CHAPTER II

LITERATURE REVIEW

1. Time-temperature indicators (TTIs)

Time temperature indicators are devices that can be used to monitor the time/temperature history, for example of foods, vaccines and other pharmaceuticals. The principle of TTI operation is either a mechanical, chemical, or enzymatic irreversible change, usually expressed as a visible response in the form of mechanical deformation, color development, or color movement. The rate if change is temperature dependent, increasing at higher temperatures in a manner similar to most physicochemical reactions as modeled by Arrhenius kinetics. The visible reading obtained gives information on the storage conditions that the tag has been exposed to. The extension which this information reflects a real time-temperature history depends on the type of indicator.

TTIs devices should fulfill the following requirements:

- React to temperature fluctuations across a wide range of temperature,
- Have high accuracy and reproductively,
- Be easy to activate, with a definitive point of activation,
- Be able to be stored prior to use, without a reaction being initiated,
- Be resistant to physical, chemical and mechanical abuse,
- The signal must be easy to read and understand,
- Track the product temperature as closely as possible

- Be indelible, tamper-proof and impossible to remove from the product.

1.1 Classification of indicators

Indicators can thus be classified according to the kind of information they convey, their functionality, and their operation principle. Taoukis, Fu and Labuza (1991) proposed a three category classification:

1.1.1 Critical Temperature Indicators. CTIs show exposure above (or below) a reference temperature. They involve a time element (a few minutes up to a few hours) but are not intended to show history of exposure above or below the critical temperature. CTIs were the earliest type of indicators developed.

1.1.2 Critical Temperature/Time Integrators. CTTIs indicate a response that reflects the cumulative time-temperature exposure above a reference critical temperature. They useful for reactions important to quality or safety that occur at measurable rates only above a critical temperature. Their response can be translated into an equivalent exposure time at the critical temperature.

1.1.3 Time-Temperature Integrators or Indicators. TTIs give a continuous, temperature-dependent response. They integrate, in a single measurement, the full time-temperature history from time of activation and can be used to indicate an "effective average" temperature (T_{eff}) during distribution with theoretically can be correlated to continuous, temperature-dependent quality-loss reactions in products.

1.2 Types of commercials TTIs.

In the past decade, Taoukis and Labuza (1989) proposed three types of commercial continuous-response TTIs were the focus of both scientific and industrial trials; they make claim to satisfy most of the requirements:

1.2.1 Type I is a diffusion-based indicator, the Monitor Mark™ (3M Co., St. Paul, Minn.) It is based on time-temperature dependent diffusion of a dyed fatty acid ester through a porous wick. Before use, the dry ester was separated from the wick by a barrier film so that no diffusion occurred. To activate the indicator, the barrier was pulled off and diffusion started, if the temperature was above the melting point of the ester.

1.2.2 Type II is the I-Point®. It is based on a color change caused by a pH decrease due to a controlled enzymatic hydrolysis of a lipid substrate. Before activation the lipase and the lipid substrate are in two separate compartments. At activation, the barrier that separates them is broken, enzyme and substrate are mixed, and the color change starts.

1.2.3 Type III is the Lifelines Freshness Monitor®. It is based on the solid state polymerization of a thinly coated colorless acetylenic monomer that changes to a highly colored polymer.

Annette and Jacques (1992) have shown that at least one polydiacetylene compound is suitable for detecting whether an article has ever exceeded a selected temperature. The polydiacetylene compound is deposited on a substrate or mixed with a thermoplastic material. The polydiacetylene compound instantaneously and irreversibly changes from blue to red at the threshold temperature.

These products have so far not been commonly used due to their expense, supply problems with raw materials, limitations to their applicability, toxicity, fragility, sensitivity and the difficulty of manufacture. Preferably, the temperature sensitive material is edible. Also, the temperature sensitive material is a lipid. Lipids are ideal materials for use in time temperature indicators for two important reasons. Firstly, a great many different lipid can be readily purchased or manufactured with different melting points. Secondly, lipids are safe to use and are generally edible.

The temperature sensitive material may provide a visual image through shape and which melts at the particular temperature, thereby losing its shape, destroying the

visual image and there by indicating that the particular temperature has been exceeded.

Yoon et al. (1994) developed to monitor quality change of frozen foods during storage by using phospholipid-phospholipase. The TTI was more reliable than the lipase system and monitored quality changes of frozen pork during storage. The TTI was designed for reactions at sub-zero temperatures because the substrate emulsion would be stable at such temperatures. The TTI contained glycerol and sorbitol as anti-freeze reagents and was designed to show distinctive color changes according to pH by mixing bromothymol blue, neutral red, and methyl red.

Richard (2000) published that Thermal history indicator, which a temperature related phase change in a material leads to an indication that a high temperature event has occurred. A preferred format has a water-soluble, lipid-insoluble dry immobilizes within the lipid selected to have melting point at a particular temperature and has all components made from edible materials. Upon melting, the dye dissolves in water present in a secondary phase or the goods themselves giving a visual indication. Another format has a primary reagent within a solid lipid and a secondary reagent held with a secondary phase such that melting of the lipid allows the primary reagent to react with the secondary reagent, providing an indication of a high temperature event.

In the same way, a visual thermal history indicator comprising a pattern produced from at least two waxes wherein one wax has a melting point that differs from the other wax, or where the waxes have the same melting point but different melt flow behavior, and wherein the pattern is adapted so that when the lower melting point wax melts or the wax with greater melt flow behavior flows, the visual appearance of the pattern changes, and wherein when the second and subsequent higher melting waxes melt, or when the lower melt flow behavior waxes flow, the visual appearance of the pattern changes as each wax melts or flows. (Vincent, John, and Christain (2007)

Other TTI systems were based on viscous elastic polymers, which also migrate into a porous matrix, was introduced. Simons (2003) has investigated that a time-temperature indicator device comprises a polymeric layer having a first and second surface and a dye composition adhered to said first surface comprising a dye which diffuses into the polymeric layer as a result of a cumulative time-temperature exposure wherein the polymeric layer is formed from a natural or synthetic rubber polymer or copolymer. The device may be attached to a product for monitoring cumulative time-temperature exposure.

Another system, Xavier (2006) shown that the reaction of the TTI system by using microorganisms is based upon anaerobic respiration of the yeast that produces acids as a product of its glucose fermentation. The reaction occurs only when the first and the second parts of the TTI system are made in contact with each other via contact adhesive/glucose. The acids are produced at a rate which is dependent upon the storage temperature of the TTI system and, therefore, that of the product to which the TTI system is affixed. Hence, the TTI systems of the present invention are most useful for a type of product whose shelf life is known at a fixed temperature. Any temperature fluctuations during storage will bring about a faster (or slower) color change than expected as a result of a pH drop.

