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QUANTIFICATION OF THE FREEZE-CONCENTRATION EFFECT ON QUALITY DEGRADATION RATES DURING FROZEN FOOD STORAGE

BY

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ABSTRACT

QUANTIFICATION OF THE FREEZE-CONCENTRATION EFFECT ON QUALITY DEGRADATION RATES DURING FROZEN FOOD STORAGE

BY

Dar-Jen Lai

The purpose of this research is to quantify the influence of the freeze-concentration effect on degradation rate acceleration of frozen foods. A prediction approach based on integration of a rate prediction model and and a concentration factor prediction model was developed to achieve this quantification. Inputs to the prediction approach include the rate constants, the activation energy, the reaction order, the mass fraction of water in system, the specific volume of solutes in solution and the effective molecular weight of solutes. Apparent reaction rates or rate constants at sub-freezing temperatures are the outputs.

Autoxidation of oxymyoglobin was used as the model reaction to verify the prediction approach. The reaction followed first order kinetics with respect to oxymyoglobin concentration in de-ionized water, in acetate buffer of different concentrations, in the liquid state before freezing and in the unfrozen solution of frozen state. A common activation energy of 118 KJ/mole was obtained for reaction in acetate buffer of various concentrations. The reaction demonstrated a 0.316 power dependency on buffer concentration when the variation in buffer concentration was large. The rate constants for reactions in supercooled solutions and in de-ionized water (frozen) coincide with the extrapolated Arrhenius plots initiated at temperatures above freezing. Increased rate due to freezing was observed for the autoxidation reaction in acetate buffer. The extents of rate acceleration were larger in less buffered systems. Local rate maxima were near -2 or -3 C. The effectiveness of the prediction approach was verified by the agreements between measured and predicted apparent reaction rate constants for this reaction.

Based on the prediction approach, the addition of inert (with respect to the object reaction) solutes can reduce or eliminate the influence of the freeze-concentration effect on the rate acceleration. The 'adverse temperature range' for storage, within which storage is unjustified from the standpoint of more energy input without better preservation, is widened by low activation energy, high reaction order and low initial solute concentration.

To my parents, my wife and my little daughter.

I love you.

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A : pre-exponential of the Arrhenius equation

Aw : water activity

(a),(b),(c)-- : concentration of components a, b, c, etc.

(a)t,(b)t,-- : concentration of components a, b, etc., based on total liquid volume

(a)u,(b)u,-- : concentration of components a, b, etc., based on unfrozen solution volume

Cf : concentration factor

dw : density of water

E : activation energy

k : reaction rate constant

kA : apparent reaction rate constant

kr : reaction rate constant at reference temperature, Tr

Ms : effective molecular weight of solutes

Mw : molecular weight of water

mb : mass fraction of bound water

mi : mass fraction of ice

ms : mass fraction of solutes

mu : mass fraction of unfrozen water

mw : mass fraction of water

na,nb,nc : reaction order with respect to components a,b,c

nt : total reaction order

R : gas constant, 8.314 KJ/mole

t : time

T : absolute temperature, K

Tr : reference (with respect to kr) absolute temperature

Vi : volume of water crystallized as ice

Vs : specific volume of solutes in solution

Vt : total liquid volume

Vu : unfrozen solution volume

Vw : specific volume of water in solution

x : concentration ratio, buffer concentration(M)/.01(M)

Xw : mole fraction of water

θ : temperature, C

I. INTRODUCTION

Frozen storage is one of the best methods for the preservation of food quality. Like other preservation methods, freezing does not eliminate deterioration of quality entirely. It does reduce the deterioration rate significantly. In general, the deterioration rate decreases with temperature according to a relationship that can be approximated by the Arrhenius equation over a moderate temperature range. In commercial applications, it is assumed that lower temperature storage improves frozen food shelf-life.

Exceptions to the Arrhenius equation have been demonstrated in foods and simple reaction systems. Within certain temperature intervals, a negative effect of temperature on shelf-life or rate acceleration of quality change was observed. This is a situation where reduction of temperature does not decrease the reaction rate. The occurrence of a negative effect of temperature indicates that lower temperature storage may not be justified for all products. Investigation of factors influencing the occurrence of the negative effect of temperature is important in light of the relationship of energy

conservation to quality preservation.

Numerous factors could cause the negative effect of temperature on frozen food shelf-life. Of particular interest to frozen food storage is the freeze-concentration effect. The freeze-concentration effect results from the progressive transformation of water into ice during the freezing process which results in a more and more concentrated unfrozen solution in the product. The concentrated reactants and other solutes then exert their influences on the reaction rate and usually a rate acceleration is observed.

Quantification of this freeze-concentration effect is necessary before investigation of other effects that influence the reaction rate in a frozen state since it is common to all reaction systems with higher than first order kinetics. In addition, prediction of this effect is of practical importance in the improvement of quality prediction models, in the development of possible accelerated shelf-life tests and in the optimization of process design for frozen food storage.

In this dissertation, a prediction approach for the quantification of the freeze-concentration effect will be developed. The overall objective is to use the prediction approach to improve process practices to be used in future investigations of quality predictions for frozen food storage. The prediction approach is based on combining a rate prediction model and a concentration factor prediction model. Corresponding to these two models, kinetic information including reaction rate constants, activation energy and reaction order, and system information including mass fraction of water , specific solutes volume in solution and effective molecular weight of solutes are needed as inputs. These types of information are obtained through laboratory determinations.

For the purpose of demonstrating and supporting the prediction approach, oxymyoglobin concentration was selected as a quality index and the autoxidation of oxymyoglobin was selected as the model degradation reaction. This reaction was chosen due to the relationship to the discoloration of red meat and some fish products, the availability of necessary background for obtaining kinetic information and the observation of a negative effect of temperature on reaction rate at sub-freezing temperatures. These make oxymyoglobin autoxidation a suitable reaction model for demonstrating the concentration effect and for investigation of the reaction in a food system. The procedures performed on the model reaction system should be applicable to other systems, possibly with some minor modifications.

The specific objectives of this study were :

(i) to develop an approach that will allow prediction of the influence of the freeze-concentration effect as a fuction of temperature on the frozen food quality degradation rates.

(ii) to experimentally verify the prediction approach by use of the autoxidation of oxymyoglobin as a model reaction system.

(iii) to evaluate the possibility of extrapolating kinetic data from temperatures above freezing to sub-freezing temperature applications, as a potential accelerated test for frozen food storage.

(iv) to reveal the influence of the freeze-concentration effect on quality degradation of frozen foods based on the prediction approach and published data.

(v) to make process recommendations by varying the input parameters to the prediction approach.

II. LITERATURE REVIEW

In the first section of this review, the occurrences of the rate acceleration and factors that influence them are discussed. In the second section, background concerning the autoxidation of oxymyoglobin is discussed. This reaction was selected as model reaction for the study of the freeze-concentration effect.

2.1 Acceleration of Chemical Reactions Due to Freezing

2.1.1 The Occurrence of Rate Acceleration in Frozen Storage

The influence of temperature on reaction rate is generally described by the Arrhenius equation. If the temperature range is not too large, this equation gives satisfactory description (Charm,1971). Based on this equation, the reaction rate decreases in an exponential relationship as temperature decreases, and follows a straight line relationship between natural log of rate constant and inverse of absolute temperature. Rate acceleration is referred to as the phenomenon that reaction rates at certain temperatures are significantly higher than those extrapolated by Arrhenius equation, or in some cases, reaction rates do not decrease as the product temperature decreases. Poulsen and Lindelov(1981) classified the rate-temperature relationship into (i) normal stability, that is when a temperature decrease results in a slower reaction rate, (ii) neutral stability, that is when temperature has no influence on the reaction rate, and (iii) reversed stability, that is when a temperature decrease results in an increased reaction rate. The latter two situations are consequences of rate acceleration.

Reactions mostly of the nonenzymatic type have been found to show rate acceleration upon freezing. In the study of the reaction of malonaldehyde with alfa-amino groups of myosin from trout, Buttkus (1967) found that the reaction rate was greater at -20 C than at 0 C and almost equal to that at 20 C. Lindelov (1976) studied the same reaction with myosin from young rabbit and found that the reaction rate decreased as the temperature decreased from 45 to 0 C. However, when the system was frozen, the reaction rate increased with decreasing temperature up to -24 C.

Behnke et al. (1973) found that the rate of adenosine triphosphate depletion and lactic acid accumulation in both chicken and beef muscles were higher in frozen state at -3 C than at 10 C.

Lovern and Olley (1962) found that the rate of phospholipid hydrolysis in cod muscle had a local maximum reaction rate at -4 C. The denaturation of alfa-chymotrypsin in frozen state was found to have a local maximum rate near -12 C (Pincock and Lin, 1973).

The reaction rate of free fatty acid with protein in cod muscle was found to be greater at -29 C than at temperatures a few degree above 0 C (Anderson and Ravesi, 1969).

Poulsen and Lindelov (1981) examined the high quality life and practical storage life of vienna sausages made of pork, fat, water and veal and found that storage at -24 to -60 C decreased product stability. In another study on the storage life of sliced bacon, Poulsen and Lindelov (1981) found that storage at -30 C was less stable than at -18 C. Bogh-Sorensen et al. (1981) found that cured meat in vacuum package showed neutral stability while those in oxygen permeable packages showed reversed stability. In smoked bacon, liver paste and chopped herring fillets, Lindelov and Poulsen (1975) also observed some reversed stability at certain temperatures. Gibbons (1953) studied the oxidative rancidity in Canadian Wilshire bacon and found that decreasing the storage temperature to -40 C accelerated the reaction rate, and concluded that bacon could be kept better near its initial freezing point than at -40 C.

Poulsen et al. (1976) studied the storage life of Danish butter varieties at various pH and salt content, a 'negative' temperature influence was observed at temperatures below -25 C.

Brown and Dolev (1963b) and Zachariah and Satterlee (1973) studied the autoxidation of oxymyoglobin in phosphate buffer solution and found a local reaction rate maximum near -12 C.

Fan and Tannenbaum (1973) studied the kinetics of the nitrosation of morpholine and found that the reaction rate showed prominent rate acceleration in the frozen state.

Other reaction systems that showed rate acceleration can be found in various published studies (Mullenax and Lopez, 1975). A series of reactions including biochemical, organic and inorganic reactions were reviwed by Pincock (1969). Fennema (1975a) also listed a number of reactions that showed rate acceleration in frozen state.

2.1.2 Factors Influencing the Rate Acceleration

Several facts are observed from rate acceleration in frozen state:

(a) Satisfactory estimates of reaction rates in frozen state usually cannot be obtained by simple extrapolation of kinetic data established from reactions in liquid state before freezing (Fennema, 1975b).

(b) Usually this rate acceleration is characterized by (i) an increase in reaction rates to values well above

those observed in the supercooled state at the same temperature, (ii) having a local reaction rate maximum at sub-freezing temperature and (iii) reaction rates decline with further decreasing of temperature (Fennema, 1973)

(c) Addition of inert solutes has an unusually great influence on the rates of many reactions in frozen state (Thompson and Fennema, 1971).

The acceleration of reaction rate in frozen state is the consequence of phase transitions in the frozen state. For a one component system, such as water, there is a sharp transition from the liquid state to the solid state at the freezing point. For multicomponent system, however, the transition of liquid solvent into solid solvent takes place progressively as the temperature decreases. In the case of aqueous solution, there is always a liquid unfrozen solution existing in equilibrium with solid ice at some sub-freezing temperature, until the temperature is lowered to the eutectic point of the system, where total solidification occurs (Pincock, 1969). During normal commercial frozen storage of food products, the storage temperatures employed never put the product in total solidification (Singh and Wang, 1977). Pincock (1969) classified a multicomponent reaction solution into three states according to temperature intervals where they can exist. These are (i) the liquid state which exists at above freezing temperatures, (ii) the frozen state, existing at temperatures between initial freezing temperature and eutectic point, and (iii) the solid state which exists at temperatures below eutectic point. While ice crystals are progressively formed as the temperature of the system decreases, the volume of unfrozen solution also decreases. The amount of ice formation or the volume of unfrozen solution in frozen state is a function of temperature and is governed by phase equilibrium. Both the ice formation and the reduced volume of unfrozen solution were suggested to influence the reaction rate in frozen state. Grant et al. (1961) studied the base-catalysis of penicillin in frozen state and suggested that the concentration of reactants upon freezing, the favorable substrate-catalyst positional constraint and possibly the high proton mobility might be factors that influence the reaction rate in frozen state.

Subsequent reports on reactions in the frozen state by the same group of authors (Alburn and Grant, 1965; Grant and Alburn, 1965a,b; Grant and Alburn, 1967; Grant et al., 1966) proposed that one or more of the following reasons in addition to the freeze-concentration effect may be involved in the rate acceleration : (i) possible catalytic effect of the ice crystal, (ii) favorable substrate-catalyst positional constraint caused by ice crystallization, (iii) greater proton mobility in ice than in water causing a rate acceleration in acid-catalyzed reactions and (iv) greater dielectric constant for water than for ice, which would favor the nucleophile association reactions.

After a review on rate acceleration in a number of reaction systems during freezing, Pincock (1969) concluded that in most cases, the acceleration is more related to the liquid part than the solid part of the total system.

Fennema (1973) examined the reaction kinetics in frozen aqueous sysems and concluded that the acceleration of non-enzymatic reactions and enzyme catalyzed reactions in non-cellular systems in the frozen state usually can be satisfactorily explained on the basis of the concentration of reactants in the unfrozen solution.

In general, the acceleration of enzyme catalyzed reactions in non-cellular systems occurs rarely and in most cases, the reaction rate declines when compared with Arrhenius plot (Fennema, 1975a). Some enzyme catalyzed reactions in cellular systems are accelerated during early stage of freezing. However, it is believed that this acceleration is due to freeze-induced enzyme delocalization rather than freeze-concentration of reactants (Fennema, 1975b).

Freeze-concentration is an indirect consequence of the ice crystallization, and is caused by the reduced volume in unfrozen solution. Based on solid-liquid equilibrium, the unfrozen phase of all solutions, after equilibrium being achieved at a given sub-freezing temperature, will contain the same ratio of solute particles to water molecules. Therefore, an originally more dilute system will be concentrated to a greater extent as compared to the original system. Consequently, rate acceleration is more obvious in dilute systems (Kiovsky and Pincock, 1966a).

When dealing with the freeze-concentration effect on rate acceleration, a presumption is that the reaction takes place in the unfrozen solution. Basically, reactions in unfrozen solution are in the same enviroment as the reactions in liquid state, only in a more concentrated form. Rate acceleration or reversed stability also occurs in liquid state before freezing. McWeeny (1968a) summarized possible factors that could result in these 'abnormal' rate-temperature relationships.

Several authors observed that the rate acceleration occured in the autoxidation of the fats in dehydrated herring and herring meal. Lea et al. (1958) reported that the rate was more rapid at 10 C than at 37 C. Banks (1950) found it was faster at 0 C than at 25 C and March et al. (1961) found it faster at -20 C than at 25.5 C. Lea (1962) suggested that concentration effects due to freezing increased adsorption of oxygen at low temperature or that development of an antioxidant at higher temperature might be the reason. The latter suggestion is supported by Harris and Olcott (1966). Development of a reaction inhibitor (in this case, antioxidant) at higher temperture caused rate acceleration at lower temperatures.

Oxidized flavor of condensed milk was shown to be more prominent at -17 C than at -7 C (Bell, 1939). Off-flavor in liquid milk was more intensive for storage at 0 C than at 8 C (Dunkley and Franke, 1967). Day (1965) concluded that off-flavor is due to a mixture of carbonyl componds, especially unsaturated aldehydes. Further oxidation of these flavor compounds (Lillard and Day, 1964) and the condensation of aldehyde with amines (Montgomery and Day, 1965) may decrease the intensity of off-flavor. It was suggested (McWeeny, 1968a) that the reactions to decrease the intensity of off-flavor may have a greater dependency on temperature than those reactions to produce off-flavor. Therefore, at high temperatures, removal of off-flavor compounds are faster than formation. The difference in temperature dependencies of competing reactions leads to this rate acceleration.

McWeeny (1968b, c, d) observed that the rate of green discoloration in beta-carotene and certain hydrogenated oil was very low at room temperature, but rapid in the temperature range of 6 to -6 C. It was suggested that this rate acceleration was due to a decomposition of a reaction intermediate, which was necessary to green discoloration and proceeded faster at high temperature.

The above-mentioned rate accelerations for reactions in liquid state generally involve the difference in the temperature dependency of competing reactions. These could add to the possible influences that freeze-concentration may impose on a reaction system.

Freeze-concentration effects may influence the rate of a reaction through the following pathways: (a) increase in reactants concentrations and cause rate acceleration on reaction with higher than first order kinetics, (b) increase in salt concentration and affect the solubilization of protein, (c) differential precipitation of the buffer salts and cause pH shift which in turn influences the reaction rate, and (d) increase in the ionic strength of the unfrozen solution and increase the rate of protein denaturation, as described follows.

An increase in reactant concentration increases the rate of reactions with reaction order higher than one (Pincock and Lin, 1973). This has been demonstrated in a number of reaction systems (Alburn and Grant, 1965; Bruice and Butler, 1964; Butler and Bruice, 1964; Grant and Alburn, 1965a, b, 1967; Pincock and Kiovsky, 1965a, b, 1966a, b, c; Kiovsky and Pincock, 1966a, b; Fennema, 1973). Pincock and Kiovsky (1966c) derived a rate equation for describing the rate acceleration for a second order reaction. The observed rate was described as:

$$\text{observed rate} = - k\cdot(a)t\cdot(b)t\cdot\frac{Vt}{Vu} \qquad (2\text{-}1)$$

That is, a concentration factor Vt/Vu has to be multiplied to the 'normal' reaction rate constants to obtain the 'apparent' reaction rate constants if measurements are based on total liquid state volume at above-freezing temperatures. The unfrozen solution volume is a function of temperature. To determine it, knowledge of the phase relationships of reactants, products, solvent and possible impurities is required (Pincock, 1969). Experiemental determination of this relationship is limited to simple systems. The phase relationship for complex systems are usually too complicated to be quantitatively determined theoretically or experimentally. Quantification of the freeze-concentration effect, therefore, has been limited to simple systems.

Protein denaturation has been shown to be the result of the freeze-concentration effect. Love and Elerian (1964) found that the rate of protein denaturation in cod reached a local rate maximum near -1.5 C. Love (1968) summarized several findings on muscle alteration including frog, cattle and a number of fish muscles and concluded that the maximum change occurred at temperatures between -1.5 and -5 C. Concentrated salt solution is believed to be directly or indirectly responsible for these

protein denaturations. Duerr and Dyer (1952) soaked cod fillets in strong salt solutions without freezing and found that when the concentration of salt in the fillet rose to 8-10 %, the structural proteins suddenly changed, losing their gelatinous properties and releasing a large volume of fluid. Recent study indicated that the denaturation or aggregation of the fish proteins also involved the free fatty acid liberated enzymatically in the frozen state, but the concentration of salt solution still played an important part (Shenouda, 1980)

The differential precipitation of buffer salt would cause a pH shift in unfrozen solution of a frozen system and affect the reaction rate. In the case of mono- and di-sodium phosphate buffer, ice precipitation alone may cause pH change up to 1 unit, salt precipitation may cause pH change up to 4 units. It was concluded that changes in pH, salt and neutral solute composition, volume of unfrozen solution, enzymatic activity and protein-salt reactions are inter-dependent in frozen foods and these changes might affect quality degradation in one way or another, especially where protein stability is involved (van den Berg, 1969). Bembers and Satterlee (1975) observed that low pH was responsible for the denaturation and oxidation and color fading in PSE pork.

2.2 The Autoxidation of Oxymyoglobin

Autoxidation of oxymyoglobin was selected as the model reaction system to reveal the freeze-concentration effect on reaction rate acceleration and to support the rate prediction approach for reactions in frozen state. Review on the kinetics of this reaction is necessary in providing sufficient background for theoretical considerations and experimental design.

Myoglobin is the major source of pigment in meat and is one of the major concerns to meat and some fish processors. The various chemical forms of myoglobin account for the changes of meat color (Fox, 1966). Two recent reviews (Livingston and Brown, 1981, and Giddings, 1977) provided comprehensive summaries of the important works done on myoglobin. In this section, discussions will focus on the works related to the kinetics of oxymyoglobin autoxidation in buffered systems.

2.2.1 General Scheme

Myoglobin is a complex protein, which can be divided into the protein portion called globin and the nonpeptide portion called heme. Heme is composed of an ion atom and a porphyrin (Clydesdale and Francis, 1976). The autoxidation reaction of oxymyoglobin is the reaction that oxidizes native ferrous form of oxymyoglobin into oxidized ferric form of metmyoglobin. The major change occurs in the ion atom. This reaction is accompanied by a discoloration from attractive cherry-red to dull brown (Fox, 1966).

It has been shown that this autoxidation reaction is first order with respect to oxymyoglobin concentration, the reaction has a local reaction rate maximum at low oxygen tension, and in most cases, the reaction is similar to the autoxidation of hemoglobin (George and Stratmann, 1952a).

2.2.2 Factors Influencing the Autoxidation Rate of Oxymyglobin

Oxygen tension, pH, buffer concentrations, metal ions, reductant levels, lighting conditions, temperature and freezing were reported to have influence on the autoxidation rate of oxymyglobin.

Using recrystallized horse metmyoglobin as the source of oxymyoglobin and sodium dithionite as the reducing agent, George and Stratmann (1952b) observed the maximum autoxidation rate at oxygen tensions where exactly half of the total ferrous myoglobin was in the deoxy form. This was at an oxygen partial pressure of about 1.0-1.4 mm Hg. The reaction rate decreased from this point until the partial pressure of oxygen was above 30 mm Hg, where the reaction rate became a constant

value. Brown and Mebine (1969) studied the autoxidation reaction with myoglobin from bovine, tuna and sperm whale origins and built a linear relationship between the reaction rate constant and the partial pressure of oxygen in a double logarithmic scale. The slopes calculated were -0.34, -0.70 and -0.68 for tuna, bovine and sperm whale myoglobins, respectively.

The dependency of autoxidation rate on pH was investigated by several authors (George and Stratmann, 1954; Brown and Mebine, 1969; Pan and Solberg, 1972; Gotoh and Shikama, 1974). In general, the autoxidation rate proceeded faster at low pH values. Brown and Mebine (1969) built a linear relationship between reaction rate constants and pH value from 5.0 to 7.0. The slopes obtained were -0.70, -0.84 and -0.84 for tuna, bovine and sperm whale myoglobins, respectively. Rate constants for reactions taking place at lower than pH 5.0 were reported unreproducible due to protein denaturation. One of the explanation for this acceleration in autoxidation rate is that low pH reduces the stability constant for the heme-globin linkages (Fronticelle and Bucci, 1963) and at pH values below 5, myoglobin could even be denatured (Appel and Brown, 1971). Any conditions that cause the heme-globin linkage to be less stable and result in greater exposure of heme group will increase the autoxidation rate (Livingston and Brown, 1981).

Kraft and Ayres (1954) showed the acceleration of discoloration of beef by both soft-white fluorescent and ultraviolet light. Lentz (1971) reported that discoloration of beef proceeded faster in display than in dark. Franke and Solberg (1971) concluded that visible light had a small but definite influence on the discoloration of beef at refrigeration storage. In model systems, Lane and Bratzler (1962) showed that the autoxidation rate of oxymyoglobin in frozen meat extract was significantly accelerated by exposure to fluorescent light. Satterlee and Zachariah (1972) showed that the autoxidation rates of porcine, ovine and bovine myoglobins in phosphate buffer systems were accelerated by exposure to fluorescent light, and the extent of acceleration was larger at higher temperatures.

Snyder and Skradlant (1966) showed that many ions accelerated the autoxidation rate of myoglobin in phosphate buffer, including copper, ion, zinc and aluminum ions. Among these,the copper ion showed the most prominent effect on the acceleration. Bember et al. (1973) indicated that 200 molar equivalents (with respect to oxymyglobin) or more of copper ions were needed to have a significant acceleration on the autoxidation rate of porcine, ovine and bovine myoglobin in phosphate buffer at pH 6.0.

Buffer concentrations also influence the autoxidation rate of oxymyoglobin. Brown and Dolev (1963a) observed that in systems with buffer strengths ranging from 0.1 to 0.6 M, the autoxidation rates for beef myoglobin decreased with increasing buffer strength, while for tuna myoglobin, the autoxidation rates were independent of buffer concentration. This is contrary to earlier report by Matsuura et al. (1962) who found that the autoxidation rate increased as the phosphate buffer concentration increased from 0.2 to 0.6 M.

