FURTHER STUDIES IN GEL PERMEATION CHROMATOGRAPHY.

A THESIS SUBMITTED

BY

TREVOR JOHN CRICHTON, B.Sc.

CANDIDATE FOR THE DEGREE OF

MASTER OF SCIENCE.

CHEMISTRY DEPARTMENT,
UNIVERSITY OF BIRMINGHAM.

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S Y N O P S I S.

Gel Permeation Chromatography has become an established method for rapid molecular weight and dispersion characterisation of polymers. The method is based on the separation of the polymer into an infinite number of narrow fractions by permeation of the polymer solution through the porous crosslinked polystyrene gel.

Fundamental studies have been carried out into the packing techniques relating to pressure and flow rate variables for the porous gel. It has been found that high constant pressure gives higher packing efficiency, but it is not as reproducible as the constant flow rate method. A study has also been made of the care and treatment of the solvent, tetrahydrofuran, with particular interest to the peroxide concentration increase, and the effect of the solvent on brass tubing over prolonged exposure.

Sample concentration effects have also been studied, paying particular interest to the low molecular weight tail which is often observed with concentrated samples. A study of different polymer concentrations, but with the same relative viscosity was also made, with particular interest in the elution volume and theoretical plate count for the polymer.

A new type of gel has been developed, which enables an aerogel to stabilise a xerogel, and hence the gel so produced has xerogel elution properties, but aerogel packing properties. The use of thin layer chromatography to characterise gels has also been considered.

A scanning electron microscope has been used to study the morphology of the new gels, and also the supramolecular structure of freeze dried polystyrene in benzene solution.

PREFACE.

The work described in this thesis is original except where due reference has been given, and was carried out at the University of Birmingham during the years 1974 and 1975.

I wish to express my sincere thanks to all who have helped me during this course of work, but especially Dr. F. W. Peaker for his supervision, Professor J. C. Robb for his continued interest in this work, Miss J. Jenkinson for some excellent photography with the scanning electron microscope, the technical staff, and finally to my research colleagues for their, occasionally, helpful discussions and suggestions.

Finally, I wish to thank Professor M. Stacey F.R.S. for making this study possible.

T. J. Crichton.

C O N T E N T S.

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CHAPTER 1.

INTRODUCTION.

The characteristics of polymers, such as high viscosity, long range elasticity and high strength, created two differing schools of thought on the subject until about 1930. The early workers, such as Harries,[1] believed the polymer to be a combination of monomer units, but they argued amongst themselves as to whether it was a covalent structure, or one held together by intermolecular "secondary valence" forces. In fact, during the 1890's, the possibility of co-ordination complexes, the concept of "partial valence" and Van de Waal's forces, were given considerable attention.

Polymers were often given cyclic structures, which was probably due to the lack of molecular weight determining techniques, and hence were given molecular weights which were often orders of magnitude in error. Further problems in the concept of a polymer arose from the fact that most of the chemists involved were organically biased, and so found difficulty in envisaging a pure compound of variable chemical formula. Hence their work was directed towards the detection of a definite molecule of precise stoichiometry which they could then regard as a polymer molecule for the particular polymer under consideration.

There were, however, some chemists who were not so restricted in their outlook, and it was these people, such as Graham,[2] who first studied the properties of polymers and tried to explain them in hitherto unknown terms, such as "colloids" or "crystalloids".

In 1920, Staudinger[3] published a condemnation of the then current trend in formulation of molecules by partial valences, and even proposed

chain structures for polystyrene and polymethylmethacrylate. His ideas were not, however, accepted until 1929 when Carothers[4] and others substantiated his chain hypotheses. It was at this period in time that the present concept of polymer chemistry began to be widely accepted.

With the rapid increase in the use of synthetic polymeric substances since the Second World War, it has become increasingly more obvious that the physical properties of the polymer, or "plastics" as they are generally known, need to be investigated. With a greater knowledge of the properties, the quality of the product can be improved or altered to fit the requirements of the product.[5], (115)

For any given polymer, the two most fundamental factors relating to its characteristics are the average chain length and the degree to which the chain lengths of individual molecules vary about the mean value. This latter relationship is known as the "distribution" of the polymer. The need for a rapid and accurate method to determine the average chain length and distribution of the molecules is, therefore, essential.

There are no quick convenient methods of determining the average chain length of a polymeric molecule, but fortunately, there are numerous ways of determining the average molecular weight of a polymer. This value is directly related to the chain length of the polymer by a factor which is dependent upon the polymer in question. The available methods can be divided into three categories depending upon their basic principles. The first category relies upon the colligative properties of the polymer in solution, and includes such techniques as osmometry, cryoscopy, ebulliometry and lowering of vapour pressure. The second category includes such methods as ultracentrifugation, light scattering and viscosity measurement of

dilute solutions of the polymer. The final category is end group analysis by physical and chemical methods.

The final method is based on the numbers of functional end groups available for detection, such as carboxylic acid groups, amino groups and other similarly reactive ones, and hence should only be employed on linear polymers, although successful results have been obtained using starch-like branched polymers.(6) This method gives the number average molecular weight of the polymer ($\bar{M}_n$) in the final result, from which can derived the number average chain length ($\bar{A}_n$). The method becomes inaccurate at high molecular weight, and in fact, seldom exceeds a value of 25,000, but with the advent of infra-red spectra(119) and radioactive labelling(118) this limit has been increased substantially. Initiators and chemical transfer agents containing groups or atoms suitable for chemical or radiotracer analysis can be used, but the molecular weight can only be accurately determined if the transfer and termination reactions are understood. The method can give a deep insight into the polymer structure and its mechanism, as for example, when used in conjunction with physically measured molecular weights to determine the number of chain end groups of a given type of molecule.

The number average molecular weight derived from colligative properties is based on the relationship that, for a dilute solution, the activity of the polymer becomes equal to its mole fraction as the concentration tends towards zero. Under these conditions, the activity of the solvent equals the mole fraction, and hence the decrease in activity of the solvent by the polymer is equal to the mole fraction of the polymer. This effect manifests itself by the lowering of the freezing point and elevation of the boiling point for solutions, compared with that of the pure solvent. It also causes the phenomenon of osmosis. It is

evident that the work has to be done with dilute solutions when the physical considerations of the nature of the randomly coiled polymer are considered. The polymer molecule may, on average, be approximated by a spherically symmetric statistical distribution of chain elements about a centre of gravity, and this spherical volume is often many times larger than that of the actual molecular volume. The whole principle of this type of technique is that it assumes all the molecules act independently of each other and not in clusters or agglomerates, hence the need for infinite dilutions.

The theory of ebulliometry and cryoscopy are basically identical, that of measuring a temperature difference of a pure solvent and a solution at their respective boiling points or freezing points. The methods are limited in their accuracy by the accuracy of the temperature sending devices and by superheating or supercooling. However, with the advent of sensitive thermocouples and thermistors, and by exercising great care, these methods are reliable up to molecular weights of 30,000.

On the other hand, osmometry can be used to estimate the molecular weight of a polymer of 10^6, but it has inherent disadvantages of diffusion through the semi permeable membrane of low molecular weight species, and should not be used for polymers of wide distribution. The theory is one of the solvent permeating through a membrane into a polymer solution, the polymer being prevented from doing this by its size. In order to prevent this, a pressure is applied to the solution in an attempt to counteract the effect. The pressure required to keep the system in equilibrium is called the osmotic pressure, and can be related to the number average molecular weight of the polymer. Although errors can be introduced by low molecular weight diffusion, by using current procedures, it can be used reliably when this does not occur with unfractionated polymers of

number average molecular weight of 50,000[28], or with polymers up to 720,000[28] [103] if the low molecular weight species are removed by fractionation. Higher molecular weights are possible if the sample has a narrower distribution.

The final methods of molecular weight determination are ones where the weight average molecular weight ($\bar{M}_w$) is determined, as in light scattering and ultracentrifugation. Values of 10^7 have been determined by light scattering, which occurs when a beam of light encounters a solution and is scattered in all directions, the wavelength of the incident and reflected ray being unchanged. The intensity of the scattering is measured at various angles and concentrations, and by extrapolation, the intensity of scattered light at zero degrees to the incident ray for zero concentration is obtained. This value can then be related to the weight average molecular weight, but the technique is exacting and lengthy.

There are two techniques for weight average molecular weight determination by ultracentrifugation; one entails the centrifugation of a polymer at relatively low speeds for a long time in order that an equilibrium is reached in which the polymer is distributed in the cell according to its molecular weight. A concentration gradient is then established in terms of the polymer concentration, and this will give the weight average molecular weight of the polymer. The other technique is to centrifuge the solution and measure the rate of movement of the polymer which was originally in solution, towards the bottom of the cell; this can also be related to the weight average molecular weight, but it requires several approximations to be made.

Molecular size can be estimated from the intrinsic viscosity of a dilute polymer solution, which is in turn empirically related to the

molecular weight of the polymer, providing it is linear. The method is not, however, absolute and needs to be calibrated, but once done it is very rapid. The resulting molecular weight is usually less than the weight average molecular weight ($\bar{M}_w$), and is called the viscocity average molecular weight ($\bar{M}_v$).

In order to determine the distribution of a polymer it had previously usually involved fractionating it into narrow distribution fractions and then measuring the molecular weights of these fractions by any of the above techniques, the most widely used probably being viscometry. As an alternative to this lengthy procedure, a non-preparative one can be employed using the equilibrium method of centrifugation. This has the advantage of not requiring fractionation and is therefore much faster. Another method is turbidmetric titrations, but this requires initial calibration for each type of polymer.

Until recently, preparative polymer fractionation was needed for distribution determination, and was achieved by one of several methods, either extraction, precipitation or elution. The foremost method involves extraction of polymer samples, usually in the form of a film, with solvents of increasing solvent power, whilst precipitation requires the polymer to be initially dissolved and then precipitated by addition of a non-solvent. Elution involves the use of column chromatography, where the polymer solution is placed on top of a packed column and the solvent becomes more powerful as it elutes through, causing fractionation to occur. A modification of this has been used by Baker and Williams[7], and employs a temperature gradient.

In recent years much progress has been made in the use of other less conventional methods of molecular weight analysis. Such methods include

spectroscopy and the use of electron microscopy. One spectroscopic method has been called "self beat spectroscopy" and is an optical technique in which the spectral distribution of light scattered from the solute molecules undergoing Brownian motion is analysed to allow the calculation of the diffusion coefficient of the molecules, and from this, the molecular weight. The range for which this method appears to be viable is between 10^4 and 10^8 daltons, and if sufficient care is taken, an accuracy of 1% is obtainable.(8) This method is similar to conventional light scattering, but much more reliable and faster. Another method is being developed, by which the number average molecular weight can be determined by mass spectrometry, and this is called "electrospray mass spectroscopy"(9) but very little work has been done on it, so no full assessment of the method is available.

Since the advent of the electron microscope, it has become possible to observe structures at a molecular level and hence to measure their sizes.(10),(11),(12) By using a very dilute solution of polymer (between 10^{-2} and 10^{-5}%) and then nebulizing the solution into small droplets, allowing the solvent to evaporate, followed by coating or "shadowing" the residue with platinum or a similar metal, it is possible to see the shape and size of the molecule. Ideally, the sample should be in a poor solvent so that the polymer chain is in a tight sphere and will, therefore, be more easily detected because of its density. Such a system needs calibration for each type of polymer and solvent used, but once calibrated, it is a rapid and simple technique which offers good results.

Once distribution data has been obtained, there are two methods of presenting it; one is an integral curve, and the other is a differential distribution curve. In the integral curve, the cumulative weight of the polymer, usually expressed as a fraction of the total weight, is plotted

against the molecular weight, while the differential distribution curve is plotted by graphically differentiating the integral curve.

During the early 1960's, a great deal of work was carried out on a new method of chromatography analysis for polymers. This is called "Gel Permeation Chromatography" (G.P.C.) and is a special type of liquid-solid elution chromatography which uses columns usually packed with an inert gel which is often a cross-linked polymer such as polystyrene, polyacrylamide,[13],[22] polymorpholine,[14] dextran,[15] but can also be silica gel,[16],[17] porous glass,[18],[19],[20] or even porous cement.[21]

The use of cross-linked polystyrene gel was introduced by Moore[23] after initial work by Vaughan,[21] Brewer,[24] and Cortis-Jones,[25] but the principle of this type of chromatography was first employed by Synge and Tiselius[26] in 1949. The theory of this form of chromatography is that large molecules are not able to penetrate the matrix of the gel, and are eluted in preference to the smaller molecules which can penetrate the matrix and are retained. In this respect it is very much like gel filtration chromatography which was initiated by Porath and Flodin,[15] who used cross-linked dextran gel in aqueous media. By this simple explanation of the mechanism it can be expected that larger molecules will be eluted in preference to smaller molecules.

The works of Porath and Flodin,[15] and Synge and Tiselius[26] were restricted in their application, and it was Moore[23] who made the advances which led to most of the present techniques in Gel Permeation Chromatography. Moore succeeded in developing techniques by which the porosity of the cross-linked polystyrene gel could be varied by the use of various solvent, non-solvent mixtures for the monomers. It is now possible to obtain gels

of maximum pore size varying between about one hundred and several millions of angstroms. Moore also noted that for any given gel pore size, the elution volume of a polymer was related to the logarithm of its molecular weight, and that if this was plotted against the elution volume (V_e), a linear plot over a range of molecular weights is usually obtained, the lower limit being nearly equivalent to the total volume of liquid in the column.

At about the same time that Moore developed his techniques, the differential refractometer was introduced as a means of continually monitoring a continuous flow system. This meant that it was no longer necessary to take individual fractions and characterise them separately. This led to the development by Waters Associates Inc. of Framingham, Mass., of a series of commercial gel permeation chromatographs. These instruments measure the difference in refractive index of the solvent and the solution of the polymer, and hence give a display of the distribution of the polymer. This is produced by assuming that the change in refractive index is proportional to the concentration of the polymer in solution for a given polymer. The difference in refractive index is then graphically portrayed, and thus the peak height of the chromatogram is an indication of the concentration of the polymer present. Hendrickson and Moore[27] were the first people to realise that the trace of the polymer concentration as a function of elution volume is a modified differential molecular weight distribution curve, and that once the system is calibrated the elution volume - molecular weight relationship can be used to calculate both weight average and number average molecular weights. Calibration of the system was achieved by plotting the elution volume of monodispersed polymers against the logarithm of the molecular weight. This has since been modified, as will be discussed in Chapter 2 in more detail.

Due to the ease and rapidity by which the molecular properties of polymers can be determined by gel permeation chromatography, it has now become an accepted technique for polymer characterisation. The ensuing work is mainly concerned with the preparation, packing and evaluation of both modified and conventional gels, and a study of these gels using a scanning electron microscope.

CHAPTER 2.

THEORY AND MECHANISMS.

2.1. Definitions and Derivations of some terms.

Before the subject can be pursued further, it is important that all terms are fully understood. As already stated, a polymer comprises many monomer units, the degree of heterogenity of the polymer with respect to its molecular weight being dependent upon the kinetics of its polymerisation. Hence, the distribution of the polymer may be either great or small, and it is important to know which it is for any given polymer before further studies can be undertaken. Any standard polymer text book[28] contains theoretical accounts of distribution, but a short summary of the principles will be outlined.

The distribution of polymer molecules over various chain lengths is described by the number of molecules, Nr, having chain length r. Therefore, Nr can be regarded as the probability of finding such a chain length in a polymer sample. When described as a molecular weight distribution curve, the probability is usually described by either the mole fraction X_r, or the weight fraction W_r, these being defined as:

$$X_r = \frac{Nr}{\sum_r Nr} \qquad 2.1.1.$$

$$W_r = \frac{r.Nr}{\sum_r r.Nr} \qquad 2.1.2.$$

When expressed as functions of molecular weight, X_r is known as the frequency distribution, and W_r as the weight distribution. The terms in the above equations are known in statistical phraseology as the "moments" of the distributions of orders zero and one respectively. In general terms,

the moment Qn, of order n, can be defined as:

$$Qn = \sum_{r} r^{n}.Nr \qquad 2.1.3.$$

where n is an integer. Hence, when n = o, Q_o is the total number of polymer molecules, and when n = 1, Q_1 is the total number of monomer units in the sample. The ratio of these is the number average chain length $\bar{r}_n$.

$$\bar{r}_n = \frac{Q_1}{Q_o} \qquad 2.1.4.$$

and is the ratio of the total chain length of each species to the number of polymer molecules.

Alternatively, the chain length of each species may be averaged on the basis of its weight fraction, which will then give the weight average chain length $\bar{r}_w$.

$$\bar{r}_w = \sum_{r} r.W_r = \frac{\sum_{r} r^2.Nr}{\sum_{r} r.Nr} = \frac{Q_2}{Q_1} \qquad 2.1.5.$$

The determination of the two averages provides some measure of the distribution; another average is the z average, which is different again.

$$\bar{r}_z = \frac{\sum_{r} r^3 Nr}{\sum_{r} r^2 Nr} = \frac{Q_3}{Q_2} \qquad 2.1.6.$$

In addition to these is $\bar{r}_v$, the viscosity average chain length which is defined as:

$$\bar{r}_v = \left[\sum_r W_r . r^{\alpha} \right]^{\frac{1}{\alpha}} = \left[\frac{\sum_r N_r . r^{1+\alpha}}{\sum_r r . N_r} \right]^{\frac{1}{\alpha}} \qquad 2.1.7.$$

where α is the exponent in the Mark-Houwink equation (2.1.8.)

$$[\eta] = KM^{\alpha} \qquad 2.1.8.$$

where $[\eta]$ is the intrinsic viscosity, M is the molecular weight, and K and α are constants for one polymer-solvent system at a stated temperature.

The value of α varies between 0.5 and 1.0, and this value can be related to the "rigidity" of the molecular chain in solution; at 0.5 it is a random coil, and at 1.0 it is linear and rigid. When $\bar{r}_v$ is compared with $\bar{r}_w$ and $\bar{r}_n$ it is found to be nearer $\bar{r}_w$, and when $\alpha = 1$, it is identical to it.

The ratio $\frac{\bar{r}_w}{\bar{r}_n}$ is a measure of the heterogenity of the polymer, and this value is often referred to as the "dispersion of the polymer" which is designated D.

$$D = \frac{\bar{r}_w}{\bar{r}_n} \qquad 2.1.9.$$

When a distribution curve is constructed for a polymer, it is assumed all fractions are monodispersed, which is erroneous, but with certain assumptions[29] an integral curve can be constructed using the equation

$$W_r = \sum_{r=1}^{r} W_r \qquad 2.1.10.$$

where W_r is the weight distribution.

Schultz and Dinglinger[29] modified equation 2.1.10. to:

$$W_r = \sum_{i=1}^{r=i} W_i + \tfrac{1}{2} W_r \qquad 2.1.11.$$

There are, however, alternative methods of construction of integral weight distribution curves, such as those proposed by Tung,[30] Taylor,[31] and Mussa.[32]

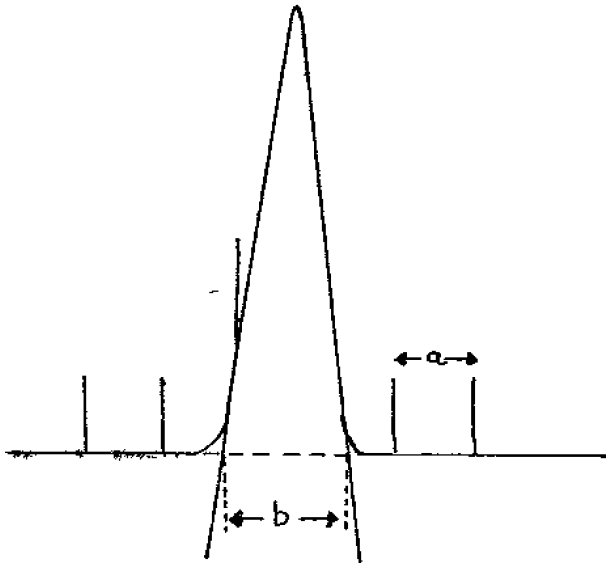
2.2 Evaluation of Column Efficiency in G.P.C.

Whenever a column series is constructed, it is necessary to evaluate the efficiency of the system; this is difficult to determine as a percentage of maximum efficiency as it is impossible to determine the maximum theoretical efficiency. There is, however, a means by which the system can be evaluated to see if it is of suitable standard. This is done by measuring the Theoretical Plate Count (TPC) or the Height Equivalent Theoretical Plate (HETP). These two terms are very closely related, the theoretical plate count being the number of plates per unit length, and the height equivalent plate count being the distance between the plates. Hence, one is the reciprocal of the other. Neither term is strictly a chromatographic phrase, in fact they are more frequently used in distillation or fractionation systems, but they do appear to suffice for gel permeation chromatography systems.

Since the two methods of evaluation are so similar, only the theoretical plate count method will be considered. The plate count is determined from the equation

$$P = \left(\frac{4d}{w}\right)^2 . \frac{1}{n} \qquad 2.2.1.$$

where P is the number of theoretical plates per unit length, d is the elution volume to the peak maximum, w is the width of the peak at the base line, which is obtained by drawing tangents to the inflection points on each side of the peak and taking the intercept with the base line, expressed in the same units as d, and n is the length of the column series. (See Fig.1).



$$P = \left(\frac{4.d}{w}\right)^2 \cdot \frac{1}{n} \qquad \text{where } w = \frac{b}{a}$$

P = No. of theoretical plates per unit length.

d = No of counts to peak.

a = Distance between counts.

b = Tangent intercept of peak with base.

n = No. of columns

FIG 1

PLATE COUNT CALCULATION.

When determining the theoretical plate count, it is important that the conditions are kept identical; that is, the temperature, the standard used to determine the value, the injection time and, to a lesser extent, the flow rate,[33] as all of these will affect the plate count and will lead to erroneous conclusions if the conditions vary.

Theoretical plate counts can also be used to compare quantitavely different method of chromatography. Giddings[34] has related the plate count to the maximum number of resolvable components (N) in a system at any one time in the form of

$$N = 1 + 0.2p^{\frac{1}{2}} \qquad 2.2.2.$$

where p is the number of theoretical plates. He also showed that gas chromatography was better than liquid chromatography which, in turn, is better than gel permeation chromatography for resolving power for the same number of theoretical plates per unit length.

Tweedale[35] has shown that the efficiency of the column is related to the diameter of the spherical particles packing the column. The smaller the gel diameter, the higher the efficiency, but in opposition to this, Whitlock and Porter[76] have shown that the pressure drop across a column is inversely proportional to the square of the particle diameter.

$$\text{Pressure Drop} \propto \frac{1}{(\text{Sphere diameter})^2} \qquad 2.2.3.$$

However, the problems of high pressure drops seem to have been partially overcome because Kato[69] et al have reported a system using 5μ particles at a flow rate of 0.5 cm^3 $min.^{-1}$ and obtaining plate count of 8,800 per foot, but the pressure drop across the column, for tetrahydrofuran, is 1,150 lbs. per square inch. The standard used by Kato in this work is 0.5% solution of benzene in tetrahydrofuran (THF).

2.3. Analysis of a Gaussian Distribution.

By the nature of the mechanism of gel chromatography, it can be expected that, in the majority of cases, the chromatogram will exhibit a Gaussian curve shape for monodispersed polymers which are not excluded by the system. Using this assumption, analysis of the curves obtained on the chromatograph involves the pre-requisite that the polymer is composed of many monodispersed samples, and that the resultant plot is a summation of all the Gaussian plots. Since the height of the peak is proportional to the change in refractive index of the solution, which is in turn proportional to the concentration of the polymer, it follows that the height of the peak is a measure of the concentration of the polymer.

In order to analyse the curve numerically, a histogram set can be drawn as in Figure 2, each block of the histogram being a species of narrow distribution. Once the column set has been calibrated, the chain lengths of each of these blocks can be found, and hence the fraction present in the whole sample. If H is the height of the interval, and i is the number of the interval, this can be expressed mathematically as

$$H_i = M_i.N_i = W_i \qquad 2.3.1.$$

where M_i is the molecular weight at point i, and N_i is the number of molecules of that molecular weight.

The weight average molecular weight $\bar{M}_w$ is defined as

$$\bar{M}_w = \sum_i \frac{W_i.M_i}{\sum_i W_i} \qquad 2.3.2.$$

so by substituting 2.3.1. into 2.3.2. the expression becomes

$$\bar{M}_w = \sum_i \frac{H_i.M_i}{\sum_i H_i} \qquad 2.3.3.$$

However, due to calibrations usually being in terms of chain length (A_i), the molecular weight has to be related to the chain length:

$$M_i = Q.A_i \qquad 2.3.4.$$

where Q is the Q factor, which is a constant for a specific polymer. Hence, equation 2.3.3. now becomes

$$\bar{M}_w = \frac{Q.\sum H_i.A_i}{\sum H_i} \qquad 2.3.5.$$

and these values can all be found from the chromatogram.

The number average molecular weight may be defined as:

$$\bar{M}_n = \frac{\sum N_i.M_i}{\sum N_i} \qquad 2.3.6.$$

but from equation 2.3.1., $N_i = \frac{H_i}{M_i}$ and by substituting $Q.A_i$ for H_i, equation 2.3.6. now becomes

$$\bar{M}_n = \frac{Q\sum H_i}{\sum \frac{H_i}{A_i}} \qquad 2.3.7.$$

As with equation 2.3.5., these values can be found on the chromatogram, so these expressions allow both weight average and number average molecular weights to be directly calculated. Therefore, from these two values, the dispersity of the polymer can be established, since dispersity D is defined as:

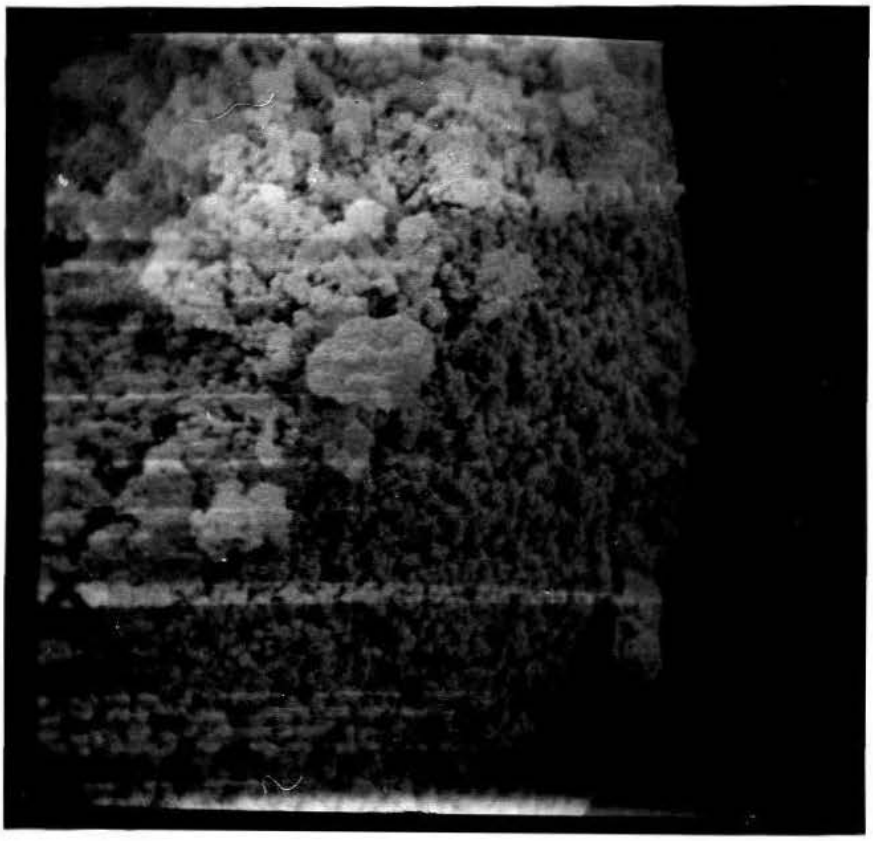
$$D = \frac{\bar{M}_w}{\bar{M}_n} \qquad 2.3.8.$$

2.4. Mechanistic Hypotheses of Gel Permeation Chromatography.

In order to be conversant with the possible mechanisms of gel permeation chromatography, a basic understanding of the gel structure is required.

