Controlled Release of Macromolecules from Ethylene-Vinyl Acetate
Copolymer Matrices: Microstructure and Kinetic Analyses

by

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CONTROLLED RELEASE OF MACROMOLECULES FROM ETHYLENE-VINYL ACETATE
COPOLYMER MATRICES: MICROSTRUCTURE AND KINETIC ANALYSES

by

RAJAN SOHANSINGH BAWA

Submitted to the Departments of Nutrition and Food Science and Chemical Engineering on January 27, 1981, in partial fulfillment of the requirements of the Degrees of Master of Science.

ABSTRACT

A novel drug delivery system comprising of ethylene-vinyl acetate copolymer-drug has recently been developed. This system has demonstrated potential for sustained delivery of macromolecules (MW < 10^7). However, the mechanism by which this system functions and parameters relevant to release have yet to be completely understood. An investigation of the microstructure and kinetics of release from these matrices would therefore aid in effective usage of this system.

Microstructural investigation of the EVA-protein system confirmed the hypothesis of "channeled diffusion" of the macromolecules through porous networks in the matrix structure. Pores of the same size range (77.0 ± 19.13 μm) as the protein particles (93.4 ± 45.4 μm) incorporated were observed in the matrix body. Their proliferation at different times of release was followed by ordinary light microscopy and Nomarski. SEM studies indicated the presence of interconnecting channels.

Kinetic investigations eliminated the possibility of external mass transfer control and generated release profiles. Release rates varied significantly with the bulk densities and the amount of protein particles cast in the matrix. This was attributed to variation of the void fraction in the matrices.

Based on the microstructural and kinetic data, a mathematical model was proposed and simulated on a computer. This model showed good agreement with the observed kinetics. Analysis of the results afforded correlations which have a predictive value in the range of high protein solubility.

Thesis Supervisor:

Robert S. Langer
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I would like to dedicate this work to the fond memory of my mother Mrs. Rajrani Bawa.
To the Memory of My Mother
Mrs. Rajrani Bawa
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CHAPTER I

INTRODUCTION

In recent years, considerable research effort has been directed towards the use of polymers to provide controlled long-term delivery of drugs and other chemicals. The use of these devices has proliferated to encompass clinical, pharmaceutical, biological, agricultural, environmental, and household applications (1).

These systems are receiving particular attention in the field of drug delivery applications that require a continuous supply of drug over a long time period. Polymer-drug systems allow drugs to be released in a more controlled and prolonged manner than enteric formulations, suspensions, emulsions and compressed tablets which are the conventional methods of slow release. This is due to the fact that the rate of release in polymer-drug systems is determined largely by the properties of the vehicle itself, whereas environmental factors greatly influence slow release formulations. Another major disadvantage of slow release systems is that the modifications necessary to achieve slow release produce new drugs with questionable efficacy (2). In controlled release this disadvantage is absent.

Though the future of polymer-drug systems looks promising, the mechanism by which they function and parameters relevant to drug release have yet to be completely understood. This thesis presents studies directed towards that goal. The work done in this field will be reviewed. This will include the development of a unique polymeric delivery system by Langer and Folkman (3) for the release of macromolecules.
Next, up-to-date studies conducted to characterize this system will be described and suggestions will be made for future efforts.
CHAPTER II

LITERATURE SURVEY

(A) Polymeric Controlled Release

(A.1) Background

The advent of polymeric systems as release matrices for the sustained delivery of drugs occurred in 1964, when Folkman and Long (4) found that triiodtrysine, isoproterenol and digitoxin diffuse slowly through silicone rubber tubing. Subsequently, the release of atropine, histamine, antimalarial drugs and general anaesthetics was demonstrated using silicone rubber (5-8). Since that time a variety of polymer-drug combinations have been exploited for slow release.

According to the classification suggested by Langer (1) there currently exist four categories of polymeric systems for the release of drugs. These are:

1) Diffusion Controlled Systems
2) Chemically Controlled Systems
3) Swelling Controlled Systems
4) Magnetically Controlled Systems.

Each of these have their peculiar advantages and drawbacks, and excellent reviews exist on the subject in the literature (1,9-11). Here we will detail only Matrix Systems, which are a subset of Diffusion Controlled Systems, since this thesis deals only with the study of a novel matrix system.
(A.2) Matrix Systems

This category comprises systems where drug is dispersed throughout the matrix and diffusion through the drug-polymer matrix is the rate limiting step. However, diffusion occurs either between the polymer segments or through channels which offer openings in the porous matrix.

In the first case the drug may be present in the matrix in quantities above or below its solubility. Analyses of these systems have appeared in the literature \(9,12,13\). Also, numerous studies have been described where the drug diffuses through pores in the drug-polymer system \(14-18\).

Matrix devices are normally capable of releasing only small molecules with molecular weights up to a few hundred. In 1976, Langer and Folkman described the first biocompatible matrix system capable of continuously releasing macromolecules \((M.W. > 1000)\) for prolonged periods \(3\). This system has demonstrated release of insulin \(19\), heparin \(20\), enzymes \(20\), DNA \(20\), tumor angiogenesis factor \(20\), and its inhibitor \(21\), and has provided the basis for various bioassays \(19\) and chemotactic studies \(22\). Potential applications include continuous insulin delivery and an immunization procedure \(19\).

So far we have reviewed the work done to date in the field of polymeric controlled release systems. The development of a novel drug-polymer system by Langer and Folkman \(3\) capable of releasing macromolecules has also been documented. Since this thesis will include investigations relating to the microstructure of this drug-polymer system, the next section will review methods available for microstructural studies.
(B) Methods for Microstructural Investigation

A variety of microscopy techniques can be used to determine the microstructure of various substances. These can be divided into three categories:

B.1) Optical Microscopy (OM)
B.2) Scanning Electron Microscopy (SEM)
B.3) Transmission Electron Microscopy (TEM)

Each of the above has its own peculiar advantages and limitations (23). Thus, OM is better than SEM for examining subsurface structure (24), while SEM is best for describing surface morphology. The greatest advantage of the TEM is its high resolution.

(B.1) Optical Microscopy (OM)

This has been used to study the structure of solid systems in widely different disciplines viz. chemical microscopy, metallurgy and the biological sciences. Hansen and Flink (24,25) have used OM to study the structure of various food systems. Numerous workers have used it in the study of polymers, including textile fibres (26,27), crystalline polymer structure (28) and the kinetics of crystallization (29).

However, only limited use of OM has been made in the investigation of drug delivery systems. In 1964, Lazarus et. al (14) used OM to examine wax cores containing tripelennamine HCl. Roseman and Higuchi (30) used OM to study release of medroxyprogesterone acetate from a silicone polymer. More recently, Hopfenberg and Hsu (31) utilized OM to study
the release of dye in a model release system. Langer and coworkers (19) have described the use of OM in studying the matrix characteristics of drug-ethylene-vinyl acetate matrices.

A recent development in OM is Nomarski Differential Contrast microscopy which is an ideal supplement to phase-contrast (diffractive) techniques (32) which exploit differences in optical path (i.e., of the product; refractive index x thickness) with respect to background to produce contrast in the image. Nomarski reveals colorless or homogeneously colored structures which would be invisible by conventional brightfield and only slightly pronounced by diffractive techniques. This is accomplished by using reflected as well as transmitted light, producing clearly defined boundaries as well as pseudo three-dimensional images.

The limitations of OM are a poor depth of focus and low resolving power ~ 0.25 μm (33).

(B.2) Scanning Electron Microscopy (SEM)

The advantages of the SEM are (23) a great depth of focus as compared to OM and TEM, the ability to switch over a wide range of magnification, higher resolution (100A) than OM and the opportunity to directly view the form of objects without the necessity of obtaining thin sections which are necessary both for OM and TEM.

In 1958, Sikorski (34) realized the importance of the use of the SEM for the study of polymers and fibres. Since these are nonconducting it is necessary to first deposit a thin layer of some conducting material on their surface (35). In 1969, Kesting (36) demonstrated the
use of the SEM in studies of membrane morphology. Subsequently, Reimschussel (29) also used this technique to characterize membranes with respect to pore size. Winram et. al (37) studied the lamellar structure of polyethylene using SEM. Okui and Kawai (38) studied the texture of ethylene-vinyl acetate copolymers, while Furuta (39) observed the microstructure of emulsion particles of ethylene-vinyl acetate with the object of investigating the mechanism of emulsion polymerization.

X-ray microanalysis, which is a particular mode of examination of SEM, has been used for the study of the distribution of elements in biological tissue (40) and for the characterization of coatings and polymers (41). This method of microanalysis is useful for determining compositional variations in the specimen which produce a distribution map of various elements (42).

(B.3) Transmission Electron Microscopy (TEM)

In principle, TEM should be able to study subsurface structures better than the SEM due to its higher resolving power - 10Å but artefacts are more likely to occur because of the error, due to the difficulty of focusing electrons transmitted through the sample as versus the incident electrons of the SEM (23). Several investigators have used the high magnifications afforded by TEM to study the crystalline structure of polymeric films (42-44). A common problem encountered in the use of TEM is the degradation of the thin sample due to the high energy of the electron beam (45).
CHAPTER III

OBJECTIVES

The novel system of drug delivery developed by Langer and Folkman (3) has found widespread application (19-22). An earlier study\(^1\) was directed towards developing a reproducible system and presenting initial kinetic data, together with a suggestion that the Higuchi model be adopted to describe the kinetics.

The objectives for this thesis are as follows:

1) Investigate the microstructure of the release matrices in order to better understand the factors governing release;

2) Conduct further kinetic studies directed towards defining the parameters affecting release; and

3) Attempt to develop and simulate a predictive model describing release.

These three broad classes of investigation will be conducted in three chapters: 5-7 respectively.

In the next chapter will be catalogued the various materials and equipment used in this thesis. The methods and procedures used in the various investigations will also be detailed.

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CHAPTER IV
MATERIALS, EQUIPMENT AND METHODS

(A) Materials

The polymer used for the controlled release matrix system studied in this thesis was ethylene-vinyl acetate copolymer (40% by weight vinyl acetate), supplied by the Alza Pharmaceutical Corp., Palo Alto, California. The manufacturer of this product is Du Pont Chemical Co., Wilmington, Delaware (product name, Elvax 40).

The proteins used for sustained release studies were obtained from the Sigma Chemical Co., St. Louis, Missouri. These proteins were bovine serum albumin (BSA, cat. no. A4503, M.W. 68,800), lysozyme (cat. no. L6876, M.W. 14,400) and β-lactoglobulin (cat. no. L2506, M.W. 37,100). Chemicals obtained from Fisher Scientific Co., Fairlawn, New Jersey were methylene chloride (cat. no. D-123, M.W. 84.93) and sodium salicylate (cat. no. 5-395, M.W. 160.11). Sodium chloride (cat. no. 7581, M.W. 58.44) was obtained from Mallinckrodt, St. Louis, Missouri. All water used was double glass distilled. Paraplast Tissue Embedding Medium (cat. no. 12-646-106) was obtained from Fisher Scientific Co., Boston, Massachusetts. This was used to coat the polymeric matrices on five sides for one-sided release studies.

The embedding medium used while cutting thin sections was obtained from Lab-Tek Products Division, Miles Inc., Naperville, Illinois (cat. no. Tissue-Tek II O.C.T. Compound 4583). Sections were mounted on glass slides obtained from Fisher Scientific Co., Boston, Massachusetts (cat.
The film used for photographing the sections using light microscopy was Ektachrome Type B:ASA 160 obtained from Eastman Kodak Co., Rochester, New York, and that used for SEM studies was Polaroid Type 52 obtained from Polaroid Corp, Cambridge, Massachusetts.

(B) **Equipment**

Protein powder was sieved using U.S. Standard Sieves and an automatic Allen-Bradley Sonic Sifter (Model L-3) marketed by Fisher Scientific Company, Boston, Massachusetts, (cat. no. 14-294-15). Drug to be released was added to polymer solution in 20 ml vials (Wheaton Scientific Co., Millville, New Jersey, cat. no. 986548). These vials were also used for release studies. Solutions were mixed on a Vortex-Genie mixer Model 12-812, (Scientific Industries, Bohemia, New York). Solutions were cast in glass molds prepared with ordinary window glass cemented with Silastic (Dow Corning, Midland, Michigan, (cat. no. 891). The molds were levelled using a circular level (Fisher Scientific Company, Boston, Massachusetts, cat. no. 12-000). Matrices were dried on wire screening (common steel mesh) and cut using a scalpel (Bard Pardner, Rutherford, New Jersey, #10 blade). Physical dimensions were measured using a micrometer (Stamet Co., Athol, Massachusetts, cat. no. 436). During release, samples were shaken on a Thomas Clinical Rotating Apparatus (Arthur H. Thomas Co., cat. no. 2995-G10), or stirred using a Lab-Line Model 1278 Multi-Magnestir (V.W.R. Scientific Co., cat. no. 58945-050). A small portable fan (Westinghouse Model R-1020) was used to keep the samples at room temperature.
In studying release under stirred conditions, the vials used were Opticlear, Flint Glass (Kimble, cat. no. 60975L, V.W.R. #66013-080). The stirring bars used were Star Head Nalgene (Nalge Co. Inc., cat. no. 6600-0010, V.W.R. #58948-502). The steel wire cloth was Stainless Steel Type 304, CX-20 Mesh 20, wire diameter = 0.015" obtained from Small Parts Inc., Miami, Florida. A stroboscope was used to measure the r.p.m. of the stirring bars. It was Strobotac Type 1531-A (General Radio Co., Concord, Massachusetts).

All volumetric glassware was grade A. Quantitative transfers were performed with Eppendorf pipettes, a L/I Repipet Jr. (Labindustries, Berkeley, California, cat. no. 701) and ordinary glass pipettes.

Samples were weighed on a Mettler 7454 electronic balance.

The vials used for estimating protein solubilities were obtained from Wheaton Scientific Co., Milreville, New Jersey, (cat. no. 986333). These were tightly capped using caps (cat. no. 240508) also supplied by Wheaton.

All spectrophotometric determinations were performed on a Gilford 24G spectrophotometer equipped with a model 410 Digital Absorbance/Concentration meter capable of reading on a linear scale up to 3.0 optical density units. The spectrophotometer was equipped with a Rapid Sampler Model 2443 containing a teflon lined cell, a Model 3021 vacuum receiver, and a Model 3022 vacuum pump (Gilford Instrument Laboratories, Oberlin, Ohio).

The machine used to obtain thin sections of the release matrix for optical microscopy was a Cryo-Cut Cryostat Microtome, Model 845 with a 4 3/4" microtome knife Type 942 manufactured by American Optical Corp.,
Scientific Instruments Division, Buffalo, New York. The machine used to obtain sections for concentration profile studies was a Minot Rotary Microtome (cat. no. 3310) Damon, IEC Division, Needham, Massachusetts. The knife used was 5" in length (cat. no. 3257).

The optical microscope, used for studying the sections of the matrix using transmitted light, was manufactured by Baush & Lomb, Scientific Optical Products Division Model BRT3Z1H obtained from VWR Scientific Co. (cat. no. 40950-162).

The optical microscope used for studying the sections of the matrix by Nomarski was manufactured by Carl Zeiss, Oberkochen, West Germany. The model was standard with attachments: Swing-out polarizer (cat. no. 470869), DIC condensor (cat. no. 473651), adapter ring (cat. no. 473695). Intermediate tube (cat. no. 473059) and Auxiliaries for color contrast (cat. no. 473701). All these were obtained from AZI, Dedham, Massachusetts.

