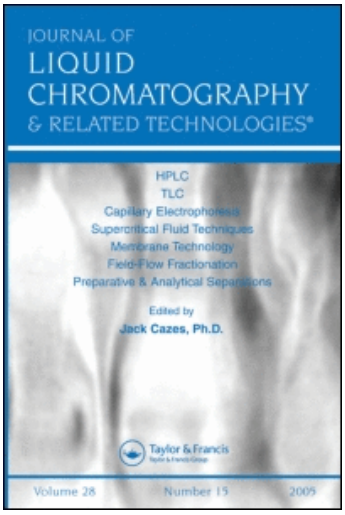

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Application of Size Exclusion Chromatography in the Development and Characterization of Nanoparticulate Drug Delivery Systems

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Abstract: The successful production of effective nanoparticulate drug delivery systems depends on maintaining the characteristics of the starting materials and the final formulation. Since compendial standards for traditional drug delivery systems do not always apply to nanosystems, analytical procedures for nanosized delivery systems must be established. This will ensure the quality of such products as they reach the marketplace. Size exclusion chromatography provides a method to continuously monitor all the development stages of nanoparticulate drug delivery systems, thereby, ensuring the quality of the starting materials used and the final product. The primary properties that can be monitored with this technique include particle size and molecular weight. Information about these properties not only serves as quality control measures, but can be used to monitor formulations for potential degradation products or impurities. Recent advances in size exclusion chromatography, such as the introduction of rapid analysis size exclusion columns and new chromatographs will make this technique even more applicable for nanosystem characterization. This

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implies that size exclusion chromatography might become an essential, economically feasible, and necessary analytical tool for ensuring the quality of nanoparticulate drug delivery systems.

Keywords: Size exclusion chromatography, Nanoparticulate, Drug delivery, Quality control

INTRODUCTION

Size exclusion chromatography (SEC) is a separation technique by which analytes are retained in a suitable polymeric packing material based solely on particle permeation into and out of the packing material.[1] This is the primary property that distinguishes SEC from other chromatographic techniques that are based on polarity and chemical interaction between the analyte and packing material. The packing material exposes a variety of pore sizes to the eluted species and, ideally, separation is imparted by molecular sieving of different particles in the sample. Particles that are larger than the pores are excluded from the pores and are not retained in the packing material, whilst very small particles are significantly retained in the pores and elute at a later stage. Particles with intermediate size ranges are separated through a combination of these sieving mechanisms and, therefore, correspond to intermediate retention times.[1,2]

The applications of SEC are manifold if coupled to appropriate detectors, e.g., light scattering photometers, viscometers, UV detectors, refractive index detectors, fluorescence detectors, NMR, FTIR, MS, scintillation counters, and various combinations of these to determine the molecular weight (ranging a few thousand to millions of gram $\cdot$ mol^{-1}), particle size (up to a few hundred nanometers), polymer chain conformation, chain composition, particle association behavior, purity and stability of polymeric systems, to name but a few.[3] These properties are all vital in the development of nanoparticulate drug delivery systems.

Nanotechnology is concerned with technological manipulation of material properties at a length scale between 1–100 nm.[4] As a restriction, nanoparticulate drug delivery systems should, therefore, also assume size aspects in this range; however, this is not always realistic, practical, and might be adjusted to fit a specific application.[5] In the day and age of nanotechnological advances, various applications have been realized in the biomedical field by nanoparticulate drug delivery systems including dendrimers,[6] core-shell nanoparticles,[7] polymeric nanocapsules,[8] and polymeric micelles.[9] The application of nanoparticulate drug delivery systems arises from their numerous advantages over their larger sized counterparts, i.e., crossing of biological barriers,[10] enhancement of solubility properties of drugs,[11] presentation of a biocompatible or targeted topography on their surfaces that could be recognized by cells, and circumvention of the abrasive nature of certain ceramic materials at a larger size scale.[12]

A wide selection of nanoparticles for drug delivery is manufactured from polymeric materials. SEC can be used to monitor at least three important characteristics of these polymers used to manufacture these delivery systems, namely, the quality of the materials employed, the quality of the derived nanoparticles, and stability of the delivery system. Quality of the employed starting material could be investigated with SEC analyses of molecular weight and particle size if suitable detection methods, i.e., light scattering is applied.[13,14]

The reason for developing quality control methods involving SEC, recalls the properties of materials at the nanoscopic level. These particles have a significantly larger surface to volume ratio compared to their macroscopic counterparts and, therefore, their behavior is fundamentally different.[15] By applying SEC, formulators can ensure that the required quality or sufficient degree of nanoscopic properties are retained for the macromolecular excipients and delivery systems by continuous evaluation of particle size and molecular weight characteristics, delivery system size characteristics, and finally time dependent size stability of the product.

ROLE OF SEC IN ENSURING DESIRED MACROMOLECULAR EXCIPIENT PROPERTIES

The properties of starting material could markedly influence the performance of the formulated nanoparticulate delivery systems. For this reason the FDA requires that all excipients that are formulated in the pharmaceutical industry have to comply with strict standards before it could be considered for use. These restrictions ensure that product properties can be correlated to the starting material properties in the situations where a product batch proved contaminated or defective.

Batch Conformity

A primary example for the need of restrictions on the use of cellulose powders was illustrated by SEC of commercially available cellulose powders.[16] Hemicellulose can be found in cellulose powders as a byproduct of the pulping and alkaline treatment of source materials to produce microcrystalline cellulose. Hemicellulose could influence the technological properties (since it is a swelling agent) of the excipient, and according to various monographs microcrystalline cellulose should contain 97–102% of cellulose. The study concluded that only two batches out of twenty two cellulose products complied with this restriction.

Careful optimization of the sampling conditions could decrease analysis time and solvent usage in industrial applications of SEC when used as a quality control measure. This was illustrated for a purification procedure for

insulin products.[17] Instead of singe batch conformity evaluations, a moving bed system could be employed by which four sampling ports were used to sample four insulin batches at selected intervals. Therefore, these batches of insulin could be evaluated by the same SEC after optimization of the sampling interval and injection gap periods to prevent mixing of different samples.