There are two classes of gel, xerogels and aerogels; the former being a rigid matrix, such as silica,[17] or porous glass spheres,[19] the latter being a cross-linked polymer which swells substantially in the elution media, such gels are Sephadex,[15] (a cross-linked dextran polymer), polyacrylamides,[22] etc. Between these two extremes are gels that do swell, but only slightly, these being usually cross-linked polystyrene[23] or similar polymers. Such gels exhibit more characteristics of aerogels than xerogels, but are nevertheless referred to as xerogel-aerogel hybrids. The advantages of a xerogel is that the apparent ratio of eluting solvent within the gel particles to that outside in a given gel bed is comparatively high, often 2:1, but in the aerogels, it is usually only 1:1. This means that for a xerogel system, the high capacity ratio will mean an increase in column efficiency; for example, doubling the capacity ratio would enable a column length to be reduced fourfold, and still obtain the same separating efficiency. The difficulty is, however, that the swollen xerogels tend to reduce the resolution of the column due to the large size of the particles which are required to negate the swelling effect on the solvent flow rate. If a small particle swells substantially, the interstitial volume is forcibly reduced and the spheres distort into a more convenient packing shape, such as cubes, hence the pressure drop across the column is increased.

In general, however, the gels can be treated as similar in their topology. A gel particle can be visualised ideally as a sphere impregnated with pores of various size, but of a predetermined maximum. These pores may be just a surface phenomen or, as indicated by electronmicroscopy,[36], [37]



2μ.

FIG. 3

SCANNING ELECTRON MICROGRAPH OF A GEL

(TYPE P10 TC_A 4/7; mag. 5,800 x)

the pores may permeate throughout the gel (Figure 3). The gel bead is comprised of much smaller spheres to form glommerates of the polymer. The principle of gel permeation chromatography can be summarised as a procedure by which the larger molecules in solution cannot penetrate the pores of the gel, and hence elute in preference to the smaller molecules which can penetrate the gel matrix, but this study will be pursued more deeply in a later section.

The gel, or beads, form a stationary phase while the solvent in which the polymer is dissolved forms the moving phase which permeates through the gel; the polymer molecules diffuse into all parts of the gel at a rate determined by the molecular dimensions of the polymer, and the pore size of the gel.[38] Porath[39] postulated the existence of conical pores which on average could be treated as identical regular pores, but this theory although basically sound, collapsed when it was realised that it is true for both spherical proteins and random coil protein polymers, which it should not be, or that the conformation of protein polymers was in error.

The elution volume of a polymer can be described mathematically as:

$$V_e = V_o + K_d.V_i. \qquad 2.4.1.$$

where V_e is the elution volume, V_o is the void volume of the column, V_i is the volume of unbound solvent internal to the gel, and K_d is the distribution coefficient.[40] Therefore, if the polymer is unable to enter the pores, V_e is equal to V_o because K_d will be zero, and if all the internal volume is available, then V_e is equal to $V_o + K_d.V_i$. However, is some cases the solutes display some affinity for the gel and K_d can exceed unity. If this happens, a new relationship is required:

$$V_e = V_o + K_d.K_p.V_i. \qquad 2.4.2.$$

where $$K_p = K'.\frac{V_o}{V_i} \qquad 2.4.3.$$

K' is called the capacity factor, and K_p is the partition coefficient. Hence, if K_p is unity, equation 2.4.2. is the same as 2.4.1., but if K_p exceeds unity, there is an apparent increase in K_d to greater than unity.[41]

Porath[39] also assumed that the effective radius of a polymer was proportional to the square root of the molecular weight, and was able to relate the volume fraction of the pores to the molecular weight. By substituting the results of Granath and Flodin,[42] Porath was able to obtain a linear plot for the cube root of the volume fraction of the gel available to the polymer to the square root of the molecular weight; hence creating a steric exclusion hypothesis.

$$(V_{iacc})^{\frac{1}{3}} = KM^{\frac{1}{2}} \qquad 2.4.4.$$

where (V_{iacc}) is the volume fraction of gel available, and K is a constant.

Brewer[43] was able to show there was a linear relationship between log M and the corrected logarithm of the elution volume in the form

$$\log M = -A \log V_e + B \qquad 2.4.5.$$

where $V_e = V_e - V_o \qquad 2.4.6.$

A and B are constants. Moore and Hendrickson[44] substantiated this work on the steric exclusion mechanism by the gel, and were able to produce a direct relationship between elution volume and log M.

$$\log M = A - BV_e \qquad 2.4.7.$$

A and B are constants.

Other workers[45], [46] supported the principle of steric exclusion, but it did not create a satisfactory overall picture, so other hypotheses were created. Altgelt[47] advocated Flodin's theories of the pore, in which he assumes a diffusion equilibrium to be in evidence. This is based on Flodin's findings of

$$D_s = 2/3\ D_m \qquad 2.4.8.$$

where D_s and D_m are diffusion coefficients for the stationary and moving phases respectively. This only applies to dilute solutions and is not flow rate dependant, which enhances the steric exclusion theory.

However, there were other hypotheses on the mechanism, such as separation by solvent flowing through the gel.[48], [49] Ackers[40] suggested restricted diffusion may be occurring, which it no doubt does, but the difficulty was that it is flow rate dependant. Yau[33] has indicated variation in elution volume with flow rate, but he does accept that both mechanisms are operative. At first, Yau believed diffusion to be the major factor, but later he noticed that the elution volume of styrene increased as the theoretical plate count of the system fall,[50], [51] which he explained by postulating the disruption of the velocity profile. This favoured steric exclusion as the predominent factor, because when styrene and polystyrene were eluted through smooth glass beads they tended to elute nearer the void volume, hence separation is not caused by the velocity profile in the interstitial spaces.

Laurent and Killander[52] also gave support to steric exclusion and assumed that the gel network could be treated as a three dimensional network of randomly distributed straight fibres or "rods", and that the volume available for a polymer in the gel determined its position in the elution diagram; this volume could be determined from the assumed physical model of the gel structure. Their hypothesis relies on the fact that the liquid phase is moving ahead of the stationary phase with the effect of a "low molecular weight tail" appearing on the peaks. This is what would be expected from equation 2.4.8., but does not take account of any diffusion in the solution, so it is a rather simplified description of the separation mechanism.

Takagi[53] applied Laurent and Killander's work, along with work by Ogston,[54] to some of his results, and by assuming for a flexible polymer

consisting of identical segments, the radius of gyration [r] is proportional to $M^{\frac{1}{2}}$, derived a relationship equating available volume within the gel to the molecular weight

$$\left(\log \frac{1}{K_{av}}\right)^{\frac{1}{2}} = KM^{\frac{1}{2}} \qquad 2.4.9.$$

where K_{av} is the fraction volume of gel available to the polymer and K is a constant. From this and other work, Takagi concluded that the molecular size is the principle parameter in separation, and so started the concept of "Universal Calibration".

If all molecules in solution exhibit a random walk configuration, their dimensions are governed by the valence angles, bond lengths and total chain lengths. Hence, if two polymers have the same backbone but different pendant groups, they may exhibit the same elution volume but different moleculer weights. Therefore, it is necessary to introduce the concept of a straight chain length which is the equivalent straight chain length, in angstroms, of the backbone of the polymer. Straight chain length can be converted to molecular weight by use of the appropriate "Q factor" which is defined as the molecular weight per angstrom length of the backbone of the extended polymer.

$$'Q' = \frac{M}{A_i} \qquad 2.4.10.$$

Alternatively, it can be described as the molecular weight of the repeating unit divided by its angstrom chain length. Typical Q factors range from 10 to 50.

There are, however, numerous difficulties in determining an effective Q factor for non-linear polymers,[55], [56], [57] although the value can be determined from a molecular model. The most probable reason for the difficulty is that the polymer probably does not exhibit this random walk

configuration in solution, and hence the molecular size will not be determined solely by the already mentioned parameters. Nakajima[57] suggests that in the case of polyethylene, a helical configuration is found which causes the 'Q' factor to be rather low.

Although the 'Q' factor concept is satisfactory for most polymers, there is still no one calibration system for all types of polymer, although the idea of Universal Calibration, using a measure of molecular volume is being sought. As has already been shown, there is evidence of a relationship between the elution volume of linear polymers and their hydrodynamic radius which is, in turn, proportional to the logarithm of the product of the intrinsic viscosity and molecular weight (eqn. 2.4.10), both of which are fundamental properties of the polymer in a given solvent. By calibrating a system using this, it has been found that the curve produced fits many other polymers, including branched polymers.[58], [59] Although Dawkins and Hemmings[60] now believe that if the α value in the Mark-Houwink equation (2.1.8) is less than 0.67 for polystyrene in the calibration medium, the graph obtained is not valid for all polymers.

$$\log [\eta] M = K.V_e. \qquad 2.4.11.$$

where $[\eta]$ is the intrinsic viscosity and K is a constant.

Coll[61], [62] has adapted the $[\eta] M$ product and related it by the Ptitsyn-Eizner approach to the hydrodynamic radius and Avagadro's Number:

$$M = \frac{10.\pi. N_A R_H^3}{3} \qquad 2.4.12.$$

N_A is Avagadro's Number, R_H is hydrodynamic radius.

Coll reports that from many trials of the equation only one sample, cellulose nitrate, appears to differ, but he suggests that this was due to the polymer being a stiff coil. However, other workers[63], [64] have

overcome this difficulty.

Although the Universal Calibration method is now accepted as being almost universal, it still has restrictions, as pointed out by Dawkins and Hemmings,[60] but they also claim that the calibration is only applicable if polymers have the same polymer/solvent interactions.[65] They also stress the need for good solvents, for if a good solvent is used, partition and adsorption mechanisms do not appear to influence the solute size separation.[94] Kubin[95] believes that the Universal Calibration concept to be generally adequate, but believes that the solvent flow rate relationship with gel size to be of greater importance than presently considered. He also considers that the mobile phase contributes to the width of the chromatographic zone. Kubin has related the HETP (height equivalent theoretical plate) to the fundamental properties of the system:

$$H.E.T.P = \frac{2D_p}{\omega} + \omega.\frac{2R_o^2}{15D}\cdot\frac{Hk(1-\rho^3)}{[1+Hk(1-\rho^3)]^3}\cdot\left[1-G(\rho)\right] \qquad 2.4.13.$$

where D_p is the coefficient of longitudinal dispersion, R_o is the diameter of the particles, w is the linear velocity of the solvent flow, ρ is the ratio of the radius of the particles available to the polymer and the radius of unavailable particles to the polymer, H is $\frac{(1-a)}{a}$ where a is the fractional free cross section of the column, k is the equilibrium partition coefficient, and G is a constant.

2.5. Method of Calibration.

Once a column, or set of columns, has been installed in the instrument, it usually requires calibration. Samples of monodispersed polymer are usually injected over a 120 second period as 0.25% solutions in tetrahydrofuran (THF), although occasionally there are exceptions to this. The solvent used in this work, in the Waters 200 instrument, is THF, although the instrument can be used with other solvents.

The count at which the injection is made is counted as zero, and all subsequent counts are numbered accordingly. Each count (or "dump index") is a measure of the eluant volume, and in this system represents 5.00 cm^3 of solvent.

All runs have been done at room temperature (24 ± 2°C.), and every attempt has been made to prevent rapid temperature variations whilst the machine is operational. All elution volumes are taken to the peak maximum.

When a columnis plate counted, the benzene or TCB (trichlorobenzene) standard is injected for 15 seconds as a 1% solution, unless stated otherwise. The flow rate is usually 1.0 cm^3 per minute, but in the case of prepared gels, it may be less, but this will be stated.

The method of calibration as shown above can be very time consuming, especially if a new gel is being prepared, so alternative calibration methods have been sought. The most efficient method time wise appears to be thin layer chromatography,[66] and this has been used yielding interesting results. It is also occasionally better to calibrate a column set with one set of standards and solvent, but then change the solvent and polymer.[67],[68] The best way to do this is to use the hydrodynamic calibration curve (universal calibration) and adjust it accordingly from the results of viscometry on the two systems. The curve can also be calculated from the Mark Houwink equation parameters.[61]

While calibrating a column series, it is most important that dilute solutions (0.25%) are used, otherwise an overload effect can arise,[70] which leads to a delay in the peak maximum appearing due to excessive elution time. This will not only cause false elution characteristics, but also lead to erroneous distribution values of dispersed polymers. If small

molecules are being used, it has been suggested[71] that the molar volume (V_m) should be used in the equation, where A and B are constants,

$$Ve = A - B \log M \qquad 2.5.1.$$

instead of molecular weight M. Hence, 2.5.1. would become

$$Ve = A - B \log V_m \qquad 2.5.2.$$

Occasionally, polymers contain groups which interact with the solvent, such as phenolic resins in THF, and the increase in apparent molecular size due to the solvent has to be taken into account.

Janus[72] recommends that to improve resolution, reduce the length of connecting piping, as there is evidence of diffusion taking place in it.

CHAPTER 3.

INITIAL INVESTIGATIONS IN GEL PERMEATION CHROMATOGRAPHY.

3.1. Packing Techniques and Column Installation.

The gel used for studying packing methods was a styragel produced in bulk by I.C.I., based on a gel by Tweedale[35] (Type P.8), the permeability of it being 3×10^4 Angstroms. The bulk gel has a particle size of not more than 90 microns, but it also has a number of "fines" present which have to be removed for satisfactory packing and good flow rates to be obtained. The "fines" are small spheres of gel less than about 10 microns in diameter.

The removal of "fines" is achieved by creating a suspension of the gel in about 1,500 cm^3 of acetone, then allowing the larger particles to sediment out. After about ten minutes the acetone is decanted off with the "fines" in suspension by means of a PTFE tube. This suspension is then filtered under reduced pressure and the acetone re-used to suspend the gel again. The remaining "fines" are then put to one side. This process is repeated at least four times, or until the suspension is translucent after five minutes standing. The remaining gel is dried at 70°C. in an oven.

In order to obtain good packing properties, the gel spheres should all be of the same size, so the gel is dry sieved using fine wire mesh sieves of 120, 90, 63, 35 micron hole sizes. Wet sieving[25], [35] is not satisfactory as the gel adheres to the bottom of the sieve. If there is not enough gel in one size range (say 63 to 90 microns), then the volume is made up with gel from an adjacent size range, but this only happened when new gels were being prepared.

Once dried and sized, the gel is mixed with a mixture of acetone and perchloroethylene (9:7 by volume), which acts as a balanced mix. To ensure the mixture is of the same density as the gel, the suspension is centrifuged

for 60 seconds, and if the gel is precipitated, more perchloroethylene is added, and if the gel rises to the surface, more acetone is added. Dawkins and Hemmings[73] have suggested using 2-chloroethanol with acetone as the balanced mix as this gives higher packing efficiency and hence higher plate count, but when this was tried it was found that the balanced mix was rather viscous in nature and difficult to handle; it is also toxic, so a great deal of care must be taken when using it.

Once the slurry is balanced it is refluxed for about half an hour to "degass" it, the mixture of the slurry being one volume of gel to two of balanced mix. The purpose of degassing is to remove as much dissolved air from the suspension as possible, as this can be released when the gel is packed under pressure. The hot slurry is then allowed to cool slowly, and is then stoppered to ensure no evaporation takes place. Once cooled, it is introduced to the packing machine.

The packing machine is fed with THF from a solvent store, as shown in Figure 4. The THF has been degassed and stored in a 5 litre flask with 20 cm^3 of 10% Stavox inhibitor (2:6 - ditertbutyl-p-cresol) added before the distilled THF. This was found to reduce the rate of peroxide build up in the solvent much better than by adding the inhibitor after the distilled THF is in the vessel.

The slurry is introduced into the packer at point A, where the column is filled up with more degassed balanced mix. Coupling A is then tightened and the pump switched on. The rate of THF introduction is governed by the stroke of the pump, and adjusted accordingly to maintain the required pressure shown on the pressure gauge at the head of the column. Once about 400 cm^3 of THF has passed through the system, the teflon tube is attached to a 10 cm^3 syringe which is allowed to partially fill. The pump is then turned

off and allowed to return to zero pressure, at which point coupling E is undone and the packed column released. Some gel is then removed from the large U bend and put into an empty end fitting (see Figure 5). This is then screwed into place similar to the other end fitting at point C. The syringe is now pressed until solvent appears out of the freshly attached end fitting, at which point some solvent filled bellows are attached. The teflon tube is now detached from end fitting C and the bellows pressed until solvent appears. A blanking off nut is inserted into the end fitting and tightened. The column is now in a suitable condition to be transported or stored.

When putting a new column into the chromatograph, it is important that no air is allowed to enter the inlet side of the column as this can disturb the packing arrangement of the gel. To ensure this the following procedure should be followed:

(1) Attach the column securely in the chromatograph.
(2) Adjust the solvent flow rate to about 0.5 cm^3 per minute.
(3) Remove the blanking nut from the column end fitting.
(4) Squeeze the bellows until solvent appears at the other end fitting.
(5) Connect the solvent inlet pipe to the opened end fitting.
(6) Remove bellows and wait until solvent appears at this outlet.
(7) Attach the column outlet to the inlet pipe to the refractometer.

If a column is to be removed and stored, the reverse procedure is followed.

When more than one column is being used, it has been the practise to put the highest pore sized column in first and then construct the set in decreasing pore size[74] but it has been suggested that better separation is obtained if the columns are put in randomly,[75] the proposed theory being that the sample "plug" is effectively segregated from the start.

Using conventional straight column sets in order to obtain a large gel bed, and hence good separation, a number of columns are needed which are joined together by fine tubing. This tubing causes a decrease in column separation efficiency,[72] so it has been suggested that a coiled column can be used[75] which is at least as efficient and more compact. These columns should have a diameter of greater than 30 cm (12 inches) to prevent a reduction in resolution due to the "race-track" effect. This effect is when the sample "plug" travels around the curve, and the portion on the inside of the curve has less distance to travel than that on the outside, causing the plug to become skew. It has also been suggested[76] that this effect can be reduced by use of fine bore tubing (0.475 cm i.d.) or by finer gel particles.

3.2. Further Packing Techniques.

Probably the most fundamental part of improving column efficiency for separation using a particular gel, is the way in which the gel is packed. Tweedale[35] reported that the theoretical plate count of a column decreased as the packing pressure increased, but this was only between 0 and 350 p.s.i. (24.4 kg/cm^2). Ouano and Barker[77] report a system similar to that described in 3.1., but using a nitrogen propelling system that produces a plate count for ethylbenzene of over 1,000 theoretical plates per foot (3,100 plates per metre). A well packed column should have an interstitial volume of 0.364 the total volume,[77], [78], [79] and a deviation from this will lead to an inefficient column. This volume is based on random packing and not on close packing, which will give a lower interstitial volume. The ideal situation is difficult to obtain, but it can be approached by using microparticle gels. Any disruption, or poorly packed gel, will cause a non-uniform velocity profile of the solvent and this will cause non-laminar flow, which in turn will cause peak spreading and tailing, and a decrease in the resolution of the column.

There are numerous ways of packing a column, but the two methods studied were that of constant packing pressure[81] and of constant flow rate. The conditions of packing were as identical as possible, that is the same gel, same end fittings and same column were used, in order that any differences in the pressure drops or bed volumes would be eliminated. The gel is Styragel of pore size 3×10^4 Å., and the column is a four foot (1.29 m) seamless stainless steel tube. The end fitting sinters are grade D, (maximum porosity 25 microns) as provided by Sintered Products. The balanced mix is acetone and perchloroethylene (9:7 by volume), and the solvent is degassed THF with inhibitor added.

To measure the efficiency of the packing, a note was made of the packing pressure at its maximum, the flow rate of the THF at its maximum, the pump stroke, the pressure drop across the column at a flow rate of 1.0 cm^3 per minute, the theoretical plate count of the column using both benzene and TCB (trichlorobenzene) and the ratio of these two. The packing process was assumed to be complete after 400 cm^3 of eluant had passed through the column, as recommended by Tweedale.[35] To ensure that the column had packed satisfactorily, it was assumed that the resolution of benzene and TCB would be very similar, and hence their plate counts would be similar; if they were not, they were disregarded as this indicated a deterioration in the column.

In Graph 1 the results of packing at constant pressure are shown. These results were obtained by allowing the pressure to reach a preset value as quickly as possible and then adjust the flow rate of the solvent to maintain the pressure, this stable flow rate being the one assumed to be related to the packing pressure. When the plate counts of the columns were calculated for benzene and TCB, it was found that occasionally the values

were widely different, indicating a change in the resolving power of the column. Arbitary values of this plate count ratio were taken and any values above 1.0 : 1.50 were disregarded, as this indicated a major discrepancy in the resolution, as since the molecules are very similar in structure and size, a similar plate count would be expected for both. A lower ratio value of 1.0 : 1.10 was also arbitarily chosen, and the results for the experiments where the ratio was between 1.0 : 1.50 and 1.0 : 1.10 are shown by a spot within a circle. Where the ratio was between 1.0 : 1.0 and 1.0 : 1.10, the results are shown by a cross within a circle.

As can be seen from the graph, the lower ratio points tend to be of higher plate count, and hence higher resolving and packing efficiency. The plot also indicates a rapid increase in packing efficiency until a pressure of 400 p.s.i. (37.8 kg/cm^2), then decreases slightly but starts to increase again at about 700 p.s.i. (48.6 kg/cm^2). The higher ratio points show a rapid increase in packing efficiency until a pressure of about 220 p.s.i. (15.3 kg/cm^2) is reached, and after this point, a less rapid improvement.

The scatter in Graph 1 indicates that this method of packing a column is not very reproducable, but this could be due to many factors, such as the fierce pulsing of the pump as it discharges its load, inefficiency due to the end fittings, disintegration of the gel due to repetitive packing or re-alignment of the packed gel (especially at low packing pressures).

It was found, however, that for most column a linear fundamental relationship exists between the flow rate of the solvent and the pressure obtained across the column while being packed. The relationship is maintained only if the porous sinters are not blocked and the gel has not

disintegrated, as these factors will increase the pressure drop across the column. When this occurred the sinters and the gel were replaced by identical substitutes. This relationship, however, is characteristic of the packing system which is inclusive of the pump, column, gel and end fittings, as well as the balanced mix. For instance, the relationship for pressure and flow rate for a balanced mix of perchloroethylene and acetone will not be the same as for 2 chloroethanol and acetone, (a mixture suggested by Dawkins and Hemmings[73]). Occasionally, however, packing pressures did not comply with their expected value for the flow rate being used in that experiment, so when this did occur, it was decided to disregard the experimental packing pressure and use the pressure expected from the fundamental relationship for that flow rate. As can be seen from Table 1, the number of results greater than the expected packing pressure is about the same as the number less than the expected packing pressure for the flow rate being used in the experiment. When the packing pressure is greater than expected, it can be explained by blocked sinters and disintegrated gel, as discussed earlier, but it is much more difficult to explain the higher than expected flow rate, as this indicates an easier than expected solvent flow. Of the results in this group, most are of less than 5% discrepancy, but there are three of much higher discrepancy, up to 30%. Such results are difficult to explain, as there is no apparent reason for it.

As can be seen from Table 1, most of the differences between theoretical and practical pressures are relatively small, but a few are in excess of 10% difference. If the theoretical plate counts are replotted against the theoretical pressure, the shape of the curve is altered. Graph 2 shows all the points on Graph 1, but plotted against theoretical pressure.

Graph 2 compliments the earlier findings of Tweedale[35] in that at low pressures (below 400 p.s.i.) and necessarily low flow rate, the packing

efficiency of the column decreases with increased packing pressure, up to a pressure of about 400 p.s.i. (37.8 kg/cm^2). Tweedale[35] has reported that such columns are unstable and liable to deteriorate rapidly once used in the chromatogram. At above 400 p.s.i. (37.8 kg/cm^2), however, the results start to show a reverse trend, and as the pressure increases so the packing efficiency increases. This indicates that the particles are physically forced together into a tightly packed matrix, as would be expected. Since the pressure drop across the column in the chromatogram can be 100 p.s.i. (6.95 kg/cm^2), it is reasonable to disregard the very low pressure results as being too variable for the system, as the packing pressure required for the solvent flow rate will be greater than the original packing pressure, and a further, or "secondary" packing process could take place in the chromatogram.

Three columns were packed at zero pressure, and this was accomplished by pouring the degassed gel slurry into a vertical column which had an end fitting on the lower end, and allowing the solvent to permeate out of the bottom, leaving the gel bed in the column. There are reports of packing by vibrating the column and the gel slurry for several hours and still obtaining efficient columns,[80], [116] but these are not with rigid gels.

The second packing method is to pack at a pre-determined flow rate and let the pressure build up accordingly. This method gives more reproducible results, as can be seen from Graph 3. However, there is still a low efficiency point at about 450 p.s.i. (31.3 kg/cm^2), which is equivalent to a flow rate of 10.5 cm^3 per minute. Again, this confirms Tweedale's[35] results for low pressure packing.

Although the two packing systems are different, the factor which relates them as being similar in the end result is that of the rate of flow

of the solvent. It appears, however, that if a column is packed by using a pre-set pressure and adjusting the flow rate accordingly, the packed column is of less predictable efficiency, but could be of greater efficiency than the one packed by continuous pre-set flow rate. The highest plate count obtained was 2,200 plates per foot (6,800 per metre) and was packed by using a constant pressure. Below is a table of the packing techniques and efficiencies for comparative purposes.

Plate Count per foot (per metre)	Constant Pressure over 600 p.s.i. (41.8 kg/cm^3) (29 Trials)	Constant Flow Rate (35 Trials)
500 (1650)	20%	29%
500 - 750 (1650 - 2310)	15%	27%
750 - 1250 (2310 - 3880)	40%	44%
1250 (3880)	25%	-

TABLE 2.