The camera, Model AT-1 and attachment, Photomicro Unit F, used for taking photographs, while using the two microscopes catalogued above, were obtained from Canon USA, Inc., Long Island, New York.

The equipment used for drying the release matrix for observation with the SEM was a Critical Point Drying Apparatus, Model 11-120A, manufactured by Balzers Union, Furstentum, Liechtenstein. The dried samples were stored in vials obtained from Fisher Scientific Co., Boston, Massachusetts (cat. no. 3-335-10A).

The evaporator used to coat the samples with carbon and gold for observation in the SEM was Economy Coater Type CVE-15 manufactured by Consolidated Vacuum Corp. Rochester, New York.
The SEM used, Model JSM-U3 was manufactured by JEOLCO INC., Medford, Massachusetts.

(C) **Methods**

(C.1) Fabrication Procedure for Release Matrices

(C.1.1) Uncoated Matrices:

Ethylene-vinyl acetate copolymer (40% vinyl acetate by weight) was dissolved in methylene chloride to give a 10% w/v solution. Protein powder was sieved to obtain particles of different size ranges (e.g., 63-149 μm). A weighed amount of protein powder from a size range was then added to 15 ml of polymer solution in a glass vial and the mixture vortexted to obtain a homogeneous suspension. This mixture was poured quickly into a glass mold (7cm x 7cm x 0.5cm) which had been previously precooled by placement on a slab of dry ice for 5 min. During precooling, the mold was covered with kimwipes to prevent excess frost formation. After the mixture was poured, the mold remained on the dry ice for 10 mins. At this time, the frozen slab was pried loose with a spatula and transferred onto a wire screen in a -20°C freezer where it was dried for two days. It was then further dried at room temperature under a mild vacuum (600 millitorr) for an additional two days. Upon drying, slabs shrank in size to approximately 5cm x 5cm x 0.1cm. The central 3cm x 3cm x 0.1cm slab was excised with a scalpel and straight edge, and then further divided into nine 1cm x 1cm x 0.1cm slabs for release studies and 0.5cm x 0.5cm x 0.1cm slabs for concentration profile studies.
(C.1.2) Coated Matrices:

For one-sided release studies and for concentration profile studies, coated matrices were used. The first part of the procedure for preparing these was the same as that detailed in the previous subsection. Described below is the procedure followed for coating these matrices.

**Coating Procedure:** Matrices were coated on five sides, using paraplast, heated to a temperature of 80°C. The matrix is placed top-side (1cm x 1cm) down on a plastic culture dish. A short-tipped pasteur pipet is equilibrated with the hot paraplast by alternate filling and emptying. Then, a thin ribbon of paraplast is applied around the edge of the matrix which coats the edges and anchors it to the dish. Next, a small amount of paraplast is dripped directly onto the matrix. On cooling, it turns opaque.

The coated matrix should be allowed to cool to room temperature. It is then removed from the dish with a scalpel. The embedded matrix with one side (1cm x 1cm) exposed is now trimmed to approximately 1.4cm x 1.4cm. Finally, for one sided release studies, two straightened stainless steel autoclips are pressed into the paraplast on the back of the slab to anchor it down when placed in the release medium.

(C.2) Assay Procedures

All spectrophotometric assays were performed at room temperature; identical readings were obtained using either the rapid sampler or cuvettes. For all spectrophotometric assays, standard curves were
obtained which are documented in Appendix A. In all release experiments, the frequency of solution changes was kept low enough to ensure spectrophotometer readings greater than 0.050 O.D. units.

Sodium salicylate concentrations were measured by direct absorbance at 294nm (15). Protein concentrations were also measured by direct ultraviolet absorbance. The early release of protein was measured at uv 280nm (46). When concentrations of protein per timepoint fell below that which would give a reading of 0.05 O.D. units at 280nm, a more sensitive assay of absorption at 220nm was used (47). The spectrophotometric assay for protein was compared with the Lowry Assay (48) and good agreement between the two was found for release using the shaker. For stirred matrices however, only the assay at 220nm was found to agree with the Lowry Assay, hence just this wavelength was used.

(C.3) Sectioning Procedure

The cryomicrotome temperature control is set at -25°C. The knife is mounted into the machine and the clean metal chucks (Fig. 1A) which are used for mounting the sample, placed on their holder. The machine is then switched on and takes about 45 minutes to attain the set temperature.

Next, about 1ml of embedding medium (which is a transparent liquid) is poured onto the chucks, and begins to harden at the low temperature. After about 30 secs. it freezes into an opaque solid. It is then planed by cutting thin sections off its top surface.

The release matrix is then placed on top of the planed embedding medium and buttressed by more (about 1ml of) embedding medium (Figure
**Fig 1a.** Chuck (Top and Cutaway Side) Views

**Fig 1b.** Chuck with Sample and Mounting Medium (Top and Cutaway Side) Views
18). For microscopy studies about 9mm² of matrix was cut out from various locations in the original 1cm² release matrix while for concentration profile studies a matrix 25mm² was used for release and for sectioning. In both cases, the original depth 1mm of the slab was maintained (Fig. 2). The matrix mounted on the chuck is allowed to cool for about 45 mins.

The required section thickness can then be set on the machine between 5 μm and 16 μm. It is easier to obtain thinner sections if the sample being sectioned is smaller in area (4mm² - 9mm² for the EVA-protein system). Since thinner sections are more desirable for transmitted light microscopy, 10 μm thick sections were obtained for microscopy. For concentration profile studies however, 16 μm thick sections were obtained. This allowed cutting a lesser number of sections, with a greater chance of recovery of each section, since each section cut may be lost by chipping.

The matrix was then cut and the sections stuck of their own accord to the knife. These were retrieved for microscopy by touching them with a glass slide which was at room temperature. On contact with the glass slide, the sections adhere to the slide and are ready for observation under the microscope. If the sections were to be used for concentration profile studies, an ordinary paintbrush was used to guide the sections from the knife to the vials, for subsequent release studies. Five sections, each 16 μm thick were put into one vial.

After each section was retrieved from the knife, a piece of lint or kimwipe was used to clean the knife and prevent carryover of protein to the next section.
DIMENSIONS IN mm (not to scale)

plane of sectioning

SECTIONS OBTAINED

Fig. 2.: Matrices used for Optical Microscopy (A) and Concentration Profile Studies (B)
(C.4) Critical Point Drying

For observation in the SEM, the release matrices, having been exposed to the release medium, had to be dried in order to permit attainment of high vacuum conditions necessary in the SEM.

Due to the effect of surface tension, great forces act on specimen structures; when pliable wet specimens are dried (49). If the object under investigation is suspected to deform under stress, a more careful drying method must be applied. It must be ensured that the vapour line of the phase diagram is not surpassed. This is achieved by the critical point drying method (Fig. 3).

The critical point method (49) utilizes the phenomenon that the phase boundary from liquid to gaseous state disappears when the critical pressure and temperature are reached. (Beyond the critical point the densities of liquid and gas are equal, i.e., the boundary between liquid and gas vanishes.)

As the critical values for water are \( T_{cr} = 374^\circ C \) and \( P_{cr} = 217.7 \) atm, which are beyond the safety limits of ordinary high pressure apparatus, water is unsuitable and must be replaced by other liquids, e.g., \( \text{CO}_2 \), \( \text{N}_2\text{O} \), or Freon 13.

For the study in this thesis \( \text{CO}_2 \) with a \( T_{cr} = 31.1^\circ C \) and \( P_{cr} = 72.9 \) atm was used. Use of \( \text{CO}_2 \) in drying, offers the advantage of a highly reproducible end-point and less susceptibility than Freon drying, to inadvertent air drying of samples.

The water in the matrix to be dried was replaced by ethanol by increasing ethanol/water of 10% to 100% ethanol. Afterwards, the speci-
K = Critical Point
A = Starting Point
E = End Point

Pressure vs. Temperature

Fig. 3: Phase Diagram and Critical Point Drying
men was transferred in the same fashion in 100% amyl acetate content which is easily miscible with liquid CO₂.

The sample in amyl acetate was placed in a cooled (about 4°C) drying chamber which was subsequently filled under pressure with liquid CO₂. CO₂ vapor was slowly bled to air while refilling the chamber with more CO₂ liquid; this was done to remove the amyl acetate from the sample. Once the amyl acetate was removed, and the sample was in CO₂, the drying chamber was heated to 31.1°C from 4°C, and the pressure increased to 72.9 atm. As soon as (15-20 mins) this temperature and pressure were achieved, the change from liquid to vapour phase of CO₂ occurred, and the sample was dry. The pressure and temperature were then decreased (15-20 mins) in that order to ambient conditions.

In practice, to be on the safe side, the temperature used was 40°C and the pressure was 85 atm, which are slightly in excess of the critical values for CO₂.

The dried samples were then stored in tightly capped vials which were placed in a dessicator.

(C.5) Determination of Protein Particle Density

The density of the protein particles incorporated into the release matrix was determined using a 5ml pycnometer with methylene chloride as the liquid.

The following weights were determined:

\[
Pycnometer + \text{cap} = W_1
\]

\[
Pycnometer + \text{cap} + 5\text{ml methylene chloride} = W_L
\]

\[
Pycnometer + \text{cap} + \text{protein powder} = W_2
\]
Pycnometer + cap + protein + methylene chloride = $W_3$

The density of the methylene chloride is first determined thus:

$$\rho_L = \frac{(W_L - W_1)}{V_T} \text{ gm/ml} \quad (V_T = 5 \text{ ml in this case})$$

The volume of the solid protein is next determined:

$$V_S = V_T - V_L = \frac{(\rho_L V_T + W_2 - W_3)}{\rho_L}$$

Finally, the density of the protein is determined by the equation:

$$\rho_S = \frac{W_S}{V_S} = \frac{\rho_L(W_2 - W_1)}{\rho_L V_T + W_2 - W_3}$$

Thus, the densities of the various protein particles were determined within a standard deviation of about 10%.
CHAPTER V

MICROSTRUCTURE

(A) Introduction

The mechanism of release of macromolecules by the ethylene-vinyl acetate copolymer-drug system is not as yet fully understood. Films of EVA copolymer are not permeable to large molecules (M.W. > 300) but will continuously release much larger molecules (300 < M.W. < 10^7) if they are incorporated into the polymeric matrix as previously described (3).

A possible hypothesis for this phenomenon is "channeled diffusion" which assumes that the macromolecules reside in the matrix in a system of interconnecting channels. Release of protein occurs through paths which lead all the way to the surface of the matrix.

This chapter describes optical microscopy and Scanning Electron Microscopy studies which establish this hypothesis as fact. They also offer evidence that suggests the "moving-front" Higuchi model may not be applicable to the EVA-protein system, and thus form the basis of the proposal of an alternative model in Chapter VII.

(B) Results and Discussion

The ethylene-vinyl acetate copolymer-protein matrices were studied by ordinary light microscopy, Nomarski and SEM, using the techniques and procedures discussed in the previous chapter. The optical techniques were used to study subsurface structure (24), while SEM was used to study surface characteristics (23). For ease of discussion, optical
Fig. 4: Particles of BSA (63-149 μm) MAG: 270X

Fig. 5: 10 μm thick section from centre of EVA matrix without protein incorporated MAG: 270X
Orientation: Perpendicular to plane of mold
studies will be discussed in a subsection separate from SEM even though the two are complementary. In both cases, control studies were conducted before the actual investigation.

(B.1) Studies Using Light Microscopy

(B.1.1) Controls:

(B.1.1.1) Rationale: In order to study the EVA-protein matrix system effectively, it was important to determine the gross particle morphology of the protein aggregates so as to enable their identification in the release matrix. Also, a study of the blank polymer matrix could ascertain whether any pores existed in the polymeric structure without any protein being incorporated in the matrix. It would also provide an indication of any artefacts which may arise due to the sectioning procedure, which could then be discounted in studies with the polymer-protein system.

(B.1.1.2) Experiments: 1) Protein particles of bovine serum albumin (BSA) of different particle size ranges obtained by sieving were studied using ordinary transmitted light microscopy. The results are shown in Fig. 4, for a particle size range 63-149 μm obtained by sieving. The size range for 10 of the particles in Fig. 4 was calculated as 93.4 ± 45.4 μm. The mean does lie within the range 63-149 μm.

2) The blank polymer matrix without any protein incorporated in it was sectioned following the procedure detailed in sub-
Fig. 6.: Schematic of Higuchi Moving-Front Theory
[Adapted from (12)]
section C.3 of Chapter IV. A large number (over 50) sections were obtained from different regions in the polymer matrix and studied by ordinary light microscopy. Fig. 5 is a photomicrograph of one of the sections which is representative of all the sections obtained.

Examining Fig. 5, it is apparent that blank EVA copolymer is a homogeneous structure without any holes being present. Investigation of Fig. 5 also reveals a series of parallel lines marking the EVA section. These were probably due to microscopic imperfections in the cutting edge of the knife, since the lines were always oriented parallel to the direction of knife movement towards the sample being cut. They serve as an important marker of the polymer and help us to distinguish holes in the polymer matrix in ordinary light microscopy studies (Fig. 8).

(B.1.2.) Studies of the Release Matrix At Progressive Times:

In this subsection are described a set of light microscopy studies of the EVA-BSA matrix at progressive times of release. The sections were obtained following the procedure described in subsection C.3 of Chapter IV, from various locations in the matrix (close to the edges as well as towards the centre). Each of the Figs. 7-18 is a photomicrograph of a section representative of over 50 sections from various locations in the release matrix.

(B.1.2.1.) Rationale: The objective of conducting this investigation was to determine whether the incorporation of protein in the
Fig. 7.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix unexposed to release medium MAG: 270X Orientation: Section cut perpendicular to plane of mold

Fig. 8.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix exposed to release medium until termination of release (960 hrs) Orientation: Section cut perpendicular to plane of mold.
matrix caused the formation of pores through which the release of the drug could occur and if so, to investigate the formation over time of voids where the protein had resided. Also, the existence of a "moving-front" parallel to the geometric boundaries of the matrix, which moved inward at successive times, was to be investigated. Such a front (Fig. 6) is the central assumption of the Higuchi model (12,13) which was used to describe kinetic data in a previous study². This model postulates a "moving-front" within the matrix, with all the solute particles on the side of the front closer to the outside surface being in solution and the particles on the inner side not subject to release at all.

(B.1.2.2.) Experiments: 1) Matrices of 75% EVA - 25% BSA (particle size 63-149 μm) w/w loaded were sectioned before being exposed to the release medium. Aggregates of BSA could clearly be discerned (Fig. 7).

2) Matrices of 75% EVA - 25% BSA (particle size 63-149 μm) w/w loaded were exposed to the release medium (distilled water), until no more protein was being released and sections were obtained. Holes were observed (Fig. 8) which were within the same size range as the original particles of protein incorporated in the matrix. The size range of 10 of the holes in Fig. 8 was calculated at 77.0 ± 19.13 μm.

3) Matrices of 75% EVA - 25% BSA (particle size 63-149 μm) w/w loaded were exposed to the release medium (distilled

Fig. 9.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 2 hours (10%) of release MAG: 270X.

Orientation: Section cut perpendicular to plane of mold.

Fig. 10.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 2 hours (10%) of release MAG: 270X.

Orientation: Section cut perpendicular to plane of mold.
Fig. 11.: 10 µm thick section from centre of 75% EVA - 25% BSA (63-149 µm) w/w matrix after 16 hours (22%) of release. MAG: 270X.
Orientation: Section cut perpendicular to plane of mold.