Selection of Polymeric Excipient

The quality of polymeric pharmaceutical excipients is routinely examined with SEC techniques, especially to select polymers that could be applied to control the residence time and pharmacokinetics of drugs formulated in a specific polymeric nanodelivery system. Generally, macromolecules with weights lower than 50 kDa and sizes below 6 nm tend to be filtered by the kidneys, whilst those exceeding 50 kDa could be affected by the liver by showing significant accumulation. Positively charged macromolecules tend to associate with negatively charged hepatic cell surfaces and the negatively charged polymeric carrier tend to escape this interaction; however, they are absorbed by sinusoidal liver cells.[18]

Therefore, the appropriate molecular weight of the polymer should be maintained in order to control the pharmacokinetics of the active ingredient or to suit the particular application as found for the multi application excipient, pectin, for which a wide size and weight distributions were found by SEC for different commercially available products. These pectin products all show differences in their swelling and mucoadhesive properties that were in some way related to their molecular weight. These properties significantly affected the residence times of drugs in the body as well as targeted tissue accumulation. Thorough weight characterization is, therefore, required before a pectin formulation is manufactured to suit the applications of this polymer in the formulation of cytotoxic drugs, mucoadhesive ophthalmic delivery systems, and prevention of pathogens to human cells.[19]

The size exclusion process is often time consuming, however, application of flow injection analysis by SEC made this analyses a feasible, time effective process as was demonstrated for poly(lactide-*co*-glycolide) polymerization and swelling characteristics. Consequently, this process ensures that a continuous analysis of the polymer could be performed to ensure that the dosage form properties will be consistent.[13]

The quality of starting materials that are used is important since they should impart the desired properties to the delivery system, such as nanosize or release control. These materials must be manipulated through some manufacturing technique to produce these nano-carriers for it to retain its properties. For example, the molecular weight of the starting material could markedly affect the functionality and drug release of the derived delivery

vehicle of implant device manufactured from poly(dimethyl siloxane)[20] and SEC could be employed to monitor the molecular weight of the starting polymer as well as to determine the purity of the material.

Quantification of Polymers in Pharmaceuticals

Quantification of functional excipients including the poloxamers 188 and 407 was aptly illustrated by employment of SEC. These excipients act as emulsifiers, surfactants, solubilizing agents, dispersing agents, as well as in vivo absorbance enhancers and their quantification in pharmaceutical products could again influence the product performance significantly. A limited number of analytical techniques were described to evaluate the content of poloxamers and some colorimetric methods[21–24] have been documented, however, application of these methods to solid pharmaceutical dosage forms proved inferior to a size exclusion method developed (Figures 1–3)[25] in the presence of various other pharmaceutical excipients, of which none showed significant interference in the analysis. These results show the linearity, specificity, and sensitivity of the SEC to distinguish between structurally similar molecules.

Dosage Form Optimization

A striking example of the application of SEC for evaluation of chitosan nanoparticles was found.[26] In this study, the effects of polymer weight and polymer salt form of chitosan on the particle size, drug encapsulation efficiency, and release properties of bovine serum albumin was determined. It could be seen from this study, that the relationships between molecular weight, particle size, drug encapsulation, and finally the release, were not that obvious and that a particular chitosan had to be chosen following after physicochemical characterization and evaluation of in vivo performance.[26] This clearly demonstrated the need for thorough polymer molecular weight characterization prior to formulation of the nanoparticles.

In a study concerning the production of gelatin nanoparticles, the production method was optimized by thorough characterization of the gelatin molecular weight as determined by SEC. It was illustrated that the size distribution of the produced particles varied with the molecular weight, temperature, ethanol concentration, and pH, resulting in significant agglomeration and polydispersity in the particle size if these manufacturing conditions were not controlled. With SEC the researchers could monitor the particle sizes and weight simultaneously and could be exploited to optimize the manufacturing conditions for these gelatin particles and preclude the solvation and swelling effects that were previously observed for agglomerates of gelatin nanoparticulates.[27]

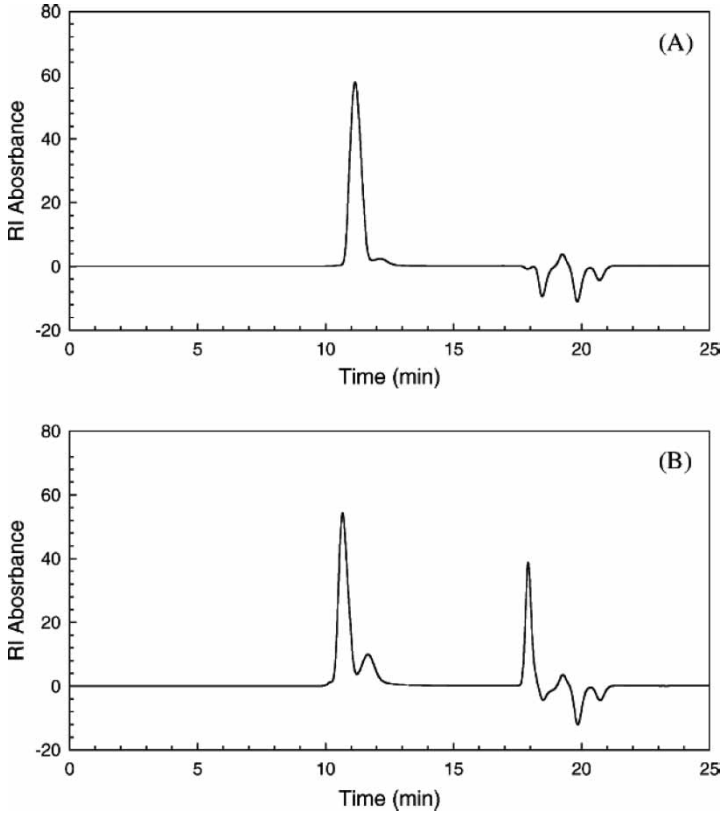

Figure 1. Size exclusion chromatograms of (A) poloxamer 188 and (B) poloxamer 407. Experimental conditions: stationary phase, two serially connected PLGel 3 μm mixed E columns (300 × 7.5 mm each); mobile phase, tetrahydrofuran; flow rate 1 mL/min; detection RI; temperature setting for the column and detector 35°C; solute concentration 2.0 mg/mL.[25]

The example of gelatin nanoparticles[27] reported that the gelatin particle could agglomerate under certain reaction conditions and a study on paclitaxel-loaded polybutylcyanoacrylate nanoparticulate drug delivery systems[28] showed that a decrease in particle size resulted in an increase in the zeta potential that disfavored agglomeration. Both these studies illustrated that SEC could clarify and develop these excipients to avoid agglomeration of particles that could potentially result in adverse effects in the body, i.e., trombi in the vasculature. Indeed, aggregation of nanoparticulates was identified as one of the challenges of designing successful nanoparticulate pharmaceutical systems; upon agglomeration the desired pharmaceutical properties could be lost during manufacturing stages and, as mentioned, result in adverse affects in the body.[5]

