CHAPTER IV

RESULTS AND DISCUSSIONS

Degradation Model of 10% w/v PVP-I Buffered Solutions

Various buffered formulations (F-1 to F-9, Table 3) were incubated at 60, 45, 40, 35°C. Typical plots of available iodine concentration remaining against time and natural logarithm of available iodine at concentration remaining against time various temperature were shown in Figure 4-7 and Figure 8-11. respectively. Table 5 was a typical data obtained from Formular F-1 kept at 60°C. The data was treated as zero order reaction and first order reaction.

The plots in Figure 4-7 and Figure 8-11 showed that the models of zero order and first order degradation were not appropriate to the whole data of this study. Zero order plots as well as first order plots showed curvature at the initial parts of the plots. The graphical character showed rapid decrease of available iodine at the initial stage beyond which the graphs displayed the linearity of both concentration and natural logarithm of concentration against time. The linearity appeared after about 10, 30, 30 and 40 days of 60, 45, 40 35°C incubation, respectively, by considering the and

graphical plots together with the pH of the solutions. The slopes and correlation coefficients of these linear portion of the plots were listed in Table 6a-6c. The values of correlation coefficient from both calculation were almost equal. It meaned that almost no difference between the two values.

However, PVP and iodine were complexed and iodine was slowly released into solutions (9). PVP acted as a stable depot for iodine (10). The concentration of free available iodine was constant throughout most of the reaction. The mechanism was shown as follows.

$$PVP-I_{3}^{-} \iff PVP + I_{3}^{-}$$

$$I_{3}^{-} \iff I_{2} + I^{-}$$

$$I_{2} + H_{2}0 \iff H^{+} + I^{-} + HOI$$

Consequently, the later linear portion of plots was postulated to be zero order degradation because of the aboved reasons and the almost constant pH of the solution at the later part of experiment.

Rapid decrease of available iodine in initial stage (about 10-15%) could be total result of the following effects:

1. It might be because of reversible reaction of iodine hydrolysis in water. At the initial state that pH

of the solution was adjust to 5.5 that was far from the nature of 10% w/v PVP-I solution, the concentration of hydrogen ion was small so the equilibria shifted to the right side.

2. There might be excess iodine left from complex formation step. This iodine would degrade quicker than iodine complexed with PVP.

3.PVP-I complex might undergo structural changes in aqueous solution . The structure of PVP-I prepared by heating PVP with iodine was postulated to be an adduct of the polymer and hydrogen triiodide in which a proton was fixed between 2 carbonyl groups of two pyrrolidone rings and the triiodide anion was bound ionically to this complex cation (37). The studies of PVP-I structure in an aqueous solution (18) supported the view of a helical model of PVP chain with iodine in hydrophobic cavity. It was not elucidated whether the chromophore was iodine molecule or triiodide chain or a mixture of iodine and iodide in varying proportions. The variation of structure might cause more released iodine than dry condition. As a result, more iodine degraded in the first period until equilibria occured.

4.PVP was polydispersed; i.e. it consisted of a mixtures of polymer molecule of different molecular

weight. The lower molecular weight PVP, because of its lower degree of polymerization, had more oxidisable fragments or end groups. PVP-I of lower molecular weight was less stable (58). These oxidisable fragments might cause rapid degradation in the initial state.

5. The impurities from the polymerization of vinylpyrrolidone such as ammonia (NH_3) and vinylpyrrolidone in PVP-I could reacted with iodine in the initial stage of incubation (61).

 $3I_2 + 6NH_4OH \rightarrow 5NH_4I + NH_4IO_3 + 3H_2O$

Accordingly, the appearance initial rapid degradation rates at four different temperature were calculated in model of cubic polynomial regression by using HP-41C STAT PAC calculator of Hewlett Packard, Oregon U.S.A. The degradation rates and correlation coefficient (r) of the data were shown in Table 7. The average r value was 0.9734.

Ultimately, the initial degradation was suspected to be cubic polynomial regression and the later degradation was postulated to be zero order.

Effect of Buffer upon the Degradation of 10% w/v PVP-I Solutions

In this study, phosphate, acetate and citrate buffers at various concentrations were used in the preparations (F-1 to F-9). Phosphate buffers were prepared in F-1 to F-3 with the concentrations of 0.05 M, 0.10 M, 0.15 M. Acetate and citrate buffers with the same concentrations as phosphate buffers were also prepared in F-4 to F-6 and F-7 to F-9, respectively. Their effects upon the stability of PVP-I solution were investigated by determining available iodine concentration and pH after incubation.

The comparison plots of the effect of various buffers at the concentration of 0.05 M, 0.10 M, and 0.15 M at various temperatures were shown in Figure 12-15, Figure 16-19, and Figure 20-23, respectively. The effects of varying the concentrations of buffers were displayed in following figures: phosphate buffers in Figure 24-27, acetate buffers in Figure 28-31, citrate buffers in Figure 32-35.

Because plots of concentration remaining against time were curved at the initial incubation period, the effects of buffers were evaluated as the appearance initial rapid degradation rates and later linear

degradation rates (shown in Table 8a-8b, Table 9a-9b, and Figure 36-37) by using ANOVA test (randomized complete block design). The 95% confidence interval of later linear degradation rates were shown in Table 10 and Figure 38-41. The available iodine remaining after 70 day incubation was displayed in Table 11. The pH of the solution after incubated for 70 days were also listed in Table 12.

The evaluation was separated into 2 parts, first, considering ANOVA test of initial rapid degradation and, second, considering that of later linear degradation.

a)ANOVA test of initial rapid degradation.

The effects of various buffers at the same concentration (Table 8a):

At the buffer concentration of 0.05 M, the appearance initial degradation rates were found to be significantly different at 95% confidence level among the three buffers. The stability of PVP-I buffered solutions decreased in the following order: phosphate buffer (most stable) > acetate buffer > citrate buffer.

At the buffer concentration of 0.10 M and 0.15M, there was no significant difference among the three buffers. However, by examining average degradation rates

of each buffer, the tendency of degradation was the same order as 0.05 M concentration.

The effect of various concentrations of the same buffer (Table 8b):

The stability of PVP-I in phosphate buffer had shown buffer concentration dependent significantly. The appearance degradation rates were in the order: 0.05 M < 0.10 M < 0.15 M.

