A COMBINED DIFFUSION AND CHEMICAL REACTION MODEL FOR SOLID PHASE PEPTIDE SYNTHESIS

By

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NOMENCLATURE AND ABBREVIATIONS

A	dimensionless diffusion parameter
B	blocking group
Boc	2-(4-biphenylyl)propyl(2)oxycarbonyl
C_A	concentration of amino terminus, mole/litre
C_B	concentration of symmetrical anhydride, mole/litre
C_{AO}	initial concentration of active sites, mole/litre
C_{BO}	initial concentration of symmetrical anhydride, mole/litre
C_O	concentration of symmetrical anhydride in bulk solution
D_{AB}	effective diffusivity coefficient in resin, cm^2/s
DCC	dichlorohexylcarbodiimide
DCM	dichloromethane
DCU	dichlorohexylurea
DMF	dimethylformamide
DVB	divinylbenzene
i	subscript for grid station in the radial direction
j	subscript for grid station in the time direction
K	ideal second order rate constant, mole/litre.sec
M	excess mole ratio, C_{BO}/C_{AO}
N	radial grid number
n	chemical reaction order

Phe	phenylalanine
P	protecting group
R_A	rate of reaction, mole/sec
r	radial coordinate
Ser	serine
SPPS	solid phase peptide synthesis
t	time, sec
TEA	triethylamine
TFA	trifluoroacetic acid
U	dimensionless concentration, $C_B/C_{B\,O}$
UV	ultraviolet (spectroscopy)
Vr	velocity in the radial direction
V_θ	velocity in the θ-direction
V_ϕ	velocity in the ϕ-direction
X_A	fraction of active sites reacted

Greek Letters

ξ	dimensionless radial coordinate, r/R
τ	dimensionless time, tD_{AB}/R^2

CHAPTER I

INTRODUCTION

The solid phase peptide synthesis (SPPS) method was conceived by R.B. Merrifield in 1959 in an effort to overcome many of the problems associated with the solution methods for peptide synthesis. The major feature of this scheme is that the peptide is anchored to a solid support at its carboxyl end by an ester bond. The peptide remains attached to the resin support throughout the synthesis as each amyl residue is added in the desired sequence. The peptide is then cleaved from the resin by a strong acid such as HF. The biggest advantage of this method is that the intermediate purifications and the accompanying steps are replaced by simple rinsing of the resin. SPPS bears an interesting similarity to the biological process of protein synthesis in which carboxyl activated amino acids are added to the N-terminus of a growing peptide chain which is bound to a solid support (polyribosome).

The classical method contains several distinctive characteristics. First, the resin which provides the solid support, is chloromethylated polystyrene crosslinked with 1-2% divinylbenzene. The chloromethylated sites provide the anchoring base, and the low crosslinking percentage allows adequate swelling of the resin. The second feature of this method is that tert-butyloxycarbonyl (t-Boc or

Boc) is used to protect the amine functionality while the amino acid is being added to the peptide. The final characteristic involves the method used for the coupling of the amino acids to form the peptide bonds. Dicyclohexylcarbodiimide (DCC) is either added directly to the reactor in order to allow the amino acid to penetrate the resin beads, or the amino acid symmetrical anhydride is made by reaction with DCC and added to the reactor subsequently. Although variations and modifications of every facet have been attempted, Merrifield's method remains the most widely employed.

Because SPPS is a repetitive addition of amino acids, the key to its usefulness is the successful completion of each repetition. One failed addition, or, more importantly, successive partially incomplete additions, results in both a low product yield and a mixture of similar peptides which are difficult to separate. To avoid these problems, many investigators allow the coupling step to continue substantially beyond the required time. Although this solution is acceptable for a lab scale synthesis, a more efficient method is desirable for industrial use.

Knowledge of SPPS chemistry has advanced substantially compared with the understanding of the reaction kinetics. The kinetic information of coupling reactions for use in SPPS reactor modelling and design is accomplished by a continuous monitoring method. In this method, we have a SPPS reactor connected to a UV-visible spectrophotometer. Kinetic data and reaction rate constants can be measured conveniently using amino acids or amino acid derivatives which have useful ultravoilet spectra. The rate of reagent concentration change in the liquid phase can be followed by

measuring ultravoilet absorbance. The resulting absorbance curves are analyzed to give the desired kinetic information.

Chen (5), in 1988, presented an empirical kinetic model and determined the rate constants over a range of reaction conditions (i.e. temperature, mixing rate,chain length, excess mole ratio, resin, etc.) for the synthesis of polyphenylalanine and polyserine. In general, the coupling rate between amino acid symmetrical anhydride and the polyamino acid decreased as peptide chain length increased, and increased as temperature increased. The higher excess mole ratio of carboxyl groups elevates the coupling rate. In addition, polystyrene crosslinked with one percent divinylbenzene showed faster coupling rates and less deviation from ideal second order rate kinetics than two percent crosslinking for the low excess mole ratios study.

For this heterogeneous reaction, film resistance to diffusion was assumed to be negligible as a result of mixing rate experiments. Approximate values of activation energy based on two different reaction temperatures implied that intraparticle diffusion may be significant. The simple reaction model proposed by Chen (5) could not fully explain the phenomena observed in the experimental study. Secondary structure of the peptide chain may have introduced an orientation problem of collision between amino acids and the amino terminus of the peptide resin; this hypothesis was used to explain the lack of agreement with the experimental data.

Previous studies have shown that diffusion is not the rate determining step. This is supported by Merrifield's calculation that the diffusion rate is 10 times faster than the reaction rate (24) at higher symmetrical anhydride concentrations. The purpose of this

study is to confirm or disprove this hypothesis. In this study a combined model for diffusion and reaction will be developed. This model will then be used to fit the experimental data obtained by Chen(5). This will show us whether diffusion plays a significant role in the kinetics of solid phase peptide synthesis.

The basic objectives of this report is to develop a mathematical model to describe this reaction. The model will be solved numerically for the general case of reaction accompanied by diffusion. Assumptions include radial diffusion in the spherical resin with second order reaction. Fraction of active sites reacted versus time will be plotted for various reaction parameters. Experimental data will be checked with model predictions to determine the validity of these assumptions for SPPS.

CHAPTER II

LITERATURE REVIEW

Mass transfer accompanied by an irreversible or reversible chemical reaction is a common process of high importance in the chemical industry. It has wide application in many processes, for example, in catalytic reactions, enzyme immobilization, and solid-gas, liquid-gas and solid-liquid absorption systems. Although there has been significant amount of research on this topic, no general mathematical model is available which can be applied to all systems.

There are many published mathematical models using analytical and numerical techniques for the problem of mass transfer accompanied with a chemical reaction. Some discuss diffusion and reaction models separately, whereas others considered the effect of chemical reaction upon the rate of diffusion.

In reaction-diffusion systems, one is frequently faced with a physical situation, the mathematical description of which requires the solution of linear or non-linear coupled differential equations. The non-linearity in these equations exist either in the source term (eg. reaction rate), in the derivative term (eg. when volume changes occur), in the coefficients of the governing equations or in the boundary conditions.

Three basic concepts of mass transfer at the interface have been proposed. They are the film concept, the penetration

concept, and the surface renewal concept. The film concept was adopted by Hatta (16) in developing the theory for mass transfer accompanied by a chemical reaction. The theory is based on the postulation that a stationary film exists at the interface. It is also assumed that mass is transported by steady state molecular diffusion through the film. Later Higbie (17) proposed the penetration theory which modifies the above postulation by assuming unsteady state molecular diffusion through a stagnant film. However, the existence of a stagnant film is not always conceivable, particularly when the fluid motion is a disrupted one. Thus Danckwerts (9) proposed, for gas absorption in a packed column, that turbulence creates numerous infinitesimal liquid elements which are constantly brought to the interface. While these elements are exposed to the opposite phase at the interface, diffusing molecules are transported by penetration or unsteady molecular diffusion into the elements. An objection to this model is that the depth of penetration or the thickness of the liquid element is assumed to be infinite. In reality, the depth of penetration or the thickness of a liquid element should have a finite value, and decreases as the turbulence is increased.

A film penetration model which describes the mass transfer mechanism in the absence of chemical reaction was proposed by Toor and Marchello (34). They showed that the film and penetration theories are not separate concepts, but merely limiting cases of the more general film-penetration model. However, the application of the film-penetration model to the mechanism of simultaneous mass transfer and chemical reaction had not been attempted. Based on the film-penetration concept, Huang and Kuo (19), formulated a general

mathematical model to describe the physico-chemical behaviour at the interface. The mechanism of mass transfer accompanied by a first-order irreversible chemical reaction was considered by them. The overall mass transfer mechanism across the interface consisted of two steps, that is surface renewal by freshly formed liquid elements and simultaneous molecular diffusion and chemical reaction with the exposed liquid elements. When a fresh liquid element is brought to the interface to be exposed to the other phase, its concentration is assumed to be equal to that of the bulk liquid phase. This assumption is valid when the bulk liquid is well mixed and uniform. The basic differential equation which gives the concentration gradient within the liquid element was solved by Laplace transforms. The solution obtained was expressed in an error function series. The nature of mass transfer behaviour and the transfer rate could be evaluated and analysed in terms of dimensionless groups. For mass transfer accompanied by higher order chemical reaction, there are solutions by numerical approximations (2,28). When the chemical reaction is instantaneous second order, the problem has been solved by a geometrical approximation (18,31).

In 1965, Huang and Kuo (20) derived theoretical equations for the rate of interphase mass transfer accompanied by a first order reversible reaction. These equations were based on the same three postulations; namely the film theory, the penetration theory and the surface renewal theory. The predicted effects of the chemical reaction on the overall mass transfer rate were indeed sensitive to the theory or the model adopted in postulating the mechanism. One

exception is when the diffusivities of the reaction and the product are nearly equal. For this special case, the three theories predict practically the same effects of a reversible reaction. Convective transport was assumed to be insignificant. If the order of the reaction is greater than one, then the material balances of each component yield a set of non-linear partial differential equations. The exact analytical solution of this set of non-linear partial differential equation has not been possible. An approximate solution for this case, based on film theory was attempted by them. According to the film theory, the steady state molecular diffusion and the reversible reaction takes place within a stagnant film. However, in many actual operations, the amount of reactant in the liquid solvent is comparatively large and its concentration remains nearly constant during the diffusion and reaction process. It was assumed to be equal to either the interface concentration or the bulk concentration. If the former is chosen the concentration profile of the reactant is a visualized step function, and at the end of the element, is changed abruptly from the interface concentration to the bulk concentration.

In 1976, a study was conducted by Ma and Lee (23) to study transient diffusion without chemical reaction in sherical pellets in a constant volume, well stirred system. Assuming that the transport processes in the macropores and micropores obey Fick's law, micropore diffusion coefficients were determined. Also a mathematical model was developed to describe the diffusion in a solid with a bipore distribution. They considered a well stirred system where the total quantity of the diffusing species is finite.

Some of the other assumptions made in the derivation were: spheres are of uniform size, and diffusion coefficients are constant. This system had a time varying boundary due to the fact that the total quantity of diffusing species is finite. Owing to the complexity involved, the inversion of the equation obtained by taking Laplace transforms required a numerical technique.

There are many situations of practical significance where an ionic or molecular species reacts with particles in suspension, and where the concentration of this species in the bulk fluid changes with time due to its limited presence. Reaction of the species with the suspended particles alters the surface chemistry and charge characteristics of those particles with time. Moreover, the solution chemistry of the species will be altered if the concentration of that species decreases in the bulk fluid of the suspension. The macroscopic result of such changes is a time dependence of the suspensions stability and settling properties. Examples include the uptake of drugs and nutrients by cells in suspension, the diffusion-reaction in suspended catalysts, etc.

Papadopoulas and Bailey (26) in 1986 extended the study done by Ma and Lee by including a chemical reaction along with diffusion in suspended particles with limited supply of reactant. The concentration decay of a species from the bulk phase of a suspension was examined. This species had limited presence in the fluid and disappeared by means of diffusion and first order reaction in the suspended spherical particles. In the absence of a chemical reaction and surface resistance to mass transfer, the model that describes the rate of disappearance of a species from a fluid phase by means of

diffusion in a solid (8) provided the basis for the experimental determination of diffusivities in porous solids. In their study, the species, which diffused in the solid, reacted with the solid and experienced mass transfer resistance in the fluid layer around the suspended particles. Therefore, measurements of fluid concentration versus time could be used in conjunction with the equations in order to determine, in addition to the diffusivity, the values of the reaction rate constants and the mass transfer coefficient. This approach was similar to the heating or cooling of a solid sphere which is immersed in a well stirred fluid. That method is also reviewed by Carslaw and Jaeger (4) and Bird, Stewart and Lightfoot (1). However, this was not extended to higher order reactions and the validity of the analytical solution, obtained by Laplace transformation, was not checked by any experimental data.

Jayaraman, Kulkarni and Doraiswamy (21) developed a simple method for the solution of a class of reaction diffusion problems. They had a general order reaction term, thereby getting non-linearity in the source term. But, they considered only the case of steady state diffusion and reaction. In the case of unsteady state, no analytical solution can be found and we have to resort to numerical analysis. The system considered coupled boundary conditions which were converted to an initial value problem. For this purpose, they transformed the two differential equations into those of reduced order. The complete trial and error procedure required in the use of the conventional method was avoided. The only limitation of the method was that it was applicable to a non-linear rate form of the type of nth order rate expression. Modifications would have to be

made for other non-linear rate forms, such as the general nth order reactions with volume change.

Grotch (15) used Galerkin's technique for the approximate solution of ordinary and partial differential equations. The problem of a tubular reactor in which axial diffusion is superimposed upon a one-dimensional flow was considered to demonstrate the technique. Since many solutions; analytical and numerical, were available for this specific case, it was easy to compare the results. The differential equations and associated boundary conditions had been investigated by Danckwerts for first-order kinetics. For non-linear kinetics, the effects of the three physical parameters of the problem; the reaction rate, the order of the reaction and the axial Peclet number, were difficult to account for. Therefore one must either numerically integrate the differential equation or interpolate by using the limited curves of Levenspiel (22). The use of Galerkins method yielded approximate solutions which were easier to utilize and showed parameter behaviour more clearly. Simple solutions were found for integral reaction orders. For general nth order kinetics, the problem was reduced to the solution of a single non-linear algebraic equation. There are a lot of advantages using the Galerkin technique over numerical integration schemes. Firstly, this is an alternative method of solution when conventional numerical methods experience difficulties, particularly for partial differential equations. Also, the results are expressed in an analytical format in terms of only a few parameters. This often provides greater insight into a problem than a tabulation of numerical results. Interpolation or extrapolation is also generally simpler and more accurate. But setting up Galerkin

equations can entail an excessive amount of algebraic manipulations, particularly if a large number of parameters are employed.

The penetration theory of Higbie (17) has been applied widely to unsteady state diffusional processes, particularly those with a chemical reaction. Obtaining an expression for the rate of diffusion of a reactant through the boundary of a semi-finite medium in general, requires the solution of a set of simultaneous partial differential equations with appropriate boundary conditions. While the solutions obtained for various combinations of reaction orders with diffusion will continue to be quite useful, many problems do not fit within any of the models for which the equations have been solved. Accordingly, it is apparent that the general solution to a model possessing a high degree of flexibility would be useful for solving many problems that are beyond the scope of existing models. It was the attainment of such a solution which made Secor and Beutler (30) work towards the mathematical model of diffusion accompanied by a single generalized, reversible chemical reaction. The partial differential equations that they obtained were non-linear, therefore analytical solutions were not expected. A variety of numerical techniques employing finite difference methods are described in the literature. An implicit method was chosen, since these techniques are inherently numerically stable over the extreme variation in space and time increments. The techniques used in the solutions of these equations were incorporated into a FORTRAN program.

The problem of predicting the effect of a simultaneous liquid phase chemical reaction on the rate of gas absorption has often been

approached by adopting a simplified model of a liquid flow pattern, which could then be treated mathematically. One interesting aspect of this problem is that the predicted answer is surprisingly insensitive to the liquid flow pattern model chosen. Several publications have considered the effect of an infinitely rapid bimolecular reaction on the rate of mass transfer from a solid surface to a fluid stream. Results for laminar and turbulent boundary layer models showed agreement with film and penetration theory results. The problem of gas absorption accompanied by a second order reaction of finite rate was solved by Brian, Hurley and Hasseltine (3). The differential equations were approximated by time centered implicit finite difference equations analogous to the equations of Crank and Nicholson (14). Linearizing by the method of Douglas, a system of simultaneous linear equations, which together with the boundary conditions were solved by the method of solving tridiagonal equations. This linearized, implicit finite difference method was chosen to avoid the severe stability limitations encountered when an explicit method is used, wherein, an exceedingly small net size was required and a very large amount of machine time was consumed in obtaining the results. Brian (2) extended the above case to an irreversible reaction of general order. He concluded that the general solution of equal diffusivities was quite insensitive to the value of n, the order of the chemical reaction with respect to the concentration of the absorbing species. The curves were found to be very sensitive to the value of diffusivity ratio when compared at a constant value of the asymtotic solution for an infinitely rapid chemical reaction.

Pearson (27) considered the same problem of diffusion with chemical reaction and solved the resulting equations numerically for intermediate cases. Analytic solutions were given for certain limiting cases and the structure of the asymtotic expansions valid near the limit was examined. The system consisted of one substance diffusing into a medium containing another substance with which it reacts according to a second order equation. The latter substance also diffused into the medium.

Enzymes, which are globular proteins, catalyze practically all of the chemical reactions which occur in living organisms. To allow repetitive use of enzymes in process applications, enzymes are often immobilized by entrapment within or attachment on insoluble supports. Immobilization of enzymes within a porous solid support of macroscopic size provide a catalyst with high activity per unit volume. Because the ultimate overall catalytic properties of the immobilized enzymes depend on the results of the immobilization process, it is appropriate to attempt to describe enzyme immobilization in porous supports. Do and Bailey (12) formulated the mathematical model for a system in which support particles are immersed in enzyme solution. They assumed the local immobilization rate to be linear with respect to the enzyme concentration in the adjacent pore fluid, which is a fairly reasonable assumption during the initial period of immobilization. The pores in the solid were of much greater diameter than the enzyme. They developed an analytical solution for this model using finite Strum Liouville integral transforms. The results obtained showed that if intraparticle diffusional resistances are not large, the intensity of

mixing has no significant influence on the bulk enzyme concentration trajectory. But for larger resistances, the response of bulk enzyme concentration is significantly influenced by the intensity of mixing. Also the enzyme immobilization rate could be determined, provided all other parameters are known. Solutions for both the enzyme concentration in the pore fluid and in the bath were obtained. This form of model describes substrate conversion to product in uniformly immobilized enzyme catalyst particles with first order local kinetics. Most of the earlier analysis of such problems provided fluid phase concentration but not intraparticle profiles. The intraparticle profiles are necessary for the enzyme immobilization model in order to determine the internal profile of enzyme activity.

Another method in which a diffusion and chemical reaction in a catalyst pore can be simulated is a Monte Carlo process. Zielinski and Petersen (35) obtained results of typical concentration profiles for first and second isothermal reactions using the method mentioned. They tried to explore the Monte Carlo method as an alternative to the more usual numerical methods for solving the governing equations of diffusion. If one applies this method in a conventional fashion to problems of simultaneous diffusion and reaction, the number of diffusion jumps required before a single reaction takes place is extremely large. This is because the reaction probibility in the time interval of one collision is small in catalysts of normal activity. A numerical algorithm would use excessive computer time. This method effectively increased the number of molecular collisions with the pore wall in a defined interval.

Carta (25) in 1989 published and showed in his article the effects of the mass transfer resistance in the continuous phase, particularly during the initial stage of contact between phases. Mass transfer is controlled entirely by the continuous phase resistance until the concentration of the reactant is reduced to zero. When the diffusivity of the second reactant is small, the introduction of a finite external mass transfer resistance has only a small effect. But for the case of diffusivity of the second reactant greater than the diffusivity of the first reactant the case is reversed.

All of the above articles discussed the general problem of diffusion with chemical reaction in various combinations. As applied to the specific case of solid phase peptide synthesis, several kinetic expressions were tried to fit the kinetic data. An apparent reaction order study done by Dietrich (11), showed an order shifting phenomena which implied that a reaction intermediate may be formed during the coupling reaction. By graphing ln (-dC/dt) versus ln (C), several conclusions were made. First, apparent zero and first order reactions tend to occur during early stages of the reaction, while higher order reactions occur in the latter stages with lower symmetrical anhydride concentrations. Also, as chain length increased, the jump to higher orders, with respect to the sites converted, occurred earlier in the reaction. This shifting order phenomena was explained by particle diffusion resistance in latter stages of coupling or at longer peptide chain length.