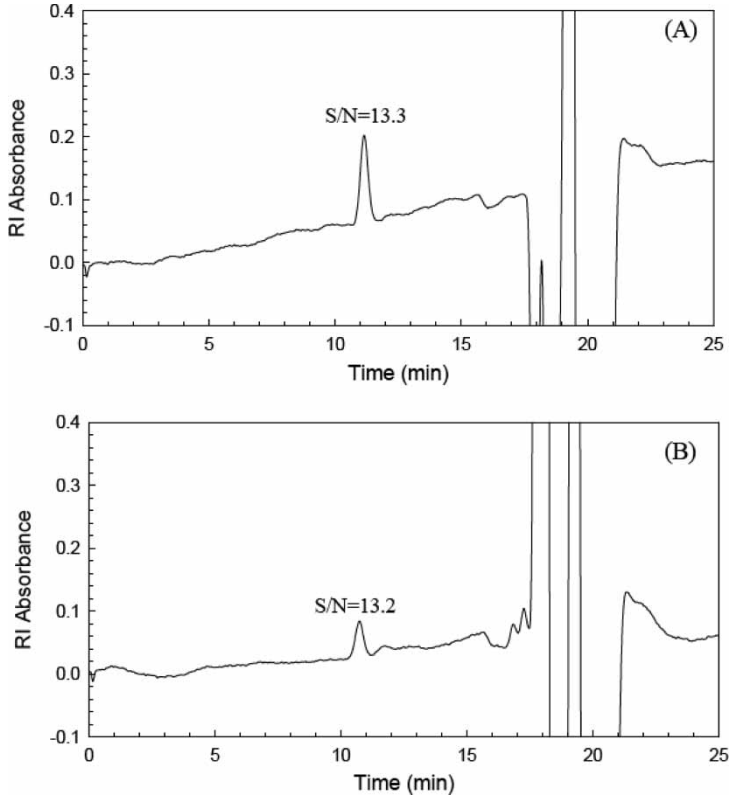

Figure 2. Size exclusion chromatograms of (A) poloxamer 188 and (B) poloxamer 407 at the limit of quantitation. Experimental conditions are the same as described before; solute concentration 0.005 mg/mL.[25]

Monitoring Synthesis

In addition to the gelatin and chitosan example characterization by SEC a polymer was synthesized by employment of an enzyme and also analyzed by SEC. The copolymer consisted of adipic acid and glycerol and was characterized through various techniques and gel permeation chromatography to monitor the molecular weight of the material and optimize the copolymerization conditions of temperature and enzyme concentration that was also time dependent. In addition, dexamethasone phosphate loading capacity of these nanoparticles were correlated to copolymer weight and size.[29]

Yet another polymer was synthesized and characterized with SEC to be evaluated for its utilization as a drug nanocarrier. An amphiphilic graft copolymer was synthesized by grafting of poly-L-aspartic acid to 1,3-trimethylene carbonate. Analysis by multi angle light scattering coupled to

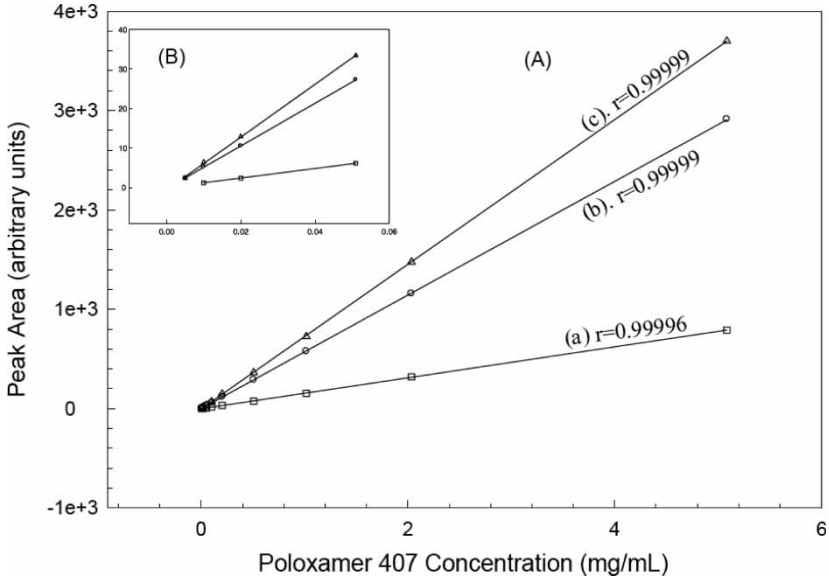

Figure 3. (Plot A) Calibration curve for poloxamer 407 with the concentration from 0.005 to 5 mg/mL. Symbol (□) and line (a): peak area for 11.69 min peak (diblock impurity peak) only; symbol (○) and line (b): peak area for the 10.71 min peak (poloxamer 407 peak) only; symbol (Δ) and line (c) sum of peak areas at 10.71 min and 11.68 min. (Plot B) Soom in plot at the lower concentrations.[25]

size exclusion chromatography revealed that the copolymer assumed a brush like structure with branching that confirmed the grafting reaction and, in addition, the conformation analysis of these polymers revealed that micelles were formed in water. The structure and composition of the polymers resulted in a complex, sustained release pattern of two drugs, i.e., prednisone acetate and tegafur from formulated nanoparticles with no detected degradation over a release period of four days.[30]

Purification

Purification and incorporation of certain materials into a specified formulation could ultimately determine its performance. The two properties are interrelated and could arise from synthesis reaction or nanoparticle loading procedures. A few examples are discussed to illustrate this point. SEC was also successfully employed to characterize and purify a novel block copolymer consisting of a poly(lactic acid) derivative and poly(ethylene glycol). The polymer weight was first characterized by a typical characterization chromatographic analysis, followed by a preparative size exclusion procedure to purify and fraction the polymer into the desired weight range. The fractions were then

employed to prepare nanoparticles that were loaded with a molecular probe to monitor uptake when passively targeted to solid cancer tumors.[31]

New Developments

A new development was recently realized in the form of size exclusion reaction chromatography, and arose from the need to reprocess and analyze materials after synthesis was completed. A size exclusion column could be utilized as the reactor in which a reaction could be performed to graft poly(-ethylene glycol) to the proteins α-lactalbumin and β-lactoglobulin. In principle, a reaction zone was created in the packing material that retained some reactants. As the reaction took place a more voluminous product formed and this product was excluded from the pores and eluted from the column. In addition, this purified the product by retention of reactants in the packing material. The method determined the ideal reactant injection conditions to ensure overlap in the column for the specified reaction time (Figure 4 shows the reaction results for the α-lactalbumin PEGylation). In conclusion, control of the introduction of reactants into the column and additional packing length could optimize the production of a mono-

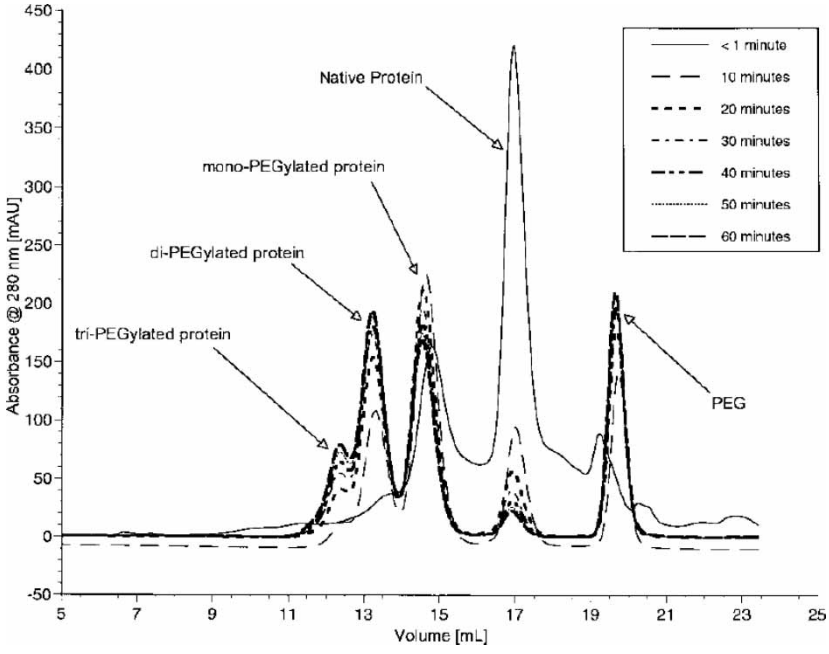

Figure 4. The effect of reaction time on the apparent molecular weight of α-lactalbumin for batch PEGylation.[32]

