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Gel and Affinity Chromatography

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GEL PERMEATION CHROMATOGRAPHY

Liquid Exclusion Chromatography

V. F. Gaylor and H. L. James

This review covers the period of approximately December 1973 through November 1975. The literature surveyed includes major analytical, chromatographic, and polymer journals, *Chemical Abstracts,* and various liquid chromatography literature abstracts.

Publication of the ASTM D-20.70.04 "Bibliography on Liquid Exclusion Chromatography" was a major event of the past two years (*13*). This document includes literature on gel permeation chromatography from the early 1960's through 1972, and will be updated regularly. Annual seminars on gel permeation chromatography, sponsored by Waters Associates, Inc., were a second major event. These meetings in late 1973, 1974, and 1975 were an excellent communications forum for specialists in the field. The American Chemical Society short course on gel permeation chromatography was made available in tape cassette form (*46*). Two additional American Chemical Society short courses on liquid chromatography also contain training material useful in the practice of liquid exclusion chromatography.

The authors of this review concur with ASTM D-20.70.04 in preferring "liquid exclusion chromatography" to the less definitive "gel permeation chromatography" nomenclature. But, nevertheless, we've used the common abbreviation, GPC, for ease of reading the review. However, we've not necessarily drawn any rigid distinction between size separations from soft gels ("gel filtration"), semi-rigid gels ("GPC") and porous, inorganic column packings ("rigid gels"). Some of each are included. Similarly, we've included some material on small molecules along with work on synthetic and natural polymers.

GENERAL REVIEWS

A number of general discussions on theory and practice of GPC and gel filtration chromatography were published (*27, 117, 124, 129, 183, 247*). Steric exclusion technology is also covered in other documents dealing with practice and applications of modern liquid chromatography (*58, 93, 306*). GPC source material is also included in general reviews of characterization methods for polymers, such as Keiner's review of automated methods (*170*). One third of the book, "Polymer Molecular Weight Methods," edited by Ezrin (*107*) is devoted to selected topics in GPC, and several of these are referenced elsewhere in this review. Chapter 6 from a book of the same name, and edited by Slade, is also recommended reading for the novice (*253*).

APPARATUS

Considerable improvement in liquid chromatography equipment was made during the past two years, and the number of suppliers of such equipment increased. Many of these developments are useful in GPC. The 1974/75 International Chromatography Guide (*147*) is a particularly useful equipment guide for both novice and experienced chromatographer. A number of general reviews (*50, 176, 221, 327*) covered all hardware requirements and available equipment, and a fifth review focused on solvent delivery systems (*150*). Additionally, Rossler, Schneider, and Halasz successfully tested a syphon-type flow meter for high pressure liquid chromatography (*285*), and Palyza described a

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simple flow adapter for gel chromatography (*257*).

Detectors for liquid chromatography were included in general reviews of chromatographic detection systems (*234, 267–269*), and Karasek specifically reviewed new detectors for liquid chromatography (*158*). New developments in ultraviolet absorbance detectors were discussed (*15, 16*). Two papers were also devoted to refractive index detectors (*335, 336*). New developments in viscometric detectors were of particular interest to chromatographers responsible for polymer molecular weight analyses. Tymczynski coupled a GPC to an automatic capillary viscometer for continuous measurement of viscosity of fractionated polymers (*321*). Ouano used a miniature pressure transducer to measure pressure drop across a capillary tube, thus effecting continuous molecular weight monitoring of column effluent (*250*). A later paper included complete instrumental design and computer interfacing for a continuous viscometric detector (*254*). Gallot, Marais, and Benoit also described a viscometric detector for GPC (*120*). And successful molecular weight monitoring of GPC eluant by low angle, laser light scattering was reported (*255*). The advantages of monitoring column effluent by infrared spectrometry, to define chemical or branching heterogeneities, were again demonstrated (*82, 225, 228, 284*).

Applicability of mini-computers in GPC was documented. A general discussion of real-time data acquisition, data processing, etc., for chromatographic systems (*136*) contains helpful background material. Hamielec, Walther, and Wright described a low-cost minicomputer for GPC data acquisition and reduction (*128*), and MacLean reported on-line data handling for high speed GPC (*205*). Development of software for data processing was also discussed (*204, 309, 310*). And Ohmori and Okuyama described a curve resolving computer program for gel filtration chromatography (*245*).

