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SEC-MALLS AND HYPHENATED ANALYTICAL TECHNIQUES IN PHARMACEUTICAL CHARACTERISATION: APPLICATIONS IN MOLECULAR WEIGHT AND STRUCTURAL ANALYSIS

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Abstract

Size Exclusion Chromatography coupled with Multi-Angle Laser Light Scattering (SEC-MALLS) and hyphenated analytical techniques have emerged as powerful analytical platforms for molecular weight determination and structural characterization of pharmaceutical and biopharmaceutical products. The increasing complexity of biologics, biosimilars, proteins, peptides, vaccines, polymers, and nanomedicine formulations has created significant demand for advanced analytical methodologies capable of simultaneous molecular separation, aggregation profiling, and structural analysis. SEC-MALLS combines chromatographic separation based on hydrodynamic volume with absolute molecular weight determination through multi-angle light scattering detection, thereby enabling comprehensive characterisation of molecular mass, size distribution, aggregation behaviour, conformational stability, and molecular heterogeneity without dependence on calibration standards. In addition to SEC-MALLS, several hyphenated analytical systems, including LC-MS, LC-MS/MS, LC-NMR, GC-MS, CE-MS, SEC-DLS, SEC-RI, and SEC-UV, provide enhanced analytical sensitivity, structural elucidation capability, and impurity profiling for complex pharmaceutical systems. The present review comprehensively discusses the principles, instrumentation, working mechanisms, applications, method development strategies, analytical advantages, and limitations of SEC-MALLS and multidetector hyphenated analytical techniques in pharmaceutical characterisation. Particular emphasis is placed on applications in monoclonal antibody aggregation studies, protein characterisation, biosimilar comparability assessment, nanoparticle analysis, liposomal formulations, dendrimers, and advanced drug delivery systems.

Keywords: *SEC-MALLS, Hyphenated analytical techniques, Molecular weight determination, Biopharmaceutical characterisation, Nanomedicine analysis, Structural characterization, multi-angle light scattering.*

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INTRODUCTION

Pharmaceutical characterization plays a critical role in the development, quality evaluation, stability assessment, and regulatory approval of modern drug products and biopharmaceutical formulations. Comprehensive physicochemical characterization is essential for understanding molecular structure, molecular weight distribution, aggregation behavior, conformational stability, and product heterogeneity, particularly for complex biomolecules such as monoclonal antibodies, peptides, proteins, vaccines, and nanoparticle-based drug delivery systems [1]. Among the various characterization parameters, accurate molecular weight determination is considered one of the most important analytical requirements because molecular size and distribution directly influence biological activity, pharmacokinetics, immunogenicity, therapeutic efficacy, and product stability [2]. Conventional analytical techniques often provide only relative molecular weight estimations and may fail to adequately characterize structurally heterogeneous or aggregated biomolecular systems. Consequently, advanced analytical methodologies capable of simultaneous molecular weight and structural analysis have become increasingly important in pharmaceutical and biopharmaceutical research.

The rapid evolution of hyphenated analytical techniques has significantly improved pharmaceutical characterization by combining chromatographic separation with highly sensitive spectroscopic and mass-based detection systems [3]. Techniques such as LC-MS, LC-MS/MS, LC-NMR, GC-MS, CE-MS, and

SEC-MALLS provide enhanced selectivity, structural elucidation capability, impurity profiling, and multi-parameter analysis compared with conventional standalone analytical methods. Among these approaches, Size Exclusion Chromatography coupled with Multi-Angle Laser Light Scattering (SEC-MALLS) has emerged as one of the most powerful and widely applied techniques for absolute molecular weight determination and structural characterization of complex pharmaceutical and biopharmaceutical products [4]. SEC-MALLS enables direct measurement of weight-average molecular weight, radius of gyration, polydispersity index, aggregation profile, and molecular conformation without dependence on column calibration standards. The technique has gained substantial importance in the characterisation of monoclonal antibodies, biosimilars, protein aggregates, polymeric nanoparticles, liposomes, dendrimers, and advanced nanomedicine formulations because of its high sensitivity, non-destructive analysis, and ability to evaluate macromolecular heterogeneity [5].

