magnesium chloride (Merck, Merck KGaA, Darmstadt, Germany), Glucose (R&M chemicals, R&M Marketing, Essex, UK), Methyl paraben (Sigma, Sigma-Aldrich CO, St Louis, MO USA).

B. Preparation of Artificial Salive

Albumin, cellulose, potassium chloride, potassium phosphate and sodium fluoride were dissolved in a small amount of water. Methyl paraben, magnesium chloride and dextrose were dissolved in warm water and then cooled down before mixing all the solutions together. Finally a flavouring agent is added to the final solution. The formulations studied containing different ratios of methyl cellulose (MC), sodium carboxymethylcellulose (CMC) and hydroxypropyl-methylcellulose (HPMC). Each of the formulations contain methyl paraben 0.2%, potassium chloride 0.062%, magnesium chloride 0.005%, potassium phosphate 0.034%, sodium fluoride 0.01%, dextrose 4.69% and flavor.are

shown in Table 1.

Table 1. Formulation of Artificial Saliva

Sample	Table of formulation studied			
	Albumin (%)	MC (%)	SCMC (%)	HPMC (%)
A	0.1	0.25	-	-
B	0.1	-	0.25	-
C	0.1	-	-	0.25
D	0.1	0.5	-	-
E	0.1	-	0.5	-
F	0.1	-	-	0.5
G	0.1	0.75	-	-
H	0.1	-	0.75	-
I	0.1	-	-	0.75

C. Evaluation of Relative Density

The relative densities of each formulations were determined using a pycnometer. The weight of the empty picnometer was measured. Each sample was then filled into the pycnometer and weighed. Readings were taken at room temperature. Each measurement was run in triplicates. Apparent density was calculated by the following equation:

$$\rho = \frac{W2-W1}{C} \qquad (1)$$

where,

ρ = apparent density

W_2 = weight of filled pycnometer (gram)

W_1 = weight of empty dry pycnometer (gram)

C = Capacity of the pycnometer in millilitre at 20°C (ml^3).

D. Evaluation of Viscosity

The viscosity of each formulation was determined using a glass U-tube viscometer, size G. Each sample was filled through tube L to slightly above the mark "G". The tube was placed vertically and the liquid was then sucked to a point approximately 5 mm above the mark E. After releasing pressure and suction, the time taken for the bottom of the meniscus to fall from the top of mark E to the top of mark F was measured. Readings were taken at room temperature 25 ± 0.1. Viscosity was calculated by the equation:

$$\eta_w/\eta_c = (\rho_w \times t_w)/(\rho_c \times t_c) \qquad (2)$$

where

η_w = viscosity of water (centipoises)

η_c = viscosity of sample (centipoises)

ρ_w = density of water (g/ml^3)

ρ_c = density of sample (g/ml^3)

t_w = time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F (minute) for water

t_c = time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F (minute) for sample

E. Evaluation of pH

The pH of the artificial saliva was determined using a pH meter (pH Meter Model 320, Mettler Toledo, Switzerland). Calibration of pH meter was done using buffer solutions. The pH obtained were the average of three determinations.

F. Analysis of Preservative Content

Analysis of preservative content was obtained using the UV-visible spectrophotometer which consisted of UVmini-1240 Shimadzu (Nakagyo-Ku, Kyoto, Japan). The methyl paraben reference standards were obtained from Reference Standard Unit, National Pharmaceutical Control Bureau (NPCB), Malaysia. Water for dilution was 0.05 M sulfuric acid buffer. All analysis was carried out at room temperature (25°C). The ultraviolet (UV) detection wave length was fixed at 254 nm.

Preparation of Standard Solution

Seven solutions were prepared with concentrations of 0.5, 1, 2, 4, 6, 8, and 10 µg/ml from standard stock solutions with dilution water (0.05 M sulfuric acid) for calibration purposes.

Preparation sample/test solution

All the test samples were developed with composition of methyl paraben at 0.2 mg/ml. The 20 µl sample was transferred to a 10 ml volumetric flask and then made up to volume with dilution water, while absorbance was measure at 254 nm.

G. Microbial Limit Test

To perform the test pour plate method was chosen. All the artificial saliva formulations have been observed for their limit of microbial contamination from *Escherichia coli*, *Pseudomonas auregenosa* and *Staphilococcus aureus* using agar media. Chromocult coliform agar was used to observe the growth of *E. coli*, centrimide agar for *Pseudomonas* and vogel-johnson agar for *Staphylococcus aureus*.