For the low excess mole ratio reaction between the amino acid anhydride and amino terminus, it is reasonable to apply ideal second order reaction rate to express the reaction process. This has been

done by Merrifield (24) and Rudinger (29). The attachment of amino acid to aminoacyl resin satisfied the second order rate expression, but when the number of amino acid residues in the peptide increased, deviation from second order kinetics occurred. A kinetic model was developed by Chen (5) for the synthesis of selected peptides, polyserine and polyphenylalanine, with the rate constants determined over selected reaction temperatures, mixing rates, peptide chain length and excess mole ratio of t-Boc amino acid. The simple shifting order model could take some observations into account, but for low excess mole ratio between the symmetrical anhydride and the reaction site on the resin, a second order reaction rate expression failed to fit the data.

CHAPTER III

SOLID PHASE PEPTIDE CHEMISTRY

The classical method of solid phase peptide synthesis.basically is repetition of three chemical reactions, as illustrated in Figure 1. The rinsed resin or peptide resin is first subjected to treatment with a 1:3 solution of trifluoroacetic acid (TFA): dichloromethane (DCM). This removes the Boc protecting group on the terminal amine. This deprotection step is followed by treatment with 10% triethylamine (TEA) in DCM. TEA neutralizes the terminal end. The final step is acylation of the terminal amine by coupling of the desired amyl residue. Six solvent rinses with either DCM or dimethylformamide (DMF) are needed between each of the above steps. The details are described in EXPERIMENTAL PROCEDURE.

Several modified solid phase procedures have also been used and studied. The chemistry background of these modifications can be discussed in three aspects, namely; polymer support, peptide-resin link, and deprotection of -amino groups.

A suitable insoluble support and a satisfactory means of attaching the first amino acid are of critical importance for successful SPPS. The standard 1% or 2% divinylbenzene crosslinked polystyrene, which is commonly used in polymer support reactions, were chosen by Merrifield and are currently the most popular polymer supports for SPPS research. But for some peptide sequences

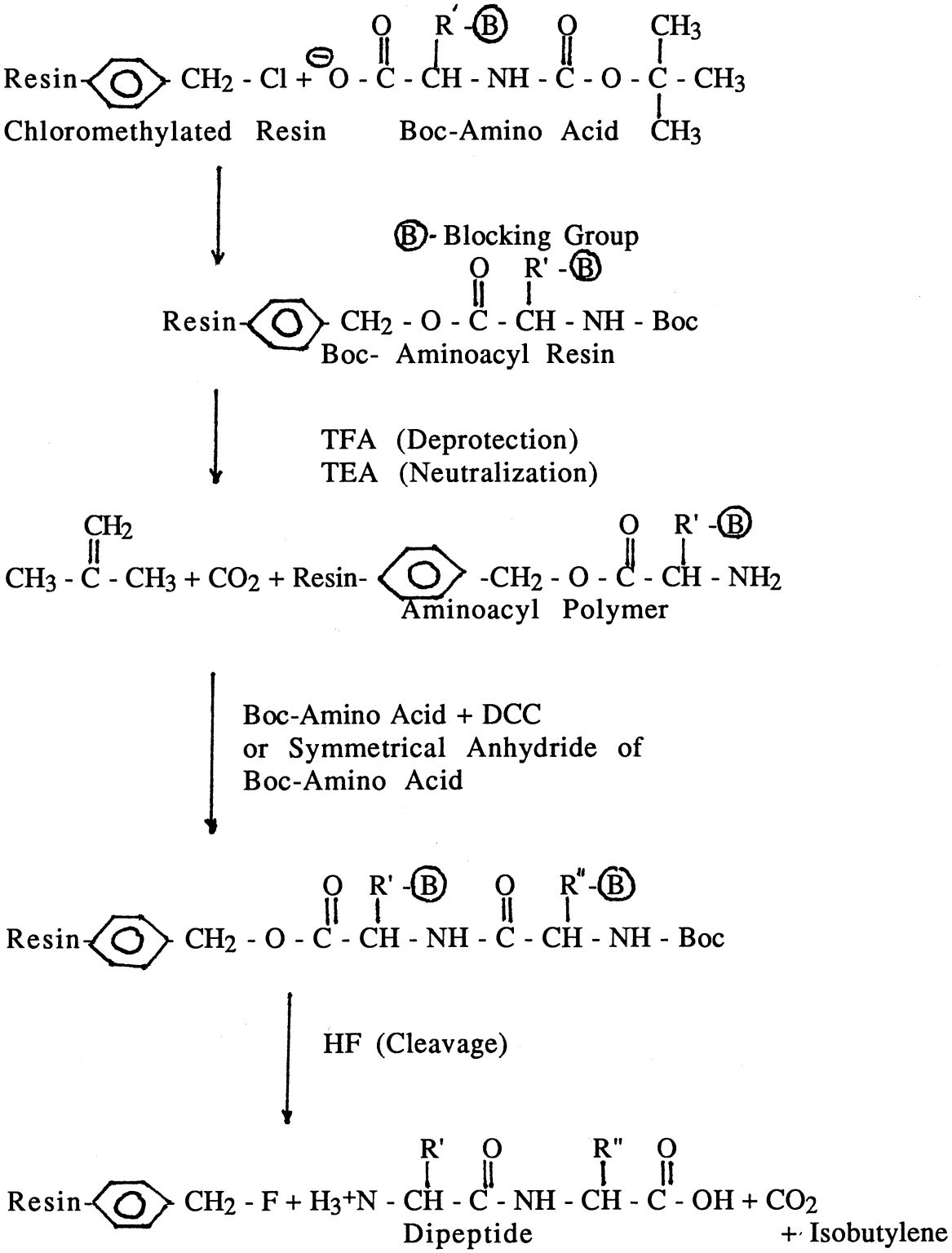


Figure 1. The Classical Merrifield Scheme of Solid Phase Peptide Synthesis (33)

solvents commonly used for SPPS, such as DCM, swell the resin effectively but would not be expected to solvate the peptide chains very well. In this case, the incompatibility between the natures of the growing peptide chain and the polystyrene resin causes a significant percentage of growing peptide termination.

In the classical SPPS system, the ester linking the peptide to the resin is only slightly labile to the reagents normally used for removal of Boc groups at each step of the systhesis. This might give a loss of about 1% per deprotection step by 25% TFA in DCM. This is not acceptable of synthesis of long peptides. A more stable peptide-resin link is needed and this can be achieved by substitution of the resin electron withdrawing groups. The choice of protecting group depends not only on the nature of the group to be protected but also on the nature of other reactants to be used later in the synthesis. Protecting groups are chosen for their chemical stability which is much lower compared to the peptide bond. The most popular - protecting group during the last two decades has been the Boc group. This group gives satisfactory lability-stability characteristics toward deprotection and cleavage reagents.

The choice for blocking groups of side chain functionalities is also subject to the stability-lability characteristic to deprotection and cleavage reagent. The blocking groups for individual amino acids are discussed in Stewart and Young (34).

Besides these aspects, several coupling reaction techniques attempting either to reduce reaction time or to improve coupling efficiency have also been tried. Basically, these techniques are devoted to improving activation of the carbonyl group by replacing -

OH with a better leaving group. The use of anhydrides, activated esters of amino acids, and various reagent additions to the coupling solution are examples of such improvements.

Experimental Apparatus

Several important considerations were involved in the developement of the experimental apparatus. The major concern, and the biggest difference between this design and most other schemes, was the desire to have a continuous stream for nondestructive monitoring of the reaction dynamics, and an alternative to the standard mixing, rocking, procedure was also needed. Other factors which influenced the final design were the chemicals involved, the small volumes to be used and the general ease of the operation. Figure 2 illustrates the final scheme used. For the description of the experimental apparatus, refer to CHAPTER III (5).

Experimental Procedure

Some preliminary work is needed before synthesis of peptides can begin. The preliminary experimentation mainly consists of preparing calibration curves for both the amino acids and their symmetrical anhydrides. A series of samples are made by diluting a solution of known amino acid or anhydride concentration. Ultravoilet absorbance of each sample is then taken at numerous wavelengths. By plotting absorbance versus concentration at several wavelengths, calibration curves with the proper absorbance scale over a range of

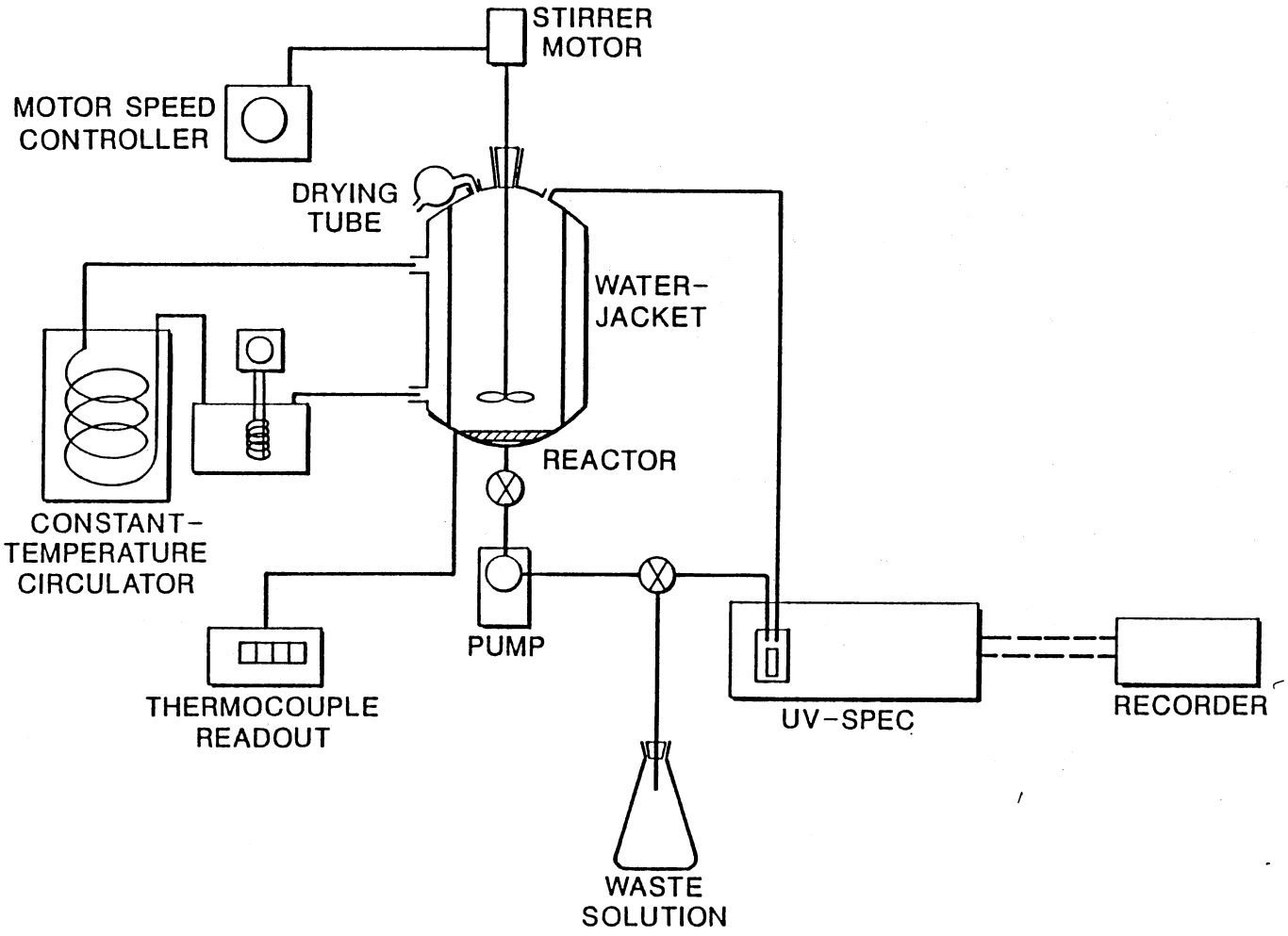


Figure 2. The experimental Set-Up for the Kinetic Study of Solid Phase Peptide Synthesis(5)

concentrations are obtained. A detailed description of anhydride preparation and spectrophotometer use are given in steps 11-15 of the following peptide synthesis procedure.

Peptide Synthesis Procedure

One to two percent divinylbenzene cross-linked polystyrene resin with the first amino acid residue already attached and analysed was used in this study. Due to this fact, many common resin preparation steps are omitted. Stewart and Young provide detailed experimental procedures for these reactions and tests (34). After a weighed sample of the resin is placed in the reactor, the repetitive synthesis steps outlined by Stewart and Young are illustrated in Table I

TABLE I

SOLID PHASE PEPTIDE SYNTHESIS STEPS WITH SYMMETRICAL ANHYDRIDE COUPLING

Step	Reagent	Volume[a](ML)	Time[b](Min)
1	DCM	15	5
2	DCM wash (3 times)	15	1.5
3	TFA/DCM	15	1.5
4	TFA/DCM	15	30
5	DCM wash (6 times)	15	1.5
6	TEA/DCM wash (2 times)	15	1.5
7	DCM wash (6 times)	15	1.5
11-13	Symmetrical Anhydride in DCM	15	c
15	DCM wash (3 times)	15	1.5

a. The volume of solvent depends on the vessel used and the mass of the resin. The volume here is based on 1 gram of resin used.
b. Time of each wash and it is approximate.
c. Time needed-until U.V. absorbance is constant.

A detailed descriptions of these reactions, based on a synthesis with one one gram of resin, is given below.(5)

1. The Boc-amyl-resin is allowed to swell in 15 mls DCM for 5 minutes before the stepwise synthesis. After the synthesis the peptide resin may be left overnight at this stage suspended in DCM.

2. Wash the resin with 15 mls DCM 3 times. Each wash is approximately 1.5 minutes in length. During this wash and all following washes, the resin is mixed by the stirrer and after the resin is all dispersed and suspended, the wash solvent is pumped out.

3. Deprotection is accomplished using 30 mls of a 1:3 solution of TFA: DCM which also contains a small amount of Indole (less than i mg/ml). Indole is essential if tryptophan is present in the peptide. But, Indole is always included to prevent any oxidative effect of the TFA on the peptide and to scavenge harmful contaminants in the TFA. The reagent is allowed to stand overnight before use. Half of the reagent (15mls) is added to the resin for a 1.5 minute pretreatment, drain and the remaining solution add to the resin for 30 minutes. Step 4 can be started during this half hour.

4. The Boc-amino acid anhydride is prepared outside of the reactor in a small vial. Weigh out an amount of Boc-amino acid which gives a desired anhydride to resin sites mole ratio. Dissolve the amino acid in minimum amount of DCM. The amino acid solution and a 1 M DCC (0.206 g DCC/ml DCM) are then cooled to 0°C. After being cooled, DCC solution is added to the amino acid solution such that the number of moles of DCC is half the moles of amino acid, i.e.

1:2 mole ratio DCC: amino acid. The solution is then kept at 0°C for at least one hour with occasional shaking.

5. Deprotection is followed by 6 DCM washes each of 15 mls. Additional washes should be added if the resin still remains purple coloring from the indole. Usually the longer the TFA/DCM with indole solution prepared, the deeper the purple color of the resin. The resin can be left overnight after this step. The final peptide-resin should be left deprotected.

6. The peptide is next neutralized by two treatments with a 1:9 solution of TEA: DCM. Each rinse is 15 mls and should be about 1.5 minutes in duration. The remaining steps should be carried out as quickly as possible.

7. Neutralization is followed by 6 DCM washes, 15 mls each. During these washes, preparations for the following steps should be made. This includes getting the spectrophotometer ready (Step 8) and having prepared the known concentration of the symmetrical anhydride solution.

8. With both channels completely empty, the ultraviolet spectrophotometer is turned on as instructed in the users' manual. This should be done 1 hour before measuring because of the time needed for warming-up the machine and background light correction. Set the UV wavelength to the appropriate value from the calibration curve. Clean both cells and fill with DCM and insert into the U.V. compartment, zero the absorbance. At this point, the front cell is ready for maesuring the initial absorbance reading of symmetrical anhydride solution. This is done in order to check with

the calibration data and give the initial point of the UV-absorbance curve.

9. Turn on the chart recorder. Adjust the zero setting, chart paper speed, recording scale and set remote control on.

10. After the six washes, dry the resin by draining as much as possible. Use the pump to remove solvent from tubing and stopcock. To have resin in swelled state and to avoid preferential solvent absorption, accurately measure amount of DCM (about half of reaction volume) was added to resin.

11. To prepare the anhydride for coupling, the dicyclohexyurea (DCU) precipitate must be removed. First, the anhydride is allowed to warm to room temperature, this prevents moisture from condensing into the anhydride solution. Anhydrides are very water sensitive and should be exposed to air as little as possible. Quickly filter the anhydride solution through filter paper with fast filter speed. Rinse the vial with DCU and DCM and bring the concentration of anhydride to desired value. Empty the front standard cell and fill with the initial concentration of symmetrical solution, record the initial absorbance reading.

12. Insert the clean flow cell which has already been connected to the reactor as shown in Figure 2. Pump the DCM in the reactor through the whole monitoring loop. Zero the absorbance reading. At this point, the environment for measuring the absorbance is the same as step 8 and the absorbance caused by DCM is zeroed. When this is all set, let the recorder paper start running. Although the UV cutoff of DCM is 233 nm, which is much lower than

the working wavelength, this act can take the noise of absorbance caused by cell or DCM out of consideration.

13. As simultaneously as possible, dump anhydride into the reactor. At this time, the stirrer speed and the reaction temperature are all at desired values. Press on the UV run key which starts the measuring of absorbance.

14. No change in U.V. absorbance for about 5 minutes is assumed to represent completion of reaction. Drain the reaction solution, clear the monitoring loop by pumping through DCM. Shut everything off. Rinse pump with ethanol.

15. Wash resin with 15 mls of DCM 3 times. Finally, the resin can be left suspended in DCM before the next synthesis step.

16. Test for completeness of reaction. A modified version of Stahl, Walter and Smith's test gives qualitative results. A 1% solution of picrylsulfonic acid is prepared in fresh DMF. Approximately a 2 mg sample is placed in a very small test tube and two drops od 10% diisopropylethylamine in chloroform solution is added. After 10 minutes at room temperature, 1 ml of ethanol is added and the resin beads are viewed through a magnifier. All colors should be associated with the beads. A positive test (little coupling) is indicated by a bright red color. (approximately 0.5 mmol/g) to a faint yellow for almost complete coupling (0.001 mmol/g).

17. The reading in step 11 is the initial absorbance reading, and the final reading in step 14 are supplied as the bases for 100% reaction as if the step 16 had a negative, complete result.

CHAPTER IV

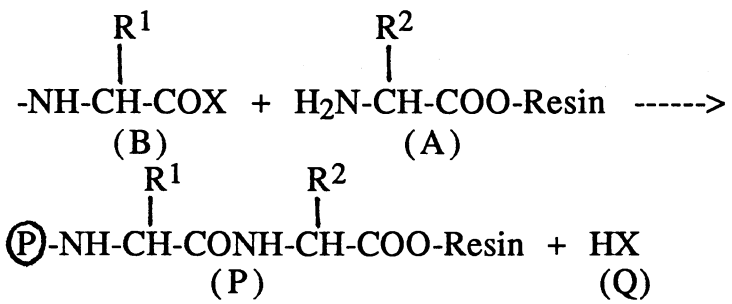
DEVELOPMENT OF THE MATHEMATICAL MODEL

As described in the Experimental Procedure in Chapter III, we have a reactor which contains the polystyrene resin beads, with the first amyl residue already attached and analysed. Then the repetitive synthesis steps outlined by Stewart and Young (33), which include swelling, rinsing, deprotection and neutralization are followed. The prepared amino acid symmetrical anhydride is then added to the reactor for the coupling reaction. The anhydride from the bulk liquid diffuses through the resin pores and a second order irreversible reaction occurs between the symmetrical anhydride and the active site on the resin. Since the symmetrical anhydride has limited presence in the fluid surrounding the resin, its concentration in the bulk fluid changes with time. The process is illustrated in Figure 3.

