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DETERMINATION OF CURCUMINOIDS STABILITY BY UV-VISIBLE SPECTROPHOTOMETRY

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INTRODUCTION

Curcuma longa Linn (commonly known as Turmeric) has revealed numerous pharmacological properties, including antioxidant, anti-inflammatory, anti-microbial, anti-carcinogenic, and anti-arthritic activities. These activities are attributable to polyphenolic curcuminoids[1-3]. Curcumin, main coloring substance in turmeric and two related compounds, demethoxycurcumin and bisdemethoxycurcumin are altogether known as curcuminoids[1, 4]. Curcuminoids are yellow-orange powder that is soluble in ethanol, dimethylsulfoxide (DMSO), and acetone but insoluble in water and ether. Curcuminoids are stable at acidic pH but unstable at neutral and basic pH, under which conditions they are rapidly hydrolyzed[5, 6]. Degradation of curcuminoids is extremely slow at pH 1-6 as normally encountered in the stomach. Furthermore, they are readily decomposed when exposed to bright light, high temperature or oxidative conditions[6].

In this study, solutions of curcuminoids were prepared and the stability of curcuminoids in 0.1 N HCl and pH 7.4 phosphate buffer at various temperatures was investigated. The simple method, UV-visible spectrophotometry was used to determine the remaining amount of curcuminoids. The objective of this study was to determine the stability of curcuminoids in 0.1 N HCl (acidic conditions) or pH 7.4 phosphate buffer (basic conditions) by UV-visible spectrophotometry.

MATERIALS AND METHODS

Materials:

Commercial curcuminoids were purchased from Government Pharmaceutical Organization (GPO), Thailand. Polysorbate 80 (Tween 80) (NOF corporation, Japan) was used as surfactant to increase solubility of curcuminionds. All other reagents were of an analytical grade.

Preparation of standard solutions:

Stock solutions of curcuminoids were prepared by dissolving curcuminoids in Tween 80 and added to 0.1 N HCl or pH 7.4 phosphate buffer (final concentration of Tween 80 was 2% v/v). Standard solutions were prepared by diluting the stock solutions with 0.1 N HCl or pH 7.4 phosphate buffer to obtain concentrations range of 1.25 - 30 µg/ml. All samples were determined by UV-visible spectrophotometry at a wavelength of 424 nm (n = 3).

Stability studies:

Sample solutions were prepared by diluting the stock solutions with 0.1 N HCl or pH 7.4 phosphate buffer to obtain concentrations of 25 µg/ml for curcuminoids. The sample solutions were filled in amber glass bottles. The bottles were then stored at room temperature (RT) and in thermostatically controlled ovens at 45 °C and 80 °C. Sample solutions were taken at predetermined time intervals. The remaining amount of curcuminoids was assayed by UV-visible spectrophotometry at a wavelength of 424 nm (n = 3). Percentage of curcuminoids remaining was calculated as a relative percentage of initial amount curcuminoids at time zero to amount of curcuminoids at time t.

RESULTS

Linearity:

The calibration curves were established by plotting the absorbance against curcuminoids concentrations. The slopes, y-intercepts, and correlation coefficients (R^2) obtained from regression analysis are shown in Figure 1. The calibration curves were linear in the tested concentration ranges. The regression equations were $y = 0.1214x + 0.0037$ ($R^2 = 0.9992$) and $y = 0.1287x + 0.0197$ ($R^2 = 0.9994$) for curcuminoids in 0.1 N HCl and pH 7.4 phosphate buffer, respectively. The correlation coefficients were all greater than 0.999, indicating high degrees of correlation and good linearity of the method.

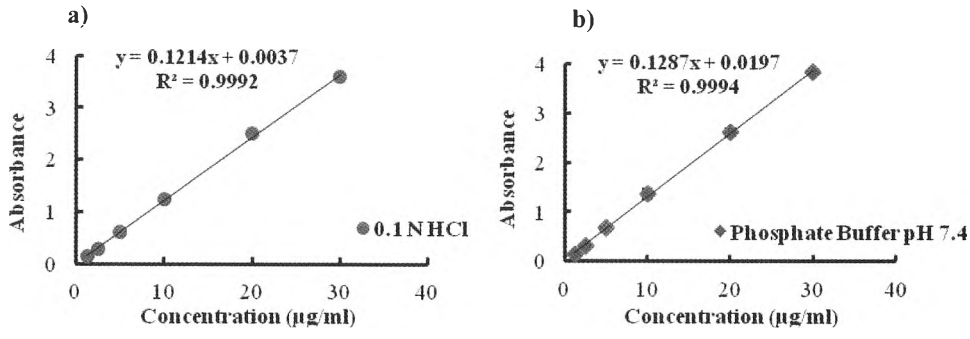

Figure 1 Calibration curves of standard solution of curcuminoids in (a) 0.1 N HCl and (b) pH 7.4 phosphate buffer determined by UV-visible spectrophotometry (wavelength 424 nm)

Stability of curcuminoids determined by UV-visible spectrophotometry:

Effect of temperature on stability of curcuminoids

The stability of curcuminoids were determined under accelerated conditions at 45°C and 80 °C, and compared to the results obtained at usual room temperature (26±2°C). The stability of curcuminoids in 0.1 N HCl and pH 7.4 phosphate buffer are shown in Figure 2. The results showed that percent remaining of curcuminoids decreased with increasing incubation temperature and turbid solution was obtained. When curcuminoids were incubated at 80 °C in 0.1 N HCl and pH 7.4 phosphate buffer, about 50% and 70% of curcuminoids decomposed within 6 hours, respectively.