Recently, some photo luminescent (PL) materials have been proposed to be useful as TTIs (Crenshaw and Weder, 2005) The approach disclosed in the prior art relies on the phase separation of initially molecularly mixed blends of excimer-forming PL dyes and amorphous host polymers with a glass transition temperature (T_g) in a temperature regime of interest. This sensing scheme involves kinetically trapping thermodynamically unstable molecular mixtures of the excimer-forming photo luminescent sensor dyes in the glassy polymer, for example by melt-processing and rapid quenching below T_g. These materials display photoluminescence spectra that are dominated by monomer emission, that is, emission from well dispersed or dissolved individual sensor molecules. Subjecting these materials to temperatures above T_g leads to permanent and pronounced changes of their PL emission spectra, as a result of phase separation and excimer formation. These prior art TTI materials and devices display the disadvantage that they are limited in application since the read-out

is a change of the photoluminescence spectrum or color of the emitted light (not absorption spectrum or color) and therefore a special light source, for example an ultraviolet lamp, required to interrogate these prior art materials and devices.

Current studies, the invention relates to a method of printing a substrate of time temperature indicator with chromic properties based on an azo coupling reaction between a capped diazonium component and a coupling component. (Hans and Bernhard, 2007)

Farid and William (2007) were improved TTIs by printing a bar code (comprising dark and light regions) or parts of such a bar code with an ink containing a dye, which changes color in the presence of an acid or a base to remove the contrast between the regions of the bar code, and a neutral compound which releases an acid or a base on exposure to energizing radiation, a bar code can be produced that becomes unreadable after a pre-determined interval. This can be used as a time/temperature indicator to ensure that products, which might be perishable, associated with such a bar code, are not used by the public.

2. Temperature-sensitive Polymers

Temperature-sensitive polymers are probably the most commonly studied class of environment-sensitive polymers systems in drug delivery research. These polymers are able to swell or deswell as a result of changing in the temperature of surrounding fluid.

The structures of some of those polymers are shown in Figure 1. The common characteristic of temperature-sensitive polymers is the presence of hydrophobic groups, such as methyl, ethyl and propyl groups. Of the many temperature-sensitive polymers, poly (*N*-isopropylacrylamide) (PNIAAm) is probably the most extensively used. Poly (*N*, *N*-diethylacrylamide (PDEAAm) is also widely used because of its lower critical solution temperature (LCST) in the range of 25–32°C, close to the body temperature. Copolymer of NIAAm can also be made using other monomers, e.g. butyl methacrylate (BMA), to alter the LCST. (Qiu and Park, 2001)

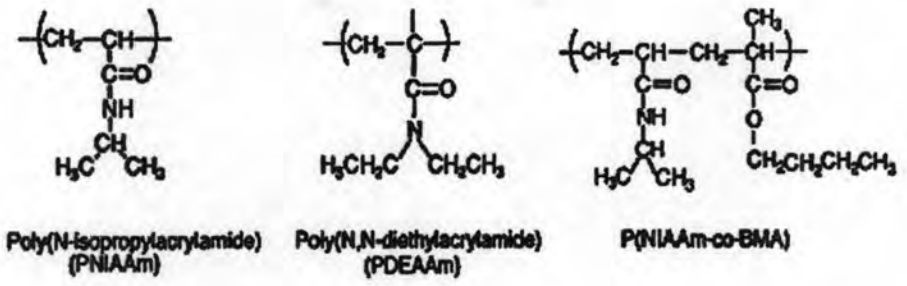

Figure 1 Structure of some temperature-sensitive polymers (Qiu and Park, 2001)

Recently, much attention has been focused on thermosensitive controlled-release systems developed from linear and crossliked polymer network with a specific temperature-dependent solubility or swelling behavior. In these systems, temperature variation triggers alteration of polymer configurations, leading to the change of release rate of compounds incorporated in the systems. For convenience, temperature-sensitive polymers are classified in to negative thermosensitive, positively thermosensitive, and thermally reversible gels (Mastekova, Chalupava and Sklubalova, 2003)

2.1 Negative Temperature-sensitive polymers have a lower critical solution temperature (LCST) and contract upon heating above the LCST. Copolymers of (*N*-isopropylacrylamide) (PNIAAM) are usually used for negative temperature release. Polymers show an on-off drug release with on at low temperature and off at high temperature allowing pulsatile drug release. LCST systems are mainly relevant for controlled release of drugs, and of proteins in particular. Thermosensitive polymers may be fixed on liposome membranes; in that case liposomes exhibit control of their content release. Another one study, has shown clear "on-off" release of indomethacin from poly (IPAAm-co-BMA) in response to a step-wise temperature change between 20°C and 30°C was reported by Okano et al. (1990)

2.2 Positive temperature-sensitive polymers have an upper critical solution temperature (UCST), such hydrogel contracts upon cooling below the UCST. Polymer networks of poly (acrylic acid) (PAA) and polyacrylamide (PAAm) or poly

(acrylamide-co-butyl methacrylate) have positive temperature dependence of swelling.

2.3 Thermoreversible gels are commonly prepared from poly (ethylene oxide)-*b*-poly (propylene oxide)-*b*-poly(ethylene oxide). Polymers solution is a free flowing liquid at ambient temperature and gels at body temperature, such a system would be easy to administer in to desired body cavity.

Figure 2 schematically illustrates the ideal MC structure designed to exhibit a positively thermosensitive drug release. The key structural feature of this MC is its composite coat consisting of nano-sized thermosensitive polymers dispersed in a thermo-insensitive ethylcellulose matrix. In this study, ethylcellulose pseudolatex, Aquacoat®, was selected as the polymer matrix. As the nano-sized thermosensitive polymers, composite latex with poly-(NIPAAm)-rich shell was newly synthesized. The detail of this latex is described later. It is well known that poly(NIPAAm) has the LCST around 32°C in water and the crossliked network (hydrogel) of this polymer shows an inverse-temperature dependence of swelling: the hydrogel swells by imbibing water below a lower gel collapse temperature (LGCT; temperature for complete deswelling), while it shrinks above the LGCT. At high temperature, therefore, the poly (NIPAAm) gels in the MC membranes should shrink, as shown in Figure 3. This shrinking would create many voids in the membranes and, consequently, the water-permeability of the membrane would increase as if a valve has been opened. Due to the voids thus formed, drug release rate at high temperatures would be expected to become higher than that at low temperature.