The coupling reaction that takes place can be expressed as

$$A + B \text{ -----> } P + Q$$

or more specifically it can be written as



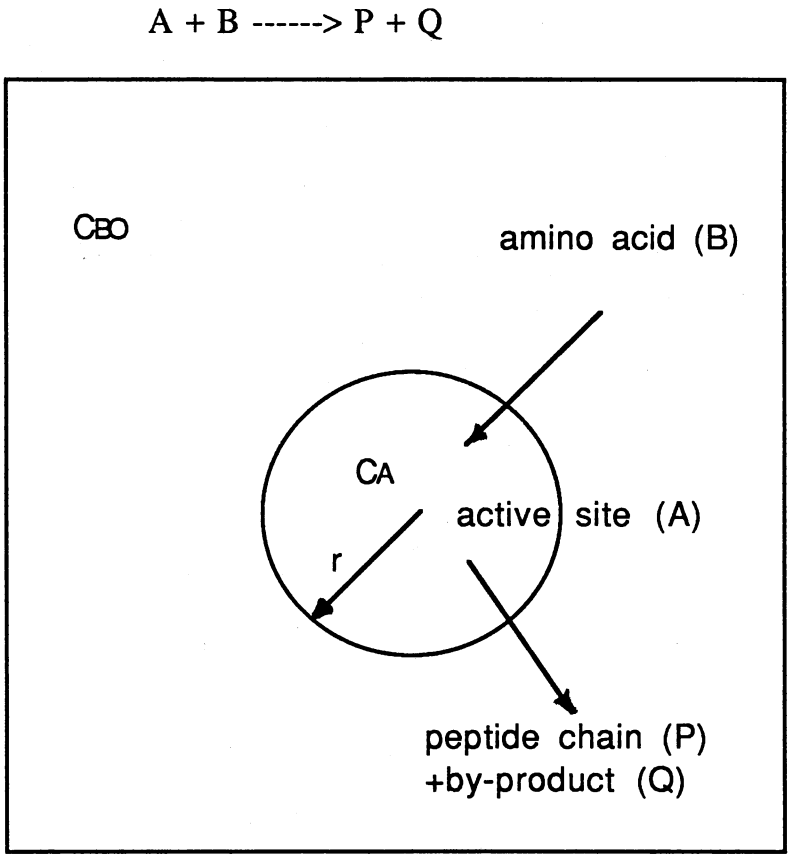


Figure 3. Schematic Illustration of the Combined Reaction and Diffusion in a Spherical Resin. The active site lies on the resin, The amino acid is added to the reactor in the solution. The peptide chain remains attached to the resin and the by-product diffuses back into the solution.

The reaction can be followed by measuring either the decrease of the amino acid derivative, (B), the decrease of the active site on the resin or the free peptide chain, (A), the increase of the newly incorporated amino acid, (P), or the increase of the by product, (Q). P represents the protection group and R^1 and R^2 are side chain groups of the amino acid.

In this study, the diffusion and reaction are assumed to proceed radially into the resin. A general diffusional model can be obtained by doing a material balance in spherical coordinates. The material balance equations contain radial diffusion, reaction term and model concentration changes with respect to position and time.

The equation of continuity is

$$\frac{\partial C_A}{\partial t} + \left(V_r \frac{\partial C_A}{\partial r} + V_\theta \frac{1}{r} \frac{\partial C_A}{\partial \theta} + V_\phi \frac{1}{r \sin\theta} \frac{\partial C_A}{\partial \phi} \right) = D_{AB} \left[\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_A}{\partial r} \right) + \frac{1}{r^2 \sin\theta} \frac{\partial}{\partial \theta} \left(\sin\theta \frac{\partial C_A}{\partial \theta} \right) + \frac{1}{r^2 \sin\theta} \frac{\partial^2 C_A}{\partial \phi^2} \right] + R_A \qquad (1)$$

In this equation, the assumptions that are made can be tabulated as

1. The density and diffusivity in the bulk phase are constant.
2. Unsteady state.
3. Isothermal reaction.
4. The fluid flow in the θ and ϕ direction is uniform or

symmetrical. The concentration change along that direction then will be zero.

5. Zero velocity. This assumption is generally valid for diffusion in solids or stationary liquids.
6. No surface resistance to mass transfer. Film diffusion is negligible.
7. Finally, the reaction rate is given by $-KC_B^n$ which is a general order reaction term. In other words, we are assuming C_A equal to C_B.

After considering these assumptions in the general equation, we may have a simplified equation, in terms of B or the amino acid symmetrical anhydride

$$\frac{\partial C_B}{\partial t} = D_{AB} \left[\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_B}{\partial r} \right) \right] - KC_B^n$$

This equation is further made dimensionless

$\xi = \frac{r}{R}$, the dimensionless radial position

$\tau = \frac{t D_{AB}}{R^2}$, dimensionless time

$U = \frac{C_B}{C_{B0}}$, dimensionless concentration

$A = \frac{KC_{B0}^{n-1} R^2}{D_{AB}}$, dimensionless diffusion parameter

Finally, for general order kinetics, the equation that describes the concentration profile inside the spherical particle is

$$\frac{\partial U}{\partial \tau} = \frac{2}{\xi}\frac{\partial U}{\partial \xi} + \frac{\partial^2 U}{\partial \xi^2} - AU^n \qquad (2)$$

This is the governing equation which will be solved later on.

Mathematically, the initial and boundary conditions can be stated as follows:

Initially, there is no reactant present in the spherical resin. The concentration at every position inside the resin is zero. If we denote the location as ξ, the initial condition will be

$$U(\xi<1,\ \tau=0) = 0 \qquad (3)$$

Since we consider diffusion to be proceeding radially inside the spherical particle, the concentration at the surface of the sphere is equal to the initial concentration in the bulk phase liquid. Our first boundary condition will then be

$$U(\xi=1,\ \tau>0) = 1.0 \qquad (4)$$

The reactant has limited presence in the bulk fluid and therefore its concentration is a function of time. As time increases, it undergoes diffusion and reaction with the active site on the resin. Accordingly, its' concentration falls by an amount given by the amount that reacts which is given by $K\ C_{BO}^2\ (1-X_A)(M-X_A)$,where K is the second order reaction rate constant, M is the excess mole ratio, X_A, is the fraction of active site reacted and C_{BO} is the initial concentration of the symmetrical anhydride.

The second boundary is that at the centre of the sphere, the concentration of the reactant will be some finite number. This finite number lies in between zero and one. This, therefore can be stated as

$$U(\xi = 0,\ \tau > 0) = \text{finite}$$

or

$$\frac{\partial U}{\partial \xi}(\xi = 0,\ \tau > 0) = 0 \qquad (5)$$

Equation (2) with the boundary conditions Equations (3) to (4) will be solved numerically. The detailed procedure will be described in the next section and a computer code is given in Appendix A.

Review of the Numerical Method Theory

There are many numerical methods for solving partial differential equations. Of these, only one stands out as being universally applicable to both linear and non-linear problems, this is the method of finite differences. In this work, only the finite difference will be considered. Since, the partial differential equation derived in the last chapter is characterised as a parabolic partial differential equation, the methods which are considered will restricted in this category.

The approach to solving a parabolic partial differential equation by a numerical method is to replace the partial derivative by finite difference approximations. The simplest form of the approximations are:

$$\frac{\partial U}{\partial \tau} = \frac{U_i^{j-1} - U_i^{j}}{\Delta \tau} \qquad (6)$$

$$\frac{\partial^2 U}{\partial \xi^2} = \frac{U_{i+1}{}^j - 2U_i{}^j + U_{i-1}{}^j}{(\Delta\xi)^2} \qquad (7)$$

$$\frac{\partial U}{\partial \xi} = \frac{U_{i+1}{}^j - U_{i-1}{}^j}{2(\Delta\xi)} \qquad (8)$$

If these expressions are substituted into Equation (2) and the boundary conditions specified in Equations (4) and (5) are applied, the values of U at the grid points can be calculated provided $\Delta\tau/(\Delta\xi)^2$ is less than or equal to one half. If this value is greater than one half, the difference equation becomes unstable. The method presented above is an explicit method because concentration at a new time can be immediately calculated from quantities that are already known (either from boundary conditions or from previous calculations). It is a simple and economical method of calculation, but the step size in the t-direction is limited to a small value. Note that a mixed order of errors was involved in equation (6) to (8). A forward difference was used to approximate $\frac{\partial U}{\partial \tau}$ while a central difference was used for $\frac{\partial U}{\partial \xi}$ and $\frac{\partial^2 U}{\partial \xi^2}$. Figure 4 shows the explicit method needs only three previous known values at time, j, to calculate the value at time, j+1. There is therefore, considerable interest in the so called implicit difference method. These implicit methods seem to have been used for the first time by Crank and Nicolson in 1974. The order of error for every term will be the same in the

implicit method, leading to better stability with bigger step size. The method is briefly illustrated below.

If the second derivative with respect to ξ is replaced by the second difference quotient, not at the $\tau + \Delta\tau$ but at $\tau + \Delta\tau/2$, then the second derivative with respect to ξ can be approximated by averaging the difference quotient at the beginning and at the end of the τ - step. Figure 5 shows the implicit method needs three previous values at time, j, and also the values at (i-1, j+1) and (i+1, j+1). However, these last two values in the implicit method introduce great difficulty since these are the values at the current time step which have not been determined yet.

$$\frac{\partial^2 U}{\partial \xi^2} \simeq \frac{1}{2}\left[\frac{U_{i+1}^{j} - 2U_i^{j} + U_{i-1}^{j}}{(\Delta\xi)^2} + \frac{U_{i+1}^{j+1} - 2U_i^{j+1} + U_{i-1}^{j+1}}{(\Delta\xi)^2}\right] \qquad (9)$$

In that case, we do consider the difference expression (6) as a central difference approximation.

Similarily, Equation (8) must be replaced by

$$\frac{\partial U}{\partial \xi} \simeq \frac{1}{2}\left[\frac{U_{i+1}^{j} - U_{i-1}^{j}}{2(\Delta\xi)} + \frac{U_{i+1}^{j+1} - U_{i-1}^{j+1}}{2(\Delta\xi)}\right] \qquad (10)$$

to show central difference approximation. After substituting these expressions [Equation (6), (9) and (10)] into the differential equation (2), it is seen that the unknowns can no longer be solved for explicitly. This is unsuitable for problems in which infinite ξ - regions appear. But for problems with a finite ξ - interval, like what we have in this study, can be handled by means of an implicit method. To that end, we can write the difference

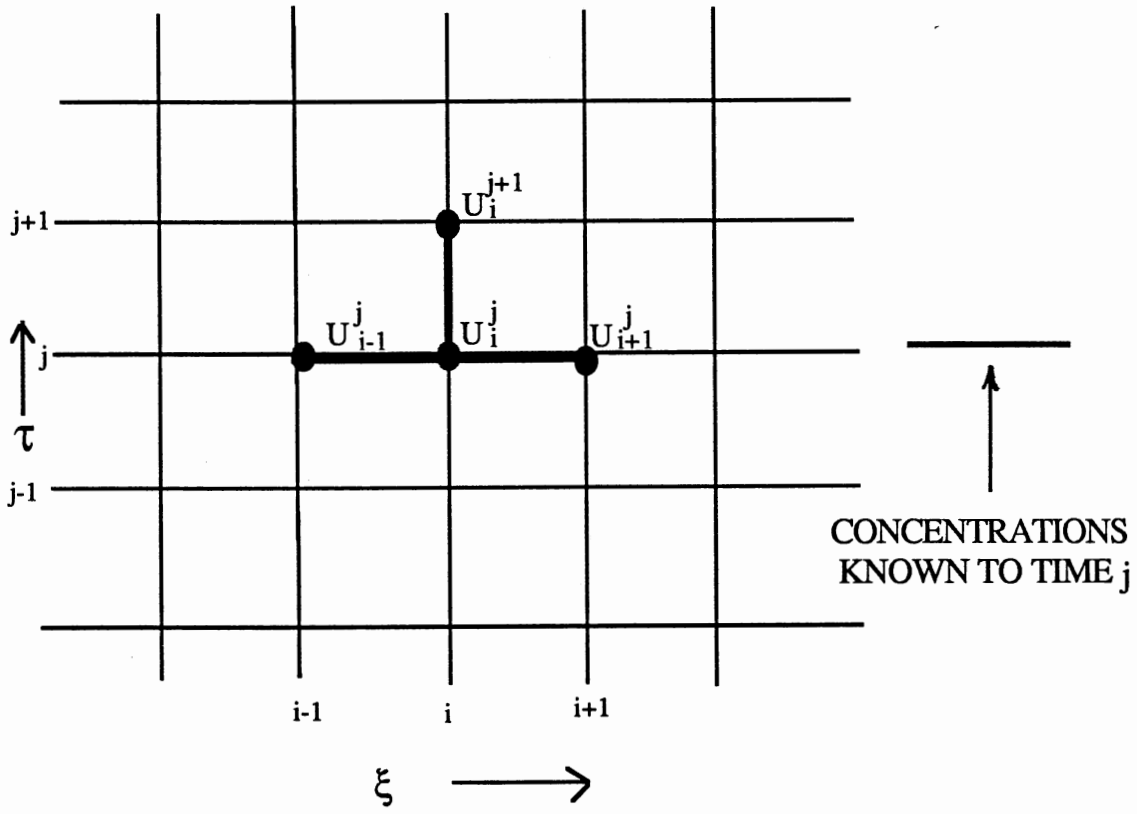


Figure 4. Computational Molecule For Explicit Finite Difference

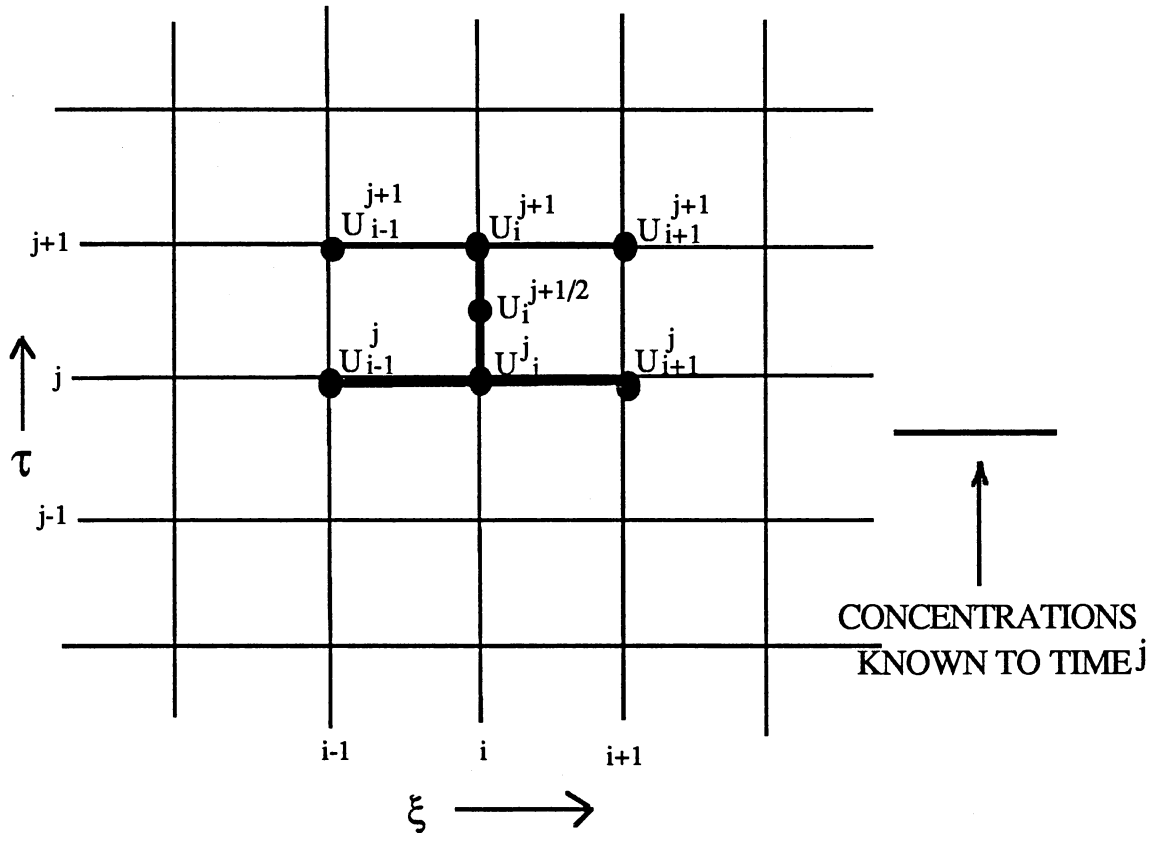


Figure 5. Computational Molecule For Implicit Finite Difference

equation for each grid point and interpret them as a system of simultaneous algebraic equations. The number of unknowns will be equal to the number of equations, which is equal to the number of grid points in each τ- step. If values of U at the grid points of the level τ are already known, the values for $\tau + \Delta\tau$ can be found by solving this algebraic system, provided the determinant is not equal to zero. The numerical solution of systems of algebraic equations is a large and widely studied subject and will not be discussed here.

Fortunately, the system of equations created by this implicit difference method is such that the matrix of the system has zeros everywhere except on the main diagonal and on the two diagonals parallel on either side. Such a matrix is sometimes called tridiagonal. This special situation saves a great deal of computational work when solving the equation set.

Calculational Procedures

The grids obtained by dividing in both τ and ξ dimensions for this derivation are shown in Figure 1. The difference equation is derived by substituting the following expressions into the partial difference equation (2):

$$\frac{\partial U}{\partial \tau} = \frac{U_i^{j+1} - U_i^{j}}{\Delta\tau}$$

$$\frac{\partial U}{\partial \xi} = \frac{1}{2}\left[\frac{U_{i+1}^{j} - U_{i-1}^{j}}{2(\Delta\xi)} + \frac{U_{i+1}^{j+1} - U_{i-1}^{j+1}}{2(\Delta\xi)}\right]$$

$$\frac{\partial^2 U}{\partial \xi^2} = \frac{1}{2}\left[\frac{U_{i+1}^{j} - 2U_i^{j} + U_{i-1}^{j}}{(\Delta\xi)^2} + \frac{U_{i+1}^{j+1} - 2U_i^{j+1} + U_{i-1}^{j+1}}{(\Delta\xi)^2}\right]$$

$$U^n = (U_i^j)^{n-1}\ U_i^{j+1}$$

After substituting Eq. (11) into Eq. (2) and simplifying as

$$\frac{U_i^{j+1} - U_i^{j}}{\Delta\tau} = \frac{2}{\xi}\frac{1}{2}\left[\frac{U_{i+1}^{j} - U_{i-1}^{j}}{2(\Delta\xi)} + \frac{U_{i+1}^{j+1} - U_{i-1}^{j+1}}{2(\Delta\xi)} + \frac{1}{2}\left[\frac{U_{i+1}^{j} - 2U_i^{j} + U_{i-1}^{j}}{(\Delta\xi)^2} + \frac{U_{i+1}^{j+1} - 2U_i^{j+1} + U_{i-1}^{j+1}}{(\Delta\xi)^2}\right] - A(U_i^{j})^{n-1}\,[U_i^{j+1}]\right]$$

Taking all j+1 terms on the L. H. S. and all the j terms on the R. H. S., we get:

$$\frac{1}{\Delta\tau}U_i^{j+1} - \frac{1}{2\xi\Delta\xi}U_{i+1}^{j+1} + \frac{1}{2\xi\Delta\xi}U_{i-1}^{j+1} - \frac{1}{2\Delta\xi^2}U_{i+1}^{j+1} + \frac{1}{\Delta\xi^2}U_i^{j+1} - \frac{1}{2\Delta\xi^2}U_{i-1}^{j+1} + A(U_i^{j})^{n-1}\,[U_i^{j+1}] = \frac{U_i^{j}}{\Delta\tau} + \frac{1}{2\xi\Delta\xi}U_{i+1}^{j} - \frac{1}{2\xi\Delta\xi}U_{i-1}^{j} + \frac{1}{2\Delta\xi^2}U_{i+1}^{j} - \frac{1}{\Delta\xi^2}U_i^{j} + \frac{1}{2\Delta\xi^2}U_{i-1}^{j}$$

Finally, the following difference equation is derived:

$$\left[\frac{1}{2\xi\Delta\xi} - \frac{1}{2\Delta\xi^2}\right]U_{i-1}^{j+1} + \left[\frac{1}{\Delta\tau} + \frac{1}{\Delta\xi^2} + A(U_i^{j})^{n-1}\right]U_i^{j+1} - \left[\frac{1}{2\xi\Delta\xi} + \frac{1}{2\Delta\xi^2}\right]U_{i+1}^{j+1} = -\left[\frac{1}{2\xi\Delta\xi} - \frac{1}{2\Delta\xi^2}\right]U_{i-1}^{j} + \left[\frac{1}{\Delta\tau} - \frac{1}{\Delta\xi^2}\right]U_i^{j} + \left[\frac{1}{2\xi\Delta\xi} + \frac{1}{2\Delta\xi^2}\right]U_{i+1}^{j} \qquad (12)$$

If the $\Delta\tau$ is thus chosen so that the coefficient of U_i^j can be equal to zero, Eq. (12) can further be simplified.