One possible explanation for these results is that the gel spheres, when packed at low pressures and flow rates, are able to orientate themselves sufficiently to obtain relatively good packing, but due to the low pressure, they are only loosely packed, hence when the pressure is increased slightly, they disorientate themselves and form loosely, or badly, packed areas, such as along the sides of the column where a channelling effect can take place.(77) However, at higher pressures or flow rates, the spheres are physically thrown at each other with such force that they are forced into packing sites and

become physically trapped in these sites by the surrounding spheres. The spheres tend to swell slightly in THF, and hence become less rigid, and are able to distort sufficiently to give a tight network of packed spaces, but at lower pressures the swelling effect is sufficient to move the spheres apart, thus giving a less efficient packing. In the case of high pressure systems, the spheres which are against the wall of the column swell and distort in such a manner that they are able to mould themselves around the topology of the wall, so reducing the channelling effect. This type of property is what makes a xerogel such a good gel, but at the same time reduces its potential use because of its high swelling ratio, when it can be over fifty times its original volume once swollen, it will cause greater resistance to flow, and even non-laminar flow, which can lead to high pressure drops and poor resolution, decreasing the efficiency of the column.

3.3. The Effect of Concentration on Elution Volume.

When a polymer is eluted on a gel permeation chromatograph, the viscosity of the solution is a very important factor in its elution characteristics, because at high concentrations the solution becomes viscous, and "viscous fingering" occurs. This is when the trace of the polymer shows an excessive low molecular weight tail. A similar effect is exhibited when the column is overloaded[70] a polymer, although it is possible to get "secondary exclusion" and hence better separation if the column is overloaded.[82] Secondary exclusion enhances separation because when it occurs, pores are blocked by small molecules and there is a decreased probability of the larger molecules finding pores of sufficient size to allow them to enter. Occasionally, it is the practise to calibrate a chromatograph by injecting different concentrations of the same standard and extrapolating the resultant elution volume to zero concentration.[5]

A study was carried out on the effect of concentration on different monodispersed polystyrene standards, as supplied by Pressure Chemicals Inc.,

Pittsburgh, Pa., U.S.A. The 0.5% and 0.25% solutions were injected for 60 seconds, and the lower concentrations for 120 seconds. The results are shown in Table 3 below.

Polystyrene Sample and Concentration (%)		$\bar{M}_w$	$\bar{M}_n$	Dispersion D	Theoretical Plate Count per foot	Elution Volume V_e (counts)
	0.5	2,530	2,057	1.23	75	31.40
900	0.25	2,008	1,718	1.17	62	31.48
	0.125	2,390	2,017	1.18	75	31.48
	0.0625	2,470	2,070	1.19	74	31.50
	0.5	11,306	6,546	1.73	86	28.40
	0.25	10,496	9,222	1.14	96	28.40
10,300	0.125	11,145	10,045	1.10	86	28.40
	0.0625	11,669	10,414	1.10	98	28.40
	0.22	10,595	9,328	1.13	77	28.35
	0.5	200,424	81,052	2.47	108	22.90
110,000	0.25	105,144	85,532	1.23	108	22.90
	0.125	108,049	94,726	1.14	108	22.87
	0.0625	107,398	93,940	1.14	99	22.83
	0.045	110,089	93,943	1.17	113	22.71

TABLE 3.

A comparison of viscometric properties was made by calculating the viscosity of 0.25% solution of polystyrene 900 (P/S 900), and then, by using the relationship below, determined the concentration required for the

other two standards to give the same viscosity, hence the fifth result for P/S 10,300 and P/S 110,000.

$$[\eta] = \left[\frac{(\ln \eta_r)}{c}\right]_{c\to 0} = \left[\frac{(\eta_r - 1)}{c}\right]_{c\to 0} \qquad 3.3.1.$$

but $$[\eta] = k M^{\alpha} \qquad 2.1.8.$$

hence $$kM^{\alpha} = \left[\frac{(\eta_r - 1)}{c}\right]_{c\to 0} \qquad 3.3.2.$$

where η_r is the relative viscosity and C is concentration. It can be seen that the concentration has little effect on either the theoretical plate count or the elution volume, but it does affect the dispersion of the polymer quite markedly. It should be noted that although P/S 900 is used as a standard, the weight average and number average molecular weights both give values of over 2,000 daltons. This is due to the fact that low molecular weight polystyrenes elute prematurely if the chain length is used as a parameter for calibration. Calibration of a column set at this low chain length region is carried out with linear hydrocarbons[116] and the lower polystyrene results are disregarded. The reason for this deviation has been explained by Tweedale[35] to be due to the pendant phenyl groups on the polystyrene backbone; since these chains are not very long, the phenyl groups will have a marked effect on the shape and volume of the molecule. This effect first manifests itself as the molecular weight of polystyrene approaches 3,000, which is equivalent to about 30 repeat units, so it is possible that the reason for the deviation is purely statistical, in that the polymer linkage is not of sufficient length to allow the statistical treatments required in solution theories.

A similar effect is noticed if the theoretical plate count is plotted against the logarithm of the chain length; below about 75 angstroms, the plate counts increase at a rapid rate. (See Figure 6).

3.4. Some Studies into the Properties of THF.

Since THF (tetrahydrofuran) is the only solvent used in this work, it is important that some of its properties are known and understood. THF is an excellent solvent for numerous polymers and oligomers, and it should be stored in clean, opaque glassware, or a clean solvent tin; it should not be left in polythene bottles as it tends to dissolve out what appears to be a low molecular weight plasticiser, which gives a substantial peak during G.P.C. analysis.

It was also noticed that the samples, if left for any length of time, started to obtain a "peroxide" peak at the low molecular weight end of the chromatogram, even though the solvent had inhibitor present, so solutions should be made up when they are needed. This peak is probably due to the build-up of peroxides created by the action of light on the THF, causing ring cleavage and subsequent reactions.

If THF is left in the presence of brass or copper, especially in the packing machine or on the column end fittings, it turns greenish blue after a few days. The pH of the THF also changes from 6.2 to about 4.5, indicating a build-up in proton concentration. If a U.V. spectrum is run of this THF it shows a peak at 305 nm, however, if some THF is left with copper wire, after it has turned blue, a peak at 301 nm can be seen, indicating a different compound being responsible. If some sodium dried THF is left with either brass or copper wire, a very slow reaction takes place, but after five weeks, it too is blue. In this case, the resultant U.V. spectra both showed two peaks, one at 265 nm and one at 292 nm. The peak at 265 nm was relatively much more intense in the case of the copper wire than with the brass. It has been proposed that this blue colour is due to a build-up of peroxides, as it does give a positive result if the peroxide test is carried out with it

using sodium iodide to detect the peroxide. It is, however, possible that the colouration is due to a chelating effect by the THF with the metals, and if this is so, it could prove very useful in the detection of these metals by G.P.C.

CHAPTER 4.

A NEW METHOD FOR CHARACTERISING GELS BY THIN LAYER CHROMATOGRAPHY.

4.1. Introduction.

In order to characterise new gels, a lengthy process of sizing has to be carried out, as already shown. However, if a new method can be employed, the characterisation process could be reduced from days to an hour or less for any gel.

One such method is thin layer chromatography (TLC), where the gel is put onto a glass plate and various standards run on the plate. The method has been used on various gels, such as silica gel,[83] dextrans,[84] and polyacrylomorpholines[66] with varying degrees of success. When a gel is used as a separating medium for TLC, it is usual for the medium to act as it would in a column; that is, the higher molecular weight species have the higher retention factor (R_f), and hence travel further along the plate towards the solvent front. Otacka[85] has suggested that the plates should be run while in a horizontal position as this, apparently, reduces the streaking associated with thin layer chromatography, but this is often difficult to arrange unless specific apparatus is built. He also comments on the lack of good resolution, but points out it can still be used for molecular weight distribution measurements, but believes the results to be about 2% higher than those obtained by conventional gel permeation chromatography. Since this method is a combination of two chromatographic techniques, it has been called "thin layer gel permeation chromatography".

4.2. Tritiation of Polymer Standards.

Since the gel being characterised is hydrophobic, the previously recommended characterisation techniques are not viable, so a new method using monodispersed polystyrene has to be instigated. Due to the low concentrations of samples being spotted onto the plate, and the nature of

the plate, it was believed that the most convenient detection method would be by radioactive tracer techniques using a spark ionisation chamber.

In order to use the polystyrenes usually used for calibration, they had to be activated without cleaving any bonds in the polymer backbone, and hence altering the molecular weight and dispersion. This restriction eliminated the use of high energy radiation to irradiate the polymer. It was decided that the best site of labelling would be the aromatic protons on the phenyl group, as this would not affect the polymer backbone.

Long et al,[86], [87] suggest using homogenous metal catalysts, such as aluminium chloride and organometalics for rapid hydrogen isotope exchange in aromatic compounds. Their results indicated a 100% yield of substituted simple aromatics after one week of reaction with all catalysts used, but the rate of reaction was dependant on the catalyst. The exchange is random with respect to aromatic protons, and is believed to follow a π complex mechanism. It is not subject to steric effects as observed in homogenous platinum catalysis. When substitution with ethyl aluminium dichloride was performed, Long[87] noticed that there was a high percentage yield of the active substrate after two minutes of reaction at room temperature using 50 mCi of tritiated water (HTO).

4.3. A modified method for polymer tritiation.

A modified method, similar to that of Long[87] was used to label polystyrene. Initial work was performed with a polystyrene of molecular weight 195,000 (P/S.195,000); this, and all monodispersed polystyrene standard preparations were conducted as follows:

25 mg. of polystyrene are dissolved in sufficient (0.25 cm^3) cyclohexane, (this has not been dried as water acts as a catalyst to the reaction) at 45°C. 0.001 gms. (0.001 cm^3) of diethyl aluminium chloride and 0.025 cm^3 of T_2O

(5mCi/gm) are added to the solution and left for half an hour. After this time the reaction is flooded with n-butanol to destroy the diethylaluminium chloride. The whole reaction is done in a glove box under nitrogen which has been passed through concentrated sulphuric acid. The cyclohexane was then allowed to cool down, whereupon the polymer precipitated out of solution. This was then collected, washed in cold cyclohexane, dried and analysed for activity. For 100% substitution an activity of 0.125 mCi for the sample would be expected, but when analysed by scintillation counting, it was found that the activity was only 40μCi/gm, indicating an efficiency of less than 1%. If the catalyst is increased to 0.005 gms., the yield of tritiated polymer is increased to slightly over 1%.

To ensure no degradation had taken place, the chromatogram of the labelled and unlabelled polymer (P/S 195,000) were compared. No change was noticed, even three months after the labelling reaction.

4.4. Preparation of TLC plates and of solutions.

Various gels were used to see if this method is feasible; the gels were mostly ones prepared by Tweedale[35] and were of known permeability. The gel was mixed with 12% $^{v}/_{v}$ of gypsum to act as a binder. The mixture was then mixed into a slurry with methanol.

The plates are washed in soapy water and then in acetone and carefully dried, ensuring no grease marks are left on the surface. They are then put into a preparation rack and the gel slurry poured into a reservoir, which is carefully but firmly run across the plates. The resulting plates are left to dry slowly in the laboratory.

The tritiated samples are dissolved in a suitable solvent, initially THF, to a concentration of 0.10%. A micropipette is then prepared by drawing

a melting point tube to a fine point, and this is used to introduce the polymer solutions to the plate.

4.5. Characterisation of the plates.

Once the plates have been prepared, they are spotted with the solution of polymer, the maximum volume of the spot being about 0.01 cm^3. The plate is then placed vertically in a shallow bath of the solvent and kept in a draught free environment, so that the atmosphere around the plate is solvent saturated. The solvent then moves up the plate carrying the various spot samples with it. When the solvent has nearly reached the top, the plate is removed and dried. The plate is then examined in a spark ionisation chamber where a polaroid film is exposed for half an hour over the plate. In order to orientate the photograph, distinct radioactive spots are put at the top of the plate and at the base line.

It was noticed that when THF was used as a solvent, the solvent front became very indistinct due to the poor solvent-vapour equilibrium at the top of the vessel. To overcome this, the solvent was changed to toluene, as this is less volatile and gives a more distinct solvent front.

The detection of the spots was very difficult, even with the spark ionisation detector, as they tended to smear, so it was decided to increase the polymer concentration to 0.3% in toluene. However, this did not give much better results, but it was noticed that polystyrene could be seen under a U.V. light (275 nm) as a light pink area on a darker pink background. To see this, however, the spot has to be rich in the polymer.

A study was also made of the effect of the dryness of the plate when it was used. It was found that the retention factor (R_f) did not alter significantly for a wet or dry plate with a wet spot, but for a wet plate with

Gel.	P.3.	P.4/7.	P.8.	P.11.	Unknown.	2 x 10^6	F/A 3536 614 40:50: II
Characterising Polymer (M_w)							
2 x 10^6	0.01	0.005	0.01	0.07	0.14	0.07	0.057
6.7 x 10^5	-	-	0.08	-	0.16	0.24	0.14
2 x 10^5	0.08	0.19	0.114	0.19	0.225	0.51	0.46
9.72 x 10^4	0.03	0.67*	-	0.30	0.25	0.73	0.57
5.1 x 10^4	0.58*	-	0.41	-	0.71	0.75	-
1.98 x 10^4	0.59*	0.29	0.58	0.66	0.78	0.87	-
10^4	0.40	-	0.85	0.73	0.76	0.84	0.86
4 x 10^3	0.50	0.52	0.87	0.88	0.84	0.85	0.89
2.1 x 10^3	0.60	0.77	0.90	0.93	0.85	0.97	0.88

TABLE 4.

R_f values of calibrants on various gels.

* erroneous results, possibly due to excessive smearing.

a wet spot, the R_f appears to be less than for a dry plate with a wet spot. However, in all the work described, the system was for a dry plate with a wet spot.

4.6. Interpretation of Results.

In order to calibrate the plates, the retention factor (R_f) has to be calculated. This is a ratio of the distance the solvent travels ($d_{solvent}$) to the distance the sample travels (d_{sample}).

$$R_f = \frac{d_{sample}}{d_{solvent}} \qquad 4.6.1.$$

This value obviously cannot exceed unity.

The distance the spot had travelled was taken to be the distance from the base line to the centre of the head of the smeared spot (see Figure 7), and the distance the solvent had travelled was from the base line to the solvent front.

A calibration curve was constructed for each gel used, plotting the R_f against the log of the chain length. Tables 4 and 5 and Figure 8 show the results.

Gel	Maximum Permeability by GPC (Å)	Maximum Permeability by TLC (Å)
P.3	-	10^3
P.4/7	2×10^3 a	5×10^2
P.8	3×10^4 a	7×10^3
P.11	5×10^5 a	10^4
Unknown	10^8 b	High
2×10^6	2×10^6 b	10^5
F/A 3536 614 40:50 II	10^4 b	4×10^4

a as determined by Tweedale[35] b as determined by author

TABLE 5.

It can be seen in Figure 8 that the separation appears to follow conventional thin layer chromatography, and not thin layer gel permeation chromatography as was expected. This is probably due, however, to the relatively high concentrations of standards needed to detect the spot, and true gel permeation could not take place due to localised sample overloading.

As shown in Table 5, the results compare favourably with each other, and with improvements to the technique, it may be possible to improve on them and use the system as an alternative to conventional gel permeation chromatography. It has been shown, however, with P.3, that gels which cannot be characterised by G.P.C. because of packing difficulties, can be by this method.

4.7. Discussion.

The low percentage yield of the tritiation reaction (4.3) is disappointing as Long et al[87] have used ethylaluminium dichloride as a catalyst and obtained favourable results, but in this study, diethylaluminium chloride which should be a stronger Lewis acid, and hence better catalyst, was used. However, Breslow and Newburg[88] believe there is no appreciable difference in the catalytic properties of the two. The reaction is not, apparently, sterically affected so another factor, hitherto unconsidered, must be responsible for the low yield. Nevertheless, it has now been established that tritiation by this method can be performed.

The chromatographs produced by the thin layer chromatography experiments did not give the expected results, but this is probably due to the relatively high concentration of polymer used in calibration, and effectively overloading the system in the specific region of the polymer. It is also possible that although the polymer spot was wet when elution started, the rate of dilution of the spot will be less for larger molecules than for smaller ones, due to

the increased viscosity of the solutions, hence the larger molecules will not be as mobile as the smaller ones. Since viscosity is related to concentration, the results can be compared with those of Belenkii,[89], who has shown that as the concentration increases, the rate of movement, and hence the R_f value decreases. Therefore, it is possible that the concentration effect could have caused the inversion of expected results.

Halaap[90] has obtained similar experimental discrepancies using silica gel and aluminium oxide as separating media, but only found the discrepancies when mixed solvents were used. He explains the results in terms of adsorption. Otacka et al[91] propose two mechanisms for phase separation and thin layer gel permeation chromatography. One is precipitation, where he suggests there is an increase in concentration of the sample as it moves up the plate, until the situation arises where the polymer cannot act as the solute and precipitates out; in other words, the solvent power decreases as elution takes place. The second is adsorption, where he suggests an equilibrium between adsorption and desorption. The preferred mechanism is that of precipitation, as macroporous adsorbants can exhibit a definite size fractionation when solvent effects have been avoided.

The unexpected results can be explained as probably being due to a combination of viscosity, overloading and solubility effects, all of which have affected the chromatograph, with the net result of giving the results above. There is, however, no reason why thin layer gel permeation chromatography cannot be resolved into a very rapid and easy technique, especially if the samples can be isolated whilst on the plate. If the streaking can be reduced, much better resolution will be possible, and better calibrations and dispersion results will be available. One way would be to use ^{14}C labelled polystyrene standards, as the detection limit is one

hundred times less than that for tritium by spark ionisation detectors (limits being 0.05 mμCi and 5.00 mμCi respectively). Conversely, if a gel can be developed which is affected photometrically by the presence of a polymer, it could be used for polymer characterisation. The use of dyed polymers would be able to do this, but the addition of a dyeing molecule will affect the chain length of the polymer.

CHAPTER 5.

THE PREPARATION AND STUDY OF SOME NEW GELS.

5.1. Introduction.

Since the introduction of gel filtration chromatography by Porath and Flodin[15] and the preparation of stable matrices by Moore,[23] many new types of synthesised gel have been prepared. Prior to these works, other media had been employed,[21], [26] but it was not until 1964 when Moore[23] suggested a method for pre-determined pore sizes in a stable gel matrix. Since then, the study of aerogels and their use in gel chromatography has increased rapidly.

It has been shown in Chapter 1 that there are two types of gel, "aerogels" and "xerogels" examples being, for xerogels, polyethyleneglycoldimethylacrylate,[92] polyvinylacetate,[93] copolymers of ethylvinyl benzene/divinyl benzene and vinylacetate/divinyladipate,[96] sephadex[15], [97], [98] and polyacrylomorpholenes.[14] Examples of aerogels are silica gel[17], [99] and porous glass.[18], [36], [100] The two types of gel are naturally different in their properties, the xerogels being soft and swellable, and the aerogels being rigid matrices. The permeability of the xerogel is dependent upon the solvent used, for the pores appear to be inter-chain spaces within the fibre of the polymer, so the more the polymer swells, the greater its pore size and permeability. In contrast, the aerogels are of fixed permeability, and are not affected in themselves by different solvents. Between these two extremes is a type of gel called a xerogel-aerogel hybrid. This gel possesses properties of both gels, they usually do not swell greatly and have a rigid matrix of known pore size, although this may alter in different solvents due to the molecular volume of the polymer in solution altering, and they tend to be used in polar organic solvents. Such a gel is the type prepared by Moore.[23]

5.2. Preparation of a gel based upon methylene bisacrylamide.

Frisque[15] patented a method describing a series of gels prepared by

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copolymerising acrylamide and methylene bisacrylamide. The gel is water compatible, but also can be used with THF.

Gel	F/A 1	F_A/A 1	F_A/A 40:50 1	F/A 40:50 1 F/A 40:50 II	F/A 80:50 1 F/A 80:50 II	F/A 20:50 1 F/A 20:50 II
Methylene NN' Bisacrylamide grms.	45	80	20	20	40	50
Acrylamide grms.	-	-	20	20	40	50
Formaldehyde mls. xx	120	-	-	100	100	500
Formic acid mls. +	-	120	100	-	-	-
n-Heptane mls.	750	750	750	750	750	750
Tween 80 mls. x	10	10	10	10	10	10
Span 80 mls. x	40	40	40	40	40	40

x supplied by Koche Light.

xx 40% soln.

\+ 90% $^{w}/_{w}$

TABLE 6.

Table 6 presents the gels prepared, which are based on the patent by Frisque. The catalyst for all the polymerisations was 20 grms. of ammonium persulphate dissolved in 40 cm^3 of water and added in a 1:5 ratio to the monomer.

A typical polymerisation reaction proceeds as follows: 750 cm^3 of n-heptane with 40 cm^3 of surface active agent, such as Span 80, is purged with nitrogen for half an hour whilst being stirred. A mixture of 20 grms. of acrylamide and 20 grms. of methylene NN'bisacrylamide is dissolved in 100 cm^3 of formaldehyde, with 20 cm^3 of surface active agent, such as Tween 80, present. This is done at pH 8-10 and at 50°C., otherwise the solution may not form. The heptane is heated to 50°C. and the formaldehyde adduct solution is added. The mixture is stirred at sufficient speed to interdisperse the aqueous and non-aqueous phases, (but not so fast that the particle size is too small.[101] The most satisfactory speed is about 500 r.p.m.) The catalyst solution, consisting of 10 grms. of ammonium persulphate in 20 cm^3 of water is added to the reaction vessel (see Figure 9) by means of the dropping funnel.

The reaction proceeds for two hours, after which time the mixture is filtered through a sintered glass filter under reduced pressure, and the remaining gel washed with copious amounts of methanol, hot, and then cold water. The gel is then dried at 50°C., sized and packed. The above method is one described for preparation of F/A 40:50. The prefixes F/A and F_A/A refer to the aldehyde and acid adducts respectively, for instead of dissolving the monomers in formaldehyde, they can be dissolved in formic, or any other similar acid. It is not a necessary requirement for a copolymer to be formed, as one with just methylenebisacrylamide can be produced as in F/A 1 and F_A/A 1, but it was found that these were not suitable for packing as the THF dissolved them. The figures after the prefix refer to the concentration of the chain monomer and crosslinking agent, for instance, 40:50 means a total of 40 grms. of monomer mixture dissolved in 100 cm^3 of solvent, and the percentage of crosslinking agent being 50% of the bulkweight. The final number refers to the preparation number of the gel.

The gels were packed in a four foot (1.29 m) column after being degassed in a balanced mix of acetone and trichloroethane in the ratio of 2:11, This choice of solvents was made because they appeared not to dissolve the gel or cause it to swell.

During packing of the prepared gels, it was noticed that the acid and aldehyde adducts of the bisacrylamide dissolved in THF, so these were discarded from any further examination. The remaining gels, however, appeared to be compatible with THF, in that they did not swell or dissolve noticeably, as described earlier. The results are shown in Table 7, where the pressure drop across the column, pore size (in angstroms) and theoretical plate count for benzene (injected for 15 secs., 1% soln. at 1 ml/min. flow rate) are shown.

	F_A/A 40:50	F/A 40:50	F/A 80:50	F/A 20:50
Pressure drop lb./sq". (kg/cm^2).	150 (10.4)	165 (11.5)	62 (4.3)	185 (12.9)
TPC (per foot) (per metre)	420 (1300)	150 (465)	317 (985)	496 (1540)
Porosity (Angstroms)	10^7	10^4	20	10^7
Swelling ratio	1.13:1	1.15:1	1.20:1	1.125:1

TABLE 7.

Figure 10 shows the calibration curves of these gels, using polystyrene standards in THF. The linear region of the graphs tend to be over a range of 10^2 Angstroms, which indicates the gel has a broad distribution in permeability. If F/A 80:50 is studied, it can be seen that the linearity

of the calibration is below 10 Angstroms, and then suddenly increases, showing no resolution at all. However, the gel does exhibit xerogel characteristics in that the elution volume of the lower chain length samples are greater than the elution volume of the column series; that is, the capacity ratio is greater than 1:1.

A study of the swelling ratio was carried out on these gels, and it was found that they compared reasonably with the ratio for Styragels, which is about 1.02:1. This was carried out by measuring a known volume of the gel in a measuring cylinder, and then filling the cylinder with THF and measuring the volume of the swollen gel. The results are shown in Table 7, and indicate that they all have a very similar swelling ratio, even though the crosslinking density is different.

5.3. Preparation and evaluation of a styrene-divinyl benzene gel.

Berger and Minder[102] have suggested a crosslinked styrene-divinyl benzene gel of variable permeability, which can be used for gel permeation chromatography.

Monomer preparation is carried out by removing the inhibitors from the styrene and divinyl benzene by washing them in 10% aqueous sodium hydroxide solution at least six times, and then four times in water. The resulting monomer is then dried over anhydrous calcium chloride and stored at -40°C. in a refrigerator. The catalyst, A.Z.B.N., is purified by recrystallisation from toluene.

One such gel is prepared by dissolving 3 gms. of polyvinyl alcohol (Gelvatol 20-30) in 3 litres of deionised water with 0.0018 gms. of sodium stearate. To this 34.5 gms. of hydrated magnesium chloride and 11.5 gms. of sodium hydroxide (dissolved in sufficient water) are added. The resultant

polymer is then filtered off, washed in 30% sulphuric acid to remove the insoluble hydroxide, followed by hot and cold water and then methanol. The gel is then dried at 50°C.

When the gel was dried, it was noticed that if the oven exceeded 70°C. the gel charred and was of no further use, so it could not be used for high temperature permeation chromatography. The swelling ratio was found to be over 2.45:1 for the gel prepared as shown above, so it was decided to increase the crosslinking density by increasing the divinyl benzene percentage to 20%, and reduce the styrene percentage to 20%. This reduced the swelling ratio to 1.7:1, but still too high for packing a column. Since the gel could not be successfully packed, no calibration was carried out and further work stopped, as the gel showed nothing new to compliment it over the established Styragel type products of Tweedale[35] and Moore.[23]

5.4. Preparation of a gel similar to that described by Altgelt and Moore.[102]

17 gms. of polyvinyl alcohol, as supplied by Fisons, was dissolved in 1200 cm^3 of deionised water and boiled. The solution was then allowed to cool down and a mixture of 83.6 cm^3 of toluene, 27.5 cm^3 styrene and 2.8 cm^3 divinyl benzene, with 0.5 gms. benzoyl peroxide was added to it. The mixture was then heated to 92°C. while being stirred at 500 r.p.m. for $6\frac{1}{2}$ hours. After this time, the polymer suspension was filtered under reduced pressure, and the resultant gel washed with hot and cold water to remove any polyvinylalcohol which may adhere to the gel. It was then dried at 50°C. The gel was then swollen in THF and the swelling ratio found to be 6.17:1, which is too much to be pressure packed.

5.5. Some studies of polymerisation of divinyl benzene.

Divinyl benzene (55% pure) is polymerised using diethylbenzene as the solvent in the volume of 40 cm^3 and 60 cm^3 respectively. The catalyst is

0.5 gms. benzoyl peroxide, and the reaction is carried out in a 3 litre round bottomed flask with 1200 cm^3 of deionised water dissolving 17 gms. of polyvinylalcohol. The temperature is 92°C. and the reaction lasts seven hours while being stirred at 500 r.p.m.