The variation of concentrations of citrate and acetate concentrations showed no significant difference on the appearance initial degradation rates. Nevertheless, the average initial degradation rates of each concentration of acetate buffer were in the order as phosphate buffer.

b)ANOVA test of later linear degradation.

The effect of various buffers at the same concentration (Table 9a):

At the buffer concentration of 0.05 M and 0.10 M, the ANOVA showed that there was significant difference among the three buffers. The stability of PVP-I was in the decreasing order when used the following buffers: phosphate buffer (most stable), acetate buffer, citrate buffer. At the buffer concentration of 0.15 M, there was no significant difference caused by varying these three buffers, but the average degradation rates of each buffered solution were also in the order as above.

The effect of various concentrations of the same buffer (Table 9b):

Varying the concentration of three buffers, linear degradation rates of PVP-I buffered solution using phosphate and citrate buffer showed buffer concentration dependent significantly, whereas that using acetate buffer did not. The degradation rates of PVP-I in first two buffers were in the order: 0.05 M (most stable) < 0.10 M < 0.15 M.

three buffers are commonly These used in pharmaceutical preparations at the pH of about 5.50 with the impression that they can keep pH constant or almost constant and that they will exhibit least effect upon the stability of PVP-I. Trubitsyma, S.N. (56) reported the effect of citric acid upon PVP-I stability. They found that there might be structural deterioration due to between PVP and citric acid. The same effect H-bonding could occured in acetate buffers because of carboxylic group but there were less carboxylic groups in acetate buffer than in citrat buffer at the same concentration.

So PVP-I in acetate buffer were more stable than in citrate buffer. In case of phosphate buffers, they always formed dimer between two phosphate molecules. Phosphate did not affect the binding of PVP and iodine.

We could conclude from Table 8a-8b and Table 9a-9b that, at the same buffer concentration, the stability of PVP-I in buffered solutions tends to be in the order: phosphate buffer (most stable) > acetate buffer > citrate buffer (the pH of solutions was not constant throughout the experimment). With the same buffers, the higher the buffer concentration, the lower the stability of PVP-I would be.

Usually, the more degradation of PVP-I would result in lowering of the pH. In case of citrate buffer, despite it caused the highest degradation, it could keep the pH of PVP-I solutions better than the other two (as shown in Table 12).

The low pH of solution would affect the skin irritation. So the next experiments were designed in order to increase the stability and buffer capacity of the solution. Phosphate buffers were mixed with citrate buffers at the different concentrations according to Table 4, F-10 to F-18).

Selection of Suitable Buffer with Appropriate Concentration

the previous sections, the effects of single In buffer on the stability of 10% w/v PVP-I solution were investigated. The selected buffers were mixed and the preparations were incubated to observe their physical change (pH change) and chemical stability (available iodine remaining). The result from the previous studies showed that 10% w/v PVP-I solution in phosphate buffer exhibited the best chemical stability, while that in citrate buffer had the best buffer capacity. In order to yield an appropriate formular, many mixtures of phosphate and citrate buffers with various proportions were prepared (Table 4). The degradation profiles of formular F-10 to F-18 were shown in Figure 42. Figure 43-45 showed the comparison of the effect of mixed buffers and single buffer at the same total concentraion. The comparison profiles, when one buffer component was fixed and another was varied, were displayed in Figure 46-51. The degradation rates and pH change after 70 day incubation were recorded in Table 13 and were graphically compared in Figure 52. The appearance degradation rates and pH after 70 day incubation of mixed buffers and single buffer at the same total concentration were compared in Table 14.

Figure 43-45 and Table 14 showed that most mixed buffers gave more appreciable result than single buffer except 0.15 M total buffer concentration.

The graphical comparison of Figure 46-51 showed that when one buffer concentration was fixed and another was increased, the degradation also increased. By examining the gap between graph when fixed one buffer component at a certain concentration, increasing citrate concentration and fixing phosphate concentration make wider gap among three graph lines than increasing phosphate buffer. As the result, increasing concentration of citrate buffer affected the degradation more than increasing that of phosphate buffer.

Table 13 showed that increasing phosphate concentration while citrate concentration was fixed scarcely affected pH maintenance. On the contrary, increasing citrate concentration while phosphate concentration was fixed enhanced better pH maintenance. However, the more citrate concentration, the more degradation would distinctly be. As the result, formular F-11 (0.025 M phosphate + 0.05 M citrate buffer) was the selected buffer. It could keep pH of 10% w/v PVP-I solution at 5.10 after 70 day incubation.

The Effect of Various Solvents (Sources of Water) on the Stability of 10% w/v PVP-I Buffered Solution

Formular F-11 from the previous section was selected and was used in this study. The degradation profiles in any source of water were graphically compared in Figure 53. Data of available iodine remaining was showed in Table 15 and was calculated to show appearance initial degradation rates and later linear degradation/ rates in Table 17. The pH of water that used in this experiment was 4.80 for single distilled water, 5.20 for DI water and 6.60 for potable water.

Varying sources of water (Table16), ANOVA test showed significant difference at 95% confidence limit in final concentration of available iodine remaining. By considering the appearance degradation rates (Table 17), 10% w/v PVP-I buffered solution in distilled water appeared to poss the greatest stability. The use of deionised water gave the better result than that of potable water (tap water).

Generally, water is a very unique solvent. Primarily because of its solvent power and physiological inertness, water is an extremely important pharmaceutical agent. In as much as water is used more cupiously and widely than other substance in pharmaceutical

manufacturing, its quality is of the utmost importance.

According to USP, the water used in this section is official in different states of purity. Supplying potable water is the starting material to prepare purified water (deionized water, distilled water). Supplying potable water is one of the most important operations in civilized communities. Potable water is usually drawn freshly from a public supply and must be suitable drinking. Potable water of satisfactory biological quality is available in many areas and may be used in preparations that are not intended to be sterile provided that the mineral impurities that it contains do not react with the medicaments or other ingredients. The overall process of local public system only involves (1)insoluble matter through removing of apropriate coaglulation, settling and filtering process; (2)destruction of pathogenic microorganisms by aeration and chlorination or other methods; (3) improvement of palatability through aeration and filtration through charcoal. Such potable water commonly contains iron, manganese, copper, zinc, calcium, magnesium, sulfate and chloride (WHO standard).

