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DEGRADATION STUDIES OF CURCUMIN

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ABSTRACT

Curcumin, a yellow phytochemical constituent obtained from *Curcuma longa* shows profound biological activities. Literature reveals that curcumin is insoluble in water and susceptible to higher pH conditions. This work is focused firstly to study the susceptibility of curcumin in water, various pH conditions in presence and absence of light by a simple UV absorption method. A series of buffer solutions of pH conditions such as 1, 1.2, 6.8, 7, and 7.4 were selected and the result showed that decomposition was pH dependent and occurs faster at neutral-basic conditions. The stability was more in acidic pH and decrease as the pH increases. It was more stable at pH of 1.2; less than 1% of curcumin decomposed within 6hrs of the total curcumin in the absence of light while it was more than 40% in the presence of light. Solution at pH of 1, 1.2, 6.8, 7.4 were studied for Thin layer chromatography (TLC) and compared with the standard curcumin. Fourier transform infrared spectroscopy (FTIR) study was also conducted.

Key words: Curcumin, Stability, pH dependent, Buffers.

INTRODUCTION

Curcumin is a polyphenolic phytochemical constituent derived from the herbal remedy and dietary spice turmeric *Curcuma longa* and it has been used from the time immemorial as a dietary supplement, coloring agent, spice and also for curing the diseases. A vast research revealed that curcumin has a wide spectrum of therapeutic effects such as anti-inflammatory [1], antibacterial [2], antifungal [3], anticancer [4], antispasmodic [5], antioxidant [6], antiamoebic [8], anti HIV [9], antidiabetic[10], antifertility [11] etc. It is also reported that the curcumin is safe up to 8g/day [12-14].

Curcumin(1,7-Bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione) is an oil-soluble pigment, practically insoluble in water at acidic and neutral pH, soluble in alkali and highly susceptible for pH change having molecular weight 368.38g/mole and a melting point of 183^0C [12].However, in aqueous systems like water, it is understood that at alkaline pH, the acidic phenol group in curcumin donates its hydrogen, forming the phenolate ion that enables curcumin into dissolution in water. It is not stable at neutral and alkaline pH for longer period of time and gets easily degraded into compounds like vanillin, ferulic acid, etc. It is stable below pH 7.0 but with decreasing the pH values, the dissociation equilibrium shifts towards the neutral form with very low aqueous solubility [13].

It is a bis-α, β-unsaturated β-diketone. It is reported that curcumin exhibits keto-enol tautomeric forms. The keto form predominates in acidic and neutral aqueous solutions and it was also reported that the same is stable in the cell membrane as compare to stability in blood [14]. It show heptadienone linkage between the two methoxy phenyl rings which contains a highly activated carbon atom, and the C–H bonds on this carbon are very weak due to delocalization of the unpaired electron on the adjacent oxygens hence it acts as an extraordinarily potent H-atom donor at pH 3–7[15](Fig. 1). In contrast, the enolate form of the heptadienone chain predominates as an electron donor and the mechanism involved is more typical as that of the scavenging activity of phenolic antioxidants above pH 8[16].

Curcumin is relatively insoluble in water, but dissolves in acetone, dichloromethane, methanol and ethanol. Curcumin as such is unstable at basic and neutral pH, and degrades within 30 min to Trans-6-(40-hydroxy-30-methoxyphenyl)-2, 4- dioxo-5- hexanal, ferulic acid,

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feruloylmethane and vanillin. The initial degradation products are formed after 5 minutes and the chromatographic pattern obtained after 28 h at pH 8.5 is representative for alkaline degradation. Ferulic acid and feruloylmethane are formed initially. Feruloylmethane rapidly forms coloured (mostly yellow to brownish-yellow) condensation products. Degradation products formed by hydrolysis of feruloylmethane are vanillin and acetone and their amount increase with incubation time. The presence of foetal calf serum, human blood, or addition of antioxidants such as ascorbic acid, N-acetylcysteine or glutathione completely blocks this degradation in culture media or phosphate buffer above pH7 [17]. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 h.