In recent years, increasing regulatory expectations for detailed biomolecular characterisation, bio similarity assessment, and quality-by-design approaches have further accelerated the application of SEC-MALLS and multidetector hyphenated analytical systems in the pharmaceutical industries [6]. These technologies play a crucial role in ensuring product consistency, process optimisation, stability monitoring, and compliance with regulatory guidelines issued by agencies such as the United States Food and Drug Administration and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Therefore, the present review comprehensively discusses the principles, instrumentation, applications, method development strategies, analytical challenges, and future perspectives of SEC-MALLS and hyphenated analytical techniques in pharmaceutical characterisation with particular emphasis on molecular weight determination and structural analysis of pharmaceutical and biopharmaceutical products.

FUNDAMENTALS AND PRINCIPLES OF SEC-MALLS

Size Exclusion Chromatography coupled with Multi-Angle Laser Light Scattering (SEC-MALLS) is one of the most powerful analytical platforms for molecular weight determination and structural characterisation of macromolecules, biopharmaceuticals, proteins, polymers, and nanoparticle-based drug delivery systems. The technique combines chromatographic separation based on molecular size with absolute molecular weight determination through laser light scattering analysis, thereby providing comprehensive information regarding molecular mass, size distribution, aggregation behaviour, conformation, and sample heterogeneity [7]. SEC-MALLS has gained extensive importance in pharmaceutical and biopharmaceutical analysis because conventional chromatographic methods generally estimate molecular weight relative to calibration standards, whereas SEC-MALLS directly determines absolute molecular weight independent of molecular standards or retention time calibration [8].

Size Exclusion Chromatography (SEC), also referred to as gel filtration chromatography or gel permeation chromatography, separates molecules according to their hydrodynamic volume or effective molecular size in solution. The stationary phase used in SEC consists of highly porous particles containing pores of controlled size distribution. During chromatographic separation, smaller molecules diffuse into the pores of the stationary phase and therefore experience a longer residence time within the column, whereas larger molecules are excluded from the pores and elute earlier [9]. Consequently, separation occurs primarily based on hydrodynamic volume rather than chemical interactions with the stationary phase. SEC is considered a mild and non-destructive separation technique because analytes are generally eluted under native solution conditions without significant structural alteration. The separation efficiency depends on factors including pore size distribution, particle size, column dimensions, flow rate, and mobile phase composition. SEC is widely employed for the characterisation of proteins, monoclonal antibodies, peptides, polymers, nanoparticles, liposomes, and biosimilar products because it efficiently resolves monomers, oligomers, aggregates, and degradation products [10].

Multi-Angle Laser Light Scattering (MALLS) is a highly sensitive detection technique that measures the intensity of scattered light produced when a laser beam passes through molecules in solution [11]. The scattered light intensity is measured simultaneously at multiple scattering angles relative to the incident laser beam, enabling direct calculation of absolute molecular weight, radius of gyration (Rg), and molecular conformation without dependence on retention time or calibration standards. The intensity of scattered light is directly proportional to molecular mass and concentration of the analyte. MALLS analysis is particularly advantageous for characterisation of heterogeneous biomolecules and aggregated systems because it provides molecular weight distribution across the entire chromatographic peak [12]. The radius of gyration (Rg) obtained from angular scattering measurements reflects the spatial distribution of mass around the centre of gravity of the molecule and provides valuable structural information regarding molecular conformation, branching, and aggregation state. Additionally, SEC-MALLS enables determination of the polydispersity index (PDI), which describes the width of molecular weight distribution and sample heterogeneity. Monodisperse systems possess PDI values close to unity, whereas highly heterogeneous samples exhibit broader molecular weight distributions and larger PDI values [13].

The combined SEC-MALLS system integrates chromatographic separation with real-time multi-angle light scattering detection for simultaneous molecular separation and structural characterization. During

analysis, analytes are first separated according to hydrodynamic size by SEC and subsequently pass through UV, refractive index (RI), and MALLS detectors connected in series [8]. The concentration of analytes is determined using UV or RI detectors, while MALLS simultaneously measures angular light scattering intensity to calculate molecular weight and structural parameters across the chromatographic profile. Online coupling of SEC with MALLS provides several analytical advantages including direct molecular weight determination, aggregate analysis, conformational assessment, and evaluation of molecular heterogeneity in a single experiment. This multidetector configuration has become especially important for characterization of monoclonal antibodies, protein aggregates, polymeric nanoparticles, lipid nanoparticles, dendrimers, and biosimilars because these systems often exhibit structural complexity and aggregation behavior that cannot be adequately characterized using conventional chromatographic methods alone [14].