It is likely that iodine in the solutions might react with Fe and Fe^{2+} and chlorine (12) as follows:

	Fe	+	I ₂		FeI2	
2FeCl ₂	+ I ₂	+	2HC1	>	2HI +	2FeCl ₃
I ₂ +	5C1 ₂	+	6H ₂ 0	>	2HIO ₃	+ 10HCl

Deionized water and distilled water are in Purified Water monograph of USP. Deionization process can remove ionisable substance, and produce a water high specific substance. Colloidal and non-ionisable impurities such as pyrogens may not be removed by this process. By distillation, the purity of water increases because it is separated as vapour from non-volatile impurities and is subsequently condensed.

As the result of different states of purity, three sources of water gave the little different result of degradation of PVP-I in buffered solution.

The Effects of Packaging Materials on the Stability of 10% w/v PVP-I Buffered Solution

According to the previous result, formular F-11 in distilled water was prepared and filled in various containers, i.e., clear glass, amber glass, low density polyethylene (LDPE), high density polyethylene (HDPE) and polypropylene (PP). The effect of the containers from various materials on the stability of PVP-I could be seen in Figure 54. The evaluation was summarized in Table 18-20.

Raw data was showed in Table 18. The result of ANOVA table in Table 19 showed significant difference at the 95% confidence interval of final available iodine concentration remaining. Table 20 displayed appearance degradation rates of the solution in each container. The degradation of PVP-I in various containers exhibited in the following increasing orders :

LDPE < HDPE < amber glass < clear glass < PP.

The containers for commercial PVP-I preparations are always made from glass and plastic. The effect of packaging material on the stability of PVP-I solution was beyond the scope of this study. It was observed that PE was the most appropriate material to contain PVP-I solution. The disadvantage of glass may due to an ion exchange process at the surface of glass and yielding an appreciable quantity of alkali to water. Soda-lime glass or NP type glass are employed in production of nonparenteral preparation. It contains the modifiers of monovalents and divalents such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Ba²⁺ that may be replaced by hydroxonium ions in acidic or neutral aqueous solution (66). In case of PP, there may be the interaction between iodine and antioxidant that is the necessary additive in PP production (67).

With regard to glass container, amber-glass bottles seemed to be more suitable than clear glass container. This finding is in agreement with the matter that light appear to have little effect on the stability of iodine solution or tincture (12).

<u>Table 5</u> Stability of Available Iodine in 10% w/v PVP-I Solution Formular 1 (F-1) at $60\degree$ C

1.2.1

Time	Conc. of	f availal	total I ₂	рН	
(days)	gm/100m1	percent	Natural logarithm	g/100m1	Solution
0.00	1.100	100.00	4.605	1.650	5.50
0.66	1.049	95.36	4.558	1.650	5.10
1.70	1.020	92.74	4.530	1.653	4.58
5.82	0.993	90.27	4.503	1.650	3.70
11.77	0.979	88.99	4.488	1.645	3.50
18.79	0.975	88.61	4.488	1.668	2.80
28.75	0.950	86.33	4.458	1.653	2.80
42.69	0.938	85.23	4.445	1.679	2.80
56.67	0.923	83.91	4.430	1.679	2.80
70.63	0.904	82.15	4.408	1.661	2.80
1					

Table 6a Slope of Regression Line and Correlation Coefficient

ਸ	Slor	e of reg	fression	line	Correlation coefficient					
•	60 °C	45 [°] C	40 °C	35 [°] C	60 [°] C	45 [°] C	40 [°] C	35 [°] C		
F-1	-0.1214	-0.0571	-0.0159	-0.0114	-0.9980	-0.9973	-0.9853	-0.9928		
F-2	-0.1705	-0.0738	-0.0424	-0.0342	-0.9937	-0.9712	-0.9752	-0.9833		
F-3	-0.1437	-0.0990	~0.0550	-0.0500	-0.9916	-0.9982	-0.9965	-0.9800		
F-4	-0.1287	-0.0749	-0.0429	-0.0637	-0.9901	-0.9624	-0.9504	-0.9988		
F-5	-0.2038	-0.0747	-0.0600	-0.0541	-0.9919	-0.9920	-0.9943	-0.9902		
F-6	-0.1767	-0.0856	-0.0730	-0.0540	-0.9934	-0.9941	-0.9971	-0.9977		
F-7	-0.1714	-0.0722	-0.0551	-0.0392	-0.9880	-0.9992	-0.9693	-0.9763		
F-8	-0.2022	-0.0833	-0.0801	-0.0445	-0.9901	-0.9930	-0.9997	-0.9184		
F-9	-0.2410	-0.0965	-0.1029	-0.0515	-0.9981	-0.9775	-0.9978	-0.9850		

When Treated Later Part of Data as Zero Order Reaction

Note F = Formular

The day to begin calculation at 60 °C is the 10th day. The day to begin calculation at 45 °C is the 30th day. The day to begin calculation at 40 °C is the 30th day. The day to begin calculation at 35 °C is the 40th day.

Table 6b Slope of Regression Line When Treated Later Part

of Data as First Order Reaction

F		Slope of regres					sion 1	ine	9			
F	6	0 '	°c	45 [°] C		40 °C		35 [°] C		'c		
F-1	-1.40	x	10 ⁻³	-6.20	x	10-4	-1.70	x	10-4	-1.20	x	10-4
F-2	-2.06	x	10 ^{-a}	-8.80	x	10-4	-4.80	x	10-4	-3.90	x	10-4
F-3	-1.72	x	10 ⁻⁸	-1.18	x	10 ^{-a}	-6.30	x	10-4	-6.70	x	10-4
F-4	-1.57	x	10 ⁻³	-8.90	x	10-4	-4.80	x	10-4	-7.10	x	10-4
F-5	-2.66	x	10 ^{-a}	-8.70	x	10-4	-6.90	x	10-4	-6.10	x	10-4
F-6	-2.23	x	10 ⁻³	-1.01	x	10-4	-8.40	x	10-4	-6.20	x	10-4
F-7	-2.14	x	10 ^{-a}	-8.50	x	10-4	-6.20	x	10-4	-4.40	x	10-4
F-8	-2.69	x	10 ⁻⁹	-9.80	x	10-4	-9.30	x	10-4	-5.10	x	10-4
F-9	-3.07	x	10 ^{-a}	-1.16	x	10 ⁻⁸	-1.21	x	10 ^{-a}	-5.90	x	10-*

Note F = Formular

The day to begin calculation at 60 °C is the 10th day. The day to begin calculation at 45 °C is the 30th day. The day to begin calculation at 40 °C is the 30th day. The day to begin calculation at 35 °C is the 40th day.