The kinetics of hydrolytic degradative reactions of compound 1 (diferuloylmethane) over the pH range 1-11 was studied. At pH<1, aqueous solutions of diferuloylmethane have a red colour which indicates the protonated form (H4A+). In the pH range 1-7, the majority of diferuloylmethane species are in the neutral form (H3A). Water solubility is very low in this pH range and solutions are yellow. At pH>7.5, the colour changes to red. The pKa values for the dissociation of the three acidic protons in compound 1 (forms H2A-, HA2- and A3-) have been determined to be 7.8, 8.5 and 9.0 respectively [18].

MATERIALS AND METHODS

Material

Curcumin extract 95% was generously gifted by Neelam phytoextract. All the other chemicals and reagents used were from LUBA chemicals and SD fine and are of analytical grade.

Method

Curcumin is reported to be highly susceptible to pH change and light [19]. In order to quantify the instability of curcumin in various pH conditions such as pH 1, 1.2, 6.8, 7 and 7.4 a simple spectroscopic method was adopted while to study the light effect it was exposed to natural light.

The stability was also found out in aqueous solution by using distilled water.A stock solution of 100µg/ml of curcumin was made by dissolving weighed amount (10 mg) of curcumin in 100 ml of methanol in a volumetric flask. Then, 1 ml of the stock solution was transferred to the 24 ml of distilled water to obtain a concentration of 4µg/ml solution. The absorbance of the resultant solution was immediately measured at 428 nm using Shimadzu UV Visible Spectrophotometer (UV-1700 pharmaspec) at different time interval against blank. The absorbance was read at zero time was considered to be as 100 %. All the solutions are then transferred to the incubator to maintain the temperature of 37 ± 0.5^0C and to avoid the interference of light. This procedure was repeated with the different buffer solutions of pH 1, 1.2, 6.8, 7, and 7.4. The effect of light and in the absence of light on buffer solutions ofpH 1, 1.2, 6.8 was studied.

Evaluation of curcumin solutions

Thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR) was performed to evaluate the formation of degraded products in the buffer solutions of curcumin. The buffer solutions of the curcumin were transferred to the petri dishes and kept in the oven for 72 hrs at 70^0C. The solidified residue was then checked for FTIR.

RESULTS AND DISCUSSION

A plot of logarithmic concentration versus time was made and from this degradation constant (K), half-life ($T_{1/2}$) was calculated [20]. The absorbance at time 0 hrs was considered to be 100%.

The results revealed that curcumin is unstable at higher pH conditions. In this study we found that more than 90% of curcumin decomposed rapidly in buffer system at neutral basic condition. The increased stability of curcumin at acidic pH may be contributed by the conjugated diene structure. However when the pH is adjusted to neutral basic condition, proton removed from the phenolic group leads to the destruction of this structure. The amount of curcumin remained after 60 min in various pH conditions as follows pH 1.2 >pH 1 > Distilled water >pH 6.8 >pH 7 >pH 7.4 as shown in table 4.However when the experiment was conducted in the absence of light or in dark the degradation of the curcumin was much lower in the pH range if 1, 1.2, 6.8 as compared to the buffers in the presence of light. The curcumin degradation in the pH of 1.2 at the time of 6 hrs was less than 1% of the total curcumin. This results shows that the degradation of the curcumin was much higher in the presence of light as shown in table 5 and figure 5. Thus curcumin should be stored in the amber coloured bottle to prevent its photo oxidation.

TLC of standard solution of curcumin (4µg/ml) and buffer solutions at the time of 60 mins were performed with the mobile phase consists of Toluene, Chloroform, and methanol in the ratio of 4:4:2 v/v for pH 1, 1.2, 6.8 and 7.4 as shown as in Figure 4 [20].Plates of buffer solution of pH1 and 1.2 shows three distinct spots of curcumin (Rf- 0.39), demethoxy curcumin (Rf- 0.35) and bis demethoxy curcumin (Rf- 0.32).this shows that there is little or no degradation at pH of 1 and 1.2.