The $\Delta\tau$ then will be

$$\Delta\tau = \Delta\xi^2$$

Subtituting this into Eq. (12) gives us the following equation:

$$[\frac{\Delta\xi}{\xi} - 1\ ]\ U_{i-1}^{j+1} + [\ 4 + A(U_i^j)^{n-1}\ 2\Delta\xi^2\ ]\ U_i^{j+1} - [\frac{\Delta\xi}{\xi} + 1\ ]U_{i+1}^{j+1} =$$

$$-[\frac{\Delta\xi}{\xi} - 1\ ]\ U_{i-1}^{j} + [\frac{\Delta\xi}{\xi} + 1\ ]U_{i+1}^{j} \qquad (13)$$

For the equations at the end points, we create fictitious boundary conditions.

So for i = 1, the equation becomes

$$[\frac{\Delta\xi}{\xi} - 1\ ]\ U_L^{j+1} + [\ 4 + A(U_1^j)^{n-1}\ 2\Delta\xi^2\ ]\ U_1^{j+1} - [\frac{\Delta\xi}{\xi} + 1\ ]U_2^{j+1} =$$

$$-[\frac{\Delta\xi}{\xi} - 1\ ]\ U_L^{j} + [\frac{\Delta\xi}{\xi} + 1\ ]U_2^{j}$$

and for i = N, where N is number of grid points, we have

$$[\frac{\Delta\xi}{\xi} - 1\ ]\ U_{N-1}^{j+1} + [\ 4 + A(U_N^j)^{n-1}\ 2\Delta\xi^2\ ]\ U_N^{j+1} - [\frac{\Delta\xi}{\xi} + 1\ ]U_{N+1}^{j+1} =$$

$$-[\frac{\Delta\xi}{\xi} - 1\ ]\ U_{N-1}^{j} + [\frac{\Delta\xi}{\xi} + 1\ ]U_{N+1}^{j}$$

For the first boundary condition

$$\left.\frac{\partial U}{\partial \xi}\right|_{\xi=0} = 0$$

$$U_2^{j+1} = U_L^{j+1}$$

$$\frac{U_2^j - U_L^j}{2\Delta\xi} = 0$$

The second boundary condition gives

$$U_{NP}^{j+1} = 1.0$$

Substituting for i = 1 and for i = N we get,

$$[\,4 + A(U_1^j)^{n-1}\,\Delta\xi^2\,]\,U_1^{j+1} - (2)U_2^{j+1} = (2)U_2^j \qquad (14)$$

and

$$[\frac{\Delta\xi}{\xi} - 1\,]\,U_{N-1}^{j+1} + [\,4 + A(U_N^j)^{n-1}\,2\Delta\xi^2\,]\,U_N^{j+1} =$$

$$-[\frac{\Delta\xi}{\xi} - 1\,]\,U_{N-1}^{j} + [\frac{\Delta\xi}{\xi} + 1\,]U_{N+1}^{j} + [\frac{\Delta\xi}{\xi} + 1\,] \qquad (15)$$

Note: U(j+1)'s are unknowns, while U(j)'s are known from boundary conditions or previous results.

To conserve storage usage in the computer, the tridiagonal matrix which is made up of the coefficients of Equations 14, 15 and 16 above together with the constant terms is compressed into an Nx4 matrix. Column one holds the coefficients to the left of the diagonal, column two holds the coefficients the diagonal terms, column three holds the coefficients to the right of the diagonal and column four holds the constant terms.

An interactive fortran program was developed to execute the above numerical job. The package is designed to run on IBM

personel computers which are inexpensive and widely available. The results are presented in the next chapter.

CHAPTER V

RESULTS AND DISCUSSION

In this section, the results of the numerical solution are presented and discussed by comparing them with experimental data. Numerical solutions for concentration of reacting species under a certain set of conditions was obtained as a function of position in the spherical particle, and time.

When using the implicit method in the numerical solution, the number of algebraic equations are equal to the number of grid. If the grid is small, the number of equations will be large. Fortunately, the set of equations thus created has a tridiagonal coefficient matrix which has zeros everywhere except on the main diagonal and on the two diagonals parallel to it on both sides. The special situation saves computation work when solving the equation set. The computer code for the numerical solution are based on some arbitrary chosen input values in the computer program. A sample output of the proposed model based on input values obtained from (5) for SPPS are presented in Appendix B.

The dimensionless bulk concentration profiles of the reactant undergoing a second order chemical reaction were made with the experimental results obtained for the reaction between the symmetrical anhydride and the active sites on the resin by Chen(5).

The experimental data was fit with the model by varying the parameters, K, the reaction rate constant and A, the ratio of the rate constant and the diffusion coefficient. The comparison of the experimental and model results are presented in Appendix C.

Effect of Input Parameters

Numerical solutions for concentration of the reacting species was obtained by first keeping the concentration of the reacting species in the bulk liquid constant. This was fixed at the initial concentration of the reactant introduced in the reactor. Based on the above boundary condition at the surface of the spherical particle, concentration versus time profiles for various A values at a fixed radial position inside the particle are shown in Figure 1 and 2. The concentration starts to increase from zero, which is the initial boundary condition, and very quickly attains a stable value of concentration. This rise in concentration takes place very quickly within a few units of dimensionless time approximately one unit of dimensionless time. In these profiles , it can be seen that the curves have the same trend but they lie very close together for values of A ranging from 0 to 5 It is evident from these plots that the value of A does not significantly change the final conversion. Figure 6. shows the model results for the case of a first order chemical reaction where as Figure 7. is for a second order reaction.

Therefore, although it would seem that diffusion is a significant phenomena in the SPPS process, the model results are not in agreement with the experimental data. This disagreement could be

This disagreement could be attributed to the assumption of a constant boundary condition at the surface of the particle. This is valid for the case when we have a arge excess of reactant in the bulk liquid or we have a continuous reactor. But SPPS is a batch process and also the reactant has limited presence in the bulk liquid. Therefore, we adjust the numerical solution by incorporating a derivative boundary condition.at the surface. So, we have a decrease in the concentration of the reactant as the time increases. The decrease in concentration is proportional to the amount that is being consumed by reaction with the active site. Now as the driving force decreases, the reaction will increase. Thus with the continuous decrease in the driving force, the time taken to reach the final concentration or to bring about the conversion will be increased. Therefore we can calculate the reaction rate constants as predicted by the model and compare them to the values that were calculated from experimental results. The value of the second order reaction rate constant calculated from experimental results is on the order of 0.5- 8 mole/litre-sec.

In Figure 8 and Figure 9, experimental data is plotted by keeping the value of the reaction rate constant at 0.01, but varying the value of the diffusivity coefficient. Since the dimensionless time is given by tD_{AB}/R^2, this changes the time as well. Therefore, we see that for a value of the diffusivity coefficient equal to $6x10^{-6}$ cm^2/s in Figure 8, the experimental points lies on the predicted curve. Figure 9 is for a value of $20x10^{-6}$ cm^2/s.

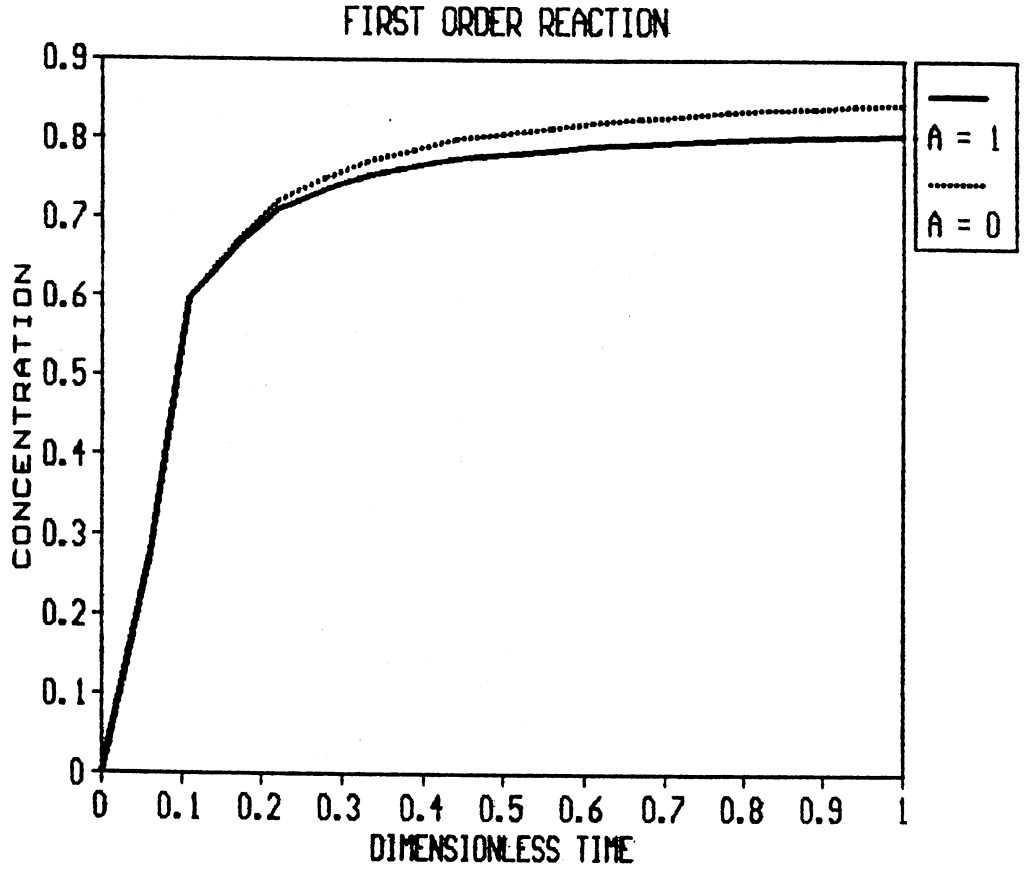


Figure 6. Dimensionless Concentration Profile for a First Order Chemical Reaction for a Constant Boundary Condition.

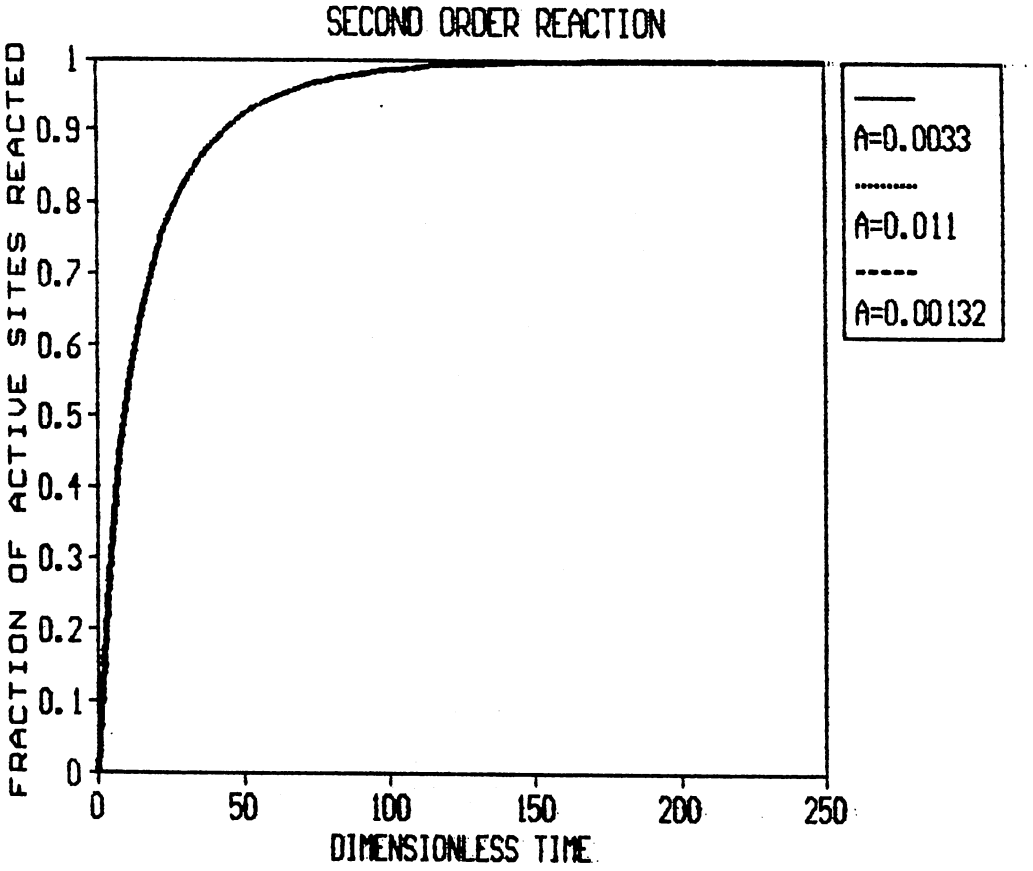


Figure 7. Dimensionless Concentration Profile for a Second Order Chemical Reaction for Constant Boundary Condition.

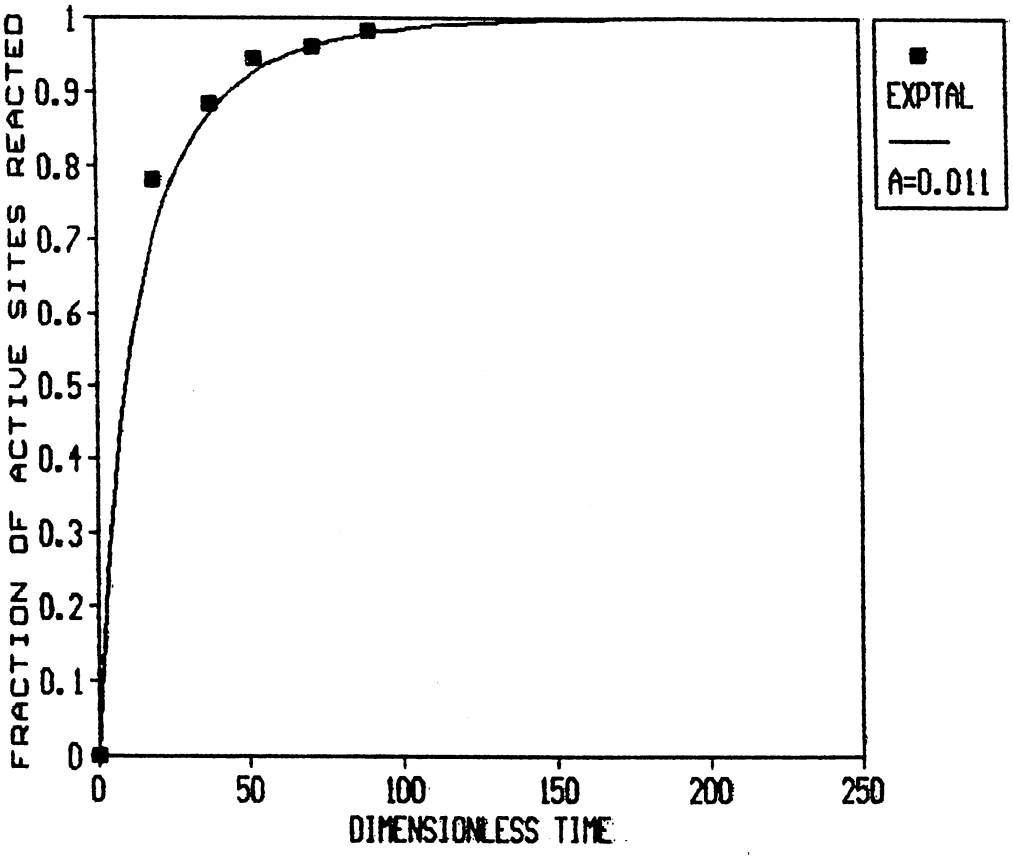


Figure 8. Model Comparison with data for Peptide I (Polyserine, n=1) for K = 0.01 and $D_{AB} = 6x10^{-6}$

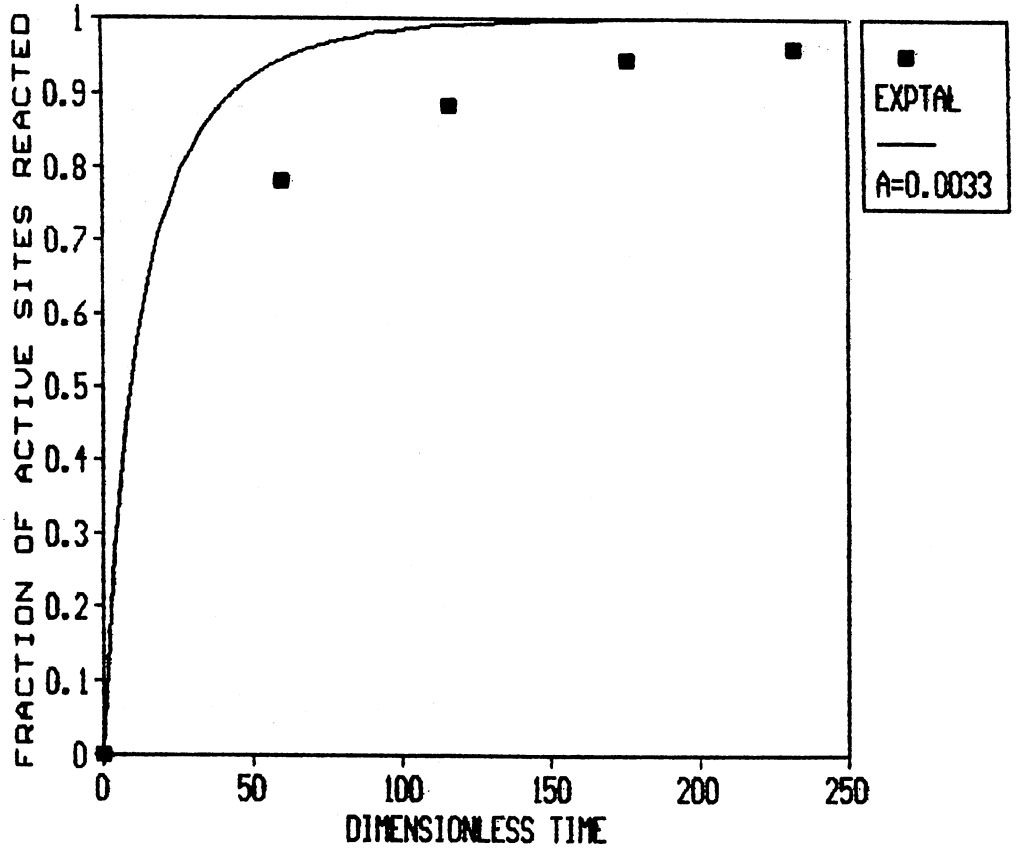


Figure 9. Model Comparison with data for Peptide I (Polyserine, n=1) for K = 0.01 and $D_{AB} = 20x10^{-6}$

Comparison with Experimental Results

Data obtained experimentally by Chen (5) was used as a check on the validity of the results obtained from the model. This would also give us an idea on the importance of diffusion in SPPS. The results from the numerical solution fit very well with the experimental data. The data compared was of a number of peptides synthesized experimentally. Table II lists the peptides sythesized and the specifications of synthesis and reaction materials.

TABLE II

LIST OF PEPTIDES SYNTHESIZED (5)

EXPT	Peptide	Reaction Temp. ^{o}C	Mixing Rate(RPM)	M.R.[a]	%CL[b]
Peptide I	$Poly(ser)_{10}$	26	200	1.5	2
Peptide II	$Poly(Phe)_{10}$	26	200	1.5	2
Peptide III	$Poly(ser)_{10}$	14	200	1.5	2
Peptide IV	$Poly(Phe)_{10}$	14	200	1.5	2
Peptide V	$Poly(Phe)_{10}$	26	200	1.2	2

a. Mole Ratio, Amino Acid Symmetrical Anhydride to Amino terminus of peptide resin.
b. Percent divinylbenzene crosslinking of polystyrene resin.

