CAHIERS MAGELLANES-NS
Volume 06 Issue 2
2024

ISSN:1624-1940
http://magellanes.com/

AN INSIGHT TO MODERN ANALYTICAL TECHNIQUES FOR DENDRIMER CHARACTERIZATION

P. Anitha[1], D. Vasavi Devi[2], B. Pooja[1], N. Sushma[1], K. Nagalakshmi[3], S.L. Manideep royal[4], Valikala Viswanath[1*], Dr.Sanapala Srinivasa Rao[5]

[1*] Department of Pharmaceutics, Annamacharya College of Pharmacy, Rajampet, India
[2] Department of Pharmaceutical Analysis, Annamacharya College of Pharmacy, Rajampet, India
[3]Department of Pharmaceutical Chemistry, Annamacharya College of Pharmacy, Rajampet, India
[4]Department of Pharmacy practice, Annamacharya College of Pharmacy, Rajampet, India
[5]Department of Pharmaceutical Analysis, Pulla Reddy Institute of Pharmacy, Domadugu village, Gummadidala Mandal, Sangareddy District, Telangana.

Corresponding Author:
Dr. Valikala Viswanath
Professor,
Annamacharya College of Pharmacy,
Rajampeta, Andhra Pradesh
India.
viswanath.prrm@gmail.com

Abstract:

The solubility and permeability of the drug have a significant impact on the bioavailability of a number of drug administration routes. Pharmaceutical formulation faces a significant difficulty in optimizing these crucial variables without making structural modifications. As a result, research has shown that dendrimers are new and offer a viable method for delivering targeted drugs. The physiochemical characteristics of dendrimers are defined by a wide range of characterization techniques and a number of preparation methods for a variety of dendrimers, each with specific benefits. It is challenging to discern between the morphological and structural characteristics of the synthesized dendrimers since there is a dearth of strong theoretical knowledge on dendrimer characterization. This study outlines an insightful literature assessment that ensures the choice of an appropriate analytical approach to support the structural characterization of dendrimers.

Key words: Dendrimers, hyper branched structures, nano composites, arborols, cascade molecules.

1. Introduction:

Dendrimers are fascinating three-dimensional polymeric architectures with well-defined molecular sizes, dense and uniform peripheral functionalities, and porosity. The dendrimers offer various reaction sites from the core to the terminal region. This excited the investigators to explore novel synthesis methods, applicability in novel drug delivery, and miscellaneous analytical techniques to justify their significance in nanomedicine. In the 20th century, polymers were structurally categorized as linear polymers, branched polymers, crosslinked polymers, and dendritics. Furthermore, the dendritic are categorized as dendrigrafs, branched polymers, and dendrimers. Dendrimers are multifaceted, and structurally well-organized with pseudo monodisperse framework. Although the dendrimers are not synthesized through polymerization techniques, they are still categorized under polymers due to their