The temperature dependency of autoxidation rate of oxymyoglobin has been reported by several authors to follow the Arrhenius equation. Gotoh and Shikama (1974) reported that autoxidation of native bovine oxymyoglobin had an activation energy value of 26.5 Kcal/mole and a $Q_{10}$of 5.3, and the value was independent of the pH. Brown and Mebine (1969) reported a range of activation energy values from 24.0 to 27.8 Kcal/mole, depending on the source of myoglobin (tuna, bovine and sperm whale myoglobins) and pH values of the phosphate-acetate buffer sytems. Activation energy values of 25.0 and 23.7 Kcal/mole were reported by George and Stratmann (1952b) and Snyder and Ayres (1961), respectively. These are for reactions occur under air-saturated conditions. For reactions taking place under low oxygen partial pressure, i.e., at 4 mm Hg, an activation energy value of 19 Kcal/mole was reported (George and Stratmann, 1952b). In addition to the normal rate-temperature relationship described by the Arrhenius equation, temperature itself may affect the stability of myoglobin and result in acceleration of autoxidation rate. Awad and Deranleau (1968) reported that at temperatures above 40 C, there were significant permanent alterations in the myoglobin spectrum and eventually the myoglobin precipitated. Atanasov et al. (1968) showed that even for a temperature as low as 33 C, there was a pre-denaturation conformational transition. These conformational changes could make the heme more susceptible to autoxidation.

2.2.3 Autoxidation of Oxymyoglobin in Frozen State

Freezing has been shown to accelerate the autoxidation rate of oxymyoglobin. Ball (1959) found that a few samples of beef stored below -18 C discolored quicker than those stored at 0 C. Sana et al. (1959) observed that less metmyoglobin was formed in tuna at 0 C than at -5 C. In phosphate buffer system, Brown and Dolev (1963b) demonstrated that the autoxidation rate of both bovine and tuna oxymyoglobins were accelerated in the temperature range from -5 to -18 C, with a local rate maximum near -15 C. As contrast to this observation, they found that the reaction rate at the same sub-freezing temperature was much greater in frozen state than in supercooled solution. They concluded that ice

formation was necessary for the rate acceleration. Zachariah and Satterlee (1973) and Satterlee and Zachariah (1972) observed the same rate acceleration phenomenon in porcine, ovine and bovine myoglobins and indicated a local maximum reaction rate near -11 or -12 C.

The autoxidation rate of oxymyoglobin in frozen state was found to decrease, slightly in the cases of bovine and ovine myoglobins and to a greater extent in porcine myoglobin, with increasing buffer concentrations (Zachariah and Satterlee, 1973). This was in agreement with the findings by Brown and Dolev (1963b).

III. THEORY

This chapter is focused on the development of the prediction approach for the quantification of the freeze-concentration effect. In the first section of this chapter, a general scheme of the reaction kinetics in the frozen state is introduced. The second section of this chapter summarizes the assumptions underlying the development of the prediction approach. In the third section, the rate prediction model of the prediction approach is developed. The fourth section of this chapter is a discussion of the concentration factor prediction model. A summary of the input and output parameters of the prediction approach is presented in the last section of this chapter.

3.1 General Scheme of the Reaction Kinetics in the Frozen State

The rate equation and the Arrhenius equation are the two primary expressions used to describe reaction kinetics. The rate equation describes the concentration dependency of reaction rate. Two parameters are determined by this equation; the reaction order and reaction rate constant. Reaction order reveals the extent of dependency

on reactant concentration while the reaction rate constant describes the magnitude of the reaction rate. Reaction order in general is independent of temperature. The temperature dependency of the reaction rate is revealed through the reaction rate constant and is described most frequently by the Arrhenius equation. Activation energy is a parameter determined by the Arrhenius equation and is an indication of the extent of temperature dependency of reaction rate. The activation energy is assumed to be constant over a given temperature range.

Theoretically, the Arrhenius equation describes 'pure' temperature effect on the reaction rate. If the reaction system changes with temperature, these additional variations have to be evaluated separately. Any discontinuity encountered in the extrapolation of Arrhenius equation from the liquid state to the frozen state is most likely due to complications from these temperature dependent reaction environment changes (McWeeny, 1968a).

Following the classification by Pincock (1969), the frozen state exists at temperatures between the initial freezing temperature and the eutectic point of a reaction system. For aqueous systems, there is always an unfrozen liquid solution in equilibrium with solid ice. The volume of the unfrozen solution and the amount of ice crystal formation are functions of temperature. Reactions in the frozen state therefore encounter these additional changes

in reaction environment besides the normal rate-temperature relationship described by the Arrhenius equation.

The reaction volume reduction and the ice crystal formation tend to accelerate the reaction rate in the frozen state (Alburn and Grant, 1965; Bruice and Butler, 1964; Butler and Bruice, 1964; Grant and Alburn, 1965 a,b, 1967; Grant et al. 1961, 1966; Kiovsky and Pincock, 1966a, b; Pincock and Kiovsky, 1965a, b, 1966a, b, c; Pincock, 1969). In most cases, however, it is believed that the freeze-concentration effect (or reaction volume reduction) can account for the rate acceleration encountered by non-enzymatic chemical reactions in food and simple reaction systems (Pincock, 1969; Fennema, 1975b).

This study is primarily interested in the quantification of the influence of the freeze-concentration effect on reaction rate. A prediction approach will be developed to allow the prediction of the 'apparent' reaction rate constants as a function of temperature for reactions taking place in the unfrozen solution of the frozen state, which takes into account the 'normal' temperature dependency of reaction rate and the influence exerted by the freeze-concentration effect. The inputs to this prediction approach are based on kinetic information from reactions in liquid states above freezing.

3.2 Summary of the Assumptions Underlying the Prediction Approach

Several assumptions are needed for the development of the prediction approach, including :

(a) In addition to the normal rate-temperature relationship, the prediction approach takes into account only the influence of the freeze-concentration effect from concentrated reactants, which is only one of the many factors that influence the reaction rate in the frozen state.

(b) The same rate equation is followed by reactions taking place both in the liquid state above freezing and in the unfrozen solution of the frozen state.

(c) Rate constants in supercooled solution can be estimated in an acceptable manner by direct extrapolation of the Arrhenius plot initiated from temperatures above freezing. The Arrhenius equation is applicable over the temperature range of interest, from about +20 to -20 C, if no complications from ice crystal formation are involved.

(d) Reaction solution follows ideal solution behavior (Roault's Law).

(e) Phase equilibrium is obtained at each storage temperature in a negligible amount of time as compared to storage period.

(f) Only pure ice, no other solute, is crystallized out during the frozen storage.

3.3 The Rate Prediction Model

Basically, the prediction approach is accomplished by adding the freeze-concentration effect to the 'normal' rate constant-temperature relationship to estimate the apparent rate constants in frozen state. In this application, the rate equation and the Arrhenius equation remain unchanged, except that the rate constant is modified to become an 'apparent' rate constant and incorporates the freeze-concentration effect into the rate equation. A concentration factor is employed to introduce the freeze-concentration effect into the apparent rate constant. Temperature dependency of the concentration factor accounts for the temperature dependency of the freeze-concentration effect.

3.3.1 Nth Order Rate Equation

The rate of a reaction can generally be described or approximated by the following nth order rate equation (Hammes, 1978):

$$\text{rate} = \frac{d(a)}{dt} = -k(a)^{na} \cdot (b)^{nb} \cdot (c)^{nc} \; ..... \qquad (3\text{-}1)$$

The reaction orders na, nb, nc with respect to component a, b, c are assumed to be constant over the temperature range studied. The reaction rate constant 'k'

is a function of temperature. Its temperature dependency is described by the Arrhenius equation.

3.3.2 The Arrhenius Equation

The Arrhenius equation can be stated as follows (Hammes, 1978):

$$k = A \cdot \exp\left(\frac{-E}{R\,T}\right) \qquad (3\text{-}2)$$

or

$$k = kr \cdot \exp\left[\frac{-E}{R}\left(\frac{1}{T} - \frac{1}{Tr}\right)\right] \qquad (3\text{-}3)$$

where the activation energy 'E' is assumed to be constant over the temperature range of concern.

3.3.3 The Concentration Factor

Equations (3-1) and (3-2) or (3-3) are combined and used for the description of the temperature dependency of reaction rates in liquid state and are applicable only to reaction systems with fixed volume.

For reactions in the frozen state, the reaction volume varies with storage temperature when dealing with reactions taking place in the unfrozen solution of the frozen state. This variable volume leads to the concentration (or dilution) of the reactants and other solutes. To account for this concentration change with

respect to the total liquid volume, a concentration factor (Cf) is defined as the ratio of the total liquid volume (Vt) to the unfrozen solution volume (Vu) (Pincock and Kiovsky, 1966c) or:

$$Cf = \frac{Vt}{Vu} \tag{3-4}$$

From the definition of molar concentration, volume change would result in variations in the concentration of reactants or solutes in the unfrozen solution of the frozen state. The concentration in unfrozen solution [(a)u, (b)u, ---] is equal to the product of the concentration in total liquid volume [(a)t, (b)t,---] and the concentration factor.

$$\begin{aligned} (a)u &= (a)t \cdot Cf \\ &\text{and} \\ (b)u &= (b)t \cdot Cf \\ &\text{etc.} \end{aligned} \tag{3-5}$$

3.3.4 The Apparent Reaction Rate Constants for Reactions in the Frozen State

At certain sub-freezing temperatures and in the unfrozen solution of the frozen state, the reaction is assumed to follow the same rate equation as in liquid state, only the reactants concentrations have been changed.

This is represented as :

$$\text{rate} = \frac{d(a)u}{dt} = -\ k\cdot(a)u^{na}\cdot(b)u^{nb}\cdot(c)u^{nc} \ldots \qquad (3\text{-}6)$$

Substituting Equation (3-5) into Equation (3-6) :

$$\frac{d(a)t}{dt} = -\ k\cdot Cf^{na\ +\ nb\ +\ ..-\ 1}\ (a)u^{na}\cdot(b)u^{nb} \ldots$$

or (3-7)

$$\frac{d(a)t}{dt} = -\ kA\cdot(a)t^{na}\cdot(b)t^{nb}\cdot(c)^{nc}\ \ldots\ldots.$$

If a kinetic study is conducted by following concentration change in thawed solution (total liquid volume) after frozen storage, the reaction rate constant obtained is the 'apparent' reaction rate constant (kA), which according to equations (3-7) and (3-1) is related to the 'true' reaction rate constant (k) in the following manner :

$$kA = k\cdot Cf^{na\ +\ nb\ +\ nc\ +\ \ldots.\ -\ 1}$$

or (3-8)

$$kA = k\cdot Cf^{nt\ -\ 1} \qquad nt \neq 0$$

where the total reaction order (nt) equals the sum

of reaction orders with respect to individual reactants, or:

$$nt = na + nb + nc + \ldots\ldots \qquad (3\text{-}9)$$

Reactions with a total reaction order of zero are concentration independent and are not described by equation (3-8).

In most cases, kinetic study on reactions in the frozen state is based on the concentration change with respect to the total liquid volume, since direct tracing of the concentration change in the unfrozen solution is difficult, if not impossible. Therefore, 'apparent' reaction rate constants (kA), instead of the 'true' rate constants (k), were reported for reactions in frozen state.

The concentration factor in Equation (3-8) is a function of temperature, which accounts for the temperature dependency of the freeze-concentration effect.

3.4 The Concentration Factor Prediction Model

For the purpose of the following derivations, several volume terms are defined in Figure 3.1. The total liquid volume of concern in Equation (3-4) is the solution volume of a system, not including the suspended particles or other insoluble parts as usually encountered in food systems. This volume is not readily measurable since the

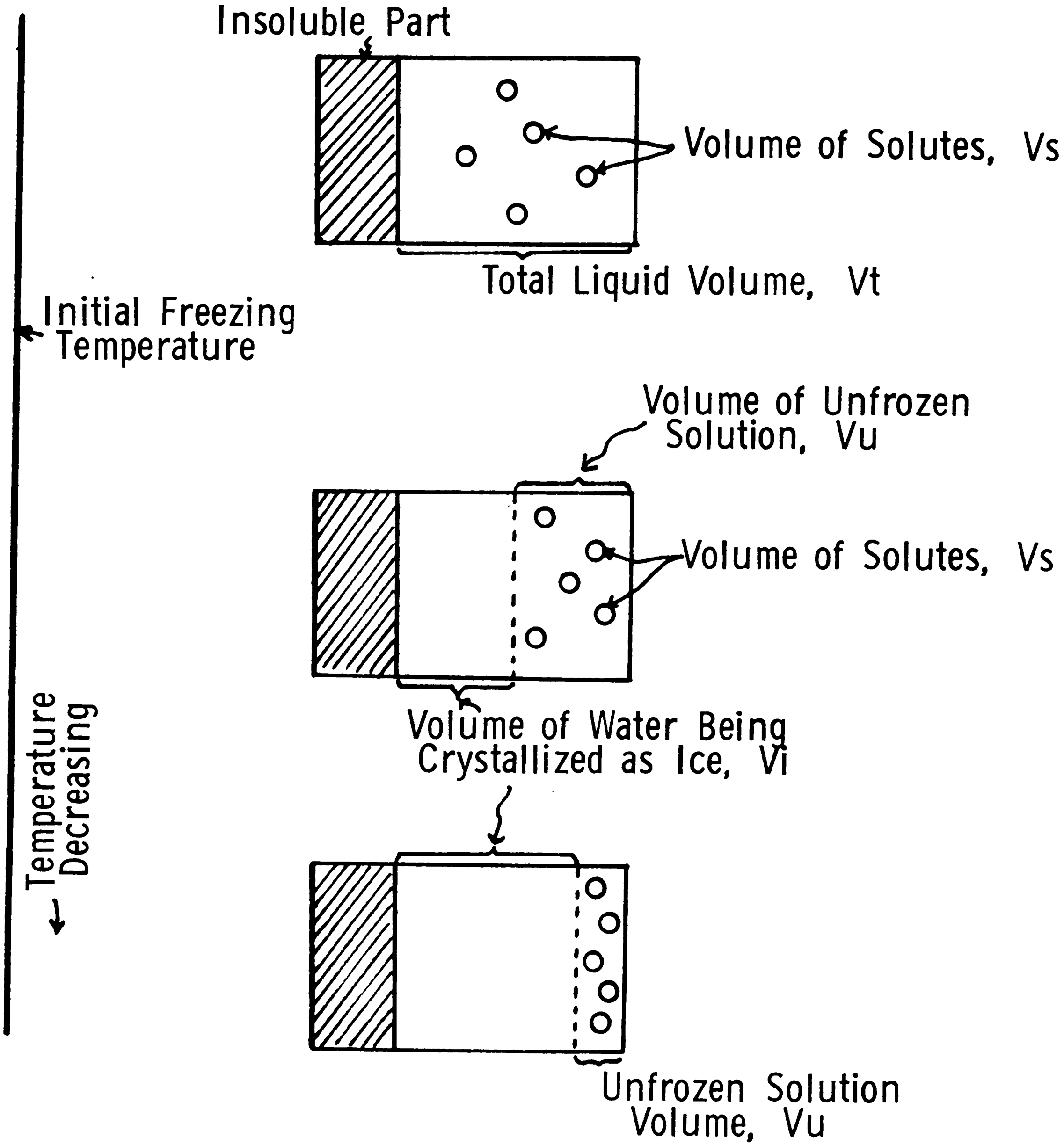


Figure 3.1 Definition of Volume Terms for a Frozen System

solution part is not easily separable from all other insolubles. In principle, the total liquid volume can be expressed as the volume of total water (Vw) presented in the system plus the volume of solutes in solution (Vs).

$$Vt = Vw + Vs \tag{3-10}$$

where

$$Vw = \frac{mw}{dw} \tag{3-11}$$

As freezing process begins, ice is progressively crystallized out and the available liquid volume (unfrozen solution) for reaction is reducing (Figure 3.1). The volume of unfrozen solution (Vu) can be determined by substracting the amount (volume) of water being crystallized as ice (Vi) from the total volume, if only pure water are crystallized out.

$$Vu = Vt - Vi \tag{3-12}$$

where

$$Vi = \frac{mi}{dw} \tag{3-13}$$

By combining Equations (3-4), (3-10), (3-11), (3-12) and (3-13)

$$Cf = \frac{mw / dw + Vs}{mu / dw + Vs} \qquad (3-14)$$

where

$$mu = mw - mi \qquad (3-15)$$

To be of dimensional consistency, the volumes (Vt, Vw, Vs) referred above are specific volumes, i.e. volumes of unit weight. In application, density of water is assumed to be independent of temperature, which is justifiable since the maximum deviation caused is less than 0.3% in the temperature range from 0 to 25 C, based on maximum density value (1.0000g/ml) at 3.98 C (Weast, 1971).

Mass fraction of unfrozen water (mu) is a function of temperature. The temperature dependency can be described by the following :

Assuming that the aqueous system during freezing is an ideal solution and represents a two component system composed of water and solutes (as a whole), the water activity of the reaction solution is equal to the mole fraction of water. By introducong the effective molecular weight of solutes (Heldman, 1974), the relationship can be represented as :

$$Aw = Xw = \frac{mu / Mw}{mu / Mw + ms / Ms} \qquad (3-16)$$

or

$$mu = \frac{Mw \cdot Aw \cdot ms \;/\; Ms}{1 - Aw} \qquad (3\text{-}17)$$

Actually, ms / Ms is the total mole fraction of solutes. The effective molecular weight of solutes (Ms) is introduced to avoid detailed quantification of effective mole numbers of each species of solute, which is difficult to achieve in complex systems.

Water activity in Equation (3-16) is a function of temperature and can be estimated through the freezing point depression equation, which being approximated numerically up to second order in freezing temperature (Θ) can be stated as (Ferro Fontan and Chirife, 1981):

$$Aw = \exp\,(9.693 \times 10^{-3} \cdot \Theta - 4.761 \times 10^{-6} \cdot \Theta^{2}) \quad (3\text{-}18)$$

The determination of the effective molecular weight of solutes can be done by using Equations (3-16) and (3-18) with knowledge of mass fraction of water and initial freezing temperature of the system.

The above-mentioned unfrozen water prediction model has been satisfactorily verified for systems with small amounts of unfreezable water (Heldman, 1974). For systems with noticeable amounts of unfreezable water, Larkin et al. (1983) proposed a modification on Equation (3-16) as :

$$Xw = \frac{(mu - mb) / Mw}{(mu - mb) / Mw + ms / Ms} \qquad (3\text{-}19)$$

3.4 Summary of the Inputs and Outputs of the Prediction Approach

(a) The concentration factor prediction model : Knowing initial freezing temperature (θ) or effective molecular weight (Ms), mass fraction of water (mw) and the specific volume of solutes in solution (Vs), concentration factor (Cf) as a function of temperature in frozen state can be determined by using Equations (3-14), (3-16) or (3-19) and (3-18).

(b) The rate prediction model : Knowing pre-exponential (A) of the Arrhenius equation or the reference reaction rate constant (kr) at reference temperature (Tr) and the activation energy (E), the reaction rate constants in supercooled solution or in liquid state before freezing at different temperatures can be predicted by Equation (3-2) or (3-3).

(c) Combined prediction approach : Knowing total reaction order (nt) of the reaction, apparent rate constants (kA) as a function of temperature can be estimated by Equation (3-8). These apparent rate constants take into account the influence of the freeze-concentration effect in addition to normal rate-temperature relationship.

(d) Quality magnitude prediction : With the apparent rate constant determined, the quality magnitude change as a function of storage time at fixed temperature can be predicted by Equation (3-7).

IV. EXPERIMENTAL MATERIALS AND PROCEDURES

Laboratory experiments were performed primarily to generate reaction rate constants at various temperatures above freezing and at sub-freezing temperatures for the oxymyoglobin autoxidation reactions. This experiment involves collection of oxymyoglobin concentation change in the reaction system as a function of storage time.

4.1 Experimental Materials

4.1.1 Reaction Tube

The autoxidation reaction was accomplished in air-saturated solution. Pyrex tubes with an inside diameter of about 15 mm were used as reaction vessels and Parafilm was used to cover the tube and prevent excessive dessication.

4.1.2 Oxymyoglobin solution

Oxymyoglobin was selected as reaction model for the study of the freeze-concentration effect in frozen state. The reaction was chosen for the following reasons :

(a) The reaction showed rate acceleration at frozen storage, and only qualitative reasonings have been proposed (Zachariah and Satterlee, 1973). Quantitative description

may be helpful in further understanding the reaction.

(b) The reaction is related to red meat discoloration; from attractive cherry red of oxymyoblobin to dull brown of oxidized metmyoglobin.

(c) The reaction has been studied to certain extent at temperatures above freezing. This provides the supporting background for quantification of the kinetics at sub-freezing temperatures.

For the preparation of oxymyoglobin solution, commercial crystallized myoglobin of horse heart origin (Sigma) was used. This purchased myoglobin was in the oxidized form of metmyoglobin. To reduce metmyoglobin to myoglobin and subsequently oxygenate the myoglobin to oxymyoglobin, the procedures reported by Brown and Mebine (1969) were adopted. Approximately 1 g of crystallized metmyoglobin was dissolved in 50 ml of de-ionized water. After mixing with about 50 mg of sodium dithionite, the solution was immediately passed through a mixed bed ion exchange column (Bio-Rad AG 501-X8) in the cold room (0 - 4 C) to remove the residual sodium dithionite and the breakdown products of it. The reduced myoglobin was oxygenated while passing through the column.

The oxymyoglobin solution was diluted with de-ionized water or buffer solution to a final concentration of about 1 mg/ml for subsequent storage tests.

4.1.3 Buffer Solution

Phosphate buffer has been used extensively in the investigation of oxymyoglobin autoxidation reaction, and it was in this buffer system that autoxidation rate acceleration due to freezing was observed (Brown and Dolev, 1963b). At sub-freezing temperatures, however, differential precipitation of the buffer salts occurs. This causes a pH shift (van den Berg, 1968) and possible myoglobin denaturation (Zachariah and Satterlee, 1973) which add complications to the quantification of the freeze-concentration effect.

Acetate buffer was selected for its high solubility in water in this study. For the preparation of acetate buffer of pH 5.6 , a volume ratio of 8.8 to 41.2 of the desired molarity of acetic acid solution to sodium acetate solution were mixed to give the specific molar concentration of buffer solution. The pH measurement was conducted by using a Corning Model 10 pH meter (Corning Scientific Instruments). Dilution of the buffer solution from 1.0 M to 0.01 M by de-ionized water did not result in noticeable pH change in the solution.

4.1.4 Storage Temperature Control

Temperature control during storage tests was accomplished by Aminco Constant Temperature Laboratory Baths (American Instrument Co., Inc.). Either ethanol (for sub-freezing temperature storage) or ethylene glycol (for above-freezing temperature storage) was used as bath liquid. A copper-constantan thermocouple was inserted into a control tube filled with buffer solution for the temperature measurement. Temperature reading was done through a digital thermometer (Omega Engineering, Inc., model 2176A). Bath temperatures were maintained within ±0.4 C.

4.2 Experimental Procedures

4.2.1 Initial Freezing Temperature Determination

Initial freezing temperature was used to determine the effective molecular weight or effective molar concentration of all solutes in the system as a whole. This information is needed as an input to the concentration factor prediction model.

In this study, a Model 130 Osmometer (Fiske Associates, Uxbridge, MA.) was used for the determination of initial freezing temperature of oxymyoglobin in buffer solutions of various concentrations.

4.2.2 Determination of Mass Fraction of Water

Mass fraction of water is another input necessary for the concentration factor prediction model. In general, it can be determined by various moisture determination methods. In this study, however, due to high moisture content and possible volatization of acetic acid in the reaction system, the determination of mass fraction of water was made through calculation based on measured solution density and known mass of solutes in the fixed solution volume.

The weight of solutes in a reaction system was known since all the solutes were added intentionally. The solutes include buffer salts and myoglobin. For 1.0 M buffer solution of pH 5.6, each liter of solution contains 79.93 g of buffer salts. To each liter of solution, about 1.0 g of myoglobin was added.

Density of the solution was obtained through measurement of the solution weight of fixed volume. This was done by putting the solution into a 100 ml volumetric flask at room temperature and determining the weight of the contents.

The mass of water in a unit volume can be calculated from the difference between the density of solution and the mass of solutes in a unit volume. Mass fraction of water can be determined by dividing mass of water by total mass.