PEGylated protein of predictable properties that was purified and separated from its reactants and undesired higher PEGylated species.[32]

A screening procedure for drug discovery compounds have also been realized based on a preparative or purification method based on size exclusion. A mixture of small molecule ligands, such as actinonin, were introduced to a macromolecular target, i.e., peptide deformylase and incubated and then followed by size-exclusion separation. Compound which showed no affinity for the protein were retained in the column, whilst the larger reaction products were eluted. The retained products were then analyzed and excluded as potential drug candidates since they did not interact with the target protein. The analyses of these unbound ligands were also considerably easier compared to analyses of the protein-ligand products.[33]

Drug-Polymer Interaction in the Dosage Form

SEC coupled to light scattering and viscometry detectors could also illuminate the effects of the assimilation of a drug into polymer chains. A water-soluble conjugate of paclitaxel and poly[*N*-(2-hydroxypropyl)methacrylamide)] (PNU) was studied and compared to unconjugated samples of poly[*N*-(2-hydroxypropyl)methacrylamide)] (PHPMA). Comparison of the polymeric carrier and the drug polymer conjugate revealed that the conformation of the polymer chains was markedly affected even though the total concentration of drug was approximately 1% of the total conjugate. However, it was also concluded that the drug moieties in the chain did not demonstrate any significant intramolecular force interactions.[33] Figure 5 shows the conformation

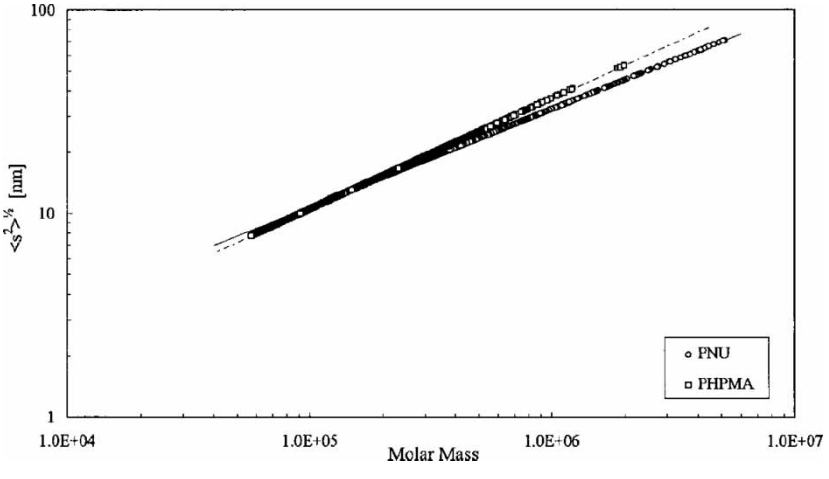

Figure 5. Comparison of the $\langle s^2 \rangle^{1/2} = f(M)$ power laws for PHPMA and PNU constructed by gathering the data of seven higher molar mass PNU fractions.[34]

plots which were derived from light scattering analysis. The figure plots root mean square radius of gyration versus molar mass.

Application of the PHPMA polymer to another antitumor drug, camptothecin,[35] again illustrated exhaustive characterization of both raw materials and the conjugated polymeric delivery systems. Narrow molar mass distribution (MMD) PHPMA polymer carrier samples were obtained and compared with a synthesized laboratory batch. The difference in retention times, as well as weight polydispersity, was determined by size exclusion chromatography for the different batches and is shown in Figure 6.

A succession of measurements followed the evaluation of molar mass for these PHPMA samples in order to determine the conformation of the polymer conjugates through off-line viscometry of 11 separate narrow standards, and subsequently evaluating the suitability of the on-line method to determine the same parameters. Figures 7 and 8 shows the results of off-line and on-line methods and generally showed excellent agreement, and the on-line method showed the suitability of deriving the plots from a broad standard.

Due to instrumental limitations, the MALS wavelength of 632.8 nm proved unsuitable for measurement of radius of gyration, $\langle s^2 \rangle^{1/2}$ for small fractions; however, the high molar weight fractions could be used to extrapolate the conformation. Figure 9 shows the extrapolation of the conformational data for four higher molar mass fractions of the conjugates. Finally, it was concluded that the laboratory batch of the PHPMA drug conjugate would be suitable for clinical use since its characteristics complied with the clinically evaluated narrow standards also evaluated in the study.

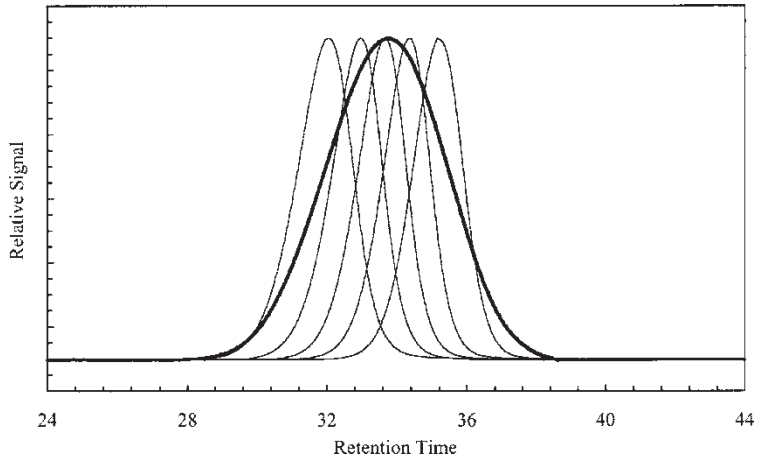

Figure 6. Comparison of the refractometer signals of five narrow MMD fractions (5–9) and of a broad MMD PHPMACPT sample (lot 5, boldface line).[35]

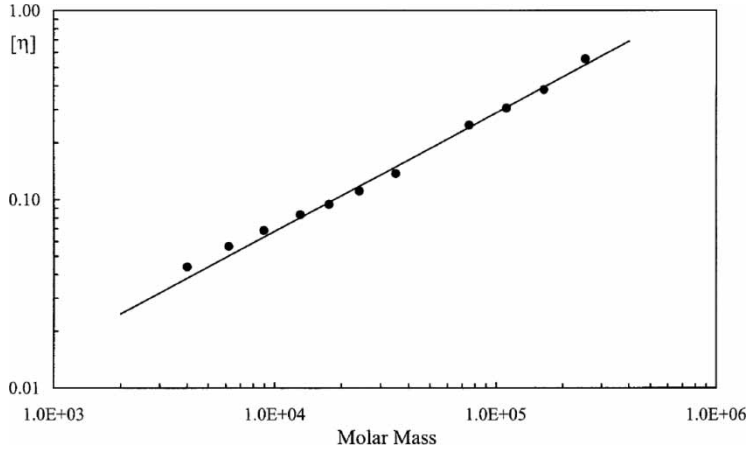

Figure 7. MHS plot for the PHPMA-CPT conjugate from 11narrow MMD fractions.[35]

These examples are but a small selection of studies pertaining to biological applications and the role that SEC has to play in the quality control of the materials and conjugates used in the production of nanoparticulate delivery systems. It was apparent that the reports here indicated a link between material properties and the final application of the material.