This experiment was repeated, but the second preparation was at 85°C. It was also repeated using four times as much catalyst (2.0 gms.), a higher temperature (98°C.) and a longer time (18 hours) to see if this affected its swelling ratio. The latter preparation was called poly DVB XTRA, and was very similar to the previous preparations.

Further polymerisation reactions were carried out using different solvents, in order to see if the solvent strength with respect to divinyl benzene affected the degree of intramolecular crosslinking. The two solvents were toluene and isoamylalcohol. The toluene reaction took place at 92°C. and took 7.5 hours, and the isoamylalcohol reaction was at 89°C. and lasted $8\frac{1}{2}$ hours. The results of all these swelling ration determinations are shown below (Table 8).

	Poly DVB (I)	Poly DVB II (II)	Poly DVB XTRA (III)	Poly $DVB_{tol.}$ (IV)	Poly DVB_{ISAA} (V)
Solvent	diethylbenzene	diethylbenzene	diethylbenzene	toluene	isoamylalcohol
Temp.	92°C.	85°C.	98°C.	92°C.	89°C.
Time of reaction.	7 hrs.	7 hrs.	18 hrs.	$7\frac{1}{2}$ hrs.	$8\frac{1}{2}$ hrs.
Swelling ratio.	1.43:1	2.00:1	1.74:1	1.50:1	1.61:1

TABLE 8.

The polydivinylbenzenes using diethylbenzene as a solvent, all appeared to be the same physically, but those using other solvents were different. The toluene solvated sample (IV), appeared much more granular than the diethylbenzene sample (1), whilst the isoamylalcohol sample (IV) was softer and not so granular as poly DVB (1). When swollen in THF, poly DVB (1) loses the granular texture and becomes much more like a suspension, while poly $DVB_{tol.}$ (IV) does to a lesser extent, and poly DVB_{ISAA} (V), still less.

It was noticed during the swelling ratio experiments that if the gel was left near the oven, it turned from cream white to light yellow, and then finally dark brown. A further study of this was made using poly $DVB_{tol.}$ kept at 120°C. for seven hours, the swelling ratio of a sample being taken at regular periods; the results are presented below in Table 9.

Time at 120°C.	Swelling Ratio (s.r.)	Log s.r.	1/s.r.
0 (hrs.)	1.50:1	0.1761	0.6667
1	1.32:1	0.1206	0.7576
2	1.26:1	0.1004	0.7940
3	1.23:1	0.0864	0.8130
4.25	1.15:1	0.0607	0.8696
5.0	1.125:1	0.0512	0.8889
7.0	1.107:1	0.0298	0.9337

TABLE 9.

The reaction is kinetically first order, and the rate of reaction equal to 0.00125 $sec.^{-1}$ As it is first order, it indicates that the reaction is intramolecular free radical, and that further crosslinking caused by a free radical mechanism will not take place until a "threshold" concentration of free radicals has been established. From the results obtained, this appears to take about 40 minutes at 120°C.

5.6. Preparation of a gel of porosity 1.2×10^4 Angstroms.

Numerous attempts were made to prepare gel 6, as described by Moore,[23] which has a porosity of 1.5×10^4, but in order to get dispersion of the gel liquor in the diluent (water) the rate of stirring had to be in excess of the recommended speed to obtain a suitable bead size.[101]

It was decided to prepare a new gel based upon Moore's[23] gels, but using a different solvent - non solvent ratio. 13.4 cm^3 (6.7%) of styrene is mixed with 66.6 cm^3 (33.3%) of divinyl benzene, both of which have had their inhibitors removed, as described in Section 5.2. 17 gms. of polyvinylalcohol are dissolved in 1200 cm^3 of deionised water and heated to 85°C. while being stirred at 500 r.p.m. A solution of 0.5 gms. of benzoyl peroxide is prepared by dissolving it in a mixture of 40 cm^3 (20%) diethyl benzene and 80 cm^3 (40%) isoamylalcohol. This solution and the styrene/divinyl benzene mixture are then added to the water and stirred for $7\frac{1}{2}$ hours at 90°C.

The gel prepared in this fashion, when calibrated, has a porosity of 8×10^3 Angstroms, and is now being used in the medium column set. The swelling ratio is 1.08:1 in THF.

5.7. Preparation of gel P.10.

Tweedale[35] describes the preparation of a series of gels, one being of very high porosity (ca. 10^{12} Å) and designated P.10. For the work described in the following chapter, much of P.10 has been produced with varying results in respect of its pore size.

The preparation of the gel is essentially the same as for the gel in Section 5.6., but the solvent - non solvent is 5% (10 cm^3) diethyl benzene and 55% (110 cm^3) isoamylalcohol, all other variables being the same. The reproducibility, however, of the preparation is not very high, although it is

quite possible to obtain consistently high porosity products. Figure 11 shows the calibration curves of five such preparations, the variability in the preparations being obvious. This, however, is of little consequence for each preparation is calibrated as standard procedure and any alteration to the gel, as is described in the next chapter, is compared with the original gel calibration.

The most probable explanation for this is that the solvent volumes are not reproduced exactly, and this will affect the products, as Tweedale[35] has shown with isoamylalcohol volumes with his preparations.

5.8. Discussion.

Whilst initial work was done using Gelvatol 20-30, when the stock had expired no more was obtainable, so alternative stocks were obtained from Fisons Limited. From the information obtained, it was decided to reduce the quantities of polyvinylalcohol from 20 gms. of Gelvatol[35] which has a degree of hydrolysis of 80%, to 17 gms. for the Fisons product which has a degree of hydrolysis of 88%. Therefore, the quantity of active emulsifier will be the same due to the increase in the degree of hydrolysis compared to Gelvatol.

It is apparent that the styrene/55% divinyl benzene/diluent ratio of 1:5:9 by volume is the most satisfactory in preparing stable copolymer gels, as suggested by Tweedale.[35] If the mole fraction of styrene and 55% divinyl benzene is calculated, it is found to be 1:4, hence it can be seen that there is an excess of divinyl benzene in the polymer, with the possibility of unreacted vinyl groups still being present. If this is so,

further monomers can be copolymerised with the vinyl groups and be held in the matrix.

If a comparison of swelling ratios is made, as in Table 10, it can be seen that the gels described in this chapter, with the exception of Sections 5.6. and 5.7., are not very satisfactory for pressure permeation chromatography.

Gel.	Swelling Ratio.
Frisque (5.2)	1.125:1 to 1.20:1 depending on gel.
Berger & Minder (5.3)	1.7:1 and 2.45:1
Altgelt & Moore (5.4)	6.17:1
Divinyl benzene (5.5)	1.11:1 to 2.00:1 depending on heat treatment.
1.2×10^4 (5.6)	1.08:1
P10 (5.7)	1.02:1

TABLE 10.

In the case of the polydivinyl benzene, the swelling ratio is quoted for both the thermally untreated gel and the treated gel, as once it is thermally treated, its permeation properties deteriorate. However, in the

next chapter, it will be shown that these high swelling gels can be stabilised and used in a rigid matrix.

CHAPTER 6.

PREPARATION OF XEROGELS GRAFTED ONTO AEROGELS.

6.1. Introduction.

By using a xerogel-aerogel hybrid such as Tweedale's P.10,[35] it may be possible to adsorb the monomer mixture of a xerogel into the very large pores of the hybrid gel, and once in the site, polymerise the xerogel so it is held into the pores and stabilised by the rigidity of the matrix.

It is believed that the matrix gel (P.10) may have a surplus of unreacted vinyl groups on the divinyl benzene molecules within the polymer, and these groups could crosslink with the xerogel and hold it in the pores by chemical bonding. This, however, assumes that the unreacted vinyl groups are suitably positioned to react in this fashion, the most easily accessible site being on the surface of the pore.

A series of "grafting" reactions will be described which indicate that such a reaction has, in fact, occurred.

6.2. Preparation of Gel P.10.

Gel P.10 is a very high porosity gel first described by Tweedale.[35] The pore size is estimated to be about 10^{12} Angstroms, so it is ideal for using as a stabilising matrix. It is prepared as follows:

Dissolve 20 gms. of polyvinylalcohol (Gelvatol) in 1200 cm^3 of deionised water and heat to 75°C. while stirring at 500 r.p.m. A mixture of 6.7% styrene, 33.3% of 55% divinyl benzene, 55% isoamylalcohol and 5% diethyl benzene is prepared. The usual total volume of monomer is 200 cm^3, so the volumes are 13.4 cm^3 styrene, 66.7 cm^3 divinyl benzene and 110 cm^3 isoamylalcohol; these are mixed together and added to the polyvinylalcohol solution. 0.5 gms. of

benzoyl peroxide is dissolved in 10 cm^3 of diethyl benzene and this is added to the liquor as soon as the monomer mixture has been added. They are not previously added together to ensure that the reaction does not start until all conditions are adequate.

The reaction is continued at 85°C. for seven hours, while being stirred at 500 r.p.m. The gel is then filtered off, washed, sized and packed. Occasionally, the gel needs to be refluxed in boiling water to remove excess polyvinylalcohol which adheres to it if the solution is allowed to cool down too much.

6.3. Addition of styrene monomer to the gel matrix.

Initial experiments were performed on a vacuum line where 100 cm^3 of pre-sized P.10 gel was put into a 250 cm^3 round bottomed quickfit flask, and all the air removed by application of a vacuum of about 10^{-4} cm. of mercury. On the same vacuum line a calibrated cylinder of styrene monomer, with its inhibitor removed, was frozen in liquid nitrogen, the vacuum was then applied to the styrene, after which the liquid nitrogen bath was removed and the styrene allowed to melt. As it melted, any dissolved air boiled out of it. This procedure was repeated until no further air appeared to be evolved.

Once both vessels had been degassed, the flask with the gel in it was put in an ice bath and the styrene vessel in a water bath at 35°C. Taps A and C were closed and B opened (see Figure 12), and the styrene monomer allowed to distil into vessel 2 from vessel 1. Initially the transfer went very easily, but after about 5 cm^3 had been distilled, the styrene surface began to oligomerise. To overcome this a magnetic stirring rod in glass was left in the styrene vessel, and during the transfer stage this rod was used to agitate the surface of the styrene to enhance distillation. Finally, 22.6 cm^3 of styrene were transferred to the P.10 gel, which was designated TC.P.10 22.6.

The styrene loaded gel was then heated in an oven for 56 hours at 95°C. to thermally polymerise the styrene in the pores. The gel was then packed in the usual way and calibrated. When calibrated (see Figure 13), it was noticed that the pore size had increased, and not decreased as expected. This indicated that either the gel had not adsorbed the styrene, and that it had polymerised on the surface of the gel, or that the styrene had effectively increased the pore size of the gel by polymerising with the vinyl groups within the gel polymer. Heating did not affect the P.10 gel whatsoever, indicating that there are no vinyl groups available for thermal polymerisation.

To give further insight into the reaction, a further addition of styrene was performed, but in a suspension system, as follows:

Dissolve 20 gms. of polyvinylalcohol (Gelvatol) in 1200 cm^3 of deionised water and add 100 cm^3 of P.10 gel to it. While stirring at 800 r.p.m. at room temperature, 20 cm^3 of inhibitor-removed styrene monomer is added, and the temperature increased to 87°C. The reaction is left for seven hours. The gel is then filtered from the liquor, dried and packed; it is designated P.10.TC_s20.

The resulting column was calibrated as described previously, and it was found that the styrene had increased the permeability of the gel, but there was no evidence for it being polymerised on the surface of the gel, as the swelling ratio and packing properties of the gel were identical to the original P.10 prepared for this experiment.

6.4. Addition of divinyl benzene to the gel matrix.

20 gms. of polyvinylalcohol (Gelvatol) were dissolved in 1200 cm^3 of deionised water by heating the solution to 75°C. and stirring at 500 r.p.m.

100 cm^3 of P.10 (preparation $P.10.TC_8$) were then dispersed in the solution, and 1 cm^3 of divinyl benzene, with its inhibitor removed, was added. The reaction was heated to 89°C. for eight hours and stirred vigourously (700 r.p.m.) The gel ($P.10.TC_D1$) was then allowed to cool, filtered and dried. The resulting calibration can be seen in Figure 14.

Due to the remarkable decrease in permeability of the gel, a second addition of 1 cm^3 per 100 cm^3 of the gel prepared above was made. The pore size was reduced again ($P.10.TC_D2$), but not as much as with the first addition, so another 3 cm^3 of divinyl benzene were added to 100 cm^3 of the gel $P.10.TC_D2$. This gel, $P.10.TC_D5$, shows a much greater decrease in pore size, as can be seen in Figure 14.

To ensure that the decrease in pore size was not due to increased crosslinking density, the gel $P.10.TC_D5$ was resuspended in 1200 cm^3 of water containing 20 gms of polyvinylalcohol (Gelvatol), and 1 gm. of benzoyl peroxide in 10 cm^3 of toluene was added. The reaction vessel was heated for six hours at 90°C. to allow further crosslinking to take place. If none did, the divinylbenzene would be shown to have been grafted in the pore surfaces and crosslinked with itself, and hence would not affect the pore size. The gel was packed and calibrated, and no difference was found between this gel and the original $P.10.TC_D5$, verifying the fact that the divinyl benzene had polymerised in the pores of the P.10 gel.

6.5. Preparation of a double gel, $P.10.TC_A4/7$.

Gel $P.10.TC_A4/7$ is a stable P.10 matrix with gel P.4/7 (prepared initially by Tweedale[35]) in the pores.

Sufficient P.10 is prepared as described in Section 6.2., and then 100 cm^3 of the presized gel is resuspended in 1200 cm^3 of deionised water with 20 gms.

of polyvinylalcohol (Gelvatol) dissolved in it. A monomer mixture of 1.34 cm^3 styrene (6.7%), 6.66 cm^3 divinylbenzene (33.3%), 3.00 cm^3 toluene (15%) and 9,00 cm^3 of n-dodecane (45%) is prepared, but the toluene is kept separately as it has 0.15 gms. of recrystallised benzoyl peroxide dissolved in it.

The mixture is added to the reaction vessel, followed by the toluene with the initiator, the temperature being 85°C. and the suspension being stirred at 500 r.p.m. The reaction continues for seven hours, after which time the gel is filtered off, washed with methanol, hot water and finally cold water, and then packed into a column. The resulting calibration curve can be seen in Figure 15, but it is worth noting at this point that there is an exclusion limit at about 2 x 10^2 Angstroms, but this will be discussed later in the chapter.

6.6. Preparation of a low crosslinked gel stabilised by P.10.

Gel SP_2 is one developed by Patel,[120] which is highly swellable due to its low crosslinking density. It is prepared by polymerising, in suspension, a mixture of 45% styrene, 15% divinyl benzene, 22.5% toluene and 67.5% n-dodecane, the initiator being 0.005% benzoyl peroxide.

A suspension of 100 cm^3 gel P.10 was prepared, as described previously, and a mixture of total volume 2.0 cm^3 was prepared of the SP_2 reactants. The initiator (0.025 gms.) was dissolved in the toluene and added immediately after the remaining reactants had been added. The reaction was left for seven hours at 90°C., being stirred at 500 r.p.m., after which time the gel was filtered off, washed, refluxed in water to remove some polyvinylalcohol, washed again and dried. The gel was packed using the method recommended by Dawkins and Hemmings.[73] The calibration curve is shown in Figure 16, and it can be seen that a 2% (2 cm^3) addition of SP_2 makes little difference to the gel, so the volume was increased to 5% (5 cm^3); this did give a larger difference

with respect to the original P.10 gel, but it was hoped a bigger difference would be available, so the volume of grafted gel was increased to 15% (15 cm^3).

6.7. Preparation of a gel described by Altgelt and Moore,[102] stabilised by P.10.

In Section 5.4 the preparation of a gel similar to one produced by Altgelt and Moore[102] is described. This gel, however, is known to swell to over six times its dry volume in THF and has consequently not been used for a packing medium. The amounts of reagents needed to produce this gel are styrene 24.8%, divinyl benzene 2.5% and toluene 72.7%, all percentage weight.

A grafting of this gel was attempted by preparing 125 cm^3 of the P.10 sample in the fashion described in 6.2., and then adding a mixture of 13.78 cm^3 of styrene and 1.36 cm^3 of divinyl benzene, followed by 41.78 cm^3 of toluene with 0.25 gms. of benzoyl peroxide dissolved in it. The resultant gel was stirred at 500 r.p.m. for $6\frac{1}{2}$ hours at 85°C., when it was filtered, washed and packed. Figure 17 shows the comparison between the initial P.10 gel and the new P.10.TC.A+M.45 gel, and as can be seen, the previously highly swellable gel can now be characterised very easily.

6.8. Preparation of a xerogel stabilised by P.10.

In the previous chapter, various gel preparations were described, one of these being based on a patent by Frisque.[13] Attempts were made to graft these gels into the pores of a stable P.10 xerogel-aerogel hybrid. The chosen gel is one in the middle of the pore size range and is designated F/A 40:50; its preparation is comprised of suspension polymerising 20 gms. methylene NN' bisacrylamide with 20 gms acrylamide, all dissolved in 100 cm^3 of formaldehyde and suspended in 750 cm^3 of heptane with 10 cm^3 Tween 80, and 40 cm^3 Span 80 present as emulsifiers. The reaction is catalysed by 50% aqueous ammonium persulphate solution in a 1:5 ratio with the monomer mixture.

The initial attempt to graft this gel onto 100 cm^3 of P.10 was carried out by suspending 100 cm^3 of P.10 in 750 cm^3 of heptane which had been flushed with nitrogen and had 40 cm^3 of Span 80 dissolved in it. To this was added a solution of 2 gms. of methylene NN' bisacrylamide and 2 gms. of acrylamide dissolved in 10 cm^3 of formaldehyde and 2 cm^3 of Tween 80. The reaction was catalysed by 2 cm^3 of 50% ammonium persulphate solution. The reaction was kept at 50°C. for six hours and was stirred at 500 r.p.m. The resultant product showed no difference from the original gel, but it was noticed that some residue was left at the bottom of the reaction vessel, and it was assumed this was the acrylamide gel.

The second attempt (P10 TC_G F/A 40:50 H_2O 10) was prepared in 1200 cm^3 deionised water with 17 gms. of polyvinylalcohol (from Fisons) dissolved in it. The acrylamide gel mixture was of 2 gms methylene NN' bisacrylamide with 2 gms. of acrylamide dissolved in 10 cm^3 of formaldehyde with 2 cm^3 of Tween 80 present. The reaction was catalysed by 2 cm^3 of 50% aqueous ammonium persulphate solution. It was stirred at 500 r.p.m. for four hours at 88°C. The resultant gel was filtered, washed, packed and calibrated. When the polystyrene standards were eluted, it was noticed that they possessed a low molecular weight tail, as if they had been retarded in their elution. In order to verify that the standard had been adsorbed onto the gel, some amines were eluted, but they did not show a tailing effect, although they did exhibit possible retention by the gel. This was slight, however, so it was decided to increase the amount of acrylamide gel in the pores.

This was accomplished as described above, but with double the quantities of polymer mixture. This gel is designated P10 TC_G F/A 40:50 H_2O 20., and was prepared at 95°C. while being stirred at 500 r.p.m. for six hours. The resulting gel was treated as before and calibrated. Unfortunately, it did not exhibit the desired effect conclusively, so alternative preparations were considered.

Tweedale(35) recommends a styrene, divinyl benzene, diluent ratio of 1:5:9, so a similar preparation was used for attempted grafting. As before, 100 cm^3 of the P.10 gel was suspended in 1200 cm^3 of water with 17 gms. of polyvinylalcohol dissolved in it. To this cooled suspension was added 2.0 gms. of acrylamide and 2.0 gms. methylene NN' bisacrylamide dissolved in 10 cm^3 of formaldehyde and 8 cm^3 of isoamylalcohol. The solution was allowed to stir at 450 r.p.m. for one hour, after which the catalyst (0.5 gms. benzoyl peroxide in 10 cm^3 of diethyl benzene) was added, and the temperature raised to 95°C. for two hours. When the gel was calibrated, it was noticed that no grafting had occurred.

Another preparation was attempted. 170 cm^3 of gel were suspended in a solution of polyvinylalcohol (17 gms. in 1200 cm^3) and a mixture of 3.4 gms. methylene NN' bisacrylamide, 3.4 gms. acrylamide dissolved in 17 cm^3 of formaldehyde, and 13.4 cm^3 of isoamylalcohol was added at room temperature. A 50% aqueous solution of ammonium persulphate was then added, and the reaction allowed to take place at 70°C. for two hours, while being stirred at 250 r.p.m. This gel was designated P10 TC_J F/A 40:50: 10. When the gel was characterised and compared with the original P.10 gel, it was noticed that it had altered, but only drastically for molecules with an amine group present, although low molecular weight tailing with polystyrenes was observed. In order to compare them numerically, the elution volume for benzene in both gels were taken as the same, and adjustments made to the other elution volumes. See Table 11.

It was possible, however, that these results could have been due to a form of affinity chromatography, so solutions of the acrylamide and methylene NN' bisacrylamide were made up in dry methanol and allowed to elute through a "deacidite FF" (now known as "Zerolite FF") ion exchange column to remove any acid which may be present. The eluant was then evaporated down on a rotary evaporator, and the product crystallised from dry ethanol.

	Elution Volume P10 TC_J F/A 40:50 10 (counts)	Elution Volume P10 (counts)	Corrected Elution Volume P10 (counts)	Difference (counts)
Aniline	5.15	5.425	5.125	0.025
Diphenylamine	5.08	5.25	4.95	0.13
1,2,Diaminoethane	6.59	5.39	5.09	1.50
o-aminophenol	5.12	-	-	-
Dry NH_3	6.44	-	-	-
.880 NH_3	6.76	-	-	-
Dianisidine	5.13	5.21	4.91	0.22
Benzene	5.18	5.48	5.18	0.00

TABLE 11.

The resulting products were used to prepare a repeat of P10 TC_J F/A 40:50 10, and it was found that there was very little difference between the two, the difference being within experimental error.

6.9. Some experiments with the P10/acrylamide gel.

A short series of characterisation experiments were carried out to obtain a better concept of the properties of the new gel. The gel was characterised using polystyrene standards, but a series of amino compounds (0.25% soln. in THF) were also used, as shown in Table 11. As can be seen from the calibration curve (Figure 18), the polystyrene characterisation is consistent with the usual type of curve, but when the amine compounds are used, there is a definite delay of elution, indicating sample/gel interaction. This is not found with untreated polystyrene gel, so it is not a solvent effect.

The gel (P10 TC_J F/A 40:50 10) was also calibrated using polyethylene glycol, as shown in Figure 18. It is of interest to note that there appears to

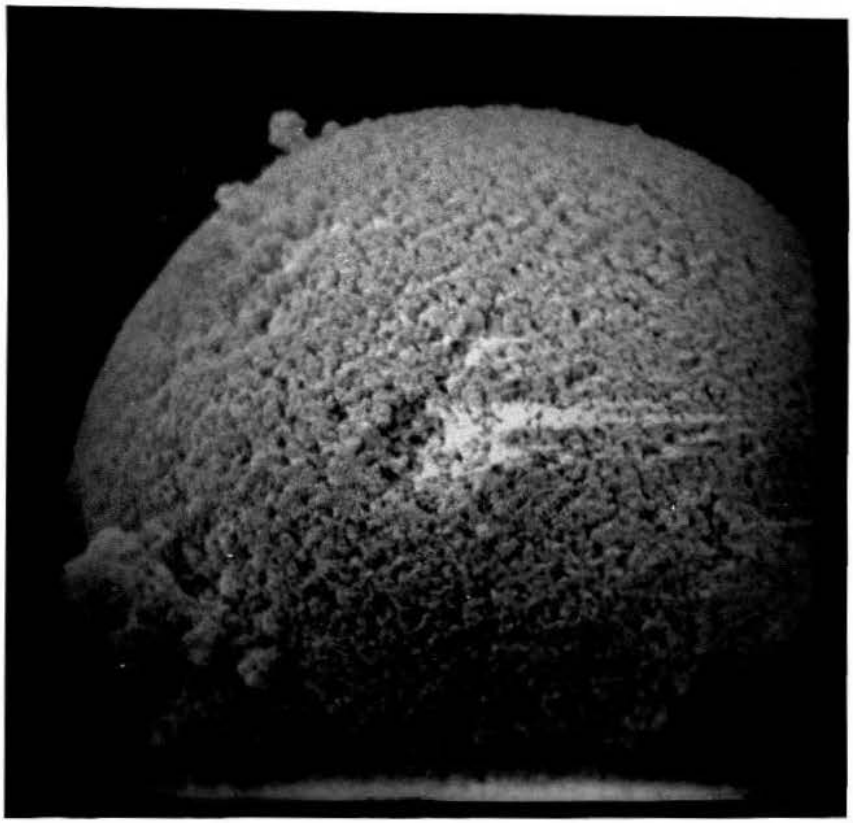
be an exclusion limit of the glycol at about molecular weight 10^3, but at higher molecular weights, further separation appears to start to take place again. This can be explained by the polymer being characterised primarily by the P.10 pores, and any polymer of small enough size is then recharacterised by the acrylamide gel. Again, no similar effects have been observed with untreated P.10 gel, or any other similar gel.

When three acids were injected, benzoic, succinic and phthallic, it was found that only benzoic acid eluted, and this elution was greater than would be expected (9.84 counts; benzene eluted at 9.80). The other two acids remained on the gel, probably because they reacted with the amide groups in the acrylamide gel, and benzoic acid does not have enough acid strength to react sufficiently for total retention to take place.

6.10. Results.

It has been shown that the concept of grafting one gel into the pores of another one is sound, and that interesting results can be obtained by doing so. It has also been shown that the pore size of the gel alters as the grafting takes place. This can be seen by comparing the photographs taken by a scanning electron microscope in Figure 19, and Figures 21 to 25. A comparison of pore sizes can also be made, as in Table 12.

All pore sizes are based on calibration by monodispersed polystyrene and linear hydrocarbons in THF, unless stated otherwise. It is difficult to compare the pore size of the gel before it is grafted onto P.10, because most of the gels have not been characterised due to their swelling capacity, but those which have are shown in Table 13.



2μ.

FIG. 19

SCANNING ELECTRON MICROGRAPH OF GEL P10 TC_S (mag. 5,500 x)

Gel	Pore size (Angstroms)	Gel	Pore size (Angstroms)
P10	Very high.	P10 TC_A 4/7	5×10^4 and 2×10^2
P10 TC 22.6	Very high.	P10 TC_B $SP_2$2	High.
P10 TC_S 20	Very high.	P10 TC_B $SP_2$5	High.
P10 TC_D 1	ca.10^6	P10 TC_B $SP_2$15	ca.10^6
P10 TC_D 2	2×10^4	P10 TC A+M 45	1.5×10^4
P10 TC_D 5	3×10^3	P10 TC_J F/A 40:50 10	High and 3×10^2 †

† as determined by polyethylene glycol calibration.

TABLE 12.