4.2.3 Determination of Specific Solutes Volume in Solution

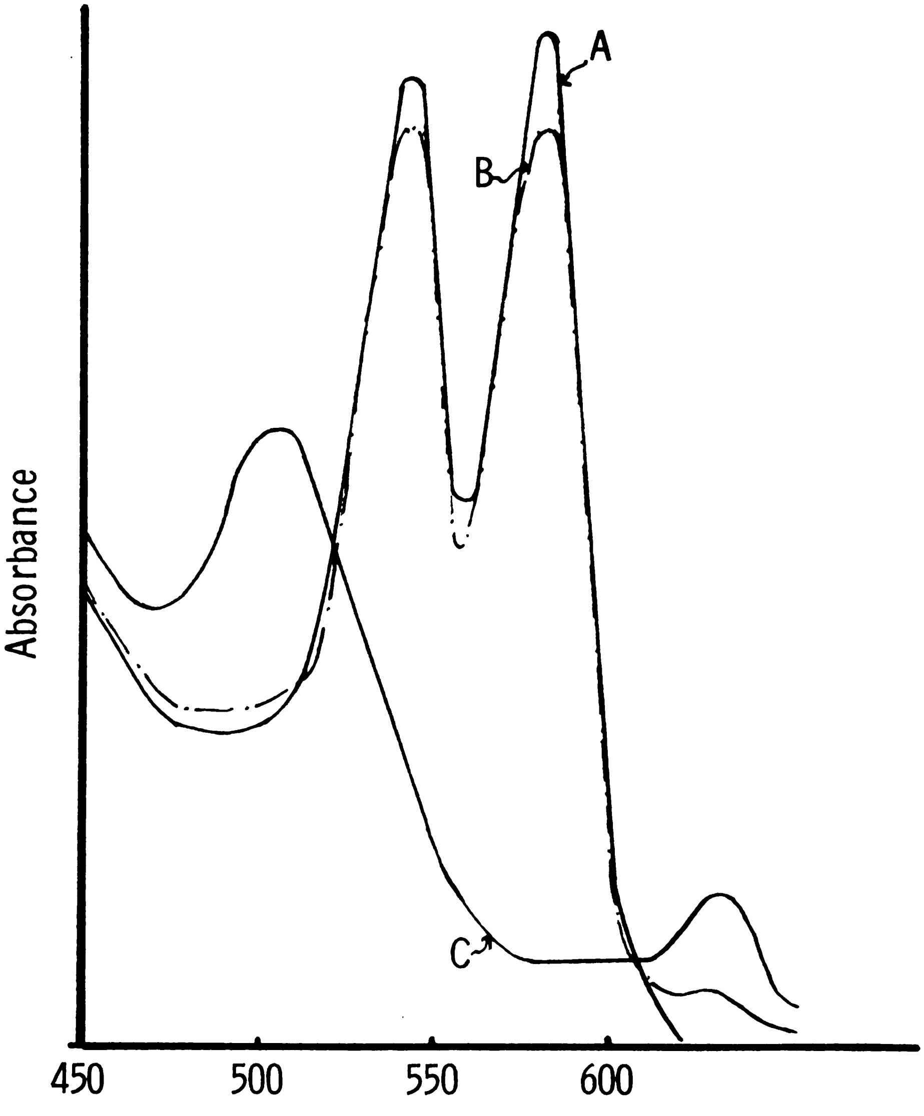
Specific solutes volume in solution can be calculated by substracting the specific volume of water in solution (assuming density of water to be equal to 1.0

g/ml) from the specific volume of solution.

4.2.4 Determination of Oxymyoglobin autoxidation rate

The rate of oxymyoglobin autoxidation was determined as the absorbancy change at alfa-maximum of oxymyoglobin. For horse myoglobin, this peak is at 582 nm (Gotoh and Shikama, 1974). The oxymyoglobin concentration change was expressed as % oxymyoglobin remaining in the reaction solution as a function of time. Freshly prepared oxymyoglobin solution, immediately after passing through the mixed bed ion exchange column and after desired dilution, was taken for the first absorbancy reading. This reading was established as 100 % oxymyoglobin. The solution, after designed storage tests, was added to a few milligrams of potassium ferricyanide to totally oxidize the remaining oxymyoglobin into metmyoglobin. The reading, after oxidation, was taken as 0% oxymyoglobin. The absorbancy reading measured during storage test could then be converted to % oxymyoglobin remaining. In pratical measurement, a spectrum scanning was done over wavelengths from 500 nm to 700 nm and the peak reading at alfa-maximum was utilized.

Typical spectra for initial reading (curve A), final reading (curve C) and reading during storage test (curve B) are shown in Figure 4.1. The myoglobin concentration in this system is about 1 mg/ml.



Wavelength, nm

Figure 4.1 Spectra of Oxymyoglobin, Metmyoglobin and the Transition Mixture of Both

4.2.5 Experimental Design

(a) Experiments for establishing input parameters to the prediction approach:

(1) To obtain reaction rate constant and reaction order, storage tests at fixed temperature in liquid state were needed.

(2) To obtain activation energy for autoxidation of oxymyoglobin, storage tests at various fixed temperatures in liquid state were performed.

(3) To quantify the influence of buffer concentration on the reaction rate, storage tests at different buffer strengths were performed.

(4) To investigate the temperature dependency of the buffer concentration effect, storage tests at various buffer strengths and at various fixed temperatures are needed.

(b) Experiments for verifying the prediction approach:

(1) To check if the same rate equation is followed both in liquid state before freezing and in unfrozen solution of frozen state, storage tests in the frozen state were performed.

(2) To check the validity of extrapolation of the Arrhenius plot from temperatures above freezing to temperatures below freezing, storage tests in supercooled solutions were performed.

(3) To examine possible complications from

different freezing medium temperatures, storage tests were performed on samples frozen by immersing in -8.0 or -70 C cold bath.

(4) To check one of the implications from the prediction approach that the rate of a first order reaction will not be influenced by the freeze-concentration effect, storage tests were performed on myoglobin autoxidation in de-ionized water in frozen state. These storage tests also give the opportunity to observe the influence of ice crystal formation on the reaction rates in frozen state.

(5) To check the effectiveness of the entire prediction approach, several sets of storage tests in the frozen state and at different buffer concentrations were performed.

4.2.6 Storage Trials

Storage trials were performed in both liquid state and frozen state. For liquid state storage, about 6 ml of oxymyoglobin solution was transferred to each tube. For each trial, 16 or more tubes were prepared. The tubes were transferred to a constant temperature bath of desired temperature immediately after preparation. Temperature reading in a control tube was taken to examine the thermal equilibrium in the tube, which was ascertained by equal temperatures observed inside the tube and outside in the bath liquid. The first reading was taken after thermal equilibrium was obtained. Subsequent readings were taken at time intervals depending on the reaction rate. Two tubes were taken for each time interval for duplicate readings. Each storage trial was conducted at certain constant temperatures. Different temperature trials were performed to determine the temperature dependency of reaction rate. Four to six temperatures were employed for this purpose.

Five storage trials of liquid state reaction were performed in supercooled solution. In this study, the supercooled solution was obtained by transferring the prepared sample to the desired sub-freezing temperature bath. Only supercooled solution with a few degrees below its initial freezing temperature can be obtained by this method. Possible ice formation was examined by visual inspection, since any trace amount of ice crystal formation in supercooled solution would initiate the whole system to freeze.

Storage trials in the frozen state were performed to examine the rate acceleration during frozen storage. The prepared test tubes with oxymyoglobin solution were transferred to a -8 C temperature bath to initiate the freezing process and then stored at desired storage temperatures. After thermal equilibrium was obtained, the test sample was thawed in a room temperature water bath (about 20 C) until all ice crystals disappeared and the first reading was taken. The storage period recorded was based on the time when the reading was taken, instead of the time when the tube was removed from the controlled temperature bath.

Four buffer concentrations were used to study the rate acceleration caused by the freeze-concentration effect. A 3.0 M acetate buffer of pH 5.6 was prepared as stock buffer solution. To the oxymyoglobin solution elucidated from ion exchange column, certain amounts of stock buffer solution and/or de-ionized water were added to obtain final buffer concentrations of 0.01, 0.05, 0.5 or 1.0 M.

A set of storage trials was conducted in de-ionized water to examine the implication from theoretical consideration that the rate of a first order reaction would not be affected by the freeze-concentration effect. Observations also can be made based on this set of trials whether complications other than the freeze-concentration effect accompanied with ice formation exert significant influence on reaction rate.

To investigate possible influence of freezing rate on autoxidation rate, two storage trials in frozen state were carried out. The freezing process of these two trials was accomplished by an acetone-dry ice bath of temperature at about -70 C. After the freezing process was completed the samples were then transferred to the desired

temperature bath. Procedures followed were the same as other storage trials in frozen state.

4.2.7 Data Analysis

(a) Least square fitting

Least square fitting was used frequently in the analysis of kinetic data. To determine the reaction rate constant at a fixed temperature, a linear or first order least square fitting was performed between natural log of % oxymyoglobin and storage time. Slope of this straight line is equal to the reaction rate constant. In the determination of activation energy, linear fitting was performed between natural log of reaction rate constant (k) and inverse of absolute temperature (1/T). Slope of this straight line is equal to activation energy divided by universal gas constant, which has a value of 8.314 kJ/mole. Another application was done in the determination of the influence of buffer strength on reaction rate. The slope of the least square fitted straight line between ln k and ln x (fold of concentration change) is similar to a reaction order, which describes the buffer concentration dependency of the oxymyoglobin autoxidation rate.

(b) Indicator variable method

The indicator variable method was proposed by Chu and Heldman (1983) in the analysis of activation energy.

This method is especially useful in the evaluation of common slope for groups of data with different intercepts. Experimental data of the autoxidation of oxymyoglobin at different buffer concentrations conform to this situation. Since reaction mode in different buffer concentrations is expected to be the same and the influence of buffer concentration exerts on the magnitude of reaction rate. A common activation energy value is therefore expected for reactions in different buffer concentrations.

The indicator variable method (Neter and Wesserman, 1974) in principle allocates an extra portion of intercept to each new coming set of data while imposing no flexiblility on the slope during a multiple linear regression process. For example, in the case when three groups of data are present, the following regression equation is used :

$$Y = B1 + B \cdot X + B2 \cdot X2 + B3 \cdot X3 \qquad (4\text{-}1)$$

where X2 , X3 are called indicator variables, which are defined as :

X2 = 1 , if data belong to second group
X2 = 0 , otherwise

and

X3 = 1 , if data belong to third group

$X3 = 0$, otherwise

Thus, for first qroup of data, Equation (4-1) reduces to :

$$Y = B1 + B \cdot X \tag{4-2}$$

and for second and third groups of data, Equation (4-1) reduces to :

$$Y = (B1 + B2) + B \cdot X \tag{4-3}$$

and

$$Y = (B1 + B2 + B3) + B \cdot X \tag{4-4}$$

respectively. It can be seen that for each new group of data, a new portion of intercept is allowed to vary, as B2 for second group of data and B3 for third group of data in the above example. While the slopes are forced to be the same, all have the same parameter, B, in the above example.

A multiple linear regression program can be used to determine best estimates of the parameters B, B1 , B2 and B3 .

V. RESULTS AND DISCUSSIONS

The primary objective of this study was to develop a prediction approach which will allow the quantification of the freeze-concentration effect in a frozen system. The first part of this section presents the experimentally determined input parameters of the oxymyoglobin autoxidation reaction necessary for the prediction approach. The second part presents the results of several experimental designs which support the prediction approach and compares the measured apparent reaction rate constants with the predicted values for oxymyoglobin autoxidation in frozen state. The third part develops process recommendations by varying the input parameters to the prediction approach. In the fourth part of this section, attempts are made to identify possible concentration effect existing in frozen food systems.

5.1 Input Parameters of the Oxymyoglobin Autoxidation Reaction

The two models (the rate prediction model and the concentration factor prediction model) that make up the prediction approach require two types of input information. One is the kinetic information which includes the reaction

rate constants, the reaction order and the activation energy. The second model requires system information which includes the mass fraction of water in the system, the specific volume of solute in solution and the effective molecular weight of solutes. This information is obtained through laboratory determinations with procedures discussed in Chapter IV.

5.1.1 The Reaction Rate Constant and the Reaction Order of Oxymyoglobin Autoxidation Reaction

The autoxidation reaction of oxymyoglobin in various buffer solutions and under 'air-saturated' conditions has been shown to be first order with respect to oxymyoglobin concentration (Brown and Dolev, 1963a; Brown and Mebine, 1969; Gotoh and Shikama, 1974), even for reactions taking place in frozen state (Brown and Dolev, 1963b; Zachariah and Satterlee, 1973). In this study, the oxymyoglobin in acetate buffers and in de-ionized water was exposed to air and the autoxidation reaction was taking place under the same 'air-saturated' conditions. Experiments were performed in four different acetate buffer concentrations including 0.01, 0.05, 0.50 and 1.00 M. Storage tests were conducted at fixed temperatures for the determinations of reaction rate constants. Results showed that the autoxidation of oxymyoglobin in acetate buffer or in de-ionized water was first order with respect to oxymyoglobin concentration at all buffer concentrations and at all temperatures investigated. This observation was based on plots of the natural log of % oxymyoglobin versus storage time to obtain straight lines for storage experiments. The plots for samples stored at 9 C and at four buffer strengths are shown in Figure 5.1. Results obtained at a buffer strength of 0.50 M and at storage temperatures of 20, 14, 9 and 3 C are shown in Figure 5.2. Figure 5.3 shows the first order kinetics for oxymyoglobin autoxidation reactions taking place in de-ionized water, and those plots for reactions in the frozen state are shown in Figure 5.4. The linear curves shown in these plots were determined by using least squares regressions. Rate constants were calculated from the slopes of the linear curves using the first order rate equation. Each straight lines was based on five or more measurements during a constant temperature storage. The rate constants with standard deviations for the reactions occurring in the liquid state for four different buffer strengths are listed in Table 5.1. Results for the reactions in frozen state are listed in Table 5.2. Percentage oxymyoglobin change for different storage times are tabulated in Appendix A. The low standard deviations for these rate constants and high correlation coefficients for the linear regressions ($r^2 > 0.95$ in most cases) confirm that the reaction follows first order kinetics with respect to oxymyoglobin

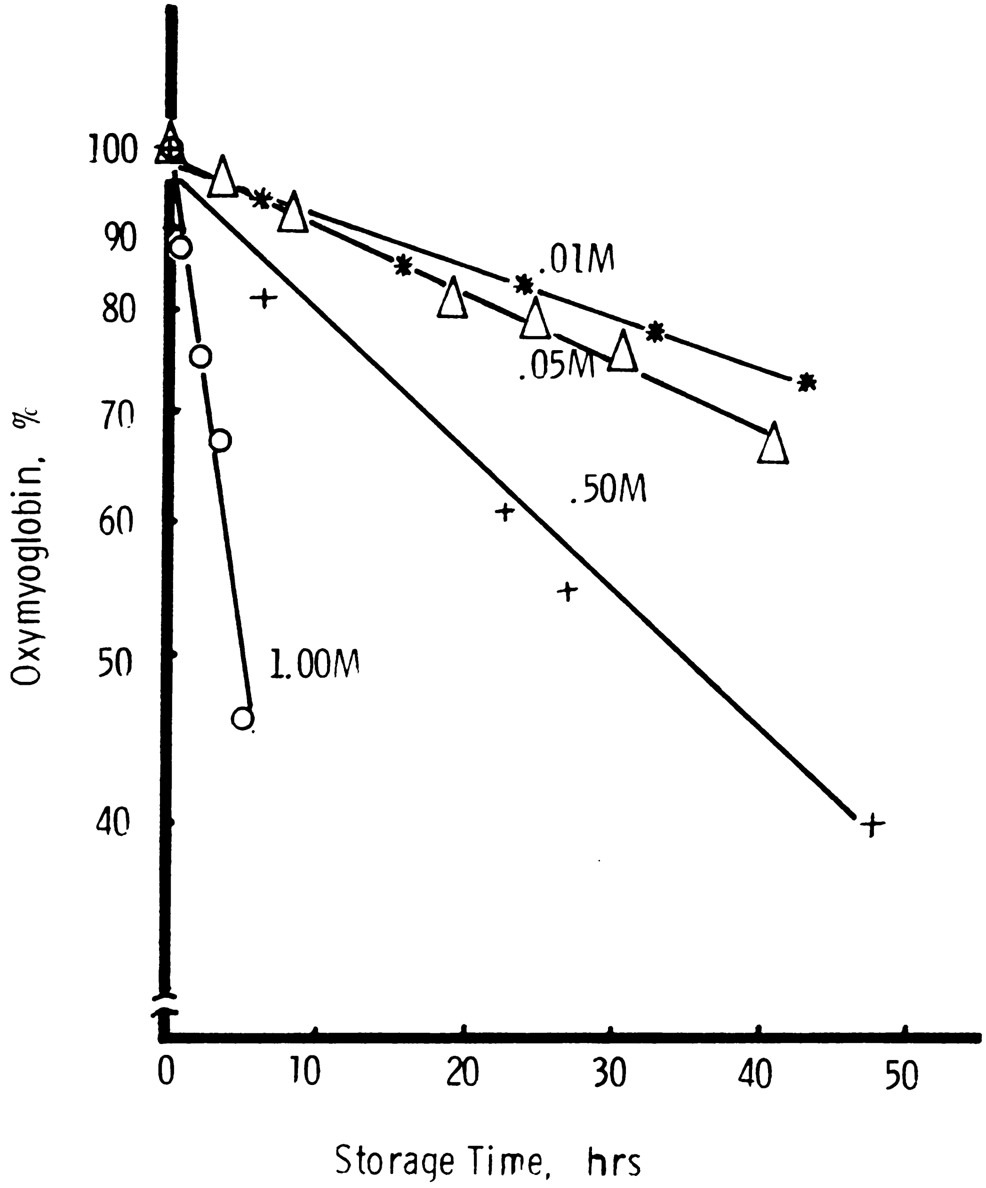


Figure 5.1 First Order Reaction Kinetics of Oxymyoglobin Autoxidation in Acetate Buffer at 9 C

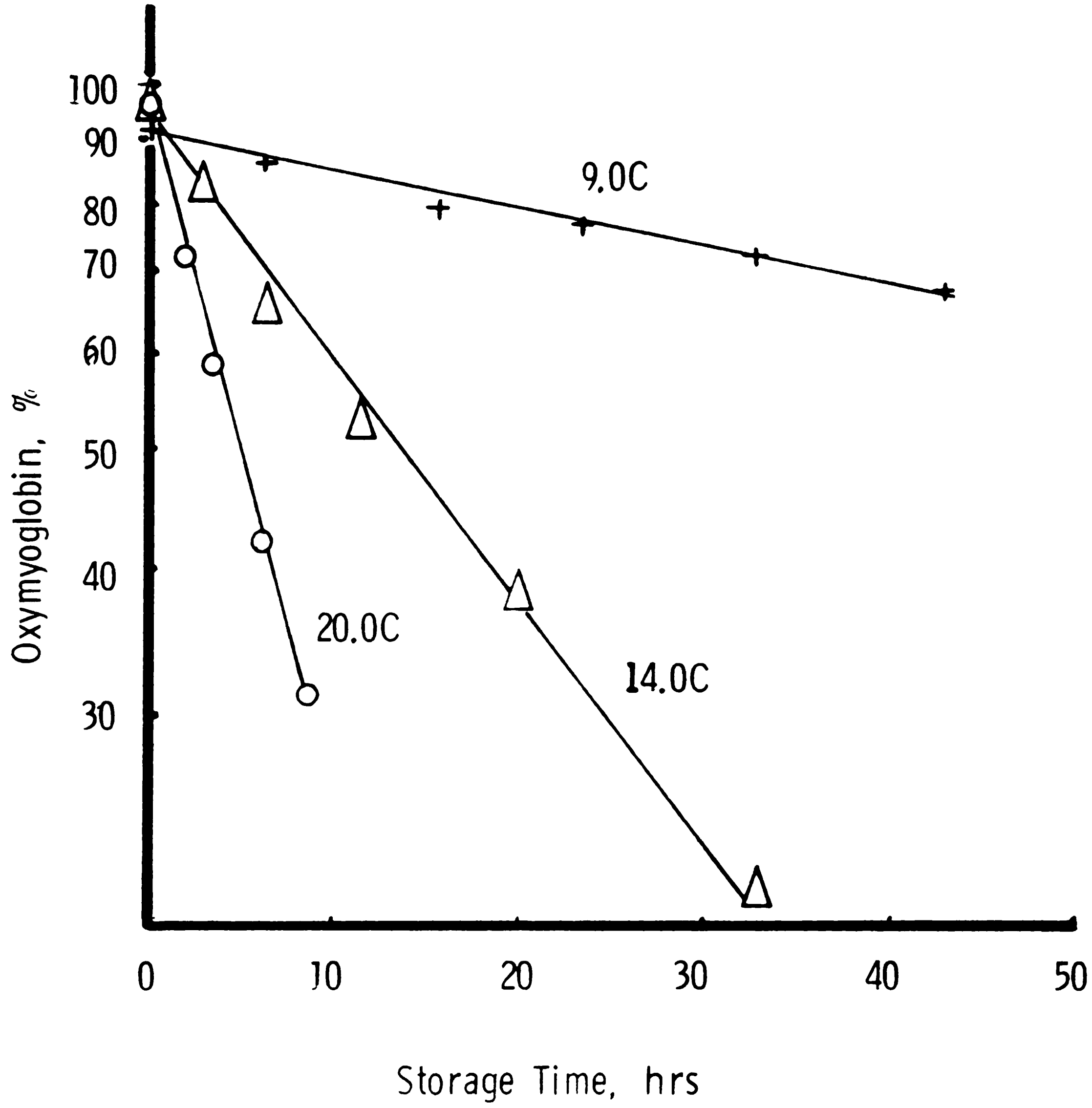


Figure 5.2 First Order Reaction Kinetics of Oxymyoglobin Autoxidation in .50 M Acetate Buffer

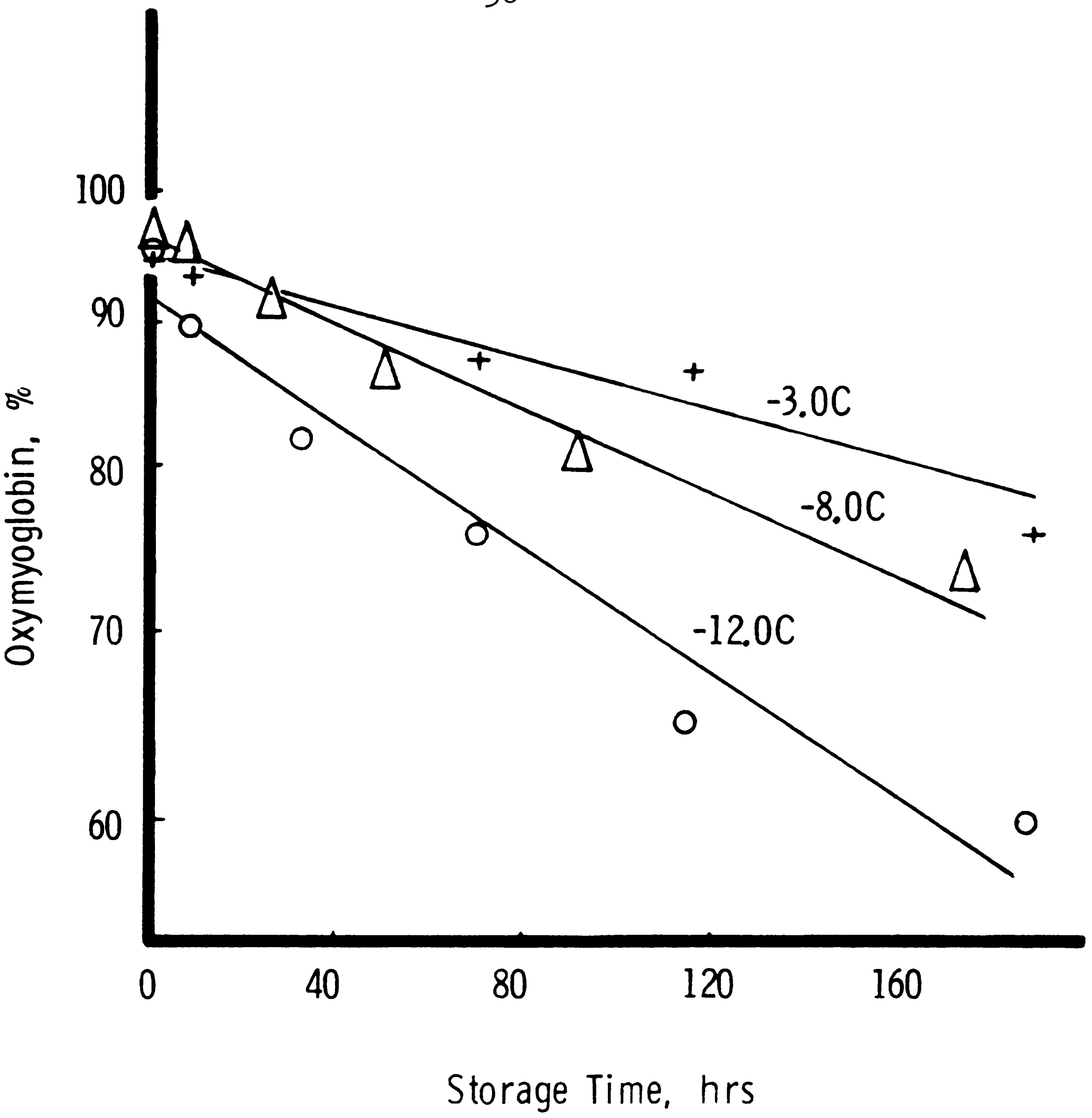


Figure 5.3 First Order Reaction Kinetics of Oxymyoglobin Autoxidation in .01 M Acetate Buffers in the Frozen State

