CHAPTER IV

SENSITIVE-DYES INCOPORATED PP/ORGANOCLAY NANOCOMPOSITES

4.1 Abstract

A novel color indicator for fresh milk packaging is developed to evaluate the degree of fresh milk deterioration during storage and distribution. The processing of pH-sensitive material used for milk packaging based on polypropylene/organoclay nanocomposites incorporated with indicator dyes was focused. The nanoclay composites with indicator dye were compounding through a twin screw extruder using Surlyn® as a reactive compatibilizer. The nanoclay composites were fabricated into the sample sheet for the color testing. Milk deterioration was assessed for titratable acidity (TA), and color changes of the indicator were measured and expressed as Hunter values as well as total color difference (TCD). TCD values of bromothymol blue (BMB) and bromocresol purple (BP) type indicator also changed continuously with the response of the nanocomposte indicator film. The color changes of the nanocomposte indicator film correlated well with TA value of fresh milk. According to the changes in Hunter color values of the nanocomposte indicator film within the packages of fresh milk during storage at ambient temperature, the results show that the color of BMB type-film turned from initially green to finally yellow whereas these of BP type-film turned from violet to light green. The color changes of the developed indicator properly represented the degree of deterioration of fresh milk. The nanocomposite indicator films could be employed as an effective smart packaging technology for evaluating fresh milk.

4.2 Introduction

Milk packaging has been newly developed to evaluate the degree of milk spoilage during storage mainly due to increased demands on product safety, environmental issues, cost-efficiency, shelf-life extension and customer convenience. According polymer/clay nanocomposites, the property improvements over polymers are high mechanical properties, excellent heat resistance, dimension stability, low gas permeability and improved die-ability. Bentonite is selected to be clay for modifying as an organoclay because of low price and widely available in the market. Due to the incorporation of organoclay and indicator not only the nanoparticles of organoclay are able to block oxygen, carbon dioxide and moisture but also make the plastic lighter, stronger and more heat resistance [1]. Moreover, the smart packing can indicate directly the quality of the fresh milk instead of following the designated expiry date [2].

The pH in normal fresh milk is approximately 6.6. The values higher than 6.7 indicate mastitis inflections, whereas the values below 6.5 indicate the presence of colostrums or bacterial deterioration. The acidity in milk is measured, for example by titration with a 0.1N NaOH solution, and indicates the consumption of NaOH to shift the pH from 6.6 corresponding to fresh milk, to pH 8.2-8.4. The determination of acidity in fresh milk by means of titration is therefore more a measure of the buffer action of milk than anything else. It is thus necessary to mention about the developed acidity, which is the result of bacterial activity producing lactic acid during milk collection, transportation and processing. In order to avoid the uncertainties about the degree of titratable acidity or developed acidity, it is important to use directly to determine lactic acid during processing.

When milk becomes curding, pH of fresh milk reduces from 6.8 to 5.2. The reason why bromocresol purple (BP) and bromothymol blue (BMB) are selected to be incorporated to PP is that their color changes are respond to pH reduction found in fresh milk. In case of BP, pH change range is 5.2-6.8 (yellow to violet) whereas this of BMB is 6.0-7.6 (yellow to blue). So BP can be performed as a sensitive dye for fresh milk package better than BMB because its color change range is more suitable than BMB's.

In present work, Na-bentonite was treated with a quaternary alkylammonium cation to obtain the organoclay for use as a nano-reinforcement in polymer matrix and substrate for dying. The indicator dyes were incorporated in nanoclay before fabricating the nanocomposites indicator film. The capability to use this film as pH sensitive packaging is demonstrated in term of change in total color difference (TCD) and titratable acidity (TA).

4.3 Experimental

A. Preparation of organomodified bentonite

350 g of Na-bentonite, (Mac-Gel® GRADE SAC) supplied by Thai Nippon Co., Ltd. Thailand, was swollen in water 1.05 liter for 24 hr. DOEM (Stepantex VP-850, Union Carbide Co., Ltd.) 285 g as an alkyl ammonium ion (1.5CEC) was dissolved in the mixture of water and methanol (1:1 v/v) 1500 ml. The whole swollen clay was then mixed with DOEM solution under vigorous stirring for 2 hr at 80°C. After that the mixture was homogenized stirring for 20 min at 80°C. The sediment was filtrated and washed with hot water several times to remove to excess salts. It was dried in a vacuum oven at 100 °C overnight and ground into powder using centrifugal ball mill (FRITSCH Peluerisette 6) for 20 min before being screened through a mesh #325.