Clearly, SEC can be employed to monitor or control the synthesis of new materials and conjugates from which nanoparticles could be prepared and should prove more vital in the future since novel polymeric materials are currently being developed.

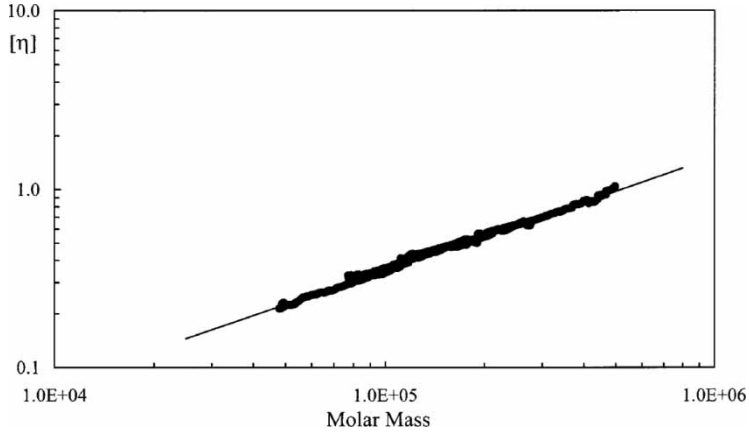

Figure 8. MHS plot for the PHPMA-CPT conjugates from online MALS and SCV data.[35]

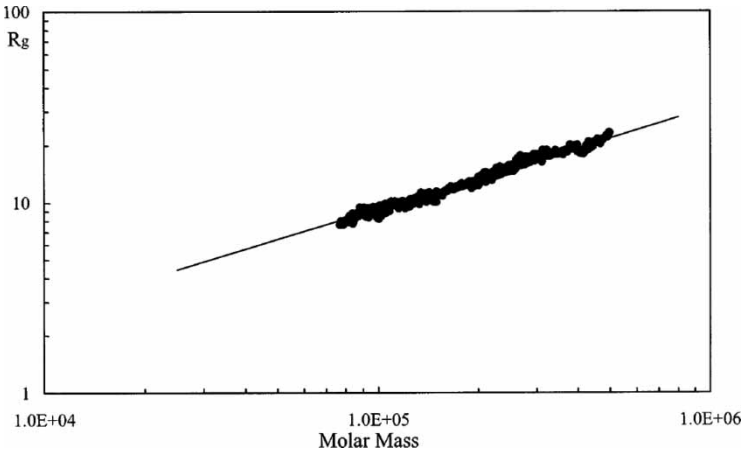

Figure 9. $R_g = f(M)$ power law for the PHPMA-CPT conjugate from on-line MALS data.[35]

CHARACTERIZATION OF NANOPARTICULATE DRUG DELIVERY SYSTEMS

SEC has also found application in the characterization of nanoparticulate delivery system performance as well as in quality control aspects of these formulations.

Liposomes

Although, liposomes have often been described as micro sized amphiphilic vesicles consisting of lipid bilayers, numerous nanosized variations have been produced over the years. An outline of the applications of SEC in the development and characterization of liposomes is illustrated in Figure 10. The differences between conventional and high performance SEC were illustrated by their specific application in liposome delivery systems. The conventional method refers to employment of the size exclusion technique with SEC columns with specific size range restrictions. The conventional techniques served best as a preparative and purification technique and, in addition could be employed for drug loading. The high performance technique incorporates a full HPLC system coupled to columns with packing materials with a wider pore size range for a relatively fast analysis compared to conventional SEC. The use of this system enables accurate size distribution, stability, and reconstitution characterization, and additionally realizes time resolved studies of release kinetics, permeability, and encapsulation stability.[36]

An example of size distribution and stability studies of liposomes was reported employing size exclusion chromatography.[37] The study exploited

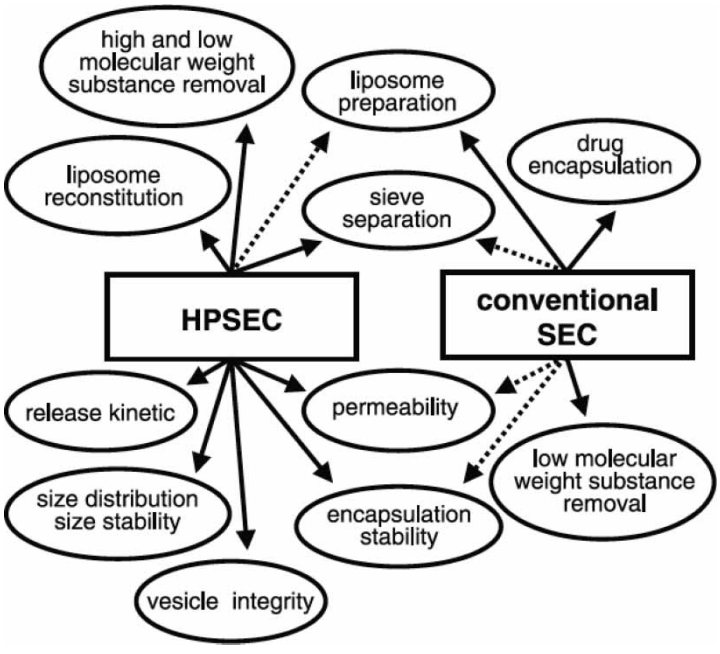

Figure 10. Application fields of HPSEC and conventional SEC in liposome technology from preparative uses to analytical ones. According to the purpose of analysis, the full arrows indicate the best potentialities of HPSEC and SEC, while dashed arrows show possible but less efficient routes.[36]

various detection methods including light scattering, refractive index, fluorescence, and radioactivity, and only the application of the light scattering technique would be highlighted here, since it could be utilized for studying aggregation of the liposomes and its time dependent evolution as demonstrated in Figure 11. Again, liposomes served only as an example, and the technique could be applied to other nanoparticulate systems to illustrate their aggregation behavior to serve as a quality control measure of any prepared nanoparticulate system.