The molecular weight and structural calculations in SEC-MALLS are based on fundamental light scattering equations. The Rayleigh scattering equation relates scattered light intensity to molecular weight, analyte concentration, refractive index increment, and scattering angle.

RAYLEIGH SCATTERING EQUATION

$$\frac{Kc}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c$$

Where:

- K= optical constant
- c= analyte concentration
- $R(\theta)$= excess Rayleigh ratio
- M_w= weight-average molecular weight
- $P(\theta)$= particle scattering function
- A_2= second virial coefficient

The Debye equation is widely applied for determination of molecular weight and intermolecular interaction parameters in dilute polymer and protein solutions.

Debye Equation

$$\frac{Kc}{R(\theta)} = \frac{1}{M_w} + 2A_2 c$$

The polydispersity index (PDI) is calculated using the ratio of weight-average molecular weight to number-average molecular weight.

Polydispersity Index Equation

$$PDI = \frac{M_w}{M_n}$$

The radius of gyration (Rg) is obtained from angular dependence of scattered light intensity and provides important structural information regarding molecular dimensions and conformation.

Radius of Gyration Relationship

$$P(\theta) \approx 1 - \frac{16\pi^2 R_g^2}{3\lambda^2} \sin^2\left(\frac{\theta}{2}\right)$$

SEC-MALLS, therefore, represents a highly advanced and versatile analytical platform for absolute molecular characterisation and structural analysis in pharmaceutical and biopharmaceutical research. The technique offers substantial advantages, including direct molecular weight determination, aggregate profiling, non-destructive analysis, high sensitivity, and simultaneous multi-parameter characterisation, thereby making it indispensable for modern pharmaceutical characterisation and quality assessment [15].

Table 01: Comparison of SEC with Conventional Chromatographic Techniques

Technique	Separation Principle	Molecular Weight Determination	Major Application
SEC	Hydrodynamic size	Relative/absolute (with MALLS)	Proteins, polymers, nanoparticles
HPLC	Polarity/interaction	Indirect	Small molecule analysis
Ion Exchange Chromatography	Charge-based separation	Not direct	Protein purification
Affinity Chromatography	Specific binding interaction	Not direct	Biomolecule isolation
Gel Electrophoresis	Charge and size	Approximate	Protein/DNA separation

HYPHENATED ANALYTICAL TECHNIQUES IN PHARMACEUTICAL CHARACTERISATION

Hyphenated analytical techniques have become indispensable tools in modern pharmaceutical characterisation because they combine the separation efficiency of chromatographic systems with the detection sensitivity and structural elucidation capability of advanced spectroscopic or mass spectrometric techniques. These integrated analytical platforms provide comprehensive physicochemical information regarding molecular structure, molecular weight, impurity profile, degradation behavior, conformational stability, and biomolecular heterogeneity, thereby significantly improving pharmaceutical quality assessment and regulatory compliance [16]. Conventional standalone analytical techniques often provide limited structural or molecular information; however, hyphenated analytical systems enable simultaneous separation, identification, and quantification of complex analytes within a single analytical run. Among chromatographic hyphenated systems, Liquid Chromatography–Mass Spectrometry (LC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) are extensively utilized for impurity profiling, metabolite identification, peptide