The presence of the organo-surfactant was measured by FT-IR. Fourier transform infrared spectra were recorded using a Nicolet Nexus 670 FT-IR spectrometer over a wave number range of 4,000-400 cm^{-1} with 32 scans at a resolution of 2 cm^{-1}.

B. Preparation of nanocomposite indicator films

The nanocomposite indicator films consisted of polypropylene (Meplen HP 400H, HMC Polymer Co., Ltd.), organomodified clay, Na salt Surlyn® (PC350, Du-Pont Co., Ltd.) as a reactive compatibilizer [3] and indicator dyes, bromothymol blue (BMB), and bromocresol purple (BP) (Labchem Chemical Co., Ltd.). They were prepared as a two-step compounding process. First, polypropylene was blending with 6%wt of compatibilizer, Surlyn® using twin screw extruder (Colin D-8017 T20 with L/D ratio of 30 and 25-mm-diameter) maintained at 80/160/180/190/200/210°C from feed to die with screw speed of 50 rpm. Then, the organoclay and the indicator dye were mixed of the weight ratio 10:1 by hand shaking, after that the 3wt% clay mixture was incorporated in to the PP/6wt% Surlyn® by twin screw extrusion. The pellets of the nanoclay composites were processed by the tubular blow-film extrusion machine (L/D ratio of 26 and screw diameter of 45 mm) to fabricate the sample film of 60 μm wide using screw speed of 50 rpm with temperature profile from hopper to die of 210°C. The resulting films were cut into small pieces (6x6cm). This could be used for the color change testing. The nanocomposite indicator films were soaked in a 2200 ml water container for approximately a week, and then the absorbance of the water at a wavelength of 609 nm was carried out to detect the presence of the indicator dyes. The fresh milk was obtained from CP-Meiji Co., Ltd. and tested 2 weeks before the expired date.

The structure of organobentonite and its PP nanocomposites was analyzed by Wide angle X-ray diffraction (WAXD) using a Rigaku Model Dmax 2002 diffractometer with Ni-filtered Cu K_α radiation operated at 40 kV and 30 mA. The experiment was performed in the 2θ range of 1.2-2.0 degrees with scan speed 2 degree/min and scan step 0.01 degree.

C. Preparation and analysis of fresh milk

The degree of deterioration of fresh milk was assessed as titratable acidity (TA), which was compared with Hunter color values of the indicators. Color changes of the indicator were measured through the lid with a Chroma Meter (ColorFlex® CX1034) and expressed as Hunter system (*L*, *a* and *b*) values and total color difference (TCD). The TCD value (ΔE) was calculated by the following equation [4]:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \qquad (1)$$

Here, ΔL is the brightness difference between sample and target, Δa the redness difference between sample and target, and Δb is the yellowness difference between sample and target. The target color is (93.13, -0.96, 1.69) corresponding to (*L*, *a*, *b*) for white standard color in Hunter system. The TA was assessed by titrating 25 ml of the milk sample kept at difference storage times with 0.1 N NaOH solution to reach end point at pH 8.3, and converting as lactic acid content (g/l) following by equation (2).

$$\text{TA (g/l)} = \frac{\text{ml NaOH} \times \text{normality NaOH} \times \text{eq. wt of lactic acid} \times 100}{\text{Sample volume (ml)}} \qquad (2)$$

The indicator dyes were dissolved in water to prepare the standard solution of 1, 2, 5 and 10 ppm. The absorbance at 609 nm of the indicator dye standard solutions was observed by UV-vis spectroscopy (SHIMADZU model UV-2550) with medium scan rate and sampling data every 1 nm.