Stimuli-Responsive Nanoparticulate Systems

Development of stimuli responsive polymeric nanoparticles is a recent development in membrane science. An example was found where a membrane was fabricated from poly(*N*-isopropylacrylamide) that was grafted onto an ion-track pore wall in another polymer support, poly(ethylene terephtalate). By exposing the membrane to various temperature levels, the pores on the membrane opened or closed in accordance to swelling of the polymer

membrane pores and allowed conductivity measures of selected ions and quantitation poly(ethylene glycol) molecules. The poly(ethylene glycol) served as a marker to determine if the membrane would allow organic molecule permeation in addition to ion diffusion, and SEC was crucial to determine the range of poly(ethylene glycol) that was allowed to permeate. The method established that organic molecules larger than 2 nm could not transverse.[38]

The example above could illustrate a very significant application in evaluating the efficiency of membranes to selectively allow permeation of certain substances from the interior of a delivery system, and thermoresponsive (and

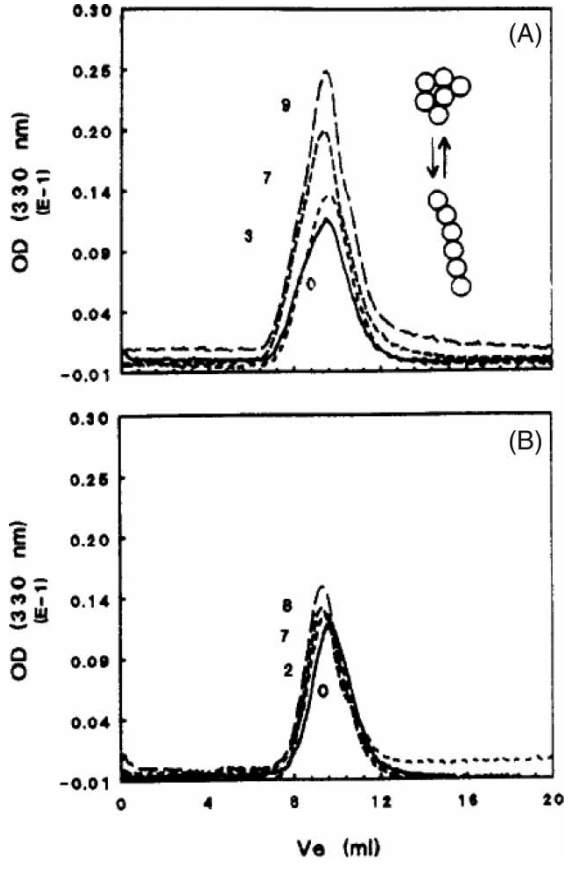

Figure 11. Study of vesicle size stability. Coupling light scattering to HPLC-GEC allows a distinction between aggregation and fusion processes to be made. Gel exclusion chromatograms were recorded by turbidity at 330 nm, of pure EPC SUV (A) and mixed EPC/EPA (91/9 mol ratio) SUV (B); (A) after 0, 3, 7, and 9 days; (B) after 0, 2, 7 and 8 days at 25°C (sample loading, 50 p,l; eluant flow rate, 1 mL/min). The eluant was the buffer (aqueous 145 mM NaCI, 10 mM HEPES, pH 7.4) used for vesicle preparation and calibration curve determination.[37]

other stimuli) nanoparticles for drug delivery would seem a very promising delivery tool. The employment of SEC could further develop future membranes that could demonstrate different permeation properties and could serve as a control measure in industry to ensure that the manufacture delivery systems comply with a certain standard that was set for the delivery system.

Physical Stability

The production of drug loaded nanoparticles is not without problems and often these particles tend to agglomerate or flocculate as found for nanosuspensions. Polymers have often been used to prevent this flocculation phenomenon by adhering these molecules to the surface of the nanoparticle. The effect of the polymer charge has been demonstrated to repel adjacent particles that were also covered with charged polymer particles. Hydroxypropylcellulose was previously used to stabilize a classified drug nanosuspension. Evaporative light scattering was combined with SEC to determine surface coverage of the nanosuspension to aid and optimize the development of the formulation employed by the pharmaceutical company. The bound and unbound fractions of the polymer could be determined by a validated method to ensure future quality control of the formulation procedure[39] as shown in Figure 12.

Biodegradation

The synthesis of biodegradable polymeric nanoparticles based on poly(ε-caprolactone) grafted to dextran chains have been described using SEC as the characterization method. Dextran grafting of the nanoparticle surface prevents uptake by macropghages, prolonging blood circulation. Additionally, polysaccharides could be used for target specific drug delivery because of recognition by surface proteins. A potential advantageous effect was also realized in that the total content of dextran significantly influenced the adsorption to bovine serum albumin (a common blood serum carrier protein) with a maximum nanoparticle adsorption seen at 33% dextran content. Different weight dextrans also influenced bovine serum album adsorption with a 40 000 $g \cdot mol^{-1}$ dextran showing 2- to 4-fold higher adsorption compared to its 5000 $g \cdot mol^{-1}$ counterpart. The pharmacokinetics of the dextran grafted poly(ϵ-caprolactone) could therefore be adjusted and SEC provided essential information to modify these properties.[40]

Target-Specific Delivery

Site specific delivery of nanoparticles has been identified as a very attractive advantage in the employment of the technology. Coating of these particles

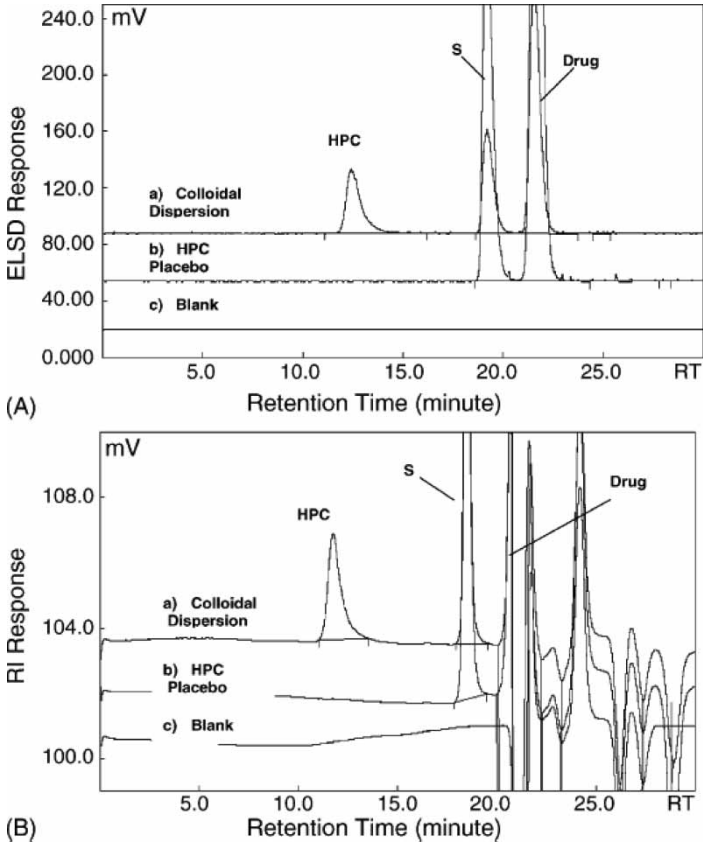

Figure 12. ELS (panel A) and RI (panel B) chromatograms showing separation of HPC from the other components of the colloidal dispersion: dispersing agent S and the drug.[39]