However at buffer solution of pH 6.8 there are two spots having Rf value of 0.31 and 0.34.thus it can be concluded that there is some amount of curcumin left behind and the spot which is there is of degraded ones. Also at buffer of pH 7.4 there is one spot having Rf value of 0.54 indicating the presence of other compounds.

The Infra-red spectrographs of pure curcumin shows the functional groups of phenolic OH stretching, C=O and aromatic C=C which shows the peaks at 3500-3300 cm^{-1}, 1625 –1640 cm^{-1} and 1520 – 1400 cm^{-1} in the IR spectrum [21]. It has been observed that at pH 7.4 of the curcumin it showed the additional peak of aldehyde at 2845 cm^{-1} due to the presence of vanillin or feruloylmethane. It also shows the less intense peak at 3508 cm^{-1} of OH stretching and the peak has been shifted from 3510 to 3508 cm^{-1}.

Table 1. Data showing the logarithmic concentration and its absorbance of DW, 1, 1.2,.8 in the presence of light.

Time	Distilled water (DW)		pH 1		pH 1.2		pH 6.8	
(hrs)	Abs	Log Conc	Abs	Log Conc	Abs	Log Conc	Abs	Log Conc
0	0.2516	0.602	0.2512	0.602	0.2514	0.602	0.2167	0.602
1	0.2412	0.583	0.2412	0.583	0.2426	0.586	0.1539	0.453
2	0.2238	0.569	0.2274	0.558	0.2197	0.579	0.1262	0.367
3	0.2016	0.551	0.2116	0.526	0.2043	0.547	0.1021	0.275
4	0.1733	0.505	0.1834	0.465	0.1872	0.509	0.0084	-0.809
5	0.1548	0.391	0.1662	0.422	60.1467	0.403	0.0067	-0.91
6	0.1207	0.282	0.1217	0.286	0.1214	0.321	0.0042	-1.113
7	0.1172	0.270	0.1148	0.261	0.1178	0.308	0.0038	-1.15
8	0.1108	0.245	0.1103	0.244	0.1121	0.287	0.0034	-1.2
9	0.0073	-0.935	0.0036	-0.057	0.0084	-0.838	0.0021	-1.42

Table 2. Data showing the logarithmic concentration and its absorbance of pH7 and 7.4.

Time	**pH 7**		**pH 7.4**	
(mins)	**Abs**	**Log Conc**	**Abs**	**Log Conc**
0	0.2126	0.602	0.2114	0.602
15	0.1937	0.561	0.1724	0.513
30	0.1476	0.443	0.1253	0.374
45	0.1123	0.324	0.1087	0.313
60	0.1018	0.282	0.0081	-0.814
75	0.0071	-0.874	0.0037	-1.15

The degradation constant (K) and Half-life of the reaction ($T_{1/2}$) was then calculated by the following equation.

K= -2.303 X –Slope

$T_{1/2}$ = 0.693 / K.

Table 3. Solution stability data of curcumin in different pH solutions in light.

Sample	**Degradation constant (k) hr^{-1}**	**Half-life (t½) hrs or mins**
Distilled water	0.1188 hr^{-1}	5.83 hrs
pH 1	0.1107 hr^{-1}	5.92 hrs
pH 1.2	0.1098 hr^{-1}	6.55 hrs
pH 6.8	0.245 hr^{-1}	2.82 hrs
pH 7	0.0145 min^{-1} or 0.87 hr^{-1}	47.7 mins
pH 7.4	0.0154 min^{-1} or 0.924 hr^{-1}	45 mins

Table 4. **Cumulative amount of curcumin remaining after 60 mins.**

pH	**% Amount of drug remained after 60 mins**
1.2	96.47%
1	95.94%
Distilled water	95.85%
6.8	71%
7	47.87%
7.4	3.83%

Table 5. Data showing the logarithmic concentration and its absorbance of pH 1, 1.2, 6.8 in the absence of light.

