## ARTIFICIAL SALIVA PREPARATION

The artificial saliva used in this present study were prepared according to Macknight-Hane and Whitford (1992) formula. The sorbital was not used because the artificial saliva would be more viscous than normal saliva when the sorbital was mixed together with sodium carboxymethyl cellulose (Levine, *et al.*,1987).

The compositions of artificial saliva (grams per liter):

Methyl-p-hydroxybenzoate	2.00
Sodium Carboxymethyl Cellulose	10.00
KCI	0.625
MgCl <sub>2</sub> . 6H <sub>2</sub> O	0.059
CaCl <sub>2</sub> . 2 H <sub>2</sub> O	0.166
K₂HPO₄	0.804
KH <sub>2</sub> PO <sub>4</sub>	0.326

The pH of artificial saliva was adjusted to 6.75 with KOH.

#### Preparation

- 1. Dissolving 2 g of methyl-p-hydroxybenzoate in 800 ml of distilled water (solubility: methyl-p-hydroxybenzoate 1 g in 400 ml of water). 20 ml of the solution was storing for other chemical agent solvent. The remaining solution was storing in refrigerator.
- 2. Boiling 200 ml of distilled water then sprinkled 10 g of sodium carboxymethyl cellulose into boiling water and stirring until total of sodium carboxymethyl cellulose was dissolved.

- 3. Pouring cold methyl-p-hydroxybenzoate solution (from item 1) into sodium carboxymethyl cellulose solution (from item 2) then Mixing them together until they are in gel form.
- 4. Dissolving 0.625 g of KCl in methyl-p-hydroxybenzoate solution (from item 1) then pouring the solution into the solution in item 3 and mixing them together.
- 5. Dissolving 0.059 g of MgCl<sub>2</sub> . 6H<sub>2</sub>O in methyl-p-hydroxybenzoate solution (from item 1) then pouring the solution into the solution in item 4 and mixing them together.
- 6. Dissolving 0.166 g of CaCl<sub>2</sub> . 2 H<sub>2</sub>O in methyl-p-hydroxybenzoate solution (from item 1) then pouring the solution into the solution in item 5 and mixing them together.
- 7. Dissolving 0.804 g of  $K_2HPO_4$  in methyl-p-hydroxybenzoate solution (from item 1) then pouring the solution into the solution in item 6 and mixing them together.
- 8. Dissolving 0.326 g of KH<sub>2</sub>PO<sub>4</sub> in methyl-p-hydroxybenzoate solution (from item 1) then pouring the solution into the solution in item 7 and mixing them together.
  - 9. Adjusting the pH of artificial saliva to 6.75 with KOH.

## **EQUIPMENTS**

1. pH meter (Precisa; model pH 900, Precisa Instruments AG, Dietikon, Switzerland)



Figure 14. pH meter (Precisa; model pH 900)

2. The rotator shaking machine

This machine consist with:

- 1. power switch
- 2. drive motor
- 3. thermal protection fuse
- 4. support pillar (bottle handler shaft supporter)
- 5. driver belt
- 6. bottle fixing lever (bottle hander)
- 7. bottle

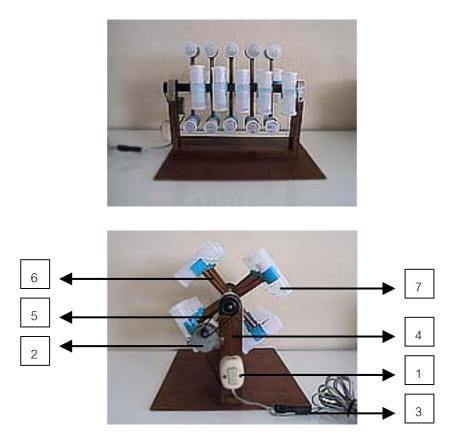


Figure 15. Rotator shaking machine and the composition of machine.

3. magnetic stirrer (Corning; model PC 420, Corning Incorporated, New York, USA)



Figure 16. Magnetic stirrer (Corning; model PC 420)

4. Inductively Couple Plasma Optical Emission Spectrometer, ICP-OES (Perkin Elemer; model Optima 4300 DV, Connecticut, USA)



Figure 17. Inductively Coupled Plasma Optical Emission Spectrometer, ICP-OES.

5. Scanning electron microscope (JEOL; model JSM 5800 LV, JEOL, Tokyo, Japan)



Figure 18. Scanning electron microscope (JEOL; model JSM 5800 LV)

## RAW DATA OF DISSOLVED CALCIUM MEASUREMENT

Table 5. Raw data of dissolved calcium measurement by ICP-OES.

Са	Group A	Group B	Group C
Tooth No.	(mg/L)	(mg/L)	(mg/L)
1	53.8	28.2	26.9
2	56.0	49.9	30.0
3	40.9	25.9	28.2
4	33.0	27.3	23.1
5	33.0	35.3	26.1
6	61.5	32.3	24.1
7	54.0	37.2	30.6
8	61.7	49.9	38.5
9	49.5	36.7	29.9
10	37.8	27.5	27.0
11	43.0	32.2	22.0
12	49.0	25.8	33.3
13	38.6	35.2	28.8
14	45.2	41.4	28.0
15	45.7	20.3	29.8
Mean	46.85	33.67	28.42
SD	9.33	8.56	4.09

- Group A Single fluoride varnish application group immersed in 0.05 mol/L acetic acid with 1 mg/L fluoride.
- Group B Single fluoride varnish application group immersed in 0.05 mol/L acetic acid with 10 mg/L fluoride.
- Group C Intensive fluoride varnish application group immersed in 0.05 mol/L acetic acid with 10 mg/L fluoride.

# **SOLUBILITY AND SOLUBILITY PRODUCT CONSTANT**

## **Solubility**

Chemical agent	Solubility
-KCI	1 g in 2.8 ml of water
-MgCl <sub>2</sub> . 6H <sub>2</sub> O	1 g in 0.6 ml of water
-CaCl <sub>2</sub> . 2 H <sub>2</sub> O	freely soluble
-K₂HPO₄	very soluble in water
-KH <sub>2</sub> PO <sub>4</sub>	1 g in 4.5 ml of water
solubility product constant (K <sub>sp</sub> )	

 $K_{sp} = 1.7 \times 10^{-10}$ -CaF<sub>2</sub>