COLUMN PACKINGS

The single, most important development in liquid chromatography during the past two years was the routine availability and use of columns packed with microparticles. Use of microparticles in exclusion chromatography has the same advantages as in other modes of liquid chromatography. Separations which often required several hours on larger size exclusion packings may now be achieved in minutes. And the higher resolution obtained with microparticle exclusion packings permits separation of molecules much closer in size. Kato and coworkers studied cross-linked polystyrene gels of 20, 10, and 5 micron sizes and reported optimum analysis conditions for 5-μ packing (*166, 168*); molecular weight distribution analysis time was 10 min and resolution was comparable to that of the sedimentation velocity method. Limpert, Cotter, and Dark used 20-μ polystyrene gel to achieve 20 000 total plates at high speeds (*199*), and Vivilecchia et al. reported up to 3300 plates per cm for 10-μ gels (*329*). The practice of high speed GPC for molecular weight distribution analyses has one potentially serious limitation. Because of the much smaller retention volumes, flow rate variations can cause serious errors in molecular weight analyses; this source of error was studied in detail by Bly et al. (*37, 38*).

Microparticles of rigid, porous column packings were also tested for GPC analyses. These studies included 10-μ porous glass (*323*), 44-μ porous glass (*70*), 20-μ porous silica (*84*), and 20-μ alumina (*85*). Majors recently reviewed commercially available microparticles (both rigid and semi-rigid) for steric exclusion chromatography (*206*).

Telepchak reported separating polymeric materials on 36–44 μ porous glass (*314*). Cooper concluded that none of the available porous glass packings have pores small enough to separate low molecular weight oligomers (*61*). Chromatographic and other properties of silica gel packings were characterized by Cooper and Barrall (*64*), and surface-modified silica gels were used for molecular weight analyses of polymers of styrene, α-methylstyrene, and butadiene (*273*).

General reviews of gels used in steric exclusion chromatography were published (*114, 184*). Cross-linked poly(vinyl alcohol) gels were tested as hydrophilic stationary phases for GPC and found to be both pressure- and biostable, and nonadsorptive (*134*). Epton and co-workers described the preparation and chromatographic characterization of cross-linked poly(acryloylmorpholine) matrices with both aqueous and organic mobile phases (*101–103*). Other gels tested as exclusion packings were polyurethane (*189*), copolymers of hydroquinone–divinyl ether and methylmethacrylate (*307*), and cross-linked acrylates (*345*).

Saunders and Rehfeldt used a calculation procedure to select a set of columns with a desired molecular weight calibration range (*291*). Heitz reported that column coiling did not significantly contribute to band spreading (*133*).

FUNDAMENTAL STUDIES

Factors which determine elution volumes of polymers in GPC were discussed by Janca and Kolinsky in a review (*152*) which included 136 references. A theoretical model describing mechanism by which macromolecules are separated on a packing with nonhomogeneous pores was developed and tested by Kubin (*185*); calculated dependence of column efficiency on elution volume agreed with experimental curves. Ouano and Barker derived a computer sim-

ulation for linear GPC (*251*) and generated chromatograms which agreed well with experimental chromatograms. Distribution coefficients for polymers on glass with cylindrical pores were calculated for the equilibrium state (*328*). The method of Kubin and Kucera was used to calculate bed void fractions, axial Peclet numbers, particle porosities, internal diffusion coefficients, and internal tortuosity factors from experimental data on four different packing-solute systems (*222*). Motozato et al. correlated average size of gel network with maximum size of permeable molecules and reported linear relationships with both root mean square of end-to-end distance and hydrodynamic radius of solutes (*236*). Mori, Porter, and Johnson eluted polymer solutions through columns composed of long glass frits to test the concept of separation by flow (*233*), and derived an equation for characterizing elution volume as a function of tube pore size and radius of solute molecules.

It is generally recognized that molecular size is not necessarily the single factor influencing elution volumes from gel-packed columns. Several pertinent studies of non-ideal effects, such as solute–solute, solute–gel, etc., interactions were reported. DeRuvo used a thermodynamic approach to derive an expression for the partitioning of solute molecules between swollen gel and free solvent (*88*). Dawkins and Hemming reported a distribution coefficient which includes partitioning and adsorption effects for polystyrene interactions with cross-linked polystyrene gel (*79*). Adsorption of other aromatic compounds on cross-linked polystyrene gel was studied by others (*188*). Adsorption on poly(vinyl acetate) gels was also reported (*143*) and the author urged caution in interpreting chromatograms from this type of gel. Similar reports of adsorption effects on porous silica gels were published (*30, 146*), and Kuzaev et al. suggested that achieving a steric exclusion separation mechanism on silica gel can depend on chemical nature of the mobile phase (*187*).