Gel	Pore size (Angstroms)	Size when grafted (Angstroms)
P4/7	2×10^3	2×10^2
F/A 40:50	10^4	3×10^2

TABLE 13.

It can be seen that usually the pore size has apparently decreased when grafting has occurred. This could be due to the unreacted vinyl groups from divinyl benzene partially crosslinking with the gel, and increasing the crosslinking ratio and hence decreasing the pore size. This indicates that there is more divinyl benzene crosslinking agent present than the original ratio would suggest, so the unreacted vinyl groups must still be active prior to grafting a new gel onto the P.10.

In Figure 15 (P10 TC_A 4/7) and Figure 18 (P10 TC_J F/A 40:50 10), an exclusion limit appears in the middle of the calibration curve; this is due to the presence of two gels of differing pore size, one having a much lower

exclusion limit than the other, but the former not completely filling the latter's pores. To ensure that the shape is not due to a heterogenous mixture, the theoretical curve for a mixture of P.10 and P4/7 was drawn. The gels were in the same proportion to accentuate the trend, and it can be seen in Figure 20 that the mixture shows an increase in expected elution volume for low molecular weight species, while the grafted gel (which is only 2% P4/7) shows a decrease in the elution volume, but the gradient of the calibration is less, hence better separation will be obtained. This shows conclusively that the grafted gel is not a mixture, or that the P4/7 or any other gel, has blocked the pores on the surface, for if it had, the characteristics of gel P.10 would be masked.

The initial studies with styrene indicate that either the crosslinking density is reduced with more styrene, or that the polymerised styrene can act as a gel of even higher porosity than the P.10. The styrene molecule is unable to crosslink with itself unless it is on the end of a chain, so either the chains undergo end to end cyclic polymerisation to form loops, or the chains are interwoven and swell in the presence of solvent to act as a filter. On the other hand, the styrene could be partially crosslinked by reaction with the vinyl groups of the partially unreacted divinyl benzene molecules, and since the styrene would probably be in excess, the co-polymer would be highly swellable. It is most likely that the latter occurs, because there is no evidence of free polystyrene being present, as some of this would dissolve in the solvent and be eluted out of the gel, and would be shown as peaks on the chromatogram.

The previously described preparation and evaluation of polydivinyl benzene indicates that the polymeric form is not soluble in THF, and that it is relatively highly crosslinked (swelling ratio 1.43:1) in comparison with other gels. The addition of 1% by volume to P.10 gel gave a substantial

change in the chromatographic properties of the gel, and if 5% is added, a completely new calibration curve results. This, however, does not give a great insight into the method by which the grafted gel is retained in the matrix; it could be either attached to the matrix by the vacant vinyl groups, or be totally separate but trapped in the pores by its size. However, taken in conjunction with the styrene results, it appears that the former is more probable, because on heating, the divinyl benzene product does not alter in character, indicating little thermal polymerisation, which would also support the hypothesis of the styrene being retained by the unreacted vinyl groups.

The success of being able to graft the SP_2 gel into some P.10 gel showed that even highly swellable gels could be used for such a system. Although the calibrations for the grafted SP_2 gel were not strikingly different from the original P.10, it was shown that some change was effected. The reason for this is possibly that the gel is so lightly crosslinked that it acts as a gel filter, as sephadex (crosslinked dextran) appears to, and the pore size is large enough to show negligible effect on the elution characteristics of the new gel.

However, the ease at which highly swellable gels can be grafted is illustrated by the preparation of P10 TC A+M 45 (Section 6.7)[102] and Figure 17 shows the calibration curve for it. In both cases, the swellable gels, once grafted onto the P.10 matrix, have been as easy to pack successfully as the original P.10 gel was, and the efficiency of the columns have been about the same, as shown in Table 14.

The efficiency of packing appears to improve slightly in some of the cases, but this is due to the fact that when the grafted gel was prepared, it was ensured that all the particles were as near to the same size as possible, consequently the standard P.10 calibration gel was a mixture of sizes, and hence less efficient.

Gel	P10 TC_D	P10 TC_D5	P10 TC_A	P10 $TC_A4/7$	P10 TC	P10 TC $SP_2$2
Plate count per foot. (per metre)	380 (1164)	540 (1654)	420 (1300)	500 (1550)	210 (650)	250 (775)

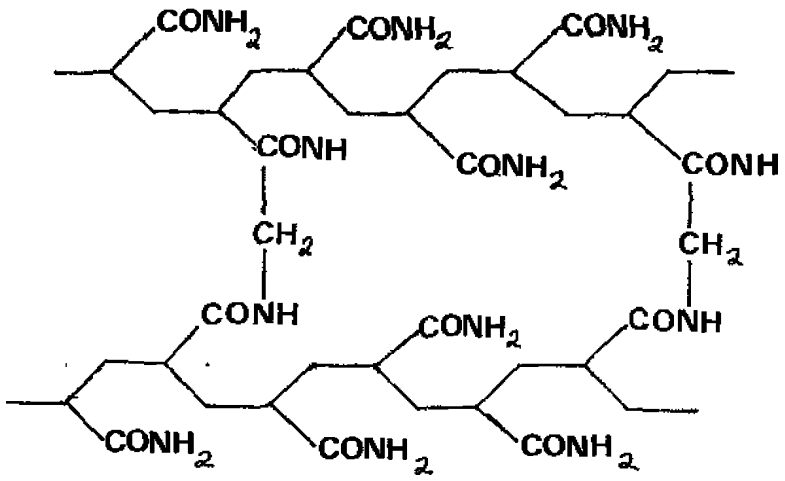
Gel	P10 TC $SP_2$15	P10 TC_S10	P10 TC_S20	P10 TC_S20 DVB	P10 TC	P10 TC A+M 45
Plate count per foot. (per metre)	200 (620)	400 (1240)	350 (1085)	210 (650)	320 (995)	270 (835)

TABLE 14.

6.11. Discussion.

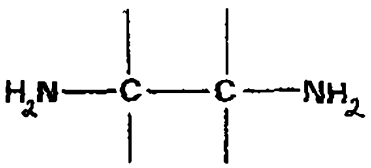
As already stated in Section 6.9., evidence has been found for a retention of certain molecules beyond the elution volume of the column system. For this to occur, there must be a sample gel interaction taking place, probably in the form of hydrogen bonding. Brook and Munday[104] have discussed such a hypothesis with regard to phenols on sephadex gels, and postulate three possible mechanisms, all of which involve hydrogen bonding, but at different sites. They also observed that similar effects are found with other groups, such as amino and carboxylic acid groups, when halogen derivatives were studied, it was found that adsorbtion was stronger than predicted by their adaptation of the Hammett equation because the halogen derivative is apparently adsorbed by the dextran chain. Jandera and Churacek[105] have verified various theoretical equations for elution characteristics in adsorption chromatography on silica gel, using four NN dimethyl-p-amino benzene azobenzoyl amides.

The work described in this chapter is related to the properties of a crosslinked polyacrylamide gel, the structure of which is shown below:

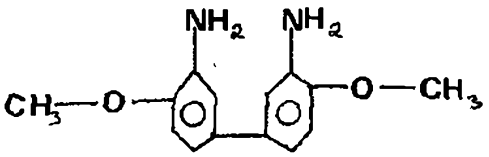


A CROSSLINKED POLYACRYLAMIDE - B10 GEL P. (106)

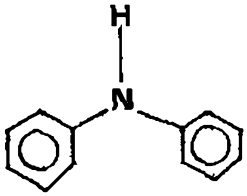
As can be seen, there are a large number of amide groups along the chain, all of which are, theoretically, capable of forming hydrogen bonds with a suitable eluting polymer. If this occurs, the elution volume will be increased, as has been shown. The samples which had greatest retention were 1,2 diaminoethane (1), dianisidine (2), and diphenylamine (3), although water and 0.880 ammonia solution also exhibited retention. The structures are shown below:



(1)



(2)



(3)

It can be seen that (1) and (2) will have quite active amino groups which can interfere with the groups on the polyacrylamide, if the amine can get inside the matrix. Similar effects have been noticed with o-aminophenol and aniline, both of which have active amino groups which will deactivate the benzene ring; the hydroxyl group in o-aminophenol will also deactivate the ring, so it would be expected that o-aminophenol will be retained more than aniline, but this is not shown on the calibration curve (Figure 18) due to the fact that the molecular volume is not used, but a measure of molecular length is, as a calibration parameter.

The elution characteristics of ethylene glycols also exhibit a retaining effect, especially at the lower molecular weights (Figure 12). The maximum molecular weight for polyethylene glycol calibration is about 5×10^4, because above this value it becomes insoluble in THF at room temperature, but there is evidence for retention even of the higher polymers. This is not expected, as the polymer has a structure:

$$HO-\left(CH_2-CH_2-O-\right)_n H$$

where n is an integer.

Hence, the hydroxyl groups can be quite a distance apart in the larger molecules, which may have difficulty in permeating the matrix, and even if they do, the active hydroxyl sites are at opposite ends of the molecule. However, in spite of this restriction, retention does take place, so it can be hypothesised that the retention is due to the amide groups on the gel matrix interacting with the oxygen in the repeat unit of the polymer chain. When ethane diol is eluted through, the peak is nearly one count (5 cm^3) overdue by the polystyrene characterisation; this is probably due to the size of the molecule, and the presence of two hydroxyl groups which are able to be attracted to the gel matrix. It is also worthy of note that both dianisidine and

acrylamide fit onto the polyethylene glycol curve. The Q factor for the glycols is taken as 15, although experimentally it has been found to be 13.6, contrary to Afifi Effat's value of 17.5.[107]

On elution of acids through the column, it was found that benzoic acid (pka 4.19)* did not elute through, but at a retained volume equivalent to the elution volume of a molecule smaller than benzene, while phthallic acid (pka 5.51)* and succinic acid (pka 5.61)* did not elute through at all. There is no detectable sign of a peak on the trace which indicates that the acids are retained on the column, but have not reacted in a way so as to give a salt with the elimination of water, as this does give a response on the chromatograph. Hence, an ionic complex may have been produced by proton donation by the acid to the amide group on the gel. This would not lead to elimination of water, so would not be detected except by lack of a peak, indicating that the acid is still in the column.

6.12. Conclusions.

It has been shown that the polyacrylamide gel prepared in Section 6.8. has the potential to retain polymers of certain molecular structures. The production of such a gel also shows that a stable matrix of an aerogel-xerogel hybrid type, is capable of stabilising a highly swellable gel, which previously could not be used in pressure permeation chromatography due to its softness. The presence of unreacted vinyl groups on the stabilising matrix, however, alters the pore size of the grafted gel, indicating that the grafted gel becomes an intricate part of the matrix.

Further studies of this type of gel should be pursued, and it may be possible to attach a heavy metal to the xerogel, either in the form of an ester, such as uranyl acetate, or in the form of an acid, such as osmic acid, and use the

* in aqueous soln.

heavy metal as a detection system to see, by using either a transmission or scanning electron microscope, where the attachment actually take place in the matrix.

CHAPTER 7.

SOME STUDIES WITH THE SCANNING ELECTRON MICROSCOPE.

7.1. Introduction.

The scanning electron microscope is an instrument which can be used for examination of the surfaces of samples at very high magnifications. Typical magnifications used in this study were in the region of 20,000 times, but on occasions, magnifications of 80,000 times were obtained, although higher (100,000) is possible.

As the name implies, the scanning electron microscope (S.E.M.) is an electron microscope which scans the surface of an object. The electrons are produced from a filament with 10 kv. voltage across it, although it can operate at 20 kv.; the higher the voltage, the greater the resolution available, but the higher energy the electrons. If the energy of the electrons is too high, or if the sample is not prepared properly, the electron beam can burn the sample and either totally destroy it or scar the surface. If the surface is scarred, the topology of that area will alter due to the intense localised energy. The electron beam is focussed by a series of electromagnets, and the beam then strikes the sample surface. The sample is mounted at an angle of 45° to the direction of the beam flow, and the electrons are reflected into a detector, which in turn converts the beam to a visual display on a cathode ray tube. A photograph can then be taken of the display.

On some scanning electron microscopes, it is possible to use x-ray detection methods to determine the distribution of a metal in a sample. This facility, however, was not used in the ensuing work, but it could be used if a heavy metal is impregnated into the polymer matrix, as suggested in the previous chapter.

7.2. Preparation of samples.

The samples studies were gels produced as described earlier, and samples

of freeze dried polystyrene, which was a digression initiated by studies on polydivinyl benzene gels.

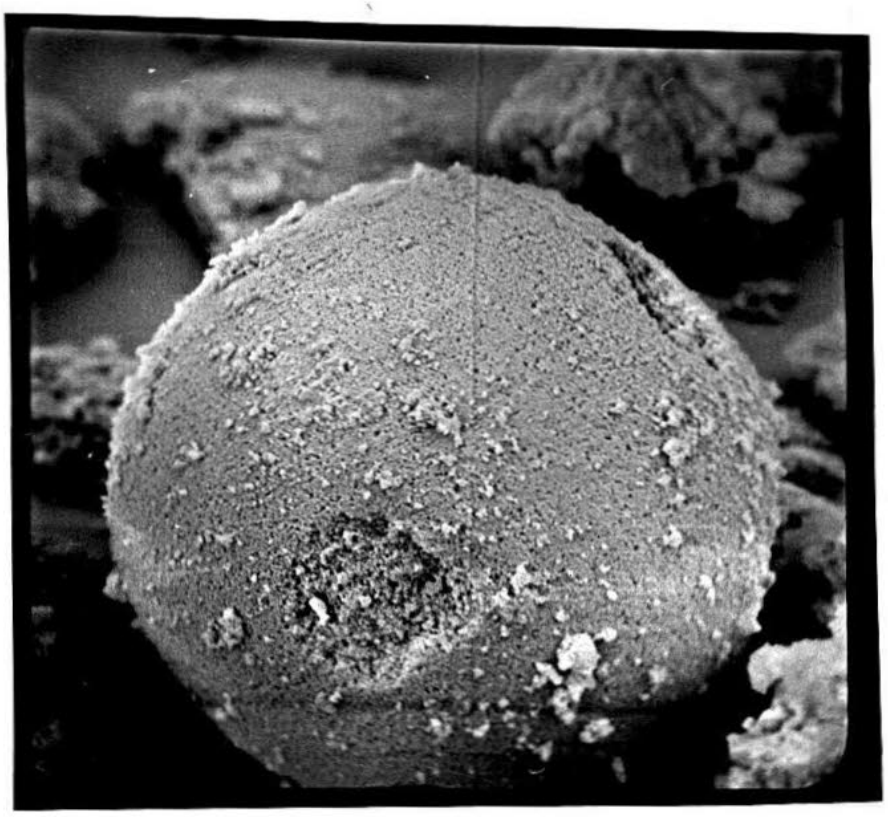
The sample is mounted onto a "stub" by means of double faced sellotape. The stub is an aluminium mounting which resembles a flat headed rivet. The mounted sample, which has to be dry, is then put into an argon atmosphere at reduced pressure and the whole mounting surface is sputtered with pure gold for four minutes at 40 M.A. current. This is sufficient to coat the sample with 400 Angstroms of gold from which the electron beam is deflected. The gold not only protects the sample from the electron beam, but it also acts as a heat sink to disperse the energy. If the gold is not thick enough the sample will "charge", and ultimately be burnt. When the sample charges, it is due to the surface becoming electrostatically charged, and the detectors cannot resolve the image, so the picture becomes very streaky. If the charging is excessive, the image shows a "herring-bone" effect and resolution of the image is impossible.

If the gold layer is too thick, the resolution is impaired due to the gold masking the contours of the surface. Hence, it can be seen that the thickness of the gold covering is critical for high standard, highly resolved photographs.

Once the sample has been coated, the stub is inserted into the base of the microscope, and the visual display is focussed. A photograph is taken of the display when required, using Ilford HP.4 film.

7.3. Some S.E.M. photographs.

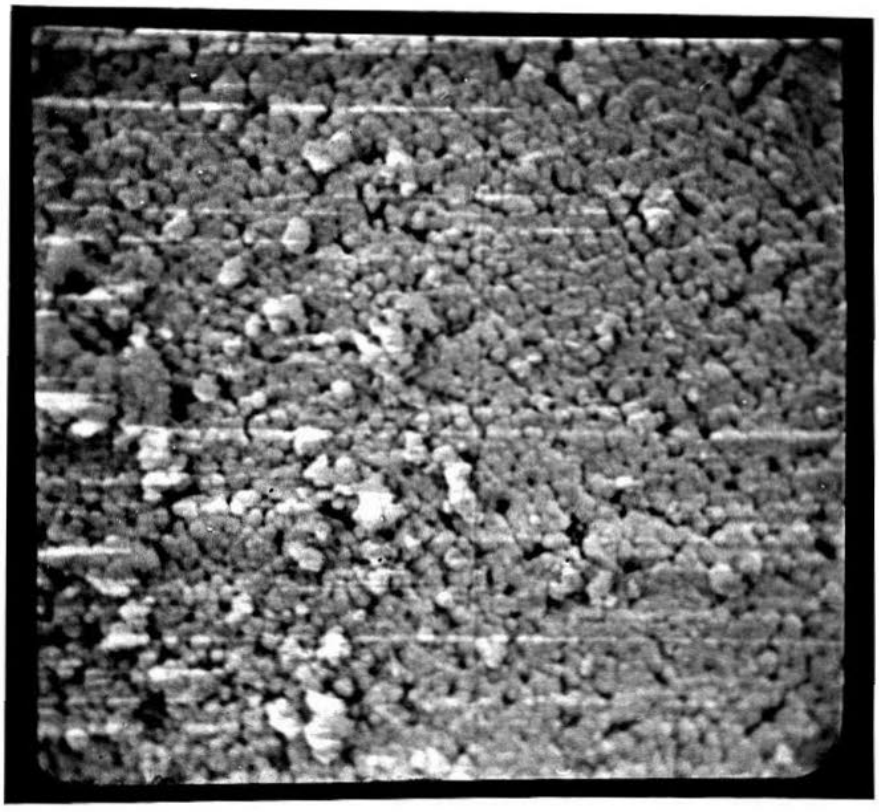
The following pages show photographs, at various magnifications, of gels described in Chapters 5 and 6, taken on the Cambridge Mk.II or S.4 scanning electron microscope.



GEL P10 (1,200 x)

10μ

FIG. 21

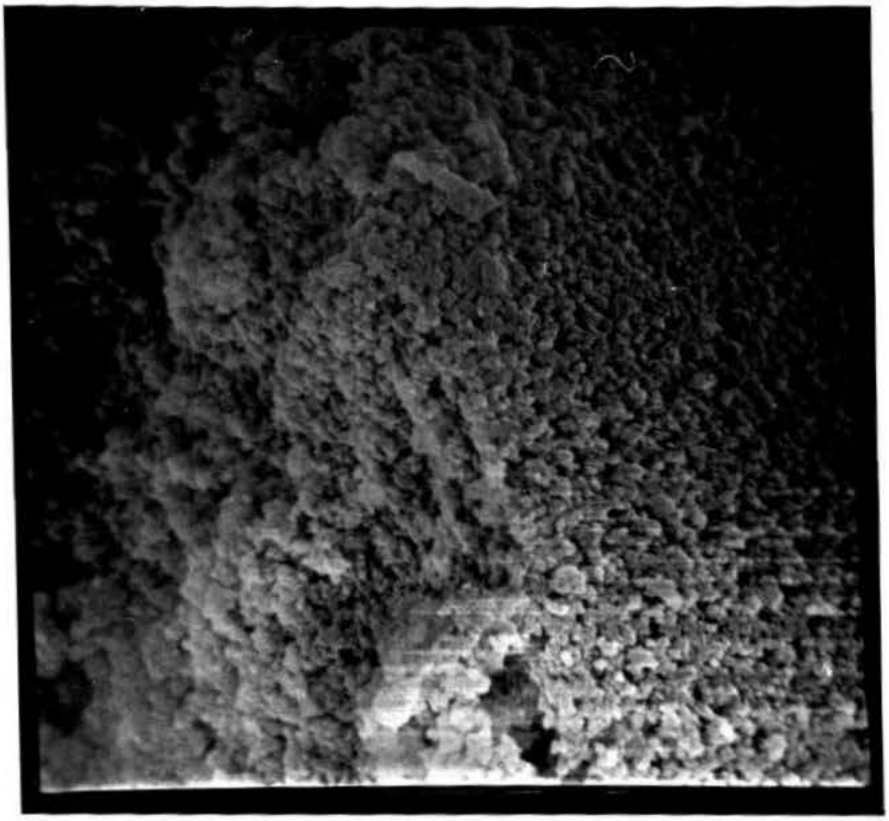


GEL P10 (6,000 x)

2μ

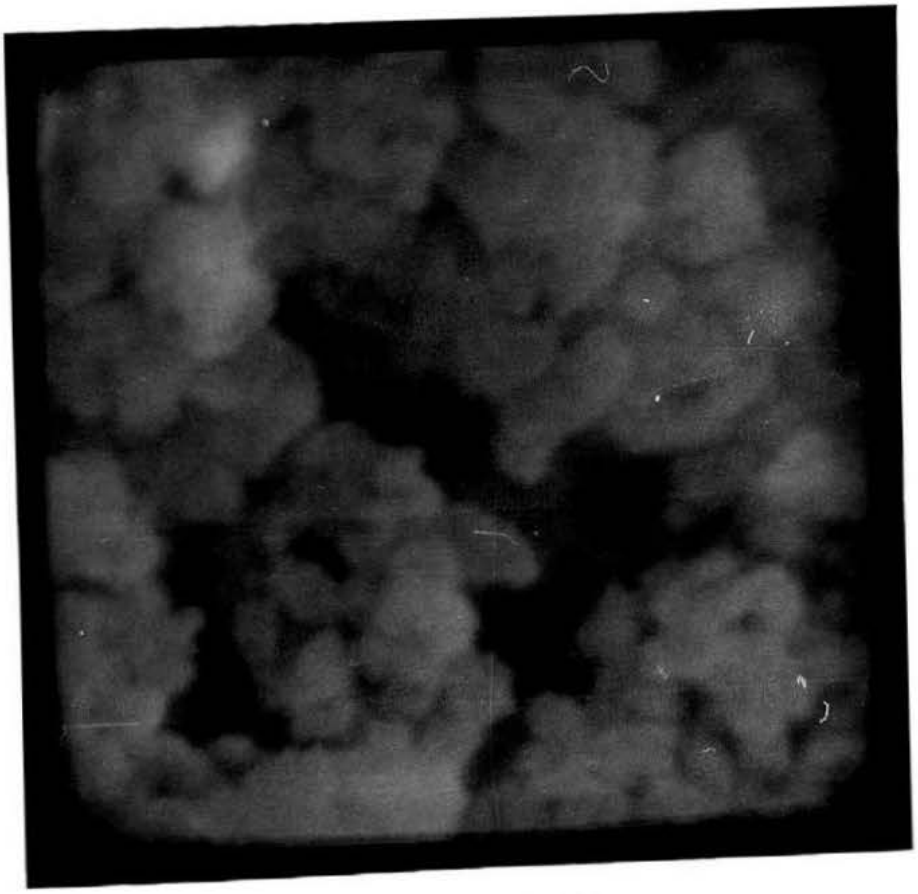
FIG. 22

GEL P10 (6,000 x)



2μ

FIG.23



GEL P10 (52,000 x)

0·5μ

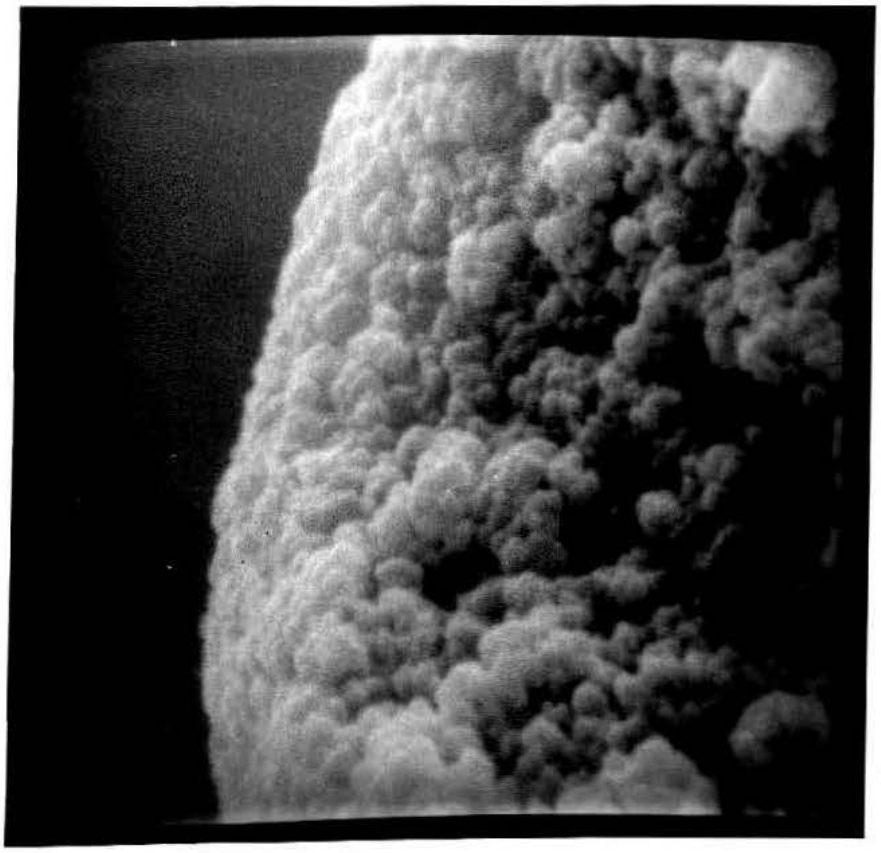
FIG.24

GEL P10 TC_S (2,250 x)

5μ

FIG. 25

GEL P10 TC_S20 (20,000 x)



0·5μ

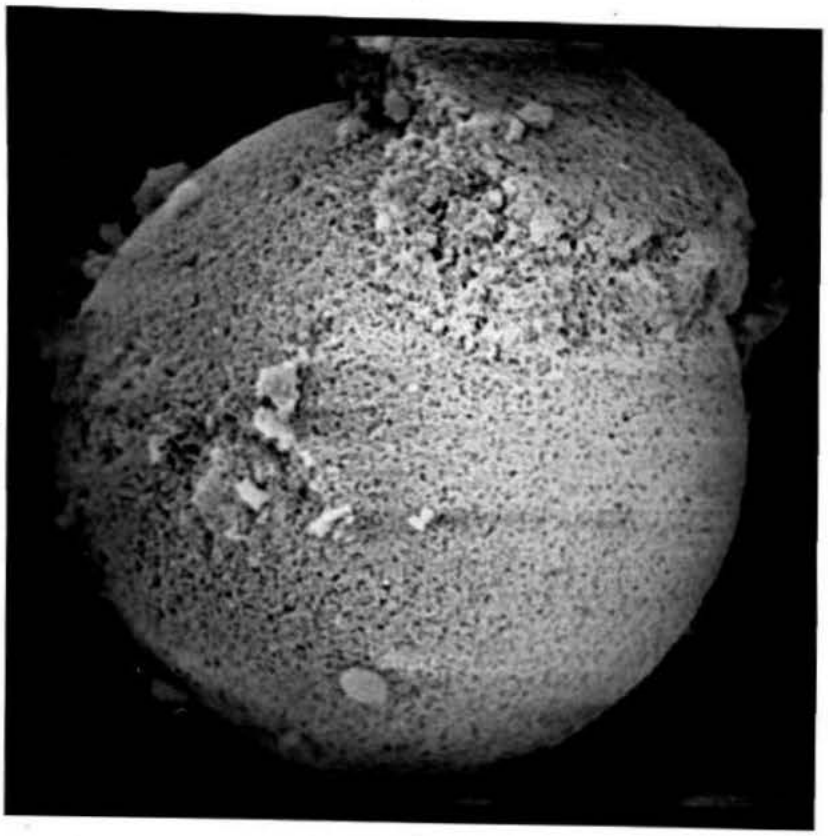
FIG.26

Figure 21 shows a typical sphere of gel P.10. The large pores of the gel appear as small black dots on the surface, but Figures 22, 23 and 24 show the pores better. Two large "holes" can be seen on the surface of Figure 21; these are due to the packing of the gel, as when the gel is packed the spheres are thrown together and tend to chip each other. As can be seen, the internal structure of the gel is not the same as the external, as is shown in Figure 36. Figure 22 shows the pores at a higher magnification, it can be seen that they are not all the same size, and in fact range from sizes of nearly 1μ across to very small pores of tens of angstroms. The streaking is due to charging of the sample. Figure 23 shows a broken sphere; the left hand side is the surface with the pores, and the right hand side is the internal structure. It should be noted that the internal pore structure of the gel is not as large as the external, but it still possesses the permeable structure of the surface. Figure 24 shows the surface of the gel when magnified 52,500 times. The composition of the surface shows that it is a number of small spheres of the gel polymer bound together.

