CHAPTER III

MATERIALS AND METHODS

Instruments

1. Chem-mate^{TM} pH Meter ; combined glass electrode (Beckman Instruments,Inc., Fullerton CA92634)
2. Pycnometer (Arthur H. Thomas Company, capac. 10 ml., No. 8350-B28)
3. Ostwald viscometer (Arthur H. Thomas Company, No. 7162-N10)
4. Thermo Incubator Model SCIXI
5. Heraeus Incubator Type FB420
6. Incubator (Laboratory Thermal Equipment Ltd., Serial No. 39384)
7. Incubator (Memmert, No. Tv4ouL998oo1)

Chemicals

1. PVP-I USP.(G.A.F.Chemicals U.S.A. Lot.cx677-8, contained 10.2% available iodine, 5.1% iodide)
2. Commercial arrow root starch (BDH Chemicals)
3. Sodium dihydrogen phosphate (Riedel-De Haenag Seelze-Hannover)
4. Disodium hydrogen phosphate (Riedel-De Haenag Seelze-Hannover)

5. Glacial acetic acid (E-Merck)
6. Sodium acetate (E-Merck)
7. Citric acid (BDH Chemicals)
8. Concentrated hydrochloric acid 37% (E-Merck)
9. Ammonium thiocyanate (May and Baker)
10. Sodium carbonate (E-Merck)
11. Sodium chloride (E-Merck)
12. Potassium iodide (E-merck)
13. Sodium bicarbonate (E-Merck)
14. Sodium bisulfite (E-Merck)
15. Eosin Y (E-Merck)
16. Ferric ammonium sulfate (E-Merck)
17. Sodium thiosulfate (E-Merck)
18. Methanol (E-Merck)
19. Silver nitrate (E-Merck)
20. Potassium dichromate (E-merck)
21. Nitric acid 65% (E-Merck)

Chemicals no.1-2 are pharmaceutical grade. The others are analytical grade.

Solutions

Volumetric solutions (according to USP XX)

0.02 N Sodium thiosulfate VS

0.1 N Silver nitrate VS

0.1 N Ammonium thiocyanate VS

Test solutions (according to USP XX)

Starch TS

Eosin Y TS

Ferric Ammonium Sulfate TS

Sodium Bisulfite TS

Analytical method

Available iodine, total iodine, and iodide ion in PVP-I USP. and 10% w/v PVP-I solution were determined according to the process in USP XX.

Experiments

1.Buffer and pH

The pH of the sample was set at 5.5 which was the normal pH of the skin. The appropriate buffers had been selected from general pharmaceutical products available at this pH. The selected buffers were phosphate, acetate and citrate buffer.

2.Preparation of 10% w/v PVP-I buffered solution

Various solutions of PVP-I were prepared with the basic formular :

PVP-I	38.5	g
buffer qs. to selected pH		
distilled water qs. ad.	350	ml

Eighteen formulations were employed in the study. The formulations differed only in buffer systems and/or concentrations of the buffers (as shown in Table 3). Formulars F-1 to F-9 were first prepared to study the effect of single buffer at different concentrations (0.05 M, 0.10 M, 0.15 M).

2.1 Make 10% w/v stock solutions of citric acid, acetic acid, sodium acetate, sodium dihydrogen phosphate and disodium hydrogen phosphate.

2.2 Pipett required volume of stock buffer solution (to make concentration as in Table 3 for 350-ml preparation) in about 250 ml water and adjust pH approximately 5.5 by 1 N sodium hydroxide.

ratio of eack buffer to make pH about 5.5

phosphate buffer : ratio of sodium dihydrogen phosphate to disodium hydrogen phosphate is 49.88.

acetate buffer : ratio of acetic acid to sodium acetate is 0.1807.

citrate buffer : use only citric acid and adjust pH with 1 N sodium hydroxide.

2.3 Add in small portions PVP-I under continuous stirring mechanically to get a homogenous solution. The pH of solution was again adjusted to 5.5. The final

Table 3 Total Buffer Concentration Added in 10% w/v PVP-I Solution.

Formular	Phosphate buffer	Acetate buffer	Citrate buffer
F-1	0.05 M	-	-
F-2	0.10 M	-	-
F-3	0.15 M	-	-
F-4	-	0.05 M	-
F-5	-	0.10 M	-
F-6	-	0.15 M	-
F-7	-	-	0.05 M
F-8	-	-	0.10 M
F-9	-	-	0.15 M

volume was adjusted to 350 ml with water.

2.4 The prepared solution was devided into 10 portions by filling in 30 ml.- ambered glass bottle with nylon closure and polypropylene screw cap.

3.Degradation study of 10%w/v PVP-I buffered solutions

The solutions of single buffer (F-1 to F-9) were kept in the constant temperature incubators at 60, 45, 40, and 35°C. The samples were analyzed for available iodine at suitable time intervals. The pH changes of each buffered solution were also recorded. The concentrations of total iodine were determined occasionally to test permeability of iodine through the surface of containers.

The first sample was considered to be 100% of remaining concentration of available iodine and the subsequent samples were calculated as a relative percentage amount of the original available iodine.

4.Evaluation of the effects of buffers upon PVP-I stability

The effects of buffers upon chemical stability of PVP-I were compared by statistical method (ANOVA test). Higher degradation would result in less stability. The

final pH of each buffer was also measured.

5.Selection of suitable buffer with appropriate-concentration.

In the previous section, the effects of various possible buffers on the stability of PVP-I solutions were thoroughly investigated. The effective buffers with appropriate concentration were assembled in order to obtain the suitable products in this section.

Formulations of mixed buffers (phosphate and citrate buffers) at various ratios (as shown in Table 4, F-10 to F-18) were prepared and kept in 35^{o}C incubator. All formulars were divided into 10 portions of 30-ml amber glass bottles with nylon closure and polypropylene screw cap. The available iodine remaining was determined at suitable intervals. The degradation rates and pH of solutions were recorded. The results were evaluated by considering the plots of available iodine concentration remaining against incubation time and the appearance degradation rates.

6.Evaluation of the effects of various solvents on the stability of 10% w/v PVP-I buffered solutions.

Based on information from the previous section, the best formular was prepared by using various solvents,

Table 4 Total Concentration of Selected Buffers Added in 10% w/v PVP-1 Solutions According to Previous Experiments

Formular	Phosphate buffer	Citrate buffer
F-10	0.025 M	0.025 M
F-11	0.025 M	0.050 M
F-12	0.025 M	0.075 M
F-13	0.050 M	0.025 M
F-14	0.050 M	0.050 M
F-15	0.050 M	0.075 M
F-16	0.075 M	0.025 M
F-17	0.075 M	0.050 M
F-18	0.075 M	0.075 M

i.e., single distilled water, deionized water, and tap water (potable water). The pH of water before preparing was measured. All preparations were kept in 35°C incubator, and were analyzed for available iodine remaining at the suitable time intervals. The data and available iodine remaining against incubation time plots were compared to evaluate the effects of various vehicle on the stability of 10%w/v PVP-I buffered solution.

7.Evaluation of the effects of packaging materials on the stability of 10% w/v PVP-I buffered solution

According to the result from 5 and 6, the effective formular in appreciable solvent was prepared and filled in containers from various materials, i.e., clear glass, amber glass, low density polyethylene (LDPE), high density polyethylene (HDPE), and polypropylene (PP). Ten bottles of each sort of containers were filled with the solution and placed in room temperature. Available iodine remaining at suitable time intervals was determined and then was evaluated by degradation plots and appearance degradation rates.