4.4 Results and Discussion

A. Characterizations of the organoclay and the nanocomposites

The layered expansions of the organoclay and the nanocomposites are presented in Fig. 4.1. It can be observed that the d_{001} peak (Fig. 4.1a, b) shift to the lower angle, corresponding to an increase on the basal spacing of the clay by exchange of interlayer with alkylammonium ions. The d_{001} peak of pristine Na-BTN is 1.49 nm ($2\theta = 5.92^{\circ}$) while those of the organoclays are presented at lower distance (lower 2θ angles). However, this modified bentonite shows broad peaks at the lower angles due to the inhomogeneous distribution of the surfactant between the layers of the clay, presenting a broader range of interlayer distances depending on the extent of ion exchange. For the PP/organoclay nanocomposites with the indicator dyes, it cannot be observed the XRD patterns suggesting that the organoclay modified with cationic surfactant has a nearly exfoliated dispersion in the polymer matrix. [5]

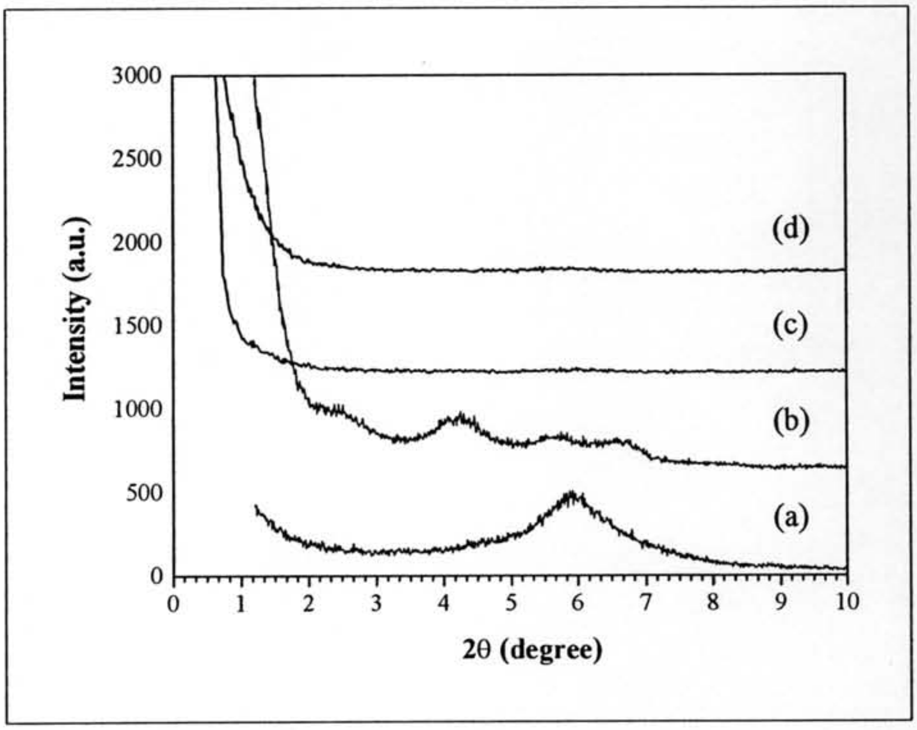

Figure 4.1 The WAXD patterns of organomodified bentonite: (a) Na-BTN, (b) DOEM-BTN, (c) PP2S6D3-BP and (d) PP2S6D3-BMB.

In order to obtain the evidence for the intercalation of the organo-surfactant into the silicate layers, FT-IR spectra were recorded in the region of 400-4000 cm^{-1}. Figure 4.2 shows the FT-IR spectra of Na-BTN and DOEM-BTN. The bands at 1092 and 1039 cm^{-1} are the characteristic absorption bands of the clay. [6] After the treatment of DOEM, not only a pair of strong bands near 2850 and 2930 cm^{-1} at each spectrum can be assigned to the symmetric and asymmetric stretching vibration of methylene group (υCH_2) of the guest molecules but the characteristic bands of amide group at 1750 and 1470 cm^{-1} are observed as well, supporting the intercalation of the surfactant molecule between the silicate layers.