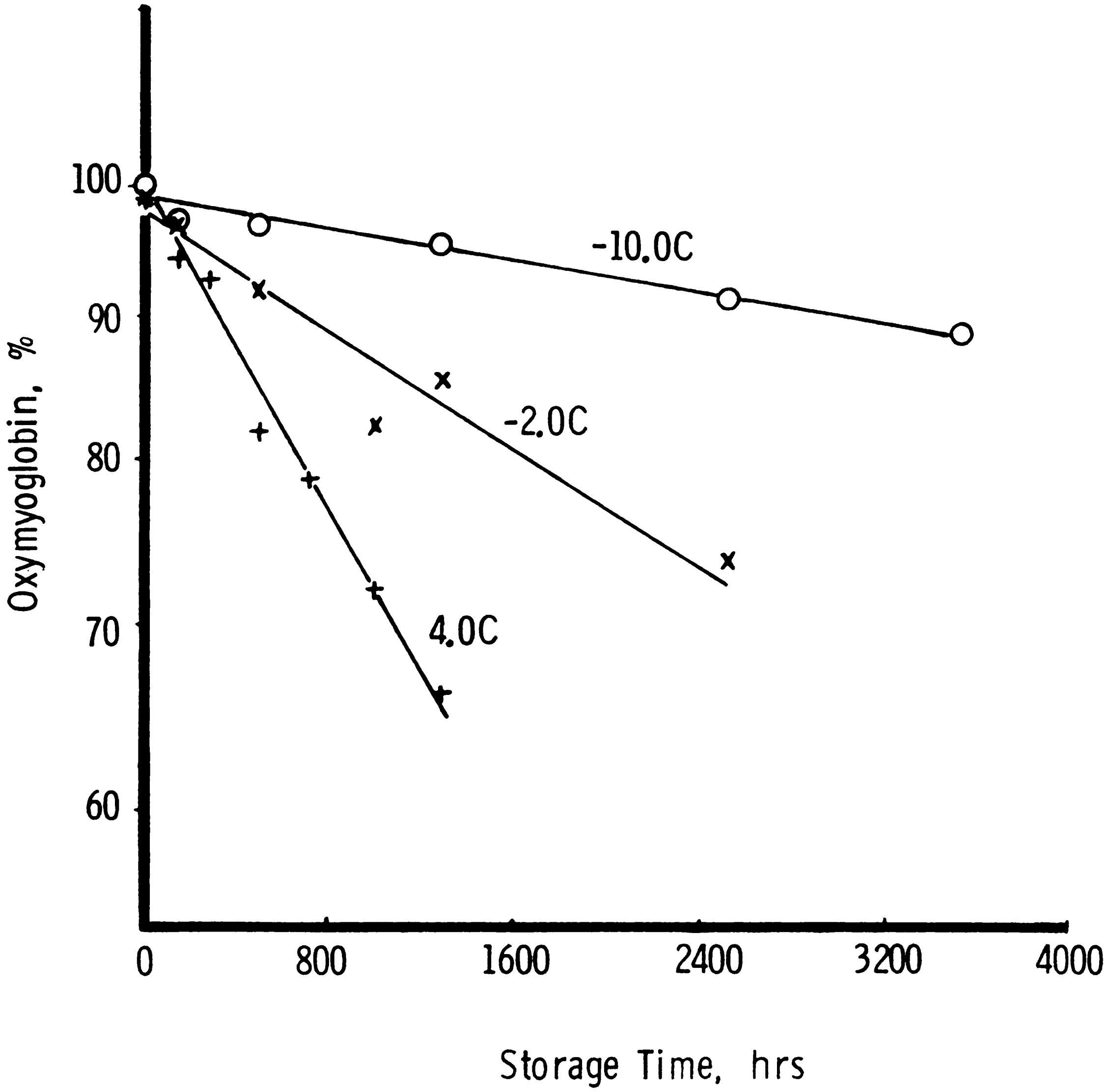


Figure 5.4 First Order Reaction Kinetics of Oxymyoglobin Autoxidation in De-Ionized Water

Table 5.1 First Order Rate Constants for Autoxidation of Oxymyoglobin in Liquid State of Four Buffer Concentrations

Temperature, C	Buffer Concentration, M			
	0.01	0.05	0.50	1.00
	Rate Constant, $k \times 10^{-3}$ hr^{-1}			
20.0	41. ± 1.	56. ± 2.	128. ± 4.	148. ± 3.
14.0	15.7 ± 0.5	23.5 ± 0.6	45. ± 2.	52. ± 2.
9.0	7.0 ± 0.4	8.8 ± 0.3	19.0 ± 0.1	23.1 ± 0.5
3.0	1.84 ± 0.06	3.0 ± 0.1	6.0 ± 0.3	8.6 ± 0.6
-1.0	*1.09 ± 0.04	-----	3.0 ± 0.2	-----
-3.0	*0.72 ± 0.05	-----	*1.8 ± 0.1	3.0 ± 0.1
-5.0	-----	-----	*1.36 ± 0.09	*1.4 ± 0.1

* Supercooled solution

Table 5.2 Apparent First Order Rate Constants for Autoxidation of Oxymyoglobin in Frozen State of Four Buffer Concentrations

Temperature, C	Buffer Concentration, M			
	0.01	0.05	0.50	1.00
	Rate Constant, $k \times 10^{-3}$ hr^{-1}			
-2.0	2.8 ± 0.2	2.2 ± 0.1	-----	-----
-3.0	2.4 ± 0.3	2.3 ± 0.2	2.3 ± 0.1	-----
-8.0	1.8 ± 0.2	1.4 ± 0.1	1.28 ± 0.07	1.3 ± 0.2
-12.0	0.91 ± 0.09	1.1 ± 0.5	0.81 ± 0.05	0.86 ± 0.02
-15.0	-----	0.55± 0.07	-----	-----

concentration.

Comparing the results listed in Table 5.1 with the first order rate constants for beef oxymyoglobin autoxidation in phosphate buffer (Brown and Dolev, 1963a, Table 5.3), it can be observed that the results are within the range of the magnitudes although variations due to difference in buffer systems do exist.

5.1.2 Activation Energy of the Oxymyoglobin Autoxidation Reaction

In general, within a reaction system, as long as a limiting reaction exists, the rate constants decrease with decreasing temperature in an exponential relationship, and this can be described by the Arrhenius equation if the temperature range is not too large. Activation energy is a parameter in this equation and its magnitude is the indication of the extent of temperature dependency of the reaction rate. Activation energy was calculated from the slope of the straight line relationship between natural log of rate constant versus inverse of absolute temperature, as described by Equation (3-2). The straight line is determined by least square regression and based on four to seven data points depending on the buffer concentration. Rate constants obtained in supercooled solutions are incorporated into the determination of activation energy. The plots of natural log of rate constants versus inverse

Table 5.3 First Order Rate Constants for Autoxidation of Beef Oxymyoglobin in Phosphate Buffers (Brown and Dolev, 1963a)

Temperature, C	Buffer Concentration, M		
	0.1	0.2	0.6
	Rate Constant, $k \times 10^{-3}$ hr^{-1}		
40	1600	655	257
37	----	296	194
30	555	170	93
	575	---	--
	515	---	--
20	357	41	34
10	161	16	9
0	54	8	4
	--	9	-

of absolute temperature for reactions in four buffer solutions are shown in Figure 5.5. The four linear curves are nearly parallel to one another. This indicates that similar or the same reaction mode is followed at all four buffer strengths. The calculated activation energy values and their standard deviations are listed in Table 5.4. The small standard deviations reveal the applicability of the Arrhenius equation in the description of the temperature dependency of oxymyoglobin autoxidation rate over the temperature interval of concern. The activation energy values obtained are close to the upper bound of the range (from 100.4 to 116.3 KJ/mole) reported by Brown and Mebine (1969).

The indicator variable method as described in section 4.2.5 was used to determine a common activation energy value for all four systems with different buffer concentrations. Corresponding pre-exponential values of the Arrhenius equation were also determined. The resulted activation energy was 118 ± 2 KJ/mole, with pre-exponentials of 4.27×10^{19}, 5.94×10^{19}, 1.22×10^{20} and $1.53 \times 10^{20} hr^{-1}$ for 0.01, 0.05, 0.50 and 1.00 M acetate buffer systems, respectively.

The assumption of the validity of the Arrhenius equation in the prediction approach imposes the necessity to check the linear relationship between natural log of rate constant and inverse of absolute temperature before

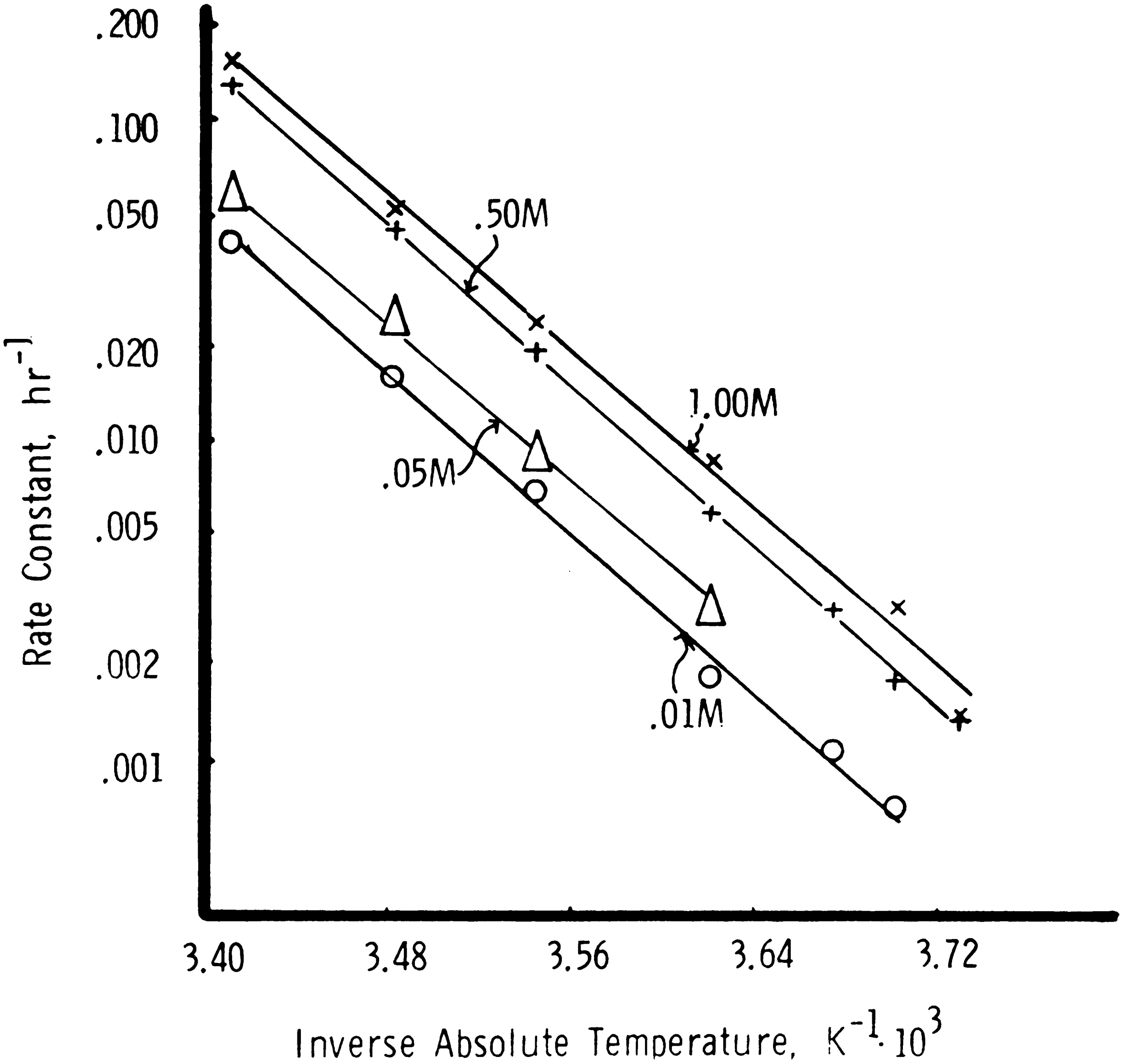


Figure 5.5 Arrhenius Plots for Autoxidation of Oxymyoglobin in Four Strengths of Acetate Buffers

Table 5.4 Activation Energies for Autoxidation of Oxymyoglobin in Liquid State of Four Buffer Concentrations

	Buffer Concentration, M			
	0.01	0.05	0.50	1.00
Activation Energy, KJ/mole	117 ± 3	117 ± 4	120 ± 1	117 ± 4

further application of the prediction approach.

5.1.3 Influence of Buffer Concentration on Oxymyoglobin Autoxidation Rate

Buffer concentration usually can be kept constant for a reaction taking place in the liquid state. In the frozen state, however, buffer solutions may be concentrated and result in large variation in the buffer strength with respect to the concentration before freezing. For example, a solution at initial concentration of 0.001 M was shown to produce an unfrozen solution in frozen state at -5 C which is 1000 times more concentrated than the original liquid state solution (Pincock, 1969).

Therefore, an investigation of the solutes (other than reactants) concentration influence on reaction rate is necessary for frozen state reaction. The solutes in the reaction system employed in this study were the buffer salts.

Phosphate buffer has been used extensively in the study of the oxymyoglobin autoxidation reaction. For frozen state, this buffer may be selectively precipitated and result in pH shift (ven den Berg, 1969) as well as protein denaturation (Zacharich and Satterlee, 1973). To avoid these complications, acetate buffer was used in this study.

Four strengths of acetate buffer solutions were

used to investigate the influence of buffer strength; 0.01, 0.05, 0.50 and 1.00 M preparations. Rate constants determined in these buffer solutions and at various storage temperatures are listed in Table 5.1, which showed a tendency that an increase in buffer strength caused an acceleration in reaction rates. This observatiton is in agreement with the results obtained by Matsuura et al. (1962).

When plotting ln k versus ln x, a straight line relationship was observed. Here "x" represents the ratio of buffer concentration to 0.01 M, the most dilute system used. By doing this, "x" can also be referred to as the folds of concentration to a standard buffer concentration. This relationship is helpful in the description of concentration effect exerted by buffer solution and can be expressed as:

$$k = ko \cdot (x)^{nx} \tag{5-1}$$

where "nx" is the slope of the ln k versus ln x plot, while ko can be referred to as the rate constant measured at standard buffer concentration when x = 1. When the buffer strength is varied to a certain fold of the standard buffer concentration, x = (new buffer concentration) / (standard buffer concentration), the rate constant in the new buffer system will be the product of ko and x to the 'nx' power as governed by Equation (5-1). If the standard value is taken as the concentration of buffer in each system before freezing, then 'x' is equal to the concentration factor as defined by Equation (3-4).

The value of 'nx' is determined from the slope of the ln k versus ln x plot. The plots for different temperature trials are shown in Figure 5.6. These straight lines are close to parallel to one another, indicating that while ko depends on temperature, nx is not a function of temperature or buffer concentration. This is in agreement with the observation made earlier that reaction mode and activation energy do not vary to a noticeable extent in systems with different buffer strengths. The determined nx values and their standard deviations are listed in Table 5.5. To determine a common nx value over different temperatures, the pre-exponentials obtained by indicator variable analysis were used to represent the "standard" rate constants. Plot of ln A versus ln x was drawn in Figure 5.7. The "A" value for 0.01 M buffer system deviated significantly from the linear relationship based on other three data points. Least square fitting based on three data points from 0.05, 0.50 and 1.00 M buffer systems yields a 'nx' value of 0.316 ± 0.004, while with the point from 0.01 M buffer system included, it yields a 'nx' value of 0.28 ± 0.02. The standard deviation significantly increased. The deviation of the 'A' value for 0.01 M

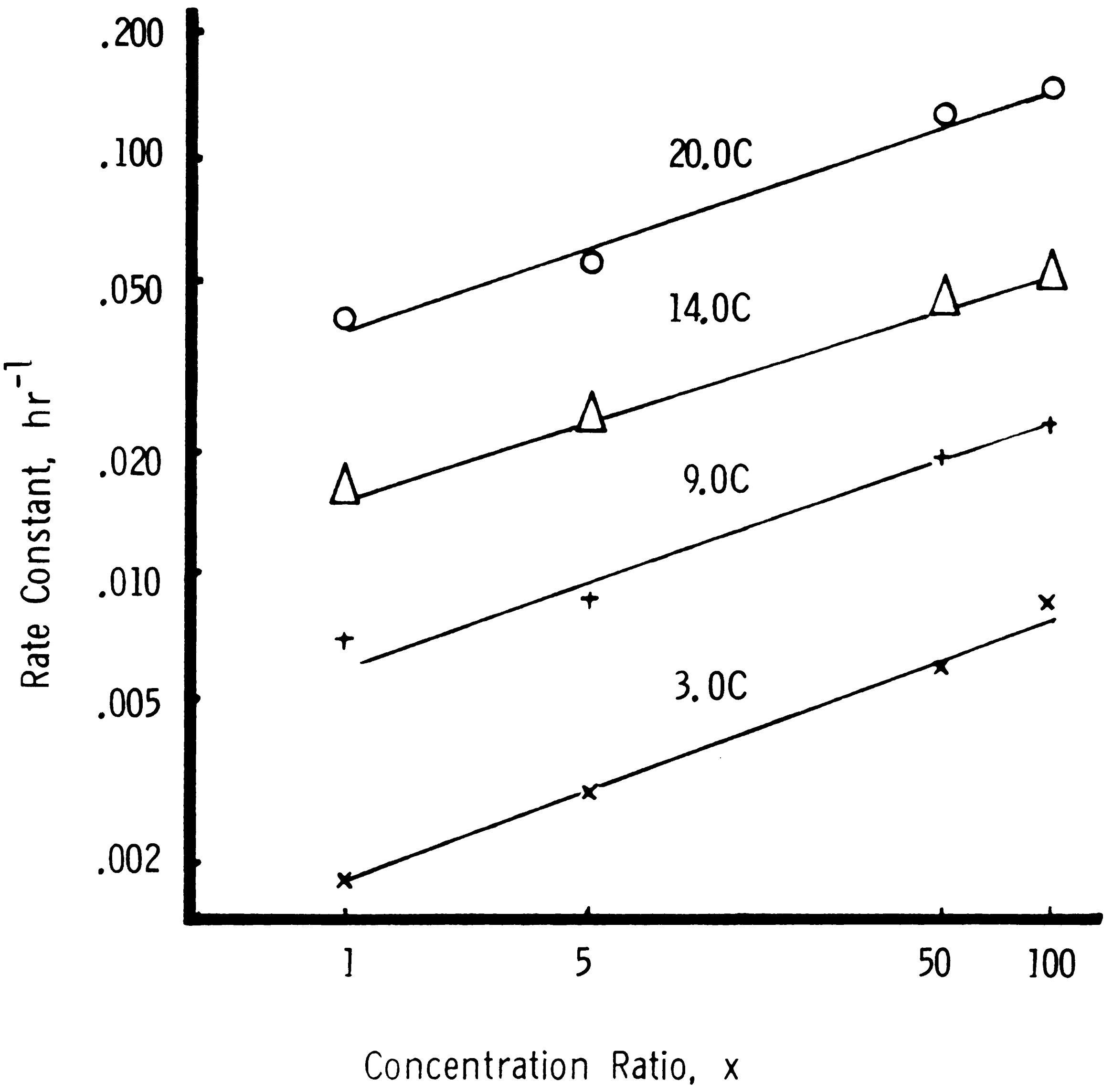


Figure 5.6 Influence of Acetate Buffer Strength on the Reaction Rate Constants of Oxymyoglobin Autoxidation

Table 5.5 Reaction Order for Autoxidation of Oxymyoglobin with respect to Acetate Buffer Concentration at various storage temperatures

Temperature, C	Reaction Order with respect to Buffer Concentration (nx)
20.0	0.29 ± 0.02
14.0	0.265 ± 0.006
9.0	0.27 ± 0.03
3.0	0.32 ± 0.02
-3.0*	0.28 ± 0.06

* Supercooled Solution

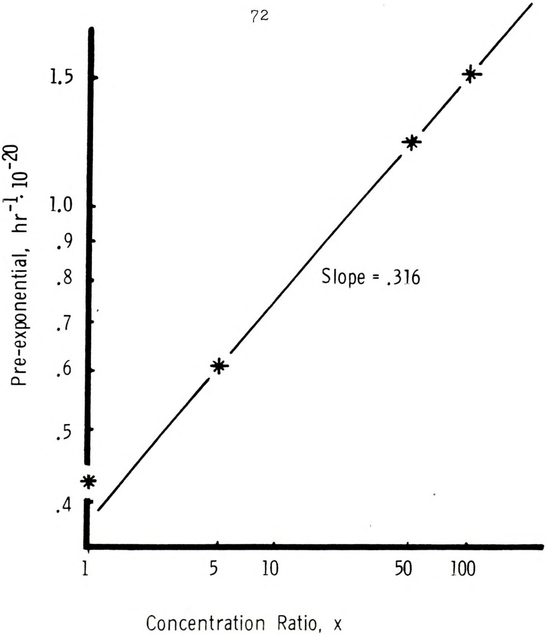


Figure 5.7 Influence of Acetate Buffer Strength on the Oxymyoglobin Autoxidation Rate

buffer system most likely is due to experimental error, since it was a 300 fold dilution from stock buffer solution, small errors in volume measurement could be magnified by small total volume of stock buffer solution used. A 'nx' value of 0.316 therefore was adopted as input to the prediction approach.

The meaning of 'nx' to the rate equation is like a reaction order. The only difference is that buffer strength does not change to a noticeable extent as the reaction proceeds. Therefore, for reaction in liquid state, the apparent reaction rate is not a function of buffer concentration and the total reaction order is equal to one. While during the freezing process, concentration phenomenon varies the buffer concentration to the extent that the influence of buffer strength has to be taken into account. In this case, the rate equation should be modified as below :

$$\text{rate} = \frac{d(\text{MbO}_2)}{dt} = k \cdot (\text{MbO}_2) \cdot (x)^{0.316} \qquad (5\text{-}2)$$

At fixed storage temperature, either above freezing or sub-freezing, the buffer concentration is fixed and therefore the reaction remains first order. This accounts for the observed first order reaction for autoxidation of oxymyoglobin even in frozen state storage trials, as reported in section 5.1.1.

5.1.4. System Properties of the Buffered Myoglobin Solutions

Inputs to the concentration factor prediction model include the mass fraction of water , the effective molecular weight of total solutes and the specific volume of solutes in solution. Effective molecular weight can be calculated from the known solutes composition or can be back calculated from the freezing point depression equation by knowing the initial freezing temperature. To account for some dissociable solutes in a system, the latter method could be better related to the true "effective" molar numbers in the solution, as long as the initial freezing tempreature can be accurately determined.

In this study, the initial freezing temperatures were determined by an osmometer. The mass fraction of water was calculated from known density and weight of solutes in a given volume. This could avoid the inaccuracy encountered for high moisture system when usual moisture determination methods are used. The determination of specific volume was done by substracting specific volume of water in solution from specific volume of solution, as described in Chapter IV.

Results for the mass fraction of water, the specific volume of solutes and the molecular weight of each reaction system are listed in Table 5.6.

As the buffer strength increased, the effective molecular weight of the system decreased. This is

Table 5.6 System Information for Oxymyoglobin Solutions of Four Acetate Buffer Concentrations

	Buffer Concentration, M			
	0.01	0.05	0.5	1.00
Mass Fraction of Water (mw)	0.9982	0.9950	0.9600	0.9220
Specific Volume of Solutes(Vs), ml/g	0.0014	0.003	0.021	0.041
Initial Freezing Temperature, C	-0.037	-0.182	-1.73	-3.59
Effect Molecular Weight, g/mole	90.48	51.22	44.31	43.17

reasonable in considering that the weight of solutes contributed by oxymyoglobin becomes more and more dominant as the buffer concentration decreases, while the contribution of oxymyoglobin to molar concentration still negligible. For example, in 1 liter of solution, buffer salts weigh about 79.93 g and 0.80 g for 1.00 and 0.01M acetate buffer systems, respectively, with added myoglobin of about the same weight of 1.00g in both systems. While the molar concentration of myoglobin of 1.00 g/l is about 6.0×10^{-5}M (taking the molecular weight of myoglobin to be 16700 g/mole), negligible to both systems, the mass fraction of myoglobin is negligible only to 1.00 M system. For 0.01 M buffer system, the weight of total solutes increases relatively from the emerging significance of the weight of myoglobin without having a significant decrease in initial freezing temperature, which causes an increase in effective molecular weight as buffer strength decreases.

5.2 The Effectiveness of the Prediction Approach

The prediction approach in this investigation includes the rate prediction model and the concentration factor prediction model. The validity of the assumptions underlying the concentration factor prediction model have been satisfactorily verified for food systems with low quantities of unfreezeable water (Heldman, 1974). For systems with significant amounts of unfreezeable water,

Larkin et al. (1983) presented a modified approach to achieve the prediction, as described in Chapter III. Those assumptions are adopted without further verification. The following sections will be devoted to the discussions of:

(a) the influence of freezing rate on the apparent reaction rate of the oxymyoglobin autoxidation reactions in frozen state.

(b) the validity of the assumption that the same rate equation is followed for reaction taking place both in liquid state before freezing and in unfrozen solution of frozen state, by checking if the same reaction order is kept for reactions in frozen state.

(c) the validity of the extrapolation of the Arrhenius plot from temperature range above freezing to temperature range below freezing, by examining rate constants in supercooled solutions.

(d) the validity of the implication from equation (3-8), that a reaction with first order kinetics is not affected by the freeze-concentration phenomenon, by observing the reaction rates in de-ionized water.

(e) the influence of the magnitude of the specific volume of solutes on the predicted values of concentration factors or apparent rate constants.

(f) the effectiveness of the total prediction approach, by comparing the predicted outputs with measured apparent rate constants of oxymyoglobin autoxidation in

frozen state.