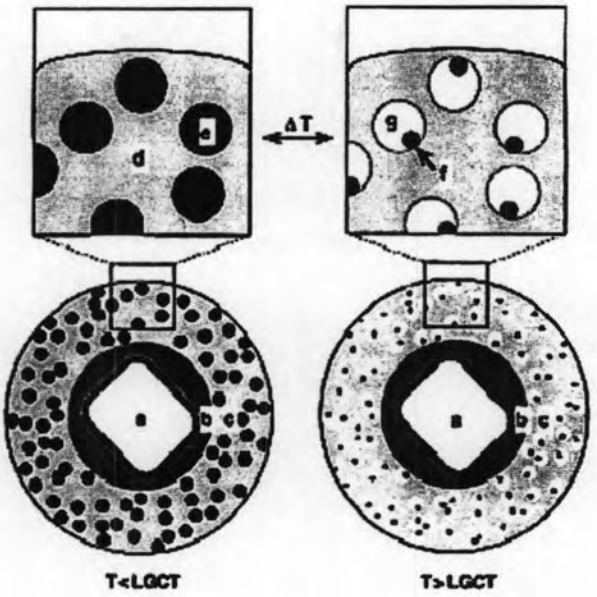

Figure 2 The ideal particle structure of the composite latex with poly(NIPAAm)-rich shell designed to be the nano-sized thermosensitive polymers is schematically. (Ichikawa and Fukumori, 2000)

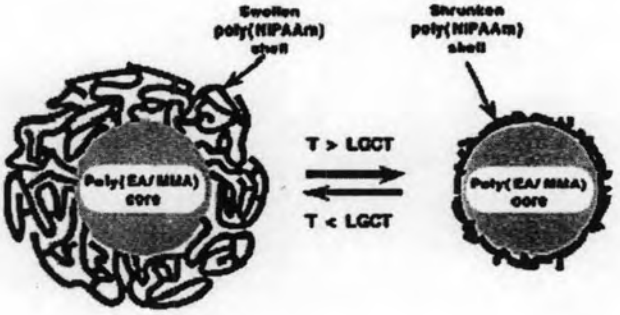

Figure 3 Schematic diagrams showing ideal particle structure of composite latex with poly (NIPAAm) hydrogel shell. (Ichikawa and Fukumori, 2000)

3. Anthocyanins

Natural Food Color is any dye obtained from any vegetable, animal or mineral, that is capable of coloring food, drugs, cosmetics or any part of human body. These natural colors come from variety of sources such as seeds, fruits and vegetables, leaves, algae & insects. According to the application a suitable Natural

Color can be achieved by keeping in mind the factors such as pH, heat, light, storage and the other ingredients. Chigurupati et al. (2002) reported that the red cabbage extract in solution was most stable at room temperature and pH 3 (percentage of degradation was only 1-5% over a period of 10 days).The color was found to drop with increases in pH, ascorbic acid, as storage time and temperature. (Walkowiak-Tomeczak and Czapski, 2007)

Anthocyanins being natural colorant appear in the exempt from certification in the FDA list but permits restricted use. They are used in beverages, fruit fillings, snacks, dairy products and confectionery.

Table 1 Anthocyanins Properties
(Available form: www.foodadditivesworld.com/anthocyanins.html, 11 Oct 07)

Color	Red, Purple and Blue
Source	Red Cabbage, Strawberries, Grape Skin, Blueberries, Raspberries
Color Pigments	Cyanidin, Delphinidin, Malvidin, Peonidin, Petunidin, Pelargonidin
Solubility	Soluble in aqueous solutions
Stability	Each pigment has different stability. Brighter in lower pH range Becomes blue at higher pH
Other Properties	Antimicrobial Properties, Antioxidant properties Anti-cancer properties

Dyrby, Westergaard and Stapelfeldt (2001) have reported that the color of anthocyanins is dependent on pH due to the existence of four different structures in aqueous solutions. The four structures have different colors and, since each of them vary in concentration throughout the pH-scale with the red flavylium cation as the dominant structure in acidic environment; the exact color of solution depends on the pH-value. Red cabbage (*Brassica oleracea* L.) is promising source of anthocyanins for coloration of foods since its anthocyanins are unique in being colored over a very broad pH-range compared to anthocyanins from, e.g. grape skin, black currant,

elderberry and red cabbage, for which they may provide a natural alternative to synthetic blue colorants.

Chigurupati et al. (2002) reported that the pH of red cabbage solution can also affect both its color and intensity. They could determine the ionization constant (pKa) of red cabbage color, the effect of pH and temperature on its stability in solution and evaluation of this natural color as a pH indicator in pharmaceutical system.

Thurman, Lohmeyer and Olds (2004) pointed out that the edible pH indicator includes any substance that changes color in response to the pH of the solution it is dissolved in. Edible indicators are non-toxic when consumed in amounts that are effective for detecting color changes due to pH variations. The pH indicator may be responsive to narrow or broad pH ranges, and may be show the greatest colorimetric response at alkaline pH ranges, acidic pH ranges, or neutral pH ranges. Particularly useful pH indicators for use in the instant beverage composition include anthocyanins. Anthocyanins are typically present in extracts of red cabbage, such as red cabbage juice, or red cabbage powder. Such extracts of red cabbage are a particularly useful edible pH indicator for inclusion in the first mixture. Red cabbage extracts are typically blue or green at high pH levels (more basic) purple at neutral pH levels, and pink to red at low pH levels (more acidic).

Chlorophyll and anthocyanin could be used as pH indicators for biodegradable materials reported by Attarian et al. (2006). Although the colorimeter has detected and correlated color variations to pH exposition, color changes were only visible with naked eyes at pH 0.0 and 14.0, indicating the new extracts sources or extraction procedures should be investigated. The natural extracts investigated as additives have affected the characterization properties of cassava starch films, and so, its utilization should be evaluated according to the type of product to be packed by the starch film material.

4. Encapsulation and matrix film

In recent years, the microencapsulation processes have been used in many industries, such as food, food additives, cosmetics, adhesives, household products and many others. Microencapsulation has been used in pharmaceutical industry for the conversion of liquids to solids, taste-masking of bitter drug, prolonged or sustained release, separated of incompatibles, reduced gastric irritation and environment al protection of labile moieties.

Micropheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes. The natural polymers include gelatin, alginate and chitosan (Ye et al. 2005, Taqieddin and Amiji, 2004, Ribeiro et al. 1999) the synthetic polymers include polylactic acid and polyglycolic acid. (Li, Wang and Wu, 1997)

The solvents used to dissolve the polymeric materials are choosing according to the polymer and drug solubilities, process safety, and economic considerations. Substance can be incorporated within microspheres in the liquid or solid state during manufacture or subsequently by absorption.

4.1 Wax coating and Hot Melt

In this method, wax is used to coat the core particles. Most commonly a simple emulsion is formed, where the drug or other substance to be encapsulated is dissolved or dispersed in the molten wax. This waxy solution or suspension is dispersed by high speed mixing into a cold solution, such as cold liquid paraffin. The mixture is agitated for at least one hour. The external phase (liquid paraffin) is then decanted and the microcapsules are washes with hexane and allowed to air-dry. Multiple emulsions may also be formed. Flow diagram of wax microcapsules was showed in Figure 4.

Wax coating provides a relatively inexpensive process and is often used. However, the microcapsules produced release drug more rapidly than polymeric microcapsules. Carnauba wax and beeswax can be used as the coating materials and these can be mixed in order to give microcapsules with desired characteristics.