Fig. 12.: 10 µm thick section from centre of 75% EVA - 25% BSA (63-149 µm) w/w matrix after 40 hours (30%) of release. MAG: 270X.
Orientation: Section cut perpendicular to plane of mold.
Fig. 13.: 10 μm thick section from centre of 75%EVA - 25% BSA (63-149 μm) w/w matrix after 158 hours (45%) of release. MAG: 270X.
Orientation: Section cut perpendicular to plane of mold.

Fig. 14.: 10 μm thick section from centre of 75%EVA - 25% BSA (63-149 μm) w/w matrix after 158 hours (45%) of release. MAG: 1080X.
Orientation: Section cut perpendicular to plane of mold.
water) for different time periods of release, and sections of the matrices were obtained corresponding to these different time periods (Figs. 9-14).

All of these above studies were performed using ordinary light microscopy. Since both the polymer and the protein (BSA) particles were colorless, Nomarski (Differential Interference Contrast) microscopy was used next to obtain better contrast. Use of a chemical staining agent (e.g., Coomassie Blue) which could dye the protein particles selectively was precluded because it would have involved exposing the thin sections to a liquid which may have caused the protein to dissolve or get dislodged from the polymer thus introducing artefacts.

Accordingly, the thin sections obtained as described under experiment 3 were studied using Nomarski (Figs. 15-18). The polymer and background appear blue and the protein particles appear yellow.

Examining Figs. 7-18 we find that at successive time intervals an increasing area or pores, formerly occupied by protein, is void. This trend has been followed progressively from 0 hours (Fig. 7) upto 158 hours (Fig. 13) of release. Also, Fig. 8 displays a section of the matrix at 960 hours when release has ceased. Investigation at intermediate times between 158-960 hours would show the same trend of increasing
Fig. 15.: 10 \mu m thick section from centre of 75\% EVA - 25\% BSA (63-149 \mu m) w/w matrix after 2 hours (10\%) of release MAG: 580X.  
Orientation: Section cut perpendicular to plane of mold.  
[NOMARSKI]

Fig. 16.: 10 \mu m thick section from centre of 75\% EVA -25\% BSA (63-149 \mu m) w/w matrix after 158 hours (45\%) of release MAG: 580X.  
Orientation: Section cut perpendicular to plane of mold.  
[NOMARSKI]
Fig. 15.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 2 hours (10%) of release MAG: 580X.
Orientation: Section cut perpendicular to plane of mold.
[NOMARSKI]

Fig. 16.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 158 hours (45%) of release MAG: 580X.
Orientation: Section cut perpendicular to plane of mold.
[NOMARSKI]
Fig. 17.: 10 µm thick section from centre of 75% EVA - 25% BSA (63-149µm) w/w matrix after 7 hours (16%) of release.
MAG: 580X.
Orientation: Section cut perpendicular to plane of mold.
[NOMARSKI] Focus adjusted to top of hole.

Fig. 18.: 10 µm thick section from centre of 75% EVA -25% BSA (63-149 µm) w/w matrix after 7 hours (16%) of release.
MAG: 580X.
Orientation: Section cut perpendicular to plane of mold.
[NOMARSKI] Focus adjusted to bottom of hole.
Fig. 17.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 7 hours (16%) of release. MAG: 580X.
Orientation: Section cut perpendicular to plane of mold. [NOMARSKI] Focus adjusted to top of hole.

Fig. 18.: 10 μm thick section from centre of 75% EVA - 25% BSA (63-149 μm) w/w matrix after 7 hours (16%) of release. MAG: 580X.
Orientation: Section cut perpendicular to plane of mold. [NOMARSKI] Focus adjusted to bottom of hole.
void area, up to the time when the protein no longer exists in a solid form. Mention must be made of the fact that a lower loading or particle size may cause a sizable fraction of the drug to be encapsulated. In that case, particles of protein may be observed even after release has ceased.

Another observation apparent from Figs. 7-18 is the absence of a "moving-front" parallel to the geometric boundaries of the matrix, which moves inward at successive times. In contrast, Figs. 9-18 indicate that holes begin to appear throughout the matrix. At a given time, protein in a pore closer to the surface may still be present even though deeper within the matrix, holes may have appeared.

To illustrate this point with a specific example, consider Fig. 10. We observe a hole (as marked) farther from the boundary of the matrix than a protein particle near the boundary. Figs. 17 and 18 which are Nomarski studies of the same section confirm this observation. Since Nomarski allows a very shallow depth of focus, one can focus at the top of the hole (Fig. 17) so that the bottom of the hole is out of focus and vice versa (Fig. 18). In Fig. 17 the protein particles which appear yellow under Nomarski are in focus and in Fig. 18 they are out of focus, since their upper surfaces lie in a plane coincident with the surface of the section and the top of the hole.
Fig. 19.: SEM micrograph of the surface of a blank EVA matrix without protein incorporated.
MAG: 100X.
Fig. 20.: SEM micrograph of the surface of a 75% EVA - 25% BSA (63-149 \( \mu \)m) w/w matrix unexposed to release medium. MAG: 100X.
Figs. 13 and 14 which are ordinary light microscopy studies of a section at two different magnifications again illustrate this observation. Fig. 14 shows an enlarged view of a rectangular pore partially filled with protein, while both Figs. 13 and 14 indicate that empty holes have appeared beyond it, deeper in the matrix.

The fact that no "moving-front" is observed in the EVA-protein system suggests that using the Higuchi model is inappropriate for this system.

(B.2.) Studies Using SEM

(B.2.1.) Controls:

(B.2.1.1.) Rationale: As in the optical microscopy studies, a study of the blank polymer could ascertain whether any pores existed in the polymeric structure without any protein being incorporated in the matrix.

(B.2.1.2.) Experiment: The blank polymer matrix without any protein incorporated in it was studied by SEM (Fig. 19). A few pores were observed, but these were only superficial surface irregularities.

(B.2.2.) Investigation of EVA-BSA Matrices:

(B.2.2.1.) Rationale: Since the optical microscopy studies had provided evidence to support the "channeled diffusion" theory, SEM
Fig. 21.: SEM micrograph of the surface of a 75% EVA - 25% BSA (63-149 μm) w/w matrix exposed to release medium until termination of release (960 hours). MAG: 100X.
Fig. 22.: SEM micrograph of the surface of a 75% EVA - 25% BSA (63-149 μm) w/w matrix exposed to release medium until termination of release (960 hours). MAG: 1000X
(Magnified view of pore marked in previous figure).
Fig. 23: SEM micrograph of the surface of a 75% EVA - 25% BSA (63-149 μm) w/w matrix exposed to release medium until termination of release (960 hours). MAG: 3000X
(Magnified view of pore marked in preceding two figures).
could be used as a totally independent means of confirming the presence of channels.

(B.2.2.2.) Experiments: 1) Matrices of 75% EVA - 25% BSA (63-149 μm) w/w loaded were studied before being exposed to the release medium (distilled water) (Fig. 20).
2) Matrices of 75% EVA - 25% BSA (63-149 μm) w/w loaded were exposed to the release medium (distilled water) for 960 hours. These were then dried using the procedure described in subsection C.4 of Chapter IV and studied under SEM (Figs. 21-23). Channels were observed which would permit the passage of protein molecules from the interior of the matrix to the surface. These were in the same size range as the original protein particles (101 ± 33.4 μm for 6 pores).

Figs. 21-23 are progressive magnifications of a particular pore on the surface of the leached-out matrix. These figures illustrate the interconnection of pores within the matrix. One can speculate that the narrow neck which is observed in the right hand corner of Figs. 22 and 23 could lead into a larger pore deeper within the matrix, thus giving rise to a continuous channel from the surface to the inside.

Thus, the optical microscopy and SEM studies complement each other, confirm the hypothesis of "channeled diffusion" and question the propriety of using the "moving-front" Higuchi model to explain release.
CHAPTER VI

KINETICS

(A) Introduction

Preliminary studies describing the kinetics of release by the ethylene-vinyl acetate copolymer-protein system have been conducted (3,50). Release rates have been found to vary with the particle size of the protein aggregates as well as percentage weight loading of protein in the matrix. The hypothesis of "channeled diffusion" proposed to explain release has been confirmed in the previous chapter.

This chapter describes kinetic studies directed towards further defining the parameters affecting release. In particular, the kinetics of stirred versus shaken matrices were compared and no difference in release rates was found. Kinetic data for matrices releasing from only one side was also collected and is described herein.

This chapter also presents preliminary studies, describing the concentration profile within the release matrix before and after exposure to the release medium.

(B) Results and Discussion

In this section are described four distinct sets of experiments conducted to characterize the kinetics of release. The first set comprises various control experiments, the second deals with investigating possible boundary layer effects, the third presents data for slabs releasing from only one side and the fourth describes studies of the concentration profile within the release matrix.
(B.1.) Controls

(B.1.1.) **Rationale:** Since ultra-violet spectroscopy was to be used in measuring the release from the ethylene-vinyl acetate-protein matrices, it was imperative to determine whether or not any substance which may leach out from the release matrix would interfere with the spectrophotometric assay. Such a substance could be a resin or plasticizer incorporated during manufacture in the polymerization process or residual methylene chloride used as a solvent during the casting procedure. Accordingly, control experiments were conducted to investigate both these alternatives of potential artefacts. Also, the stainless steel mesh to be used in the study of release under stirred conditions (described in subsection B.2.2.) could conceivably be oxidised and thus release some substance which could interfere with the assay. Hence, a control experiment was conducted to eliminate possible artefacts due to the mesh. Paraplast, which was used to coat the matrices for one-sided release studies was also investigated.

In addition to these various control experiments conducted to eliminate possible artefacts due to the intrusion of any substance during assay, yet another control experiment was performed to validate the assumption of perfect sink conditions in the release medium. Such an assumption was necessary in the Higuchi model and convenient in the model proposed to explain release kinetics as detailed in section D of Chapter VII.
All the control experiments conducted are described in the following subsection.

(B.1.2.) **Experiments:**
1) A few drops of methylene chloride were mixed with 10 ml of distilled water. The mixture did not exhibit any absorbance at 220 or 280 nm which were the wavelengths of ultra-violet light to be used in the assay for protein.
2) The blank ethylene-vinyl acetate copolymer matrix was exposed to the release medium (distilled water) for several weeks. No measurable change in optical density units was observed.
3) The stainless steel mesh (claimed by the manufacturer as being corrosion-resistant) was submerged in the release medium (distilled water) which was stirred, using a teflon stirrer, for several weeks. There was no measurable change in the optical density of the medium. In addition, the mesh was exposed to a concentrated protein solution and on subsequent exposure to release medium caused no change in its optical density. This observation eliminated the possibility that protein molecules could adsorb on the surface of the mesh and hence introduce artefacts during assay.
4) Paraplast, on exposure to the release medium (saline) did not cause any measurable change in its optical density.
5) Release was monitored for two sets of matrices 67% EVA - 33% BSA (180-250 μm). One set was exposed to 10 ml of release medium (saline) while the other was exposed to 15 ml. Solution
was changed in both these cases at time periods greater than 6 hrs. No difference in release kinetics was found in the two cases. This indicates that 10 ml of release medium generates a solution (released protein + release medium) as dilute as does 15 ml. Thus exposing the slabs to 10 ml of release medium is sufficient to approximate ideal sink conditions. If any difference in release kinetics had been discerned in the two cases, the volume of release medium would have had to be increased in steps of increasing volume until no difference would be discerned in two subsequent steps.

Having conducted these controls, the kinetic experiments could then be performed with confidence.

(B.2.) Studies Investigating Boundary Layer Effects

(B.2.1.) Rationale: Investigators have documented the effect of the boundary layer on rates of release from reservoir systems (51). The Biot number (52) which is the ratio of the external to the internal mass transfer rates, serves as a guide in deciding whether or not boundary layer effects are significant. In general, a value of the Biot number less than 0.1 indicates that the resistance to diffusion is mainly in the boundary layer, while a value greater than 25 indicates that the predominant resistance is within the matrix. Intermediate values however, demand experiments to determine which resistance is controlling.
In general the Biot number can be written as:

$$Bi = \frac{k_C d}{D_{int}}$$  \hspace{1cm} (1)

where $d$ is a critical dimension, $D_{int}$ is the internal diffusivity

$k_C$ is the external mass transfer coefficient.

If $d$ is expressed in cm, $D_{int}$ in cm$^2$/sec, the units of $k_C$ are cm/sec. To obtain an estimate of $k_C$ we use the Sherwood number obtained from the Rantz correlation (53).

$$Sh = \frac{k_C d}{D_{ext}} = 2.0 + 1.8 \text{Sc}^{1/3} \text{Re}^{1/2}$$  \hspace{1cm} (2)

where Sc is the Schmidt number and Re the Reynold's number.

Under stagnant conditions, which essentially is the case for unstirred slabs, the Reynold's number approaches zero and the Sherwood number approaches 2.0. Hence, by transposition,

$$k_C = 2.0D_{ext}/d$$  \hspace{1cm} (3)

Substituting equation (3) in (1) we get:

$$Bi = 2.0 \frac{D_{ext}}{D_{int}}$$  \hspace{1cm} (4)

Next, estimating: $D_{int} = D_{ext} \varepsilon^2$ as recommended by Walker et. al (54) where $\varepsilon$ is the porosity, we obtain:

$$Bi = 2.0/\varepsilon^2$$  \hspace{1cm} (5)

For the system under consideration, taking for example the case
Fig. 24: Schematic of device used for Boundary Layer Experiment
of 50% EVA - 50% BSA (75-250 μm) w/w the porosity $\epsilon = 0.65^3$. The calculated value of the Biot number is 4.73.

From the foregoing discussion it emerges that we cannot decide on the basis of theory alone whether the controlling resistance lies within the matrix or outside it. However, since the value calculated for the Biot number is in excess of 0.1, this suggests that boundary layer resistance is not solely controlling. However the value of the Biot number: 4.73 (less than 25) does not establish internal resistance to diffusion as being totally controlling either. It is evident that experimental means for determining the controlling resistance are justified.

(B.2.2.) **Experiment:** Three sets of matrices of 75% EVA - 25% BSA (63-149 μm) w/w loaded were exposed to the release medium (distilled water). Each individual matrix was exposed to a constant volume (10 ml) of medium which was replaced frequently to approximate ideal sink conditions. The first set was neither shaken nor stirred, the second set was subjected to shaking, while the third was subjected to a high rate of stirring (greater than 1000 rpm) in vials equipped with teflon stirrers (Fig. 24). Release kinetics were measured for the three cases. The results are displayed in Fig. 25. It is evident that the release kinetics for matrices under stirred conditions coincides with that for matrices subjected to shaking only, and those not subjected to shaking or stirring.

Fig. 25: Comparison of release kinetics of matrices containing BSA (63-149 µm) 25% loaded under stirred, shaken and stationary conditions. The curves all have a standard deviation of 10-20%.
This observation implies that the predominant resistance to protein mass transfer lies within the matrix, with the boundary layer resistance being insignificant. If this were not the case, all release experiments to determine the intrinsic kinetics of the matrices would have to be conducted under rates of stirring high enough to eliminate the boundary layer resistance. Thus, the results displayed in Fig. 25 allow release experiments to be conducted subjecting the matrices to merely shaking.

(B.3.) One-Sided Release Studies

(B.3.1.) Rationale: The motivation for these studies stemmed from the fact that release from only one side of the matrix versus release from all six sides would afford a simpler system to study the release kinetics. This could then help to characterize the release process better.