H. Stability Test

A stability chamber (CLIMACELL MMM Medcenter, German) was used to conduct the stability test. It was set with temperature ranging from 0°C to 99.9°C and relative humidity (RH) from 10% to 95% in various time modes. The hydrophilic and lipophilic base nasal lubricant solutions were packed in 5 ml plastic drop bottles and stored at 40°C and 75% RH (both ± 1) while gel and ointment were packed in 7.5 g ointment tube and stored at room temperature. Samples were analyzed for their appearance, odour, colour,

ISSN : 9-772476-969006

pH, density, viscosity and stability indicating assay of preservative content at intervals of 1 month, 2 months and 3 months.

I. Statistical Analysis

This study statistically used bivariate correlation statistical treatment. The statistical analysis was calculated using the statistical software SPSS version 10.

III. RESULT AND DISCUSSION

Viscosity

The different viscosity of the artificial saliva formulations is shown in Table 2. Formulae using methylcellulose (formula A, D and G) 0.25, 0.5 and 0.75% have viscosity values of 1.3491, 2.2812 and 5.0717 cPs respectively. Formulae using sodiumcarboxymethyl cellulose (formula B, E and H) 0.25, 0.5 and 0.75% have viscosity values of 1.3881, 2.6657 and 6.3918 cPs, respectively, while the formulae using hydroxypropylmethylcellulose (formula C, F and I) have viscosity values of 1.4927, 4.2316 and 10.4278 cPs. It was found that formula using HPMC showed a higher viscosity compared to MC and SCMC with the same concentration. In general, an increase concentration of MC, SCMC and HPMC increased the solution's viscosity (Table 2). Pearson correlation coefficient indicated that HPMC concentration was positively correlated with viscosity ($p<0.05$).

Hatton and colleagues (1987) found that viscosity measurements of mucin-based substituts (5.0 ± 1 cPs) are most closely approximated to whole human saliva (6.0 ± 2 cPs). They also found that there is little positive correlation between the apparent viscosity of the saliva substitute and natural saliva and its relative lubricating ability. The mucin-based substitutes showed a greater ability to lubricate the interface than most of the CMC based substitutes (generally prepared in a 1 % aqueous solution) although its viscosity was lower (10 – 15 cPs).

The result obtained showed that 0.75% MC, SCMC 0.75% and HPMC 0.5% (formula G, H and F) have viscosities closest to human saliva and mucin-based saliva substitutes. This finding suggests that cellulose-albumin mixture in lower concentration of cellulose have viscosity properties closest to whole human saliva and mucin-based saliva substitutes.

The addition of albumin in our formulations may have important clinical implications for salivary substitutes. Perhaps residual salivary function in xerostomic individuals can be augmented by the addition of biologically ubiquitous entity (i.e., albumin) in a substitute fluid. Reducing the concentration of cellulose in our formulation, compared to 1 % CMC in available saliva substitutes can also reduce the complaint of patients about sticky accumulation at particular sites in the mouth when using a CMC-based saliva substitutes. In addition, sodium fluoride is included for mineralization of the dentition (Alpöz et al., 2008).

Table 2. Physical Properties of Artificial Saliva

	Formula	pH	Density (g/ml^3)	Viscosity (cPs)
A	0.25% MC	7.03	1.092	1.3491
		-0.115	-0.0002	-0.005
B	0.25% SCMS	7.04	1.1637	1.3881
		-0.115	-0.0003	-0.0812
C	0.25% HPMC	7.03	1.192	1.4927
		-0.115	-0.0003	-0.0003
D	0.5% MC	7.05	1.0539	2.2812
		-0.1	-0.0002	0.01
E	0.5% SCMC	7.05	1.0584	2.6657
		-0.057	-0.0002	-0.0005
F	0.75% HPMC	7.05	1.0639	4.2316
		-0.1	-0.0005	-0.0508
G	0.25% MC	7.04	1.0961	5.0717
		-0.057	-0.0004	-0.006
H	0.5% SCMC	7.05	1.1072	6.3918
		-0.057	-0.0005	-0.0046
I	0.75% HPMC	7.04	1.0989	10.4278
		0.037	-0.0003	-0.0126

pH

As can be gleaned from Table2, the pH value for all the formulations were ranging from 7.03 to 7.05. From this it can be concluded that there was no pH difference among the different bases (methyl cellulose, sodium carboxymethylcellulose and hydroxylpropyl-methylcellulose) of formulation. According to Smith and Morton (2001), the alkalinity or acidity of saliva depends on the rate of flow but the pH is usually within the range 6.2 – 8.0.