Table 6c Correlation Coefficient When Treated Later Part of Data as First Order Reaction

R	Correlation Coefficient										
F	60 [°] C	45 [°] C	40 °C	35 [°] C							
F-1	-0.9919	-0.9975	-0.9855	-0.9928							
F-2	-0.9935	-0.9730	-0.9759	-0.9834							
F-3	-0.9921	-0.9979	-0.9969	-0.9810							
F-4	-0.9905	0.9648	-0.9516	-0.9980							
F-5	-0.9923	-0.9928	-0.9944	-0.9906							
F-6	-0.9925	-0.9944	-0.9972	-0.9978							
F-7	-0.9895	-0.9990	-0.9702	-0.9766							
F-8	-0.9932	-0.9936	-0.9996	-0.9185							
F-9	-0.9983	-0.9789	-0.9971	-0.9852							

Note F = Formular

The last

The day to begin calculation at 50 °C is the 10th day. The day to begin calculation at 45 °C is the 30th day. The day to begin calculation at 40 °C is the 30th day. The day to begin calculation at 35 °C is the 40th day. Table 7 Appearance Initial Degradation Rates Calculated By Using Cubic Polynomial Regression.

	60	°c	45	°c	40°C		35	້ດ		
	r	b	г	b	r	b	г	b		
F - 1	0.9488	-0.7686	0.9781	-0.3372	0.9574	-0.3440	0.9667	-0.4361		
F-2	0.9583	-0.7797	0.9344	-0.5594	0.9644	-0.4857	0.9801	-0.5513		
F-3	0.9670	-1.0535	0.9641	-0.8323	0.9744	-0.5783	0.9774	-0.5589		
F - 4	0.9690	-1.2016	0,9645	-0.5654	0.9663	-0.4894	0.9909	-0.4212		
F-5	0.9774	-1.1220	0.9890	-0.7346	0.9749	-0.5782	0.9732	-0.3769		
F-6	0.9696	-1.2365	0.9804	-0,8278	0.9709	-0.5839	0.9869	-0.4915		
F-7	0.9797	-1.2314	0.9835	-0.7634	0.9857	-0.5940	0.9849	-0.5038		
F-8	0.9700	-1.2272	0.9874	-0.7391	0.9714	-0.5841	0.9794	-0.4956		
F-9	0.9756	-1.0897	0.9753	-0.9042	0.9805	-0.6641	0.9799	-0.5173		
		[l		
	average r value = 0.9734 <u>+</u> 0.0038 = 0.9696 - 0.9772									

Note F = Formular

- r = Correlation coefficient
- b = Initial degradation rate (% / day) calculated by assuming b value from equation y = a + bx + cx² + dx² of cubic polynomial regression.

Table 8a Analysis Of Variance Table For Evaluation Of The Effects Of Used Buffers On Initial Rapid Degradation.

CODO		Degradation Rates in							- v
	Tempi (0)	Phosphate	A	cetat	ve.	Cit	rate		
0.05M.	60	0.7686	1.2016		6 1.2314			1.0672	
	45	0.3372	0.5654		4 0.7634			0.5554	
	40	0.3440		0.489	94	0.	5940		0.4758
	35	0.4361		0.4212		0.5038			0.4537
	у.	0.4715		0.669	€4	0.	7732		0.6380
	source of	f variatior	2	df	Ş	55	MS		V.R.
	-average			1.	4.8	3848			
	-between	block	3 0.		7539	0.25	13	20.7730	
	-between	treatment	2 0		0.	1879	0.09	40	7.7667**
	-residual			6	0.0	0726	0.01	21	
	Total			12	5.	8993			
1	1				1				

Note conc. = total buffer concentration

The appearance initial degradation rate (%/day)

F = 5.14

The buffers show significant effect on the initial rate.

There is no significant difference.

CODCA	Temp. (°C)	Degrada			- Y				
		Phosphate	A	cetat	e,	Cit	rate		
0.10M.	60	0.7797*		1.122	20	1.	2272		1.0430
	45	0.5594		0.734	16	ο.	7391		0.6777
	40	0.4857		0.578	32	0.	5841		0.5493
	35	0.5513		0.376	59	0.	4956		0.4746
	У _{е.}	0.5940		0.702	29	0.	7615		0.6862
	source of	f variatior	3	df	ć	6 S	MS		V.R.
	-average			1	5.0	5498			
	-between	block		3	0.	5726	0.190	80	12.0946
	-between t	treatment		2	0.0	0578	0.028	39	1.8310
	-residual			6	0.0	0947	0.01	58	4
	Total			12	6.	3748			
1				1					

Table 8a (continued)

Note conc. = total buffer concentration

The appearance initial degradation rate (%/day)

- F = 5.14
- The buffers show significant effect on the initial rate.
- There is no significant difference.

CODC.	Temp. (°C)	Degradation Rates in							- Y
conc.	Tempi (0)	Phosphate	A	cetat	,e	Cit	rate		1
0.15M.	60	1.0535	-	1.2365		1.0897			1.1266
	45	0.8323		0.827	78	0	9042		0.8548
	40	0.5783		0.583	39	0	6641		0.6088
	35	0.5589		0.4915		0.5173			0.5226
	у.	0.7558		0.784	49	0	7938		0.7782
	source of	f variation	1	df	ę	5 S	MS		V.R.
	-average			1	7.2	2664			
	-between 1	lock		3	0.6	5683	0.22	13	50.6570
	-between t	creatment		2	0.0	032	1.6x10) - 2	0.3629
	-residual			6	0.0	0262	4.4×10	с - а	-
	Total			12	7.9	9596			

Table 8a (continued)

Note conc. = total buffer concentration

The appearance initial degradation rate (%/day)

- F = 5.14
- ** The buffers show significant effect on the initial rate.
- There is no significant difference.