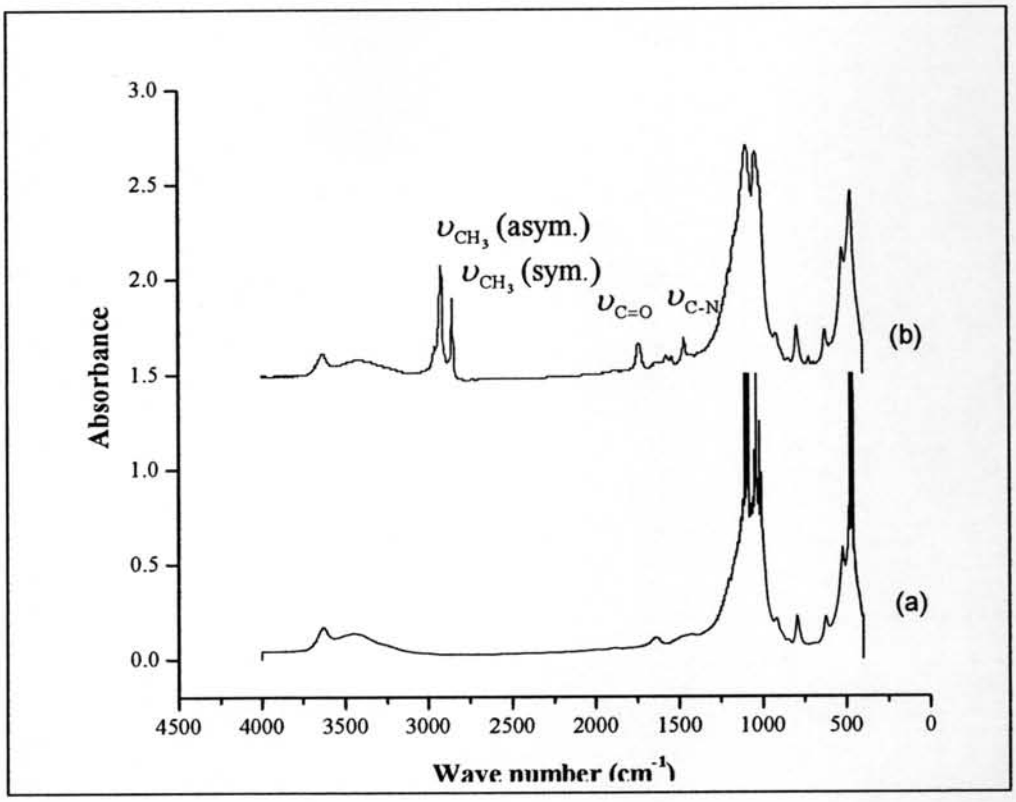

Figure 4.2 Infrared spectra of organomodified bentonite: (a) Na-BTN and (b) DOEM-BTN.

B. Milk deterioration and color changes of the nanocomposite indicator films

TA and pH are usually chosen as the major quality attributes and deterioration indexes of fresh milk because they can represent the characteristic sourness and carbonated taste of the spoiled milk. The changes in TA of fresh milk during storage at room temperature were measured as presented in Fig. 4.3. The TA values of fresh milk gradually increased at the initial step, steeply increased at the medium step, and remain constant at the final step of milk deterioration. This shows a sigmoid increase in TA with increasing storage time.

The sudden increase of TA on the third day of measurement corresponds to change in pH from 6.7 to 5.9 and the milk start to be curding. At this point, the change in TA is only about 0.14 g/l or about 50% increase in TA. In other words, the critical TA content of milk spoilage is 0.36 g/l.