ISSN:1624-1940
http://magellanes.com/

repetitive branching. Nearly fifty families of dendrimers exist today with unique structural features that can be tailored for miscellaneous applications in various allied fields. The exclusive properties such as multifunctional terminal region, molecular uniformity, and internal cavities for encapsulation of various drug candidates make them suitable for industrial and biomedical applications. Dendrimer chemistry is the most fascinating and rapidly developing field in modern chemistry. The aforementioned unique structural characteristics have clinched the attention of investigators in the academia and industrial sectors and have widespread applicability to pharmaceuticals, medicinal chemistry, and nanotechnology [1]. Dendrimers pertain to polymer chemistry because of repetitive structural branching and molecular chemistry because of their organized synthesis. Thus, they benefit from both fields and are widely recommended in the modern biopharmaceutical sector. The current literature survey is focused on the delineation of various characterization techniques of dendrimers excluding the interactions within themselves, bulk solutions, concentrated solutions, and interactions about their generation. Despite their numerous advantages, they suffer from poor drug loading, purity, structural homogeneity, and toxicological issues about cationic architecture. The investigators resolved the issue through structural and peripheral functional group modifications via ligand and folic acid anchoring. Conventional dendritic architectures possess limited applications because of their identical terminal end moieties and conjugated with miscellaneous components that result in the loss of functional moieties. Therefore, a demand for sophisticated characterization techniques that can justify the structural composition is necessitated. Although an abundant literature survey about the current discussion is available on various platforms, the present paper furnishes precise information that reflects its significance in biomedical applications. Dendrimers are functionally versatile and expressed as highly engineered biomacromolecules with peripheral functional groups that mark their significance in supramolecular chemistry. Macromolecular carriers are widely recommended in pharmacokinetics and targeted drug delivery as they can be synthesized in a reiterative manner and the comprehensive knowledge of various complementary analytical techniques helps generate an enhanced therapeutic output. Furthermore, the relationship between dendritic nano scaffolds, biocompatibility, drug delivery, and retention must be precisely elucidated to expose its prominence in nanotherapeutics which can be hastened through extensive knowledge of modern characterization techniques [2]. Therefore, we have comprehensively enumerated the scientific literature survey on dendrimer characterization that serves as a shred of authentication for the investigators to trigger its technical applicability and biomedical advancements.

2. Characterization of dendrimers:

2.1 NMR spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most common and sensitive techniques in the characterization of dendrimers. NMR spectroscopy allows the determination of the structure and dynamics of molecules in solution. Rotational-Echo Double Resonance (REDOR) solid-state NMR spectroscopy is used to characterize the PAMAM dendrimers and complex PAMAMs. One-dimensional (1D) and two-dimensional (2D) NMR studies are used to probe the conformation of a melamine dendrimer bearing unique NMR signals from the core to the periphery. (2D)-NMR, (3D)-NMR Multidimensional NMR spectroscopy is also acquiring increasing importance in the characterization of dendrimers. The information regarding the composition of the dendrimer is found by using mono- and bi-dimensional analysis of different nuclei. Quantitative determination of internuclear distances for nuclei in different parts of the dendrimer molecule is determined by NOESY experiments. Besides its use in the detection of each step completion, it has the drawbacks of not detecting structural defects and external impurities and lacks high sensitivity [3].

ISSN:1624-1940
http://magellanes.com/

2.2 Mass spectroscopy

Mass spectroscopy plays a crucial role in dendrimer chemistry because it is extensively preferred for the analysis of dendrimer purity and structural defects in higher generations. The spectrophotometer allows the particles to suspend in a gaseous phase and creates an isolated environment for the interpretation of host-guest interactions and critical properties that are not easily predicted in the solution phase. Mass spectroscopy serves as an important tool in evaluating the structural integrity of dendrimers or any structural deformations that occur during synthesis such as protonation, self-assembly, and host-guest interactions in dendritic complexes. Apart from the above, there are a few uncertainties such as the formation of supplementary signals because of fragment ions or due to dendrimer defects. Hence, to overcome the above impediments, tandem mass spectrometry is advised. In the case of dendrimers, the generated molecular mass is exponentially proportional to the number of generations synthesized and the function groups present in them. Furthermore, the synthesis produces several potent byproducts with varied substitutions due to incomplete reactions and demands sophisticated analytical techniques for characterization [4]. The Electro-spray ionization (ESI) mass spectroscopy is a sophisticated ionization technique that functions on the principle of multiple charging and uses less energy for ionization, which does not initiate fragmentation when ionized [5]. The electron spray ionization is coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to extend the potentiality of mass spectrometry for the characterization of various dendrimers [6]. In addition, Fast atom bombardment spectroscopy (FABMS) is used for establishing the purity and molecular weight of dendrimers and is highly recommended for the characterization of nano architectures whose molecular weight is less than 3000da [7]. Furthermore, the Matrix-assisted laser desorption ionization (MALDI), whose collaboration with electron spray ionization has reformed the fundamentals of analysis in various disciplines. The recent advances in mass spectroscopy such as time-of-flight mass spectroscopy (TOF-MS) in association with MALDI serve as a potent tool in the design of crucial instruments and for the challenges in biochemistry. However, the metastable ions are not observed in linear MALDI TOF-MS because the fragmented ions in the free field regions possess the same velocity as the stable ions and cannot be distinguished. In addition, the investigative report of Mamyrin [8] elucidated that the laser assistant *reflectron time-of-flight mass spectroscopy* in which reflectron is magnet-free TOF-MS that can diverge the ion energy from its origin and be applied in numerous fields [9].