The influence of hydrogen bonding on exclusion chromatography behavior of small molecules was studied on small pore polystyrene gel (*115*). Forss and Stenlund studied the influence of charge effects on elution behavior of polyelectrolytes and used the results to improve resolution (*113*). Bakos and Berek compared calibration curves for polystyrene and poly(methyl methacrylate), and for mixtures of the two, and attributed increased elution volumes for the mixtures to incompatibility of the two polymers (*17*). A report that polystyrene of molecular weight above 10^7 shear degrades in the chromatographic system was published (*299*).

The effect of operating variables on separation efficiency was studied in a number of ways. Two general studies and a review of the subject were published (*55, 56, 155*). Kato and Hashimoto discussed the various factors contributing to band broadening and concluded that broadening is best suppressed by use of high efficiency columns (*159*). Cooper and Bruzzone studied effect of temperature on efficiency of separating polymers on porous glass columns (*65*). Separation efficiency on polystyrene gel columns was shown to be highly dependent on flow rate in the range 0.05–1.5 ml/min (*68*). Cooper, Johnson, and Bruzzone also studied effect of flow rate on calculated molecular weight averages and found the resolution parameter is molecular weight dependent at 1.0 ml/min but independent at 0.2 ml/min (*69*). With porous glass columns, peak widths increased with increasing flow rate in the range 1.0–4.5 ml/min, and higher flow rates produced unresolved molecular weight distribution analyses for polystyrene of molecular weight greater than 411 000 (*62*). Cooper compared efficiency of several commercially available column packings and proposed the ratio $\bar{M}_w/\bar{M}_n$ for polymer solutes is a convenient parameter for comparing column efficiencies (*63*). Ishida et al. found that good resolution could be achieved at fast flow rates by reducing column dead volume, and by using small diameter columns and gel with a narrow particle size distribution (*148*). Effect of sample size on efficiency was also reported (*57*).

The effect of sample size on elution volume has been a vexing problem in the chromatography of polymers. Kato and Hashimoto studied solvent dependency and found that elution volumes are generally independent of sample concentration when a theta solvent is used (*160, 161*). The same authors verified this practical solution to the sample size effect by comparing accuracy of molecular weight averages measured in both good and theta solvents (*162*). Berek et al. also minimized dependency of elution volume on sample size by use of a theta solvent with porous silica gel columns (*31*).

Axial dispersion was measured by Berger with refractionated polystyrene standards (*32*). Suzuki investigated effects of axial dispersion in dextran gel with NaCl and blue dextran (*308*). Experiments with mixtures of ^{14}C-labeled, monodisperse polystyrene and inactive polystyrene showed that a molecularly nonuniform polymer acts like the sum of molecularly uniform samples (*33*). Ehrlich and Smith used elution and reverse phase chromatography, as well as GPC, to show that peak widths of monodisperse polymers are independent of polymer type and depend only on elution volume (*99*); they therefore concluded that all polymer chromatograms can be treated as summations of narrow fraction chromatograms. Fourier transform techniques were used to derive a simple correction for some sources of peak broadening which are treated empirically in other correction methods (*207*). A method which assumes skewed chromatograms can be represented as resultant halves of two different Gaussion peaks, was used to calculate real molecular weight averages, where spreading functions are skewed and concentration effects exist (*241*). Smith showed that the Hamielec and Ray method for correcting molecular weight averages can be combined with the resolution index concept of Smith and Feldman to provide a simple and accurate resolution correction (*302*). Timm and Rachow also reported a resolution correction procedure (*316*).