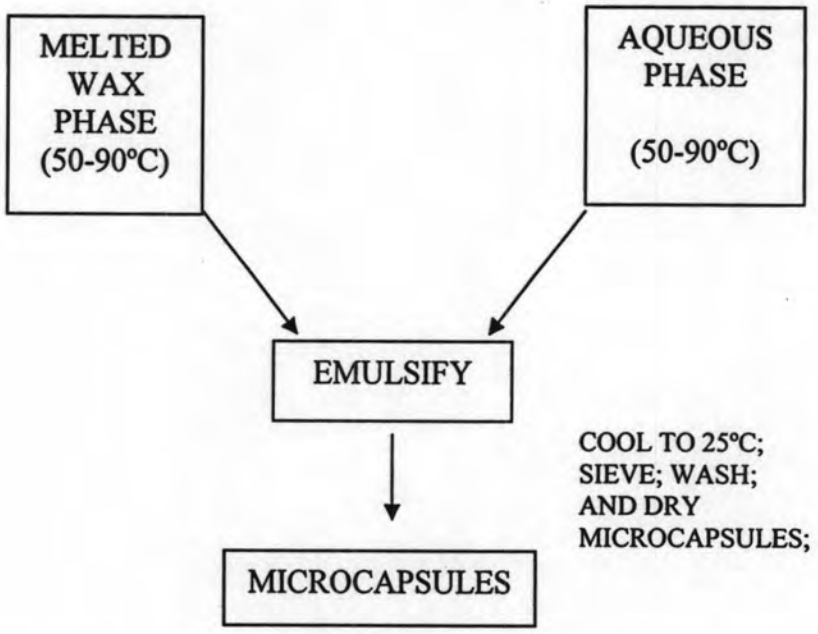

Figure 4 Flow diagram of wax microcapsules manufacturing method, oil –in-water melt-dispersion technique (Thawatchai Phaechamud and Garnpimol Ritthidej, 1996)

4.2 Spray Coating and Pan Coating

Spray coating and pan coating employ heat–jacked coating pans in which the solid drug core particles are rotated and into which the coating material is sprayed. The core particles are in the size range of micrometers up to a few millimetres. The coating material is usually sprayed at an angle from the side into the pan. The process is continued until an even coating is completed. This is the process used to coat tablets and capsules.

4.3 Coacervation / Phase separation

Encapsulation by coacervation is an uncomplicated process which does not involve any elaborate manufacturing equipment. It can be used to microencapsulate a large number of liquids, solids and gases. The polymers used to coat the core material can be both water soluble and water insoluble. Water-soluble core materials are microencapsulated with water insoluble polymer in organic solvent, on the other hand water-insoluble core materials are microencapsulated with water soluble polymer.

In the present of only one macromolecule, this process is referred to as simple coacervation. When two or more macromolecules of opposite charge are present, it is referred to as complex coacervation. Simple coacervation is induced by a change in conditions which results in molecular dehydration of the macromolecules. This may be achieved the addition of nonsolvent, the addition of micro-ions, or a temperature change, all of which promote polymer-polymer interactions over polymer-solvent interactions. Complex coacervation is driven by electrostatic interactive forces between two or more macromolecules. The large number of variables involved in complex coacervation (pH, ionic strength, macromolecule concentration, macromolecule ratio, and macromolecular weight).

Yeo et al.(2005) have encapsulated flavour oil in complex coacervate microcapsules using gelatin and gum arabic. When heated particles to 100°C or higher, univesicular microcapsules released almost all of the encapsulated oil, while multivesicular microcapsules had lesser degrees of release.

4.4 Calcium alginate Microcapsules

These are made by dropping or spraying a sodium alginate solution into a calcium chloride solution. The divalent calcium ions cross-link the alginate, forming gelled droplets. These gel droplets can be permanently cross-linked by addition to a polylysine solution. Variations on this method with different polymers

have been developed. Chitosan in preferred, because it has a better bio-compatibility than alginate. Traditionally alginate beads were formed by dropping the alginate solution into the calcium chloride with a fine bore pipette. However, the droplets were relatively large, because the drops do not fall until they reach a critical mass. Smaller droplets can be formed by using a pump to force the alginate through the pipette, a vibration system to help remove the drops from the end of pipette and air atomization method. The particle size can be reduced to 300μm with the vibration method and to less than one micrometer with the air atomization method. The particle size range obtained by this method depends on the air pressure, the nozzle size, the spraying height, and the rate of flow from the infusion pump. The substance to be encapsulated is suspended or dissolved in the sodium alginate solution.

Many studies (Kim and Lee, 1992; Kikuchi et al.,1999; Chretien, C.,2005) have investigated applicability of alginate gel beads could transport and delay the release of a macromolecular compounds. Blue dextran was used as the model of macromolecular drugs.

Alginate beads have the following advantages:

(1) Alginate is known to be nontoxic when taken orally, and also to have a protective effect on the mucous membranes of the upper gastrointestinal tract (Koji et al., 1981a-c, 1982)

(2) Since dried alginate beads have the property of reswelling, they can act as a controlled-release system;

(3) Since their property of reswelling is susceptible to environmental pH, acid-sensitive drugs incorporated into the beads would be protected from gastric juice.

Another approach, Gaserod, Smidsrod and Skjak-Braek (1998) prepared the alginate-chitosan capsules by dropping a solution of sodium alginate through a syringe connected to a coaxial airflow which gives the possibility of regulating the size of the droplets. The gelling bath contained an aqueous solution of 50 mM calcium chloride and 200 mM sodium chloride in the gelling solution. An

exact number of beads were transferred directly from the gelling solution to a solution of 0.15% (w/v) chitosan in a sodium acetate buffer and incubated for 24 hour on a shaker. They previously have shown that the binding and stability of alginate-polycation capsules depends on both the composition of the alginate gel and on the molecular weight, flexibility and charge density of the polycation.

Kim, Kim, Lee et al. (2005) have synthesized the graft copolymer (APN) of alginate and poly-*N*-isopropylacrylamide (PNIAAM) and APN beads were prepared by dropping the aqueous solution of the copolymer into an aqueous solution of Ca2+ solution. Alginate chains were employed to play a role in forming beads by electrostatic interactions with a multivalent ion, Ca2+. The percent of release of blue dextran from APN beads was higher at 40 ◦C than at 25 ◦C. The difference in the release between two temperatures became more distinguishable when the content of PNIAAm in APN beads is higher. Below lower critical solution temperature (LCST), the expanded PNIAAm would close the pores of the beads.

4.5 Micromatrices

Matrix systems are composed of drug molecules or particles homogeneously distributed through a polymer meshwork. A variety of methods have been employed to produce small spherical matrix systems. Kim, Lee, Kim et al.(2002) have prepared hydrogels composed of temperature sensitive PNIAAm and pH-responsive alginate that have macropores and a comb-type grafted structure using Sodium chloride particles as a porogen. The porous hydrogels rapidly reached their equilibrium swelling and deswelling states, and from their large surface area and free chain mobility, showed a fast response to changes in pH and temperature.

There is interest in systems like PNIAAm-based hydrogels for drug delivery applications. To Change in the swelling ratio of PNIAAm gels by raising temperature. Membranes of cross-linked polyacrylamide having poly (N-isopropylacrylamide) included, like semi-IPN hydrogels, were studied by Muniz and Geuskens. The permeability to Orange II was determined at temperatures ranging

from 25 to 40 8C. Around 32°C the permeability to Orange II increases sharply, indicating that the PNIAAm influences the average mesh size of the membrane, in comparison to PAAm hydrogels without PNIAAm, used as control.