(B.3.2.) Experiment: Matrices of EVA-protein (BSA/lysozyme/β-lactoglobulin) were prepared using protein of various size ranges and percent w/w loadings. The dimensions of these matrices were carefully measured using a micrometer (Table 1). Next, the matrices were coated as described in subsection C.1.2. of Chapter IV. These were then exposed to the release medium (saline) and the kinetics of protein release was determined as in subsection B.2.2. above. The slabs were subjected to merely shaking, based on the results of Fig. 25. The
### TABLE 1
Physical Parameters of the EVA-Protein Matrices Used for Release Studies

<table>
<thead>
<tr>
<th>Protein</th>
<th>Particle Size (μm)</th>
<th>Weight% Loading</th>
<th>Average Volume A. &amp; (cc)</th>
<th>Average Area A.(cm²)</th>
<th>Average Thickness &amp; (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>106-150</td>
<td>10</td>
<td>0.068</td>
<td>1.0</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>106-150</td>
<td>20</td>
<td>0.087</td>
<td>0.99</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>106-150</td>
<td>25</td>
<td>0.084</td>
<td>0.96</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>106-150</td>
<td>30</td>
<td>0.089</td>
<td>1.0</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>105-180</td>
<td>20</td>
<td>0.068</td>
<td>0.88</td>
<td>0.077</td>
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<tr>
<td></td>
<td>105-180</td>
<td>25</td>
<td>0.083</td>
<td>1.06</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>105-180</td>
<td>30</td>
<td>0.078</td>
<td>1.0</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>250-425</td>
<td>10</td>
<td>0.071</td>
<td>1.08</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>250-425</td>
<td>25</td>
<td>0.084</td>
<td>1.10</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>250-425</td>
<td>30</td>
<td>0.086</td>
<td>1.04</td>
<td>0.082</td>
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<td>β-lactoglobulin</td>
<td>75-150</td>
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<td>0.931</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>75-150</td>
<td>25</td>
<td>0.067</td>
<td>0.96</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>75-150</td>
<td>40</td>
<td>0.09</td>
<td>1.0</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>75-150</td>
<td>50</td>
<td>0.105</td>
<td>1.0</td>
<td>0.105</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>106-150</td>
<td>15</td>
<td>0.065</td>
<td>1.0</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>106-150</td>
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<td>0.09</td>
<td>0.90</td>
<td>0.1</td>
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<tr>
<td></td>
<td>106-150</td>
<td>50</td>
<td>0.099</td>
<td>0.90</td>
<td>0.11</td>
</tr>
</tbody>
</table>
results obtained from these studies appear in Figs. 26-30. Rates of release can be seen to vary with particle size and weight loadings for all the proteins studied. In Chapter VII will be presented a mathematical model to explain these results.
Fig. 26: One sided release kinetics for matrices containing BSA of particle size 106-150 μm, at various loadings. Each curve represents the mean of at least 8 samples.
Fig. 27.: One sided release kinetics for matrices containing BSA of particle size 150-180 µm, at various loadings. Each curve represents the mean of at least 8 samples.
Fig. 28.: One sided release kinetics for matrices containing BSA of particle size 250-425 μm, at various loadings. Each curve represents the mean of at least 8 samples.
Fig. 29.: One sided release kinetics for matrices containing β-lactoglobulin of particle size 75-150 μm, at various loadings. Each curve represents the mean of at least 8 samples.
Fig. 30: One sided release kinetics for matrices containing lysozyme of particle size 106-150 μm, at various loadings. Each curve represents the mean of at least 8 samples.
(B.4) Concentration Profile Studies

(B.4.1.) Rationale: Since the microstructural studies detailed in Chapter V provided evidence which cast doubt on the applicability of the "moving-front" Higuchi model (Fig. 31) to the EVA-protein system, it was felt that an investigation of the concentration profile within the release matrix would prove advantageous. Accordingly, studies were undertaken to generate these profiles.

(B.4.2.) Experiments: 1) Matrices of 75% EVA - 25% BSA (180-250 μm) w/w loaded were coated on five sides as described in subsection C.1.2. of Chapter IV. These were then sectioned as described in subsection C.3 of Chapter IV, without being exposed to the release medium (saline). The sections thus obtained were collected in vials and subjected to release for one week (i.e., 168 hrs). Five consecutive sections each 16 μm thick were put in the same vials and exposed to 5 ml of release medium (saline). Other vials also containing 5 ml of release medium were used to release protein from five sections (16 μm x 5 = 80 μm) consecutive to the previous five along the length of the matrix.

Thus, the concentration profile in matrices unexposed to release medium was generated as displayed in Fig. 32 which represents the mean of 5 matrices. The points on the curve correspond to the release from each set of five 16 μm thick
MATRIX ON EXPOSURE TO RELEASE MEDIUM AT TIME 't'

Fig. 31: Schematic of Higuchi Moving-Front Theory
[Adapted from (12)]
slices (i.e., 5 x 16 μm = 80 μm) along the EVA-BSA matrix. These points generate a profile which is essentially flat. However, a number of peaks and valleys do appear. These can be explained by the fact that the protein resides in pores within the matrix, rather than as an even dispersion throughout the matrix structure.

In Fig. 32, the cross-hatched regions of the profile which slope down towards the edges are an artefact due to the fact that only incomplete sections of the matrix were obtained in this region. This occurs probably because the release matrix may have slight departures from ideality in not having totally flat surfaces. Thus the areas of the face of the slab which are slightly raised with respect to the rest of the surface are sectioned first and afford incomplete sections. These obviously release less protein than full sections of the entire slab cross-section would, and thus cause the concentration profile to slope down towards the edges. About 25% of the total area under the concentration profile lies in this region.

2) Matrices identical to those used in the previous experiment were exposed to the release medium (saline) for 10 days (240 hrs). Release was from one large (1cm x 1cm) face. About 50% of the protein originally present in the slabs was released during this time. The slabs were then patted dry on a kimwipe and then sectioned. A concentration profile was obtained (Fig. 33) as in the previous experiment. This represents the mean of
Fig. 33: Concentration profile of matrices containing BSA (180-250 μm) after release for 240 hrs. Profile represents the mean of 2 samples.
two slabs. This profile appears to be different from that envisaged by the "moving-front" Higuchi theory (Fig. 31).

Figs. 32 and 33 represent preliminary results of investigations relating to concentration profile characterization. At present, they serve as a qualitative guide to visualize the concentration profile within the release matrix.

After having presented the results and discussion of the microstructural and kinetic investigations, as detailed in Chapters V and VI, theoretical analyses of these will be undertaken in the next chapter.
CHAPTER VII

MODELLING AND COMPUTER SIMULATION

(A) Introduction

The microstructural and kinetic investigations of the ethylene-vinyl acetate-protein system have been documented in Chapters V and VI. The limitations of the "moving-front" Higuchi model (12,13) with regard to this system will be discussed in this chapter.

An alternate model which successfully explains the kinetic data will then be presented, together with its computer simulation. Correlations will then be developed which have a predictive value.

Also described in this chapter are procedures used to estimate some physical parameters relevant to the theoretical analysis.

(B) Determination of Physical Parameters

In this section are described procedures to estimate various physical parameters of the polymer-protein system for use in the subsequent analysis. These include the protein solubility $C_s$, the bulk diffusion coefficient of the proteins in water $D_0$ and the matrix porosity $\epsilon$. Each of these will be dealt with in a separate subsection.

(B.1.) Estimation of Protein Solubility

The solubility of the proteins was estimated by equilibrating an excess of solid protein powder with 1 ml of distilled water in tightly capped vials and assaying aliquots at large times (several weeks) after
dilution.

The experiment was performed in duplicate and the solutions were analyzed at 280 nm.

Application of this method yielded a solubility value for BSA in water at room temperature (18-24°C) of 563 mg/ml. This agrees quite well with the value determined by Kozinski (55), who found the solubility of BSA in pure water to be about 585 mg/ml at 25°C.

The solubility estimates for the proteins studied in this thesis are displayed in Table 2.

(B.2.) Estimation of Protein Diffusivity

The classical method for determination of protein bulk diffusivity D involves free diffusion from a solution of the molecule being studied into a layer of solvent above the first solution (56). Concentration distributions are determined by measuring refractive index distributions originating at the interface of the two solutions. At various times the refractive index profiles can be used to yield values of the diffusion coefficient D based on the following equation:

$$\frac{\partial n}{\partial x} = \frac{n_1 - n_0}{(4\pi D t)^{1/2}} \exp(-x^2/4D t)$$

where $n_0$ and $n_1$ are respectively the index of refraction of solvent and protein solution.

Values of the diffusion coefficient of various proteins based on this method are well documented in the literature. It has become standard practice to express all diffusion coefficients as if they had been measured in pure water at 20°C for which the viscosity $\eta = 0.01005$
<table>
<thead>
<tr>
<th>Protein</th>
<th>Particle Density (gm/c.c)</th>
<th>Solubility Cs (mg/ml)</th>
<th>Weight % Protein</th>
<th>Concentration Co = A (mg/ml)</th>
<th>Porosity ε</th>
<th>Volume % protein (100·ε)</th>
<th>Higuchi Parameter (3·ε·Cs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (106-150µm)</td>
<td>1.24</td>
<td>563</td>
<td>10</td>
<td>97</td>
<td>0.0782</td>
<td>7.82</td>
<td>137.24</td>
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<td></td>
<td></td>
<td>20</td>
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<td></td>
<td></td>
<td>25</td>
<td>263</td>
<td>0.2121</td>
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<td></td>
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<td>0.2588</td>
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<td>193</td>
<td>0.1625</td>
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<td>25.26</td>
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<td>0.1095</td>
<td>10.95</td>
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<td>25</td>
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<td>0.267</td>
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<td>267</td>
<td>0.3286</td>
<td>32.86</td>
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<td>β-Lactoglobulin (75-150µm)</td>
<td>1.128</td>
<td>390</td>
<td>15</td>
<td>157</td>
<td>0.1392</td>
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<td>240</td>
<td>0.2127</td>
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<td>363</td>
<td>0.3218</td>
<td>32.18</td>
<td>376.51</td>
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<tr>
<td>Lysozyme (106-150µm)</td>
<td>1.125</td>
<td>385</td>
<td>15</td>
<td>132</td>
<td>0.1173</td>
<td>11.73</td>
<td>135.48</td>
</tr>
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<td>27.06</td>
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</tbody>
</table>
poise. The product $Dn/T = constant$ is calculated for the actual conditions under which $D$ was determined; and this product is set equal to $D_{20}$ (0.01005)/293.16, thus yielding values for $D_{20}$ commonly reported. Alterations in $D_{20}$ are minor in the case of water or dilute salt solutions at temperatures between $0^\circ C$ and $25^\circ C$ (57).

In the investigations in our laboratory, the ambient temperature varied between $18^\circ - 24^\circ C$ with a mean around $21^\circ C$. Thus the values of the bulk diffusion coefficients used in analyzing the experimental data were taken as the reference values at $20^\circ C$. Table 3 displays these values for BSA (58), $\beta$-lactoglobulin (59) and lysozyme (57).

(B.3.) Calculation of Matrix Porosity

The density of different size ranges was determined for various proteins, following the method detailed in subsection C.5 of Chapter IV. The results are displayed in Table 2 together with the protein concentrations for the various slabs.

The porosity for a particular matrix is then computed thus:

$$
\varepsilon = (c.c \text{ protein})/(c.c \text{ matrix}) = C_0/(\rho_s \times 1000) = \frac{(\text{protein concentration in slab})}{(\text{protein density}) \times 1000}
$$

where $C_0$ is in mg/cc matrix, and $\rho_s$ is in gm/cc protein.

This method of porosity estimation makes certain assumptions:

1) The volume of air trapped in the matrix is negligible compared to the volume occupied by the protein. Examination of Fig. 7 in
Simulated and Experimental Parameters of the EVA-Protein Matrices Used for Release

<table>
<thead>
<tr>
<th>Protein</th>
<th>Bulk Diffusion Coefficient (cm²/hr)</th>
<th>Volume % Protein (100·ε)</th>
<th>Effective Diffusion Coefficient (cm²/hr)</th>
<th>Factor F Simulated</th>
<th>Factor F from equation</th>
<th>Root Mean Squared [Mt - (M_∞)Exp^-(Mt/M_∞)*t]</th>
<th>Maximum Experimental Std.Dev. SDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (106-150µm)</td>
<td>2.52 E-3</td>
<td>7.82</td>
<td>0.2 E-7</td>
<td>0.8 E-5</td>
<td>0.71 E-5</td>
<td>3.66</td>
<td>1.72</td>
</tr>
<tr>
<td>(58)</td>
<td></td>
<td>16.04</td>
<td>0.2 E-6</td>
<td>0.8 E-4</td>
<td>1.5 E-4</td>
<td>2.5</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.21</td>
<td>0.88 E-6</td>
<td>0.35 E-3</td>
<td>0.49 E-3</td>
<td>1.54</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.88</td>
<td>0.19 E-5</td>
<td>0.75 E-3</td>
<td>1.2 E-3</td>
<td>1.49</td>
<td>8.15</td>
</tr>
<tr>
<td>BSA (150-180µm)</td>
<td>2.52 E-3</td>
<td>16.25</td>
<td>0.2 E-6</td>
<td>0.8 E-4</td>
<td>1.6 E-4</td>
<td>3.50</td>
<td>3.49</td>
</tr>
<tr>
<td>(58)</td>
<td></td>
<td>21.05</td>
<td>0.16 E-5</td>
<td>0.65 E-3</td>
<td>0.48 E-3</td>
<td>0.74</td>
<td>4.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.26</td>
<td>0.63 E-5</td>
<td>0.25 E-2</td>
<td>0.11 E-2</td>
<td>2.03</td>
<td>14.23</td>
</tr>
<tr>
<td>BSA (250-425µm)</td>
<td>2.52 E-3</td>
<td>10.95</td>
<td>0.2 E-6</td>
<td>0.8 E-6</td>
<td>0.3 E-6</td>
<td>6.20</td>
<td>5.99</td>
</tr>
<tr>
<td>(58)</td>
<td></td>
<td>26.7</td>
<td>0.71 E-5</td>
<td>0.27 E-2</td>
<td>0.13 E-2</td>
<td>2.98</td>
<td>18.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.86</td>
<td>0.1 E-4</td>
<td>0.4 E-2</td>
<td>0.33 E-2</td>
<td>1.32</td>
<td>19.54</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>2.82 E-3</td>
<td>13.92</td>
<td>0.65 E-7</td>
<td>0.23 E-4</td>
<td>0.83 E-4</td>
<td>1.37</td>
<td>2.34</td>
</tr>
<tr>
<td>(59)</td>
<td></td>
<td>21.27</td>
<td>0.85 E-6</td>
<td>0.3 E-3</td>
<td>0.5 E-3</td>
<td>1.11</td>
<td>3.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.06</td>
<td>0.59 E-5</td>
<td>0.21 E-2</td>
<td>0.14 E-2</td>
<td>2.36</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.18</td>
<td>0.11 E-4</td>
<td>0.38 E-2</td>
<td>0.3 E-2</td>
<td>2.42</td>
<td>2.77</td>
</tr>
<tr>
<td>Lysozyme (106-150µm)</td>
<td>3.74 E-3</td>
<td>11.73</td>
<td>0.28 E-6</td>
<td>0.75 E-4</td>
<td>0.4 E-4</td>
<td>0.46</td>
<td>2.36</td>
</tr>
<tr>
<td>(57)</td>
<td></td>
<td>27.06</td>
<td>0.56 E-5</td>
<td>0.15 E-2</td>
<td>0.14 E-2</td>
<td>1.48</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.13</td>
<td>0.74 E-5</td>
<td>0.2 E-2</td>
<td>0.38 E-2</td>
<td>3.12</td>
<td>3.13</td>
</tr>
</tbody>
</table>
Chapter V (photomicrograph of the 75% EVA - 25% BSA (63-149 µm) w/w loaded matrix before exposure to the release medium) justifies this assumption.