Figure 25 shows a particle of styrene added to P.10 gel (P10 TC_S) Notice should be drawn to the fact the pores are still visible, and also that a piece has been removed from the surface due to packing. The residue on the lower left hand side of the sphere is surface debris. Figure 26 shows the edge of a hole in the surface of the gel, magnified 20,000 times. It is very obvious in this picture that the sphere is composed of a number of small spheres. Two pores can be seen just below the centre of the photograph, although it is difficult to see others due to the angle at which the photograph is taken.

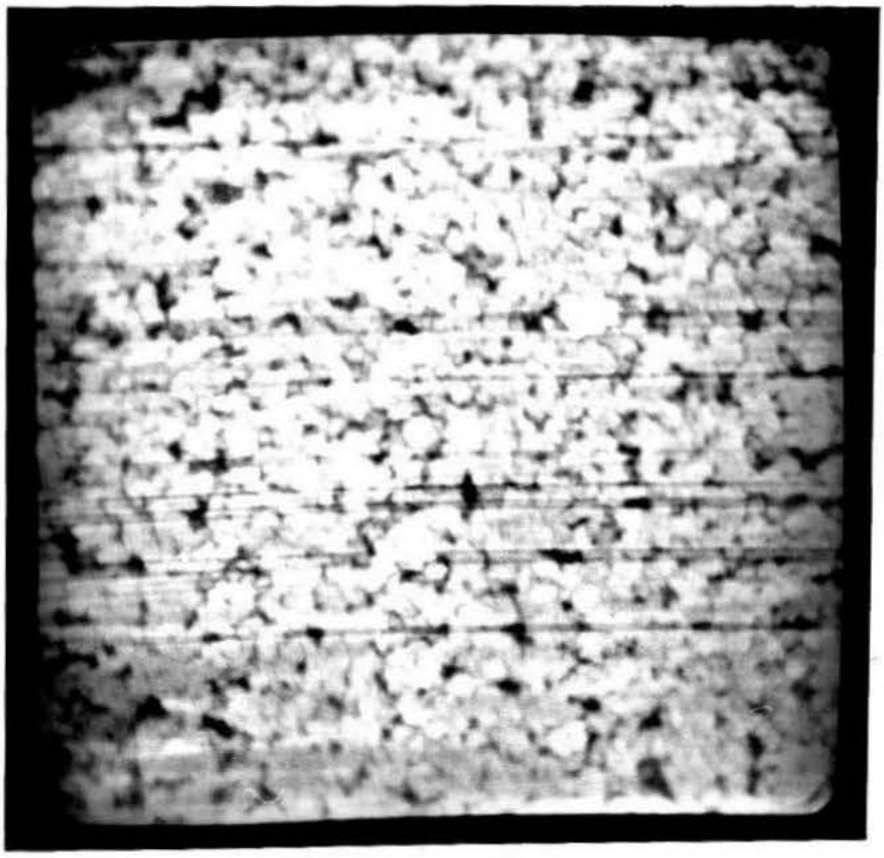
Figure 27 shows a particle magnified approximately the same as Figure 25. Although the particle sizes are different, the pore sizes are very similar on the surface. Figure 28 also shows the surface pores when magnified 9,000 times.

GEL P10 TC_{DVB} (1,800 x)



5μ

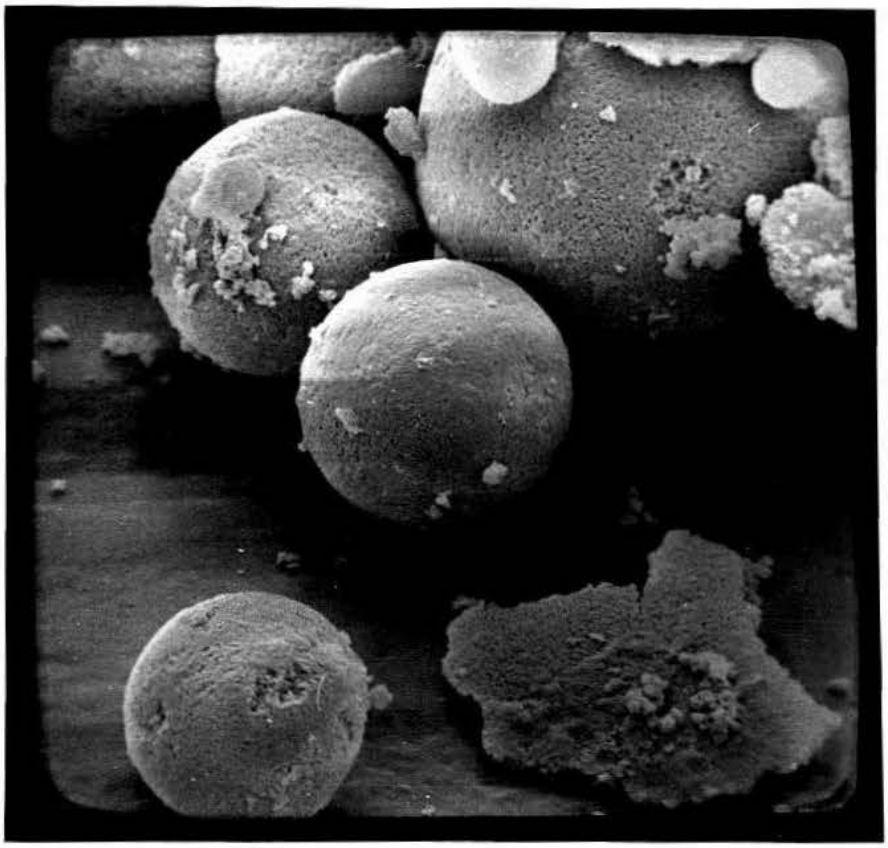
FIG. 27



GEL P10 TC_{DVB} (9,000 x)

1μ

FIG. 28



GEL P10 TC_A 4/7 (2,200 x)

5μ

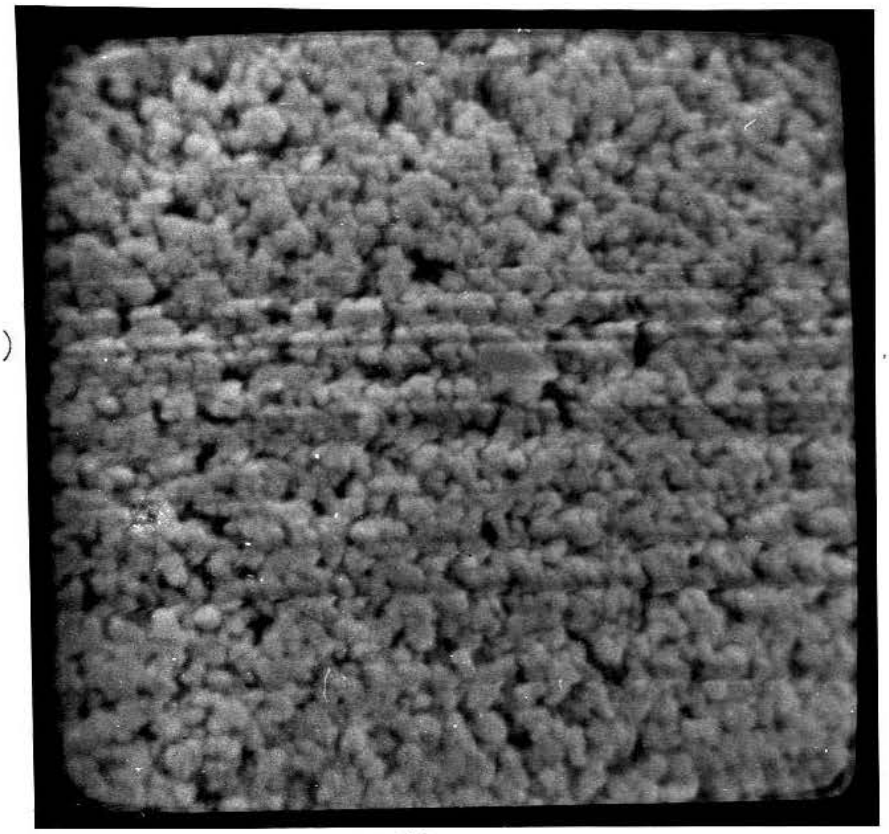
FIG. 29

GEL P10 TC_A 4/7 (11,000 x)

1μ

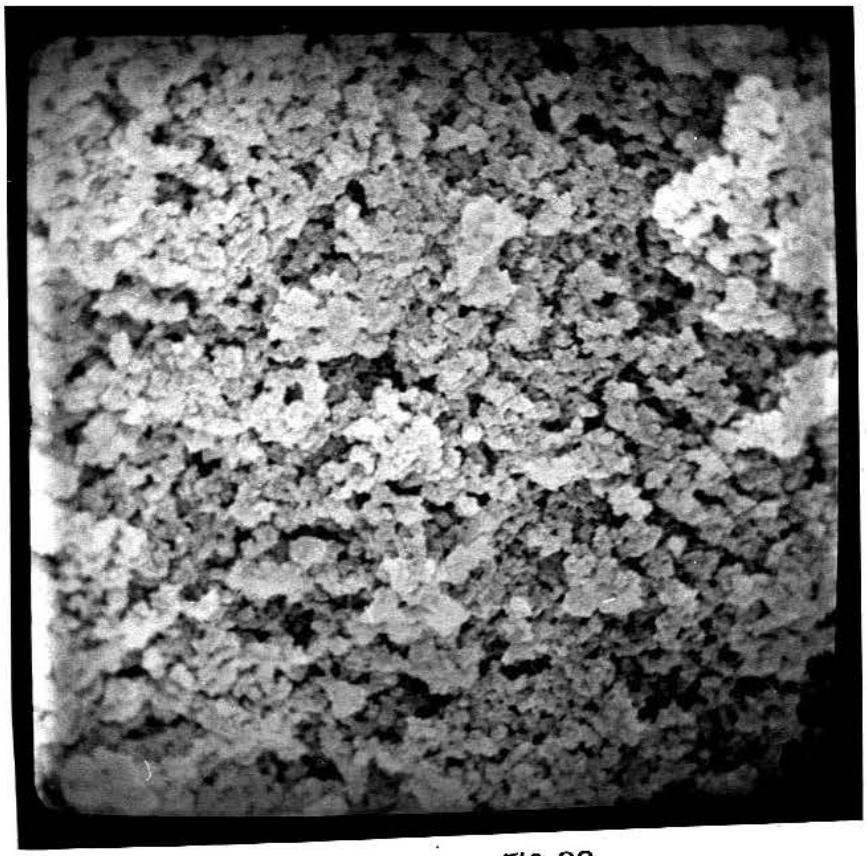
FIG. 30

GEL P10 TC_A 4/7 (22,000 x)



0.5μ

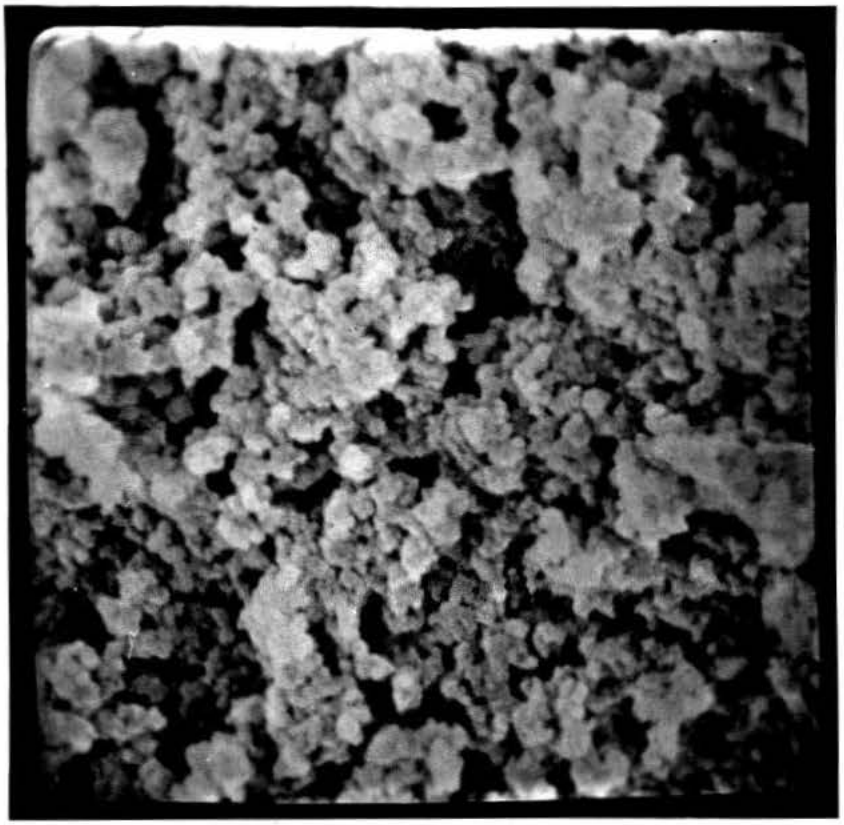
FIG.31



GEL P10 TC_D SP_2 15 (5,000 x)

2μ

FIG.32

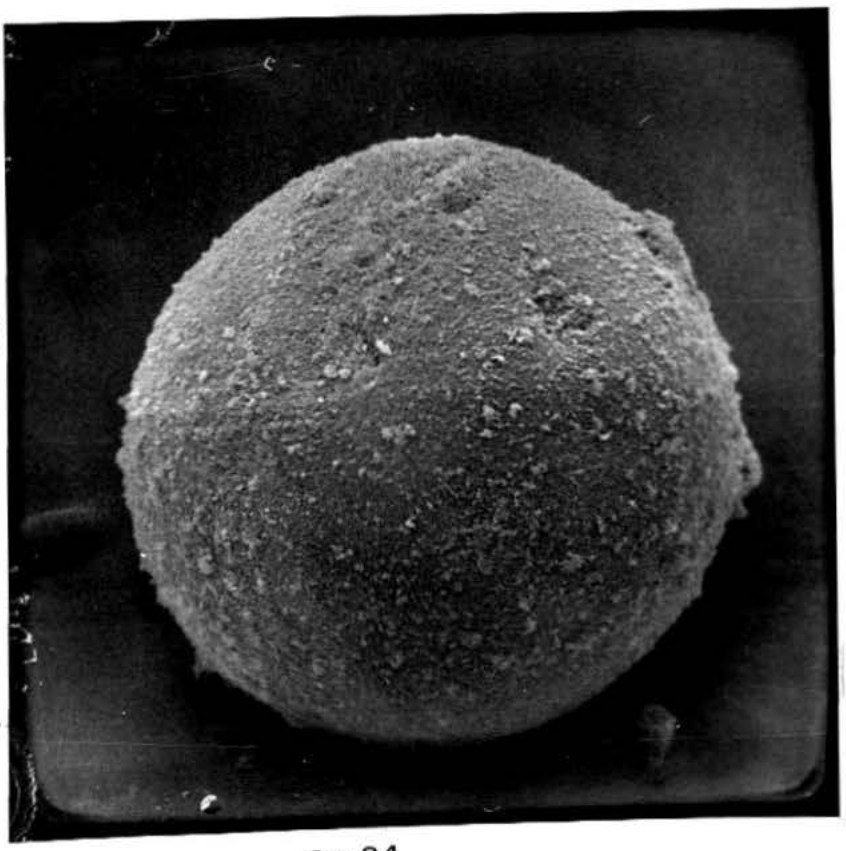


GEL P10 TC_D SP_2 15 (10,000 x)

1μ

FIG.33

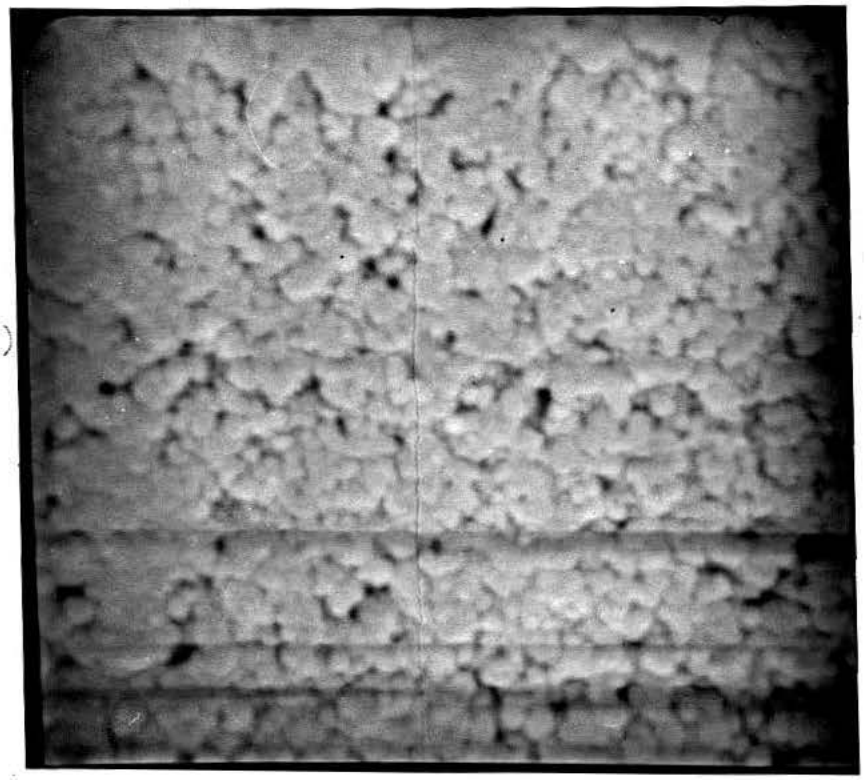
GEL P10 TC_F A+M 45 (1,100 x)



10μ

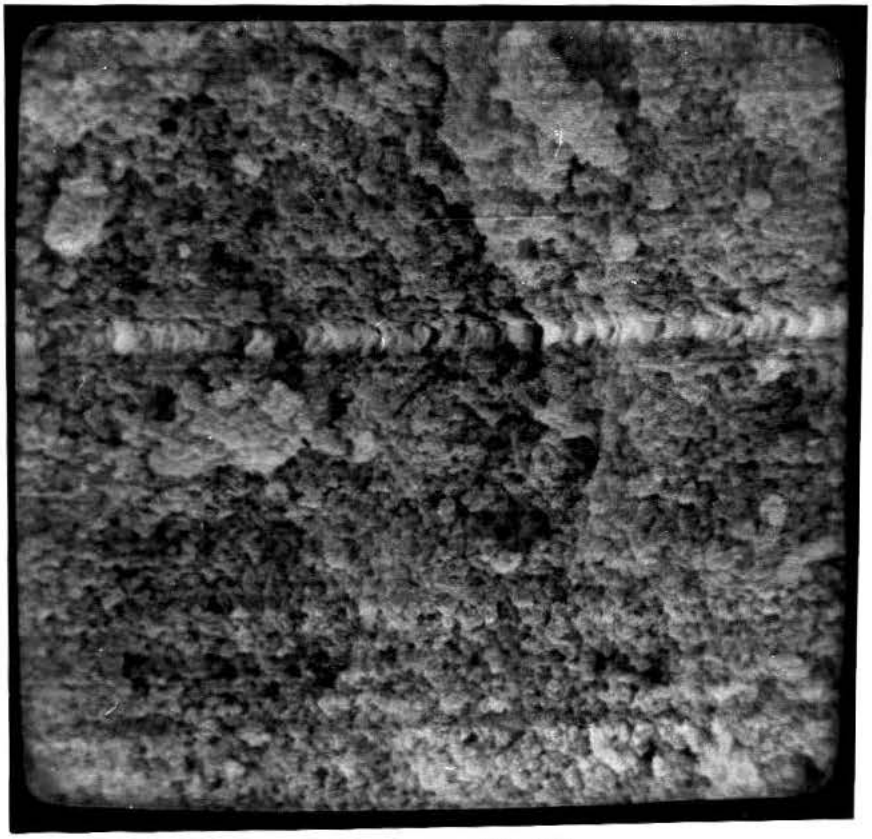
FIG.34

GEL P10 TC_F A+M 45 (11,000 x)



1μ

FIG.35



GEL P10 TC_F A+M 45 (5,7000 x)

2μ

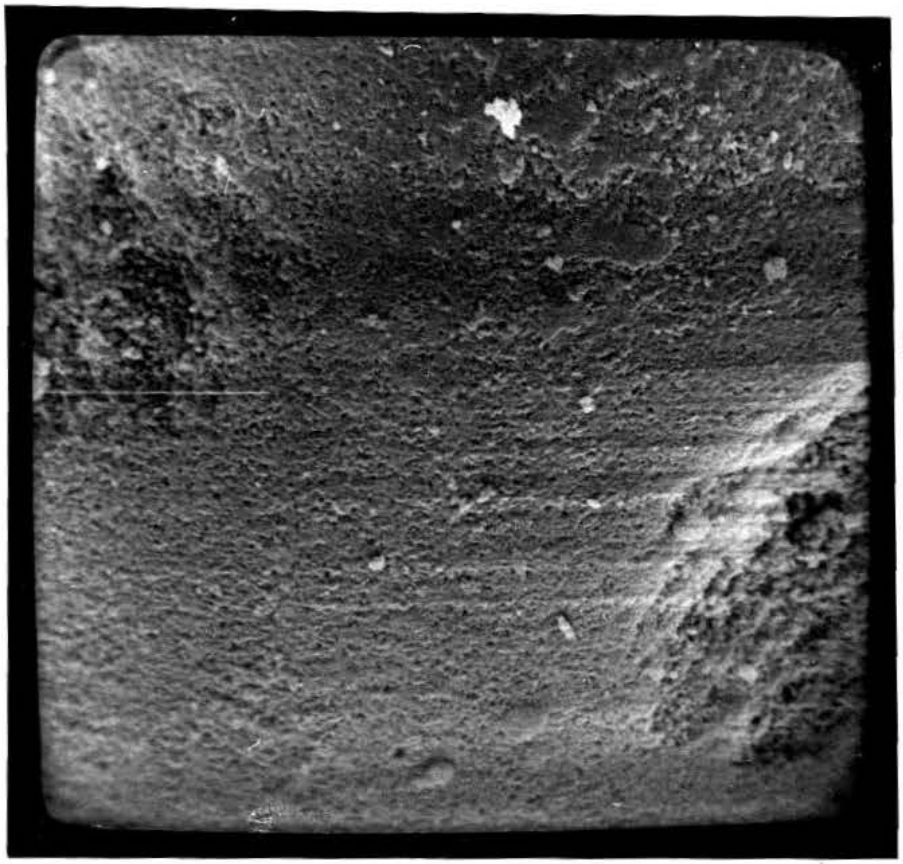
FIG.36

Even though the gel has had divinyl benzene added and polymerised in it, the pore structures still appear the same as in Figure 22.

Figure 29 shows various spheres of P10 TC_A 4/7. The surface concentration of the pores appears to have decreased at low magnifications, but when magnified 11,000 times, the familiar "orange peel" texture reappears, as shown in Figure 30. Figure 31 shows the surface of the gel magnified 22,000 times, and the gel spheres are obvious. It will be noted that the sample is beginning to charge, and that a herringbone effect can be seen.

Figures 32 and 33 show the surface of P10 TC_D $SP_2$15 (see 6.6.), but the resolution is not very good due to slight over-exposure of the film. However, the large pores are still visible, but they appear to be larger and more granular than other photographs of the same magnification. This, however, is probably due to the increased contrast accentuating the light differences as the pores have actually decreased in size.

Figure 34 shows a typical sphere of gel P10 TC A+M 45. The surface is coated in superficial debris and slight dents in the surface are visible. By magnifying the gel 11,000 times (Figure 35) the pores are clearly visible, but appear to be smaller than in the corresponding P10 photographs. The surface also appears smoother, but this is probably due to lack of resolution. Figure 36 shows the edge of a hold made by the packing process; the surface of the gel is on the left hand side and the hole is the remainder of the photograph. The material in the gel appears much finer than the surface material, as observed previously (Figures 23 and 26). The sample is also beginning to charge in Figure 36, as shown by the brighter band across the picture. Figures 37 and 38 show the gel surface at 2,200 and 32,000 magnifications respectively, and shows convincingly that the gel is comprised of a number of little spheres held together in such a fashion as to make the matrix porous.