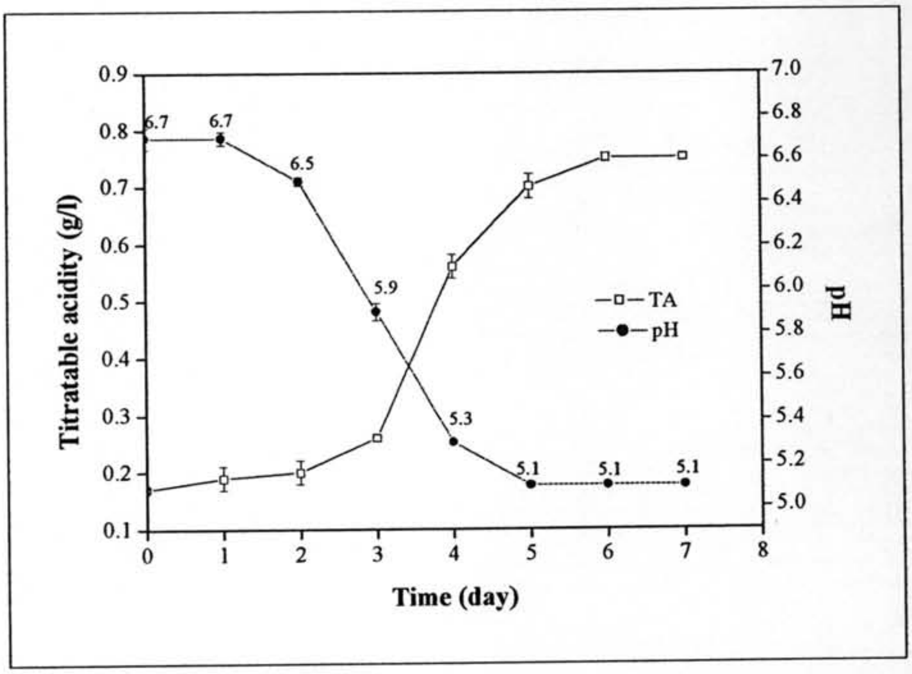

Figure 4.3 Changes in titratable acidity (TA) and pH of fresh milk during storage at ambient temperature.

The changes in Hunter color values for both indicators of packaged films upon direct contact of the milk during storage at room temperature are compared in Fig. 4.4. Hunter *L* increases slowly with storage time, Hunter *b* slightly increases at the initial stage, gradually increases at the middle stage and remain constant at the final stage while Hunter *a* decreases with time and remain constant. In case of BMB type film, the color of the film turns from initially green to finally yellow, whereas that of BP type film turns from violet to light green. The color changes of the nano-composite indicator films are not only by the chemical change of the chemical dyes but also by the response the organoclay.