5.2.1. The Influence of Freezing Rate on Autoxidation Rate of Oxymyoglobin

It is believed that the rate of freezing may affect the extent to which solutes are concentrated; the concentration effect is larger for systems with slower freezing rates (Tappel, 1966). The influence of freezing rate on apparent rate constants of oxymyoglobin autoxidation was checked for possible deviations came from variations in freezing procedures. Reactions in 0.01 M acetate buffer were desinged for this purpose.

Two storage temperatures, -2.0 and -8.0 C were tested. Two kinds of freezing processes were performed. One was by immersing sample tubes into an acetone-dry ice bath (about -70 C) to complete freezing process. The other was accomplished by immersing sample tubes at -8.0 C bath. No attempts were made to quantify the absolute magnitudes of freezing rate because this design was made to detect possible source of complications.

Apparent rate constants determined at -2.0 C were 0.0028 ± 0.0002 and 0.0025 ± 0.0002 hr^{-1} for -8.0 C and -70 C freezing treatments, respectively. While those determined at -8.0 C were 0.0018 ± 0.0002 and 0.0016 ± 0.0001 hr^{-1}, respectively. There was a slight tendency for samples frozen at -70 C to have smaller rate constants.

However, the difference were within one standard deviation of the determined values. Therefore, it is concluded that the influence of freezing process on apparent reaction rate in this study is negligible.

5.2.2. Rate Equation for Reactions in Unfrozen Solution of Frozen State

The autoxidation of oxymyoglobin in frozen state maintains first order kinetics with respect to oxymyoglobin concentration. The same rate equation was followed for reactions taking place both in liquid state before freezing and in unfrozen solution of the frozen state. The first order plots of the reaction in 0.01 M buffered system at three sub-freezing temperatures in frozen state have been shown in Figure 5.3. The apparent rate constants determined in four buffer systems have been listed in Table 5.2.

5.2.3 Rate Constants in Supercooled Solution

Supercooled solution is a solution at temperatures below its initial freezing point but without ice crystal formation. Ice formation is the cause of the freeze-concentration effect. Without ice formation, the rate-temperature relationship is expected to follow the Arrhenius equation. Accurate prediction of reaction rates in supercooled solution assures the effectiveness of the prediction approach.

Supercooled solutions of several degrees below their initial freezing points were obtained by placing the sample tubes directly into the cold baths of desired temperatures. Possible ice formation was checked by visual examination. At supercooled temperatures, any trace amount of ice crystal formation would initiate freezing of the entire system.

Five supercooled solutions were observed, including -1.0 and -3.0 C for 0.01 M buffer; -3.0 and -5 C for 0.5 M buffer and -5.0 C for 1.0 M buffer. Plots of ln k versus 1/T are shown in Figure 5.8, 5.9 and 5.10. For each set of data, two straight lines are determined by least squares regression. One is based purely on data points obtained at above-freezing temperatures. The other includes data points from reactions in supercooled solutions. As observed from these results, the two lines do not deviate significantly from each other. It is concluded that data points from the supercooled solutions do not differ from the Arrhenius plots initiated from the above-freezing temperatures. Slopes of these straight lines are listed in Table 5.7. Small difference among these values further supports the observation.

In principle, the Arrhenius equation is able to describe the relationship between reaction rate and temperature, as long as system or reaction mode changes accompanied with temperature variations can be separated

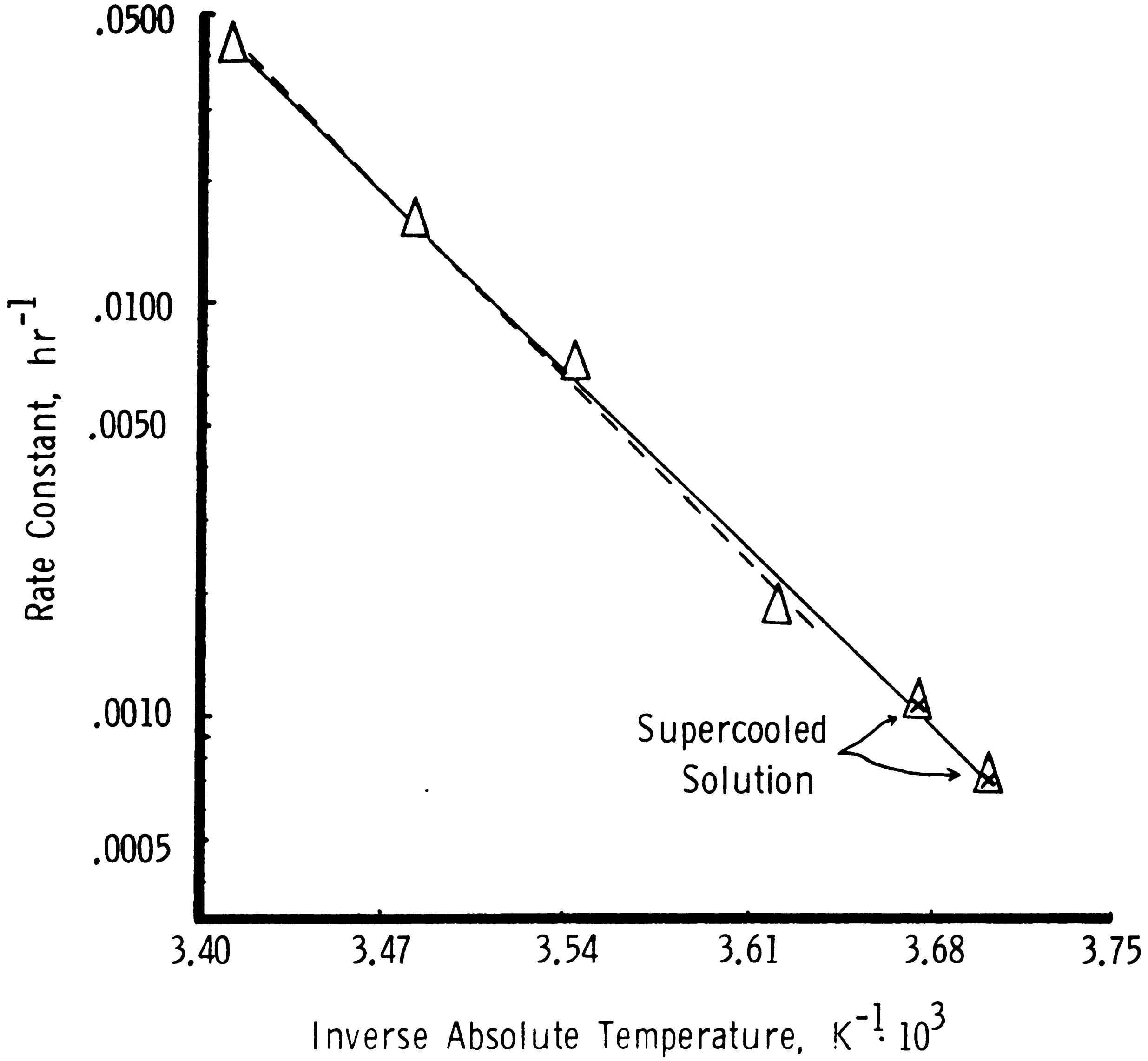


Figure 5.8 Arrhenius Plots for Autoxidation of Oxymyoglobin in .01 M Acetate Buffer

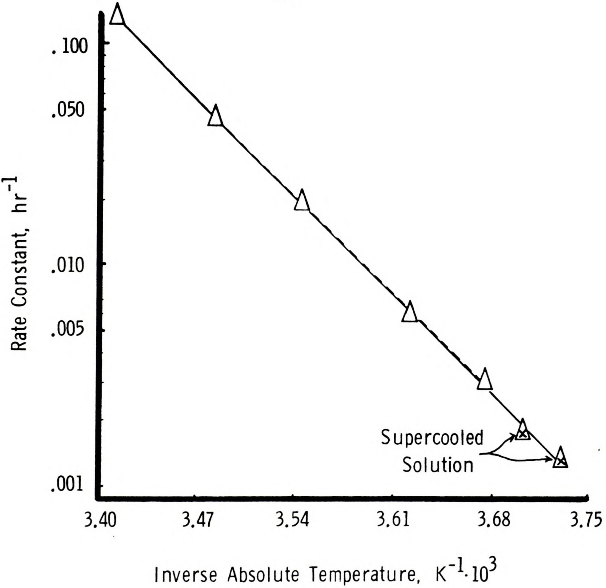


Figure 5.9 Arrhenius Plots for Autoxidation of Oxymyoglobin in .50 M Acetate Buffer

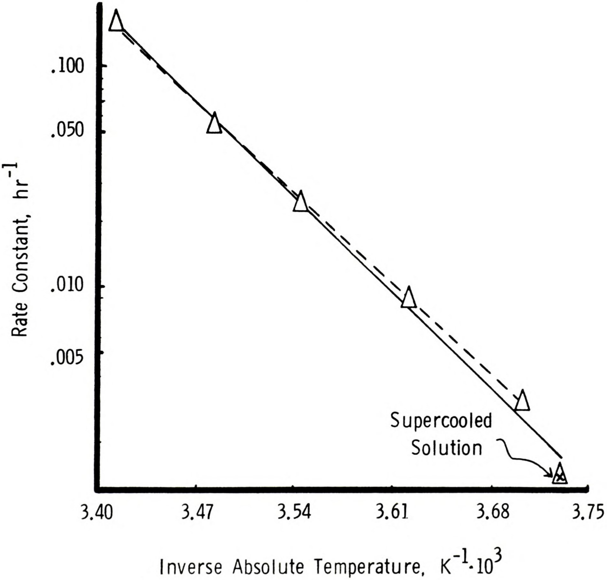


Figure 5.10 Arrhenius Plots for Autoxidation of Oxymyoglobin in 1.00 M Acetate Buffer

Table 5.7 Slopes of Arrhenius Plots for Autoxidation of Oxymyoglobin in Buffer Solutions with or without Data Points from Supercooled Solutions

	Buffer Concentration, M			
	0.01	0.05	0.5	1.00
Slope Determined with Points from Supercooled Solution	14100 ± 400	-----	14400 ± 100	14000 ± 500
Slope Determined without Points from Supercooled Solution	14700 ± 700	14100 ± 500	14300 ± 100	13300 ± 200

from the 'true' temperature effect. In other words, extrapolation of the Arrhenius plot from above freezing temperature range to sub-freezing temperature applications is justifiable if other influences can be taken into account separately.

5.2.4 Reaction Rates in De-ionized Water

Autoxidation of oxymyoglobin in acetate buffer was confirmed as first order reaction with respect to oxymyoglobin concentration in section 5.1.1, and was shown to have a 0.316 order dependency on acetate buffer concentration when its variatin was significant (section 5.1.3.).

Storage tests performed in de-ionized water have shown first order kinetics with respect to oxymyoglobin concentration, as could be seen from the natural log of % oxymyoglobin remaining versus storage time plots in Figure 5.4. The calculated reaction rate constants are tabulated in Table 5.8.

Reaction in de-ionized water eliminates the buffer salts from the system and is close to a 'true' first (total) order reaction. In a reaction system of first order kinetics, the concentration phenomenon imposes no influence on reaction rate according to the theoretical considerations described in chapter III. In this type of system, Arrhenius plots should not show a discontinuity

Table 5.8 First Order Rate Constants for Autoxidation of Oxymyoglobin in De-ionized Water

Temperature C	30.0	24.6	16.4	4.0	-2.0*	-10.0*
Rate Constant $k \times 10^{-4}$ hr^{-1}	358	148	27.4	3.19	1.15	0.313

* Frozen State

upon freezing.

Storage tests were performed at four temperatures above freezing; 30.0, 24.6, 16.4 and 4.0 C, and at two sub-freezing temperatures; -2.0 and -10.0 C (frozen state). When plotting natural log of rate constant versus inverse of absolute temperature (Figure 5.11), the two data points from frozen state did not deviate significantly from the extrapolated straight line relationship based on data from liquid state reaction. The slope was 15300 + 600 without including these two points into linear fitting. When the two points were included in the analysis, a slope value of 14200 + 500 is closer to those values obtained for reactions in liquid state (Table 5.7). Theoretically, the slope will not deviate much if reaction follows the same mode. Therefore, the two data points obtained from frozen state reaction can be considered as in agreement with the extrapolated Arrhenius plot. No rate acceleration was observed although the concentration phenomenon should be extensive in dilute solution. This observation supports the rate prediction model; the rate of a first order reaction is not influenced by the freeze-concentration phenomenon.

No detectable pH change was observed in the thawed solution throughout the storage period. Based on the results, the dissolved oxygen concentration did not vary to such an extent that would cause the rate constants to

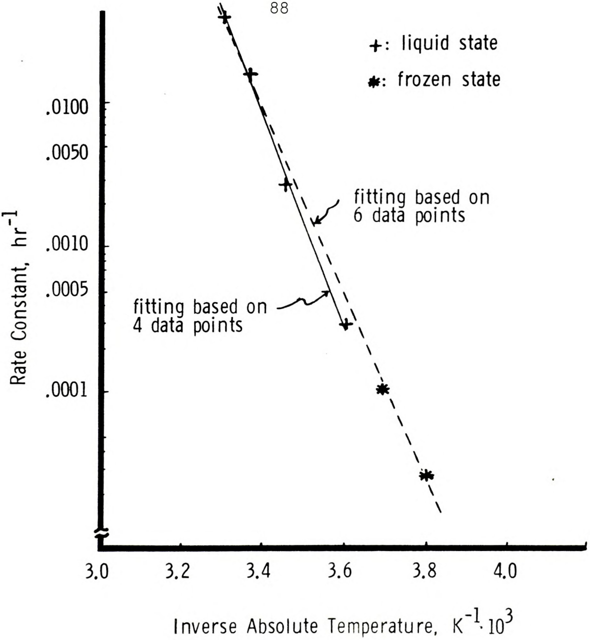


Figure 5.11 Arrhenius Plots for Autoxidation of Oxymyoglobin in De-Ionized Water

deviate from Arrhenius plot when the transformation of water to ice occured, and ice crystal did not influence the oxymyoglobin autoxidation rate to a noticeable extent, since ice crystals were formed in de-ionized water system but did not cause rate constants to deviate from the extrapolated Arrhenius plot.

5.2.5 Influence of the Specific Volume of Solutes on the Predicted Concentration Factors

Suspended particles or insoluble solids are common constituents of food products. These components make the determination of accurate aqueous solution volume difficult to achieve. An approximation was made at the begining of this study to bypass this difficulty. That was done by assuming the aqueous volume to be equal to the volume of water presented in the system, or equivalently, the solutes occupied no volume in the solution. This assumption, however, introduces errors that in some cases make the prediction approach unacceptable. The errors occurred in the prediction of concentration factor and propagated to the predicted apparent rate constants.

The assumption imposed the specific volume of solutes (Vs) in Equation (3-11) to be equal to zero, while in practical situations, Vs usually has a value greater than zero. Based on Equation (3-11) and from the following inequality,

$$\frac{mw / dw + Vs}{mu / dw + Vs} \leq \frac{mw / dw}{mu / dw} \qquad (5-2)$$

where $mw > mu$

it is clear that the assumption gives a predicted concentration factor higher than practically encountered values.

As an example, for the oxymyoglobin system in 0.01 M acetate buffer, with the solution properties listed in Table 5.6 as inputs, the predicted concentration factors at various temperatures are compared with those values predicted by replacing the input specific volume of solutes from 0.0014 to 0 ml/g. The results (Table 5.9) show that concentration factors for the group with Vs equals 0 ml/g are greater than those for the group with Vs equals 0.0014 ml/g, and the difference becomes greater at lower storage temperatures.

The errors will be magnified by greater deviation in Vs values and also by higher reaction order when apparent rate constants are predicted. Therefore, a proper estimation of solutes volume in solution is necessary for better prediction of concentration factor and apparent rate constants.

5.2.6 Rate Acceleration of Oxymyoglobin Autoxidation in Acetate Buffer by Freeze-Concentration Phenomenon

It was shown that the Arrhenius equation could be

Table 5.9 The Concentration Factors Determined with or without the Assumption that Solutes Occupy No Volume in Solution

Temperature C	Concentration Factor		
	Solute Volume Vs = 0.0014 ml/g	Solute Volume Vs = 0.0 ml/g	*Deviation %
-1.0	26.2	27.2	3.8
-5.0	116.3	138.8	19.3
-10.0	204.0	285.2	39.8
-15.0	272.4	439.7	61.4
-20.0	327.1	602.8	84.3

$$^{*}\text{Deviation \%} = \frac{\text{Value with Vs} - \text{Value without Vs}}{\text{Value with Vs}}$$

extrapolated to predict the rate constants of oxymyoglobin autoxidation in supercooled solution. Those rate constants are referred to as 'supercooled' rate constants and are free from the complications by ice formation. The rate constants for reactions taking place in frozen state are termed 'apparent' rate constants in this study.

The influence of the freeze-concentration effect on reaction rate can be observed by comparing the difference between apparent and supercooled rate constants at the same temperature. This comparison was conducted by taking the ratio of apparent rate constant to its corresponding (temperature) supercooled rate constant which was determined by direct extrapolation of the Arrhenius plot. As can be observed from Table 5.10, the ratio tends to increase with decreasing storage temperature and to decrease with increasing buffer strength. This indicates that concentration effect is more prominent in dilute systems and is more extensive as temperature decreases.

5.2.7 Comparison between Predicted and Measured Apparent Reaction Rate Constants

Fourteen experimentally determined apparent reaction rate constants for oxymyoglobin autoxidation in four buffer systems are compared with the computer predicted results. Input parameters including solutes volume in solution, effective molecular weight, mass

Table 5.10 The Concentration Effect as Shown by the Ratio of Apparent Rate Conatant to Supercooled Rate Constant

Temperature	Ratio *			
	Buffer Strength, M			
C	0.01	0.05	0.50	1.00
-2	3.33	1.83	-	-
-3	3.48	2.40	1.15	-
-8	6.92	3.93	1.75	1.41
-12	8.27	7.01	2.52	2.12
-15	-	6.59	-	-

$$* \text{ Ratio} = \frac{\text{Measured apparent rate constant}}{\text{Extrapolated supercooled rate constant}}$$

fraction of water, activation energy, pre-exponentials of the Arrhenius equation and total reaction order are summarized in Table 5.11.

Basically, the prediction approach estimates the supercooled rate constants and concentration factors separately, and the apparent rate constants were calculated by multiplying the supercooled rates by concentration factors to the power of total order less one. The temperature dependency of the supercooled reaction rate was discussed in section 5.2.3, and that of the concentration factors at four strengths of buffer system is shown in Figure 5.12. The predictions were done through the rate prediction model and the concentration factor prediction model as described in Chapter III.

The predicted apparent rate constants versus temperature relationship for reaction in 0.01 M acetate buffer system is shown in Figure 5.13. Four laboratory determined aparent rate constants are also plotted. The deviations based on measured value range from 2.14 to 34.07 % with absolute differences ranging from .06 to .31 x $10^{-3}hr^{-1}$. The calculated percent deviation is based on the difference between predicted and measured values divided by the predicted value. The prediction is good when considering that most of the deviation is within one standard deviation of the experimental determined value. In most cases, only two significant digits can be

Table 5.11 Summary of Input Parameters to the Prediction Approach for Prediction of Apparent Rate Constants

	Buffer Concentration, M			
	0.01	0.05	0.5	1.00
Effect Molecular Weight, g/mole	90.48	51.22	44.31	43.17
Mass Fraction of Water (mw)	0.9982	0.9950	0.9600	0.9220
Specific Volume of Solutes(Vs), ml/g	0.0014	0.003	0.021	0.041
Activation Energy, KJ/mole	118	118	118	118
Pre-exponentials (A) x 10^{19}	4.267	5.947	12.23	15.33
Total Reaction Order (nt)	1.316	1.316	1.316	1.316

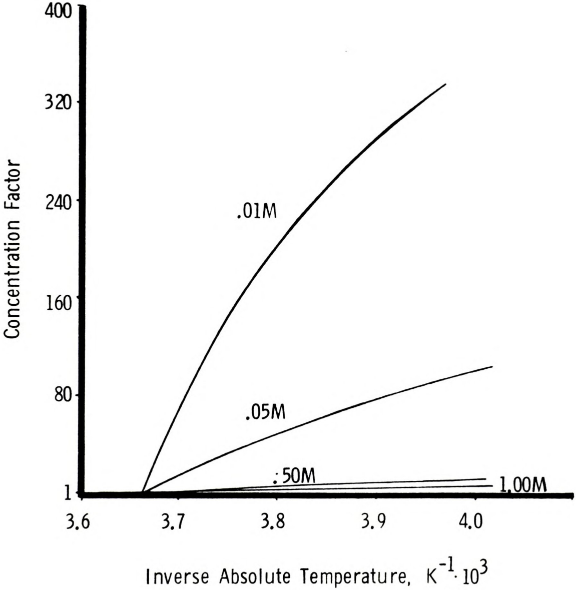


Figure 5.12 Concentration Factor as a Function of Temperature

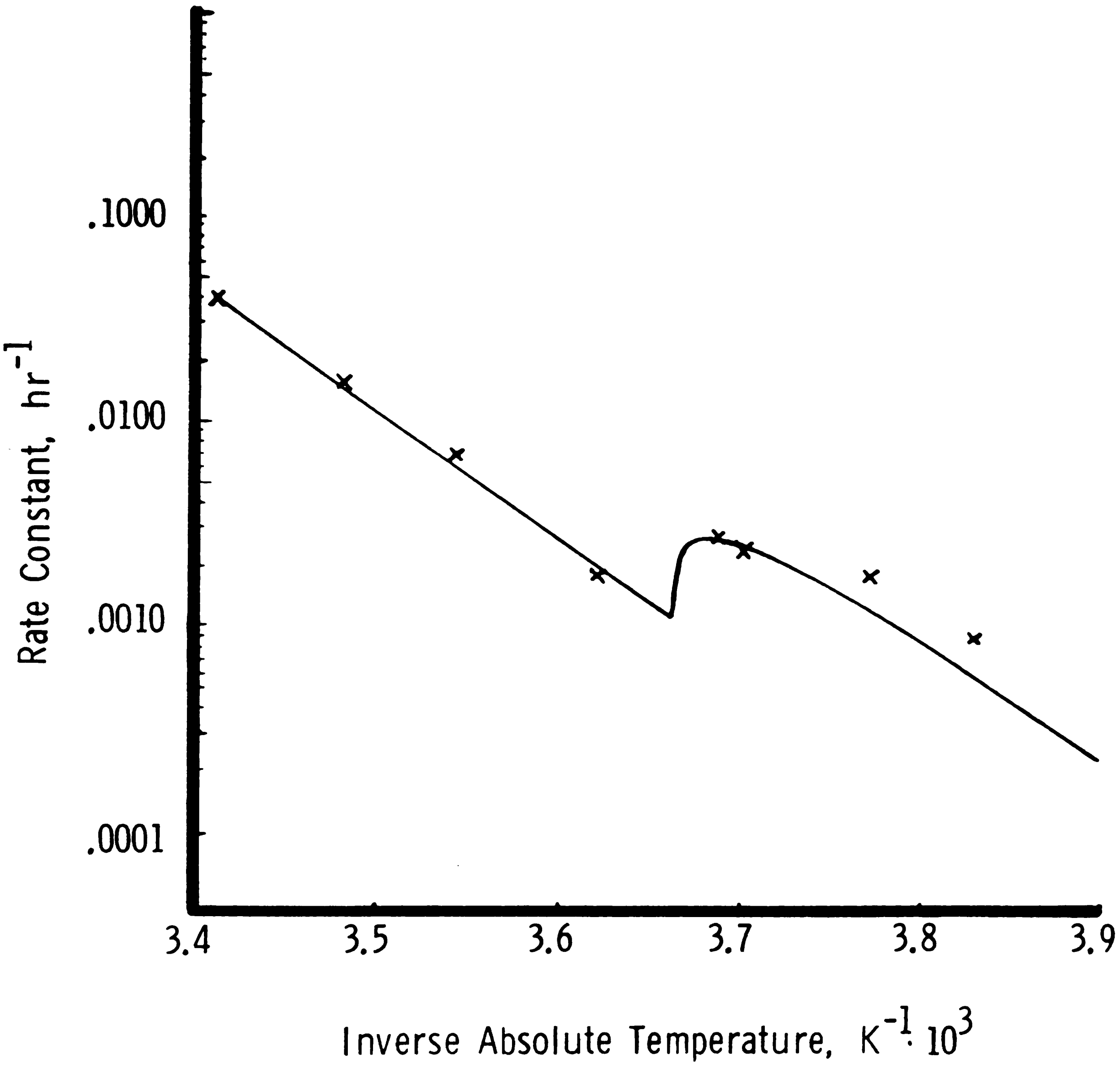


Figure 5.13 Influence of the Freeze-Concentration Effect on the Oxymyoglobin Autoxidation in .01 M Acetate Buffer

considered for the experimental results, and the predicted values differ in second digit, except the one at temperature of -12.0 C.

Figure 5.14 shows the predicted rate-temperature curve for reaction in 0.05 M buffer, 5 data points are compared. The deviations based on measured value range from 1.30 to 48.18 % with absolute differences ranging from .02 to .11 x $10^{-3}hr^{-1}$. Maximum deviation occurs in the trial of storage temperature at -12.0 C. This and the one at -15.0 C are the two values that have the difference in double digits.