Calibration of columns and equipment for molecular weight analyses continued to receive considerable attention in the literature. Calibration and calculation procedures were reviewed in some depth (*25, 106, 279*). The Benoit hydrodynamic volume concept, commonly called the universal calibration method, continued to be widely used. Spatorico verified the universal calibration concept for poly(methyl methacrylate), poly(vinyl chloride), and polyester on both porous glass and polystyrene gel columns (*304*). Otocka and Hellman verified universal calibration on surface treated porous glass with four different solvents (*248*). Spatorico and Beyer also verified a universal calibration with porous glass for four different hydrophilic polymers in three different solvents (*305*). Dawkins and Hemming concluded that deviations from universal calibration in theta solvents were due to polystyrene gel–solute interactions (*78, 80*). Hydrodynamic volume plots for linear and comb-shaped, branched polystyrenes did not agree but radius of gyration calibration plots for the two were identical (*164*). Comparison of calibration data for rigid rod and random coil polymers suggested that hydrodynamic volume is a valid universal calibration parameter (*81*). Dondos, Rempp, and Benoit confirmed validity of the universal calibration method for a variety of random and block copolymers (*92*). Chang constructed a copolymer calibration curve from the calibration curve of one homopolymer and the Mark–Houwink parameters (*51*); validity of the technique for $\bar{M}_n$ values was verified by osmometry. The universal calibration method was tested on a variety of other polymers, including polyacrylates (*153, 278*), poly(vinyl chloride) (*177*), polysulfones and polycarbonates (*83*), polyester (*303*), oligomers of polyglycols (*26*), asphaltenes and bituminous resins (*280*), and cellulose and cellulose derivatives (*52, 252, 293, 312, 325, 332*), and used for analysis of polyolefins (*232, 301*). Belenkii and co-workers tested four different water soluble polymers on a Sephadex gel and attributed deviations from the universal calibration principle to differences in polymer–gel interactions (*28*). Abdel-Alim and Hamielec reported a calibration method for polyacrylamide on a porous glass–silica gel column bank (*3*). Brussau found that measured viscosities of chromatographic fractions from a polydisperse polystyrene yielded a calibration curve which differed from that obtained from monodisperse polystyrene standards (*43*). Nichols commented on $\bar{M}_w/\bar{M}_n$ ratios obtained by universal calibration (*242*).

Reproducibility of chromatographically measured molecular weight distribution analyses was tested in a ten-labo-

ratory round-robin (*4*); a coefficient of variation of 6.2 ± 0.7% for $\bar{M}_n$ was reported. The two samples of linear polyethylene distributed by the Macromolecular Division of IUPAC were fractionated and characterized by gel permeation chromatography, osmometry, viscosity, and infrared techniques (*239*). Ambler and Mate compared chromatography and osmometry for evaluating $\bar{M}_n$ of poly(vinyl chloride) and polybutadiene and obtained good agreement after correcting for the low molecular weight error inherent in osmometry (*7*). Tung and Runyon compared chromatography and sedimentation velocity analyses on Pressure Chemicals polystyrene standards, and concluded that the high molecular weight standards are skewed in distribution (*320*). Molecular weight distributions of two samples of poly(vinyl chloride), representing two extremes in polydispersity, were characterized by both chromatography and precipitation fractionation (*11*). Giddings, Yoon, and Myers compared chromatography and thermal field-flow fractionation for polymer separations and found the two techniques about the same in number of theoretical plates (*123*).

TECHNIQUES

The effect of branching on molecular weights derived from GPC of polymers, and the measurement of branching by combining chromatography with other techniques were again studied. One review included methods for determining branching (*227*). Ambler, Mate, and Purdon combined GPC and viscosity to characterize branching (*8*) and published branching measurements for star-branched copolymers of styrene and butadiene and randomly branched polybutadienes and SBR (*9*). Kraus and Stacy also used viscometry and GPC and compared branching results from two simplified methods applied to polyethylene, star-branched polybutadiene, and block copolymers of styrene and butadiene (*181*). Similarly, Servotte and DeBruille characterized long-chain branching in polyethylene by viscometry and GPC (*295*). Branching in bisphenol-A polycarbonate was characterized by the same two techniques, and light scattering (*20*). The Drott method for estimating branching was evaluated on unfractionated and fractionated polyethylene (*48, 341*). Ross and Shank used an infrared detector on the GPC column effluent to monitor methyl groups in polyethylene and thereby measure branching as a function of molecular weight distribution (*284*). Additional methods and chromatographic studies of branching in polyethylene (*333, 339, 346*), styrene–butadiene rubber (*324*), epoxide resins (*23*), and star- and triblock polymers (*34*) were also published. Kato et al. showed that the GPC peak for a star-shaped poly(α-methyl styrene) with three branches of equal molecular weight can be separated from that for a linear polymer comprising two of the same branches by using high efficiency columns (*163*). GPC has also been used in the characterization of a number of graft polymers (*60, 105, 171, 172, 193, 194*).