Table 8b Analysis Of Variance Table For Evaluation Of The Effect Of Buffer Concentration On Initial Rapid Degradation.

Buffer	Temp. (°C)	Degra	dat	ion Rate	s in	- Y
burrer		0.05 M	C	D.10 M	0.15 M	1
Phosphate	60	0.7686		0.7797	1.0535	0.8673
	45	0.3372		0.5594	0.8323	0.5763
	40	0.3440		0.4857	0.5783	0.4694
	35	0.4361		0.5513	0.5589	0.5154
	у.	0.4715		0.5940	0.7558	0.6071
	source of	f variat ⁿ	df	SS	MS	V.R.
	-average		1	4.4227		
	-between I	block	3	0.2880	0.0960	11.5835
	-between t	treatment	2	0.1626	0.0813	9.8112
	-residual		6	0.0497	8.3×10^{-3}	
	Total		12	4.9231		
1						

<u>Note</u> The appearance initial degradation rate (%/day) source of variatⁿ = source of variation

F = 5.14
The buffers show significant effect on the initial rate.
There is no significant difference.

Buffen		Degra	dat	tion Rate	- Y	
Durrer	Tempi (0)	0.05 M	0	0.10 M	0.15 M	* 1
Acetate	60	1.2016		1.1220	1.2365	1.1867
	45	0.5654		0.7346	0.8278	0.7093
	40	0.4894		0.5782	0.5839	0.5505
	35	0.4212		0.3769	0.4915	0.4299
	, ,	0.6694		0.7029	0.7849	0.7191
	source of	f variat ⁿ	df	SS	MS	V . R .
	-average		1	6.2051		
	-between 1	block	3	0.9925	0.3308	75.3010
	-between	treatment	2	0.028 3	0.0141	3.2158
	-residual		6	0.0264	4.4×10^{-3}	40
	Total		12	7.2522		

Table 8b (continued)

Note The appearance initial degradation rate (%/day) source of variatⁿ = source of variation

Fo.95(2.6) = 5.14 The buffers show significant effect on the initial rate. There is no significant difference.

Buffen		Degra	Degradation Rates in					
Durrer		0.05 M		D.10 M	0.15 M	1		
Citrate	60	1.2314		1.2272	1.0897	1.1828		
	45	0.7634		0.7391	0.9042	0.8022		
	40	0.5940		0.5841	0.6641	0.6141		
	35	0.50381		0.4956	0.5173	0.5056		
	- y.	0.7732		0.7615	0.7938	0.7762		
	source of	f variat ⁿ	df	SS	MS	V.R.		
	-average		1	7.2291				
	-between 1	block	3	0.7965	0.2655	51.8892		
	-between	treatment	2	2.1×10 ⁻³	1.1×10 ⁻⁹	0.2090		
	-residual		6	0.0307	$5.1_{X}10^{-3}$	a di		
	Total		12	8.0584				

Table 8b (continued)

Note The appearance initial degradation rate (%/day) source of variatⁿ = source of variation

- F = 5.14
- The buffers show significant effect on the initial rate.
- There is no significant difference.

Table 9a Analysis Of Variance Table For Evaluation Of The Effects Of Used Buffers On Later Zero Order Degradation.

conc.	Temp.(°C)	Degrada	ition		- Y			
Ϋ́Τ.		Phosphate Acetate Citrat		trate				
0.05M.	60	0.1214	0.1287		0.1714			0.1405
	45	0.0571	0.0	0749	0	0722		0.0680
	40	0.0159	0.0	0429	0	0551		0.0380
	35	0.0114	0.0637		0.0392			0.0381
	– بر	0.0514	0.0775		0	.0845		0.0712
	source of	variation	df	SS	6	MS		V.R.
	-average		1	0.00	607			
	-between block		3	0.03	210	7.0×10	ວ ີ ອ	30.2203
	-between	treatment	2	2.4x	10 ⁻³	1.2x10	с з	5.2309
	-residual	ua l		1.4X	10 ⁻³	2.3x10	o ⁻ ↑	144
	Total		12	0.0	856			

Note conc. = total buffer concentration

- The appearance later zero order degradation rate(%/day) $F_{0.95(2.6)} = 5.14$
- The buffers show significant effect on the later rate.
- There is no significant difference.

CODO	Temp ([°] C)	Degradation Rates in						- Y	
conct		Phosphate	Acet	late	Cit	trate			
0.10M.	60	0.1705	0.2038		0.2022			0.1922	
	45	0.0738	0.0	0747	0	.0833		0.0773	
	40	0.04244	0.0	0600	0	.0801		0.0608	
	35	0.0342	0.0541		0.0445		0.0443		
	y	0.0802	0.0982		0.1025			0.0936	
	source of	variation	df	SS	5	MS		V . R .	
	-average		1	0.10	052				
	-between 1	block	3	0.04	405	0.013	35	148.298	
	-between (treatment	2 1.1x		10-7	5.6x 10	5-4	6.140	
	-residual		6	5.5x	10-4	-4 9.1x 10 ⁻⁵			
	Total		12	0.14	474			00	
				1					

Table 9a (continued)

Note conc. = total buffer concentration

The appearance late zero order degradation rate (%/day)

- F0.95(2,6) = 5.14
- The buffers show significant effect on the later rate. There is no significant difference.

Ta	b	1	е	9a	(cont	i	nued)
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		Degrada		- v				
conc.	Temp:(C)	Phosphate	Acet	tate Citrate			• s	
0.15M.	60	0.1438	0.1767		0.2410			0.1872
	45	0.0990	0.0	0856	0.0965		1	0.0937
	40	0.0550	0.0	0730	0	.1029		0.0770
	35	0.0580	0.0540		0.0515		0.0545	
	у.	0.0889	0.0973		0	.1230		0.1031
	source of	variation	df	SS	5	MS		V.R.
	-average		1	0.12	275			
	-between 1	olock	3	0.03	306	0.010	02	16.7057
	-between t	creatment	2	2.5x	10-3	1.3x10	- 3	2.0572
	-residual		6	3.7 <u>x</u>	10-3	6.1 _X 10	0-4	
	Total		12	0.1	643			30

Note conc. = total buffer concentration

The appearance later zero order degradation rate (%/day)

F = 5.14

- The buffers show significant effect on the later rate.
- There is no significant difference.