Commercially available divinylbenzene(DVB) polystyrene copolymer resin with the first amino acid attached were used as starting materials for the synthesis. The dry resin size was 200-400 mesh and the loading of the first amino acid on the resin varies, which is specified by the supplier, SIGMA Chemical Company. The relative volume of the resin and external solution was about 1:6 for 1.3 g resin in 30 ml reactor volume, depending on the peptide content. For a detailed description of experimental procedures, refer to Chapter III.

By varying the values of the reaction rate constant, the model was used to fit the experimental data of the previously mentioned peptides under the specified conditions. Table III-Table VII give a comparison of the values of percent of active sites reacted versus time between the experimental results and those predicted by the model. These tables are given in Appendix C.

This information in these tables can be shown with the help of plots also. (See Figures 6 to 25). Hence it is evident that the second order reaction rate constants fall in the range of 0.01-0.1 mole/litre.sec. The experimentally calculated second order reaction rate constant is 0.5-8 mole/litre.sec. The difference in the order of magnitude is due to the phenomena of diffusion being significant. The experimentally calculated rate constant takes diffusion as well as reaction into account.

Reaction Parameters

The reaction parameters that will be discussed in this section include chain length of peptide, reaction temperature, excess mole ratio between the amino terminus and the symmetrical anhydride. Emphasis will be to see if the phenomena observed experimentally can be explained by the
model results.

Peptide Chain Length

Results of the synthesis of homopolyserine and homopolyphenylalanine to residue number 7 were compared with numerical results (Peptide I, II ,III and IV). Figure 10 to Figure 13 illustrated that the reaction rate decreases as the chain length increases. This phenomena is true for both polyserine and polyphenylalanine with the reaction rate for serine being faster. The side chain of each amino acid has the ability of affecting electron transfer, and the impact of secondary structure of peptide is also to be considered on peptide chain length on coupling rate. As the peptide chain length increases, it forms a spiral around the resin. There is no indication that the resin matrix becomes filled with peptide as it grows, nor does the efficiency of the synthesis become limited by steric effect. The steric hindrance may be because of diffusion limitation in the polymer network. The coupling rate also depends on the reactivity of the amino acid, for instance , the high reactivity of Ser result in faster reaction rate for n=1 than n=5.

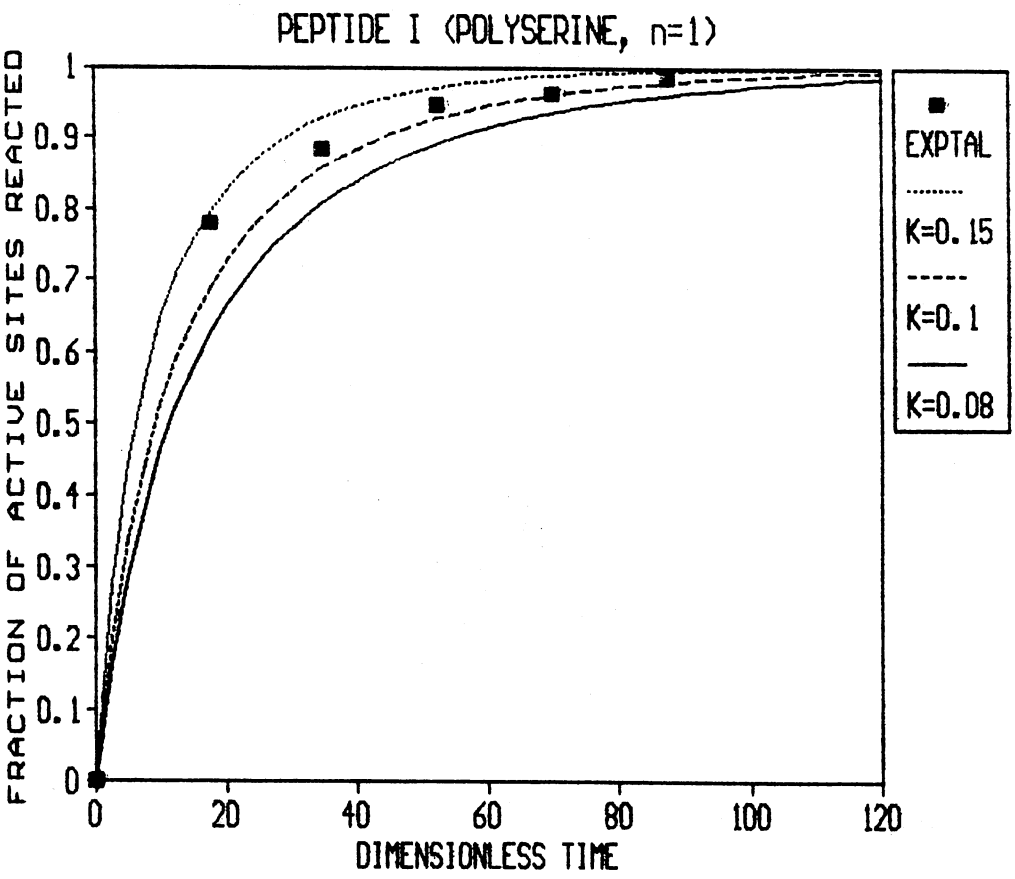


Figure 10. Model Comparison with Data for Peptide I(polyserine, n=1)

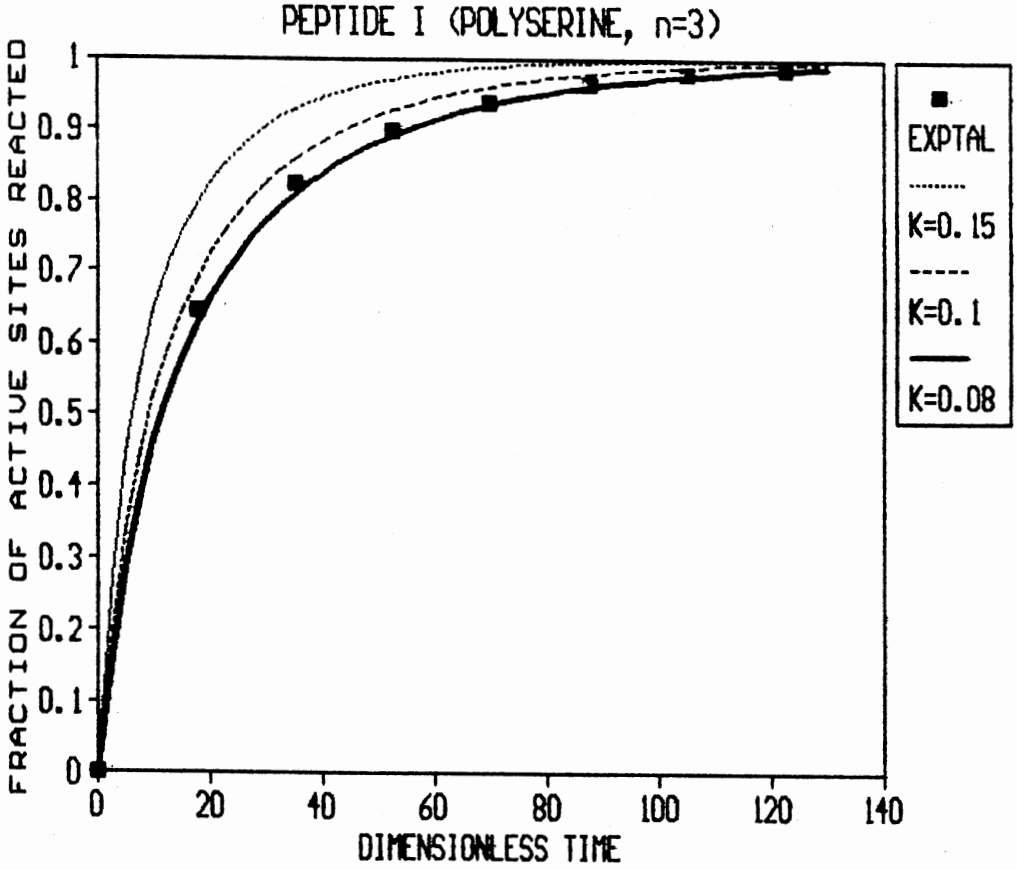


Figure 11. Model Comparison with data for Peptide I(Polyserine, n=3)

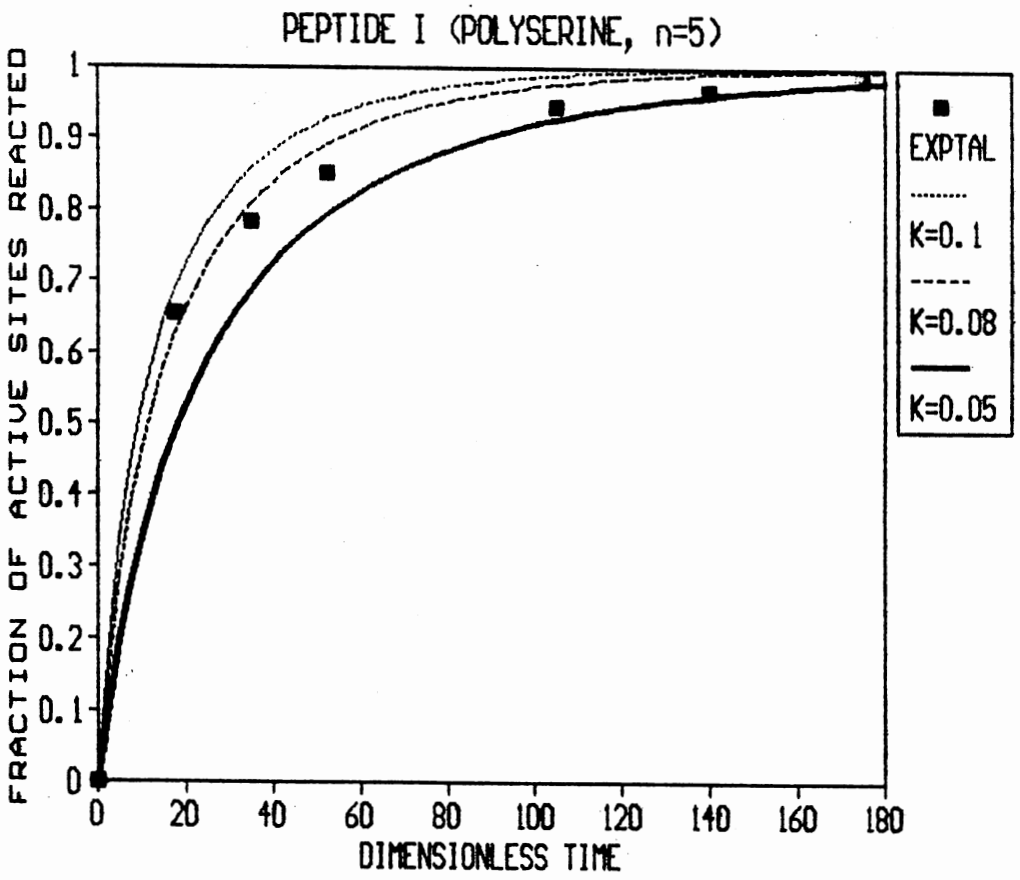


Figure 12. Model Comparison with Data for Peptide I(Polyserine, n=5)

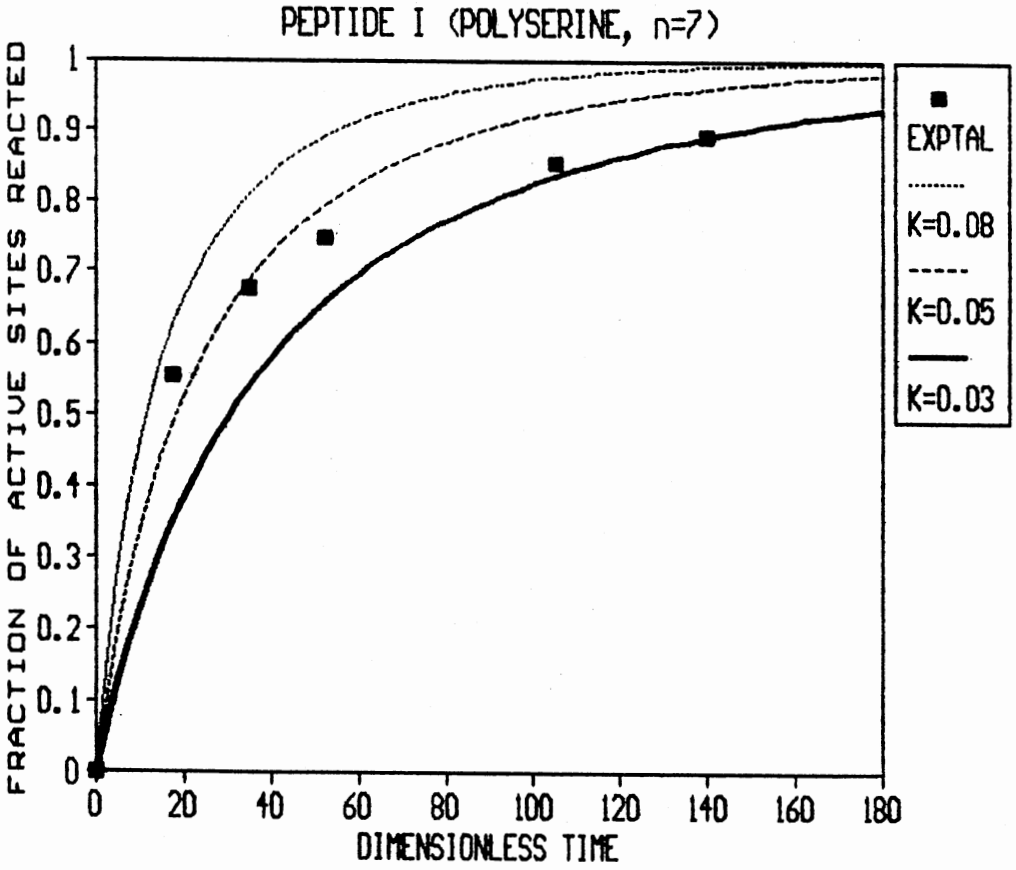


Figure 13. Model Prediction with Data for Peptide I(Polyserine, n=7)

Reaction Temperature

Experimental results of Peptide I and Peptide II were repeated at a lower reaction temperature of 14°C. These were compared with and fit with the model results in Figures 14 through 17 and Figures 22 through 25. Similar chain length effects were observed and by comparing figure 14 and figure 22, the lower the temperature, the lower the reaction rate. This effect was enhanced as the chain length was increased. Thus we can say that a lower temperature decreases the coupling rate with the effect enhanced as the peptide chain grows. This phenomena provides indirect evidence for the microphysical nature of coupling difficulties such as the freedom of peptide chain vibrations and physical aggregates due to hydrogen bonding. Based on an ideal second order reaction rate approximation, the apparent activation energy for the synthesis of polyphenylalanine at residue number 3 is 2.9 Kcal/gmole (see Reference 5, Appendix D) which indicated a temperature insensitive, intraparticle diffusion, may be the reaction control step. However, a sharp increase in reaction time at a latter stage of coupling and attachments imply a higher value of activation energy which may be attributed to the collision rate decrease caused by secondary structure.

Mole Ratio

Excess mole ratios of 1.5 and 1.2, symmetrical anhydride to

reactive site or amino terminus, were performed for polyphenylalanine synthesis (Peptide II and V). These were fit by the model in Figures 14-17 and Figures 26-29. The excess carbonyl groups increase the coupling rate between amino acids, which indicates that the reaction step does not dominate the whole reaction process. In other words, the diffusion process of the reactant, including film diffusion and particle diffusion, are significant in reaction rate for this heterogeneous reaction, especially at the latter stage of synthesis of polyphenylalanine at $n \geq 4$. The film diffusion resistance has been ruled out in the range of mixing rate under investigation in this study. According to the model results, for greater excess mole ratio, the reaction rate was lower.

From the predictions of the model, we were able to calculate the values of the second order reaction rate constants. This was done by fitting the experimental data by varying values of K. Table III lists the reaction rate constants as they changed for different number of residues for the peptides that were synthesized experimentally.

TABLE III

RESULTS OF THE COMPARISON OF EXPERIMENTAL DATA WITH MODEL PREDICTIONS

Peptide No.	Number of residues attached (n)	Reaction Rate Const. (mole/l.sec)
I	1	0.1
	3	0.08
	5	0.05
	7	0.03
II	1	0.08
	3	0.05
	5	0.05
	7	0.01-0.03
III	1	0.08
	3	0.05
	5	0.03-0.05
	7	0.03-0.05
IV	1	0.05
	3	0.03
	5	0.01-0.03
	7	0.01
V	1	0.1
	3	0.03
	5	0.03

Therefore, a number of conclusions can be made from the above results. Firstly, this model for an ideal second order reaction rate can be fit with all the experimantal data upon variation of parameters. Second, K is a function of the amino acid, peptide chain length, reaction temperature and excess mole ratio. It also follows all the experimental trends and explains all the phenomena observed experimentally. Finally, it leads us to the conclusion that diffusion is a significant consideration in the SPPS process.

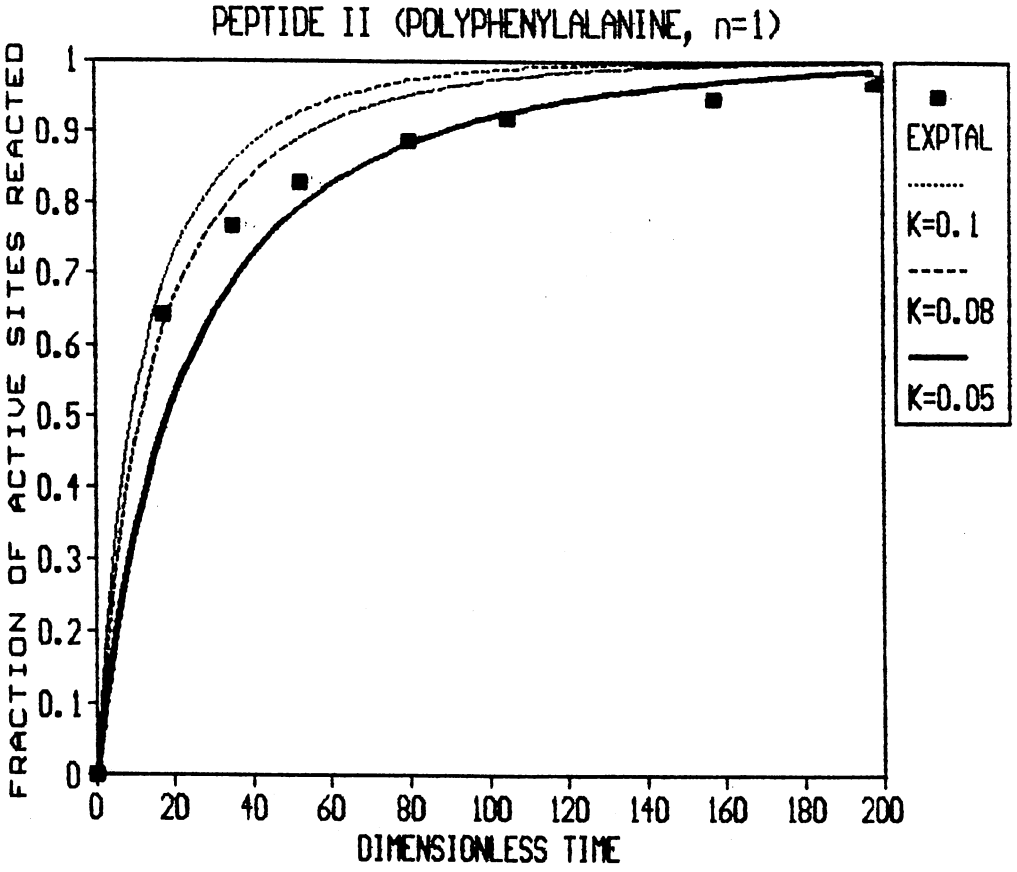


Figure 14. Model Comparison with Data for Peptide II(Polyphe, n=1)