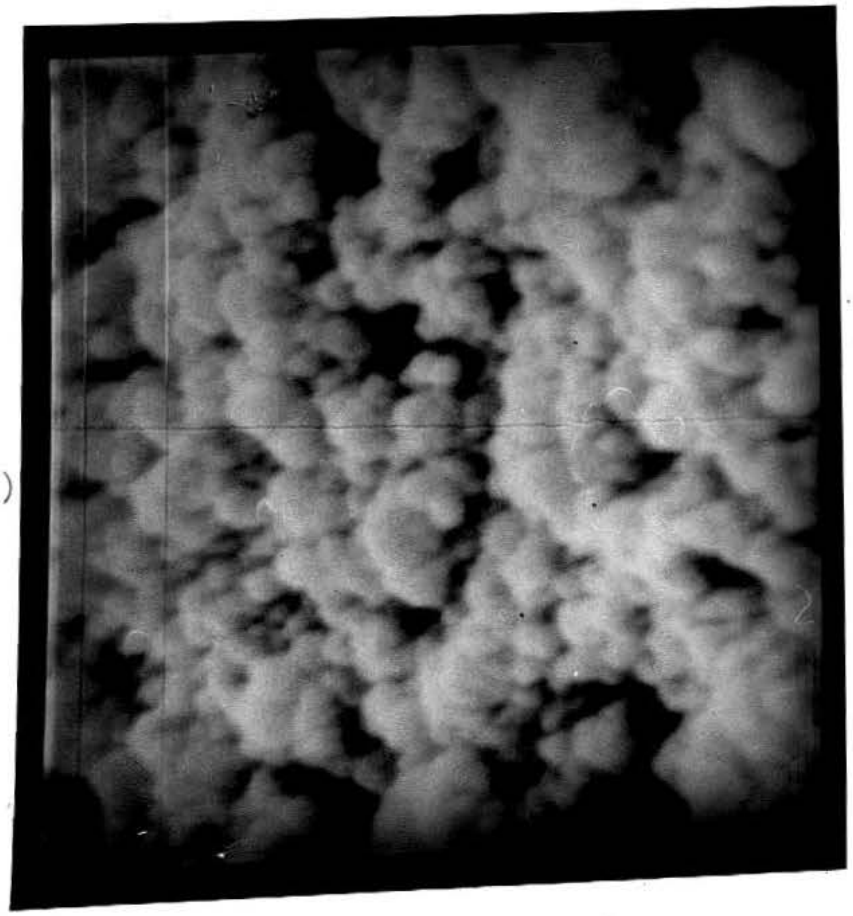


GEL P10 TC_F A+M 45 (2,200 x)

2μ

FIG. 37

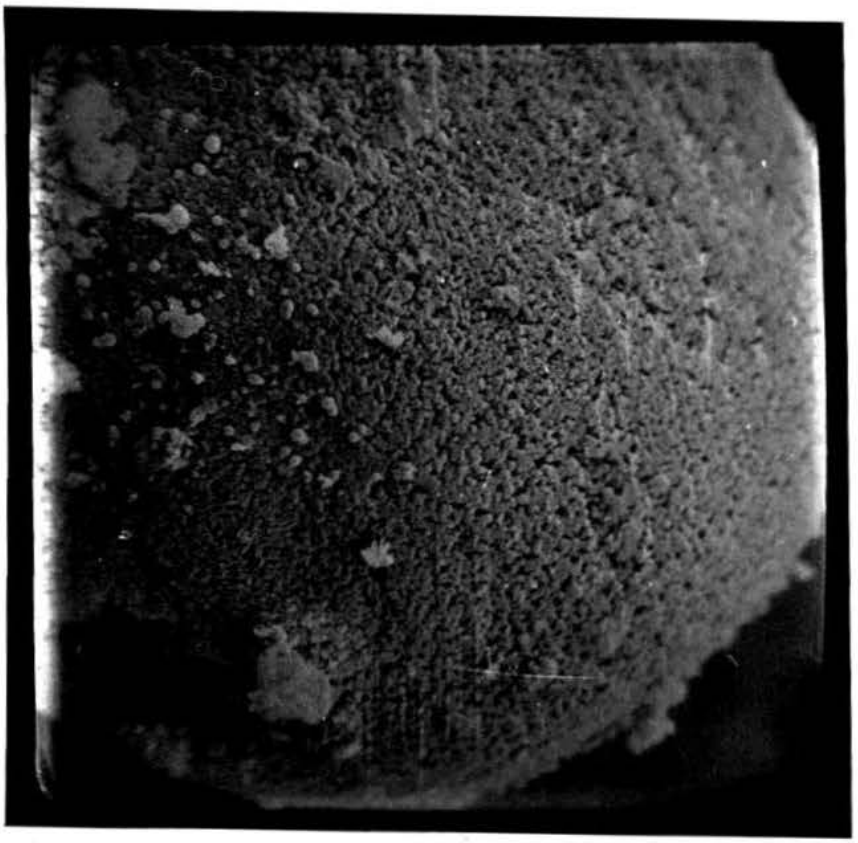
GEL P10 TC_F A+M 45 (32,000 x)



0.5μ

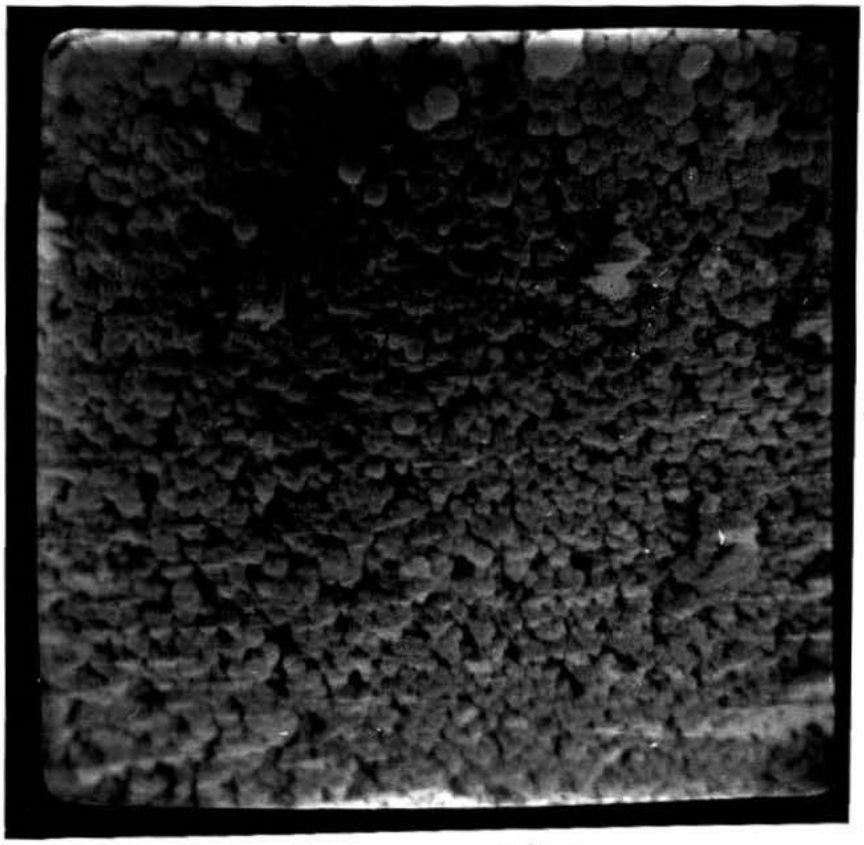
FIG. 38

GEL P10 TC_J (2,700 x)



4μ

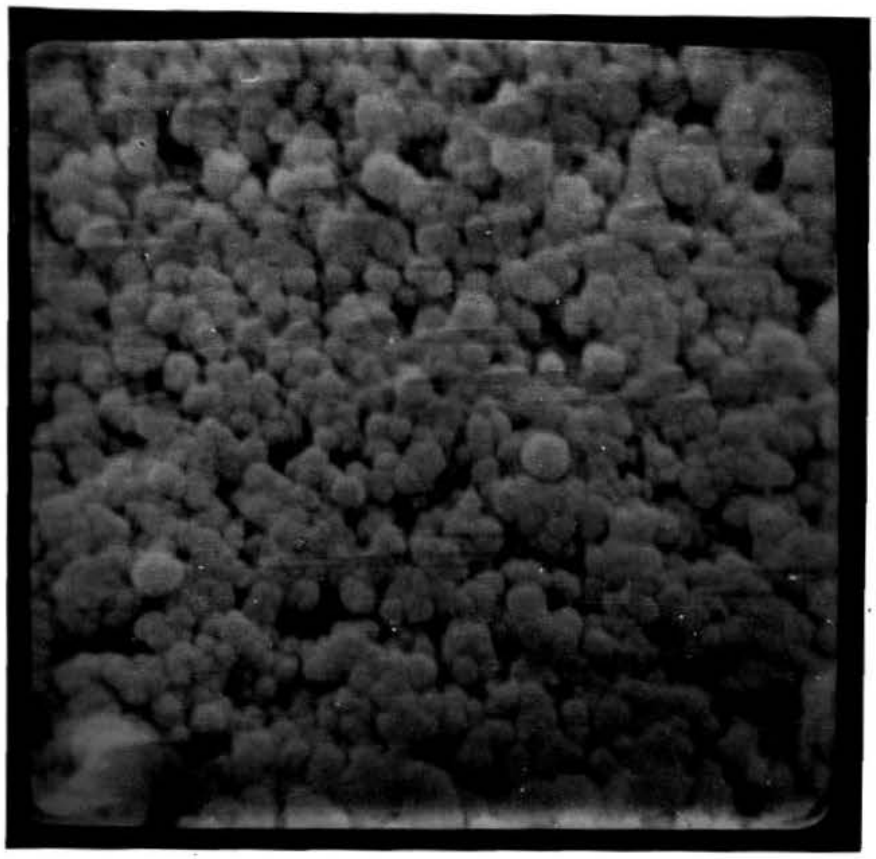
FIG. 39



GEL P10 TC_J (6,700 x)

2μ

FIG.40

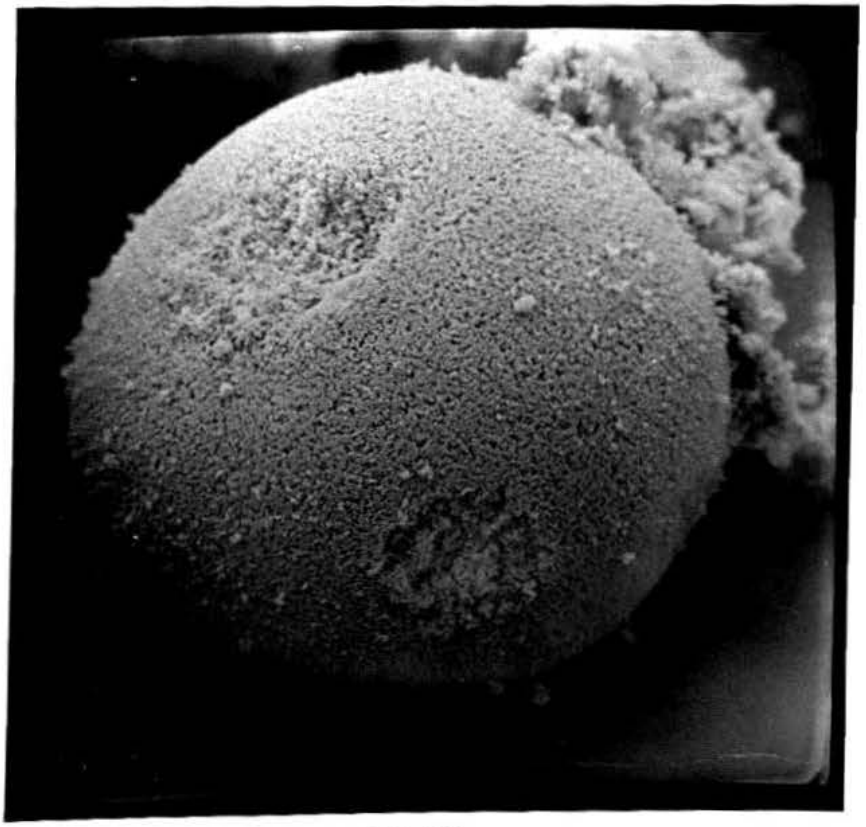


GEL P10 TC_J (13,500 x)

1μ

FIG.41

GEL P10 TC_J F/A 40:50 10
(1,350 x)

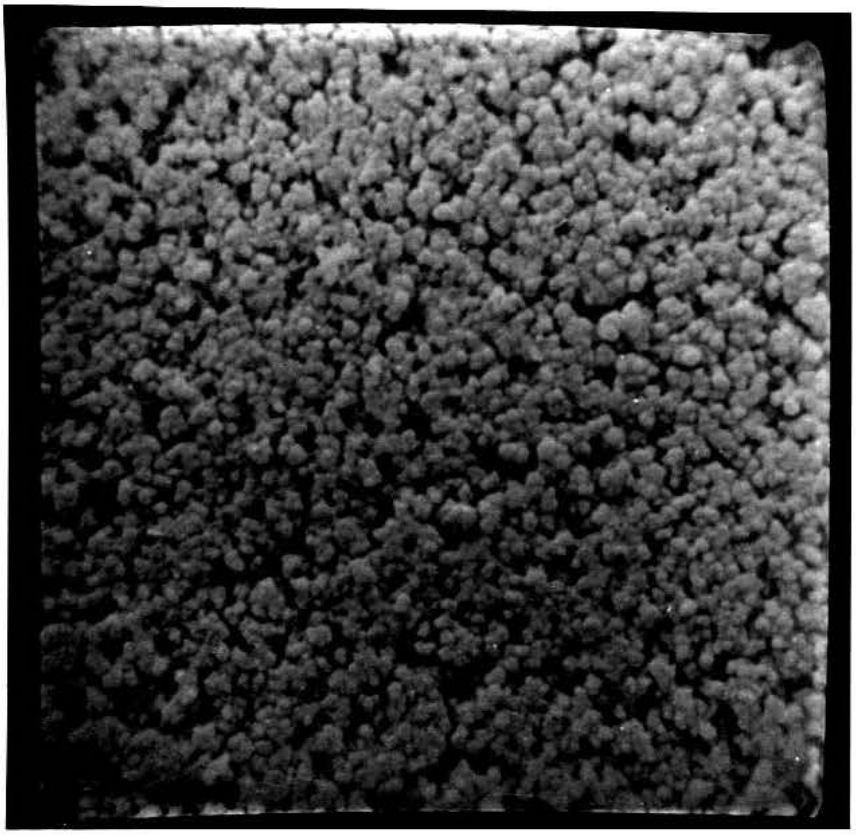


10μ

FIG.42

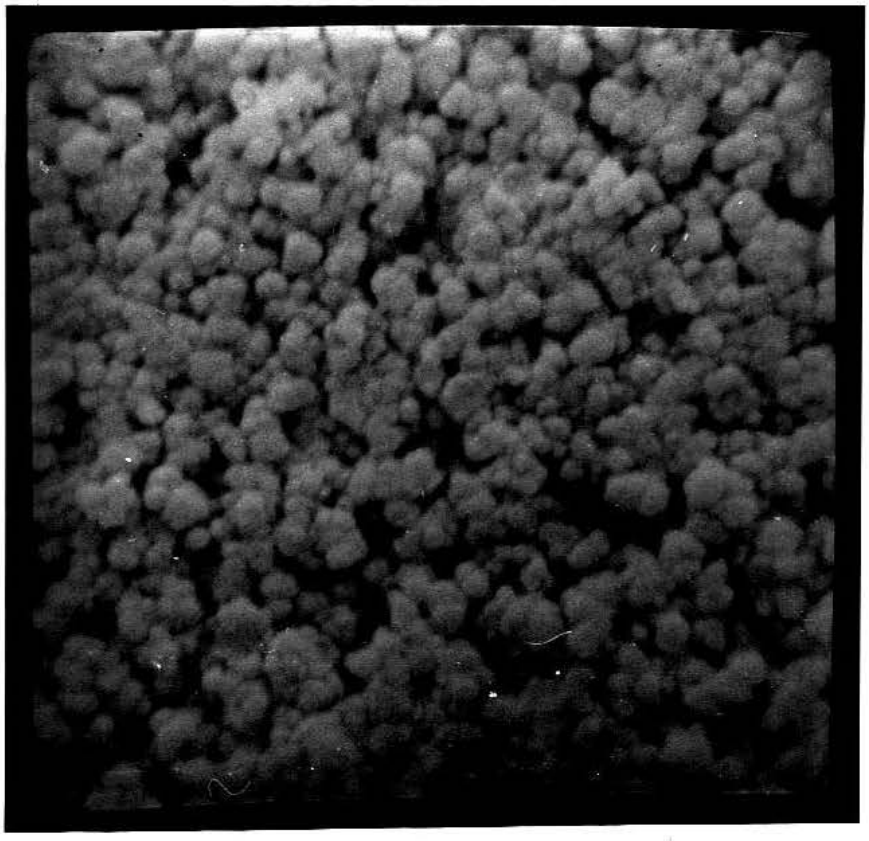
GEL P10 TC_J F/A 40:50 10

(6,700 x)



2μ

FIG.43

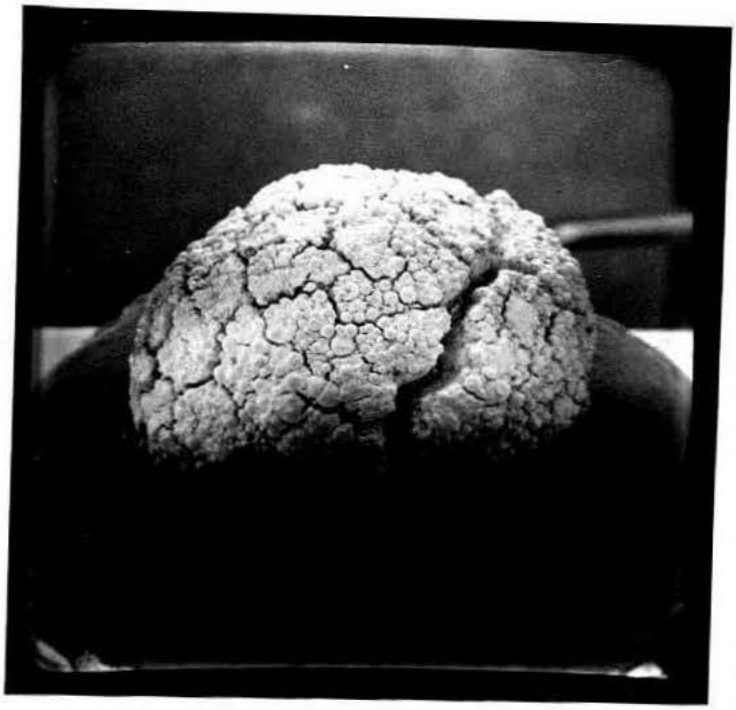


GEL P10 TC_J F/A 40:50 10

(13,500 x)

1μ

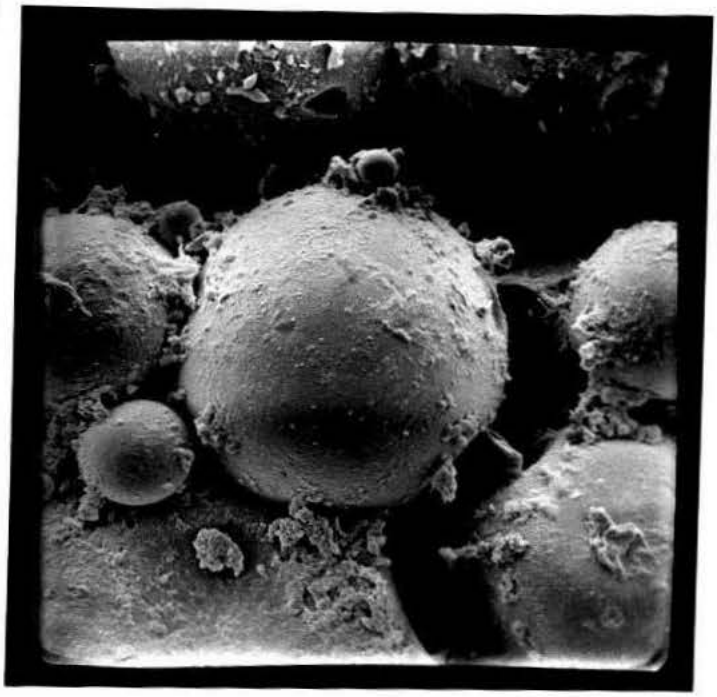
FIG.44



a

400μ

23 x



b

20μ

575 x

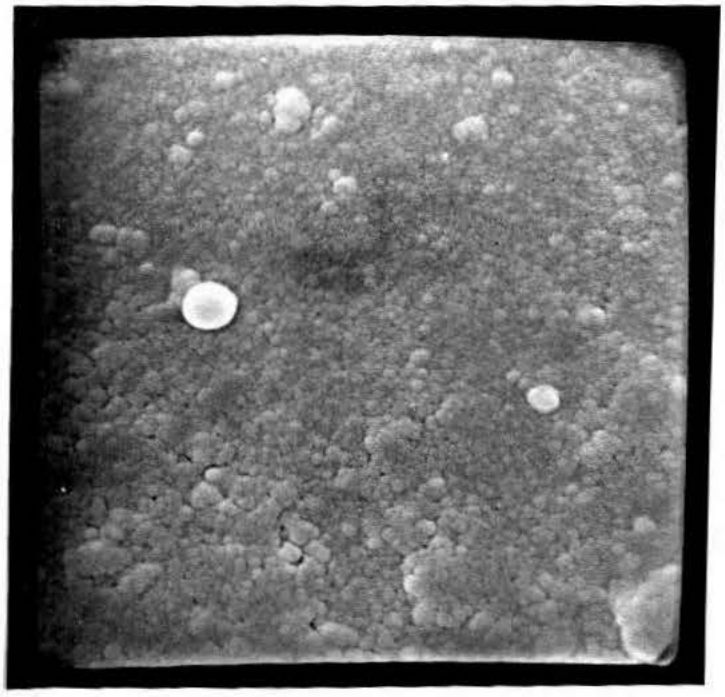
FIG 45

Poly Divinyl Benzene.

11,700 x

1μ

c



23,000 x

d

0·5μ

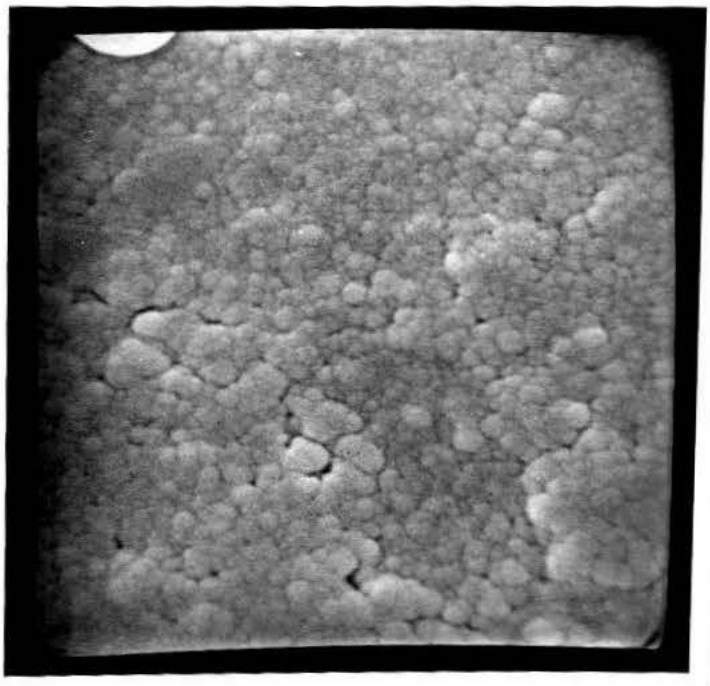
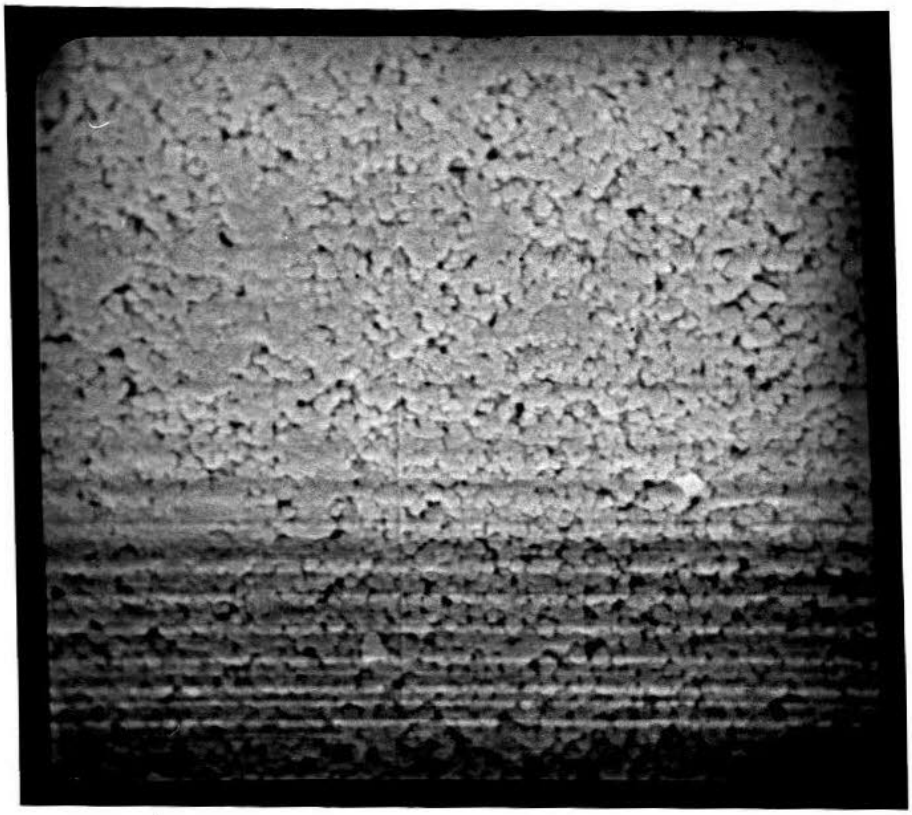


Figure 39 shows a sphere of gel P10 TC_J at a magnification of 2,700, Figures 40 and 41 show the same sphere but at higher magnifications, and it can be seen that it is very similar to Figures 21, 22 and 23 in structure. When these are compared with the photographs of P10 TC_J F/A 40:50 10 in Figures 42, 43 and 44, the differences are negligible, but the latter pictures are of the added xerogel as discussed in Chapter 6. This shows that, although the elution characteristics are different, the morphology of the gel remains the same, indicating that the xerogel has been adsorbed into the pore matrix inside the particle, as has been suggested earlier. These photographs therefore confirm the hypothesis proposed in the preceding chapter that the xerogel is grafted internally in the xerogel-aerogel hybrid.

Figure 45a shows a sphere of polydivinyl benzene, as described in Section 5.5, the particle is about 2 mm. in diameter, but the composition is of interest. Figure 45b shows the same sphere, but magnified 575 times, and it can be seen that, as expected, it is composed of smaller spheres. If these smaller spheres are magnified to 11,700 times other smaller spheres become apparent, and at a magnification of 23,000 times, these spheres become obvious. This indicates that the gel is comprised of very small spheres which conglomerate to give larger spheres, which in turn conglomerate to give particles. With the previous gels, only the particle sphere and the spheres creating the particle were visible, but this series of photographs suggest that these small spheres may be composed of even smaller ones, but the resolution on the S.E.M. is not of high enough quality to detect them, although it is possible to see shapes of 800 Angstrom diameter.