A statistically significant difference was observed among the pH values of the various sodium carboxymethylcellulose (SCMC) and hydroxypropylmethylcellulose) HPMC concentrations ($p<0.05$). Statistical result also shows that there was little increase in pH value when concentration of SCMC increased. In contrast, pH value decreased when concentration of HPMC increased. pH value of human saliva is 5.5

– 7.5. Therefore our formulations can be accepted.

Preservative Content

Methyl paraben was used as the preservative in all the formulations. As part of the physico-chemical tests, the determination of methyl paraben content was carried out using uv-spectrofotometric system. The wavelength of 254 nm was selected in the determination of methyl paraben in all formulations because methyl paraben is shown to have maximum absorption in this wavelength.

The calibration curve was obtained for the concentration ranging from 0.5 to 10 µg/ml. The coefficients of correlation were obtained with a mean r value of 0.9971 (should more than 0.99, though >0.95 is still acceptable). For intra-day coefficient variant values (C.V), precision, recovery and accuracy assays were carried out. Three concentrations were chosen to represent the entire range of the calibration curve, with 0.5 µg/ml representing low, 4 µg/ml representing medium, and 10 µg/ml representing high range). The CV, precision, recovery and accuracy were determined using three readings for each chosen concentrations. The precision around the mean value should not exceed 15% of CV and the mean values should within ± 15% deviation of the nominal value for accuracy. The CV values of sample tests varied from 0 to 1.66% that's mean the CV values are within the acceptance of range. The data are presented in Table 3.

Results obtain from this method showed individual recovery value varied from 84.75% to 116.89%, with The British Pharmacopoeia recommending 80% to 120%. Therefore, from this analysis, we can conclude that methyl paraben is very stable in the new artificial saliva formulations with the average recovery within the acceptance range. The uv-spectro-photometer method showed a good result in the analysis of methyl paraben in that formulations study.

Microbial Limit Test

To perform the test pour plate method was chosen. All the artificial saliva formulations have been observed for their limit of microbial contamination from *Escherichia coli*, *Pseudomonas auregenosa* and *Staphilococcus aureus* using agar media. Chromocult coliform agar was used to observe the growth of *E. coli*, centrimide agar for *Pseudomonas* and vogel-johnson agar for *Staphylococcus aureus*.

All samples test at all concentrations were observed. No microbial colonies were recovered from the dishes representing the initial one in ten dilution of the specimen, express the results as less than 10 microorganisms per gram or per mL of specimen. It was therefore concluded that the test specimen meets the requirements for freedom from *E. coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Table 3. Recovery tests and coefficients of variation using the uv-visible spectrophotometer (n=3)

Sample	Expected value (µg/ml)	Value obtained (µg/ml)	Inaccuracy (%)	Recovery (%)	CV
A	0.5	0.529	5.84	105.84	0.29
	4	4.140	3.35	103.35	0.09
	10	9.026	9.74	89.74	0.23
B	0.5	0.574	14.79	114.79	1.66
	4	3.660	8.57	91.43	0.15
	10	9.425	5.75	94.25	1.42
C	0.5	0.584	16.89	116.89	0.26
	4	3.923	1.93	98.07	0.12
	10	9.702	2.98	97.02	0.78
D	0.5	0.554	10.78	110.78	0.34
	4	4.104	2.59	102.59	0
	10	9.733	2.67	97.33	0
E	0.5	0.502	0.46	100.36	0.42
	4	4.013	0.33	100.33	0.16
	10	9.671	3.29	96.71	0.99
F	0.5	0.493	1.47	98.53	0.49
	4	4.040	1.01	101.01	0.28
	10	9.578	4.22	95.78	0.79
G	0.5	0.514	2.84	102.84	0.18
	4	3.850	3.83	96.25	0.21
	10	9.425	5.76	94.25	1.42
H	0.5	0.499	0.22	99.82	0.28
	4	3.928	1.81	98.20	0.16
	10	9.425	5.77	94.25	1.42
I	0.5	0.424	13.91	84.75	0.43
	4	3.789	5.28	94.72	0.16
	10	9.344	6.56	93.44	1.06

Stability Test

The stability study for physical factors for the new formulation includes observation for changes in odour, appearance, color, pH, density and viscosity. Study of chemical stability includes determination of preservative content. As a result of visual observation for physical factors like odor, appearance and color, no change was observed in all the formulations after three months of storage at 40°C and 75% RH. However, Pearson's correlation coefficients indicated that time storage was positively correlated with pH, density and viscosity ($p<0.01$). pH, density and viscosity of all sample artificial saliva formulations will decrease when storage time increases.