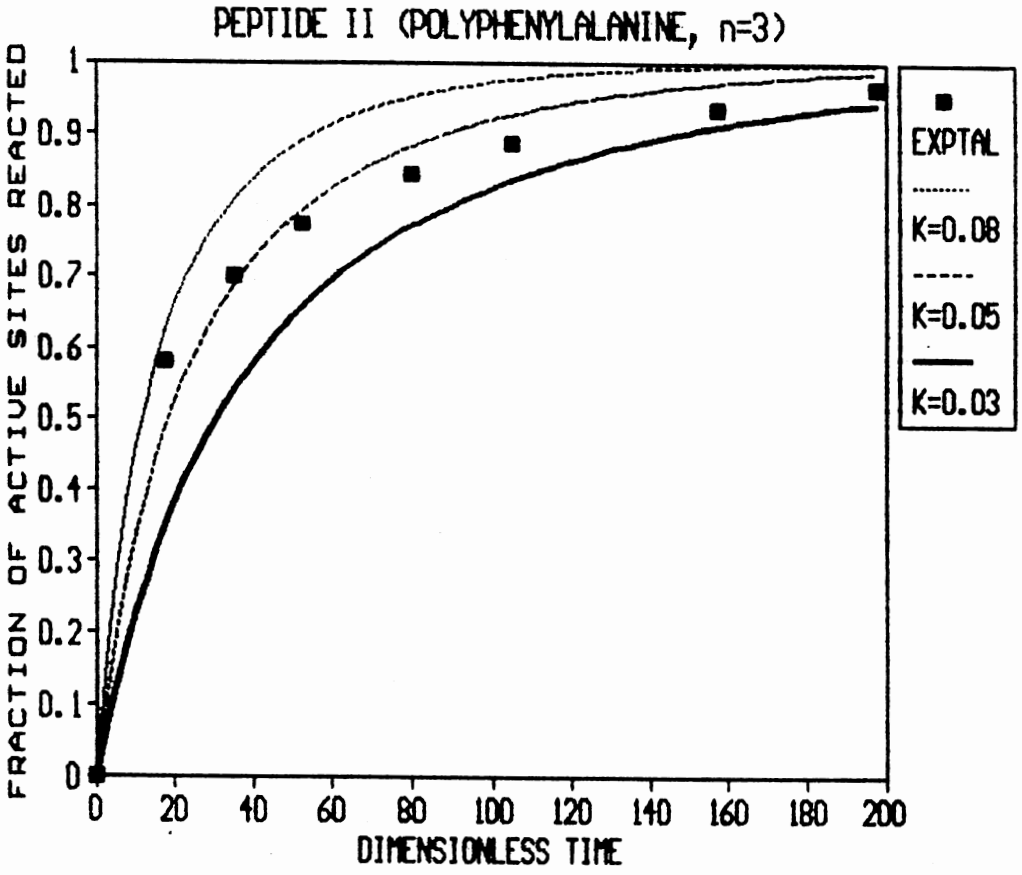


Figure 15. Model Comparison with Data for Peptide II(Polyphe, n=3)

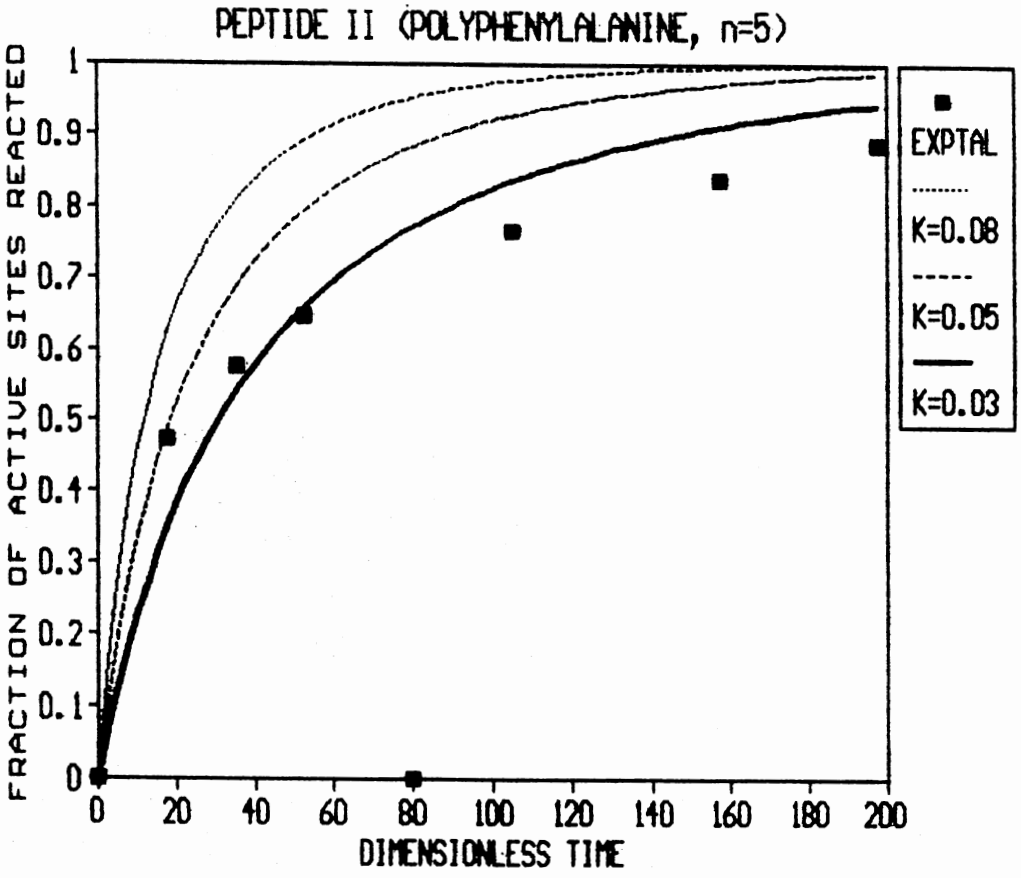


Figure 16. Model Comparison with Data for Peptide II(Polyphe, n=5)

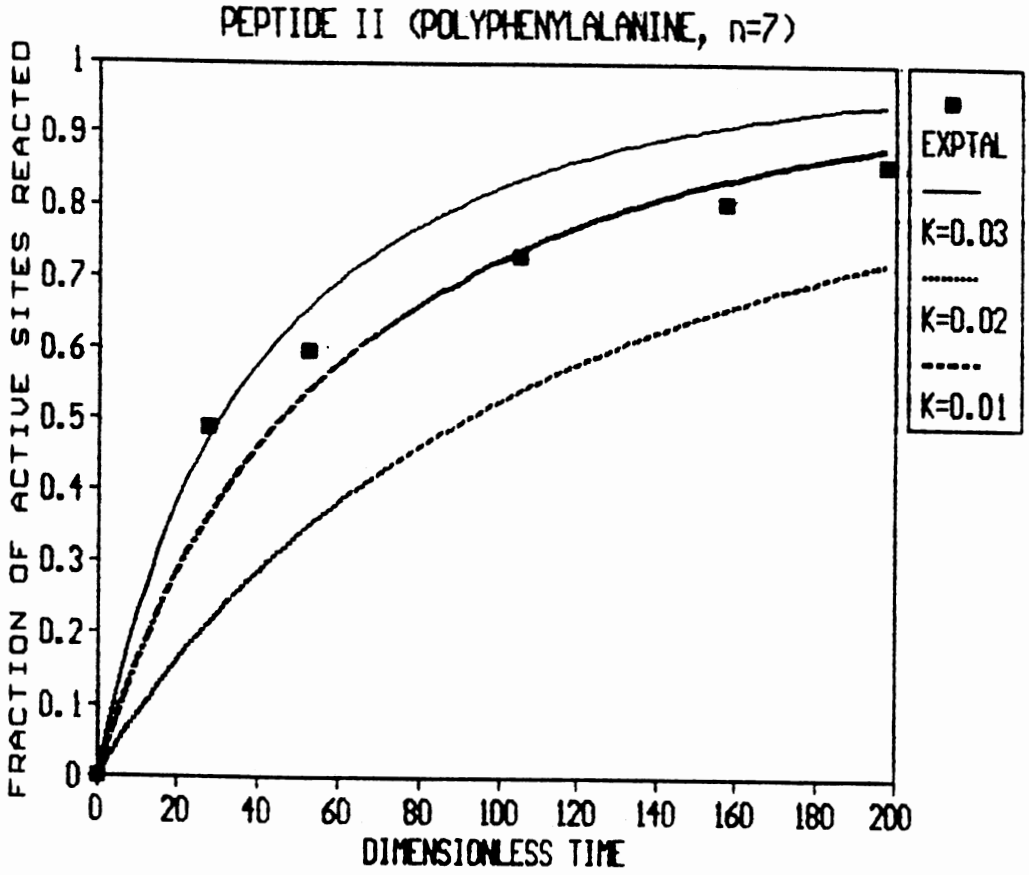


Figure 17. Model Comparison with Data for Peptide II(Polyphe, n=7)

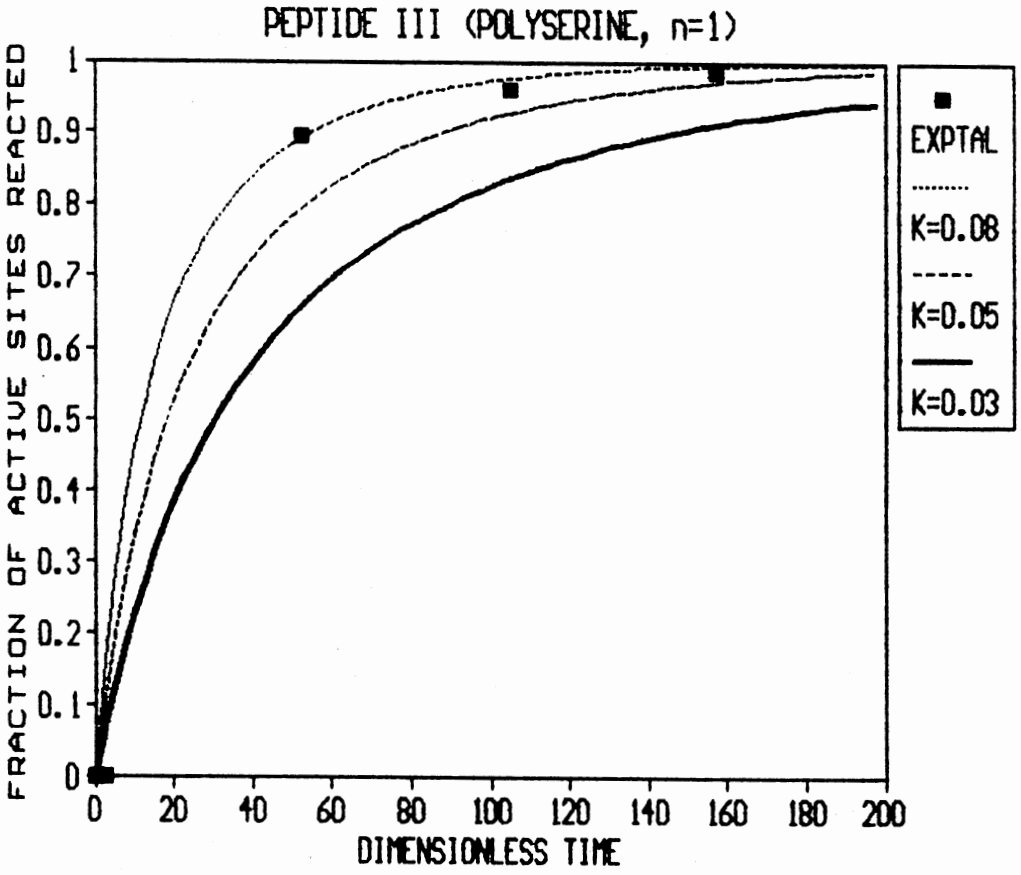


Figure 18. Model Comparison with Data for Peptide III(Polyserine, n=1)

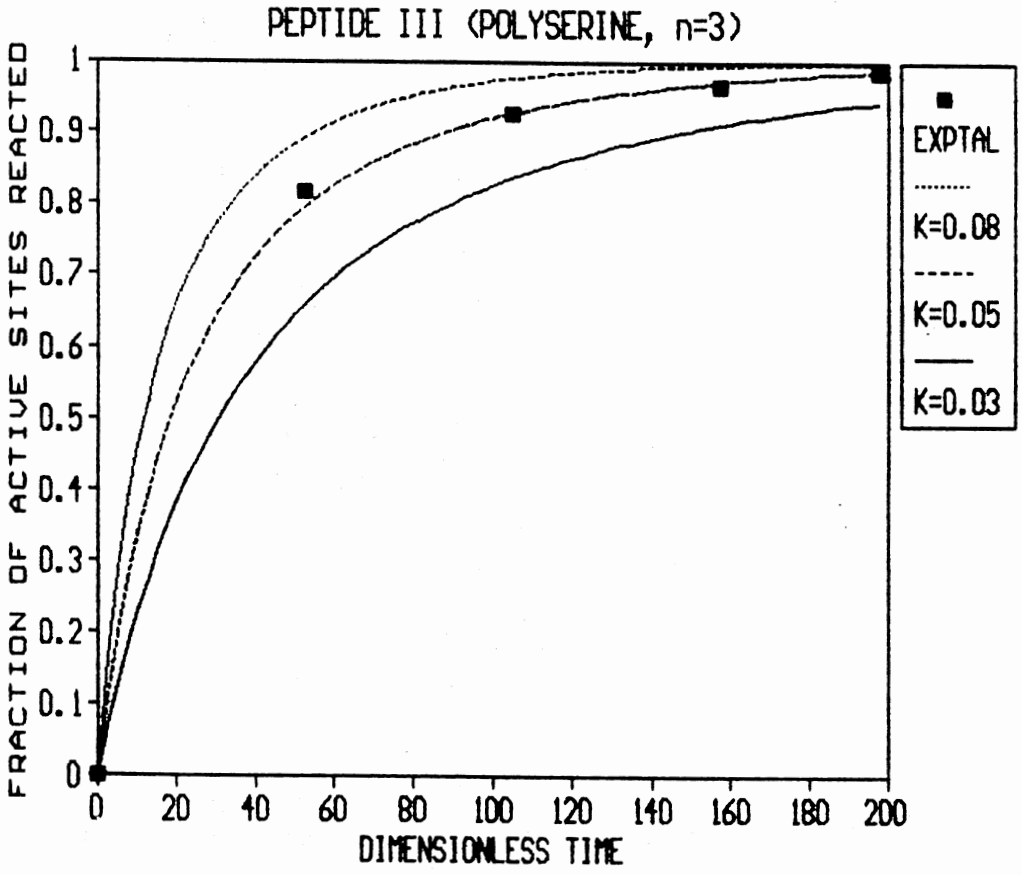


Figure 19. Model Comparison with Data for Peptide III,(Polyserine, n=3)

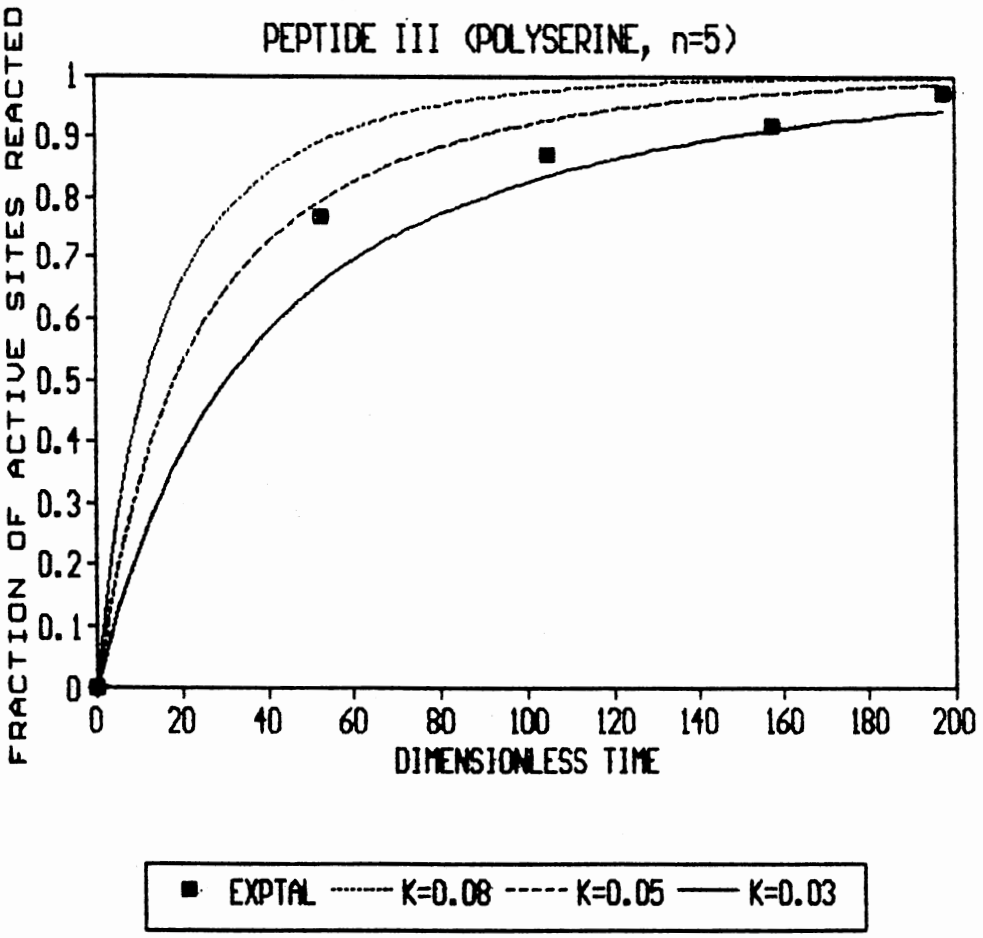


Figure 20. Model Prediction with Data for Peptide III(Polyserine, n=5)

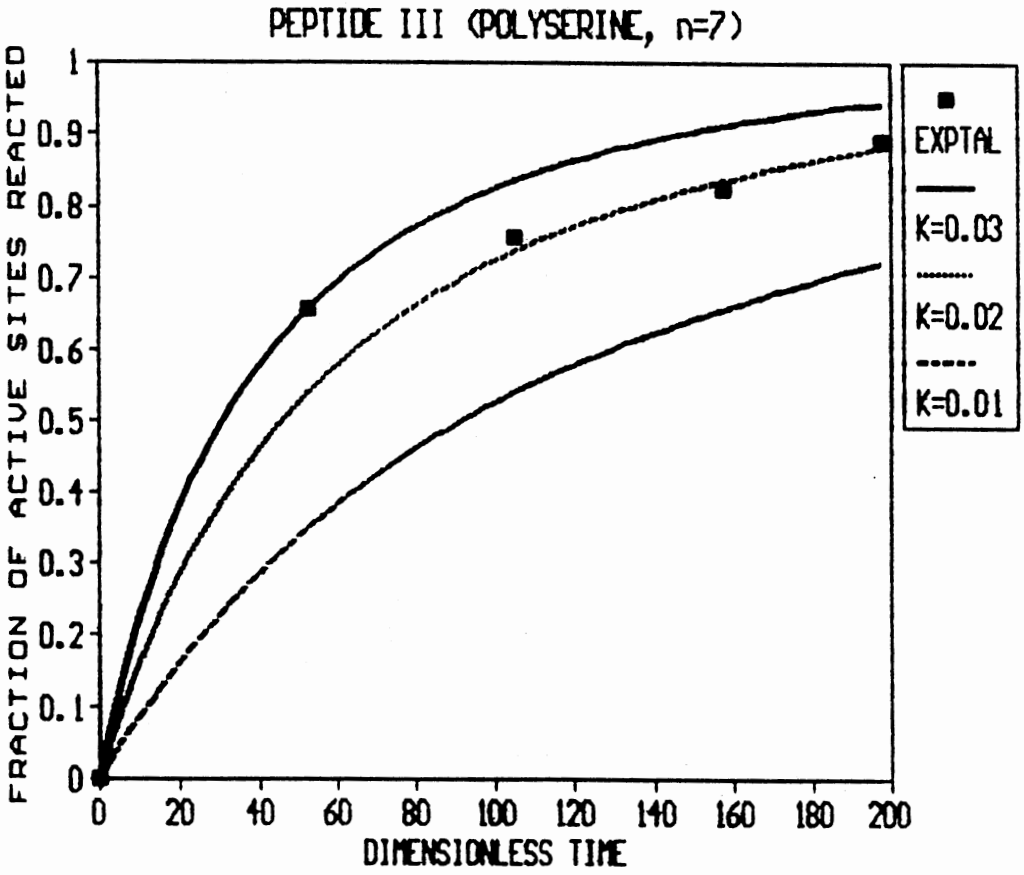


Figure 21. Model Comparison with Data for Peptide III(Polyserine, n=7)