Compositional heterogeneity of copolymers was monitored in a number of cases by using one or more selective detectors with GPC. Harmon and Folt used a combination of refractometer, ultraviolet, and infrared detectors to measure average styrene content, sytrene distribution, and oil content of oil extended SBR (*131*). Mirabella, Barrall, and Johnson used stop-and-go infrared detection to measure compositional changes with molecular weight for a copolymer of vinyl chloride and vinyl stearate (*225*). And Ross and Shank also used infrared detection on ethylene–ester copolymers to define compositional heterogeneities as a function of molecular weight (*284*). Ultraviolet absorbing derivatives of hydroxyl-terminated polybutadienes were prepared to measure functionality distribution with a uv detector (*10, 14*). Radiotracer techniques were applied to polymers containing carboxyl or hydroxyl groups to evaluate compositional homogeneity or heterogeneity (*292*).

Recent applications of preparative scale GPC were reviewed (*67*), and a two-part review of techniques used in preparative scale liquid chromatography included steric exclusion techniques (*89, 90*). Montague and Peaker described design and operation of a preparative scale instrument using 2.34-cm i.d. columns (*231*). Effects of operational variables on fractionation efficiency of preparative scale GPC were studied by two different groups (*66, 169*). Kato et al. used 10-μ gel particles for the preparative scale fractionation of NBS 706 polystyrene and also commented on effects of operational variables with the high resolution column packing (*165*). Martin and Johnson compared both cost and efficiency of preparative scale GPC to other methods of preparing narrow distribution polymers (*216*). Others reported use of preparative scale techniques (*244, 263*).

The recycling technique was used by several to improve resolution of small molecules separated by GPC. Recycling was used to separate configurational isomers of hydrocarbons (*195*), diastereoisomers (*196, 197*), phenolic compounds (*156*), and oligomers and polymer additives (*240*). Kalasz, Nagy, and Knoll derived an expression for calculating optimum number of cycles for the required resolution (*157*).

A variety of miscellaneous techniques were reported. Patel proposed using an oligomer to eliminate molecular weight analysis errors due to effects of flow rate and syphon temperature variations on elution volumes (*258*). As an example, *n*-decane was used as internal standard for polyethylene analysis (*259*). Total polymer was measured in a variety of industrial materials, as an example of "exclusion limit chromatography" (*137*). Small chromatographed aqueous latex on solid glass beads and achieved a particle size separation in the interstitial volume of the column (*300*); he called this technic "hydrodynamic volume chromatography". Gaylor, James, and Herdering solubilized aqueous lattices of nitrile rubbers in a solvent for the non-cross-linked fraction and separated suspended gel particles from solubilized rubber by GPC (*122*); this technique was used for rapid measurement of gel contents. Vacancy permeation chromatography, where the mobile phase contains a small amount of the solute, was studied by Otocka and Hellman (*249*). And Wormser used GPC to study chemical equilibrium during migration of one reactive species in a mobile phase containing the second reactant (*343*). Chromatography on a Sephadex gel was used to clean up vegetable extracts for insecticide analysis (*264*). Copolymers of acrylic acid were esterified for GPC analysis on polystyrene-type gels (*311*).

Size separations were also achieved by thin layer chromatography (TLC). Epton, Holding, and McLaren developed TLC techniques and standards for rapid screening of porous gel candidates for aqueous GPC columns (*100*). Other TLC applications included size separations of SBR (*179*) and hydrolysis polymers of metal ions (*298*).

APPLICATIONS

The number of reports of using GPC for separating small molecules increased. And this applications area is likely to increase even more with the high resolution columns now available. GPC techniques were used extensively in the characterization of fossil fuels, refined products, and by-products (*18, 59, 76, 95–97, 138–140, 219, 220, 223, 271, 272, 274, 294, 315, 350, 351*), other fats and oils (*35, 132, 261, 266*), additives in plastics (*41, 75, 167, 275–277, 286, 318, 319, 331*), inorganic mixtures (*72, 86, 246, 288, 352*), and other kinds of materials (*73, 175, 208–210, 260, 264, 270, 289, 296, 340, 342*). The separation of low molecular weight substances on organophilic gels was also reviewed (*224*).