Stability-indicating assay of methyl paraben in all new artificial saliva formulations using uv-visible spectrophotometer method shows that individual recovery value vary from 88.30% to 117.18 %. In the one-month stability study, the recovery for methyl paraben was within the range of 88.30% to 117.18%, where as in the two-months study it was 88.30% to 117.04 and in the three-months study the recovery was 88.30% to 117.04%. The results obtained showed that concentrations of methyl paraben lie within the

range of 80% to 120% as recommended by The British Pharmacopoeia (2000). The result also showed that methyl paraben is very stable in the new artificial saliva formulations as shown by the average recovery which was found to be within the acceptance range.

IV. CONCLUSION

The new artificial saliva formulations containing various ratios of SCMC, MC and HPMC have been developed. All the formulations show high standard of quality. This can be gleaned from the description of appearance and physical properties where all the ingredients were found chemically and physically compatible. Combination of cellulose derivatives and albumin in these formulations resulted in the physical properties of these new artificial saliva substitutes closely resembling human saliva and mucin-based saliva substitutes. Formulations E, G and H were found to be the most suitable formulae due to their viscosity properties which closely resemble human saliva and mucin based saliva substitutes. The pH of all formulations fall within the range of the normal pH of human saliva. Incorporation of orange flavour and dextrose increased the taste of the formulation. The addition of electrolytes that mimic those found in natural saliva and fluoride as protective element can help not only to maintain the integrity of teeth but also to increase the clinical performance of these preparations.

Acknowledgment

The author research programs are supported by FELDA grants 4111169 "Penyelidikan Felda" and University Malaya.

References

[1]. Ali, MS, Chaudhary, MS, & Takieddin, MA 1999, 'Simultaneous determination of metronidazole benzoate, methylparaben and propylparaben by high-performance liquid chromatography', *Drug Development on Industrial Pharmacy*, **vol.25, No. 10**, pp. 1143-1147.

[2]. Aulton, ME 1988, *Pharmaceutics: the Science of Dosage Form Design*, ELBS, Churchill Livingstone, United Kingdom.

[3]. Allal, AS, Dulquerov, P, & Bieri, S 2000, 'Assessment of quality of life in patients treated with accelerated radiotherapy for laryngeal and hypopharyngeal carcinomas', *Head Neck*, **vol22**, pp. 288-293.

[4]. Alpöz, E, Guneri, P, Onder, G, Cankaya, H, Kabasakal, Y, & Kose, T 2008, 'The Efficacy of Xialine® in patients with Sjogren's syndrome: a single-blind, cross-over study', *Clin Oral Invest*, **vol.12**, pp. 165-172.

[5]. British National Formulary 2009, *British National Formulary*. London. BMJ Group & RPS Publishing.

[6]. Bolton, S 1997, *Pharmaceutical Statistics Practical and Clinical Applications*, Marcel Dekker Inc., New York.

[7]. Burket, LW, Greenberg, MS, Glick, M, & Ship, JA 2008, *Burket's Oral Medicine*, BC Decker Inc., Ontario.

[8]. Davies, A & Finlay, IG 2005, *Oral care in advanced disease*. Oxford University Press London.

[9]. Duxbury, AJ, Thakker, NS & Wastell, D G 1989, 'A double-blind cross-over trial of a mucin-containing artificial saliva', *British Dental Journal,* **vol**. **166**, **pp. 115-120.**

[10]. Fox, PC 1997, 'Management of dry mouth', *Dental Clinics of North America,***vol.41, no. 4, pp.863-875**.

[11]. Grotz, KA 2003, 'Dental care for patients with antineoplastic radiotherapy of the head and neck', *Strahlenther Onkol,* **vol.179, pp. 275-278**.