mapping, degradation studies, and quantitative pharmaceutical analysis because of their high sensitivity, selectivity, and accurate mass determination capability [17]. Gas Chromatography–Mass Spectrometry (GC-MS) is widely employed for volatile and semi-volatile compounds, including residual solvents, extractables, leachables, and low molecular weight impurities. Liquid Chromatography–Nuclear Magnetic Resonance (LC-NMR) combines chromatographic separation with detailed structural characterisation and is highly useful for identification of unknown impurities, natural products, and complex pharmaceutical degradants [18]. LC-FTIR integrates chromatographic separation with infrared spectroscopic detection for functional group analysis and structural confirmation, whereas Capillary Electrophoresis–Mass Spectrometry (CE-MS) offers high-resolution separation of charged biomolecules including peptides, proteins, oligonucleotides, and monoclonal antibodies. In addition to chromatographic hyphenated systems, multidetector analytical platforms such as SEC-MALLS, SEC-DLS, SEC-RI, and SEC-UV have gained major importance in biopharmaceutical and polymer characterisation because they provide simultaneous information regarding molecular weight, hydrodynamic radius, aggregation state, particle size distribution, concentration, and conformational properties [19]. SEC-MALLS enables absolute molecular weight determination and aggregation analysis, while Dynamic Light Scattering (DLS) coupled with SEC provides hydrodynamic size measurements for proteins and nanoparticles. Refractive Index (RI) detectors support concentration analysis, whereas UV detectors provide chromophore-based quantification of analytes. These multidetector systems are particularly valuable for characterisation of monoclonal antibodies, biosimilars, vaccines, polymeric nanoparticles, liposomes, dendrimers, and advanced drug delivery systems [20]. Hyphenated analytical techniques therefore play a central role in structural elucidation, impurity profiling, biomolecular analysis, polymer characterization, formulation development, process optimization, and quality control in modern pharmaceutical industries. Continuous advancements in multidetector instrumentation, high-resolution mass spectrometry, and computational data analysis are further expanding the analytical capability and industrial importance of hyphenated techniques in pharmaceutical characterization [20].

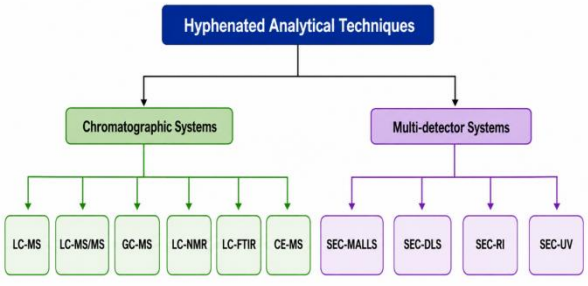

Figure 01: Classification of Hyphenated Analytical Techniques

Table 20: Comparison of Major Hyphenated Analytical Techniques

Technique	Principle	Major Application	Advantages	Limitations
LC-MS	LC coupled with mass spectrometry	Drug analysis, impurities, metabolites	High sensitivity and specificity	Expensive instrumentation
LC-MS/MS	Tandem mass spectrometry	Quantitative bioanalysis	Ultra-trace detection	Complex optimization
GC-MS	Gas chromatography with MS	Volatile compounds analysis	Excellent separation	Limited to volatile analytes
LC-NMR	LC coupled with NMR	Structural elucidation	Detailed structural information	Low sensitivity
LC-FTIR	LC with infrared detection	Functional group analysis	Structural confirmation	Limited sensitivity
CE-MS	Capillary electrophoresis with MS	Biomolecule analysis	High-resolution separation	Interface complexity
SEC-MALLS	SEC with light scattering	Absolute molecular weight analysis	Aggregate characterization	High instrumentation cost
SEC-DLS	SEC with dynamic light scattering	Particle size analysis	Hydrodynamic size measurement	Sensitive to aggregates
SEC-RI	SEC with refractive index detector	Concentration analysis	Universal detection	Lower sensitivity
SEC-UV	SEC with UV detection	Protein quantification	Simple and reliable	Requires chromophore presence

INSTRUMENTATION AND WORKING MECHANISM

The instrumentation and working mechanism of Size Exclusion Chromatography coupled with Multi-Angle Laser Light Scattering (SEC-MALLS) involve the integration of advanced chromatographic separation systems with multidetector analytical platforms for comprehensive molecular characterisation of

pharmaceutical and biopharmaceutical products. SEC-MALLS instrumentation is specifically designed to provide simultaneous information regarding molecular weight, molecular size, aggregation profile, concentration, hydrodynamic radius, and molecular conformation in a single analytical run. The SEC system consists of several essential components, including solvent delivery pumps, autosampler, chromatographic columns, detector systems, and computerised data acquisition software. High-performance solvent delivery pumps are responsible for maintaining constant and pulse-free mobile phase flow throughout the chromatographic system, thereby ensuring stable separation efficiency and detector performance. Autosamplers provide accurate and reproducible sample injection while minimising sample carryover and manual handling errors. The chromatographic separation is achieved using SEC columns packed with porous stationary phase materials such as silica, agarose, dextran, polymethacrylate, or hybrid polymeric particles containing well-defined pore size distributions. Column chemistry plays a critical role in separation performance because pore size, particle diameter, surface chemistry, and hydrophilicity directly influence molecular diffusion, retention behaviour, and resolution of analytes. During SEC separation, larger molecules are excluded from the pores and elute earlier, whereas smaller molecules diffuse into the pores and therefore exhibit longer retention times. The separated analytes subsequently pass through a series of coupled detectors, including UV, refractive index (RI), dynamic light scattering (DLS), viscometer, and Multi-Angle Laser Light Scattering (MALLS) detectors connected online for simultaneous multi-parameter characterisation [21-24].