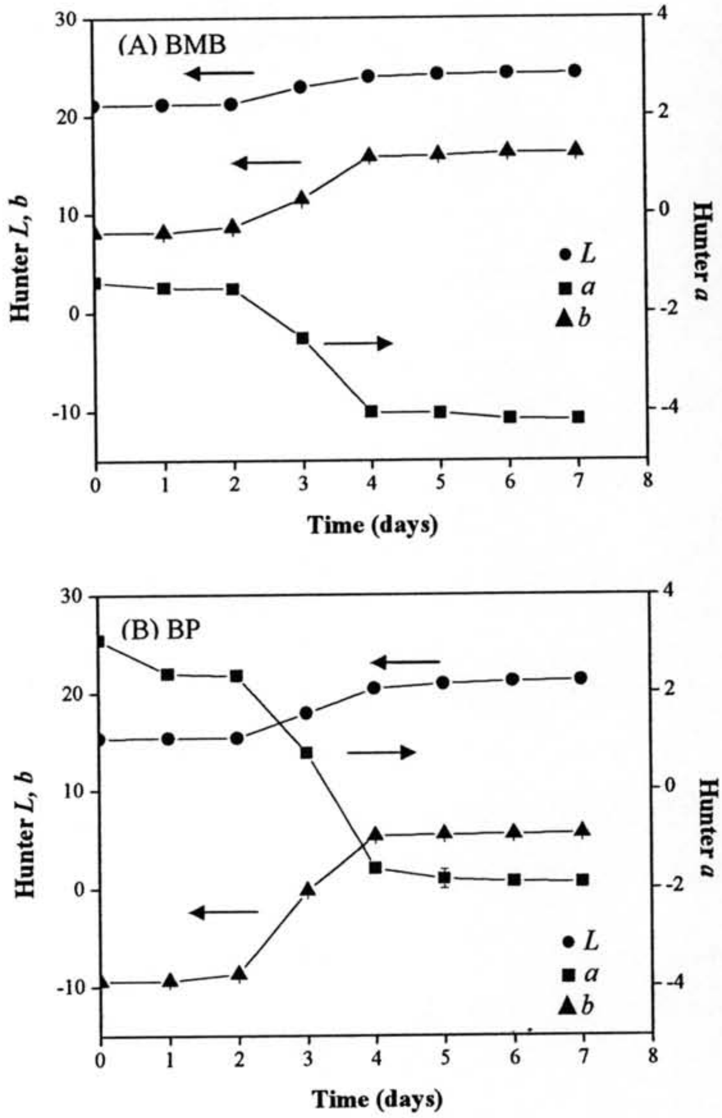

Figure 4.4 Changes in Hunter color values of the indicator films in the milk during storage at ambient temperature: (A) BMB type, (B) BP type.

C. Response function of the nanocomposite indicator films

The TCD values (ΔE) of the nanocomposite indicator films for fresh milk packaging during storage at ambient temperature are calculated and depicted in Fig. 4.5. The TCD value also changes continuously with the response of the nanocomposite indicator films. The TCD values significantly increased with time when fresh milk became bad. It is generally known that TCD values more than 5.0 can be easily detected by human eyes and TCD more than 12.0 may imply absolutely different color spaces.

From the color data below, when fresh milk started curding on the third day, TCD values of BP type film were about 10. The change of the film's color is thus easy to notice by the different color spaces (from violet to light green). However, that of BMB type film is around 4, which means that it is rather difficult to detect the color change. The milk is completely spoiled, the BMB type film shows TCD around 8 and thus it is effective enough to indicate the bad milk.

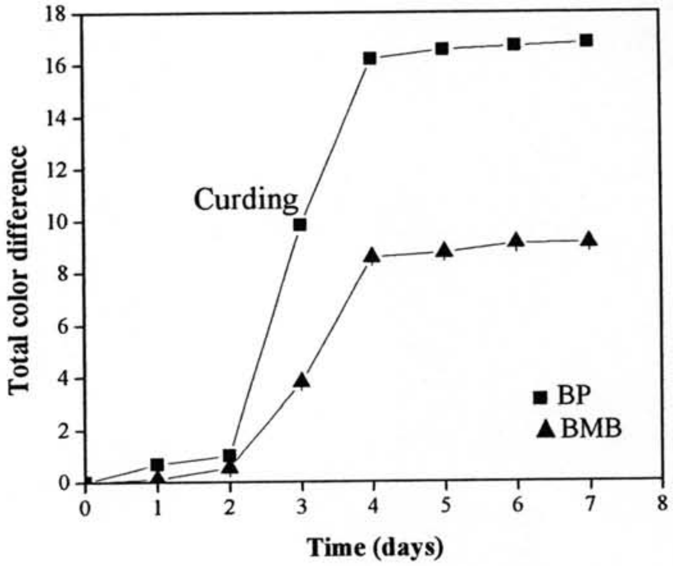

Figure 4.5 Changes in TCD values of the indicator films in the milk during storage at ambient temperature.

D. Correlation of response of the nanocomposite indicator films to milk spoilage

The main purpose for the application of the nanocomposte indicator film to fresh milk packaging is to provide an easy and reliable method to evaluate the degree of the deterioration of fresh milk in non-destructive way during distribution and retail sale. The TA values of fresh milk are hence compared with the TCD values of the indicator as illustrated in Fig. 4.6.