Applications to synthetic macromolecules included polyolefins (*6, 12, 36, 45, 180, 337*), polystyrenes (*1, 49, 118, 119, 265, 283, 322*), condensation polymers (*22, 24, 44, 54, 77, 98, 108–111, 135, 149, 174, 178, 202, 203, 256, 282, 317, 354*), acrylic resins (*173, 217, 218*), halogen-containing polymers (*2, 200, 338, 349*), silicon-containing polymers (*142, 151, 191, 297*), celluloses (*42, 53, 211–213, 326, 334*), styrene copolymers (*94, 141, 179, 215, 235, 348, 353*), and others (*5, 29, 39, 40, 47, 104, 144, 145, 186, 190, 192, 226, 238, 281*). Applications of GPC to thermosetting resins were also reviewed (*313*).

Biochemical materials studied included peptides (*121, 214*), proteins (*116, 127, 198, 330, 344*), sugars (*74, 87, 91, 154, 287*), dextran (*19, 21, 243*), and others (*71, 112, 125, 126, 130, 182, 201, 210, 229, 230, 237, 262, 290*).

Affinity Chromatography

Howard H. Weetall

Affinity Chromatography or biospecific adsorption chromatography is a specific type of separation technique which takes advantage of the biological specificity of an enzyme, antibody, or binding protein for its specific substrate, inhibitor, or receptor.

The technique generally takes advantage of a biospecific ligand covalently attached to some water-insoluble matrix. A solution containing the species to be recovered is passed through a column containing the bound ligand. The protein to be isolated binds to the ligand and is thus retarded in its passage through the column. The binding affinity between the ligand and the protein to be recovered may vary from very weak, in which case only retardation of the species occurs, to very strong, in which case the species is immobilized. Elution is accomplished by changing one or more of the adsorption parameters, e.g., pH, ionic strength, temperature. In some systems, addition of a competitive molecule of greater binding affinity will cause release of the bound species.

During the past two years, little major progress has been made in affinity chromatography. A few new support materials have joined those already in use. The most unusual material to find use has been the inorganic support (*6A*, alogues (*19A*). These derivatives have been utilized for the separation of several dehydrogenases and kinases from the same samples by sequential elution with NAD^+ or NADH (*21A*). Recently, lactate dehydrogenase isoenzymes have been separated by affinity chromatography (*24A*).

Studies of the purification of chymotrypsin (*32A*) were reported as was the isolation of DNA and RNA polymerase (*3A, 30A*). Other enzymes purified by affinity chromatography include plasminogen (*16A*), transcarboxylase (*7A*), and sweet potato β-amylase (*36A*). Concanavalin found one more unique application, for the isolation of collagen fractions by affinity techniques (*1A*).

Although no large scale industrial applications of affinity chromatography were reported, there was a report of a pilot plant scale-up for the isolation of β-galactosidase (*29A*).

During the past two years, a great deal of attention has been paid to the mechanism of affinity binding to ligands. Data on the binding of chymotrypsin to a specific ligand indicates that binding does not occur at the active site (*32A*). Additional studies on the hydrophobicity or hydrophilicity of the spacer arm has shown that a great deal of adsorption of enzymes and other proteins is due to nonspecific hydrophobic interactions (*22A*). Studies on hydrophobic interaction has led to what is now called, "Hydrophobic Chromatography".

REVIEW AND GENERAL STUDIES

A large number of reviews and general type studies have appeared over the past two years. Several books have also been published containing sections on affinity chromatography (*11A, 13A–15A, 21A, 23A, 27A, 31A, 34A, 37A–40A*).

AREAS OF APPLICATION

Over the past two years, major emphasis has been placed on the use of immobilized enzyme co-factors and co-factor analogues for the isolation and purification of dehydrogenases and kinases. These studies have utilized various forms of NAD^+ (*4A, 5A, 9A, 33A*), and AMP (*2A, 5A, 7A, 10A, 17A, 28A, 33A*). Other co-factors used include UDP (*33A*), ATP (*5A*), and pyridoxyl-5′-phosphate and its an-

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A large number of papers have appeared describing techniques of isolation which take advantage of hydrophobic adsorbents for protein isolation (*8A, 12A, 22A, 23A*).

Many of these adsorbents have been found to still contain ionic groups, which diminishes the truly hydrophobic binding (*23A, 24A*). This technique of affinity chromatography may become more useful in the near future.

One of the most important finds to occur was the discovery that CNBr coupled ligands are not totally stable. It was clearly shown that CNBr coupled compounds are susceptible to nucleophilic attack by amino groups releasing N_1–N_2-distributed guanidines (*41A*). Similarly, it has been shown that isoproterol bound to CNBr activated agarose is released in soluble form by hydrolysis of the arm-linked derivative from the agarose (*35A*).

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Gel Permeation Chromatography

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