The MALLS detector represents the central analytical component of SEC-MALLS systems and operates based on the principle of laser light scattering. A monochromatic laser source illuminates analyte molecules as they pass through the detector flow cell, and the intensity of scattered light is measured simultaneously at multiple scattering angles relative to the incident laser beam [5]. Typical detector arrangements include multiple photodiodes positioned at angles ranging from low forward angles to high backward angles such as 18°, 45°, 90°, 135°, and 165°. The angular dependence of scattered light intensity enables direct calculation of absolute molecular weight, radius of gyration (Rg), molecular size distribution, and conformational properties without the need for calibration standards. The UV detector measures absorbance of analytes at selected wavelengths and is primarily used for concentration determination of chromophore-containing molecules such as proteins and peptides. Refractive Index (RI) detectors provide universal concentration detection by measuring changes in refractive index relative to the mobile phase and are particularly useful for polymers, carbohydrates, and analytes lacking UV chromophores. Dynamic Light Scattering (DLS) detectors determine hydrodynamic radius (Rh) by analyzing fluctuations in scattered light intensity caused by Brownian motion of particles in solution, while online viscometers measure intrinsic viscosity and provide valuable information regarding molecular conformation, branching, and solution behavior. The combined multidetector configuration therefore enables simultaneous characterization of molecular weight, size, aggregation, concentration, and conformational properties of complex pharmaceutical systems.

Modern SEC-MALLS instrumentation additionally incorporates advanced computerized data processing software for signal integration, chromatographic peak analysis, molecular weight calculations, and multidetector data synchronization. The software integrates detector responses from UV, RI, MALLS, DLS, and viscometer systems in real time and applies mathematical light scattering models including Rayleigh and Debye equations for molecular characterization. Sophisticated algorithms are utilized for baseline correction, peak deconvolution, aggregation analysis, radius of gyration determination, and molecular weight distribution profiling. Data processing systems also support automated calibration verification, detector normalization, dn/dc correction, and quality control evaluation to ensure accurate and reproducible analytical performance. These advanced software platforms enable comprehensive characterization of proteins, monoclonal antibodies, biosimilars, vaccines, nanoparticles, liposomes, polymers, and advanced drug delivery systems with high analytical precision and reliability. Consequently, SEC-MALLS instrumentation has become one of the most powerful multidetector analytical systems for pharmaceutical characterization, structural analysis, and quality assessment in modern pharmaceutical and biopharmaceutical industries[24-26].

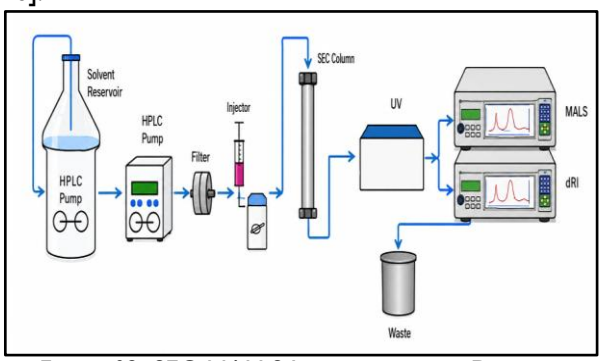

Figure 02: SEC-MALLS Instrumentation Diagram

MOLECULAR WEIGHT AND STRUCTURAL CHARACTERIZATION

Molecular weight and structural characterization represent some of the most critical analytical requirements in pharmaceutical, biopharmaceutical, and polymer science because molecular size, structural organization, aggregation behavior, and conformational stability directly influence biological activity, pharmacokinetics, immunogenicity, formulation stability, and therapeutic efficacy of pharmaceutical products. SEC-MALLS has emerged as one of the most powerful analytical platforms for comprehensive