[12]. Hatton, MN, Levine, MJ, Margarone, JE, & Aguirre, A 1987, 'Lubrication and viscosity features of human saliva and commercially available saliva substitutes', *Journal Oral Maxillofac Surg*, **vol.45**, **pp. 496-499.**

[13]. Hahnel, S, Behr, M, Handel, G & Bürgers, R 2009, 'Saliva substitutes for the treatment ofradiation-induced xerostomia-a review', *Support Care Cancer,* **vol.17, pp. 1331-1343.**

[14]. Joyston-Bechal, S & Kidd, EAM 1987, 'The effect of three commercially available saliva substitutes on enamel in vitro',*Br. Dent. J,***vol.63, pp. 187.**

[15]. Kelly, C 2001, 'Sjogren's syndrome and the respiratory system', viewed 12 September 2002, <http://www. webhealth.co.uk /WbHealth_A-Z of Health/About Sjogren_s Syndrome>.

[16]. Lund, W, (ed) 1994, *The Pharmaceutical Codex,* 12th edn. The Pharmaceutical Press, London.

[17]. Levine, MJ 1993, 'Development of artificial salivas', *Critical Reviews in Oral Biology and Medicine*, **vol.4, no. 3-4, pp.279-286**.

[18]. Leung, VWH & Darvell, BW 1997, 'Artificial salivas for in vitro studies of dental materials', *Journal of dentistry,* **vol.25, no. 6, pp. 475-484.**

[19]. Myers, EN & Ferris, RL 2007, *Salivary Gland Disoreders,* Springer, New York.

[20]. Momm, F, Muller, M, & Tsekos, A 2001, 'Xerostomia after radiotherapy: more effective treatment by a mucin-containing spray, *HNO,***vol.49**, **pp. 831-836**.

[21]. Momm, F, Volegova-Neher, NJ, Schulte-Monting, J & Guttenberger, R 2005, 'Different Saliva Substitutes for Treatment of Xerostomia Following Radiotherapy', *Stralenther Onkol,***vol. 181, pp. 231-236.**

[22]. Philip, DDS 1997, 'Management of dry mouth', *Dental Clinic of North America*, **vol. 41, no. 4, pp. 863 –875.**

[23]. Ramachandran, S, Chen, S & Etzler F 1999, 'Rheological characterization of hydroxypropylcellulose gels', *Drug Development and Industrial Pharmacy,***vol. 25, no. 2, pp. 153-161.**

[24]. Radus, TP & Gretchen, G 1983, 'Determination of antimicrobial preservatives in pharmaceutical formulations using reverse-phase liquid chromatography' *Journal of pharmaceutical sciences,* **vol. 72, no. 3, pp. 221-224.**

[25]. Regelink, G, Vissink, A, Reitsema, H & Nauta, JM 1998, 'Efficacy of a synthetic polymer saliva substitute in reducing oral complaints of patients suffering from irradiation-induced xerostomia', *Quintessense International,***vol. 29 no. 6, pp. 383-387.**

[26]. Sidney, HW & James, RS 1993, *Good Manufacturing Practices for Pharmaceuticals*. Marcel Dekker, Inc., New York.

[27]. Smith, ME, & Morton, DG 2001, *The Digestive System,* Elsevier Health Sciences.

[28]. Srivastava, LM 2004, *Textbook of Biochemistry and Human Biology*, Prentice-Hall of India Private Limited, New Delhi.

[29]. Sweetman SC (edt.) 2007, *Martindale, The Extra Pharmacopeia,* Pharmaceutical Press, Council of the Royal Pharmaceutical Society of Great Britain.

[30]. The United States Pharmacopeial convention 1995, *The United States Pharmacopeia. The National Formulary*, The United States Pharmacopeial Convention, Inc., Reckville.

[31]. Tenevuo, JO 1981, *Human Saliva: Clinical Chemistry and Microbiolog,* CRC Press.
[32]. Troy, DB (ed) 2006, *Remington: The Science and Practice of Pharmacy*, Lippincot William & Wilkins, Philadelphia.
[33]. Vissink, A, 's-Gravenmade, EJ, Panders, AK, Vermey, A, Petersen, JK, Visch, LL & Schaub, RMH 1983, 'A clinical comparison between commercially available mucin- and CMC- containing saliva substitutes', *Int. J. Oral Surg*, **vol. 12, pp. 232-238.**
[34]. Vissink, A, Waterman, HA, 's-Gravenmade, EJ, Panders, AK. and Vermey, A 1984, 'Rheological properties of saliva substitutes containing mucin, carboxymethylcellulose or polyethylenoxide', *Journal of Oral Pathology*, **vol. 13, pp. 22-28.**