The predicted rate constant-temperature relationship for reaction in 0.5 M buffer is drawn in Figure 5.15. Three data points are compared. Maximum deviation is 28.40 % for the case at 12C.

For the reaction in 1.0 M acetate buffer, the predicted rate constant-temperature relationship is shown in Figure 5.16. Two measured apparent rate constants are compared with the predictions. The freeze-concentration effect is small for high concentration systems, especially for the acetate buffered oxymyoglobin system, only 0.316 power of the concentration factor can be shown in the rate acceleration. The maximum deviation observed was 36.05 % at -12.0 C.

The values of measured and predicted apparent rate constants are tabulated in Table 5.12a, b, along with the

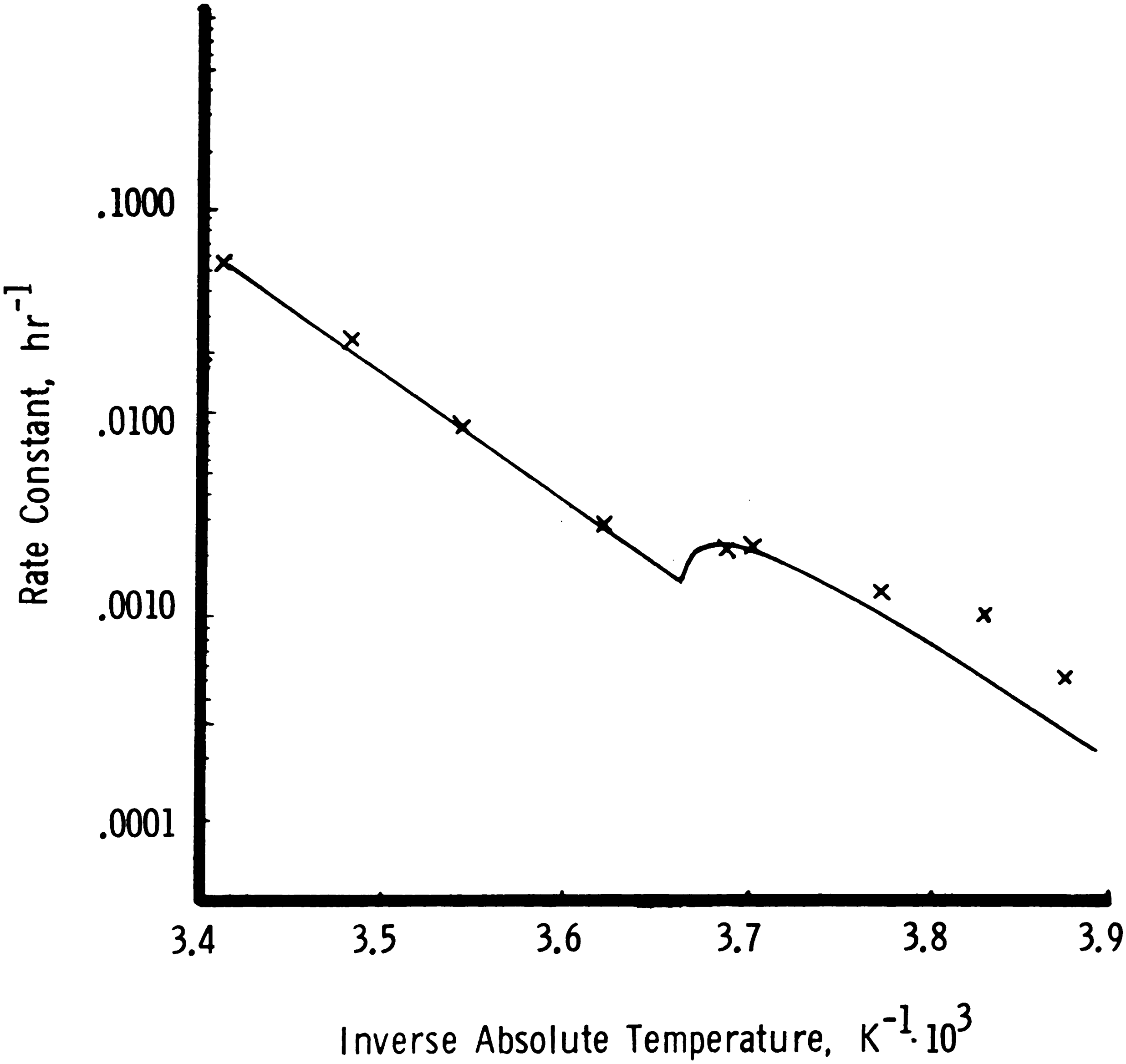


Figure 5.14 Influence of the Freeze-Concentration Effect on the Oxymyoglobin Autoxidation in .05 M Acetate Buffer

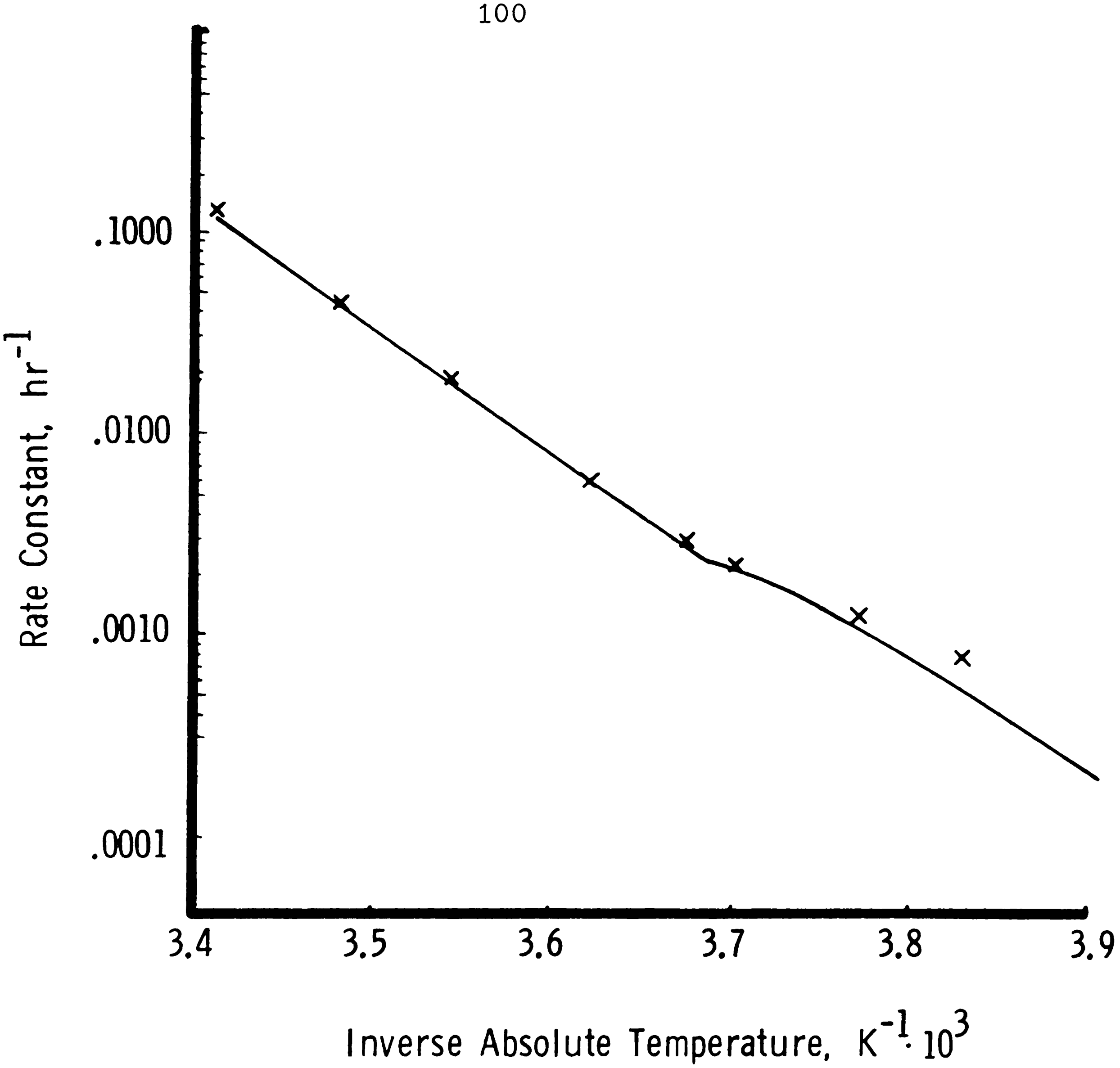


Figure 5.15 Influence of the Freeze-Concentration Effect on Oxymyoglobin Autoxidation in .50 M Acetate Buffer

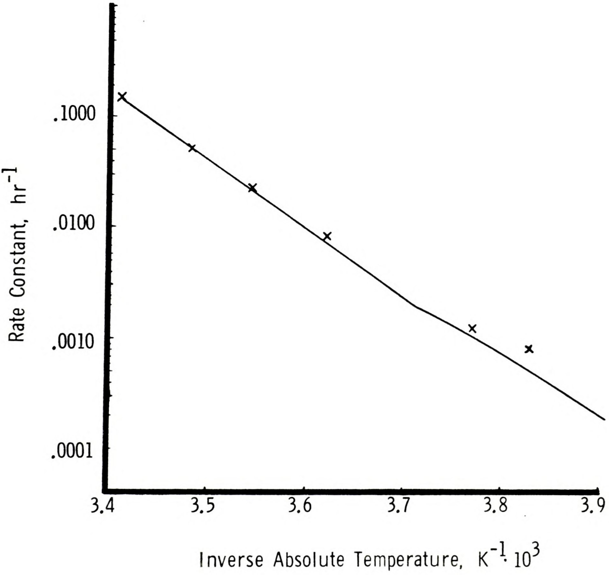


Figure 5.16 Influence of the Freeze-Concentration Effect on the Oxymyoglobin Autoxidation in 1.00 M Acetate Buffer

Table 5.12a Comparison between Predicted and Measured Apparent Rate Constants for Oxymyoglobin Autoxidation in Frozen State

Temperature	Buffer Strength, M					
C	0.01			0.05		
	Rate Constants, $k \times 10^{-3}$ hr^{-1}			Rate Constants, $k \times 10^{-3}$ hr^{-1}		
	Measured	Predicted	% Deviation	Measured	Predicted	% Deviation
-2.0	2.8	2.74	2.14	2.2	2.34	6.36
-3.0	2.4	2.54	5.83	2.3	2.27	1.30
-8.0	1.8	1.23	31.67	1.4	1.09	22.14
-12.0	0.91	0.60	34.07	1.1	0.54	48.18
-15.0	---	---	---	0.55	0.30	41.82

$$\% \text{ Deviation} = \frac{\text{Predicted Value} - \text{Measured Value}}{\text{Measured Value}} \times 100 \%$$

Table 5.12b Comparison between Predicted and Measured Apparent Rate Constants for Oxymyoglobin Autoxidation in Frozen State

Temperature	Buffer Strength, M					
C	0.50			1.00		
	Rate Constants, $k \times 10^{-3}$ hr^{-1}			Rate Constants, $k \times 10^{-3}$ hr^{-1}		
	Measured	Predicted	% Deviation	Measured	Predicted	% Deviation
-3.0	2.3	2.22	3.48	---	---	---
-8.0	1.28	1.11	13.28	1.3	1.11	14.62
-12.0	0.81	0.55	28.40	0.86	0.55	36.05

$$\% \text{ Deviation} = \frac{\text{Predicted Value} - \text{Measured Value}}{\text{Measured Value}} \times 100\ \%$$

percent deviations. The predictions show greater deviations at lower temperatures.

The deviations between the predicted and the experimentally determined apparent rate constants are due to one or more of the following reasons: (1) the validity of the extrapolation of the influence of buffer concentration, which was based on the experimental data from 0.01 to 1.0 M of buffer concentrations. At -12.0 C, for instance, the concentration of buffer is about 3.0 M, the extrapolation may result in some deviations. (2) the inherent errors in experimental measurements, including both the input parameters and the apparent rate constants, (3) the deviations in least square regression analysis, including the determination of rate constants, activation energy and the dependency on buffer concentration, (4) as concentration phenomenon proceeds, highly concentrated solution may deviate from ideal solution behavior as assumed in the unfrozen water prediction model and (5) factors other than freeze-concentration effect may come into play.

The four myoglobin systems discussed were different in the amount of buffer salts only. The amount of myoglobin in each system is of about the same value of 1.00 mg/ml. Considering the molecular weight of myoglobin, about 16700 g/mole, the molar concentration it contributes to the system is negligible, even for the most dilute

system of 0.01 M. Therefore, the higher buffered system can be considered as the concentrated form of the less buffered system. Based on the freezing point depression equation, systems with higher initial molar concentration will retain more water in the unfrozen solution and maintain a final molar concentration the same as that of initially less concentrated system at fixed sub-freezing temperatures. Since the relative (molar) proportions of solutes in four buffer strengths are approximately the same, the unfrozen solutions will have similar compositions and concentrations at fixed sub-freezing temperature, and the apparent reaction rate constants should be close to equal.

Figure 5.17 compares the predicted rate-temperature curves in four buffer strengths. The differences in rate constants among these systems disappear as all systems are frozen. The higher apparent rate constants predicted for system with 0.01 M buffer concentration is due to that higher (than the linear extrapolation of the rate constant-buffer concentration relationship) pre-exponential constant obtained experimentally was used as input to the prediction approach.

5.3 Process Recommendations

One of the practical applications from the study of the rate acceleration phenomenon for reactions in frozen

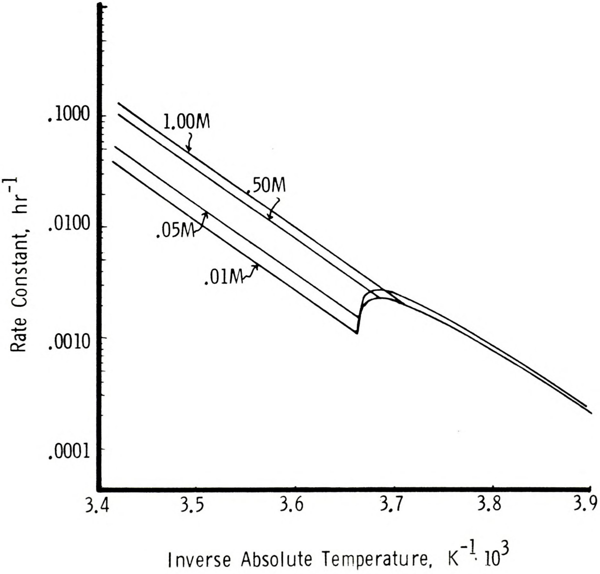


Figure 5.17 Influence of the Initial Buffer Concentration on the Rate Acceleration Caused by the Freeze-Concentration Effect

state is to avoid the 'adverse temperature range' for a degradation reaction. The 'adverse temperature range' is defined as the temperature range within which lower temperature storage can not achieve better preservation effect than some higher temperature storage. As shown in Figure 5.18, temperature range from t1 to t2 is an example of the 'adverse temperature range' defined above. A local maximun reaction rate usually can be observed in this range, as at temperature 'tm' indicated in the same illustration, which represents the least desirable temperature for storage.

Frozen storage at temperatures within this range results in wasting energy for maintaining lower storage temperature without an improvement in product shelf-life. By varying inputs to the prediction approach, some process recommendations with respect to quality preservation can be made for frozen storage.

Inert solutes have been added to food stuffs to produce final products with reduced or no ice crystal formation and with flow properies at freezer temperatures. This kind of process can save energy for the removal of latent heat and eliminate the time for thawing (Kahn and Eapen, 1979). The first part of this section will discuss the influence of this process on food quality from the concentration point of view.

Concentration before freezing or dehydro-freezing

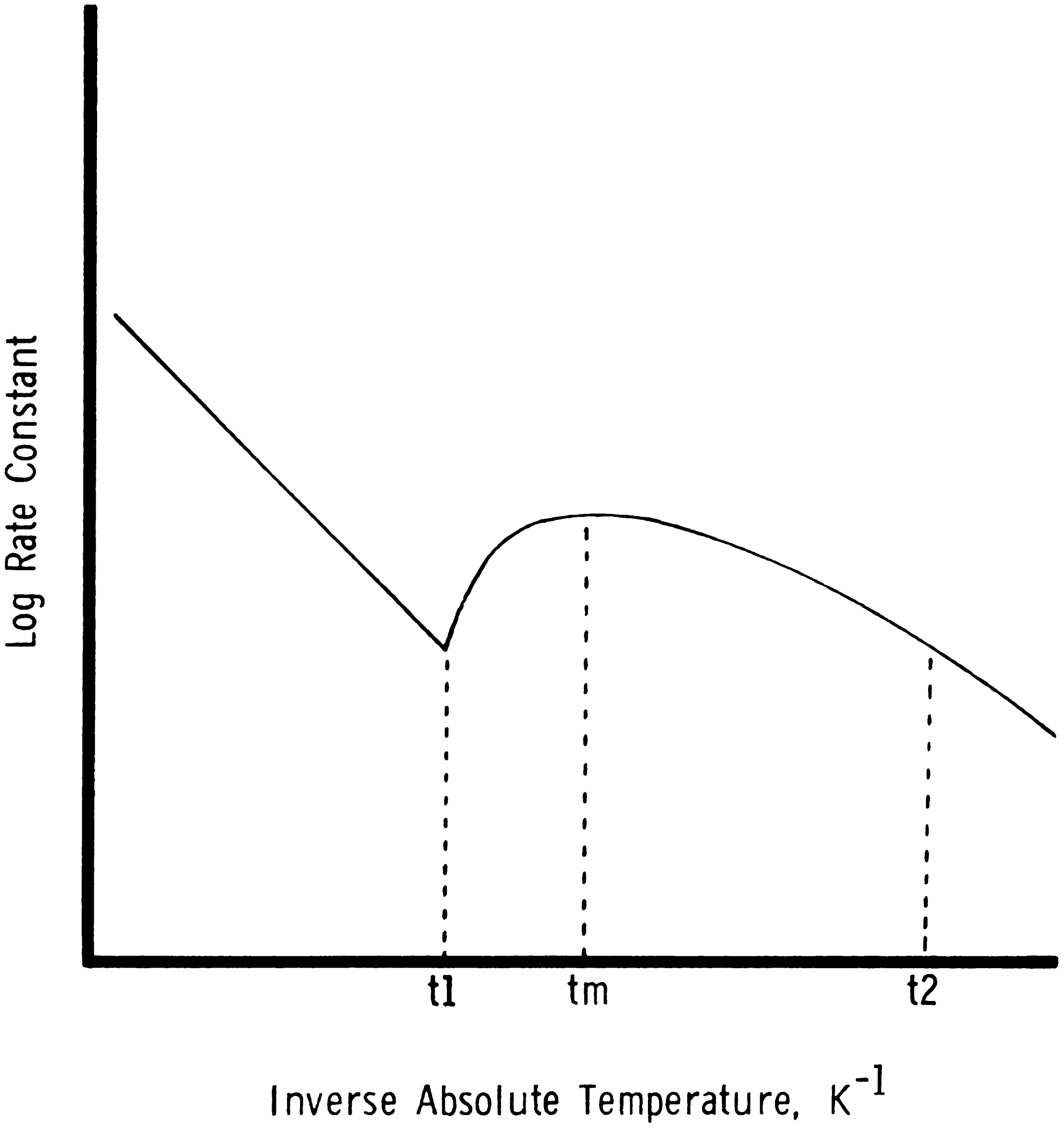


Figure 5.18 Definition of the 'Adverse Temperature Range' for Storage and the Temperature of Local Rate Maximum

is an process proposed to conserve energy and save the volume for transportation (Huxsoll, 1982). The influence of this process on quality degradation is discussed in the second part.

The third part of this section is a discussion of the influence of input parameters on the characteristics of the rate acceleration phenomenon, including the adverse temperature range and the local rate maximum.

5.3.1 Addition of Inert Solutes to Reaction System

Addition of inert solutes to a reaction system does not affect the reaction rate in liquid state before freezing. The addition will depress the initial freezing temperature of the system. As shown in Figure 5.12, the concentration factor decreases as the solute concentration increases. Consequently, apparent rate constants are lower for systems with more inert solutes added if all systems acquire their frozen states. And the addition of inert solutes tends to narrow down the adverse temperature range. These observations are shown in Figure 5.19.

Solutes of smaller molecular weight are more effective in depressing initial freezing temperature and therefore more effective in reducing the freeze-concentration effect. Addition of inert solutes to food systems before freezing in principle will eliminate or reduce possible freeze-concentration effect on quality

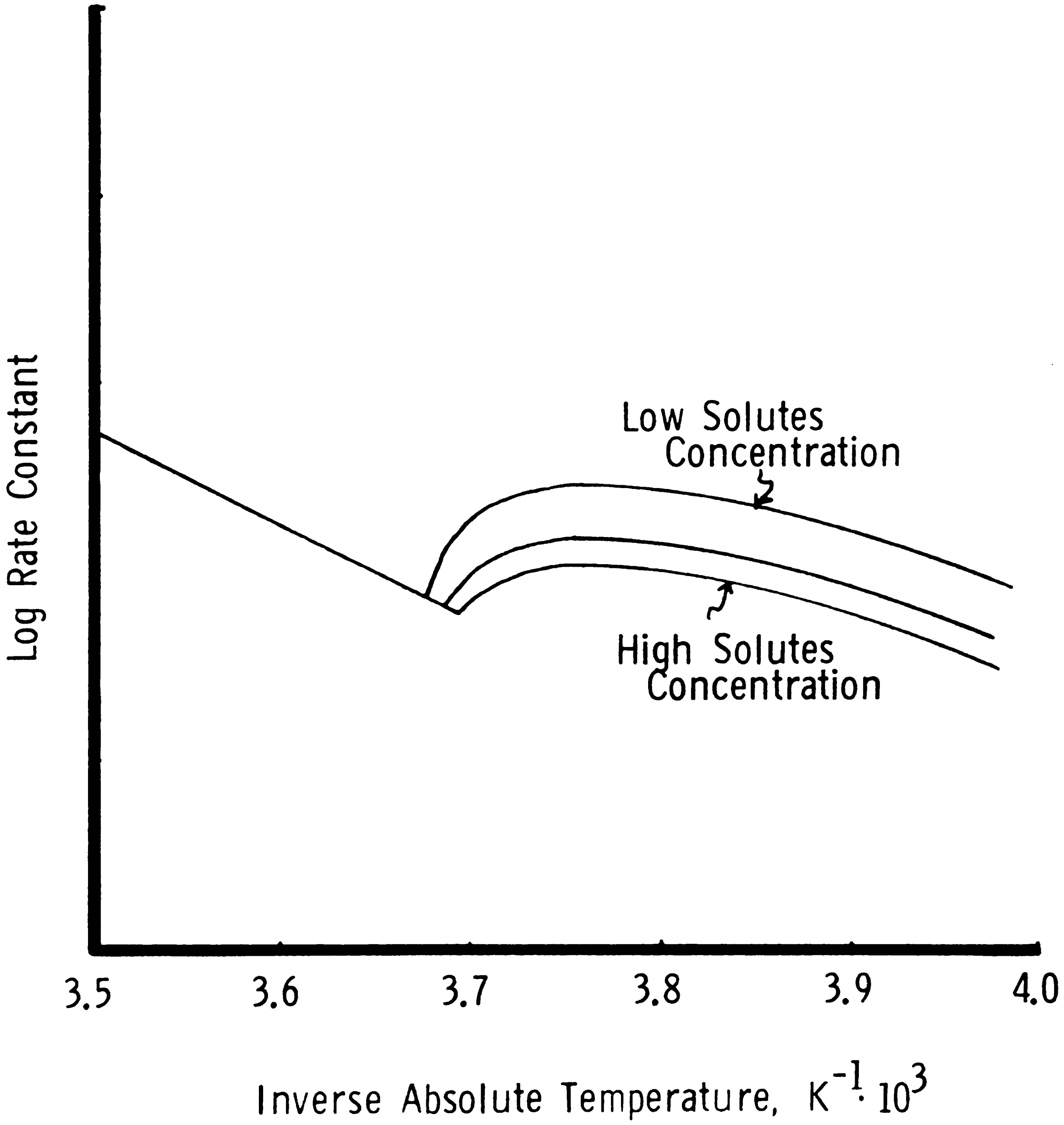


Figure 5.19 Influence of the Added Inert Solutes on Rate Acceleration Caused by the Freeze-Concentration Effect

degradation rate.

5.3.2 Concentration Before Freezing or Dehydro-freezing

Concentration processes increase the concentratiton of solutes and depress the initial freezing temperature of the reaction system. However, dehydro-freezing will increase the concentration of reactants and increase the reaction rate in liquid state before freezing. As long as all solutes remain in unfrozen solution, the apparent reaction rate in frozen state will be the same in concentrated system as in original system, based on the reasoning given in section 5.2.6. The rate-temperature relationship of a concentrated system as compared to its original system is shown in Figure 5.20.

Therefore, if storage is done at liquid state, concentration process will accelerate the reaction rate, while if the storage is done at frozen state, the process does not influence the reaction rate, as compared to the original system.