Table 9b Analysis Of Variance Table For Evaluation Of The Effect Of Buffer Concentration On Later Zero Order Degradation.

Buffor		Degra	- Y			
burrer	Temp: (C)	0.05 M	(D.10 M	0.15 M	1.1
Phosphate	60	0.1214		0.1705	0.1438	0.1452
	45	0.0571		0.0738	0.0990	0.0766
	40	0.0159		0.0424	0.0550	0.0378
	35	0.0114		0.0342	0.0580	0.0345
	y	0.0514		0.0802	0.0889	0.0735
	source of	r variat ⁿ	df	SS	MS	V.R.
	-average		1	0.0649		
	-between h	olock	3	0.0238	7.9×10^{-3}	53.0330
	-between t	reatment	2	3.8 _x 10 ⁻³	$1.5x 10^{-3}$	10.2847
	-residual		6	9.0x10 ^{-*}	1.5 10-4	196
	Tota 1		12	0.0927		

<u>Note</u> The appearance later zero order degradation rate(%/day) source of variatⁿ = source of variation

- F = 5.14
- The buffers show significant effect on the later rate.
- There is no significant difference.

Table 9b (continued)

Buffor		Degre	- v			
burren		0.05 M	(D.10 M	0.15 M	19
Acetate	60	0.1287		0.2038	0.1767	0.1698
	45	0.0749		0.0747	0.0856	0.0784
	40	0.0429		0.0600	0.0730	0.0587
	35	0.0637		0.0541	0.0540	0.0572
	<u>у</u> ,	0.0775		0.0982	0.0973	0.0910
	source of	^r variat ⁿ	df	SS	MS	V . R .
	-average		1	0.0994		
	-between h	olock	3	0.0256	8.5x10 ⁻²	21.3152
	-between t	reatment	2	1.1x10 ⁻³	5.5x10 ⁻⁴	1.3618
	-residual		6	2.4×10^{-3}	4.4×10^{-4}	
	Total		12	0.1285		

Note The appearance later zero order degradation rate(%/day) source of variatⁿ = source of variation

Fo.95(2.5) = 5.14
The buffers show significant effect on the later rate.
There is no significant difference.

Table 9b (continued)

Buffor				Degradation Rates in				
Burrer	Temp: (C)	0.05 M	(D.10 M	0.15 M	19		
Citrate	60	0.1714	•	0.2022	0.2410	0.2049		
	45	0.0722		0.0833	0.0965	0.0840		
	40	0.0551		0.0801	0.1029	0.0794		
	35	0.0392		0.0445	0.0515	0.0451		
	ӯ	0.0845		0.1025	0.1230	0.1033		
	source of	f variat ⁿ	df	SS	MS	V.R.		
	-average		1	0.1281				
	-between h	block	3	0.0440	0.0147	89.4287		
	-between t	creatment	2	$3.0_{K}10^{-a}$	1.5x10 ⁻³	9.0549		
	-residual		6	9.8x10 ⁻⁴	1.6x10 ⁻⁴			
	Total		12	0.1760		a l		

<u>Note</u> The appearance later zero order degradation rate(%/day) source of variatⁿ = source of variation

 $F_{0.95(2.6)} = 5.14$

The buffers show significant effect on the later rate.

There is no significant difference.

Table 10 Effect Of Buffer On Degradation Of PVP-I (available iodine)

न	The 95% conf	idence interve	al for slope of	f later part	
•	60 [°] C	45 [°] C	40 [°] C	35 [°] C	
F - 1	0.1023-0.1404	0.0493-0.0648	0.0108-0.0209	0.0089-0.0139	
F-2	0.1484-0.1925	0.0487-0.0990	0.0291-0.0558	0.0226-0.0458	
F-3	0.1222-0.1654	0.0879-0.1100	0.0486-0.0615	0.0363-0.0797	
F-4	0.1033-0.1540	0.0455-0.1042	0.0234-0.0624	0.0580-0.0694	
F-5	0.1676-0.2401	0.0573-0.0922	0.0511-0.0690	0.0401-0.0680	
F-6	0.1484-0.2051	0.0685-0.1027	0.0653-0.0808	0.0473-0.0607	
F-7	0.1342-0.2086	0.0669-0.0775	0.0357-0.0745	0.0232-0.0551	
F-8	0.1625-0.2420	0.0650-0.1015	0.0773-0.0829	0.0093-0.0798	
F-9	0.2205-0.2614	0.0582-0.1348	0.0935-0.1123	0.0349-0.0681	

Note F = Formular

The slope was calculated from later linear regression part. and shown in positive value to express the appearance later zero order degradation rates (%/day). Table 11 % Of Available Iodine Remaining After 70 Day Incubation.

Formular	Temperature(°C)							
r or indiar	60	45	40	35				
F - 1	82.147	90.369	91.243	90.917				
F-2	77.815	82.245	87.553	87.096				
F-3	78.328	81.915	86.516	84.855				
F-4	77.526	82.634	87.070	88.899				
F-5	71.212	84.972	85.903	87.278				
F-6	73.711	82.408	85.581	86.638				
F-7	74.697	83.489	86.777	87.191				
F-8	69.799	83.439	85.181	85.726				
F-9	71.145	80.821	84.178	86.265				

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Table 12 pH Of 10%w/v PVP-I Solution After 70 Day Incubation.