molecular characterization because it enables direct determination of absolute molecular weight, molecular size distribution, radius of gyration, aggregation state, branching behavior, and conformational properties without dependence on calibration standards. Several molecular weight parameters are commonly utilized during SEC-MALLS analysis, including weight-average molecular weight (Mw), number-average molecular weight (Mn), Z-average molecular weight (Mz), and polydispersity index (PDI). The weight-average molecular weight (Mw) reflects the contribution of larger molecules within the sample and is highly sensitive to aggregates and high molecular weight species . In contrast, number-average molecular weight (Mn) represents the arithmetic average molecular weight of all molecules present in the sample and is influenced primarily by low molecular weight species. Z-average molecular weight provides greater emphasis toward larger particles and aggregates and is especially important for characterization of heterogeneous polymeric and protein systems. The polydispersity index (PDI), calculated as the ratio of Mw to Mn, describes the width of molecular weight distribution and overall sample heterogeneity. Monodisperse systems exhibit PDI values close to unity, whereas highly heterogeneous formulations display broader molecular weight distributions and elevated PDI values. SEC-MALLS therefore provides detailed insight into molecular distribution profiles and sample uniformity in complex pharmaceutical systems [27,28].

Weight-Average Molecular Weight Equation

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

Number-Average Molecular Weight Equation

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

Z-Average Molecular Weight Equation

$$M_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2}$$

Polydispersity Index Equation

$$PDI = \frac{M_w}{M_n}$$

Structural characterization using SEC-MALLS additionally provides valuable information regarding molecular dimensions, conformation, branching behavior, and aggregation state of biomolecules and polymers. One of the most important structural parameters is the radius of gyration (Rg), which describes the spatial distribution of molecular mass around the centre of gravity of the molecule. Rg is determined from the angular dependence of scattered light intensity and provides insight into molecular shape, compactness, and conformational architecture. The hydrodynamic radius (Rh), typically obtained using Dynamic Light Scattering (DLS), represents the effective hydrodynamic size of molecules in solution and reflects molecular diffusion behaviour. The ratio of Rg to Rh is widely applied for conformation analysis because it provides structural information regarding whether molecules exist as compact spheres, random coils, rigid rods, or branched structures. Branching analysis is particularly important for polymers, conjugates, dendrimers, and nanoparticle systems because branching significantly influences solution behaviour, viscosity, drug release properties, and therapeutic performance. SEC-MALLS, combined with online viscometry and DLS, therefore, enables simultaneous evaluation of molecular size, branching density, conformational state, and structural heterogeneity in pharmaceutical formulations.

RADIUS OF GYRATION RELATIONSHIP

$$P(\theta) = 1 - \frac{16\pi^2 R_g^2}{3\lambda^2} \sin^2\left(\frac{\theta}{2}\right)$$

Aggregation analysis represents another major application of SEC-MALLS in pharmaceutical characterisation because protein aggregation and oligomerisation significantly affect product stability, efficacy, immunogenicity, and regulatory acceptance. SEC-MALLS enables highly sensitive detection and quantification of monomers, dimers, oligomers, and high molecular weight aggregates within pharmaceutical and biopharmaceutical formulations. Monomer/dimer detection is particularly important during characterisation of monoclonal antibodies, therapeutic proteins, and biosimilars because low levels of aggregation may substantially influence product safety and biological activity. Oligomer analysis and aggregate profiling additionally provide important information regarding degradation pathways, formulation stability, storage-induced aggregation, and manufacturing consistency. Unlike conventional SEC methods that rely solely on retention time, SEC-MALLS directly determines molecular weight across the chromatographic peak and therefore accurately distinguishes co-eluting species, aggregates, and structurally heterogeneous populations. Consequently, SEC-MALLS has become an indispensable analytical tool for molecular weight determination, structural analysis, aggregation studies, and quality assessment in pharmaceutical, polymer, and biopharmaceutical research.