7.4. Some S.E.M. studies using freeze dried gel spheres.

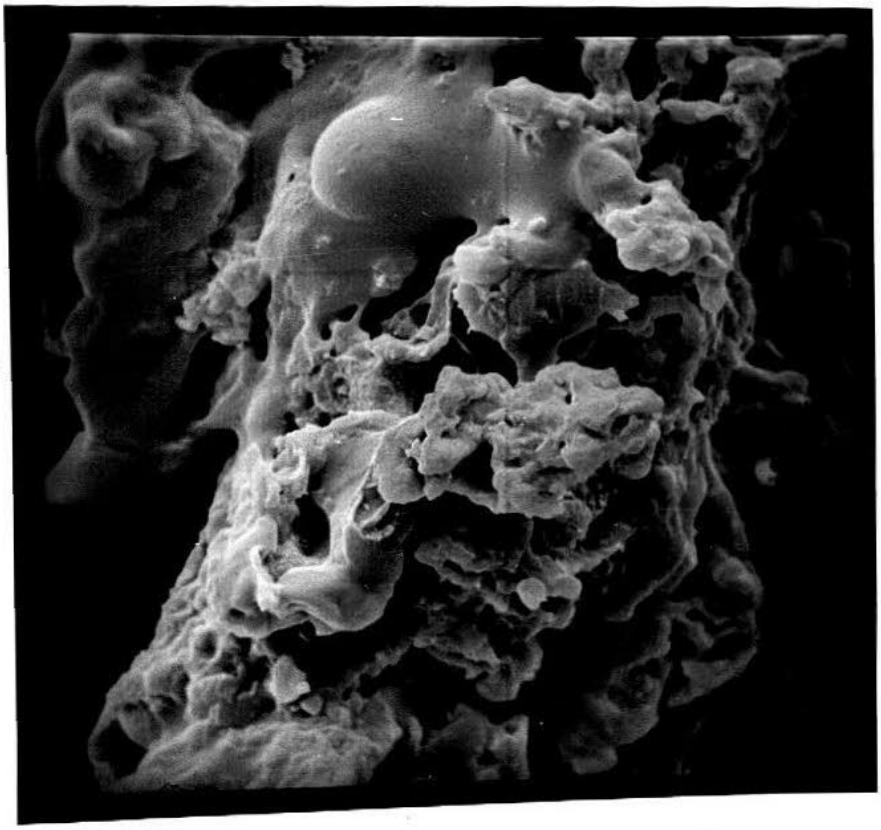
If a polymer, such as polystyrene, is frozen in a solution, and the solvent is vacuumed off whilst still frozen, the resulting matrix of the polymer will retain virtually all the original volume of the solution. It would be expected,



P10 TC A+M 45 freeze dried
F (5,600 x)

2μ

FIG.46a



GEL 'A+M' freeze dried

(2,300 x)

5μ

FIG.46b

then, that if the low density crosslinked gel had not been adsorbed into the pores, but was just on the particle surface, if it is freeze dried, a difference in surface structure will become apparent.

When this was applied to a gel, however, there was no sign of alteration in the surface morphology, as can be seen by comparing Figures 46a and 34 to 38. Figure 46b shows a freeze dried sample of the gel designated "A + M", as described in Section 5.4. It can be seen that the morphology is totally different from that of a gel and, in fact, resembles that of a freeze dried polymer.

Whilst pursuing this course of study, it was noticed that polystyrene, when studied with the S.E.M., had a supramolecular structure unlike other polymers studied.

This has shown that the gel grafted onto the P.10 matrix is not on the surface of the sphere, but is actually inside the pores, as was expected.

7.5. A short investigation into polystyrene morphology.

Initial investigations were performed with atactic polystyrene of molecular weight 391,000, dissolved in benzene at an unknown concentration. The polystyrene is dissolved, and then the solution frozen in ice, after which the sample has the solvent removed by means of vacuum freeze drying. This entails keeping the frozen solution in an ice bath to prevent meling, and removing the solvent by applying a vacuum of about 10^{-4} cm. of mercury to it. After six hours under vacuum, the resulting polymer matrix is transferred to a S.E.M. stub and prepared as discussed in 7.2.

Figure 47 shows the fibre structure obtained from such a preparation when magnified 200 times. Figure 48, however, shows one strand magnified 20,000

FREEZE DRIED P/S 391,000

(200 x)

50μ

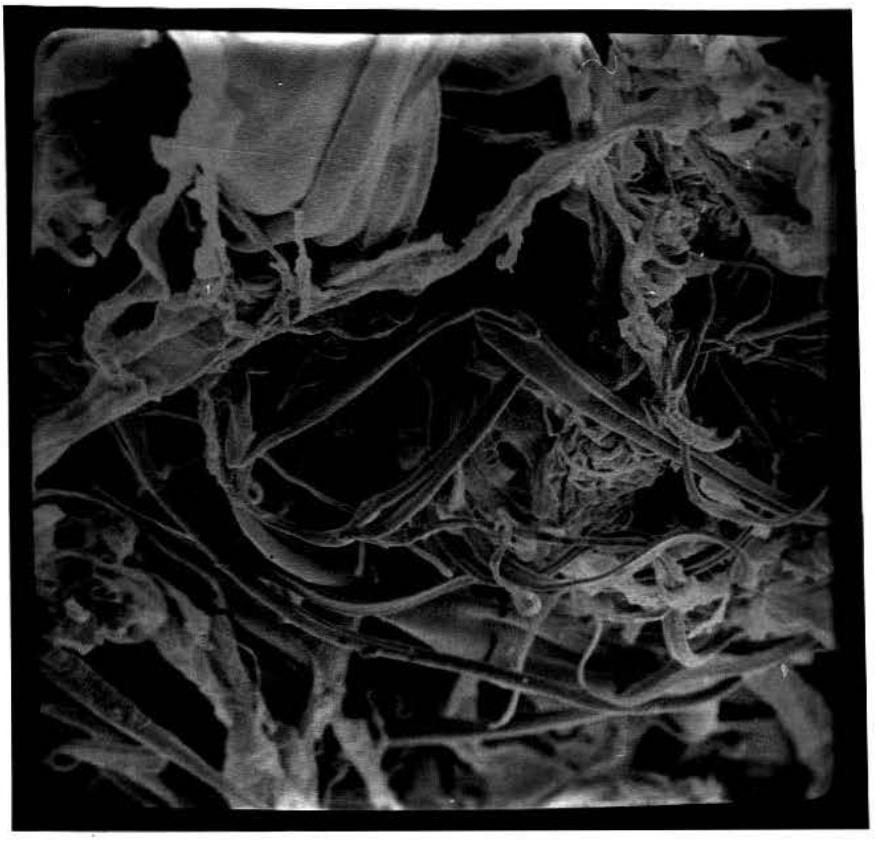
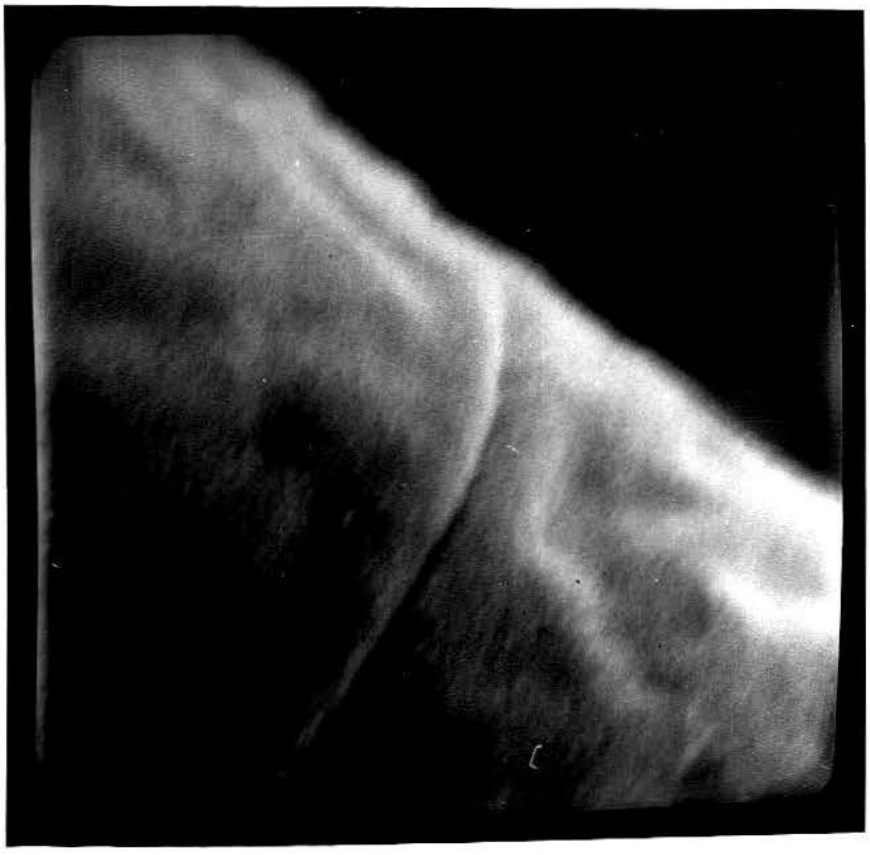


FIG.47



FREEZE DRIED P/S 391,000

(20,000 x)

0.5μ

FIG.48

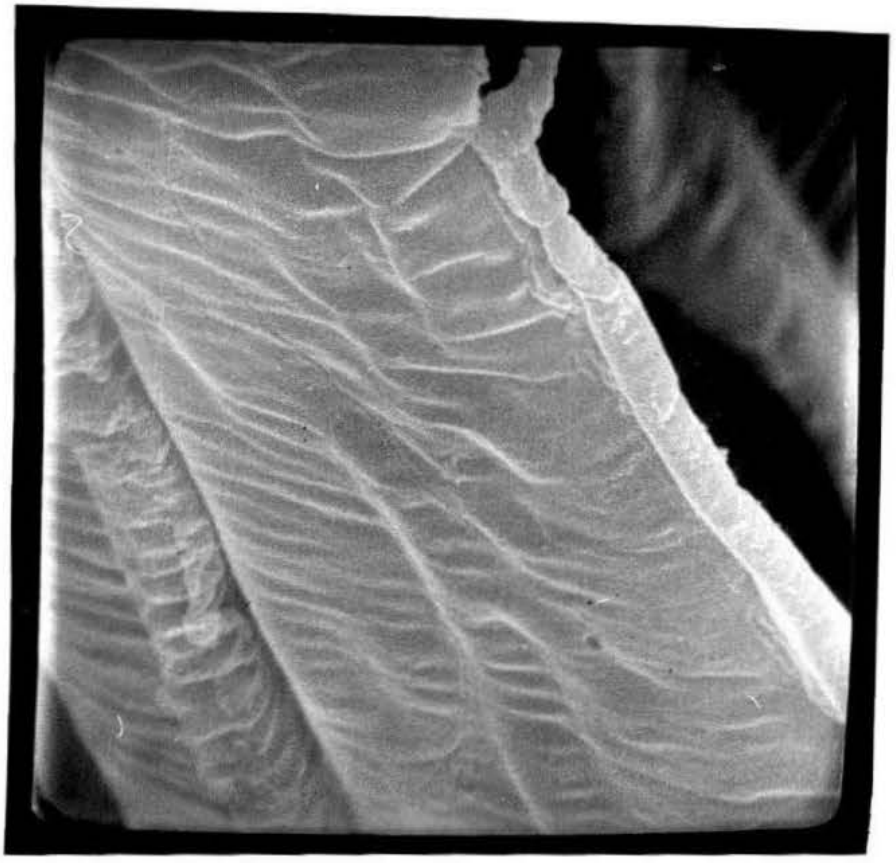
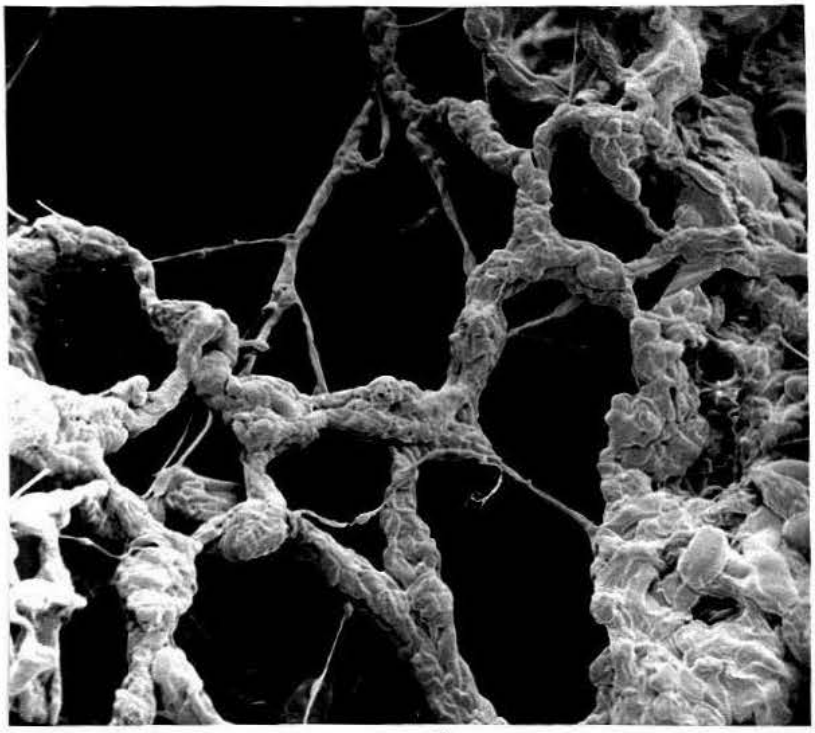


FIG. 49

FREEZE DRIED P/S 2×10^6

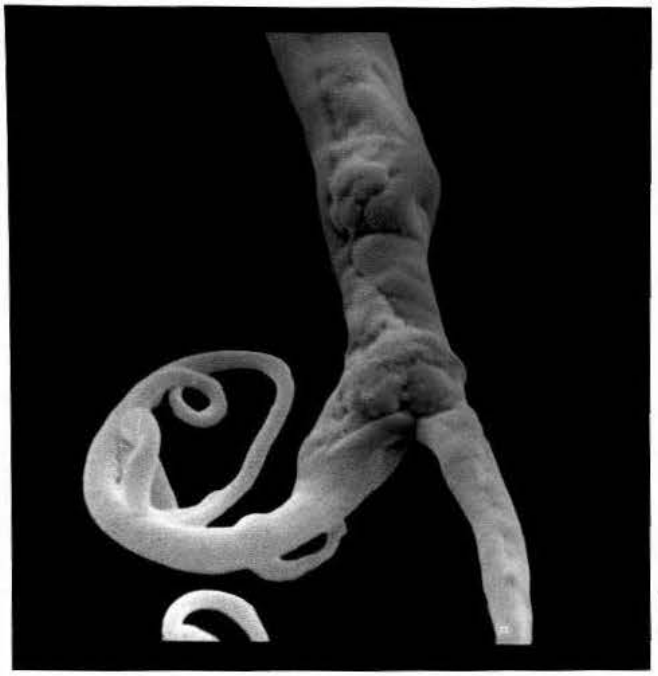
(10,000 x)



a

40μ

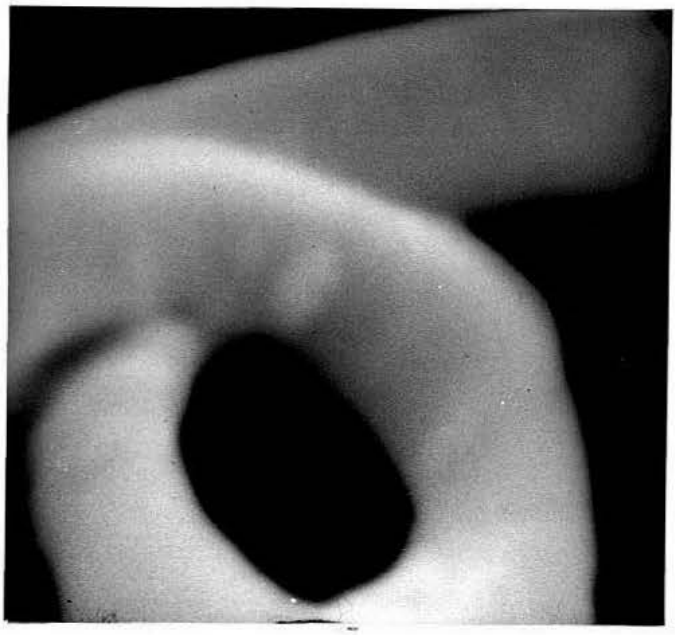
240 x



b

7,000 x

1μ



0.5μ.

70,000 x

FIG. 50

FREEZE DRIED P/S 2 x 10^6 FROM 0.015% SOLN. IN BENZENE.

times, and it can be seen that a surface morphology is present.

To ensure that this effect was not restricted to just this polymer, a series of other polystyrenes were treated in a similar way. Figure 49 shows the surface structure of polystyrene 2 x 10^6 magnified 10,000 times. As can be seen, the surface appears to have rifts covering it, travelling in one direction. Later work indicates that these rifts, or "creases" appear to originate from a point of stress. There appears to be no relationship between the extent of the rifting effect and molecular weight, but there is a relationship with concentration.

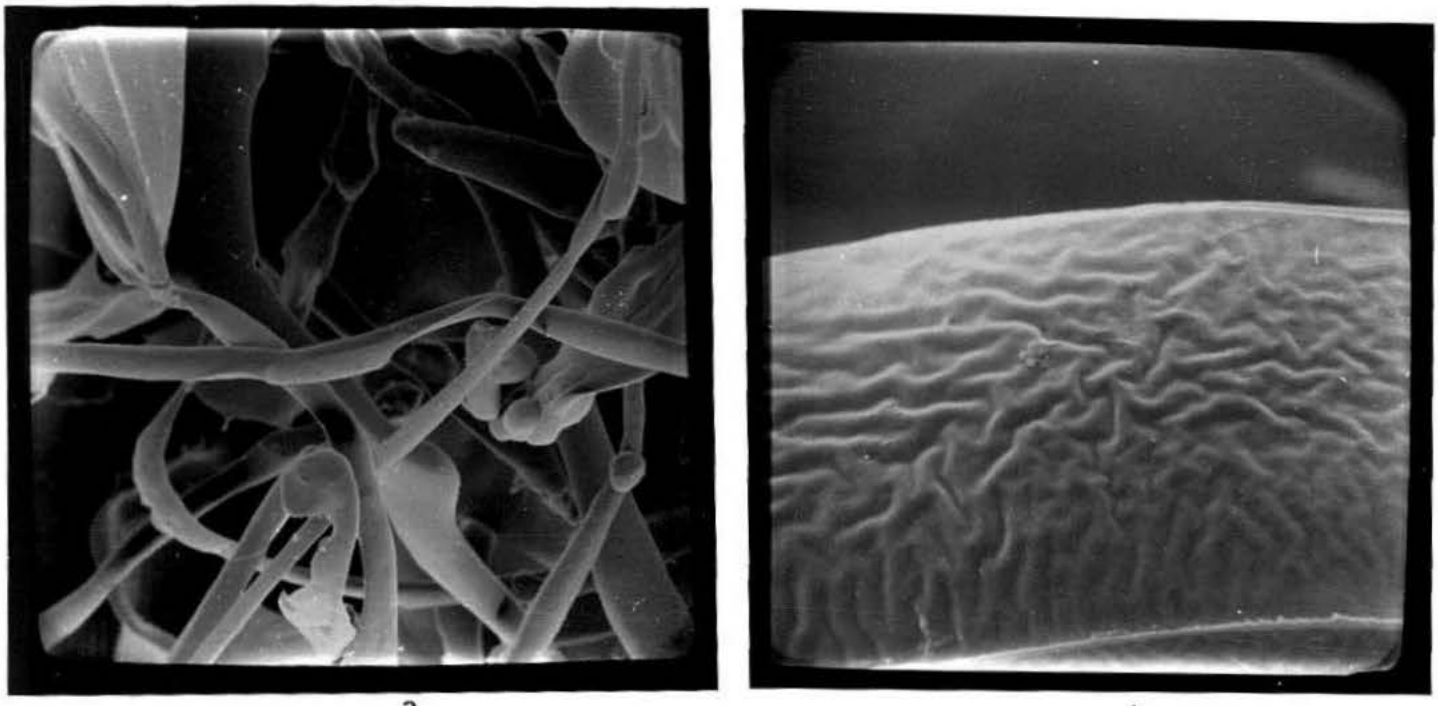
If a solution of 0.015% polystyrene of molecular weight 2 x 10^6 is freeze dried, a fine matrix of fibres is produced, as shown in Figure 50, but there is no surface morphology visible, even at magnifications of 70,000 times, as is shown in Figures 50b and 50c.

However, if a solution of 1.5% polystyrene is freeze dried, the resulting fibres are much thicker, as shown in Figure 51a. Further magnification shows rifting visible, not only on fibres, but also on plates of polystyrene, as shown in Figures 51b, 51c and 51d.

At present, there is no explanation for these "rifts" or supramolecular structures of polystyrene.

7.6. Possible explanations of the observed supramolecular structure.

Kargin[108] has reported the existence of "stripes" in elastic polymers; these stripes seem to be secondary formations and appear in the preparation of samples for electron microscopy at low temperatures, and also at the very initial stages of crystallisation. These stripes appear to be similar to those shown in Figures 49 and 51, but smaller. Arzhakov et al[109] proposes a



a

20μ 500 x

b

2,000 x 5μ

FIG. 51

FREEZE DRIED P/S 2 x 10^6 1.5% SOLN. IN BENZENE.

5,000 x

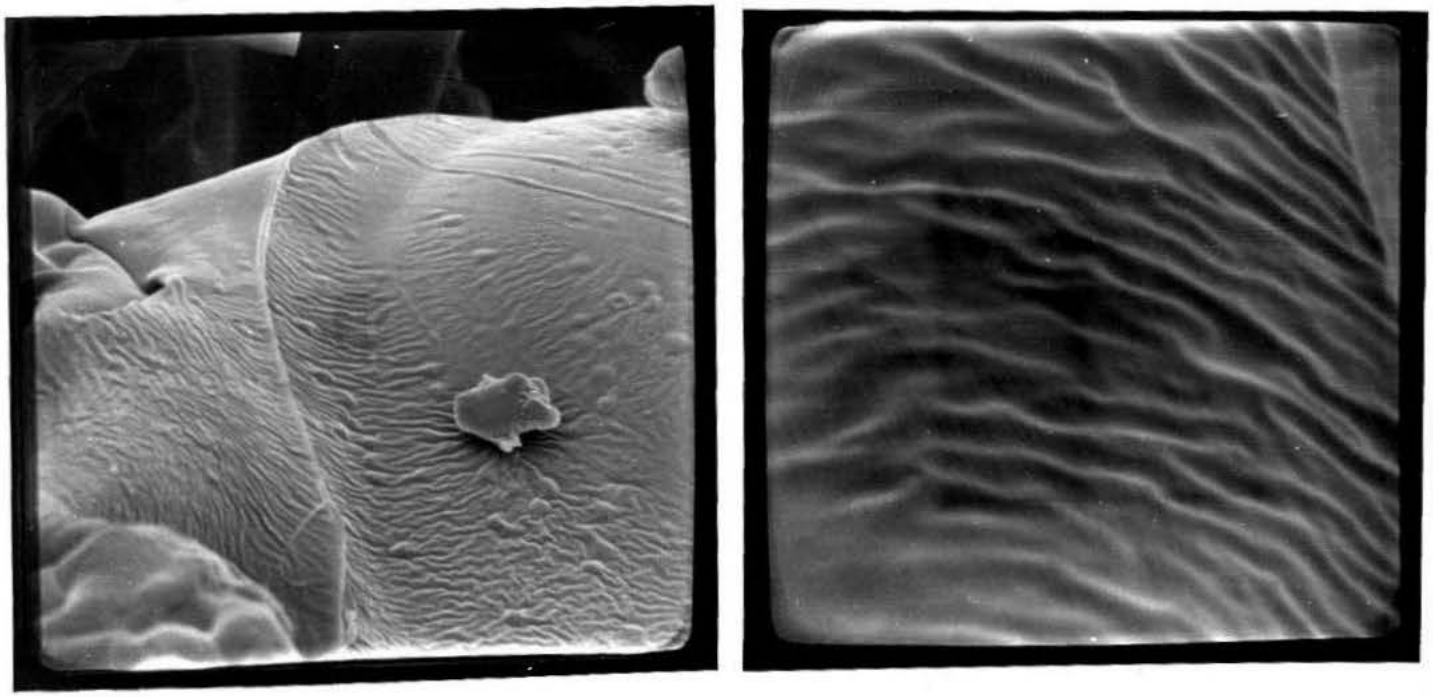
c

2μ

10,000 x

d

1μ



supramolecular structure in amorphous polymers, which atactic polystyrene is, suggesting that a major morphological element of the structure is a fibril consisting of folded chain domains linked with communicating chains. The fibrils are tightly packed in large structures called superdomains. Kargin,[110] however, disagrees with this hypothesis and suggests that amorphous polymers are composed of either chains coiled into globules, or of packets of uncoiled chains, the mechanical properties of the polymer being dependant on which form it is in.

Yeh[111] disagreed with Kargin, and suggested that the major elements of the polymer are in the grain, which consists of an ordered domain and a distinct grain boundary, and the intergrain region, which consists of molecules that truly random conformation. In the ordered system the domain, which is 20 to 40 Angstroms in size, has parallel segments.

Stuart[112] has been more specific, and has suggested that amorphous polymers are, in fact, less ordered crystalline polymers. If this is true, it would be expected that ordered regions would be observed in the polymer, even if it has previously thought to be totally amorphous, such as atactic polystyrene. Yeh[113] has reported the existence of such regions in atactic polystyrene of molecular weights ranging from 4,800 to 1,800,000. These ordered regions range in size between 15 and 45 Angstroms, and are due to long chain polymer molecules which tend to align more or less parallel and at constant average spacing. Such a phenomenon is seen in a larger size in the photographs in Figures 49 and 51. It is therefore possible, if the above literature is to be regarded as worthy, that the supramolecular structures shown in the figures could be due to crystallisation of the atactic, amorphous polystyrene into regular ordered regions, possibly with less ordered regions between them.

However, the size of the regions in the photographs appear to be in the

order of microns, while the size of the regions reported by Yeh,[113] are in the order of angstroms. The difference is so large that the theory is probably not applicable in this situation.

The rifts could, however, be due to a polystyrene-benzene crystallite boundary,[114] the polymer acting as a cold surface on which the solvent crystals are formed when freezing occurs. The polymer is forced to orientate itself with the benzene crystallite structure, and is hence moulded around it. If the concentration of the polymer is low, it will not be able to act efficiently as a cold surface, so no orientation of the polymer will be required, and hence no suprastructure. However, considerably more work has to be carried out before any definite conclusions can be drawn about the suprastructure of these polymers.

7.7. Discussion.

The photographs used in this chapter show that the surface of the gel does not appear to alter greatly during the grafting of new gel onto it. This indicates that the xerogel is, in fact, polymerized inside the gel matrix, as was proposed in Chapter 6. The gel surface resembles an orange peel at low magnifications due to the pores being visible all over the surface, but at higher magnifications, the particle can be seen to be comprised of a number of small spheres, bound together in such a fashion as to make the matrix permeable.

From this work, it is apparent that the scanning electron microscope is a very versatile instrument of wide capability, and has proved itself invaluable in the work described in this thesis.

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