	Temperature(°C)															
Formular	60	45	40	35												
F – 1	2.80	3.25	3.10	4.10												
F-2	2.80	3,50	3.70	4.80												
F-3	3.29	3.80	4.20	5.00												
F-4	4.00	4.10	4.10	4.50												
F-5	4.50	4.22	4.40	5.20												
F-6	4.30	4.30	4.50	5.30												
F-7	4.82	4.32	4.60	5.05												
F-8	5.10	4.50	4.90	5.20												
F-9	5.18	4.60	5.20	5.40												
Table	13	The	Degradation	Rates	of	Avsilable	lodine	in	F-10	ta	F-18	and	рH	after	70	Day
-------	----	------	-------------	-------	----	-----------	--------	----	------	----	------	-----	----	-------	----	-----
		Incu	bation.													

phosphate	0.025 N				0.050 N			0.075 M				
citrate	न	initial	later	Нq	F	initial	later	рH	न	initial	later	Hq
0.025 M	F-10	0.4233	0.0375	5.00	F-13	0.4410	0.0375	4.90	F-16	0.4326	0.0376	5.00
0.050 M	F-11	0.4659	0.0392	5.10	F-14	0.4691	0.0383	5.10	F-17	0.4884	0.0393	5.20
0.075 M	F-12	0.4843	0.0397	5.20	F-15	0.4875	0.0396	5.20	F-18	0.5869	0.0597	5.40

Note F = Formular.

init = The appearance initial degradation rates (%/day) calculated

by assuming b value from equation $y = a + bx + cx^{2}$

+ dx of cubic polynomial regression.

later = The calculation of appearance later linear degradation rates

(%/day) was begun at the 40th day.

pH = pH after 70 day incubation.

Table 14 The Appearance Degradation Rates of Available Iodine in 10% w/v PVP-I Buffered Solutions and pH after 70 Day Incubation When Total Buffer Concentration were 0.05, 0.10, 0.15 M (only phosphate and citrate buffer).

Total Conc.	Composition	Initial	Later	рH
0.05 M	0.05 M phosphate (F-1)	0.4361	0.0114	4.10
	0.05 M citrate (F-7)	0.5038	0.0392	5.05
	0.025 M phosphate + 0.025 M	0.4233	0.0375	5.00
	citrate (F-10)			
0.10 M	0.10 M phosphate (F-2)	0.5513	0.0342	4.80
	0.10 M citrate (F-8)	0.4956	0.0445	5.20
	0.025 M phosphate + 0.075 M	0.4843	0.0397	5.20
	citrate (F-12)			
	0.050 M phosphate + 0.050 M	0.4691	0.0383	5.10
	citrate (F-14)		÷	
	0.075 M phosphate + 0.025 M	0.4326	0.0376	5.00
	citrate (F-16)			
0.15 M	0.15 M phosphate (F-3)	0.5589	0.0580	5.00
	0.15 M citrate (F-9)	0.5173	0.0515	5.40
	0.075 M phosphate + 0.075 M	0.5869	0.0597	5.40
	citrate (F-18)			
L				

Table 14 (continued)

Note Total Conc. = Total buffer concentration of preparation Init. = The appearance initial degradation rates of preparation Later = The appearance later linear rates of preparation pH = pH after 70 days incubation

Table 15 Raw Data of Available Iodine Remaining in 10% w/v PVP-I Buffered Solution (F-11) Prepared with Various Sources of Water.

Time [®]	% available iodine remaining						
	in distilled water	in DI water	in potable water				
0.00	100.00	100.00	100.00				
0.86	99.40	99.38	99.03				
2.92	98.68	98.31	98.00				
6.11	97.43	97.21	97.08				
13.11	95.61	95.47	95.29				
27.13	93.37	93.31	93.29				
41.11	92.38	92.32	92.04				
55.11	91.74	91.54	91.44				
69.11	91.11	90.95	90.84				
83.11	90.73	90.55	90.24				

Note

Incubation time

Table 16 The Result of ANOVA Table for Final Concentration of Available Iodine Remaining in 10% w/v PVP-I Buffered Solution F-11 with Three Different Sources of Water.

Result of ANOVA test (SPS Program)	F = 20 DF = 2, F.o.96.0)•5888 4 :a,4, = 6•9	94			
Fisher's LSD	Fisher's LSD = 0.2143					
	VAR	Potable	DI	Distilled		
	Potable	0	0.31	0.49		
	DI	0	0	0.18		
	Distilled	0	0	0		

Note VAR = variable

= Significant different at the 95% confidence interval

Table 17 The Appearance Degradation Rates of Available Iodine in 10% w/v PVP-I Buffered Solution F-11 Prepared with Various Sources of Water.

water	initial degradation rates (%/day)	later linear rates (%/day)
in distilled water	0.3778	0.0400
in Di water in potable water	0.3779	0.0427

Table 18 Raw Date of Available Iodine Remaining in 10% w/v PVP-I Buffered Solution F-11 Stored in Various Types of Packaging Materials.

Time [®]	% available iodine remaining						
(ddy)	Amber glass	Clear glass	LDPE	HDPE	PP		
0.00	100.00	100.00	100.00	100.00	100.00		
2.92	98.03	98.01	98.13	98.25	97.56		
8.09	96.60	96.11	97.00	96.45	95.81		
13.11	95.62	95.22	95.69	95.69	94.94		
27.13	93.70	93.58	94.62	94.16	93.53		
41.11	92.85	92.80	93.97	93.41	92.61		
55.11	92.43	92.21	93.60	92.85	92.00		
69.11	91.75	91.62	93.05	92.30	91.38		
83.11	91.50	91.15	92.59	92.02	90.89		

Note Incubation time

Table 19 The Result of ANOVA Table for Final Concentration of Available Iodine Remaining in 10% w/v PVP-I Buffered Solution F-11 Stored in Five Different Packaging Material

Result of A (SPS Prog	ANOVA test gram)	F = 27 DF = 4 F(0.98)	745.1428 ,8 (4,8) = 3.6	84			
Fisher's LS (SPS Prog	SD gram)	Fisher's	Fisher's LSD = 0.2143				
VAR	PP	Clear Gl.	Amber Gl.	HDPE	LDPE		
PP Clear Gl. Amber Gl. HDPE LDPE	0 0 0 0 0	0.263 0 0 0 0	0.617 0.353 0 0 0	1.133 0.87 0.517 0 0	1.703 1.44 1.087 0.57 0		

Note VAR = Variable

*

= Significant different at the 95% confidence interval

Table 20 The Appearance Degradation Rates of Available Iodine in 10% w/v PVP-I Buffered Solution F-11 Stored in Various Type of Packaging Material.

packaging material	initial degradation rates (%/day)	later linear rates (%/day)
amber glass bottle	0.3585	0.0339
clear glass bottle	0.3837	0.0396
LDPE bottle	0.3286	0.0334
HDPE bottle	0.3448	0.0336
PP bottle	0.3841	0.0414



Figure 4 Concentration remaining versus time plot of available iodine in 10%w/v PVP-I buffered solution F-1 to F-9 at 60°C. □ F-1, w F-2, Δ F-3, +F-4, • F-5, × F-6, Q F-7, ◆ F-8, ⊽ F-9.