2) The protein density remains the same after the casting procedure. This can be justified by the fact that the holes found in the matrix after release were in the same size range as the original particles incorporated in the matrix as discussed in subsection B.1 of Chapter V.

3) The porosity is uniform throughout the matrix body. Examination of Fig. 8 in Chapter V (photomicrograph of the 75% EVA - 25% BSA (63-149 µm) w/w loaded matrix after 960 hours of exposure to release medium) indicates that the pores are uniformly distributed throughout the matrix structure.

The calculated values of porosity ε are listed in Table 2, together with the percentage volume loading (100ε).

(C) Discussion of the Applicability of the Higuchi Model

One assumption of the Higuchi model (12,13) is the concept of a "moving-front" within the release matrix parallel to the geometric boundaries of the matrix, with the solute particles on the side of the front closer to the outside surface being in the process of release and the particles on the inner side not being subject to release at all (Fig. 34).

For the EVA-protein system as discussed in Chapter V, there is no
Fig. 34.: Schematic of Higuchi Moving-Front Theory
[Adapted from (12)]
evidence of such a front, rather release appears to occur from throughout the matrix structure.

Secondly, as stressed by Higuchi (13), the "moving-front" model proposed by him can apply only to systems in which $A$ (the total amount of drug present in the matrix per unit volume) is greater than $C_s$ (the drug solubility) or $\varepsilon C_s$ (where $\varepsilon$ is the porosity) by a factor of three or four. Desai et. al (15-18) when using the Higuchi model used drugs of low solubility with a range between 10.8-116 mg/ml. The solubility of proteins is comparatively very high. Indeed BSA is considered to be soluble in water in all proportions according to some investigators (56). Kozinski (55) found the solubility of BSA in pure water to be about 585 mg/ml at 25°C. It can be seen from Table 2 that the matrix concentrations for the EVA-BSA system do not comply with the requirement stressed by Higuchi, that $A$ be greater than $C_s$ or at least $\varepsilon C_s$ by a factor of 3 or 4. In fact, Table 2 shows that $A$ is less than $C_s$ for all loadings of BSA (e.g., 97 < 563 mg/ml). $A$ is also less than $3\varepsilon C_s$.

A trend similar to that exhibited by BSA is observed for the other proteins. Thus, in all the cases protein solubility is in excess of the concentration of protein in the matrix.

Thus, clearly, in these EVA-protein systems the major premise of the Higuchi model is not satisfied.

Thirdly, the Higuchi model predicts linear release kinetics when cumulative percentage release is plotted against $(time)^{1/2}$ (14). Though some of the curves presented in Chapter VI appear linear, many of them show significant curvature which increases with loading. This observation indicates that the linear curves are probably degenerate instan-
ces of a more general non-linear behaviour and linearity should be considered not the rule but rather a simplification. A good model would seek to simulate all the observed experimental data both linear and non-linear. Viewed in this perspective, application of any linear model (like Higuchi's) would not be correct. However, an attempt was made to do this. The results are contained in Appendix B.

Clearly, a need emerges for a model consistent with all the experimentally observed phenomena and one which effectively explains the release kinetics.

(D) Proposed Mathematical Model

In this section is described a model for the diffusion kinetics presented in Chapter VI. The assumptions for this model are as follows:

1) Diffusion of the protein occurs through liquid-filled pores in the matrix structure, consistent with the microstructure results in Chapter V.

2) Perfect sink conditions prevail, i.e., the solution in which the matrix is immersed is essentially at zero concentration as discussed in subsection B.1 of Chapter VI.

3) The rate-limiting step for mass transfer is diffusion within the matrix, i.e., the boundary layer effects are insignificant, consistent with the results presented in subsection B.2 of Chapter VI.

4) The protein concentration in the matrix is below its solubility limit (Table 2).
5) The bulk diffusion coefficient $D_0$ is independent of concentration. This assumption is often made (60). In particular, in drug controlled release systems analysis, researchers assume that the diffusivity is not a function of concentration (61). Even the commonly used Higuchi equation (12,13), together with its extensions to complicated geometries by Baker and Lonsdale (9) and Roseman and Higuchi (30) is based on this assumption (61).

However, when considering the diffusion of proteins (which often have high solubilities) the diffusion coefficient does vary with concentration (62,63). Keller et. al (62) described this variation for BSA thus:

$$\frac{D}{D_i} = \frac{\tanh (21.3\phi)}{21.3\phi}$$

where $D$ is the diffusion coefficient at high concentrations, $D_i$ is the diffusion coefficient at infinite dilution and $\phi$ is the volume fraction (dimensionless) of solute in the solution. At a concentration of BSA of 315 mg/ml this ratio was found to be nearly 4.9.

Anderson (63) developed a simpler expression to describe the variation of the diffusion coefficient for macromolecules:

$$\frac{D}{D_i} = (1 - \phi)^{6.5}$$

Applying this equation to the experimental data of Keller (62) he got a value of $D/D_i = 5$. 

In general then it is not strictly correct to make the assumption that the bulk diffusion coefficient $D_0$ is independent of concentration. However, making this assumption a model describing the observed kinetics is developed. The implications of this will be discussed in subsection E.4 of this chapter.

6) All the protein incorporated into the matrix is available for release. This is probably a good assumption for sufficiently high loadings of protein. However, at very low loadings this may not be the case, because some of the protein may be encapsulated by the impermeable polymer. One may then expect discrepancies between experiment and theory at very low loadings.

No assumption of a "moving-front" is necessary.

The EVA-protein system can be treated as a plane sheet.

Fick's second law of diffusion states:

$$\frac{\partial C}{\partial t} = D_0 \frac{\partial^2 C}{\partial x^2}$$ for one dimension.

The assumptions are:

1) The initial concentration $C_0$ is uniform throughout the matrix.

2) Release occurs from only one face of the slab, i.e., the face at $x = 0$ is an impermeable boundary. This yields the first boundary condition:

$$\frac{\partial C}{\partial x} = 0 \text{ at } x = 0 \text{ (impermeable boundary).}$$
3) The solution outside the slab is at zero concentration. This translates as the second boundary condition:

\[ C = 0 \text{ at } x = \lambda \text{ (perfect sink)}. \]

4) Diffusion of solute occurs across the entire face \( x = \lambda \) of the slab.

In the EVA-protein system this assumption is modified by the fact that release occurs through pores and thus a modified effective diffusion coefficient \( D_e \) should be used in lieu of \( D_0 \). Thus:

\[ \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial x^2} \text{ for one dimension} \]

where \( D_e \) is a function of \( D_0 \), the bulk diffusivity and some unknown function of porosity \( F \).

Crank (60) presents the solution to this problem as:

\[
C(x,t) = 4C_0/\pi \sum_{n=0}^{\infty} \left\{ \frac{(-1)^n}{(2n + 1)} \right\} \exp \left\{ -D_e(2n + 1)^2 \pi^2 t / 4\lambda^2 \right\} \\
\cos \left\{ (2n + 1)\pi x / 2\lambda \right\}
\]

where \( C \) is the concentration in the slab at any time \( t \), \( n \) is the running index; \( D_e \) is the diffusivity within the slab; \( \lambda \) is the slab thickness; and \( x \) is the dimension for release.

To determine the cumulative percent release, equation (1) must first be differentiated with respect to the dimension \( x \) and then
integrated over the releasing area. Since only the cos term in equation (1) is a function of \( x \), differentiation is quite simple:

\[
\frac{\partial}{\partial x} \cos Gx = -G \sin Gx \text{ where } G \text{ is a constant}.
\]

Differentiating (1), and substituting \( x = \ell \), the following equation is obtained:

\[
-\frac{\partial G}{\partial x} = \frac{\partial}{\partial x} \left[ \frac{\partial}{\partial x} \right] \sum_{n=0}^{\infty} \frac{(-1)^n \sin \left( \frac{2n+1}{2} \right) \exp(-\alpha t)}{2 \ell} \quad (2)
\]

where \( \alpha = \left[ \frac{\pi^2 D_e/4}{(2n+1)/2} \right] \)

As \( \sin \left( \frac{2n+1}{2} \pi/2 \right) = (-1)^n \), equation (2) reduces to:

\[
-\frac{\partial G}{\partial x} \bigg|_{x=\ell} = 4C_0 \sum_{n=0}^{\ell} (-1)^{2n} \text{ exp} (-\alpha t) \quad (3)
\]

Also \((-1)^{2n} = 1\)

The flux at time \( t \) across the face exposed to the medium is then defined as:

\[
M_t = -D_e \frac{\partial G}{\partial x} \bigg|_{x=\ell} \quad \text{Ay}z = 4C_0 D_e A_{yz} \sum_{n=0}^{\ell} \text{ exp} (-\alpha t) dt \quad (4)
\]

Since \( A_{yz} = 2a \times 2b \) is the area of the releasing surface, equation (4) reduces to:
\[ M_t = \frac{8C_0Deb}{\ell} \sum_{n=0}^{\infty} \frac{1}{\alpha} [1 - \exp(-\alpha t)] \]

\[ t \quad t \]

{since: \[ \int_0^t \exp(-Gt)dt = -1/G [\exp(-Gt)] = 1/G [1 - \exp(-Gt)] \]}

Resubstituting the value of \( \alpha = \frac{\pi^2D_0}{4} \left[ \frac{(2n + 1)}{\ell} \right]^2 \)

and simplifying yields:

\[ M_t = \frac{8C_0}{\ell} \sum_{n=0}^{\infty} \frac{4}{\pi^2} \left[ \frac{\ell}{(2n + 1)} \right]^2 [1 - \exp \left( -\frac{\pi^2D_0(2n + 1)}{4} \right)t] \]

when \( t \rightarrow \infty \), (6) reduces to:

\[ M_t \rightarrow \infty = \frac{8C_0ab}{\ell} \sum_{n=0}^{\infty} \frac{4}{\pi^2} \left( \frac{\ell}{2n + 1} \right)^2 \]

which is the quantity of solute released at infinite time.

The cumulative percentage release at time \( t \) is then:

\[ \frac{M_t}{M_\infty} \times 100 \]

(E) Results and Discussion

(E.1) Simulation of Experimental Results

The model described in the previous subsection was simulated on the computer. The program is listed in Appendix C. To simulate the release kinetics using equations 6-8, the following parameters are required:

\( a, b \) and \( \ell \) (\( \ell = c \) in the computer program): the dimensions of
Fig. 35.: One sided release kinetics for matrices containing BSA of particle size 106-150 μm, at various loadings. Comparison of theory (curves) with experiment (points). The experimental points have a standard deviation between 10-20%.
Fig. 36.: One sided release kinetics for matrices containing BSA of particle size 150-180 μm, at various loadings. Comparison of theory (curves) with experiment (points). The experimental points have a standard deviation between 10-20%.
Fig. 37.: One sided release kinetics for matrices containing BSA of particle size 250-425 μm, at various loadings. Comparison of theory (curves) with experiment (points). The experimental points have a standard deviation between 10-20%.
Fig. 38.: One sided release kinetics for matrices containing β-lactoglobulin of particle size 75-170 μm, at various loadings. Comparison of theory (curves) with experiment (points). The experimental points have a standard deviation of 10-20%.
Fig. 39: One sided release kinetics for matrices containing lysozyme of particle size 106-150 μm, at various loadings. Comparison of theory (curves) with experiment (points). The experimental points have a standard deviation of 10-20%.
the matrix in cm (Table 4)

\[ C_0: \text{the initial concentration of protein in the matrix} = \frac{\text{percentage weight loading} \times \text{weight of matrix}}{\text{volume of matrix}} \text{ in mg/ml (Tables 2, 4)} \]

\[ D_0: \text{the bulk diffusion coefficient in cm}^2/\text{hr (Table 3)} \]

\[ F (F = \text{EPT 1} = \text{EPT 2} = \text{EPTAU in the computer program): the unknown factor of the effective diffusivity } D_e. \]

It is apparent that F is as yet unknown. However, estimates of F were determined by varying F such that curves of cumulative percent release \((M_t/M_\infty \times 100)\) were generated by equations 6-8. These were compared with the kinetic data in subsection B.3 of Chapter VI, over the entire range of varying time t. Best fits were thus obtained by minimizing the root mean squared error (Table 3). The F values which generated the least error were considered best estimates. These are also listed in Table 3 together with the estimates of the effective diffusivity \(D_e = D_0 \times F\) generated by the computer program.

The results for the simulations are displayed in Figs. 35-39 which also exhibit the kinetic data of Chapter VI for comparison. It is apparent that the experimental and theoretical results agree quite well.

Thus using the computer simulation, values of the effective diffusion coefficient \(D_e\) within the matrix were obtained for various particle sizes and weight loadings for different proteins. These were then plotted versus percentage weight loadings in Fig. 40. Different curves were obtained for different particle sizes even for the same protein.

However, since the bulk densities of the protein aggregates was found to be a function of the particle size (Table 2), the volume occupied within the release matrix by protein particles of different
Fig. 40: Plot of effective diffusion coefficient in cm$^2$/hr vs. percent weight loading for various proteins and particle sizes. (Cross: BSA particle size 106-150 μm, plus: BSA particle size 150-180 μm, circle: BSA particle size 250-425 μm, diamond: β-lactoglobulin particle size 75-150 μm, triangle: lysozyme 106-150 μm).
sizes and proteins would be different. Therefore, the effective diffusion coefficient (Table 3) was next plotted versus percentage volume loading \((100 \times \varepsilon)\) for various particle sizes of the different proteins (Fig. 41). It is apparent from Fig. 41 that the points for various particle sizes and proteins now lie on a single smooth curve.

The previously observed fact that the rate of release was found to vary with the particle size and loadings is now easily explainable because the percentage volume loading \((100 \times \varepsilon)\) would be different for different particle sizes even for the same percentage weight loading (as explained before). This would result in a different pore volume (i.e., open space) within the matrix and therefore a different rate of release.

Next, the same point were plotted on a log-log plot as displayed in Fig. 42. The points now appear to generate a straight line. Performing a linear regression on the points we get a correlation coefficient of 0.9426 for the straight line:

\[ \log (D_e) = 4.314 \log (100 \times \varepsilon) - 11.58 \]  

(1)

Also plotting the estimated factor \(F\) (Table 3) of the effective diffusivity \(D_e\) versus the percentage volume loading for different proteins and particle sizes another smooth curve (Fig. 43) is obtained.

Again, plotting \(\log F\) versus \(\log (100 \times \varepsilon)\) in Fig. 44 a straight line is apparently generated. Once more, performing a linear regression on the points the straight line:

\[ \log (F) = 4.263 \log (100 \times \varepsilon) - 8.957 \]  

(2)

with correlation coefficient = 0.9424 is obtained.