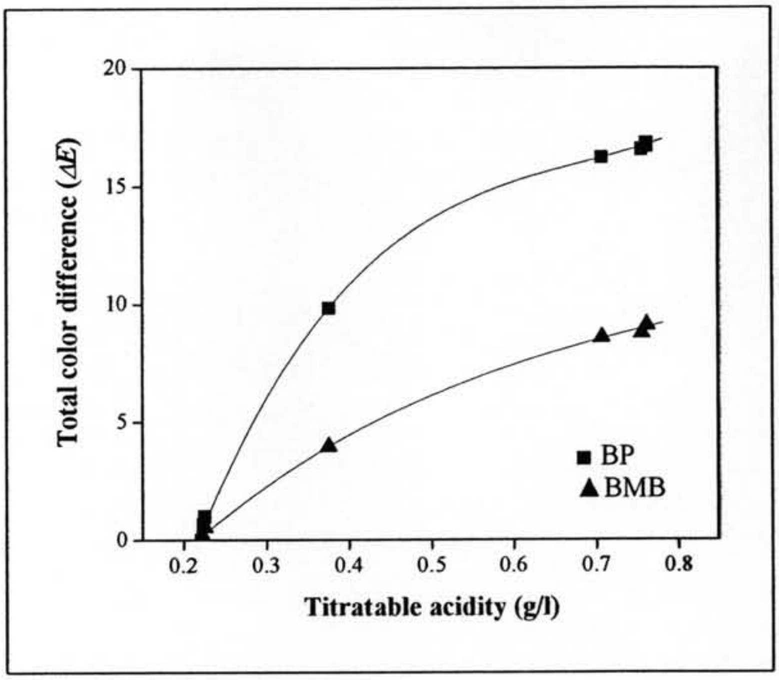

Figure 4.6 Correlation between TCD values of the indicator films and TA of fresh milk at ambient temperature.

The TCD values of both indicators give a square curve over the TA values. Correlation between the TCD values of fresh milk and the TA values of fresh milk was obviously proved by a regression analysis. The determination coefficients (r^2) for TCD of the indicators with respect to TA of fresh milk were 0.9994 for BP type film and 0.9987 for BMB type film. Present results suggest that BP can be used as fresh milk deterioration indicator due to the TCD values nearly 10 at TA of 0.4. However, the TCD values of the BMB type film were less than 5 so its color change could not be easily detected by human eyes. Thus when milk starts curding only BP type film can provide the color changes enough to indicate milk curding responding to TA value of 0.4.

E. The study on the leakage of the indicator dyes

The leakage of the indicator dyes incorporated into the nanocomposite indicator films observed by UV-vis spectrometer in form of calibration curve at 609 nm is shown in Fig. 4.7. The amount of BMB and BP leaked to water is just only 1.68 and 1.80 ppm, respectively because PP incorporated with bentonite modified by the quaternary alkylammonium cation can prevent the leakage of the water soluble dyes due to the formation of an ion pair and create a lipophillic film. [7-9]

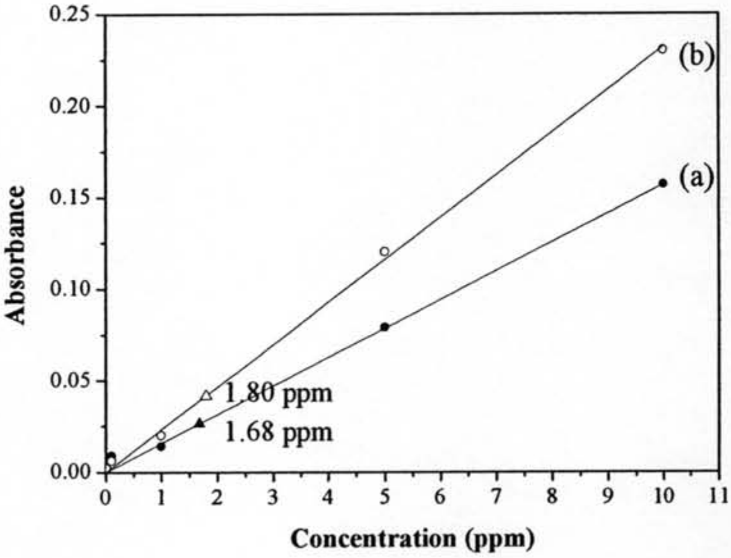

Figure 4.7 The leakage of the indicator dyes incorporated into the nanocomposite indicator films (a) BMB type film and (b) BP type film.

4.5 Conclusions

The color changes of the developed nanocomposite indicator film properly represent the degree of deterioration of fresh milk. The nanocomposite indicator film fabricated from polypropylene, modified nanoclay and bromocresol purple could be employed as an effective smart packaging for evaluating fresh milk. However, BMB type indicator film cannot be used as an effective packaging since the TCD value is less than 5 at TA of 0.4 so it cannot be observed easily by human eyes.

4.6 Acknowledgements

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4.7 References

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