Coughlan, Quilty and Corrigan (2004) developed a system for studying the effect of drug physicochemical properties on swelling/deswelling kinetics and pulsatile drug release from a thermoresponsive hydrogel. Hydrogels were loaded with drug and thermally triggered swelling/deswelling and release experiments were performed. Two series of drugs of contrasting hydrophilicity and varying physicochemical properties were examined. Benzoic acid (BA), its methyl and propyl esters, and diltiazem base were used as model hydrophobic drugs. Sodium benzoate (NaB), diltiazem HCl (DHCl), vitamin B_{12} (VB_{12}) and various dextrans (MW 4300, 10,200, 42,000, 68,800) were used as model hydrophilic agents of increasing size. The hydrogel swelling rate was slowed by the presence of the hydrophobic drugs and this decreased rate was solubility dependant for the benzoates. The hydrophilic series increased the rate of swelling compared to the unloaded system. In all cases, the magnitude and rate of hydrogel contraction were proportional to the extent of swelling prior to temperature switch. Drug release was by diffusion below the lower critical solution temperature (LCST), while a solubility-dependent drug pulse release on temperature switch was observed for the hydrophobic series. Effectiveness of thermal control of hydrophobic drug release increased with increasing solubility. The hydrophilic series produced a molecular size-dependent drug pulse on temperature switch above the LCST. Pulsatile on–off drug release was shown with DHCl, VB_{12} and the various dextrans. Drug solubility, size and chemical nature were shown to be of particular importance in the control of hydrogel swelling and drug release from thermosensitive hydrogels.

4.6 Spray drying

Spray drying is a single step, Closed-system process applicable to a wide variety of materials, including heat sensitive materials. The drug and the

polymer coating materials are dissolved on a suitable solvent (aqueous or nonaqueous) or the drug may be present as a suspension in the polymer solution.

4.7 Solvent Evaporation

This is one of the earliest methods of microsphere manufacture. The polymer and drug must be soluble in an organic solvent, frequently methylene chloride. The solution contains the polymer and the drug may be dispersed in an aqueous phase to from a droplet. Continuous mixing and elevated temperature may be employed to evaporate the more volatile organic solvent and leave the solid polymer-drug particles suspended in the aqueous medium. The particle finally filtered from suspension. Lin and Wu (1999) were developed a microspherical dosage form for a highly water soluble drug, by using the water insoluble, non-biodegradable polymer, ethylcellulose.

4.8 Precipitation

A variation on the evaporation method is the precipitation method. The emulsion consists of polar droplets dispersed in a non polar medium. Solvent may be removed from the droplets by the use of a cosolvent. The resulting increase in the polymer drug concentration causes a precipitation forming a suspension.

Wu et al.(2003) used water-insoluble polymer ethyl cellulose as retardant to prepare sustained release of potassium chloride micorspheres in this manner. Both poly (lactic acid)(PLA) and poly(D,L-lactic –co-glycolic acid)(PLGA) could be used by accurately choosing the polymer solvent to encapsulation of hydrophilic drugs.. The particles were prepared from the method obtained ranged from about 85-560 nm. (Bilati, Allemann and Doelker, 2005)

5. Degradation of pharmaceutical product and kinetics

The chemical stability of a drug is of great importance since it becomes less effective as it undergoes degradation. They decompose to form one or more degradation products, and the rate of degradation depends on their chemical nature and on conditions of storage and manipulation. Some drugs are reasonably stable and decompose only under adverse conditions (extremes of temperature, humidity, light and presence of oxygen). Also, drug decomposition may yield toxic by-products that are harmful to the patient. These researches have focused on effect of deferent temperature on the stability of pharmaceuticals product.

As temperature is increased, there is a greater available amount of free energy, which tends to increase the rate of degradation. Typically a 10°C increase produces a 2 or 3 fold increase in the rate of reaction. In the 1870s, Arrhenius pointed out that rate constants varied with temperature according to an equation.

$$K = Ae^{-Ea} / RT \quad \text{or} \quad \log K = \log A - (Ea/2.303R)/T \text{ ---------- Eq. 1}$$

Where A is a constant that is termed the frequency factor, Ea is the energy of activation, R is the gas constant and T is the temperature. Plotting the log of the rate of reaction K, against 1/T produces a straight line and thus the constants Ea and A may be determined from the slope and intercept of this line respectively.

Prediction of shelf life (t_{90}, time to 90% potency) can also be predicted. This forms the basis of many accelerated stability tests. However the mechanism or pathway of the chemical breakdown often changes with temperature. This can often be observed by a discontinuity and knee joint in the Arrhenius plot but often it is not easily detected. This can lead to wrong conclusions based on elevated temperatures for storage at room temperature or under refrigeration.

These researches were performed to investigate the stability of glibenclamide and aspirin tablet by measuring the polymorphism transformations and the degradations as function of temperature. Glibenclamide was characterized polymorphic form by differential scanning calorimetry (DSC), X-ray powder diffraction (XRD) and solubility studies. Aspirin was determined chemical degradation of acetylsalicylic to salicylic acid.

5.1 Glibenclamide polymorphisms

Glibenclamide is a sulfonylurea derivative that is orally active as a hypoglycemic drug. It exists as a crystalline powder which is sparingly soluble in water. Sulfonylureas have been shown to exhibit polymorphism. As different polymorphs or pseudo-polymorphs of drug substance may vary in their physical and chemical stability and bioavailability, which is attributed to poor dissolution. (Petterson et al., 2005)

Chemical name : (5-chloro-N- {2-[4-({[(cyclohexylamino) carbonyl] amino} sulfonyl) phenyl] ethyl}-2-methoxybenzamide)

Chemical formula : $C_{23}H_{28}ClN_3O_5S$

Structural formula :

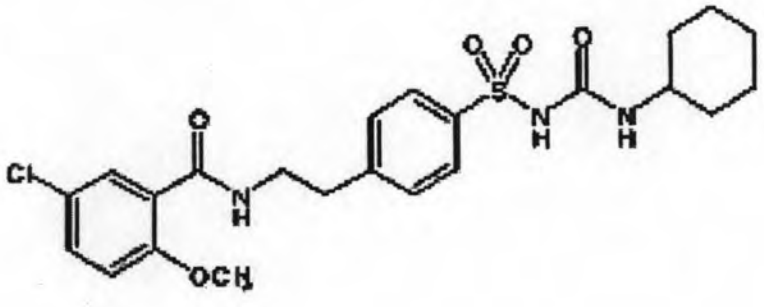

Figure 5 The chemical structure of glibenclamide. (Petterson et al., 2005)

Molecular weight : 494.004

Description : A White or off-white, odorless, crystalline powder

Melting point : 69-174°C depending on the polymorphism, Glass transition temperature (Tg) 73°C

Solubility : solubility (37°C/pH7.4) 0.01 mg/ml and pKa 5.3

Several factor influencing dissolution and bioavailability of glibenclamide have been examined, such as micronisation, molecular dispersion, incorporation of surfactants, inclusion complexation with cyclodextrin, solid dispersion (Dastmalchi et al.,2005), crystal modification (Suleiman and Najib, 1989) and glass formation (Hassan, Najib and Suleiman, 1991). It has been reported that crystallization of glibenclamide from different solvents gave two polymorphic forms and pseudo-polymorphs (solvates), which were significantly different with regard to solubility, melting properties, DSC thermograms and X-ray diffraction pattern. (Suleiman and Najib,1989).