The implication of the foregoing discussion is obvious. Using Figs. 41-44 or equations 1 and 2, the release from EVA-protein matrices
Fig. 41.: Plot of effective diffusion coefficient in cm²/hr vs. percent volume for various proteins and particle sizes (Cross: BSA particle size 106-150 μm, plus: BSA particle size 150-180 μm, circle: BSA particle size 250-425 μm, diamond: β-lactoglobulin particle size 75-150 μm, triangle: lysozyme, 106-150 μm).
Fig. 42.: Plot of log (effective diffusion coefficient) vs. log (percent volume) for various proteins and particle sizes (Cross: BSA particle size 106-150 μm, plus: BSA particle size 150-180 μm, circle: BSA particle size 250-425 μm, diamond: β-lactoglobulin particle size 75-150 μm, triangle: lysozyme 106-150 μm).
Fig. 43.: Plot of factor F vs. percent volume for various proteins and particle sizes (cross: BSA particle size 106-150 μm, plus: BSA particle size 150-180 μm, circle: BSA particle size 250-425 μm, diamond: β-lactoglobulin particle size 75-150 μm, triangle: lysozyme 106-150 μm).
Fig. 44.: Plot of log (F) vs. log (percent volume) for various proteins and particle sizes (cross: BSA particle size 106-150 μm, plus: BSA particle size 150-180 μm, circle: BSA particle size 250-425 μm, diamond: β-lactoglobulin particle size 75-150 μm, triangle: lysozyme (106-150 μm).
could be predicted as will be demonstrated in the following sub-
section.

(E.2) Demonstration of the Use of Predictive Correlations

In this subsection will be demonstrated the predictive value of
the correlations 1 and 2 developed above. The release kinetics of the
EVA-protein matrices can be generated using the same measurable para-
meters as in the previous subsection (viz. a, b, \( \lambda \), and \( C_0 \)).

In addition, to estimate the effective diffusivity from equations
1 or 2 we need to know the density \( \dot{\alpha}_s \) (gm/cc) of the protein particles
incorporated in the matrix. From the particle density we can calculate
the porosity \( \varepsilon \) and hence the percentage volume loading 100 . \( \varepsilon \) as
detailed in subsection B.3 of this chapter. Using this value of the
percentage volume loading (100 . \( \varepsilon \)) in equation 1 or 2 we can determine
the value of the effective diffusivity. Equation 1 yields this value
directly while equation 2 estimates \( F \) which is then multiplied by \( D_0 \)
the known bulk diffusivity documented in the literature. If a value of
the bulk diffusivity is available in the literature, equation 2 should
be used in preference to equation 1.

Since all the terms in equations 6-8 of section D are known, the
cumulative percent release \( M_t/M_\infty \times 100 \) can thus be determined for
various selected times.

Using this procedure and equation 2 the kinetic data of Chapter
VI can thus be simulated. The results are displayed in Figs. 45-49.
Fig. 45.: Prediction of one sided release kinetics for matrices containing BSA (106-150 μm) at various loadings. Theory: curves, experiment: points. The experimental points have a standard deviation between 10-20%.
Fig. 46. Prediction of one sided release kinetics for matrices containing BSA (150-180 μm) at various loadings. Theory: curves, experiment: points. The experimental points have a standard deviation between 10-20%.
Fig. 47.: Prediction of one sided release kinetics for matrices containing BSA (250-425 μm) at various loadings. Theory: curves, experiment: points. The experimental points have a standard deviation between 10-20%. 
Fig. 48.: Prediction of one sided release kinetics for matrices containing β-lactoglobulin (75-150 μm) at various loadings. Theory: curves, experiment: points. The experimental points have a standard deviation between 10-20%. 
Fig. 49.: Prediction of one sided release kinetics for matrices containing lysozyme (106-150 μm), at various loadings. Theory curves, experiment: points. The experimental points have a standard deviation between 10-20%.
(E.3) Limitations of the Proposed Model

Though the model proposed does explain the experimentally observed release kinetics fairly well, it does have certain limitations:

1) There is a slight discrepancy between the observed experimental data and the theoretical curves for low loadings. This may be due to the fact as explained in section D, that at low loadings a significant amount of protein gets encapsulated. One way to correct for this is to monitor release of protein until no more is released and then determined, both the calculation and re-dissolving the matrix, how much protein is left in the slab. The real initial concentration could then be determined:

\[ C_0 \text{ mg/ml} = \frac{\text{weight of protein in matrix} - \text{weight of trapped protein}}{\text{volume of matrix}}. \]

This could then be used in the equations 6-8 of section D.

2). It was assumed that the rate-limiting step for mass transfer is diffusion within the matrix. It may be possible that other factors like the rate of dissolution may be controlling. A model which takes this into account is discussed in Appendix D.

3). The model proposed in this chapter holds only when the protein solubility is higher than its concentration in the matrix. If the protein solubility is extremely low, this may not be true, and the model may be expected to be inadequate. In such cases some other model, like Higuchi's which assumes a solute concentration in the matrix far in excess of the solute solubility may explain the observed kinetics more effectively.
(E.4.) Discussion of the F Factor

In subsection E.1. of this chapter an unknown factor of the effective diffusivity $D_e$ was introduced. Subsequently, it is of interest to note how this factor $F = D_e/D_o$ yielded a smooth curve when plotted against the percentage volume loading $100.\varepsilon$ (effectively against porosity). Furthermore, when plotted on a log-log plot a straight line with correlation coefficient 0.9424 was obtained.

Equation (2) can be written as:

$$\log F = \log (100.\varepsilon) \times 4.263 - 8.957 \quad \text{(since $G \log x = \log x^G$)}$$

Also, since $\log (9.057 \times 10^8) = 8.957$

$$\log F = \log [100^{4.263} \times \varepsilon] - \log [9.057 \times 10^8].$$

This reduces to $\log F = \log [(100^{4.263} \times \varepsilon)/9.057 \times 10^8]$ (since $\log A - \log B = \log A/B$).

Simplifying yields: $\log F = \log (0.371 \times \varepsilon 4.263)$.

Finally taking the antilog: $D_e/D_o = F = 0.371 \times \varepsilon 4.263$.

Many similar expressions exist in the literature relating the ratio of the effective to the bulk diffusivities with dimensionless constants. Notable among these are:

$$D_e/D_o = \varepsilon \quad \text{the Buckingham correlation} \quad \text{(64)}$$

$$D_e/D_o = \varepsilon [1 - (1 - \varepsilon)^{0.67} \quad \text{the Dumanski correlation} \quad \text{(64)}$$

$$D_e/D_o = 2\varepsilon/(3-\varepsilon) \quad \text{the Maxwell correlation} \quad \text{(65)}$$

$$D_e/D_o = \varepsilon/(2 - \varepsilon) \quad \text{the Rayleigh correlation} \quad \text{(65)}$$

$$D_e/D_o = \varepsilon^{3/2} \quad \text{the Bruggeman correlation} \quad \text{(65)}.$$

The Buckingham correlation is the earliest and simplest of all these. His observations followed from observations on the diffusion of
carbon dioxide and oxygen through soil. Dumanski measured the transport of aqueous sodium sulphate and sodium chloride through gelatin. Maxwell and Rayleigh based their derivations on observations with dilute suspensions of particles of simple geometric shapes. Maxwell considered uniform spheres while Rayleigh considered infinite cylinders normal to the direction of flow. Bruggeman in turn extended the range of validity of the Maxwell expression to higher values of $\varepsilon$.

Also, Michaels [66] suggested a value of:

$$D_e/D_0 = [1 - (R -1)^2/R]^{-1}$$

where $R = r_1/r_2$ is the ratio of the cross sections of maximum to minimum pore diameter in a porous matrix.

In section D of this chapter an assumption was made (while proposing a model) that the diffusion coefficient was independent of concentration. Subsequent simulation showed good agreement between the experimental data and theoretical analyses based on this assumption. The effective diffusion coefficient was however found to be a function of the variable $F$. It is quite conceivable that this factor $F$ besides being a function of porosity $\varepsilon$ and tortuosity $\tau$ is also a function of concentration. Thus, though the proposed model does not explicitly account for the concentration dependence of the diffusion coefficient, this variation could be accounted for implicitly by the factor $F$.

Finally, it can be concluded that the model proposed in this chapter can effectively explain the release kinetics of Chapter VI. In the next chapter will be presented a summary of the work conducted in this thesis together with the conclusions deduced thereof.
CHAPTER VIII

SUMMARY AND CONCLUSIONS

(A) Introduction

The investigations undertaken in this thesis can be classified under three broad headings:


2). Studies of the Release Kinetics from these matrices.

3). Mathematical Modelling and Computer Simulation of the release process.

Each of the above has been discussed in detail in the preceding three chapters. In this chapter will be reviewed briefly the salient features of each of these investigations.

(B) Microstructure

The microstructural studies conducted in this thesis were of three types, viz. ordinary transmitted light studies, Nomarski studies and SEM studies. These served to complement each other and presented corroborative results.

The release matrix was studied at various times of release together with an extensive set of controls to eliminate the effect of any artefacts.
On the basis of these experiments it is concluded that due to the process of incorporation of the solid protein powder channels are formed in the matrix body which permit the diffusion of macromolecules to the outside. However a Higuchi type "moving-front" was not discerned in the matrix. Instead, the protein appeared to be diffusing from throughout the matrix structure. This was indicated by the fact that holes appeared throughout the matrix structure at any given time of release.

(C) Kinetics

The kinetic studies conducted in this thesis were aimed at defining the parameters affecting release.

Careful control studies were performed to eliminate the effects of any possible artefacts during the spectrophotometric assay of released protein.

The results of the kinetic investigations indicated that the resistance to diffusion of the macromolecules from the matrix is independent of any boundary layer effects. In addition, the release of BSA, lysozyme and β-lactoglobulin was monitored for various particle sizes and weight loadings and was found to vary with these parameters in confirmation of previous kinetic studies.

(D) Modelling and Computer Simulation

Based on the results of the microstructural and kinetic investigations, a theoretical analysis was undertaken and a model consistent with the experimental data was proposed.

The salient equations describing this model are:
\[ M_t = 8C_0 a b / \lambda \sum_{n=0}^{\infty} \frac{4/\pi^2}{\left[ \lambda / (2n + 1) \right]^2} \left[ 1 - \exp \left( -\pi^2 D_e / 4 \frac{(2n + 1)^2}{\lambda^2} t \right) \right] \]

the quantity of solute released at time \( t \),

\[ M_{t,\infty} = 8C_0 a b / \lambda \sum_{n=0}^{\infty} \frac{4/\pi^2}{\left[ \lambda / (2n + 1) \right]^2} \]

the quantity of solute released at infinite time and \( M_t / M_{\infty} \cdot 100 \) the cumulative percentage release at time \( t \).

This model was simulated on the computer and curves generated which showed good agreement with the kinetic data collected. The fact that rates of release were dependent on the particle size of the incorporated protein was attributed to variation in the density for different particle sizes. This variation caused changes in the porosity of the release matrix which yielded the factor: \( F = 0.371 \epsilon^{4.263} \).

This in turn varied the effective diffusivity: \( D_e = D_0 F \), which generated the different rates of release for various proteins, loadings and particle sizes.

Thus, using the computer model, plots and correlations were generated which have a predictive value.

In the next chapter will be discussed some suggestions for future research.
CHAPTER IX

SUGGESTIONS FOR FUTURE RESEARCH

This thesis has aimed at presenting detailed investigations to date of the unique drug-delivery system developed by Langer and Folkman (3). This chapter offers suggestions to further characterize this system.

1). The homogeneity of the slabs should be investigated by redissolving them in methylene chloride and water. It is anticipated that most of the protein would dissolve in the water and the polymer alone would dissolve in methylene chloride. However, experiments to determine partitioning of protein between the two solvents should be conducted as a control. One could then determine the weight of protein and polymer in the slab and check whether it tallies with the postulated loading.

2). Careful measurements of the dimensions of the release matrix should be performed after release and compared with those before release. These would give an estimate of the extent of pore collapse (if any) during release.

3). Studies should be conducted to characterize the concentration profile at sequentially increasing times of release within the release matrices for different particle sizes, proteins and percentage volume loadings. Especially important would be the concentration profile of encapsulated protein for low loadings. This could be obtained by following the procedure detailed in subsection B.4 of
Chapter VI, by sectioning matrices after termination of release. This concentration profile of trapped protein would then aid in eliminating limitation 1 in subsection E.3. of Chapter VII.

4). A method described by Macritchie (67) to measure protein solubility should be considered. Weighed samples of protein powder of known moisture content were placed in a vacuum desiccator containing pure water. The protein samples are weighed at intervals. After several weeks, the powder begins to be replaced by a semitransparent gel. When the transition from gel to liquid is complete, the solution is weighed for the final time. The moisture content of the powder is then easily determined, and solubility estimated.

\[ g_1 = \text{initial weight of protein powder with moisture}; \]
\[ m_1 = \text{moisture content as fraction of total weight}; \]
\[ g_1(1 - m_1) = \text{weight of drug protein}; \text{ and} \]
\[ g_2 = \text{final weight of solution}. \]

\[ C_s \text{ (mg/mL H}_2\text{O)} = g_1 (1 - m_1)/[(g_2 - g_1) + g_1 m_1]. \]
NOMENCLATURE

The following is a list of generalized nomenclature used in this thesis. Some temporary definitions are specified within the text.

A = weight loading of protein in matrix (mg/cc)
a = dimension of matrix (cm)
b = dimension of matrix (cm)
Bi = Biot number
C = concentration of protein in matrix at any time (mg/cc)
C_o = initial concentration of protein in matrix (mg/cc)
C_{or} = corrected value of initial concentration of protein in slab (mg/ml)
C_s = solubility of protein in water (mg/cc)
D = diffusion coefficient (cm^2/sec)
D_e = effective diffusion coefficient of protein within the matrix (cm^2/hr)
D_{ext} = external diffusion coefficient (cm^2/sec)
D_i = diffusion coefficient at infinite dilution (\ell^2 t)
D_{int} = internal diffusion coefficient (cm^2/sec)
D_o = bulk diffusion coefficient of protein in water at 20^\circ C (cm^2/hr)
d = critical dimension of matrix (cm)
F = factor of effective diffusion coefficient varying with porosity
k_c = external mass transfer coefficient (cm/sec)
\ell = thickness of matrix (cm)
M_t = amount of protein released at time t (mg)
M_\infty = amount of protein released at time \infty = amount of protein present (mg)
NOMENCLATURE (Continued)

\( n_i \) = index of refraction of solvent

\( n_o \) = index of refraction of protein solution.

\( P_{cr} \) = critical pressure of carbon dioxide (atm).

\( Re \) = Reynold's number

\( Sc \) = Schmidt number

\( Sh \) = Sherwood number

\( t \) = time (hours)

\( T_{cr} \) = critical temperature of carbon dioxide (°C).

\( x \) = direction of protein flux (\( \phi \))

Greek Symbols

\( \varepsilon \) = matrix porosity

\( \eta \) = viscosity (centipoise)

\( \rho_L \) = density of liquid (gm/cc)

\( \rho_S \) = density of sieved protein powder (gm/cc)

\( \tau \) = matrix tortuosity


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APPENDIX A

Standard Curves of Spectrophotometric Assays

This appendix comprises the standard curves for all spectrophotometric assays used in this thesis. Every point represents the mean of at least two readings.
Fig. A-1. Standard Curve for BSA in saline, at 220 nm.