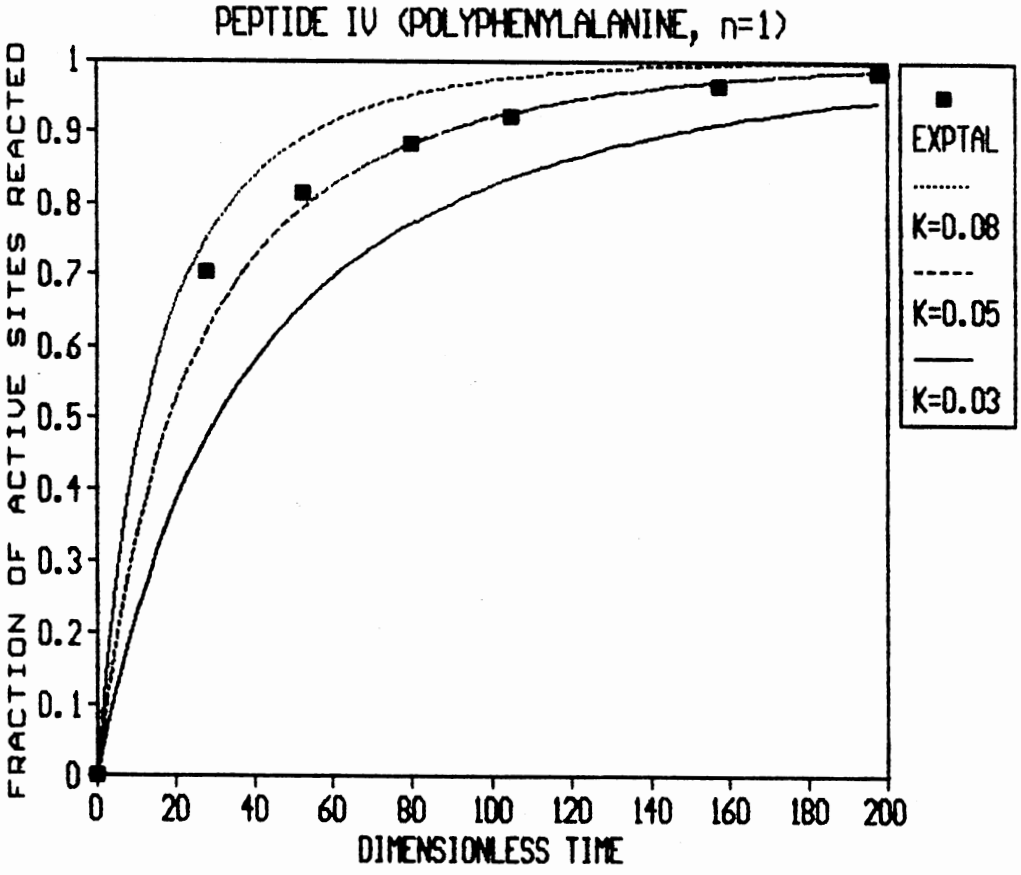


Figure 22. Model Comparison with Data for Peptide IV(Polyphe, n=1)

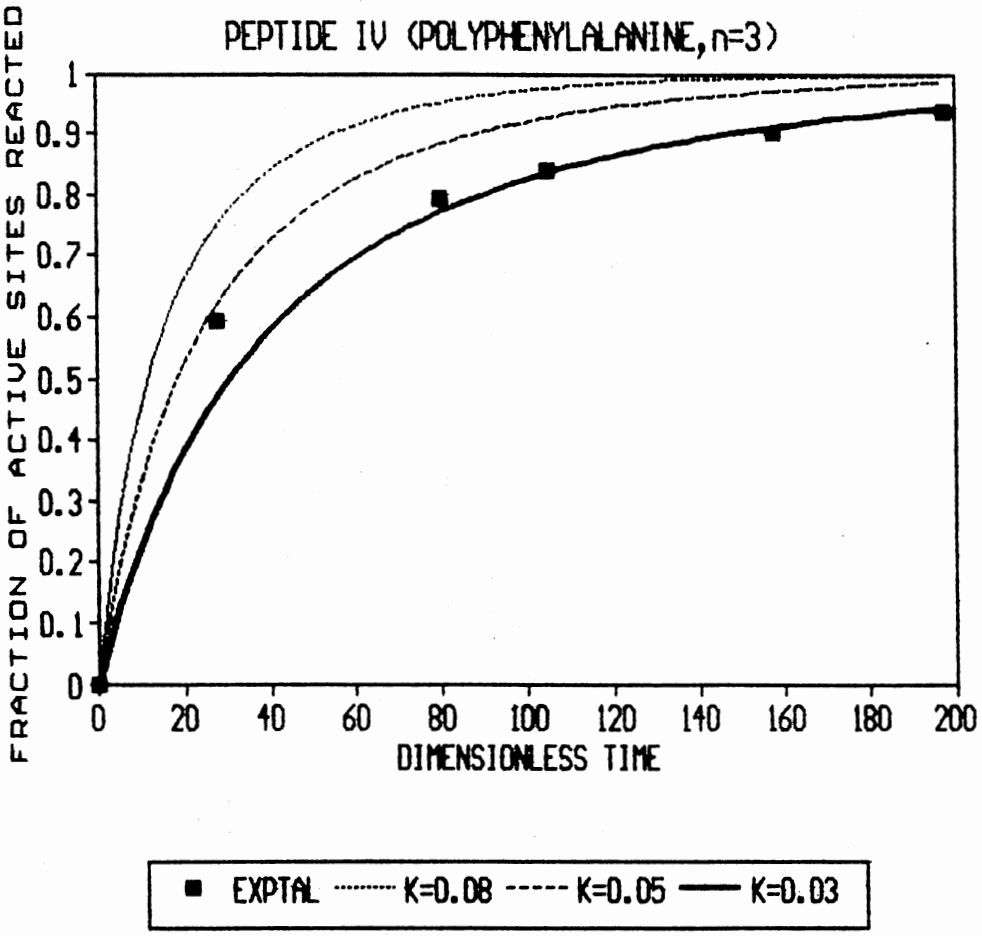


Figure 23. Model Comparison with Data for Peptide IV(Polyphe, n=3)

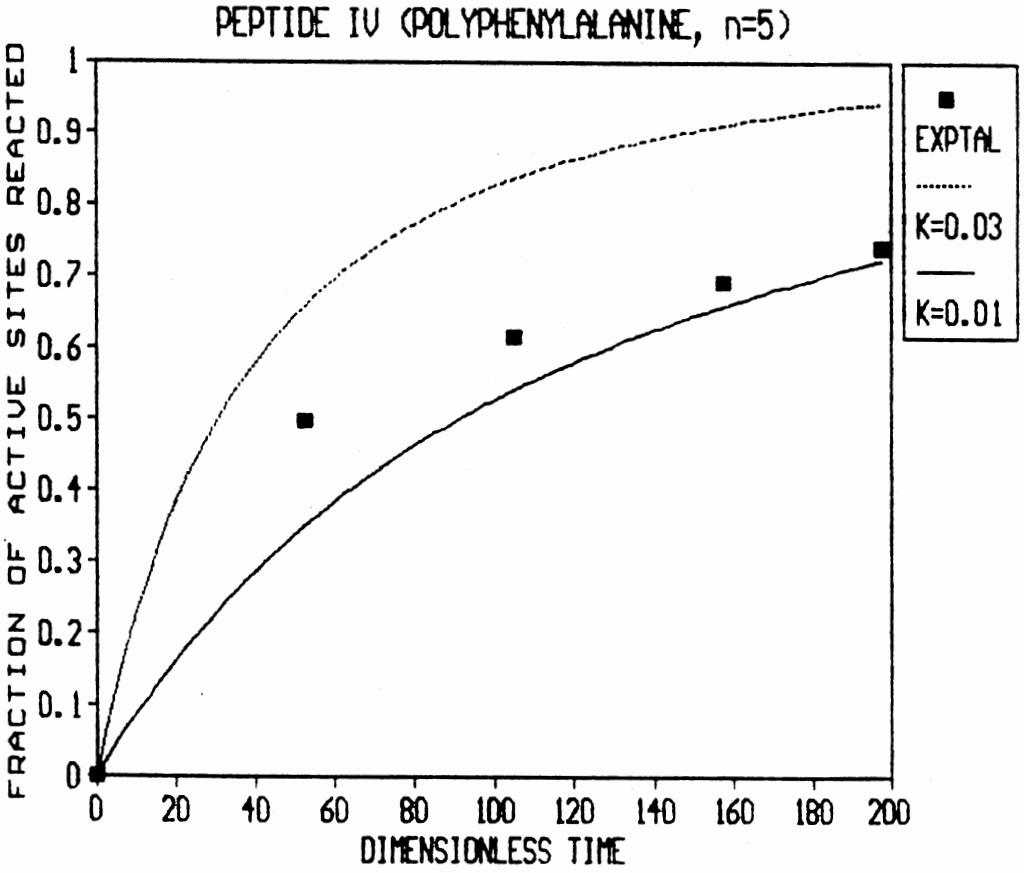


Figure 24. Model Comparison with Data for Peptide IV(Polyphe, n=5)

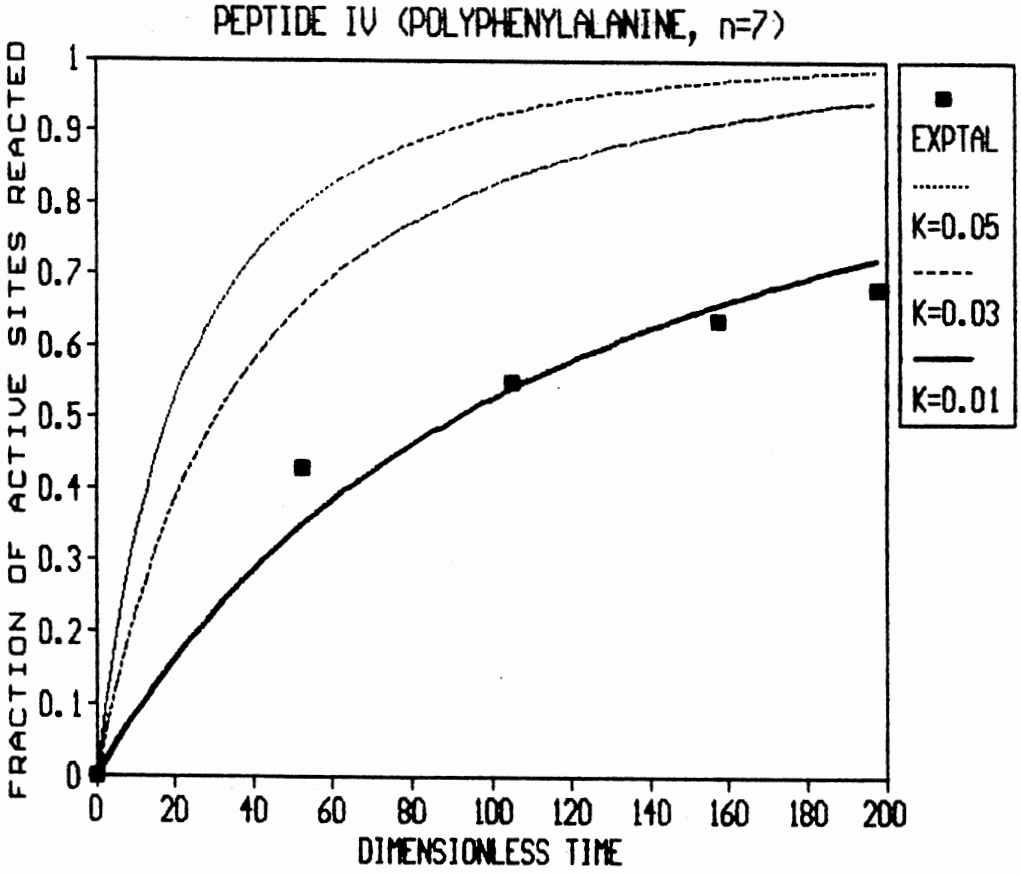


Figure 25. Model Comparison with Data for Peptide IV(Polyphe, n=7)

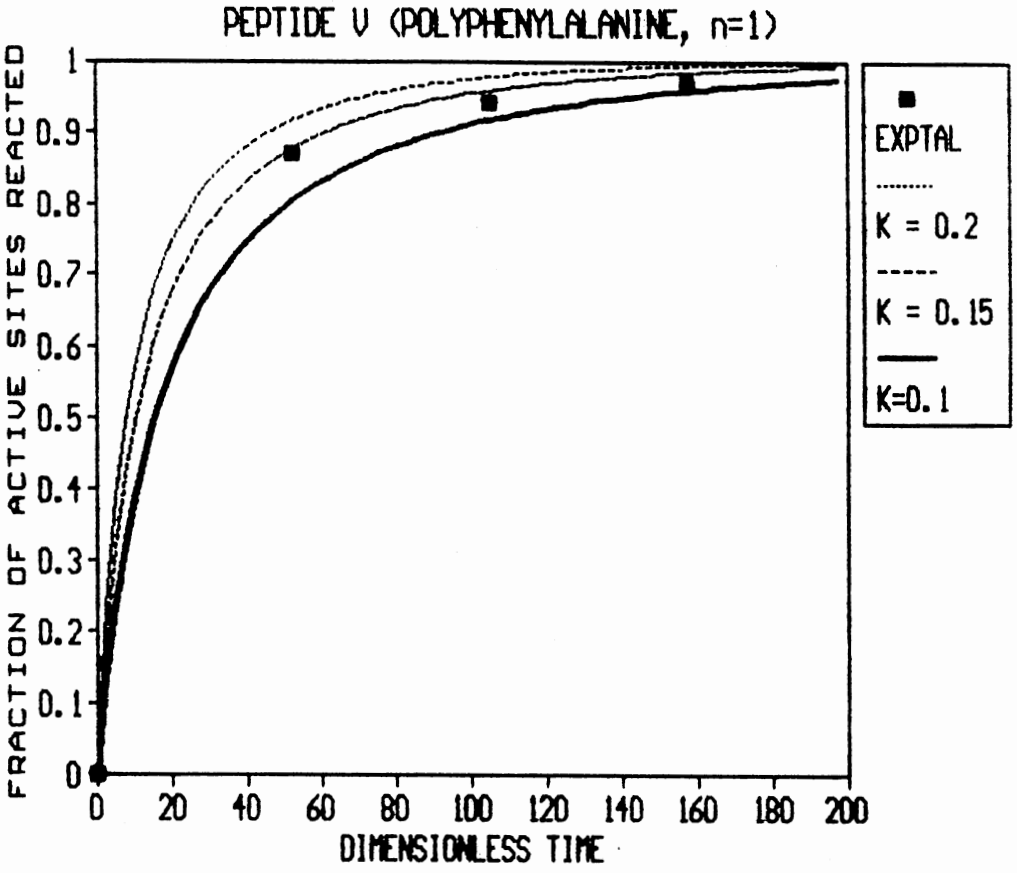


Figure 26. Model Comparison with Data for Peptide V(Polyphe, n=1)

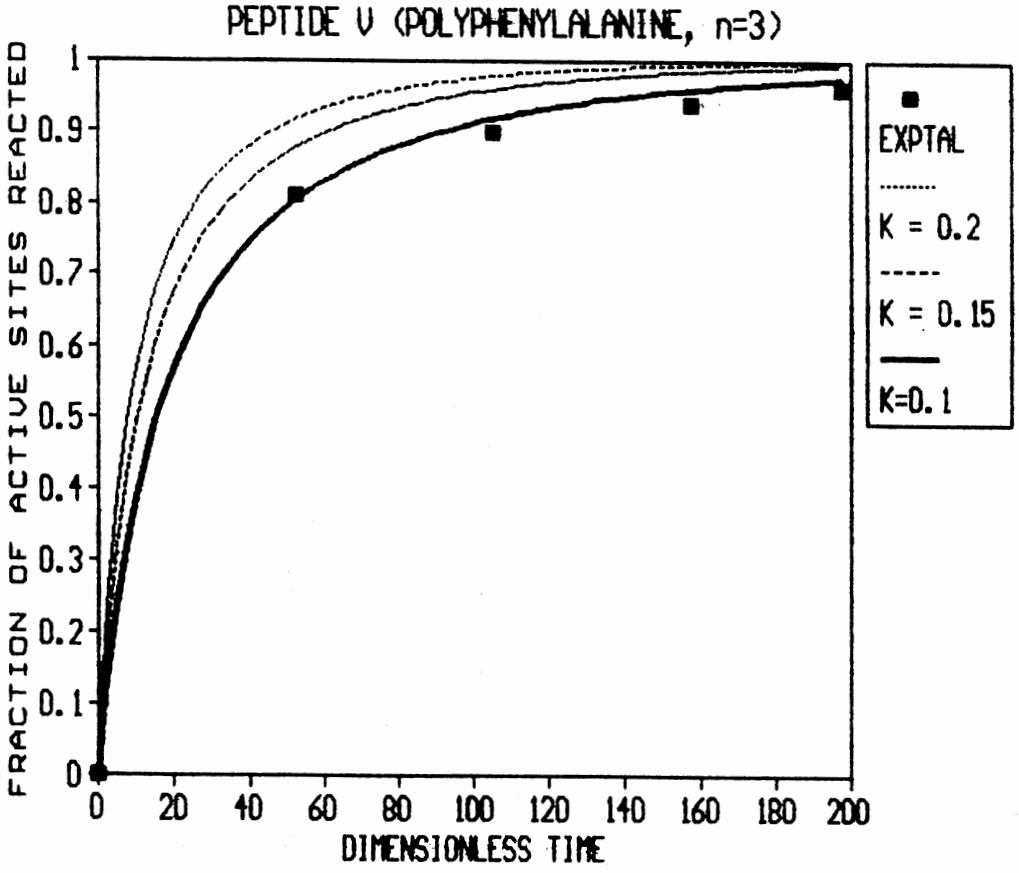


Figure 27. Model Comparison with Data for Peptide V(Polyphe, n=3)

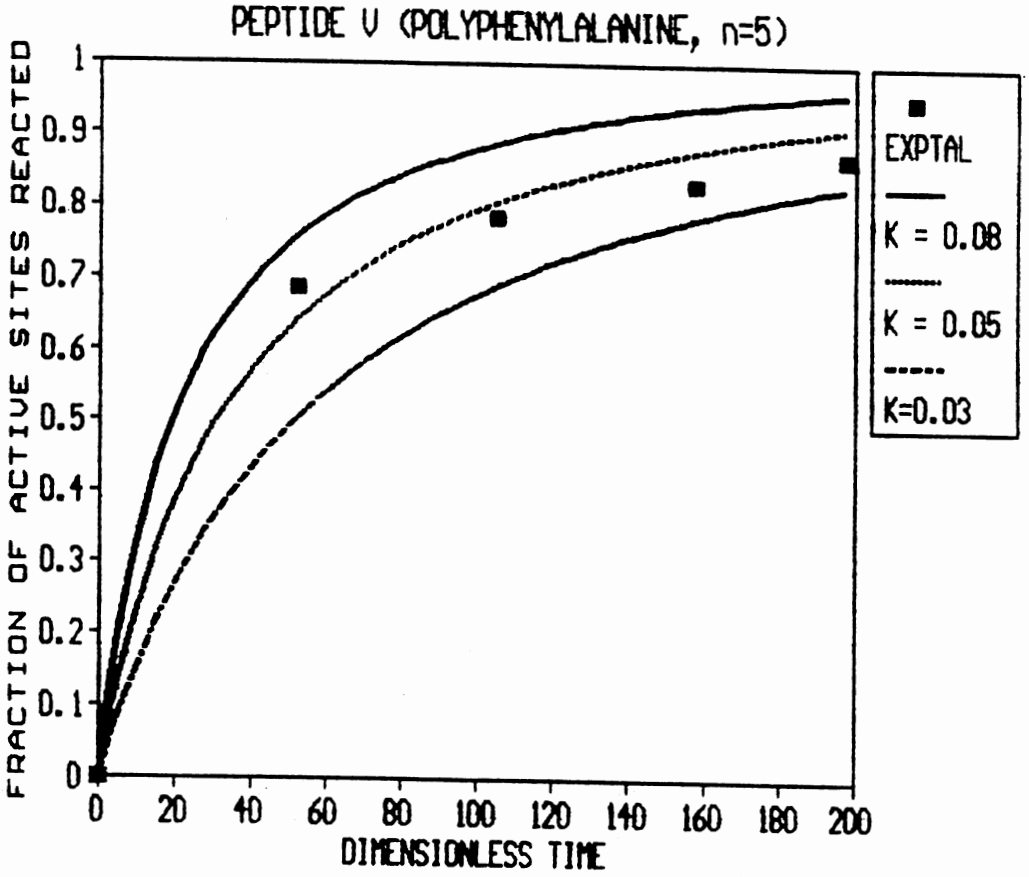


Figure 28. Model Comparison with Data for Peptide V(Polyphe, n=5)