APPLICATIONS IN BIOPHARMACEUTICAL CHARACTERISATION

- SEC-MALLS is extensively used to detect and quantify monomers, dimers, oligomers, and high molecular weight aggregates in monoclonal antibody formulations to ensure product safety, efficacy, and immunogenicity control.
- The technique enables accurate absolute molecular weight measurement of therapeutic proteins without dependence on calibration standards, thereby supporting structural characterisation and quality assessment.
- SEC-MALLS combined with multidetector systems provides information regarding peptide folding, conformational stability, and structural integrity under different formulation and stress conditions.
- SEC-MALLS plays a critical role in evaluating structural equivalence, aggregation profile, and

molecular weight consistency between biosimilar products and reference biologics.

- The technique is widely employed for particle size analysis, aggregation monitoring, and structural evaluation of protein-based and nanoparticle-based vaccine formulations.
- SEC-MALLS supports detailed characterization of glycosylation-related heterogeneity, branching behavior, and molecular distribution in complex glycoprotein therapeutics.
- The method enables sensitive detection and characterization of oligomeric species that may influence biological activity, stability, and therapeutic performance of biopharmaceutical products.
- SEC-MALLS is routinely applied during accelerated and long-term stability studies to monitor aggregation, degradation, and structural changes occurring during storage.
- The technique is used in quality control laboratories to ensure lot-to-lot consistency in molecular weight distribution, aggregation levels, and structural integrity of biologic products.
- SEC-MALLS provides highly sensitive aggregate analysis and quantification, which is essential because even low aggregate levels may induce immunogenic responses in patients.

APPLICATIONS IN NANOTECHNOLOGY AND DRUG DELIVERY SYSTEMS

- SEC-MALLS is widely used for determination of molecular weight, particle size distribution, aggregation state, and polymer architecture in nanoparticle drug delivery systems.
- The technique enables evaluation of liposome size distribution, structural integrity, aggregation behavior, and formulation stability under different storage conditions.
- SEC-MALLS plays a major role in characterization of lipid nanoparticles used in advanced drug delivery and mRNA vaccine systems by determining size heterogeneity and aggregation profile.
- The method supports structural analysis of micellar systems including determination of molecular organization, hydrodynamic size, and self-assembly behavior.
- SEC-MALLS is employed for evaluation of dendrimer branching density, molecular architecture, size distribution, and structural uniformity in nanomedicine applications.
- The technique enables characterization of polymer-drug conjugates by determining conjugation efficiency, molecular weight distribution, and aggregation behavior.
- SEC-MALLS provides detailed aggregation analysis of nanocarrier systems to ensure physical stability, uniformity, and controlled drug delivery performance.
- Combined SEC-MALLS and DLS systems enable accurate determination of nanoparticle hydrodynamic size and size distribution in pharmaceutical formulations.
- The technique is extensively applied for monitoring structural stability, aggregation tendency, and degradation behavior of nanocarrier systems during storage and formulation development.
- SEC-MALLS serves as an important analytical platform for routine quality assessment, batch consistency evaluation, and regulatory characterization of nanomedicine products.

SEC-MALLS METHOD DEVELOPMENT AND VALIDATION

Method development and validation of SEC-MALLS analytical procedures are essential for achieving reliable, reproducible, and accurate molecular characterisation of pharmaceutical, biopharmaceutical, and nanomedicine products. Effective method development begins with appropriate column selection based on pore size distribution, stationary phase chemistry, particle size, and separation range to ensure efficient resolution of monomers, oligomers, aggregates, and high molecular weight species. Mobile phase optimisation is equally critical because buffer composition, ionic strength, pH, and solvent compatibility significantly influence analyte stability, aggregation behaviour, detector response, and chromatographic performance. Flow rate optimisation is carefully performed to maintain adequate separation efficiency while minimising shear-induced degradation and detector noise. Detector calibration and normalization are necessary to ensure accurate light scattering measurements, molecular weight calculations, and detector synchronisation across UV, refractive index, dynamic light scattering, and MALLS systems. Sample concentration optimisation is also important because excessively concentrated samples may induce aggregation and intermolecular interactions, whereas very dilute samples may reduce analytical sensitivity and signal quality. Method validation is performed according to regulatory expectations and includes evaluation of accuracy, precision, specificity, robustness, sensitivity, and reproducibility to confirm method suitability for routine analytical applications. Accuracy studies assess the closeness of measured molecular weight values to reference standards, while precision evaluates repeatability and intermediate reproducibility under multiple analytical conditions. Specificity ensures adequate separation of analytes from impurities, aggregates, and degradation products without analytical interference. Robustness studies determine the effect of small variations in chromatographic conditions, whereas sensitivity evaluates the lowest detectable analyte concentration. Reproducibility confirms consistency of analytical performance across instruments, analysts, and laboratories. Regulatory agencies, including the International Council for

Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and the United States Food and Drug Administration, emphasise compliance with ICH validation guidelines and Good Manufacturing Practice (GMP) requirements to ensure analytical reliability, data integrity, and quality control during pharmaceutical characterisation and product development [29].