CONCENTRATION (µg/ml)

OPTICAL DENSITY 0.0

UNIT S
Fig. A.2: Standard Curve for BSA in saline, at 280 nm.
Fig. A.3: Standard curve for ß-lactoglobulin in saline, at 220 nm.
Fig. A-4.: Standard curve for β-lactoglobulin at 280 nm.
Fig. A-5.: Standard curve for lysozyme in saline, at 220 nm.
Fig. A-6.: Standard curve for lysozyme in saline, at 280 nm.
Fig. A-7.: Standard curve for sodium salicylate in water, at 294 nm.

CONCENTRATION (µg/ml)
APPENDIX B

Application of the Higuchi Model to the Observed Experimental Data

The Higuchi model (12,13) predicts solute release using the following equation:

\[ Q = [D \cdot Cs \left( \epsilon / \tau \right) (2A - \epsilon Cs)t]^{1/2} \]  

(1)

where:

Q: the amount of solute released at time t, in mg
\( \epsilon \): the porosity
Cs: the solubility in mg/cc
\( \tau \): the tortuosity
A: the concentration of solute in the matrix (mg/cc).

Differentiating equation (1) with respect to \( t^{1/2} \):

\[ \frac{dQ}{dt^{1/2}} = [D \cdot Cs \left( \epsilon / \tau \right) (2A - \epsilon Cs)]^{1/2} \]  

(2)

Following the procedure of Desai et al (15), polymer matrices were soaked (for 3 weeks) in a 5% sodium salicylate solution after release had terminated. The release of sodium salicylate in fresh saline was then followed for 3 weeks.

The total sodium salicylate released was then divided by its original concentration (50 mg/ml) to yield an estimate of the volume occupied by the salicylate solution. This volume divided by the total volume of the matrix estimated porosity \( \epsilon \).

Since the release of salicylate is given by:
\[ Q = 2 \co \epsilon (Dt/\pi \tau)^{1/2} \]  

the slope \( f \) of the release rate per unit area versus \( t^{1/2} \) gives tortuosity:

\[ \tau = \co^2 \epsilon^2 D/\pi f^2 \]

The bulk diffusion coefficient of sodium salicylate is \( = 9.7 \times 10^{-6} \text{ cm}^2/\text{sec}^4 \). Thus all the parameters in equation (2) are known (Table 5,3) and the slope \( dQ/dt^{1/2} \) can be estimated.

Further, to predict cumulative percent release this slope is modified thus:

\[ S = \frac{dQ}{dt^{1/2}} \times \text{Releasing Area/mg protein in slab}(P) \]  

This modified slope when used in equation (1) gives:

\[ \frac{Q}{P} \times 100 = \frac{dQ}{dt^{1/2}} \times \text{Releasing Area/mg protein in slab}(P) \]

which reduces to:

\[ \frac{Mt}{M_{\infty}} \times 100 = \text{Area/P} \times [DCs (\epsilon/\tau) (2A - \epsilon Cs)t]^{1/2} \]

which is the percent cumulative release at time \( t \).

Based on these predictions plots were generated which are displayed (together with the kinetic data of Chapter VI for comparison) in Figs. A-8 - A-12.

It is apparent that the theoretical Higuchi curves do not correspond to the observed kinetics effectively.

## Parameters Used in the Higuchi Model

<table>
<thead>
<tr>
<th>Protein</th>
<th>Weight % Protein</th>
<th>Concentration A(mg/cc)</th>
<th>Porosity $\epsilon$</th>
<th>Tortuosity $t$</th>
<th>Slopes(s) $\frac{dQ}{dt}1/2$</th>
<th>Area cm$^2$</th>
<th>Weight Protein P present (mg)</th>
<th>Slope ($\frac{dQ}{dt}1/2 \times \frac{Area}{P} \times 100$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (106-150µm)</td>
<td>10</td>
<td>97</td>
<td>0.085</td>
<td>358</td>
<td>0.184</td>
<td>1.0</td>
<td>6.67</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>199</td>
<td>0.099</td>
<td>153</td>
<td>0.445</td>
<td>0.99</td>
<td>17.26</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>263</td>
<td>0.227</td>
<td>399</td>
<td>0.473</td>
<td>0.96</td>
<td>21.96</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>321</td>
<td>0.414</td>
<td>436</td>
<td>0.645</td>
<td>1.0</td>
<td>28.61</td>
<td>2.3</td>
</tr>
<tr>
<td>BSA (150-180µm)</td>
<td>20</td>
<td>193</td>
<td>0.121</td>
<td>364</td>
<td>0.318</td>
<td>0.88</td>
<td>14.85</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>250</td>
<td>0.489</td>
<td>535</td>
<td>0.505</td>
<td>1.06</td>
<td>19.59</td>
<td>2.73</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>300</td>
<td>0.486</td>
<td>375</td>
<td>0.701</td>
<td>1.0</td>
<td>23.45</td>
<td>2.99</td>
</tr>
<tr>
<td>BSA (250-425µm)</td>
<td>10</td>
<td>89</td>
<td>0.115</td>
<td>569</td>
<td>0.157</td>
<td>1.08</td>
<td>5.94</td>
<td>2.85</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>217</td>
<td>0.377</td>
<td>460</td>
<td>0.431</td>
<td>1.10</td>
<td>16.37</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>267</td>
<td>0.374</td>
<td>247</td>
<td>0.467</td>
<td>1.04</td>
<td>22.04</td>
<td>2.2</td>
</tr>
<tr>
<td>$\beta$-Lactoglobulin</td>
<td>15</td>
<td>157</td>
<td>0.238</td>
<td>577</td>
<td>0.252</td>
<td>0.931</td>
<td>10.29</td>
<td>2.28</td>
</tr>
<tr>
<td>(75-150µm)</td>
<td>25</td>
<td>240</td>
<td>0.518</td>
<td>532</td>
<td>0.459</td>
<td>0.96</td>
<td>16.81</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>308</td>
<td>0.594</td>
<td>160</td>
<td>1.03</td>
<td>1.0</td>
<td>27.69</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>363</td>
<td>0.643</td>
<td>107</td>
<td>1.44</td>
<td>1.0</td>
<td>38.13</td>
<td>3.78</td>
</tr>
<tr>
<td>Lysozyme (106-150µm)</td>
<td>15</td>
<td>132</td>
<td>0.146</td>
<td>489</td>
<td>0.184</td>
<td>1.0</td>
<td>6.27</td>
<td>2.93</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>304</td>
<td>0.506</td>
<td>82.15</td>
<td>1.255</td>
<td>0.9</td>
<td>28.86</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>384</td>
<td>0.542</td>
<td>60</td>
<td>1.71</td>
<td>0.9</td>
<td>38.14</td>
<td>4.03</td>
</tr>
</tbody>
</table>
Fig. A-8. Application of Higuchi Model (---) to one sided release kinetics of matrices containing BSA (106-150 μm) at various loadings. Solid curves represent experiment.
Fig. A-9.: Application of Higuchi Model (--) to one sided release kinetics of matrices containing BSA (150-180 μm) at various loadings. Solid curves represent experiment.
Fig. A-10.: Application of Higuchi Model (--) to one sided release kinetics of matrices containing BSA (250-425 μm) at various loadings. Solid curves represent experiment.
Fig. A-11: Application of Higuchi Model (--) to one sided release kinetics of matrices containing β-lactoglobulin (75-150 μm) at various loadings. Solid curves represent experiment.
Fig. A-12.: Application of Higuchi Model (--) to one sided release kinetics of matrices containing lysozyme (106-150 μm) at various loadings. Solid curves represent experiment.
Discussion:

1). The porosity $\epsilon$ determined by liquid leaching was estimated by using the equation:

$$\epsilon = \frac{\text{Weight of drug released}}{\text{concentration of drug} \times \text{volume of slab}}$$

where the drug was sodium salicylate. This method does not account for the volume of the matrix occupied by trapped air or protein particles.

2). The MW of sodium salicylate (160.11) is much lower than that of proteins (~1000s). Use of a macromolecule, of the same MW range as the proteins, for liquid leaching may be desirable.

3). The paraplast used for coating the release matrices on 5 slides was subsequently found to absorb sodium salicylate. This absorption would affect the estimation of porosity. Ideally, a molecule which is not absorbed by paraplast should be used for liquid leaching experiments.

Thus the parameters (Table 5) used in the Higuchi model to predict release cannot be considered totally reliable without further investigation.
APPENDIX C

Computer Program for Modelling Release

This appendix contains the computer program DVARE1 and the subroutine ARE2 written in Fortran which were used to model the release kinetics of the EVA-protein matrices. Also listed is a typical output of the program.

Input to the program is provided in a file 'INPUT' and comprises the following:

1). a, b, ε, the dimensions of the slab. A constant TY which when set = 1 causes the program to simulate one-sided release. If set = 2 the program simulates two-sided release in one dimension. TY is input on the same line as the dimensions of the slab, immediately after ε. In this thesis TY was set = 1 in all cases.

2). On the next line of 'INPUT' are typed in:

Co: the initial concentration;
Do: the bulk diffusivity;
EPT1, EPT2: the factor F;
PW, PRO: two constants (irrelevant for the purposes of this thesis); and
NVT: the number of time points.

3). On the next line of 'INPUT' is typed in a constant other than 1.0.

4). Finally, the release data is input, one time-point to a line: Time point in hours, cumulative release Mt/M∞.
CUMAIN AL1(I100) + HO + FRAU(I100) + EPS1 + EPS2 + NVT + PKU + W + L(I100) + DEF(I100) + SAK(EA(I100)
DIMENSION ET(I100) + LH(I100)
CALL FOPM(E1 + RH(I100))
CALL FOPM(E2 + NUH(I100))
CALL FOPM(F17 + GRAHM)
READ(S5) AM, K, E, Y
PI = 3.14152
READ(S5) PC, PG, EPS1, EPS2, PKU, PV, NVT
NT = NVT
READ(S5) AM
PIU = PIU
DO 5 J = 1, 100
Y(J) = 0.0
E(J) = 0.0
EN(J) = 0.0
ETL(J) = 0.0
DEFY(J) = 0.0
SAKE(J) = 0.0
EPIAU(J) = 0.0
5 CONTINUE
DO 10 I1 = 1, NT
10 READ(S5) (I1), E1(I1)
CALL ANE
ENFIN = 0.0
IF (I1.GT.1) GO TO 12
C1 = 1.0
GO TO 13
12 C1 = 0.0
13 C1 = 0.0
CALL
13 C1 = 0.0
C2 = (PI80.5(2)+82
DO 20 L = 1, 100
L1 = 1
AL1 = (2.0*PI1111.0)*1
ALPHA = C2/AL1
ARCH = ARC
DO 50 K = 1, NT
ARKA = K
ALPHA = ALPHA
IF (ABS(ALPHA), LE.1/2.0) GO TO 75
EF = 0.0
GO TO 85
75 EF = EXP(ALPHA)
55 W1A = (1.0 - EF)/ALPHA
50 EN(IN) = EN(IN) + ABCRABBITA
Y1 = RCRAB/ALPHA
ENW = ENFIN + Y1
IF (Y1.LE.1.0 - EN) GO TO 65
GO TO 100
20 CONTINUE
85 CUNITINUE
WRITE(4, 209)
WRITE(4, 210) AM + CO
IF (AL1.EQ.1.0) GO TO 10
WRITE(4, 260)
WRITE(4, 261) EPS1 + EPS2
GO TO 10
10 WRITE(4, 259)
Portions of the text on the following page(s) are not legible in the original.
SUBR0MAIIOWME

! WRITE(6,214)PM,PKO
! WRITE(6,240)WM,PKN
! WRITE(6,240)EN,IN
! WRITE(6,220)
! DO 70 JJ=1,WT

NAT0=100+0.0H(JJ)/KFI1
IF (ALT(JJ,1,0),0,10)25
EPTAU(JJ)=EFF(JJ)/60

25
R=1F(JJ)80,5
WRITE(7,240)MT,KAI1D
WRITE(6,230)JJ,EN(JJ),KAI1D,EPTAU(JJ),HEFF(JJ),ETI(JJ)
SRC=0.0
WRITE(7,240)
DO 80 J3=1,NVT
PHL=100.0*ETI(J3)
BRX=BRX+PHEL
WRITE(7,240)BRX,PHL
IF (ABS(FJ,75,0),10,79)
SRC=SRC+5.0
GO TO 80
79
IF (SRC<.5,0,10)81
SRC=SRC+1.0
GO TO 80
81
SRC=SRC+5.0
80
CONTINUE
20
FORMAT(1X, 'S664000H',DIMENSION=4888',//)
210
FORMAT(1X,'S664000H',DIMENSION=4888',//)
220
FORMAT(1X,'S664000H',DIMENSION=4888',//)
230
FORMAT(1X,'S664000H',DIMENSION=4888',//)
240
FORMAT(1X,'S664000H',DIMENSION=4888',//)
250
FORMAT(1X,'S664000H',DIMENSION=4888',//)
260
FORMAT(1X,'S664000H',DIMENSION=4888',//)
270
FORMAT(1X,'S664000H',DIMENSION=4888',//)
280
FORMAT(1X,'S664000H',DIMENSION=4888',//)
290
FORMAT(1X,'S664000H',DIMENSION=4888',//)
STOP
END
SUBROUTINE ARE
COMMON ALT,T(100),DO,EPTAU(100),EPT1,EPT2,NVT,PROJ,FW,ETL(100),DEFF(100),SAREA(100)
IF(ALT.EQ.1)60 TO 95
DEFF(1)=0.0
SAREA(1)=0.0
EPTAU(1)=0.0
DO 80 K=2,NVT
   K1=K-1
   IF(T(K).GT.85.0) GO TO 21
   EPTAU(K)=EPT1
   DEFF(K)=DO*EPTAU(K)
   SAREA(K)=DO*EPTAU(K)*T(K)
GO TO 80
21 EPTAU(K)=EPT2
   DEFF(K)=DO*EPTAU(K)
   SAREA(K)=DO*EPT1*85.0+DO*(EPTAU(K)*T(K)-85.0))
80 CONTINUE
RETURN
95 DEFF(1)=0.0
SAREA(1)=0.0
DO 400 K=2,NVT
   DEFF(K)=PROJ*(ETL(K))*FW
   M1=M-1
   SAREA(M)=SAREA(M1)+0.5*(T(M)-T(M1))*(DEFF(K)+DEFF(M1))
400 CONTINUE
RETURN
END
<table>
<thead>
<tr>
<th>TIME</th>
<th>CAL VALU</th>
<th>PER REL</th>
<th>EP1AU</th>
<th>EFF</th>
<th>EXPER</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000000E+0</td>
<td>0.000000E+0</td>
</tr>
<tr>
<td>1.00</td>
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<td>0.3200000E-5</td>
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<td>0.4750000E-5</td>
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<td>0.4950000E-5</td>
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<td>0.5100000E-5</td>
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<td>0.1500000E+2</td>
<td>0.5610000E-5</td>
<td>0.5400000E-5</td>
</tr>
</tbody>
</table>
The program generates two outputs. One is stored in a file called 'OUT', an example of which follows. The other output is in a file 'GRAPH' which generates curves of the predicted and experimental values of Mt/M∞ versus (hours$^{1/2}$). Both 'OUT' and 'GRAPH' contain essentially the same information.

By typing in 1.0 in (3) above the constant PW and PRO are used to vary the diffusion coefficient thus:

$$D_e = PRO^{PW}$$

This was not done in this thesis.
APPENDIX D

Mathematical Model Accounting for the Rate of Dissolution

The mathematical models (12-18,30) used to describe release of a substance in drug-delivery systems uniformly assume that the rate limiting step in drug release is diffusion-limited. The model proposed in section D of Chapter VII is no exception to this assumption.