with biodegradable and biocompatible poly(lactic acid)-fetuin could circumvent problematic uptake of these particles by macrophages, a problem that is compounded by particle size aspects as well as surface characteristics.[41] The successful employment of nanoparticles therefore necessitates meticulous characterization of particle size distribution to establish the in vivo correlation to their effects. Fetuin coated poly(lactic acid) particles were prepared in a size range of 50–200 nm. The poly(lactic acid) was radiolabeled with ^{14}C and fetuin with ^{125}I to determine the concentration of the particles in each fraction that was collected from the size exclusion chromatograph. These samples were analysed with photon correlation spectroscopy and the results revealed the amount of fetuin bond to the nanoparticles as a function of particle diameter Figure 13.[42]

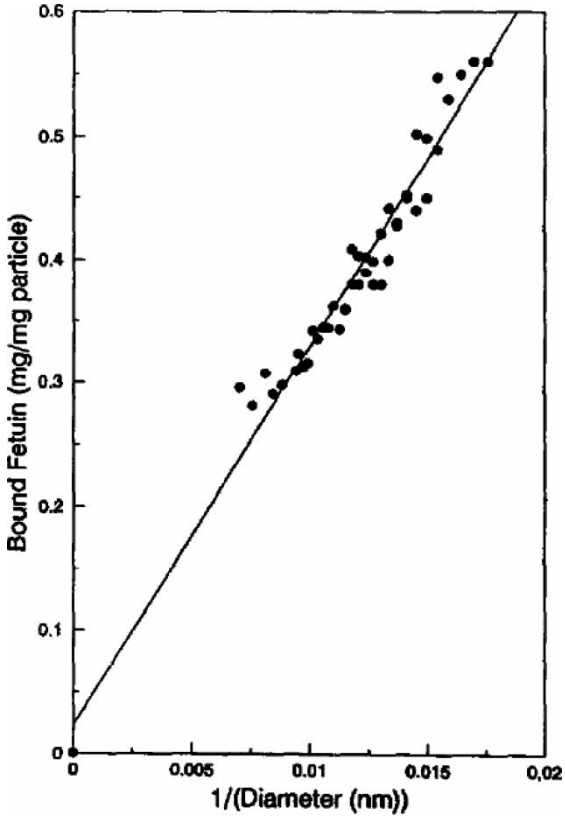

Figure 13. Amount of bound protein per unit mass of the polymer as a function of the inverse diameter.[42]

Substrate-Nanoparticle Interaction

This method could illustrate a useful tool to analyze particle size and the relationship of substrate binding as a function of nanoparticle size. It could be suggested that future studies could exploit similar techniques in order to establish the optimum particle size to bind or incorporate a specific active ingredient at a selected dose. Additionally, the method could also be employed to estimate or assay a specific product to determine both particle size and drug loading whether the drug is a protein or polymeric material.

Protein Delivery

Dwelling on the topic of proteins and their ever increasing role in pharmaceutical applications, we discuss yet another protein related study.

Nanoparticles were prepared by polymerization of human serum albumin and an antibody, IgG1 to result in polymeric colloidal carrier matrix. This nanoparticle antibody polymerization was optimized by optimization of the degree of thiolation of IgG1, which was responsible for the cross linking polymerization with the human serum albumin particle.[43] SEC was used to optimize the modification of the antibody by thiolization of the antibody surface. The thiolation results obtained with SEC are shown in Figure 14. The results suggested that a 2 hour period was sufficient for production of thiolated particles in a 50-fold excess without significant dimerization of the antibody. These particles were then compared to several controls and it could be demonstrated that the particles prepared from thiolated IgG1 showed higher biological efficiency than those prepared without thiolation, or those coated by PEG. This study showed a full design of a nanoparticulate system and evaluated its biological activity and stability. SEC proved vital for optimization of the system and linking its performance to basic material properties.

The coating of poly(lactic acid) nanoparticles ranging 90–250 nm with human serum albumin was also determined by employing a SEC analysis with a turbidity detection method based on Mie's law that could easily indicate linearity of turbidity as function of the square of the mean population diameter.[44] This analysis provided proof that the albumin coated the nanoparticles and did not get incorporated into the polymer matrix, furthermore proving that the particles which were coated could be related to their increased residence time in the body compared to uncoated particles.

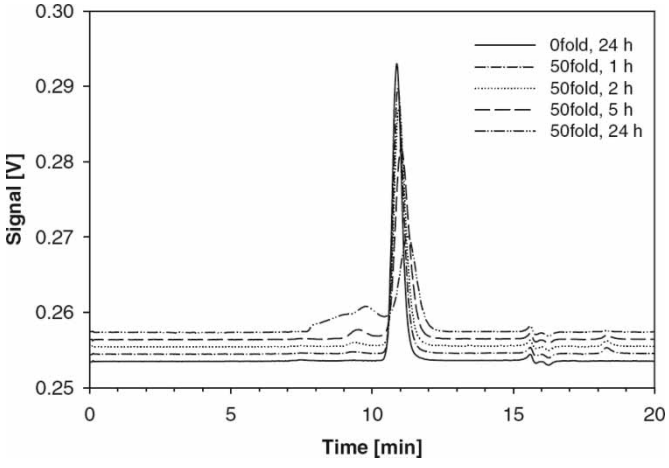

Figure 14. Thiolation of trastuzumab with 50-fold molar excess of 2-iminothiolane. The antibody was analyzed by SEC after 1, 2, 5, and 24 h of reaction time. Tastuzumab was detected at a retention time of about 11 min whereas higher conjugates were detected at shorter times.[43]

Biodistribution

Nanoparticles were also developed from synthetic monomer polymerization to reach the target size below 50 nm in order to ensure the extravasation of nanoparticles from the blood vessels to a selected target. Isohexylcyanoacrylate and isobutylcyanoacrylate particles were prepared and loaded with the drugs ampicillin and dexamethasone.[45] The effects of pH showed that more acidic conditions resulted in larger particles, whilst addition of MeOH or acetone resulted in a decrease in particle size. The SEC analysis of polymer chain weight indicated that the weight was virtually unchanged after loading, indicating that the drug was mechanically trapped in the particle matrix and did not react with the monomer units. Large amounts of the drug were released despite this entrapment and confirmed that esterases could result in bioerosion of the polymer matrix. In addition, the particle size demonstrated that these particles would deliver drugs by crossing the blood vessel walls.