Table 2 Equilibrium solubility's and Heats of solution for the crystal forms of glibenclamide (Suleiman and Najib, 1989).

Crystal form	Equilibrium solubility at 37°C(mg/100ml)	Heat of solution(kcal/mol)
I	0.66	18.15
II	1.06	15.70
Pentanol solvate	33.70	5.25
Toluene solvate	2.51	18.00

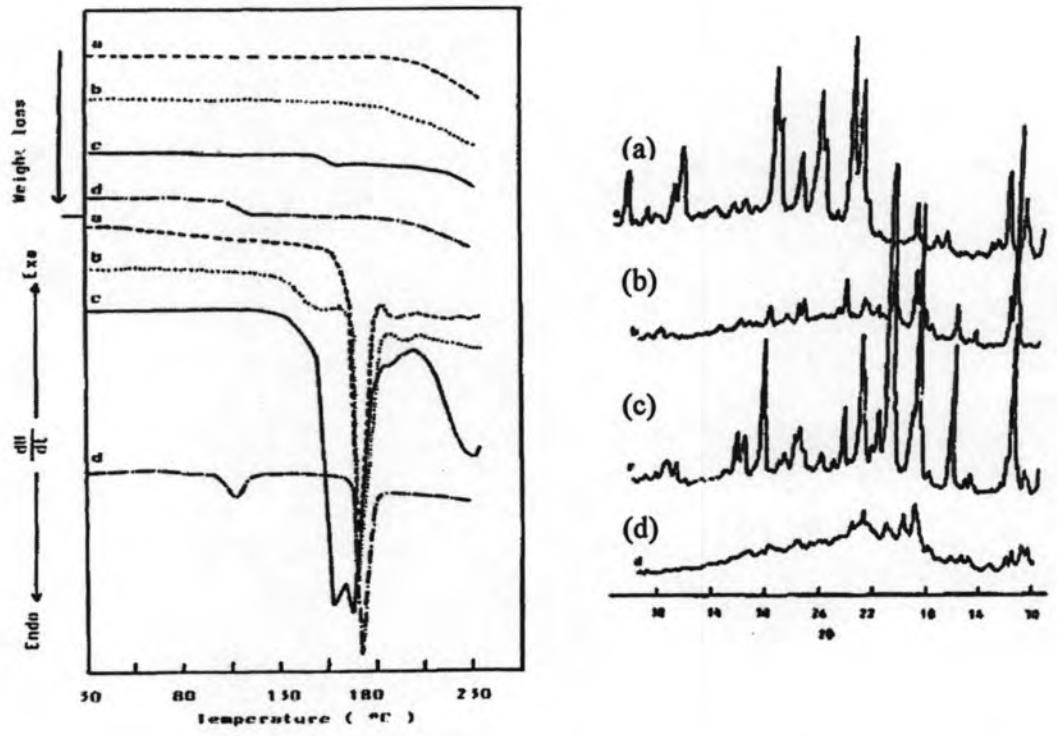

Figure 6 TG, DSC thermograms and X-ray diffraction patterns of form I(a), II (b), the solvates pentanol(c) and Toluene(d) (Suleiman and Najib,1989).

Also, glassy form of glibenclamide was obtained by quick cooling of melt, showing changes in solubility with storage at different temperatures attributed to transformation from glassy to crystalline form. (Hassan, et al., 1991) The DSC and X-ray diffraction pattern of the crystalline and glassy state are presented in Figure 7 The DSC thermogram (Figure 7 a) of crystalline glibenclamide shows one endothermic peak at 174.4°C corresponding to its melting point. The DSC thermogram of solidified melt (Figure 7 b) shows one shallow and broad peak. This shown that a glassy was had glass transition temperature peak at 71.3°C. The X-ray diffraction pattern of the crystalline glibenclamide (Figure 7) shows strong and sharp peaks. Meanwhile, the diffraction pattern of the amorphous state produced weak and diffuse diffraction spectra. These results further confirm that the solidify melt was a glass and of an amorphous nature.

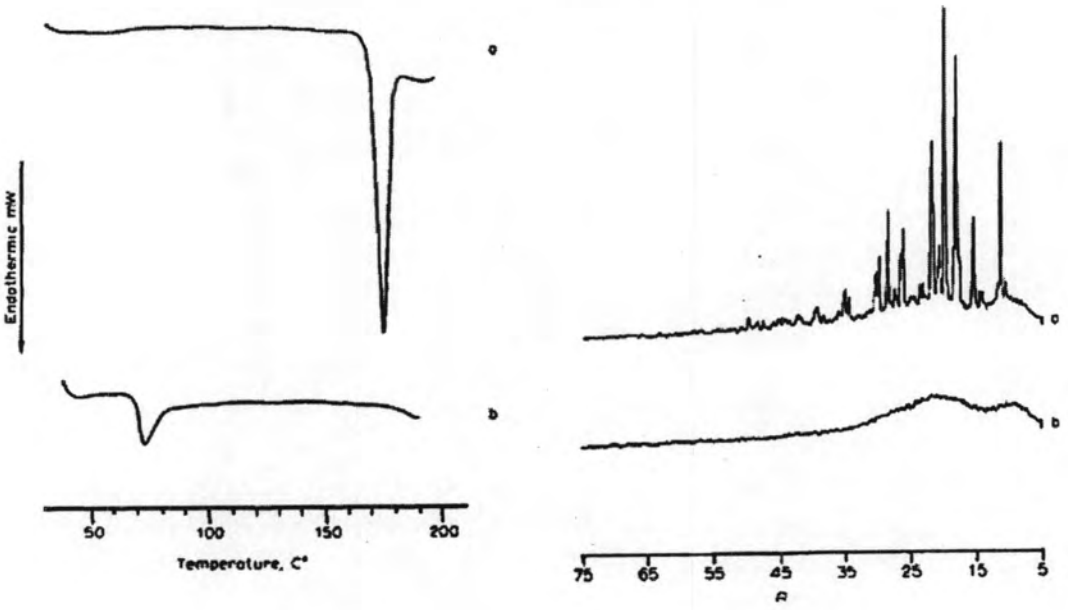

Figure 7 DSC curves and X-ray diffraction patterns of glibenclamide: crystalline (a) ; glass (b). (Hassan, et al., 1991)

Panagopoulou-Kaplani and Malamataris (2000) have shown that cooling of melted glibenclamide and subsequent storage conditions are very important factors for the development of a new crystal form and reduction of solubility.