Time	**pH 1**		**pH 1.2**		**pH 6.8**	
(hrs)	**abs**	**Log conc**	**abs**	**Log conc**	**abs**	**Log conc**
0	0.2512	0.602	0.2517	0.602	0.2468	0.602
1	0.2511	0.601	0.2514	0.6015	0.2466	0.6017
2	0.2504	0.600	0.2511	0.6010	0.2466	0.6017
3	0.2497	0.599	0.2507	0.6003	0.2461	0.6008
4	0.2496	0.599	0.2498	0.5987	0.2458	0.6002
5	0.2491	0.598	0.2496	0.5984	0.2457	0.6001
6	0.2489	0.598	0.2402	0.5977	0.2457	0.6001
7	0.2487	0.597	0.2492	0.5977	0.2452	0.5912
8	0.2486	0.597	0.249	0.5973	0.2447	0.5988
9	0.2456	0.592	0.2489	0.5972	0.2446	0.5981

Fig 1. Curcumin in Acidic and Basic condition.

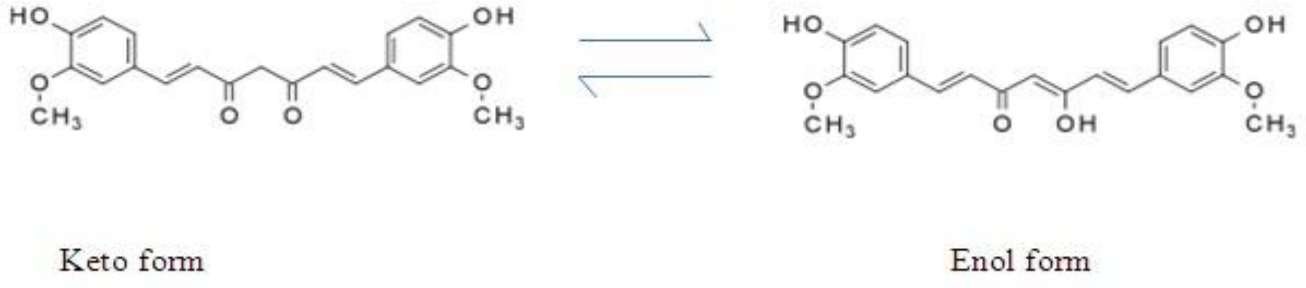


Fig 2. Degradation products of curcumin at basic condition.

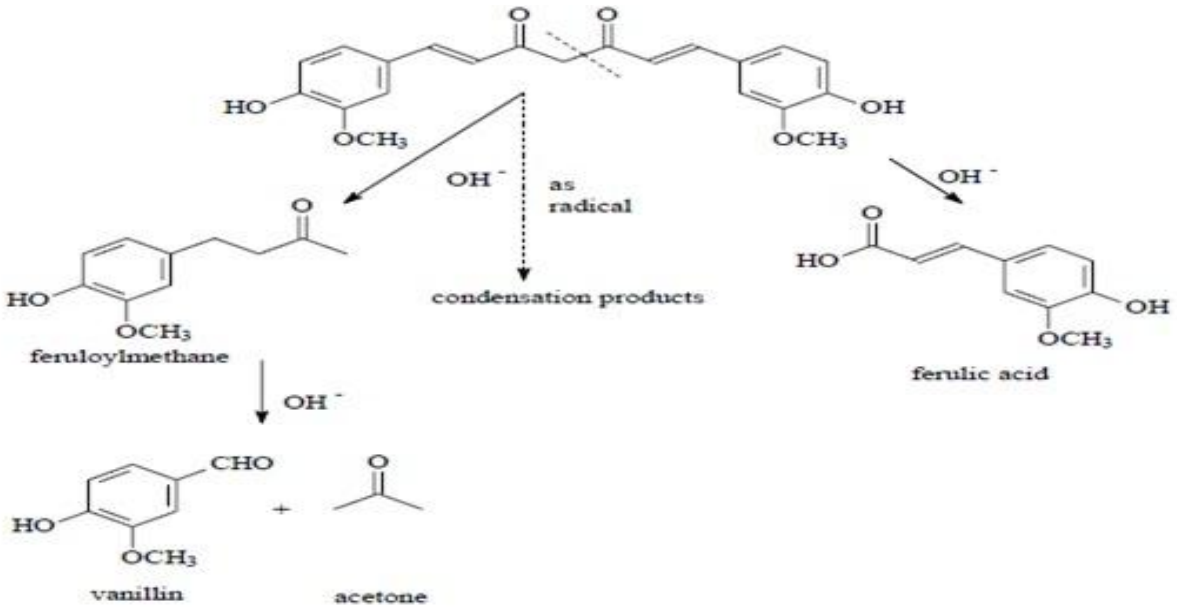


Fig 3. Red coloured compound formed above pH 7.