5.3.3 Influence of Input Parameters on the Characteristics of Rate Acceleration

Total reaction order is a key parameter that influences the extent of concentration effect on reaction rate. Pincock and Kiovsky (1966c) derived the rate equation for second order reaction in frozen state. According to their work, the apparent rate constant is

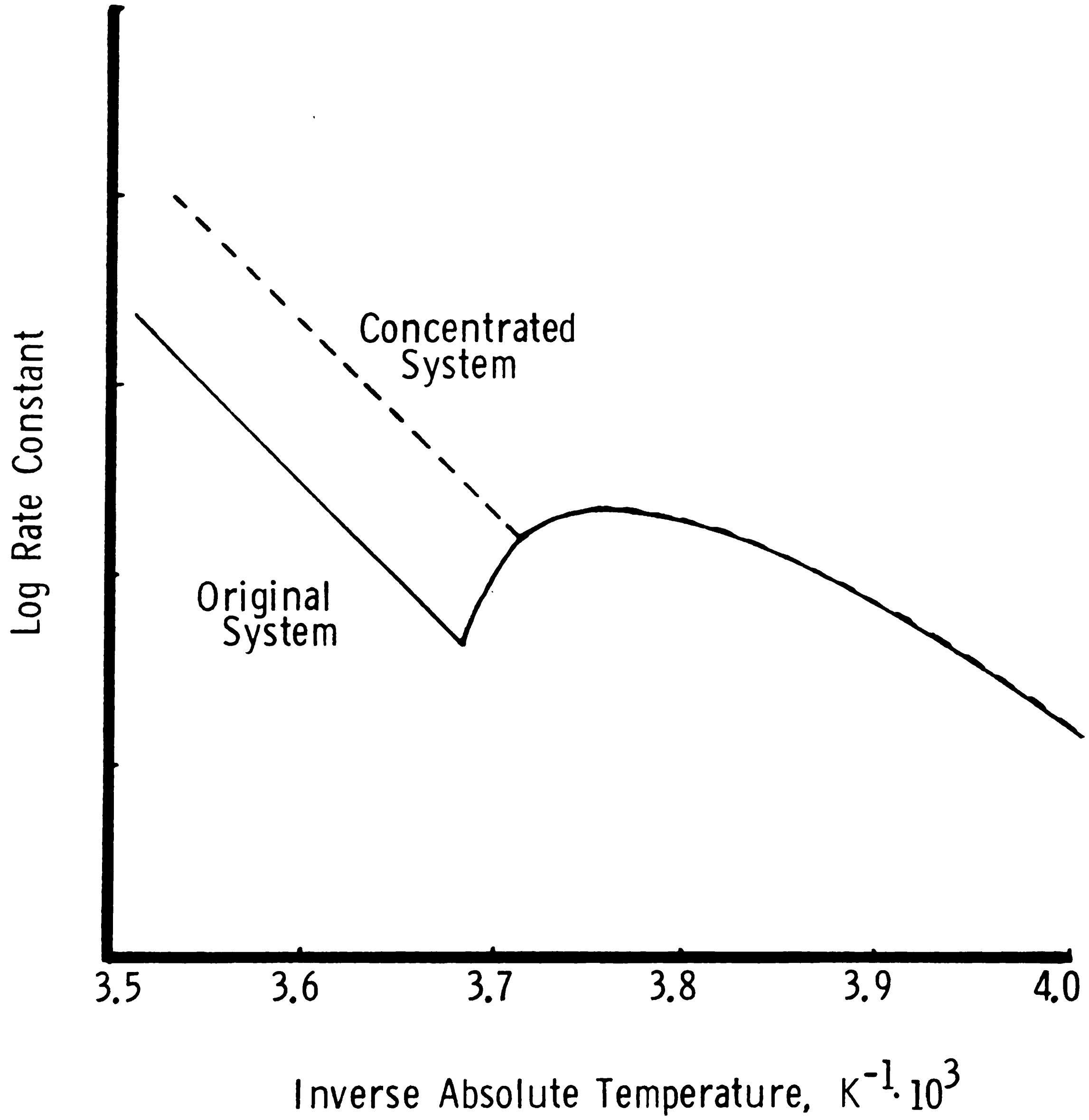


Figure 5.20 Influence of Dehydro-Freezing on the Rate Acceleration caused by the Freeze-Concentration Effect

equal to the supercooled rate constant multiplies the concentratiton factor. This observation is in agreement with the theoretical considerations of this study that the rate acceleration is a multiplier which equals the concentration factor to the power of total reaction order less one. A controversial conclusion, however, was obtained by Fan and Tannenbaum (1973), who stated that a third order reaction would have a rate acceleration equal to the concentration factor to the power of three, instead of two as suggested in this study.

Total reaction order influences the extent of concentration effect in a power format as indicated by Equation (3-8). The influence is only on magnitude of the rate and the range of the adverse temperature for storage, but not on the position of the local reaction rate maximum. In Figure 5.21, the concentration effects on second order and third order reaction are shown for comparison. An increase in reaction order widens the adverse temperature range, and also increase the magnitude of reaction rates.

Activation energy is the parameter that influences the temperature at which a local rate maximum occur. A decrease in activation energy also widens adverse temperature range. Figure 5.22 represents the rate-temperature relationship of a reaction system with the same mass fraction of water (.6) and initial freezing temperature (-2.22 C) as fresh pork (Mohsenin, 1980). Here

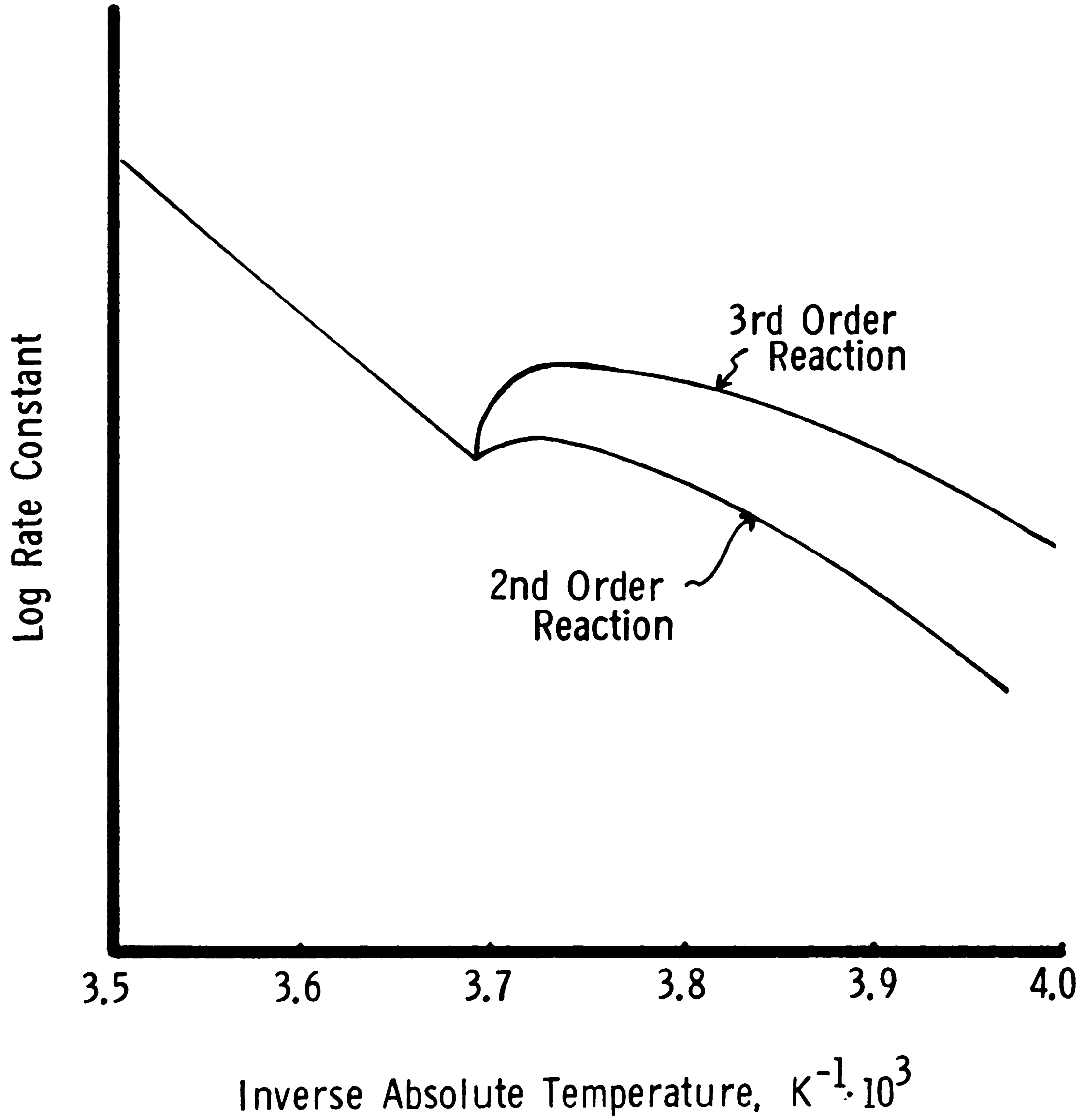


Figure 5.21 Influence of the Reaction Order on the Rate Acceleration Caused by the Freeze-Concentration Effect

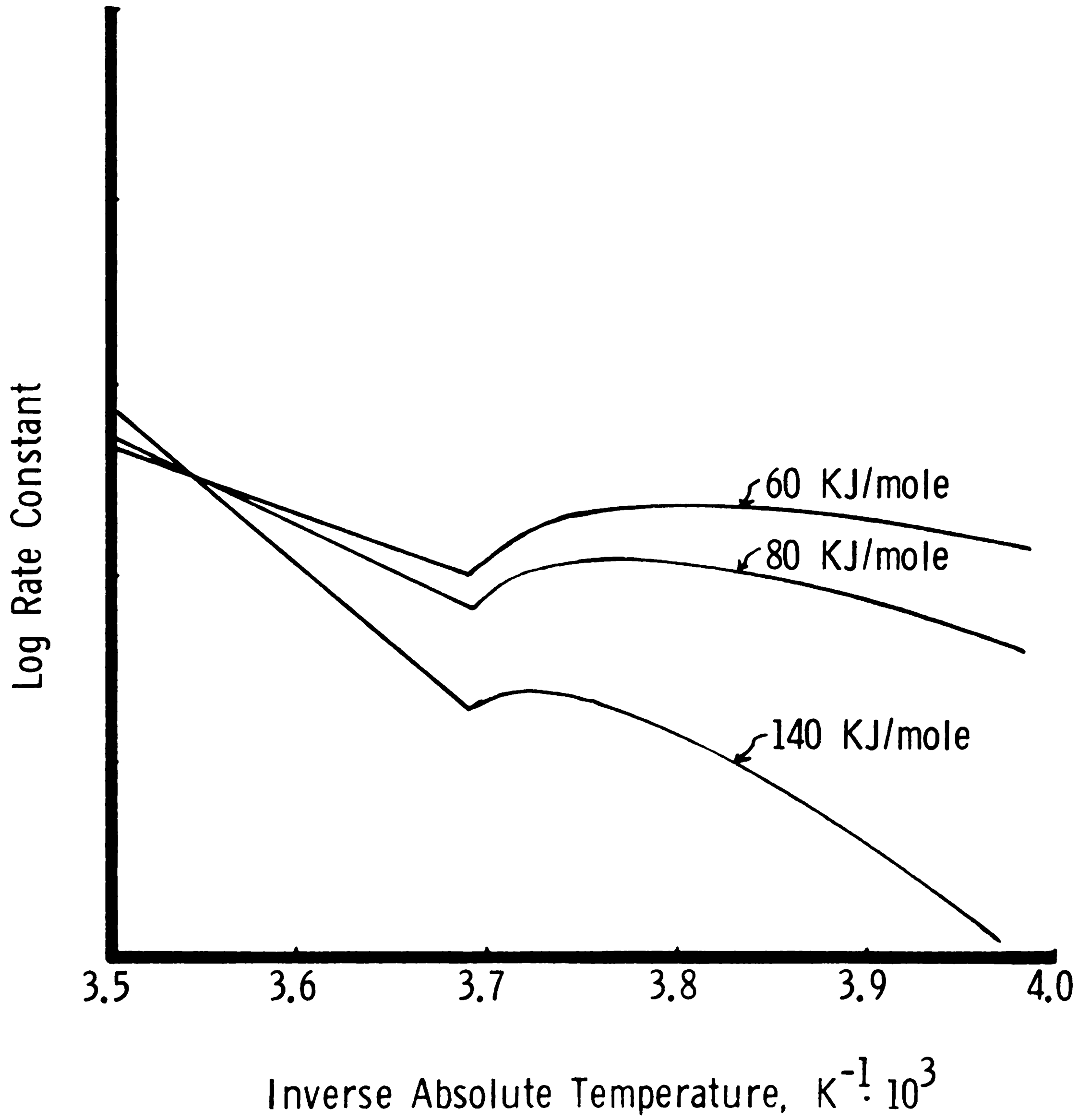


Figure 5.22 Influence of the Activation Energy on the Rate Acceleration Caused by the Freeze-Concentration Effect

the specific volume of solutes is assumed equal to zero. Three activation energy values are assumed, i.e. 60, 80 and 140 kJ/mole. The rate maxima shifted from -4, -7 to -11 C for increasing activation energy value in the above order. One important point to note is that lower activation energy widens the adverse temperature range for storage. In this example, storage at -21 C does not have better quality preservation effect than that stored at 0 C. Frozen storage in this case is hardly beneficial.

Reference rate constants do not affect the characteristic of the rate acceleration. The absolute magnitudes of the rate constants are increased when reference rate constant is increased. As shown in Figure 5.23, two parallel curves are obtained when varying reference rate constant.

An increase in solutes (molar) concentration or decrease in initial freezing temperature will narrow the adverse temperature range, as illustrated by the example given in Figure 5.18.

5.4. Possible Freeze-Concentration Effect Existing in Food Systems

The occurrence of rate acceleration caused by the freeze-concentration effect has been demonstrated in a number of simple reaction systems, mostly of non-enzymatic chemical reactions. In food systems, the occurrence has

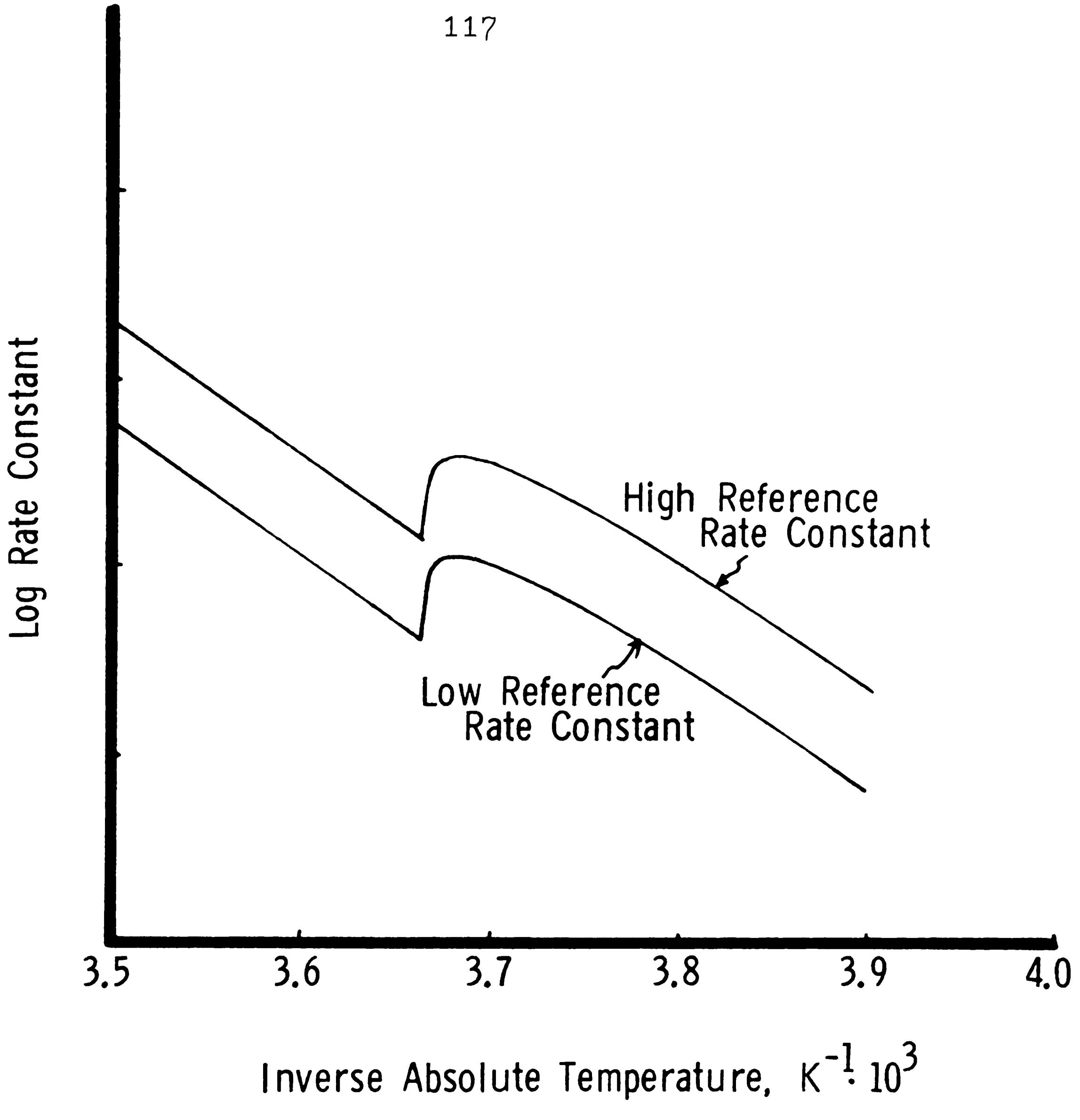


Figure 5.23 Influence of the Reference Rate Constant on the Rate Acceleration Caused by the Freeze-Concentration Effect

been reported on a limited basis.

One of the major reasons for limited reports is due to the reasonably high initial concentrations of solutes resulting in small change in reaction rate during freezing of food and with the influence extending over a rather narrow temperature range. In any event, rates of deterioration at normal frozen storage temperature (-18 C) of food products should be, and usually are, much less than at 0 C (Fennema, 1975b).

In addition to the initial concentration of solutes, other factors such as the reaction order and activation energy must be taken into account, as described in section 5.3. The following example demonstrates the possibility of rate acceleration by the freeze-concentration effect in systems with the same solutes concentrations as several food products.

Figure 5.24 presents the rate acceleration predictions for spinach, fresh beef and fresh pork. The mass fraction of water and initial freezing temperature input to the prediction approach are .923 and -.94 C for spinach, .68 and -1.67 C for beef and .60 and -2.22 C for pork, respectively. For the purposes of this demonstration, a second order reaction and an activation energy value of 83.6 KJ were assumed for all cases. Magnitudes of rate constants are not accounted for since the interest is the relative comparison.

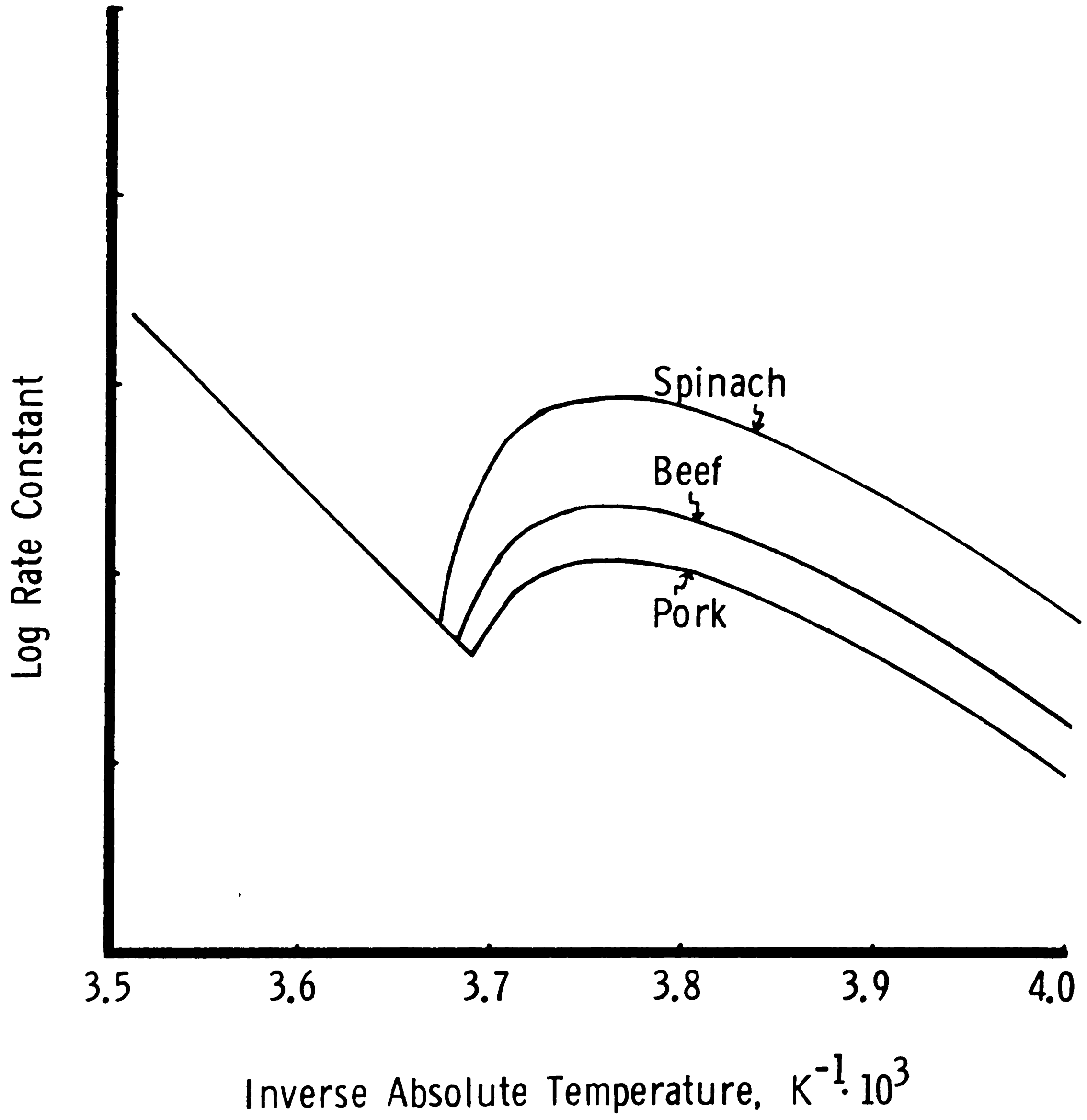


Figure 5.24 The Rate Acceleration of an Assumed 2nd Order Reaction in Food Systems

All three systems have a local rate maximum at -7 C, due to that the same activation energy value is used as input. Activation energy is the only parameter that affects the temperature where local rate maximum occurs. The 'adverse temperature ranges' for storage are from the initial temperature of each product to about -17 C, -11 C and -9 C, for spinach, beef, and pork, respectively. The wider rage of 'adverse temperatures' and the more extensive of the rate acceleration for spinach is the consequence of the higher initial freezing temperature (or lower initial solutes concentration) it has. The example indicates that under the same initial concentration of solutes as three typical food products, the rate acceleration caused by the freeze-concentration effect is still possible for some degradation reactions.

Taking all factors that influence the characteristics of the rate acceleration into account, and considering that most foods have an initial freezing temperature arround -0.5 to -2.8 C (Fennema, 1973), we cannot be sure that all frozen foods are stored outside the adverse temperature range.

Several changes in muscle or muscle juice were found to have local reaction rate maxima at temperatures near initial freezing temperatures during frozen storage, as summarized by Love and Elerian (1964) and listed in Table 5.13. Two typical rate-temperature relationships of

Table 5.13 Temperatures of Local Maximum Changes in Muscle or Muscle Juice (Love and Elerian, 1964)

Sample	Temperatures of Local Maxmum change, C	Change Followed
Frog muscle	-2 to -3	Lactic acid production
Frog muscle	-2.2 to -2.5	Lactic acid production
Cattle muscle	-1.5	Drip exudation after thawing
Cattle muscle juice	-2 to -3	Flocculation of 'myogen'
Haddock muscle	near -3	Drip exudation after thawing
Haddock muscle	-2 to -4	Disappearance of gloss on surface of smoke-cured fillet
Halibut muscle juice	-2	Flocculation of protein
Cattle and fish muscle and probably other tissues	-2 to -3	Drip exudation, formation of lactic acid, protein denaturation, breakdown of 'phosphagen' and growth of ice crystals
Fish muscle	-2 to -5	Toughness (organoleptic) and drip exudation
Carp and trout muscle	near -1.5	Breakdown of adenosine triphosphate
Cod muscle	-4	Phospholipid hydrolysis

these reactions are shown in Figures 5.25 and 5.26, for phospholipid hydrolysis in cod muscle (Lovern and Olley, 1962) and the protein denaturation of frozen cod (Love and Elerian, 1964), respectively. These relationships show great similarity with the rate acceleration phenomenon caused by freeze-concentration effect. As observed by Lovern and Olley (1962), the rate of phospholipid hydrolysis had a local rate maximum at -4 C and the rate at -7 C is faster than that at 0 C. It is believed that these rate acceleration are caused mainly by the freeze-concentration effect on soluble salts in system (Love, 1968).