Quench cooling of the melt and Ball milling of glibenclamide resulted in amorphous also be use as a preparative technique however it can result in chemical degradation. (Patterson et al., 2005)

5.2 Aspirin and salicylic acid (Merck Index, 14th edition, 2006)

5.2.1 Aspirin

Chemical name : Acetylsalicylic acid; 2-(acetyloxy) benzoic acid

Chemical formula : $C_9H_8O_4$

Structural formula :

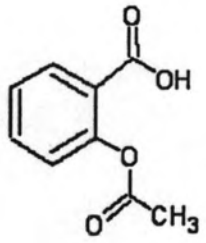

Figure 8 The chemical structure of Acetylsalicylic acid.

Molecular weight : 180.16

Description : a white crystalline powder, odourless or with a faint odour of acetic acid.

Melting point : 135°C

Solubility : 1 in 300 ml water (at 25°C), 1 in 5 ml alcohol.

5.2.2 Salicylic acid

Chemical name : 2-hydroxy benzoic acid

Chemical formula : $C_7H_6O_3$

Structural form :

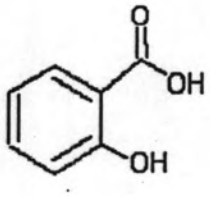

Figure 9 The chemical structure of salicylic acid.

Molecular weight	:	138.12
Description	:	a white crystalline powder.
Melting point	:	159°C
Solubility	:	0.2 in 100 ml water (at 20°C)

Aspirin is an inherently unstable phenolic ester and in presence of H_2O it hydrolysis rapidly. Its hydrolysis is accelerated greatly by extremes of pH and elevated temperature, and thus there is no aqueous formulation of aspirin. Even in the solid state, aspirin will hydrolyze slowly under warm humid conditions and badly decomposed aspirin tablets have the small of acetic acid.

Drug degradation occurs by four main processes:

1. Hydrolysis due to H_2O, H_3O^+, OH^-, pH.
2. Oxidation due to O_2.
3. Photolysis due to UV and visible.
4. Trace metal ion catalysis due to Fe^{2+}, Fe^{3+}, Cu^{2+}, etc.

Hydrolysis of the drug entity can be a major factor in the instability of solutions. Aspirin, for example, undergoes hydrolysis with the resultant degradation products being salicylic acid and acetic acid.

$$\text{Aspirin} \rightarrow \text{Salicylic Acid} + \text{Acetic Acid}$$

The kinetics of aspirin degradation was studied in these experiments by using HPLC for chemical analysis, and thus though analysis of kinetics data, determine the experimental rate constants and order of chemical reaction. For Temperature 30°C, 37°C, 45°C, 50°C and 60°C the experiment rate constants determined by Arrhenius plots.

It is desirable to determine the stability of the active ingredient in the drug so that a shelf life or expiration date may be assigned to the product. The shelf life is the length of time required for the product potency to be reduced to some percentage of its original value. For most products, this is the t_{90} or time at which the product retains 90% of its original potency. But aspirin tablets is focus on quantity of degradation, the limit is not more than 0.3 %.

6. Measuring the pH

There are substances which have the property of changing their color when they come in contact with an acidic or basic environment. These substances are called pH indicators. Usually, they are used as dissolved substances, as for instance phenolphthalein and bromothymol blue. Often, to measure the pH, special papers which have been soaked with indicators are used. These papers change color when they are immersed in acidic or basic liquids. This is the case of the well-known litmus paper (Figure 10) More recently, it has become possible to measure the pH with electrical instruments like the pH meter

Litmus paper

Litmus is a substance obtained from certain lichens. It has the property of changing its color to red with acidic substances and to blue with basic ones. On the packet of the litmus paper, there is a color scale which indicates the color assumed by the paper as a function of the pH.

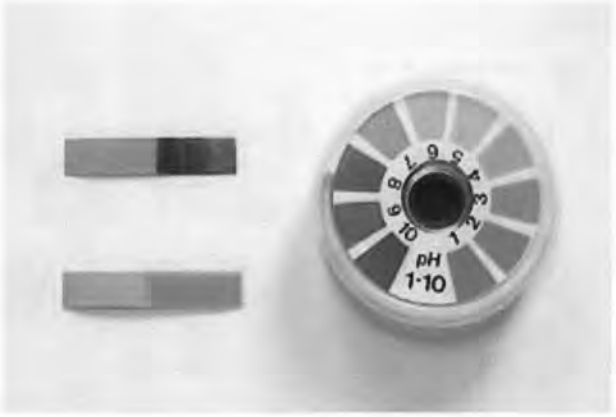

Figure 10 The packet of the litmus paper

7. Color measurement of thin films indicator

The determination of color can be carried out by visual (human) inspection or by using a color measuring instrument. Although human inspection is quite robust even in the presence of changes in illumination, the determination of color is in this case, subjective and extremely variable from observer to observer. In order to carry out a more objective color analysis, color standards are often used as reference material.

At present, color spaces and numerical values are used to create, represent and visualize colors in two and three dimensional space. Three color models are used to define color in this paper: the RGB (red, green, and blue) model, the CMYK (cyan, magenta, yellow, black) model, and the L*a*b* model. Among them, the L*a*b* model has the largest gamut encompassing all colors in the RGB and CMYK gamut (Adobe Systems, 2002).

The RGB model is an additive color model that uses transmitted light to display colors. Various proportions and intensities of three primary colors (red, green, and blue) are used to create cyan, magenta, yellow, and white. The model is used for television and computer screens, in which colored pixels are produced by firing red, green, and blue electron guns at phosphors on the screens. The model relates closely

to the way human perceives color in the retina. The model is device dependent, since its range of colors varies with the display device.

The CMYK is a color model based on the light absorbing quality of ink printed on paper (Adobe Systems, 2002). As white light strikes translucent inks, certain visible wavelengths are absorbed while others are reflected to the eyes. Three primary ink colors (cyan, magenta, and yellow) are used to create other colors. In theory, these three primary colors should combine to absorb all light and produce black; however, a muddy brown is produced instead because all printing inks contain some impurities. Thus, the fourth primary ink color (black) is needed to produce a true black. The CMYK model is also device dependent and is used in four-color process printing.

The L*a*b* model is an international standard for color measurement developed by the Commission Internationale d'Eclairage (CIE) in 1976. The L*a*b* color consists of a luminance or lightness component (L* value, ranging from 0 to 100), along with two chromatic components: the a* component (from green to red) and the b* component (from blue to yellow). The L*a*b* color is device independent, providing consistent color regardless of the input or output device such as digital camera, scanner, monitor, and printer. The L*a*b* values are often used in food research studies. It is important to reiterate that the RGB and CMYK models are device dependent. For example, the food image appears darker on a Windows system than on a Mac OS computer, because the standard RGB color space is darker in Windows than in Mac OS (Adobe Systems, 2002). Also, the RGB and CMYK gamut are smaller than the L*a*b* gamut, and thus there are out-of gamut colors that cannot be display on-screen or print.