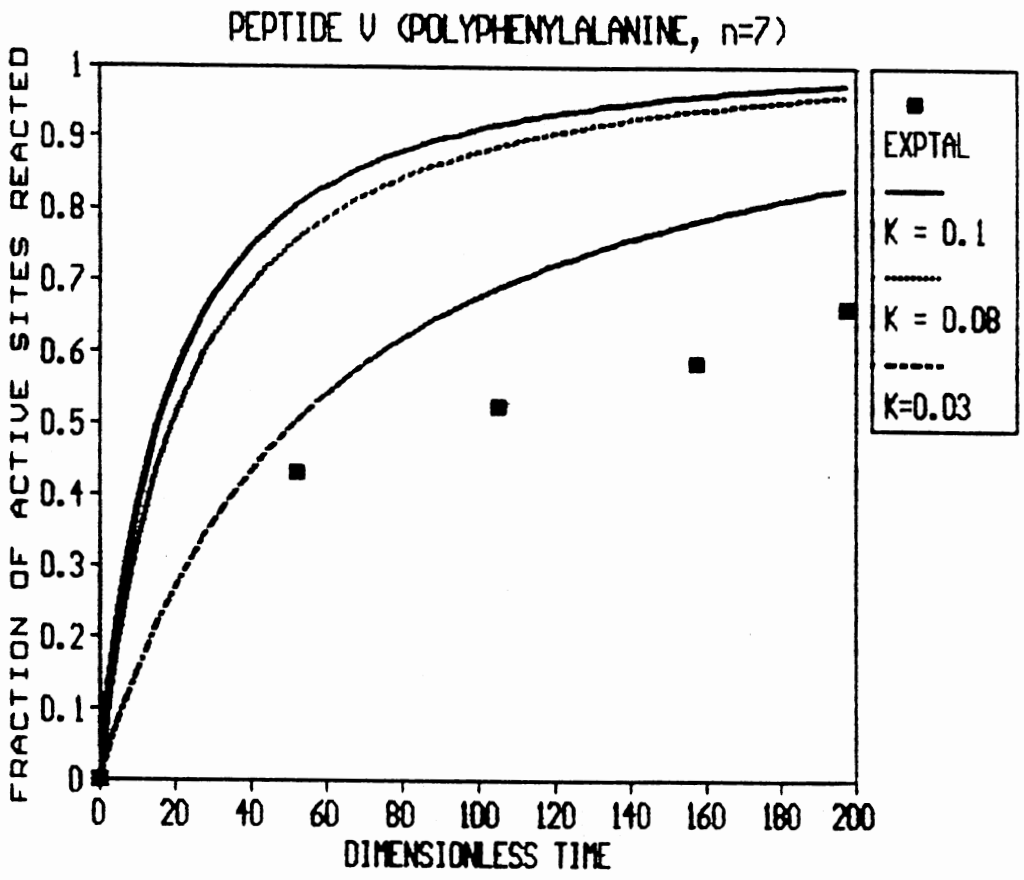


Figure 29. Model Comparison with Data for Peptide V(Polyphe, n=7)

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The program derived in this study provides a solution for diffusion problems accompanied with a general order chemical reaction. A concentration profile can be displayed and saved in data files to produce demonstrative graphs, if numerical data is not illustrative enough. Overall, the program is simple and can be applied to general diffusion reaction problems by changing according to the specified system.

With the results generated by this model, we can conclude that diffusion may be a significant step in Solid Phase Peptide Synthesis. Its validity is confirmed because it fit all the experimentally generated data. The reaction rate constant for a second order chemical reaction is lowered considerably by taking diffusion into account. It lies in the range of 0.01 to 0.1. The fortran program that was derived is very flexible and can be used for a number of different systems for different boundary conditions. Also it can fit any data obtained experimentally and can generate values of the reaction rate constants. This can give us an idea of the reaction times which can help design industrial reactors on a large scale.

Recommendations

There are always limitations implied when assumptions are made. The same is true for this study. The following recommendations are listed to improve the results.

The parameter A did not significantly effect the final conversion of the reactant. This was probably because it was the ratio of the reaction and diffusion term. If this term could be separated into two parts, then it would be possible to see exactly to what extent diffusion is important ranging from the diffusion being equal to zero to it being its maximum value. This would probably more indepth knowledge about the mechanism and improve the results obtained.

To improve the accuracy of the results, the radial grid size number could be increased. It was fixed at 20 in this program due to the consideration of saving array memory usage for Personel Computers.

Results of the numerical solution could be checked by deriving the analytical solution.

The concentration of the symmetrical anhydride was assumed equal to the concentration of the active sites. This assumption was used to simplify the equation. This could affect the results to a considerable extent.

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APPENDIX A

LISTING OF COMPUTER PROGRM

```
C     THIS PROGRAM COMPUTES THE UNSTEADY STATE DIFFUSION
C     INSIDE A SPHERICAL PARTICAL WITH A GENERAL ORDER
C     CHEMICAL  REACTION USING CRANK NICOLSON IMPLICIT
C     METHOD.
C
C-----------------------------------------------------------------
-
C     PARAMETERS ARE :
C     U           -- VALUES OF DIMENSIONLESS CONCENTRATION
C     UOLD              -- PREVIOUS VALUE OF CONCENTRATION
C     DX          -- RADIAL DIRECTION INTERVAL
C     DT          -- TIME INTERVAL
C     T           --  TIME
C     A           -- DIMENSIONLESS PARAMETER
C     N           -- NUMBER OF X INTERVAL
C     P           --EXCESS MOLE RATIO
C     Q           --INITIAL CONCENTRATION OF ACTIVE SITES
C     R           --REACTION RATE CONSTANT
C
C-----------------------------------------------------------------
C

      DIMENSION U(50,4), UOLD(50)
      OPEN (UNIT = 1, FILE = 'DATA.OUT' , STATUS = 'NEW' )
C
C-----INPUT SOME INITIAL VALUES
C
      WRITE(*,*) 'RXN ORDER = '
      READ(*,*) OR1
      WRITE(*,*) 'A = ''
      READ(*,*) A
      WRITE(*,*) 'N = '
      READ(*,*) N
      WRITE(*,*) 'T = '
      READ(*,*) T
      WRITE(*,*) 'P = '
      READ(*,*) P
      WRITE(*,*) 'Q = '
      READ(*,*) Q
      WRITE(*,*) 'R = '
      READ(*,*) R
      T = 0.0
```

```
      NP = N+1
      DX = 1./N
      DT = DX**2

      DO 10 I = 1,NP
   10 UOLD(I) = 0.0
      WRITE (1,200) T, (UOLD(I), I = 2,NP)
   21  X = 0.01


C
C-----SETTING UP THE COEFFICIENT MATRIX
C

      U(1,1) = 0.0
      U(1,2) = 4 + A * 2 * (DX**2) * POWER(UOLD(1),OR1-1)
      U(1,3) = -2

      U(N-1,1) = (DX/(X+(N-2)*DX)) - 1
      U(N-1,2) = 4 + A* 2 * (DX**2) * POWER(UOLD(N-1),OR1-1)
      U(N-1,3) = 0.0

      L = N-2
            DO 30 I = 2,L
      U(I,1) = (DX/(X+(I-1)*DX)) - 1
      U(I,2) = 4 + A* 2 * (DX**2) * POWER (UOLD(I), OR1)
      U(I,3) = -((DX/(X+(I-1)*DX)) + 1)
   30 CONTINUE

C
C-----ESTABLISH RIGHT-HAND SIDE OF THE MATRIX
C

      U(1,4) = 2*UOLD(2)

      L = N-2
            D0 40 I = 2,L
      U(I,4) = ((-(DX/X)) + 1)*UOLD(I-1) + ((DX/X) + 1)*UOLD(I+1)
   40 X = X + DX

      U(N-1,4) = (((- (DX/X)+1)*UOLD(N-1) + ((DX/X)+1)*UOLD( N+!)
      + (((DX/X))+1)
      T = T + DT
```

```
C
C-----CALL SUBROUTINE TRDG TO PERFORM GAUSSIAN
C      ELIMINATION ON A TRIDIAGONAL MATRIX
C

       CALL TRDG (U,N-1)

C
C-----SET CURRENT RESULTS AS NEW STARTING VALUES
C

            DO 50 I = 1,N-1
   50  UOLD(I+1) = U(I,4)
       UOLD(1) = U(1,4)
       XA = (1- UOLD(NP))*P
       UOLD(NP) = UOLD(NP) - R*Q*(1-XA)*(P-XA)

C
C-----OUTPUT CURRENT RESULTS
C

       WRITE(1,200) T, (UOLD(I), I = 2,NP)

       IF (T .LT. 1.0) GO TO 21

  200  FORMAT (/1X, 'T  = ' , F9.4/4X,21F7.4/)
       STOP
       END

C-----THE COEFFICIENTS OF N EQUATIONS ARE STORED IN THE N BY 4
C-----ARRAY, X.  THE FIRST COLUMN OF X HOLDS THE ELEMENTS TO
C-----THE LEFT OF THE DIAGONAL, THE SECOND HOLDS THE
C-----DIAGONAL ELEMENTS, AND THE THIRD HOLDS THE ELEMENTS
C-----TO THE RIGHT.  THE FOURTH COLUMN HOLDS THE RIGHT HAND
C-----SIDE TERMS.

       SUBROUTINE TRDG(X,N)
       DIMENSION X(50,4)
            DO 10 I = 2,N
       X(I,1) = X(I,1)/X(I-1,2)
       X(I,2) = X(I,2)-X(I,1)*X(I-1,3)
       X(I,4) = X(I,4)-X(I,1)*X(I-1,4)
```

```
 10   CONTINUE


C
C-----NOW PERFORM THE BACK SUBSTITUTIONS
C

      NM1 = N-1
      X(N,4) = X(N,4)/X(N,2)
            DO 20 I = NM1, 1, -1
      X(I,4) = (X(I,4) - X(I,3)*X(I+1,4))/X(I,2)
 20   CONTINUE
      RETURN
      END

      FUNCTION POWER(X,Y)
      IF (Y .EQ. 0 .AND. X .EQ. 0) THEN
      POWER = 0.0
      ELSE IF (Y .EQ. 0) THEN
      POWER  = 1.0
      ELSE POWER = X**Y
      ENDIF
      RETURN
      END
```

Enter Reaction Order

2

Enter value of A, the diffusion parameter

0.0022

Enter the X interval

20

Enter the maximum time

200

Enter the excess mole ratio

1.5

Enter the initial concentation

3.22X10-2

Enter the reaction rate constant

0.02

Example of Input Data

APPENDIX B

SAMPLE OUTPUT DATA

TIME	FRACTION OF ACTIVE SITES REACTED	TIME	FRACTION OF ACTIVE SITES REACTED
.0000	.0000	127.5491	.7865
2.5025	.0453	130.0516	.7913
5.0026	.0874	132.5540	.7958
7.5026	.1265	135.0565	.8003
10.0023	.1629	137.5589	.8046
12.5091	.1969	140.0613	.8088
15.0015	.2287	142.5638	.8129
17.5016	.2586	145.0662	.8168
20.0022	.2866	147.5687	.8202
22.5027	.3130	150.0711	.8246
25.0032	.3379	152.5735	.8282
27.5038	.3614	155.0760	.8318
30.0043	.3836	157.5784	.8353
32.5045	.4047	160.0809	.8387
35.0031	.4246	162.5833	.8420
37.5017	.4436	165.0858	.8452
40.0003	.4616	167.5882	.8484
42.4990	.4787	170.0906	.8514
44.9976	.4951	172.5931	.8544
47.4962	.5106	175.0955	.8573
49.9948	.5255	177.5980	.8602
52.4935	.5397	180.1004	.8630
54.9921	.5533	182.6028	.8657
57.4907	.5663	185.1053	.8683
59.9893	.5788	187.6077	.8709
62.4880	.5907	190.1102	.8734
64.9881	.6022	192.6126	.8759
67.4905	.6132	195.1151	.8783
69.9930	.6237	197.6175	.8806
72.4954	.6339		
74.9979	.6437		
77.5003	.6531		
80.0028	.6621		
82.5052	.6709		
85.0076	.6793		
87.5101	.6874		
90.0125	.6952		
92.5150	.7028		
95.0174	.7101		
97.5198	.7172		
100.0223	.7241		
102.5247	.7307		
105.0272	.7371		
107.5296	.7433		
110.0321	.7493		
112.5345	.7551		
115.0369	.7608		
117.5394	.7662		
120.0418	.7715		
122.5443	.7767		
125.0467	.7817		

TIME	BULK CONCENTRATION
.0000	1.0000
2.5025	.9700
5.0026	.9423
7.5026	.9165
10.0023	.8925
12.5019	.8700
15.0015	.8490
17.5016	.8293
20.0022	.8108
22.5027	.7934
25.0032	.7770
27.5038	.7615
30.0043	.7468
32.5045	.7329
35.0031	.7197
37.5017	.7072
40.0003	.6953
42.4990	.6840
44.9976	.6732
47.4962	.6630
49.9948	.6532
52.4935	.6438
54.9921	.6348
57.4907	.6262
59.9893	.6180
62.4880	.6101
64.9881	.6026
67.4905	.5953
69.9930	.5883
72.4954	.5816
74.9979	.5752
77.5003	.5690
80.0028	.5630
82.5052	.5572
85.0076	.5517
87.5101	.5463
90.0125	.5411
92.5150	.5361
95.0174	.5313
97.5198	.5266
100.0223	.5221
102.5247	.5178
105.0272	.5135
107.5296	.5094
110.0321	.5055
112.5345	.5016
115.0369	.4979
117.5394	.4943
120.0418	.4908
122.5443	.4874
125.0467	.4841
127.5491	.4809
130.0516	.4778
132.5540	.4747
135.0565	.4718
137.5589	.4689
140.0613	.4662
142.5638	.4635
145.0662	.4608
147.5687	.4583
150.0711	.4558
152.5735	.4534
155.0760	.4510
157.5784	.4487
160.0809	.4465
162.5833	.4443
165.0858	.4421
167.5882	.4401
170.0906	.4381
172.5931	.4361
175.0955	.4342
177.5980	.4323
180.1004	.4304
182.6028	.4287
185.1053	.4269
187.6077	.4252
190.1102	.4235
192.6126	.4219
195.1151	.4203
197.6175	.4188

APPENDIX C

LITERATURE EXPERIMENTAL DATA

TABLE IV

COMPARISON OF EXPERIMENTAL RESULTS WITH MODEL PREDICTIONS FOR PEPTIDE I

Time	Fraction of Active Site Reacted		Rxn Rate Const. (mole/l sec)
	Experimental	Model	
Peptide I (Polyserine, n=1)			
0.00	0.000	0.000	
17.52	0.780	0.687	
35.04	0.885	0.857	
52.56	0.946	0.926	
70.08	0.961	0.960	
78.84	0.984	0.971	0.1
n=3			
0.00	0.000	0.000	
17.52	0.644	0.623	
35.04	0.826	0.808	
52.56	0.898	0.891	
70.08	0.939	0.935	
87.60	0.964	0.960	
105.12	0.979	0.975	
122.64	0.984	0.984	0.08
n=5			
0.00	0.000	0.000	
17.52	0.654	0.487	
35.04	0.782	0.687	
52.56	0.850	0.793	
105.12	0.943	0.926	
140.16	0.967	0.960	
175.00	0.983	0.977	0.05

TABLE IV (Continued)

Time	Fraction of Active Site Reacted		Rxn Rate Const. (mole/l.sec)
n=7			
0.00	0.000	0.000	
17.52	0.554	0.349	
35.04	0.676	0.539	
52.56	0.747	0.657	
105.12	0.854	0.835	
140.16	0.894	0.891	
210.24	0.943	0.942	0.03

TABLE V

COMPARISON OF EXPERIMENTAL RESULTS WITH MODEL PREDICTIONS FOR PEPTIDE II

Time	Percent of Active Sites Reacted		Rxn Rate Const. (mole/l.sec)
Peptide II (Polyphenylalanine, n=1)			
0.00	0.000	0.000	
17.52	0.641	0.623	
35.04	0.767	0.808	
52.56	0.828	0.891	
78.84	0.887	0.960	
105.12	0.919	0.977	
157.68	0.948	0.983	
210.24	0.971	0.997	0.08
n=3			
0.00	0.000	0.000	
17.52	0.579	0.487	
35.04	0.700	0.687	
52.56	0.774	0.793	
78.84	0.845	0.882	
105.12	0.887	0.926	
157.68	0.934	0.970	
210.24	0.964	0.986	0.05
n=5			
0.00	0.000	0.000	
17.52	0.472	0.349	
35.04	0.575	0.539	
52.56	0.646	0.657	
105.12	0.767	0.835	
157.68	0.838	0.911	
210.24	0.886	0.942	0.05

TABLE V (Continued)

Time	Percent Of Active Sites Reacted		Rxn Rate Const. (mole/l.sec)
n=7			
0.00	0.000	0.000	
26.28	0.489	0.3498	
52.56	0.596	0.6576	
105.12	0.730	0.8353	
157.68	0.803	0.9110	
210.24	0.857	0.9422	0.01-0.03

TABLE VI

COMPARISON OF EXPERIMENTAL RESULTS WITH MODEL PREDICTIONS FOR PEPTIDE III

Time	Fraction of Active Site Reacted		Rxn Rate Coonst. (mole/l.sec)
Peptide III (Polyserine, n=1)			
0.00	0.000	0.000	
52.56	0.896	0.891	
105.12	0.959	0.975	
157.68	0.985	0.993	0.08
n=3			
0.00	0.000	0.000	
52.56	0.817	0.793	
105.12	0.925	0.926	
157.68	0.965	0.970	
210.24	0.985	0.9846	0.05
n=5			
0.00	0.000	0.000	
52.56	0.767	0.657	
105.12	0.870	0.835	
157.68	0.920	0.970	
210.24	0.971	0.984	0.03-0.05
n=7			
0.00	0.000	0.000	
52.56	0.659	0.657	
105.12	0.758	0.835	
157.68	0.823	0.911	
210.24	0.890	0.942	0.03-0.05

TABLE VII

COMPARISON OF EXPERIMENTAL RESULTS WITH MODEL PREDICTIONS FOR PEPTIDE IV

Time	Fraction Of Active Sites Reacted		Rxn Rate Const. mole/l.sec)
Peptide IV (Polyphenylalanine, n=1)			
0.00	0.000	0.000	
26.28	0.704	0.618	
52.56	0.814	0.793	
78.84	0.884	0.882	
105.12	0.922	0.926	
157.68	0.964	0.970	
210.24	0.983	0.984	0.05
n=3			
0.00	0.000	0.000	
26.28	0.591	0.470	
78.84	0.793	0.771	
105.12	0.840	0.835	
157.68	0.901	0.911	
210.24	0.935	0.942	0.03
n=5			
0.00	0.000	0.000	
52.56	0.497	0.349	
105.12	0.613	0.539	
157.68	0.691	0.657	
210.24	0.740	0.720	0.01-0.03
n=7			
0.00	0.000	0.000	
52.56	0.430	0.349	
105.12	0.548	0.539	
157.68	0.636	0.657	
210.24	0.681	0.720	0.01

TABLE VIII

COMPARISON OF EXPERIMENTAL RESULTS WITH MODEL PREDICTION FOR PEPTIDE V

Time	Fraction of Active Site Reacted		Rxn Rate Const. (mole/l.sec)
Peptide V (Polyphenylalanine, n=1)			
0.00	0.000	0.000	
52.56	0.870	0.926	
105.12	0.941	0.987	
157.68	0.969	0.997	0.1
n=3			
0.00	0.000	0.000	
52.56	0.811	0.657	
105.12	0.899	0.835	
157.68	0.939	0.911	
210.24	0.961	0.942	0.03
n=5			
0.00	0.000	0.000	
52.56	0.687	0.657	
105.12	0.782	0.835	
157.68	0.831	0.911	
210.24	0.868	0.942	0.03

VITA

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