Some useful applications of SEC in the performance of nanoparticulate drug delivery systems were demonstrated by appropriate detection methods coupled to SEC. The development of these systems was often described as an extension of the development of a selected starting material or characterization thereof. Some instances could clearly illustrate a link, and it should perhaps be suggested that all nanoparticulate systems that are developed should follow a route of investigation at the starting material level and then continued at the application level to optimize the product performance.

STABILITY ISSUES MONITORED WITH SEC

Aggregation and Complexation

SEC techniques could be combined with appropriate detection methods, i.e., light scattering, UV detection, and refractive index measurements to study aggregation behavior and complexation[46] in addition to chemical instability, and subsequent biodegradation and biodistribution of a polymeric drug carrier could affect the drug delivery system adversely. It was previously proven that polystyrene nanoparticles ranging 50 nm–3 μm showed a particle size dependent distribution in mice[47] with particles exceeding 300 nm not entering the blood circulation as was also evident from polyalkylcyanoacrylate nanocapsules studies following X-ray analysis.[48] Concerning the polystyrene nanoparticles, the 50 nm fraction was absorbed to the highest extent (34% of dose), whilst 100 nm particles ranked second (26% of dose). Particles larger than 100 nm did not reach the bone marrow, whereas up to 7% accumulation of the 50 nm doses were observed in organs. SEC was employed to determine the particle absorptions and this study provided very significant information for the development of future nanoparticulates and

should be considered in the design of these delivery systems and also the stability of these delivery systems. It could be required by a certain application that a localized effect is desired or the polymeric material should not accumulate in the body as consequence of degradation of the original polymeric delivery system to smaller particles.

Physiological Stability

A stability study was performed on poly(lactic acid) particles coated with albumin or poly(vinyl alcohol) to determine the effect of the polymer coating on particle degradation in simulated gastric fluid containing pepsin and simulated intestinal fluid containing pancreatin.[49] No degradation was illustrated for both coatings in pepsin containing fluid, however, pancreatin enriched medium degraded the albumin coating significantly. Despite the degradation no particles accumulated since the poly(lactic acid) was readily metabolized to lactate. The size exclusion chromatograms are shown in Figure 15 to illustrate the effect of digestive fluid on polymer degradation and subsequently on polymer weight as the time dependent function.

SEC was also used to study the incorporation of cyclosporine A loaded into poly(lactic acid) particles that were coated with poly(ethylene glycol).[50] Cyclosporine A could be considered as one of the model drugs that pose all the problems encountered in pharmaceutical science. It is a poorly water-soluble, biodegradable peptide and has shown poor

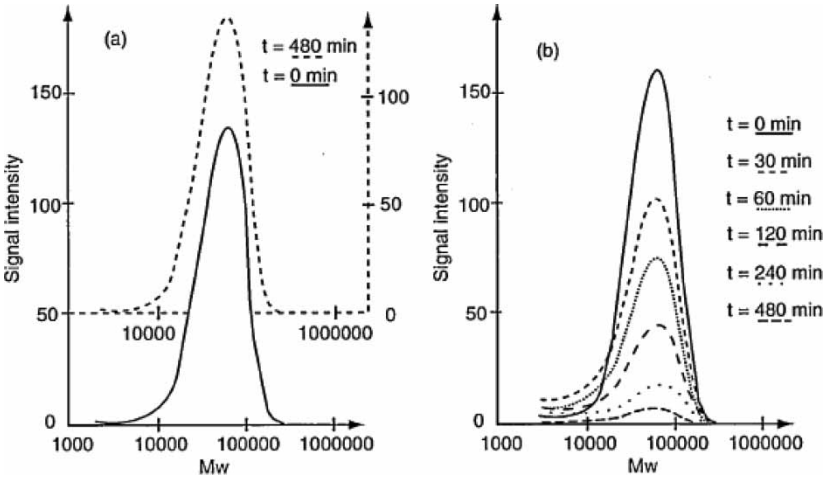

Figure 15. Size exclusion chromatogram of albumin coated PLA_{50} nanoparticles after 0 and 480 min in incubation of simulated gastric fluid (a) and simulated intestinal fluid (b) (USP XXII).[49]

bioavailability in several studies.[51,52] Nanoparticles were developed that contained this troublesome drug and evaluated by SEC to characterize particle weight and stability of the particles.

The study[50] showed that poly(ethylene glycol) was an effective control measure to control release of the drug compared to uncoated poly(lactic acid) loaded particles. Stability analysis showed that the ester linkage between the PEG and poly(lactic acid) did not degrade significantly even over prolonged periods of time. In addition, it could be illustrated that the surface loading of cyclosporine into the PEG layer of nanoparticles was also higher than that of microparticles, again emphasizing the difference in the surface volume ratio encountered between nano- and microparticulates. The entrapped cyclosporine then diffused through the PEG layer with controlled release. This study is one of the ultimate examples of nanotechnology applied in pharmaceutical science and the application of SEC chromatography to evaluate the delivery system from the top down.

Product Storage

A study performed on human serum albumin indicated the adverse effects of temperature on the protein stability in pharmaceutical preparations. The size exclusion analysis of the preparation indicated that protein concentration decreased significantly at 55 and 70°C. A dimer also seemed to form with a weight double that of the original protein. Monomeric species could also be observed and higher aggregates were found in the different formulations. No specific pattern or kinetics could be established; however, it was concluded that the formulation variables significantly influenced stability at various temperatures and storage times.

Some examples have been discussed to illustrate the application of SEC in establishing the integrity of nanoparticulate delivery systems after manufacture, however, time dependent stability aspects could also be identified, including agglomeration and biodegradation behavior.

CONCLUSION

Pharmaceutical standards have been documented in compendial format for various medicinal products. Specifications are set for starting materials and excipients as well as the active ingredients. However, some excipients or products require characterization beyond compendial standards and for some instances techniques that are not commonly used, i.e., SEC, capillary electrophoresis, and supercritical fluid chromatogrpahy.[53] Recently, some challenges have been identified in the pharmaceutical industry regarding chromatography, and these include suitable evaluation procedures for nanosized drug delivery systems.

The studies that were discussed in this review illustrated that nanoparticulate drug delivery systems already employ SEC in the developmental, characterization, and stability evaluation phases of these products. Many of these products were shown to be polymeric in nature and included several proteins and the development of even more of these products seems very probable. In addition, some problematic, as well as characteristic aspects in the development of these nanodelivery systems can already be identified by SEC when analyzing the starting materials. It would seem that both starting material characterization in conjunction with delivery system evaluation by SEC can greatly increase the success of nanoparticulate drug delivery systems. The implementation of SEC as a quality control procedure for nanosized delivery systems is constantly enhanced by improvement in SEC, such as columns,[54] which can be used under more extreme conditions such as high flow rates. Shortening analysis times has developed to a point where we can now employ superspeed SEC.[55]

Finally, we would like to suggest that improved standards for SEC might need to be developed to accommodate nanoparticulate drug delivery systems, since it is their specific size related properties that distinguish them from other medicinal products.

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