The color of many foods has been measured using computer vision techniques. A computational technique with a combination of a digital camera, image processing software has been used to provide a less expensive and more versatile way to measure the color of many foods than traditional color-measuring instruments (Yam and Papadakis, 2004). With a digital camera it is possible to register the color of any pixel

of the image of the object using three color sensors per pixel. The most often used color model is the RGB model in which each sensor captures the intensity of the light in the red (R), green (G) or blue (B) spectrum, respectively.

Today the tendency is to digitally analyze the images of food items in order to firstly carry out a point analysis, encompassing a small group of pixels with the purpose of detecting small characteristics of the object, and secondly to carry out a global analysis of the object under study such as a color histogram in order to analyze the homogeneity of the object. (Brosnan and Sun, 2004) The color and other optical properties that contribute to the appearance of food products can be assessed using video image analysis (VIA). The images are captured with a CCD camera and saved in the RGB (red, green, blue) format. (Du and Sun, 2004)

Hatcher, et al. (2004) showed that the appearance of the noodles prepared from the white and red seed coated wheat was measured using two scanner based image analysis systems differing in their means of colors correction. Speck size and gray level density parameter combinations were found to influence the red, green and blue, RGB, values of the detected specks. Unique differences in both alkaline and white salted noodle color components were observed using the inexpensive, color calibrated color system for noodles prepared from either white or red seed coated wheat flour. This system can use for monitoring their noodle's appearance.

In quantitative analysis, L*a*b* values were used because they are device independent and cover a larger gamut than RGB and CMYK. The published computational approaches that convert RGB into L*a*b* units use an absolute model with known parameters. However, the parameters of the models vary from one case to another because RGB is a non-absolute color space, i.e., the RGB color measurement depends on external factors (sensitivity of the sensors of the camera, illumination, etc.). Also, the conversion from RGB to L*a*b* cannot be done directly using a standard formula, like a conversion from centimeters to inches. Leon et al. (2006) demonstrated that the conversion from RGB to L*a*b* can be done with quadratic and neural network model, both of which show small error.

This research use a simple method that uses a digital camera to measure color, and the graphics software Photoshop (Adobe Systems Incorporated, San Jose, CA) to analyze color.

PhotoShop® is standard software used primarily by graphics producers and photographers for photo retouching and image editing (Adobe Systems, 2002). However, the software also has several features that may be adopted for analysing color of food samples.

There are several reasons for choosing PhotoShop for this method. The software is rich in image editing features, and its color analysis capability is comparable to the more expensive color analysis software. The software provides more sophisticated capability for managing color and producing consistent color than other graphics software. The software is also available in many laboratories, and it is strongly supported by the manufacturer and users.

The term ''measure'' means that the digital camera is used to obtain the color values of the pixels on the food surface. The term ''analyze'' means that Photoshop is used to manipulate those color values to obtain color distribution, averages, and so on. In this digital imaging method, the required equipment and software costs are low, the experimental setup and operating are simple, and the measurements and analysis are often adequately sophisticated for food engineering research. (Yam & Papadakis, 2004)

Photoshop can display L*a*b* values (also RGB and CMYK values) in the Info Palette and Histogram Window. Three different methods were used to determine the L*a*b* distribution of the samples. (Yam and Papadakis, 2004)

The first method used the Info Palette to determine the color distributions along the x-axis and y-axis, where the origin was located at the center of the samples. By turning on the Grid feature in Photoshop, a grid was superimposed on the thin films samples. As the computer pointer was placed at a grid point along the x-axis or

y-axis, the L*a*b* values corresponding the pixel of that grid point were obtained from the Info Palette. However, the plots of L*a*b* values as a function of the x-axis or y-axis obtained in this way had a high level of noise. The noise was due to the small dark spots in the sample and the selection of a single pixel in the determination of the L*a*b* values. It is worth mentioning that the Info Palette can also be used to identify out-of-gamut colors. The CMYK values are normally displayed in percentage. When the computer pointer is placed at a pixel whose color value is outside the CMYK gamut, the percentage is replaced with an exclamation mark.

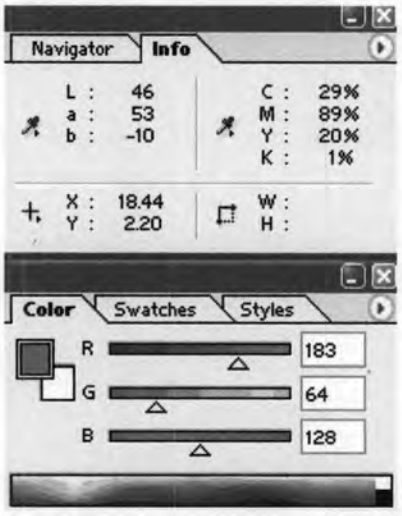

Figure 11 The Info palettes in Photoshop.

The second method used the Histogram Window to determine the color distributions along the x-axis and y-axis. The Histogram Window displays the statistics (mean, standard deviation, median, percentage, and so on) of the color value, Lightness, for a selected area in the image. The Histogram Window can also display the statistics for two other color values (a and b), which is done by selecting a and b under the Channel drop-down menu. Hence, the average color of a sample or any portion of it can be obtained easily using the Histogram Window. The Lightness, a, and b in the Histogram Window are not standard color values. However, they can be converted to L*, a*, b* values.

To determine the color distribution using the second method, average values for multiple pixels were used to reduce the noise in the plots. Again a grid was superimposed on the sample as before. Instead of the grid points, a small square along the *x*-axis and *y*-axis were selected. The histogram Window was used to provide he average values for the squares. The advantage of the second method is that it provides detailed description of the color distribution of individual samples. The disadvantage is that it is not suitable for comparing two or more samples due to the waviness of the plots.

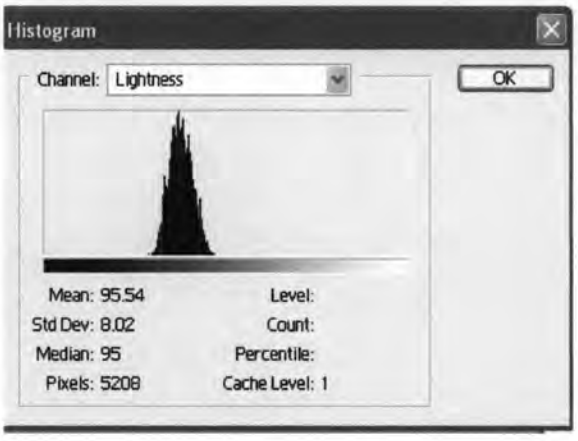

Figure 12 The Histogram Windows in Photoshop

The third method was designed for comparing multiple samples. Instead of small squares, much larger circular areas were selected. Photoshop was used to separate each sample into ten circular sections. The first section was created by removing a circle of dimensionless radius r/R = 0.9 from the sample (the remaining annulus was the first section). The second section was created by removing a circle of r/R = 0.8 from the circle that was removed from the previous step (the remaining annulus was the second section). For each circular section, the average L*, a*, b* values were obtained using the Histogram Window. The method made the assumption that the color distribution was symmetrical along the radial direction.