Though this is a good assumption in the case of high protein solubility, in the range of very low solubility accounting for the rate of dissolution of the diffusing drug may yield a model which is more appropriate.

As before, the EVA-protein system can be considered a plane sheet with a uniform initial concentration A (where Cs the protein solubility < the protein loading A) and known boundary conditions.

The pertinent equation is:

\[ \frac{\partial C}{\partial t} = D_e \frac{\partial^2 C}{\partial x^2} - \frac{\partial S}{\partial t} \]  

(1) (60)

where the rate of dissolution is:

\[ \frac{\partial S}{\partial t} = \lambda C \]  

(2)

where \( \lambda \) is the rate constant of dissolution.

\[ C = C_0, S = S_0, -a < x < a, t = 0 \]

\[ \pm \frac{\partial C}{\partial t} = \pm D_e \frac{\partial C}{\partial x}, x = \pm a, t > 0 \]

(the rate at which solute enters the solution is equal to that at which it leaves the sheet over the surfaces \( x = \pm a \).)
Here the sheet occupies the space $-a < x < a$ and the solution occupies the space $-a < x < a, a < x + a$.

We next make the transformation:

$$C_1 = C_0 - C, S_1 = S_0 - S.$$  

The solution of (1) is then:

$$C_1 = \sum_{n=1}^{\infty} \frac{C_0 \exp(pn t)}{2[a/2\lambda + pn/2De\kappa_n^2 + pn^2\lambda a/2De^2\kappa_n^2]} \cos \kappa_n x$$  \hspace{1cm} (3)

where the $p_n$ s are the non-zero roots of $\pm p_n/De = k_t \kappa_n a$, $\kappa_n^2 = -p_n/De$.

The equation for $S_1$ differs from equation (3) only by having an extra factor $\lambda/p_n$ multiplying the $n$th term.

The cumulative percent release is given by $100 \times M_t/M_\infty$ where:

$$M_t/M_\infty = 1 - \sum_{n=1}^{\infty} \frac{\exp(pn t)}{2[a/2\lambda + pn/2De\kappa_n^2 + pn^2\lambda a/2De^2\kappa_n^2]}$$  \hspace{1cm} (4)

where $M_\infty = 2C_0$.

The application of this model requires that experimental data on the rate of dissolution of the protein residing in the polymeric matrix be available, i.e., $\lambda$ be known. This in turn may require knowledge of protein-polymer interactions at the molecular level.

It is of interest to note that when the loading of protein in the matrix (A) lies below the protein solubility (C_s) and diffusion is rate limiting, this model reduces to the earlier, simpler model presented in
Chapter VII. The model described in this appendix may therefore be considered an extension of the previous one.
APPENDIX E

Kinetic Data

This appendix contains all the kinetic data used in Chapter VI.
<table>
<thead>
<tr>
<th>Hr</th>
<th>Stirred at 1000 rpm</th>
<th>Shaken</th>
<th>Stationary</th>
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<td>9.38 ± 0.87</td>
<td>9.18 ± 1.1</td>
<td>9.01 ± 1.3</td>
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<td>4</td>
<td>13.93 ± 1.78</td>
<td>13.86 ± 1.6</td>
<td>13.54 ± 1.7</td>
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<td>9</td>
<td>17.94 ± 2.44</td>
<td>17.61 ± 2.32</td>
<td>17.58 ± 2.34</td>
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<tr>
<td>25</td>
<td>25.94 ± 4.82</td>
<td>25.21 ± 3.67</td>
<td>25.19 ± 3.77</td>
</tr>
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<td>35</td>
<td>29.4 ± 5.46</td>
<td>29.04 ± 4.43</td>
<td>28.94 ± 4.41</td>
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<td>54</td>
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<td>33.61 ± 5.32</td>
<td>33.55 ± 5.28</td>
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<td>37.06 ± 5.88</td>
<td>36.90 ± 5.77</td>
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<td>102</td>
<td>42.74 ± 6.81</td>
<td>40.33 ± 6.55</td>
<td>40.30 ± 6.23</td>
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<td>126</td>
<td>45.18 ± 7.22</td>
<td>43.03 ± 6.83</td>
<td>42.81 ± 6.53</td>
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<tr>
<td>153</td>
<td>47.31 ± 7.55</td>
<td>45.37 ± 7.52</td>
<td>45.37 ± 7.54</td>
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<td>181</td>
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<td>47.9 ± 7.83</td>
<td>47.11 ± 8.10</td>
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<td>52.84 ± 8.24</td>
<td>52.15 ± 8.38</td>
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<tr>
<td>339</td>
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<td>55.01 ± 8.36</td>
<td>54.95 ± 8.45</td>
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<tr>
<td>387</td>
<td>57.98 ± 8.55</td>
<td>57.06 ± 8.58</td>
<td>56.5 ± 8.58</td>
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<tr>
<td>458</td>
<td>61.03 ± 8.72</td>
<td>60.45 ± 8.92</td>
<td>59.84 ± 8.89</td>
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</table>

Data points are cumulative percent protein release from matrices with bovine serum albumin, particle size 63-149 μm, 25% loaded. Values given are the means and standard deviations of at least 6 samples.
TABLE 7
RELEASE KINETICS OF BSA (Bovine Serum Albumin)

Particle Size 106-150 \( \mu m \)

<table>
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<tr>
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<th>20% Loading</th>
<th>25% Loading</th>
<th>30% Loading</th>
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<td>4.21 ± 1.54</td>
<td>5.51 ± 1.13</td>
<td>6.19 ± 2.68</td>
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<tr>
<td>24</td>
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<td>5.57 ± 2.08</td>
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<td>5.24 ± 0.6</td>
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<tr>
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<td>7.94 ± 2.72</td>
<td>13.99 ± 1.97</td>
<td>19.48 ± 6.73</td>
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<tr>
<td>172</td>
<td>6.50 ± 0.9</td>
<td>8.87 ± 2.92</td>
<td>16.30 ± 2.10</td>
<td>23.11 ± 7.38</td>
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<td>242.5</td>
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<td>9.78 ± 3.06</td>
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<td>26.93 ± 7.93</td>
</tr>
<tr>
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<td>10.51 ± 3.12</td>
<td>20.51 ± 2.21</td>
<td>29.83 ± 8.15</td>
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<tr>
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<td>11.17 ± 3.13</td>
<td>22.11 ± 2.15</td>
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<tr>
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<td>11.68 ± 3.15</td>
<td>23.59 ± 2.1</td>
<td>35.40 ± 8.10</td>
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</table>

Data points are cumulative per cent protein released from matrices with bovine serum albumin, particle size 106-150 \( \mu m \), incorporated at various loadings as noted. Values given are the means and standard deviations of at least 8 samples.
# Table 8

**Release Kinetics of BSA (Bovine Serum Albumin)**

**Particle Size 150-180 µm**

<table>
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<td>7.65 ± 2.19</td>
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<td>22</td>
<td>7.12 ± 1.78</td>
<td>8.99 ± 2.63</td>
<td>16.50 ± 5.20</td>
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<tr>
<td>46</td>
<td>8.21 ± 2.04</td>
<td>12.13 ± 3.32</td>
<td>23.85 ± 7.4</td>
</tr>
<tr>
<td>70</td>
<td>8.76 ± 2.19</td>
<td>14.50 ± 3.76</td>
<td>29.45 ± 8.93</td>
</tr>
<tr>
<td>94.5</td>
<td>9.18 ± 2.13</td>
<td>16.69 ± 4.10</td>
<td>34.64 ± 10.15</td>
</tr>
<tr>
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<td>22.47 ± 4.66</td>
<td>47.12 ± 12.12</td>
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<tr>
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<tr>
<td>502</td>
<td>13.41 ± 3.49</td>
<td>41.95 ± 4.89</td>
<td>72.31 ± 14.23</td>
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</table>

Data points are cumulative percent protein released from matrices with bovine serum albumin, particle size 150 - 180 µm, incorporated at various loadings as noted. Values given are the means and standard deviations of at least 8 samples.
TABLE 9
RELEASE KINETICS OF BSA (Bovine Serum Albumin)
Particle Size 250-425 μm

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<td>5.5</td>
<td>9.54 ± 2.61</td>
<td>9.43 ± 1.65</td>
<td>9.36 ± 1.94</td>
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<td>24</td>
<td>14.27 ± 4.19</td>
<td>18.82 ± 4.3</td>
<td>20.20 ± 4.39</td>
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<tr>
<td>49.5</td>
<td>16.88 ± 4.91</td>
<td>26.38 ± 6.92</td>
<td>30.76 ± 7.78</td>
</tr>
<tr>
<td>120</td>
<td>20.65 ± 5.61</td>
<td>42.70 ± 12.37</td>
<td>49.44 ± 14.0</td>
</tr>
<tr>
<td>172</td>
<td>22.59 ± 5.78</td>
<td>51.33 ± 14.57</td>
<td>58.89 ± 16.15</td>
</tr>
<tr>
<td>242.5</td>
<td>24.32 ± 5.8</td>
<td>59.41 ± 16.61</td>
<td>68.01 ± 17.78</td>
</tr>
<tr>
<td>313.5</td>
<td>25.45 ± 5.8</td>
<td>65.26 ± 17.85</td>
<td>74.72 ± 18.62</td>
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<tr>
<td>390.5</td>
<td>26.23 ± 5.95</td>
<td>69.30 ± 18.66</td>
<td>79.08 ± 19.24</td>
</tr>
<tr>
<td>481.5</td>
<td>26.63 ± 5.99</td>
<td>72.87 ± 18.47</td>
<td>82.51 ± 19.54</td>
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</tbody>
</table>

Data points are cumulative percent protein released from matrices with bovine serum albumin, particle size 250-425 μm, incorporated at various loadings as noted. Values given are the means and standard deviations of at least 8 samples.
TABLE 10
RELEASE KINETICS OF β-LACTOGLOBULIN

Particle size 75-150 μm

<table>
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<th>25% Loading</th>
<th>40% Loading</th>
<th>50% Loading</th>
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<tr>
<td>4</td>
<td>1.74 ± 0.51</td>
<td>2.02 ± 0.35</td>
<td>2.54 ± 0.25</td>
<td>1.59 ± 0.22</td>
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<tr>
<td>23.5</td>
<td>2.86 ± 0.88</td>
<td>4.91 ± 0.57</td>
<td>11.48 ± 0.74</td>
<td>11.49 ± 0.95</td>
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<tr>
<td>50</td>
<td>3.66 ± 1.11</td>
<td>7.41 ± 0.75</td>
<td>18.12 ± 0.98</td>
<td>20.21 ± 1.25</td>
</tr>
<tr>
<td>78.5</td>
<td>4.39 ± 1.27</td>
<td>9.70 ± 0.94</td>
<td>24.25 ± 1.14</td>
<td>27.98 ± 1.59</td>
</tr>
<tr>
<td>134.5</td>
<td>5.66 ± 1.48</td>
<td>13.51 ± 1.27</td>
<td>34.62 ± 1.89</td>
<td>42.05 ± 2.42</td>
</tr>
<tr>
<td>234</td>
<td>6.37 ± 1.82</td>
<td>19.72 ± 1.67</td>
<td>48.20 ± 2.93</td>
<td>56.31 ± 2.76</td>
</tr>
<tr>
<td>328.75</td>
<td>7.12 ± 2.05</td>
<td>23.25 ± 2.31</td>
<td>57.29 ± 3.13</td>
<td>65.28 ± 2.22</td>
</tr>
<tr>
<td>398</td>
<td>7.82 ± 2.16</td>
<td>26.07 ± 2.79</td>
<td>61.63 ± 3.08</td>
<td>69.03 ± 1.99</td>
</tr>
<tr>
<td>494.5</td>
<td>8.60 ± 2.34</td>
<td>29.57 ± 3.39</td>
<td>66.02 ± 2.88</td>
<td>72.54 ± 1.73</td>
</tr>
</tbody>
</table>

Data points are cumulative percent protein released from matrices with β-lactoglobulin, particle size 75-150 μm, incorporated at various loadings as noted. Values given are the means and standard deviations of at least 8 samples.
**TABLE 11**

**RELEASE KINETICS OF LYSOZYME**

*Particle size 106-150 µm*

<table>
<thead>
<tr>
<th>Hr.</th>
<th>15% Loading</th>
<th>40% Loading</th>
<th>50% Loading</th>
</tr>
</thead>
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<tr>
<td>4</td>
<td>3.32 ± 0.44</td>
<td>4.03 ± 0.88</td>
<td>5.36 ± 0.9</td>
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<tr>
<td>22</td>
<td>5.84 ± 0.76</td>
<td>11.82 ± 1.78</td>
<td>16.65 ± 2.33</td>
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<tr>
<td>46</td>
<td>7.51 ± 0.92</td>
<td>17.06 ± 1.89</td>
<td>24.26 ± 2.63</td>
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<tr>
<td>70</td>
<td>8.52 ± 1.09</td>
<td>20.49 ± 1.92</td>
<td>29.01 ± 2.83</td>
</tr>
<tr>
<td>94.5</td>
<td>9.46 ± 1.24</td>
<td>23.90 ± 2.05</td>
<td>33.37 ± 3.13</td>
</tr>
<tr>
<td>165.75</td>
<td>11.77 ± 1.59</td>
<td>32.13 ± 2.65</td>
<td>43.16 ± 3.04</td>
</tr>
<tr>
<td>216.75</td>
<td>13.11 ± 1.77</td>
<td>38.29 ± 2.77</td>
<td>48.08 ± 2.36</td>
</tr>
<tr>
<td>288.25</td>
<td>14.51 ± 1.90</td>
<td>44.91 ± 2.64</td>
<td>52.11 ± 1.48</td>
</tr>
<tr>
<td>358.5</td>
<td>15.69 ± 2.02</td>
<td>49.81 ± 2.27</td>
<td>54.37 ± 0.85</td>
</tr>
<tr>
<td>435.75</td>
<td>16.69 ± 2.17</td>
<td>53.67 ± 1.77</td>
<td>55.63 ± 0.44</td>
</tr>
<tr>
<td>502</td>
<td>17.91 ± 2.36</td>
<td>56.40 ± 0.37</td>
<td></td>
</tr>
</tbody>
</table>

Data points are cumulative percent protein released from matrices with lysozyme, particle size 106-150 µm, incorporated at various loadings as noted. Values given are the means and standard deviations of at least 8 samples.
APPENDIX F

This appendix contains Figs. A-13 - A-17 which are the release kinetics (Mt/M∞) plotted against time in hours.
Fig. A-13.: One sided release kinetics for matrices containing BSA (106-150 μm) at various loadings. Fractional release $M_t / M_\infty$ plotted against time in hrs.
Fig. A-14.: One sided release kinetics for matrices containing BSA (150-180 µm) at various loadings. Fractional release $M_t/M_\infty$ plotted against time in hrs.
Fig. A-15.: One sided release kinetics for matrices containing BSA (250-425 μm) at various loadings. Fractional release $M_t/M_{∞}$ plotted against time in hrs.
Fig. A-16.: One sided release kinetics for matrices containing β-lactoglobulin (75-150 μm) at various loadings. Fractional release Mt/M∞ plotted against time in hrs.
Fig. A-17.: One sided release kinetics for matrices containing Tysozyme (106-150 μm) at various loadings. Fractional release Mt/M∞ plotted against time in hrs.