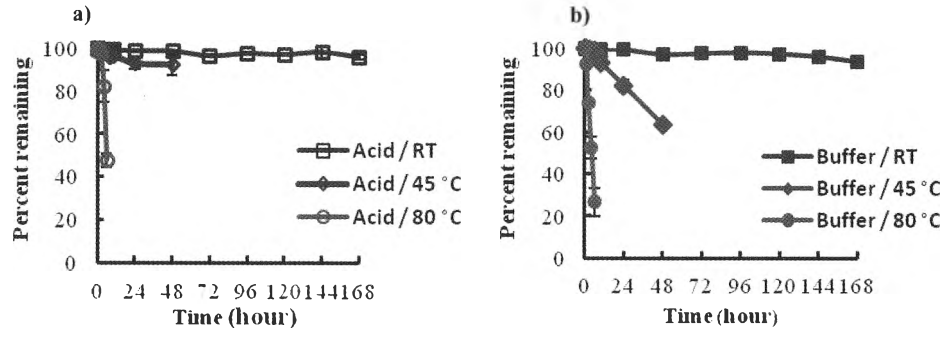

Figure 2 Effect of temperature on stability of curcuminoids in (a) 0.1 N HCl and (b) pH 7.4 phosphate buffer determined by UV-visible spectrophotometry (424 nm)

Effect of type of medium on stability of curcuminoids

The effect of type of medium on stability of curcuminoids was investigated. Figure 3 shows stability of curcuminoids in 0.1 N HCl and pH 7.4 phosphate buffer at RT, 45 °C and 80 °C. The percent remaining of curcuminoids in 0.1 N HCl was higher than that in pH 7.4 phosphate buffer, indicating that curcuminoids were more stable in 0.1 N HCl than pH 7.4 phosphate buffer.

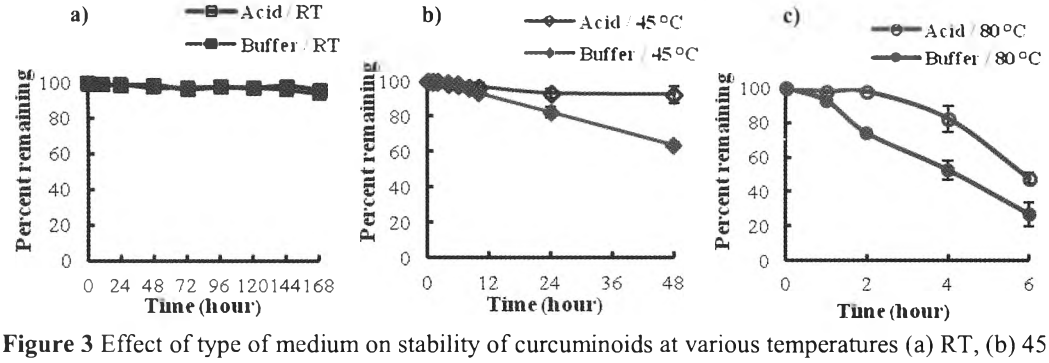

Figure 3 Effect of type of medium on stability of curcuminoids at various temperatures (a) RT, (b) 45 °C and (c) 80 °C determined by UV-visible spectrophotometry (wavelength 424 nm)

Kinetics studies on the stability of curcuminoids

The graphs of remaining curcuminoids concentration in 0.1 N HCl and pH 7.4 phosphate buffer against time were reasonably linear at all temperatures tested, indicating that degradation of curcuminoids followed apparent zero-order kinetics. Table 1 shows the observed rate constants of curcumionoids degradation (K) at each storage temperatures.

Table 1 Observed rate constants (K) for curcuminoids degradation in 0.1 N HCl and pH 7.4 phosphate buffer during storage at various temperatures

Temperature (°C)	0.1 N HCl		pH 7.4 Phosphate buffer	
	K x 10^2 (μg/ml h^{-1})	R^2	K x 10^2 (μg/ml h^{-1})	R^2
80	855.35	0.8636	1133.2	0.9875
45	16.57	0.8254	78.42	0.9917
RT (26±2)	2.85	0.8233	2.85	0.8637

DISCUSSION

The results showed that the percent remaining of curcuminoids decreased with increasing incubation temperature and turbid solution was observed, indicating that high temperature accelerated the decomposition of curcuminiods. These findings are in accordance with the results of other research [6]. Kinetics studies on the stability of curcuminoids showed degradation of curcuminoids followed apparent zero-order kinetics. The observed rate constants (K) increased with increasing temperature, indicating that curcuminoids degradation rate increased with increasing incubation temperature.

Furthermore, in this study, it was found that percent remaining of curcuminoids in 0.1 N HCl was higher than curcuminoids in pH 7.4 phosphate buffer. The results suggested that degradation of curcuminoids were pH-dependent and occurred faster at basic conditions. The increased stability of curcuminoids in acidic condition may be contributed by the conjugated diene structure. In addition, when the pH was adjusted to neutral-basic conditions, proton removed from the phenolic group, leading to the destruction of this structure. These results are consistent with the previous study of Wang *et al.*[5], which reported that curcumin is unstable in 0.1 M phosphate buffer, pH 7.2, at 37 °C. Degradation of curcumin was more than 90% in 0.1 M phosphate buffer.

CONCLUSION

The UV-visible spectrophotometry of curcuminoids showed the high degrees of correlation and good linearity. Thus, this method is possible to use for the determination of curcuminoids. Under acidic condition (0.1 N HCl), curcuminoids were stable. The percent remaining of the curcuminoids in 0.1 N HCl was higher than that in pH 7.4 phosphate buffer. Moreover, temperatures affected curcuminoids stability. It was found that the stability of curcuminoids decreased with increasing incubation temperatures.

ACKNOWLEDGMENTS

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