Concentration remaining , 🛛







Figure 7 Concentration remaining versus time plot of available iodine in 10%w/v PVP-I buffered solution F-1 to F-9 at 35°C. □ F-1, + F-2, ◊ F-3, △ F-4, × F-5, ∇ F-6, ■ F-7, • F-8, ♦ F-9.



Figure 8 Natural logarithm of concentration remaining versus time plot of available iodine in 10%/v PVP-I buffered solution F-1 to F-9 at 60° C. \Box F-1, \triangle F-2, \blacksquare F-3, + F-4, \times F-5, \bullet F-6, \Diamond F-7, ∇ F-8, \diamondsuit F-9.

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Figure 9 Natural logarithm of concentration remaining versus time plot of available iodine in 10%w/v PVP-I buffered solution F-1 to F-9 at 45°C. □ F-1, + F-2, ◇ F-3, △ F-4, × F-5, ⊽ F-6, S F-7, ● F-8, ◆ F-9.



Figure10 Natural logarithm of concentration remaining versus time plot of available iodine in 10%w/v PVP-I buffered solution F-1 to F-9 at 40° C. \Box F-1, + F-2, \diamondsuit F-3, \triangle F-4, \times F-5, ∇ F-6, \square F-7, • F-8, \diamondsuit F-9.



Figure 11 Natural logarithm of concentration remaining versus time plot of available iodine in 10%/v PVP-I buffered solution F-1 to F-9 at 35° C. \Box F-1, + F-2, \diamond F-3, \triangle F-4, \times F-5, ∇ F-6, \blacksquare F-7, \bullet F-8, \diamondsuit F-9.



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Figure 12 Comparison of degradation profile of available iodine in 10%w/v PVP-I solutions with 0.05 M. buffer at $60^{\circ}C$. Phosphate(F-1), + Acetate(F-4), \Diamond Citrate(F-7) \Box







 \square Phosphate(F-1), + Acetate(F-4), \Diamond Citrate(F-7)



Figure 15 Comparison of degradation profile of available iodine in 10%w/v PVP-I solutions with 0.05 M. buffer at 35°C. □ Phosphate(F-1), + Acetate(F-4), ♦ Citrate(F-7)

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Figure 16 Comparison of degradation profile of available iodine in 10%w/v PVP-I solutions with 0.10 H. buffer at 60°C. □ Phosphate(F-2), + Acetate(F-5), ◇ Citrate(F-8)

Concentration remaining , 🛛





Concentration remaining .. 🛪



Concentration remaining , 🛛



10%w/v PVP-I solutions with 0.10 M. buffer at $35^{\circ}C$. Phosphate(F-2), + Acetate(F-5), 🛇 Citrate(F-8)

Concentration remaining , X



Figure 20 Comparison of degradation profile of available iodine in 10%w/v PVP-I solutions with 0.15 M. buffer at 60°C.
□ Phosphate(F-3), -+ Acetate(F-6), ◇ Citrate(F-9)



 \square Phosphate(F-3), + Acetate(F-6), \Diamond Citrate(F-9)





Concentration remaining , \mathbf{X}



Figure 23 Comparison of degradation profile of available iodine in 10%w/v PVP-I solutions with 0.15 M. buffer at $35^{\circ}C$. \Box Phosphate(F-3), + Acetate(F-6), \Diamond Citrate(F-9)



Figure 24 Comparison of degradation profiles of available iodine in 10%w/v PVP-I solutions with various concentrations of phosphate buffer at 60⁰C. □ 0.05 M.(F-1), 0.10 M.(F-2), 🔇 0.15 M.(F-3) +

115

Concentration remaining



Figure 25 Comparison of degradation profiles of available iodine in 10%w/v PVP-I solutions with various concentrations of phosphate buffer at 45°C. □ 0.05 M.(F-1), + 0.10 M.(F-2), ◊ 0.15 M.(F-3)



Figure 26 Comparison of degradation profiles of available iodine in 10%w/v PVP-I solutions with various concentrations · 🗆 0.05 M.(F-1), of phosphate buffer at 40° C. 0.10 M.(F-2), 🛇 0.15 M.(F-3) +

117





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Concentration remaining , X









+ 0.10 M.(F-5), ◊ 0.15 M.(F-6)


of Acetate buffer at 35° C. \bigtriangleup 0.05 M.(F-4),

× 0.10 M.(F-5), ∇ 0.15 M.(F-6)

Concentration remaining

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Concentration remaining , Z

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Concentration remaining . Z

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Figure 35 Comparison of degradation profiles of available iodine in 10%w/v PVP-I solutions with various concentrations of Citrate buffer at 35°C. □ 0.05 M.(F-7), + 0.10 M.(F-8), ◊ 0.15 M.(F-9)

Concentration remaining , Z



Initial degradation rates









Figure 39 The later linear degradation rates of available iodine in 10%w/v PVP-I buffered solution at 45°C; The bars repressent the 95% confidence limit.







Figure 41 The later linear degradation rates of available iodine in 10%w/v PVP-I buffered solution at 35°C; The bars repressent the 95% confidence limit.



Figure 42 Comparison profiles of available iodine in 10% w/v PVP-I solutions F-10 to F-18. \Box F-10, +F-11, \Diamond F-12, \triangle F-13, \times F-14, ∇ F-15, \blacksquare F-16, \bullet F-17, \diamondsuit F-18.



□ 0.05 M phosphate (F-1), ◊ 0.05 M citrate (F-7),

△ 0.025 M phosphate and 0.025 M citrate (F-10)







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buffered solutions with 0.15 total buffer Μ Concentration. (only phosphate and citrate buffer) 0.15 M phosphate (F-3), + 0.15 M citrate (F-9), 0.075 M phosphate and 0.075 M citrate (F-18)













Concentration remaining ,





Concentration remaining 🗸









Concentration remaining , Z

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Concentration remaining



Figure 54 Comparison profiles of available iodine in 10% w/v PVP-I buffered solution (F-11) stored in various packaging material. □amber-glass, + clear - glass, ◇ LDPE, △HDPE, × PP.

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