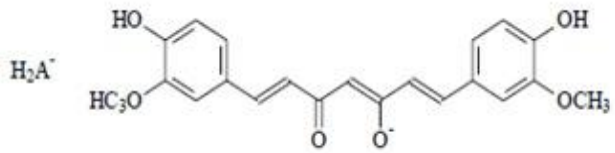


Fig 4. Comparative degradation constant profile of curcumin in different buffers.

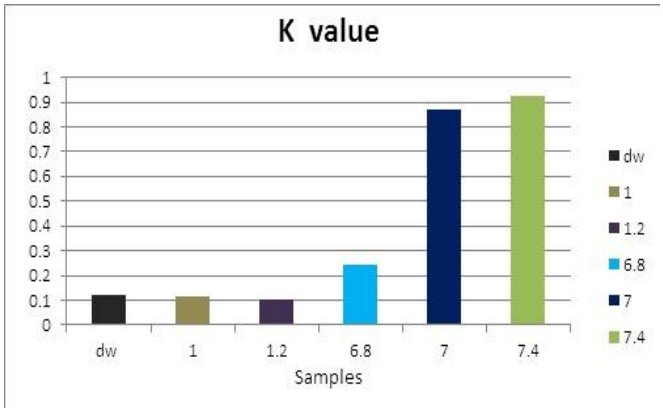


Fig 5. Comparison between the buffers in the presence and absence of light.

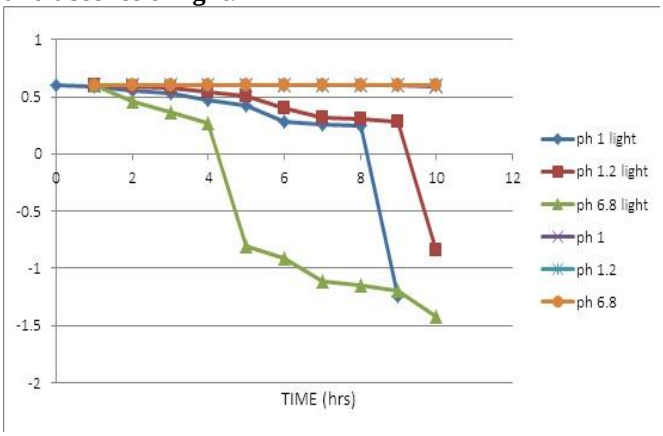
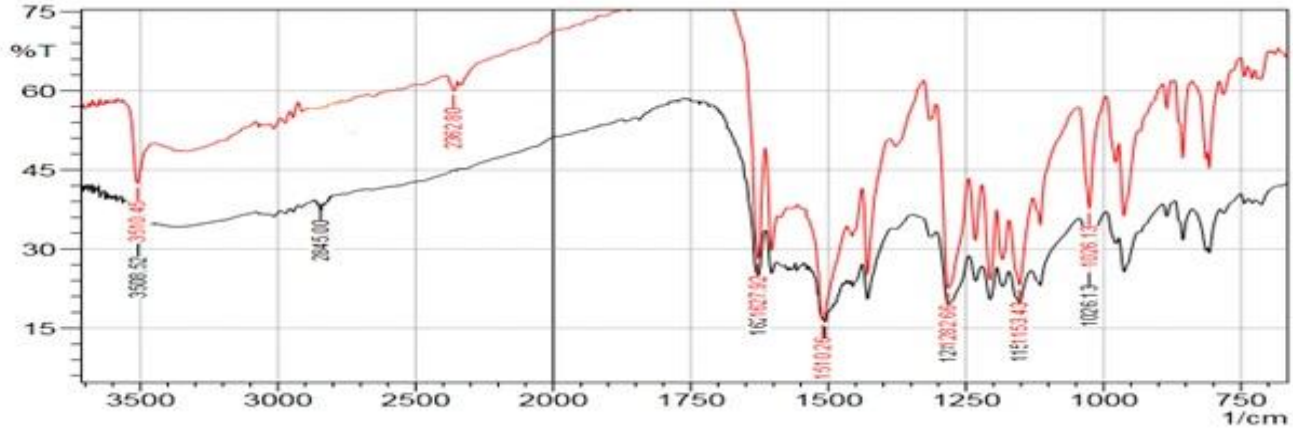
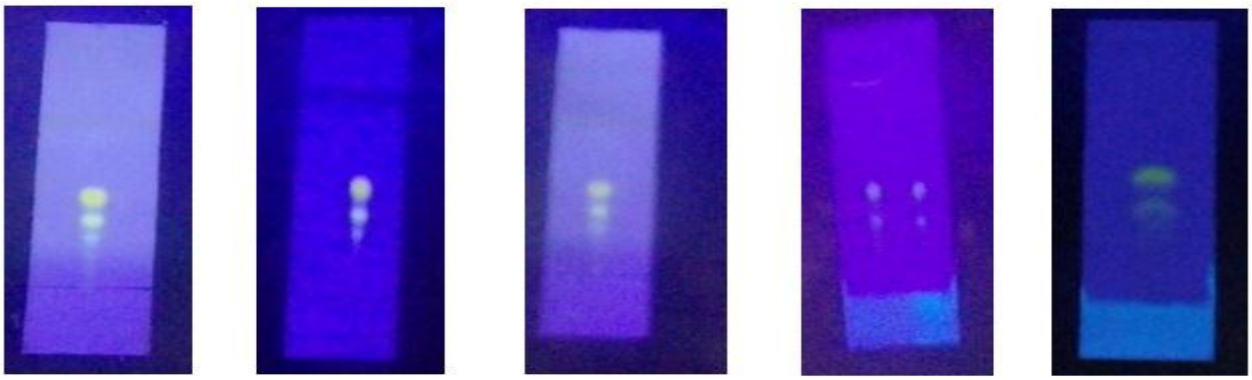