ADVANTAGES OF SEC-MALLS AND HYPHENATED TECHNIQUES

- Absolute molecular weight determination without calibration standards
- Non-destructive analysis under native solution conditions
- Simultaneous multi-parameter characterisation in a single run
- High sensitivity for aggregate and oligomer detection
- Real-time molecular and structural analysis
- Minimal sample preparation requirements
- Broad applicability for proteins, polymers, and nanoparticles
- Accurate aggregation and heterogeneity profiling
- High reproducibility and analytical reliability
- Online coupling with multiple detector systems
- Detailed conformational and branching analysis
- Effective characterisation of biosimilars and biologics
- Rapid analysis with reduced experimental variability
- Improved structural elucidation capability
- Suitable for complex pharmaceutical formulations [30].

FUTURE PERSPECTIVES

Future developments in SEC-MALLS and hyphenated analytical technologies are expected to significantly improve pharmaceutical and biopharmaceutical characterization through integration of advanced multidetector systems, artificial intelligence, and automated data analysis platforms. Emerging UHPLC-MALLS systems provide faster chromatographic separation, enhanced resolution, reduced sample consumption, and improved analytical sensitivity for complex biomolecular systems. Integration of MALLS with dynamic light scattering, viscometry, fluorescence spectroscopy, and high-resolution mass spectrometry is further expanding the capability for simultaneous structural and conformational analysis of proteins, polymers, nanoparticles, and advanced drug delivery systems. Artificial intelligence and machine learning approaches are increasingly being explored for automated molecular profiling, aggregation prediction, peak deconvolution, and multidimensional dataset interpretation. Real-time process analytical technology (PAT) and continuous manufacturing approaches are also expected to accelerate adoption of SEC-MALLS in pharmaceutical industries for in-line quality monitoring and process optimization. Additionally, increasing applications in biosimilars, gene therapy products, lipid nanoparticles, mRNA vaccines, and nanomedicine formulations are anticipated to further expand the industrial and regulatory importance of SEC-MALLS. Continuous improvements in detector sensitivity, software automation, miniaturization, and multidetector integration will likely establish SEC-MALLS as a central analytical platform for future pharmaceutical characterisation and quality assessment.

CONCLUSION

SEC-MALLS and hyphenated analytical techniques have become indispensable tools for comprehensive molecular weight determination and structural characterisation of pharmaceutical and biopharmaceutical products. The ability of SEC-MALLS to provide absolute molecular weight measurement, aggregation profiling, conformational analysis, and molecular heterogeneity assessment without dependence on calibration standards makes it highly valuable for the characterisation of proteins, monoclonal antibodies, biosimilars, vaccines, polymers, nanoparticles, and advanced drug delivery systems. Hyphenated analytical systems, including LC-MS, LC-MS/MS, LC-NMR, CE-MS, SEC-DLS, and SEC-RI, additionally provide enhanced structural elucidation, impurity profiling, and multidimensional analytical capability for complex pharmaceutical formulations. These techniques play critical roles in pharmaceutical development, quality control, stability assessment, biosimilar comparability studies, and regulatory compliance. Despite analytical challenges such as aggregation artifacts, detector limitations, data interpretation complexity, and high instrumentation cost, continuous advancements in multidetector integration, AI-assisted analytics, UHPLC-MALLS systems, and real-time analytical monitoring are significantly improving analytical performance and industrial applicability. Increasing regulatory emphasis on detailed biomolecular characterisation and nanomedicine evaluation is expected to further strengthen the importance of SEC-MALLS in pharmaceutical sciences.

AUTHOR CONTRIBUTIONS

Venkatanarayana Bypaneni conceived and drafted the manuscript. Jayaram Kamma contributed to the literature collection, analytical and regulatory sections. Murugesan Palanivelu contributed to manuscript review, editing, and technical revision. All authors approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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