The ascorbic acid retention in frozen concentrated citrus juices also showed possible rate acceleration phenomenon by the freeze-concentration effect. Experimental results of percent ascorbic acid retention after twelve month storage (Huggart et al., 1954) were transformed to rate constants or apparent rate constants by assuming a first order reaction kinetics with respect to ascorbic acid concentration. The rate constants versus temperature relationships for pineapple orange, dancy tangerine, duncan grapefruit and marsh grapefruit are shown in Figure 5.27. Similar trends were observed that the reaction rates were accelerated at or below temperature of -6.7 C.

On the study of high quality life and practical

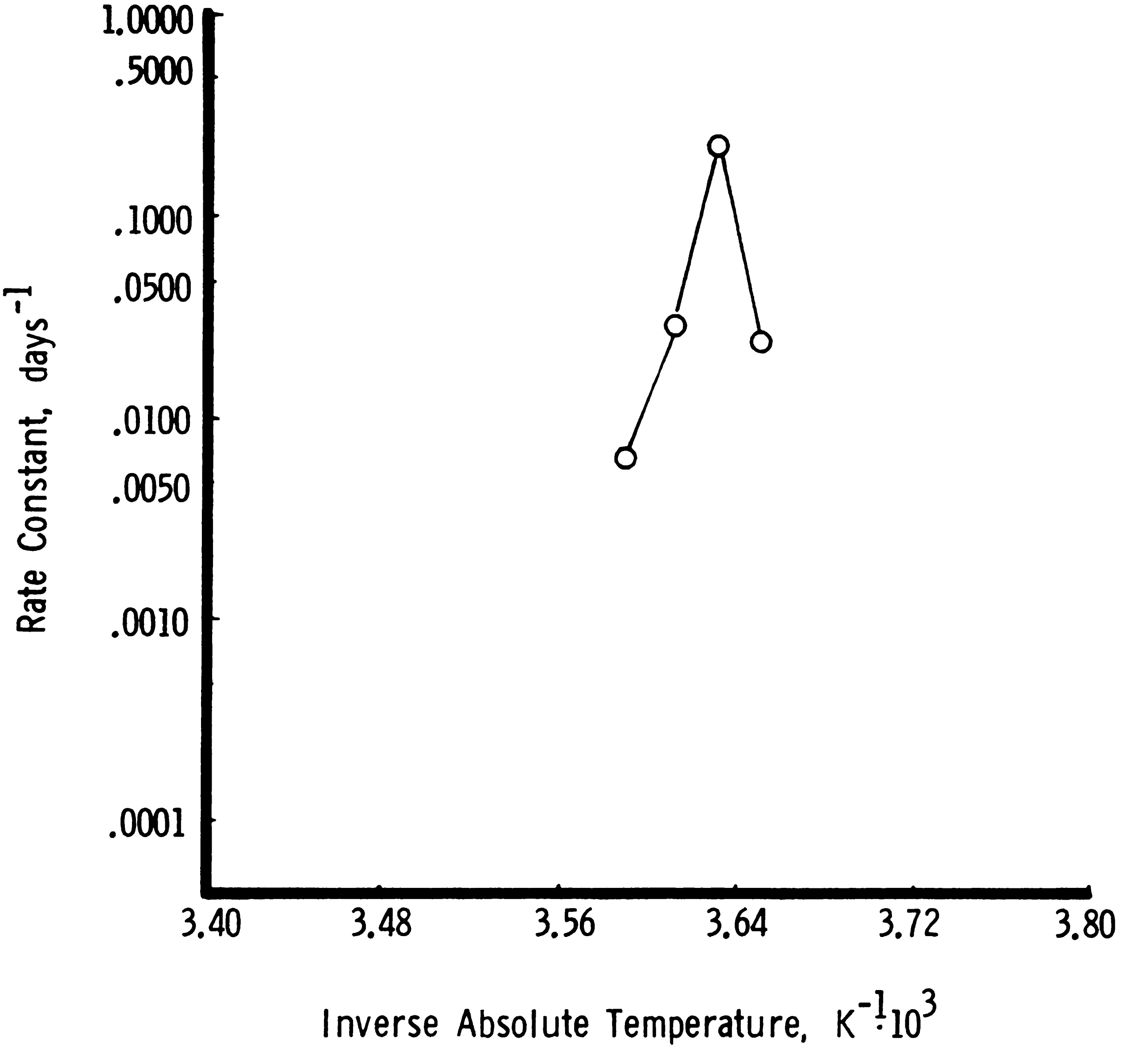


Figure 5.25 The Rate Acceleration of Phospholipid Hydrolysis in Cod Muscle (Lovern and Olley, 1962)

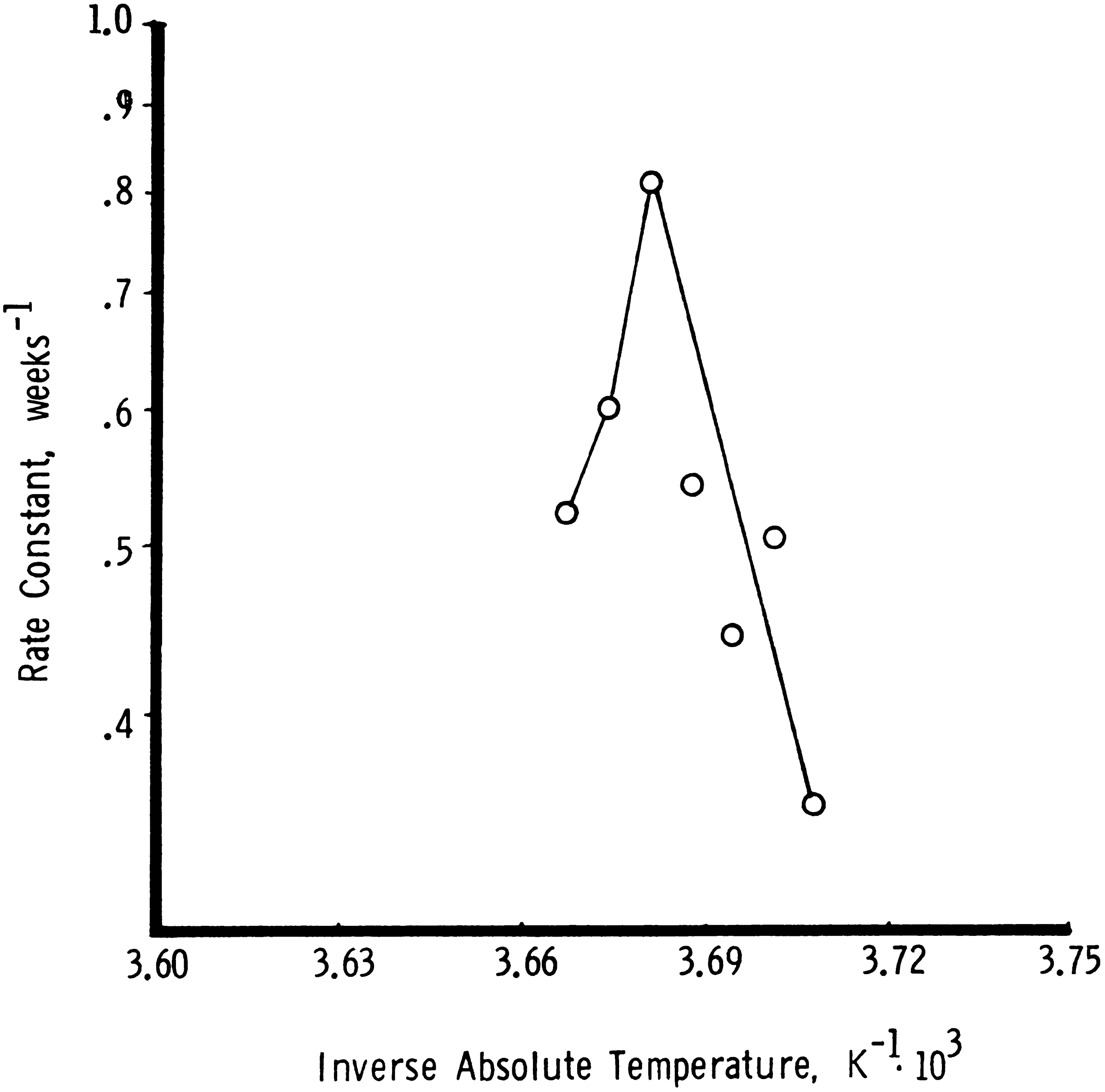


Figure 5.26 The Rate Acceleration of Protein Denaturation in Cod Muscle (Love and Elerian, 1964)

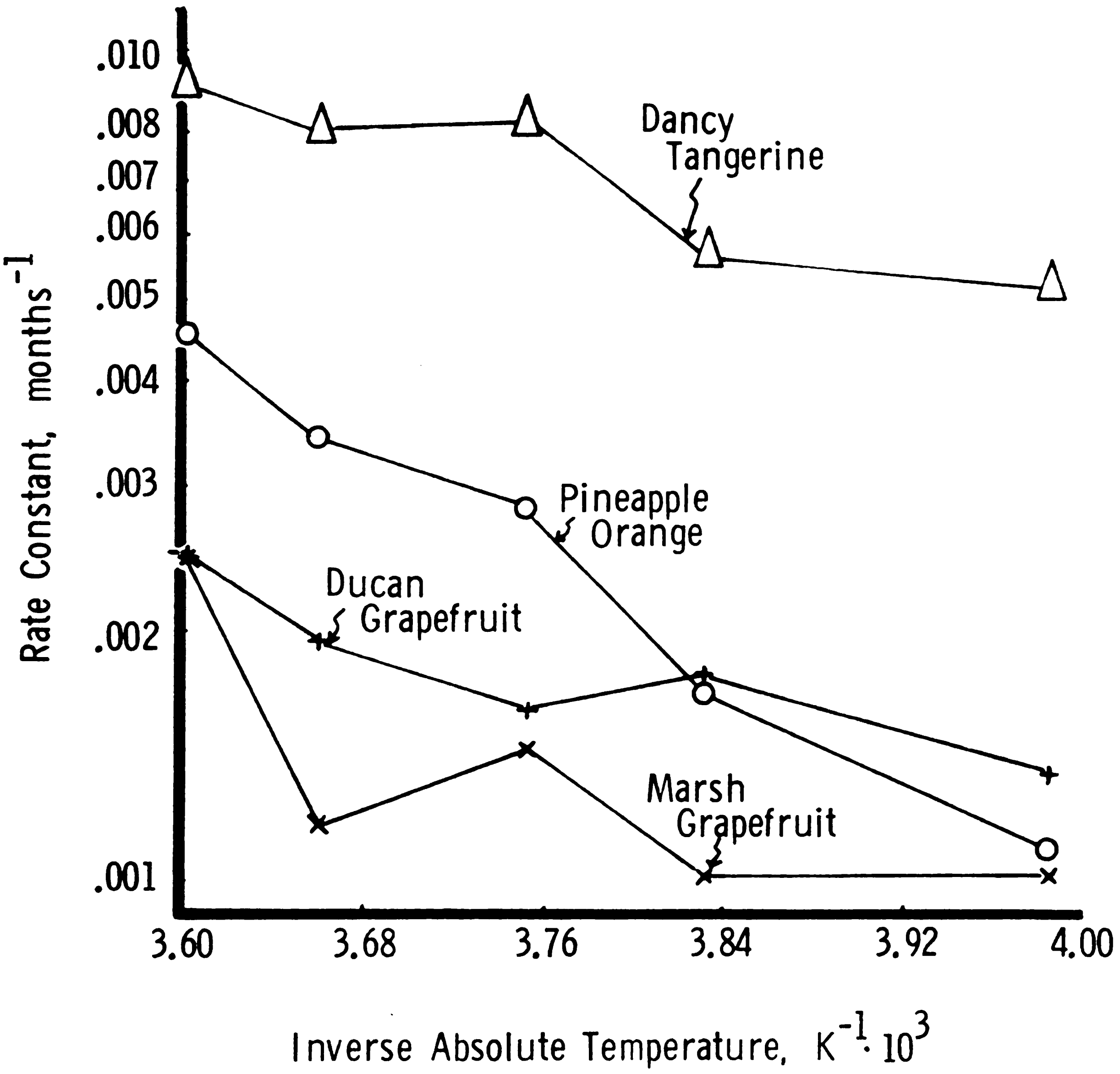


Figure 5.27 The Arrhenius Plots for Ascorbic Acid Retension in Concentrated Citrus Juices (Data from Huggart et al., 1954)

storage life of Danish butter varieties, Poulsen et al. (1976) observed rate acceleration at storage temperatures below -25 C and proposed that the freeze-concentration effect might be the reason. Lindelov and Poulsen (1975) studied the stability of smoked bacon, liver paste and chopped herring fillets and found that these products, especially with salt added, showed rate acceleration phenomenon at certain sub-freezing temperature range. Bogh-Sorensen et al. (1981) observed that cured meat in vacuum package showed neutral stability while cured pork in oxygen permeable package showed reversed stability. In other studies on Vienna sausage, smoked pork bellies, unsmoked and smoked bacons and apple aroma, Poulsen and Lindelov (1981) observed several forms of rate acceleration including neutral stability and reversed stability at certain temperature ranges. These studies are mostly based on overall quality change, which might be contributed by several quality changes (or degradation reactions). Therefore, the relationships between rate conatants and temperature are not so easily identified as similar to those exerted by the freeze-concentration effect.

The following example gives an illustration of how an overall quality change determined by following two degradation reactions can complicate the normal picture of the rate acceleration caused by the freeze-concentration effect. Curves 'A' and 'B' in Figure 5.28 represent

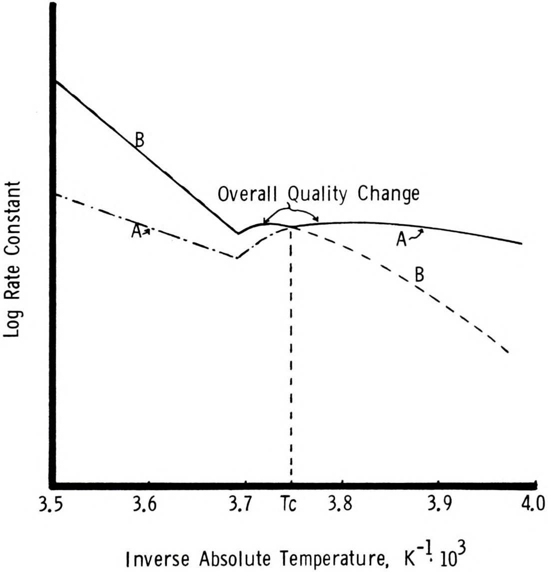


Figure 5.28 The Rate Acceleration of an Overall Quality Change Contributed by Two Degradation Reactions

rate-temperature relationships of two degradation reactions that contribute to the oveall quality change. Either one of the degradation reactions can cause the overall quality to be viewed 'unacceptable', or 'end of quality life'. At temperatures above 'Tc', reaction represented by curve 'B' proceeds faster and is the determining factor for judging the end of quality life, while at temperatures below 'Tc', the determining factor shift to curve 'A'. The overall apparent quality life therefore shows a neutral or slightly reversed stability. The example gives a possible explanation to the results observed by Poulsen and Lindelov (1981) and Bogh-Sorensen et al. (1981).

Rate acceleration caused by the freeze-concentration effect has been found in many food or other biological systems (Section 2.1). Possibly, with more attention paid to it, cool storage at near initial freezing temperature may replace frozen storage for some food products based on process optimization between energy conservation and quality preservation.

VI. CONCLUSIONS

1. Experimental results indicated that the autoxidation of oxymyoglobin followed first order kinetics with respect to oxymyoglobin concentration in deionized water; in acetate buffer of different concentrations; in liquid state before freezing or in unfrozen solution of frozen state. The reaction had a 0.316 order dependency on buffer concentration when the variation in buffer concentration was large.

2. Experimental results based on oxymyoglobin autoxidation in supercooled solutions and in de-ionized water confirmed the validity of extrapolation of the Arrhenius equation from temperatures above freezing to temperatures below freezing, as long as other reaction environment changes or reaction mode changes accompanied with the temperature variations can be separated from the 'true' temperature effect.

3. Experimental results of oxymyoglobin autoxidation in de-ionized water confirmed one of the implications from theoretical consideration that the rate of a first order reaction was not influenced by the freeze-concentration phenomenon.

4. Experimental results based on autoxidation of oxymyoglobin at various acetate buffer concentrations verified the effectiveness of the prediction approach. Deviations between measured and predicted apparent reaction rate constants were greater at lower storage temperatures.

5. Accelerated shelf-life tests are possible by quantification of major factors that influence the reaction rate, as revealed by the procedures for building up the prediction approach in this study.

6. The predicted results showed that the addition of inert (with respect to the object reaction) solutes could reduce or eliminate the influence of freeze-concentration phenomenon on the reaction rate acceleration.

7. Concentration before freezing cannot reduce the influence of the freeze-concentration effect on reaction rate acceleration during frozen storage as compared to the original system based on predicted and experimental results of oxymyoglobin autoxidation at four buffer concentrations.

8. The adverse temperature range for storage, within which storage is unjustified from the standpoint of more energy input without better preservation effect, is widened by low activation energy, high reaction order and high initial freezing point, based on the predicted results.

9. Based on the initial freezing temperatures of

most food products, the occurrences of rate acceleration by the freeze-concentration effect are not unusual, especially for degradation reactions with low activation energy or high reaction order.

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Table A.1 Autoxidation of Oxymyoglobin in 0.01 M Acetate Buffer in Liquid State Before Freezing

20 C		14 C		9 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	88.78	0.0	95.63	0.0	92.21
1.3	81.94	1.5	91.06	6.3	86.50
2.6	77.19	5.1	88.40	15.7	79.28
4.7	70.34	10.2	81.56	23.4	76.81
8.4	61.60	19.2	69.20	32.6	72.24
17.8	42.40	28.8	60.65	42.8	67.68

3 C		-1 C*		-3 C*	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	98.63	0.0	100.00	0.0	99.22
8.5	94.91	33.5	95.69	32.9	97.06
34.0	85.13	71.1	91.98	70.6	93.35
115.7	79.06	115.8	88.45	115.3	90.61
188.3	68.88	188.7	81.21	188.0	86.89

* Supercooled Solution

Table A.2 Autoxidation of Oxymyoglobin in 0.05 M Acetate Buffer in Liquid State Before Freezing

20 C		14 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	98.96	0.0	96.02
1.2	89.62	2.2	89.27
2.8	79.58	6.0	84.08
4.6	70.59	10.9	74.05
6.3	65.57	18.4	60.90
8.0	59.86	28.9	48.96
19.1	33.22		

9 C		3 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	98.96	0.0	95.67
3.3	94.29	7.8	92.21
8.1	89.97	18.5	88.41
19.0	80.44	46.4	83.56
24.6	78.03	72.0	77.68
30.5	74.57	114.1	66.78
44.6	66.26		

Table A.3 Autoxidation of Oxymyoglobin in 0.5 M Acetate Buffer in Liquid State Before Freezing

20 C		14 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	96.96	0.0	95.83
1.9	72.11	2.9	82.16
3.5	58.63	6.4	65.09
6.2	41.94	11.5	52.18
8.8	31.31	20.0	37.57
		32.9	21.44

9 C		3 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	92.21	0.0	98.63
6.3	86.50	8.5	94.91
15.7	79.28	34.0	85.13
23.4	76.81	115.7	79.06
32.6	72.24	188.3	68.88
42.8	67.68		

Table A.4 Autoxidation of Oxymyoglobin in 0.5 M Acetate Buffer in Liquid State Before Freezing

-1 C

Storage time,hr	Oxymyoglobin remained,%
0.0	98.46
10.2	91.75
35.4	86.18
73.0	78.69
117.7	67.56

-3 C*

Storage time,hr	Oxymyoglobin remained,%
0.0	96.55
10.4	91.94
35.0	87.33
72.2	81.38
117.2	75.43
189.7	67.53

-5 C*

Storage time,hr	Oxymyoglobin remained,%
0.0	94.31
24.0	89.18
53.0	85.58
91.3	80.46
137.0	76.28
190.2	72.49

* Supercooled Solution

Table A.5 Autoxidation of Oxymyoglobin in 1.0 M Acetate Buffer in Liquid State Before Freezing

20 C		14 C		9 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	82.27	0.0	94.60	0.0	94.61
0.8	71.87	0.7	89.98	2.7	88.82
1.9	62.04	1.5	85.16	7.9	76.49
2.7	55.68	2.7	82.27	22.2	54.91
5.2	38.15	4.7	73.60	44.9	33.53
		7.7	63.00		

3 C		-3 C		-5 C *	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	94.77	0.0	94.58	0.0	93.45
2.6	90.84	5.7	90.65	20.5	86.70
6.0	88.22	30.9	85.98	43.2	84.01
13.7	80.37	55.5	79.07	88.9	78.03
23.5	75.89	83.7	72.15	142.5	73.60
37.1	68.41	130.5	63.18	222.3	66.47

* Supercooled Solution

Table A.6 Autoxidation of Oxymyoglobin in 0.01 M Acetate Buffer in Unfrozen Solution of Frozen State

- 2 C		- 3 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	96.67	0.0	95.30
8.5	92.28	8.1	89.63
26.4	88.77	32.3	81.80
50.3	84.04	69.9	75.73
91.2	72.81	114.9	65.17
173.6	62.63	187.4	60.08

- 8 C		- 12 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	96.67	0.0	94.49
7.2	95.26	8.7	93.35
25.9	91.05	70.4	87.26
50.5	86.14	115.7	86.50
91.3	80.53	188.5	75.86
173.7	73.16	355.9	69.01

Table A.7 Autoxidation of Oxymyoglobin in 0.05 M Acetate Buffer in Unfrozen Solution of Frozen State

- 2 C		- 3 C		- 8 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	97.23	0.0	96.19	0.0	97.23
21.6	88.58	20.9	91.87	21.6	90.83
47.4	81.49	46.8	82.35	47.4	86.85
72.4	77.34	72.4	79.76	76.5	82.18
114.8	69.20	114.6	72.15	114.8	73.18
160.8	63.32	160.9	66.96	160.6	73.01
214.3	56.75			213.9	68.86
294.0	49.48			294.0	62.98

- 12 C		- 15 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	98.79	0.0	97.92
48.4	92.04	46.4	93.60
95.1	86.16	114.3	88.24
166.7	81.49	214.2	85.81
240.8	73.88	315.6	81.83
311.9	69.72		

Table A.8 Autoxidation of Oxymyoglobin in 0.50 M Acetate Buffer in Unfrozen Solution of Frozen State

- 3 C

Storage time,hr	Oxymyoglobin remained,%
0.0	76.01
7.8	71.79
32.2	67.95
32.5	66.99
69.7	61.04
114.6	56.62
187.2	47.98

- 8 C

Storage time,hr	Oxymyoglobin remained,%
0.0	93.74
24.2	90.70
53.1	86.53
91.2	80.46
137.1	77.80
190.2	71.73
270.5	66.60

- 12 C

Storage time,hr	Oxymyoglobin remained,%
0.0	95.78
10.4	96.16
71.4	89.06
116.7	85.99
189.4	80.81
356.9	72.36

Table A.9 Autoxidation of Oxymyoglobin in 1.00 M Acetate Buffer in Unfrozen Solution of Frozen State

- 8 C		- 12 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	92.49	0.0	94.61
43.0	85.55	71.9	87.28
89.1	82.27	144.0	82.66
142.2	79.38	240.0	75.72
220.4	67.44	358.9	68.98
		480.8	62.04

Table A.10 Autoxidation of Oxymyoglobin in 0.01 M Acetate Buffer in Unfrozen Solution of Frozen State and Frozen in Acetone-Dry Ice Bath

- 2 C		- 8 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	96.67	0.0	96.67
8.5	92.28	7.2	95.26
26.4	88.77	25.9	91.05
50.3	84.04	50.5	86.14
91.2	72.81	91.3	80.53
173.6	62.63	173.7	73.16

Table A.11 Autoxidation of Oxymyoglobin in De-Ionized Water

30.0 C		24.6 C		16.4 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0.0	95.18	0.0	95.18	0.0	96.01
0.6	91.53	1.9	91.86	22.6	89.53
1.7	86.21	6.0	85.38	67.2	81.89
3.7	80.56	11.2	79.57	137.9	69.10
6.7	71.10	16.4	73.26	219.2	54.49
11.6	60.13	19.7	68.44	286.0	42.86
16.7	50.33	25.1	64.45		
20.2	44.51	20.0	52.82		
25.5	38.04				

4.0 C		- 2.0 C		- 10.0 C	
Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%	Storage time,hr	Oxymyoglobin remained,%
0	99.67	0	99.00	0	100.00
142	94.35	142	96.84	140	97.18
287	92.69	502	91.86	501	96.84
501	81.89	1007	82.21	1296	95.18
718	78.74	1295	85.38	2518	91.03
1007	71.93	2517	73.59	3524	88.54
1295	66.11				