Fig 6. IR spectra of standard curcumin (red) and curcumin at pH 7.4 (black)



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1026.13	26.35	9.971	1043.49	995.27	23.559	2.446
2	1153.43	19.731	6.095	1172.72	1132.21	25.972	2.16
3	1429.25	19.574	12.1	1303.88	1244.09	36.814	6.343
4	1506.41	16.101	1.154	1508.33	1498.69	7.379	0.123
5	1627.92	24.765	12.713	1681.93	1614.42	27.659	2.338
6	2845	38.69	0.815	2856.58	2777.5	31.795	0.187
7	3508.52	31.792	1.517	3539.38	3502.73	17.047	0.432
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1026.13	37.629	20.089	1045.42	995.27	15.034	3.149
2	1153.43	23.117	14.18	1172.72	1128.36	22.943	4.054
3	1429.25	22.531	29.926	1303.88	1244.09	28.457	10.405
4	1510.26	16.457	19.841	1541.12	1465.9	43.036	9.638
5	1627.92	28.096	27.055	1674.21	1614.42	16.583	3.74
6	2362.8	60.003	2.199	2389.8	2349.3	8.607	0.306
7	3510.45	42.428	11.827	3545.16	3460.3	26.671	3.599



Standard curcumin pH 1 pH 1.2 pH 6.8 pH 7.4

Infrared spectroscopy of the Standard curcumin and curcumin at pH 7.4 was taken and compared the spectral bands and its shifts.

CONCLUSION

From the above experiment, curcumin is found to be unstable in solution form. Stability increases in acidic and decreases as the pH increases. Also in the presence of light the degradation was much higher as compared to in the absence of light. It has been found that that curcumin at pH 1.2 is highly stable in the absence of light as compared to pH 1.2 in the presence of light. Stability decreases as the pH of the solution increases with the formation of its degraded products such as ferulic acid and vanillin.

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