2.3 FTIR spectroscopy:

IR spectroscopy is a nondestructive technique for the analysis of sensitive and selective chemical constituents. The IR spectrum produces a specific bandwidth in the fingerprint region that enables the identification of distinct chemical species and their corresponding isomers. The vibrational frequencies are highly sensitive to the geometrical alignment and bonding of atoms and furnish information about the molecular orientation and conformations in liquid crystals. An FTIR spectrum provides accessibility to understand the hydrogen bonding interactions, and the influence of temperature over structural, conformational, and orientational changes in the dendritic architecture. It can also be used to monitor the fluctuations in the spectrum due to various parameters such as concentration, temperature, pressure, and time for complex biomolecules. The current technique simplifies the interpretation through attenuation of the selected characteristics and completely filtering the unnecessary portions of the output. The FTIR is used in genetic engineering to investigate the structural components of DNA and for surface mapping in biological research. In pharmaceutical preparations, it is used to assess the drug-excipient compatibility, pharmaceutical composition, and therapeutic activity of drug, moieties [10].

2.4 UV-VIS spectroscopy:

UV-VIS spectroscopy is used to detail the interactions between functional groups and metal ions of

ISSN:1624-1940
http://magellanes.com/

hyperbranched structures. It is a facile and inexpensive technique for monitoring the synthesis of dendritic architectures where the number of chromophore units concerning generation is proportional to the absorption band intensity and is used in the estimation of the purity and structural imperfections of dendrimers. Apart from the above, UV-VIS spectroscopy is used to define the morphological characteristics of dendrimers by analyzing the absorption maxima between various generations. UV-VIS spectroscopy is used to study the radial complexity phenomenon in dendrimers through an in-depth analysis of the isosbestic point and its variability between various generations. Furthermore, UV-VIS spectroscopy is employed to investigate the structural characteristics of florescence dendrimers because the characteristic property is instigated through irradiation at a specific wavelength and gets absorbed by the compound under investigation [11].

2.5 Raman Spectroscopy

The Raman spectroscopy serves as a valuable tool to enumerate the active positions in the dendritic architecture and to detail the structural characterization of non-crystalline nano architectures. The bandwidths in the Raman spectra are used to analyze the conformational changes in hyperbranched structures and furthering the Raman spectroscopy is used to detail the flexibility of dendrons and terminal functionalities of hyperbranched structures. The conjugation of dendrons has led to the emanation of various generations that are distinguished through the lines assigned to specific molecular entities in the dendrimers. However, the decreased sensitivity of Raman spectroscopy has led to the disclosure of surface-enhanced Raman spectroscopy (SERS) that intensifies the signal to multiple magnitudes and refers exclusively to biological sensing. The SERS can generate the signal intensity to 1014 within a minute or not without any sample development. The peculiarity of SERS in the characterization of biomolecules is that it can carry out analysis in the aqueous phase while the same is not feasible with Raman spectroscopy as it is weakly scattered [12].

2.6 Florescence spectroscopy

Fluorescence quenching is an interdisciplinary province in the biomedical sectors such as photodynamic therapy, gene therapy, drug delivery, and cancer markers. Its versatility lies in providing information regarding the polarity, shelf life, and intensity of the sample. The fluorescent dyes are conjugated at various positions in the sample such as core, terminal, end region, and branches to detail the optical features of dendrimers. In addition, optical sensing techniques are used to detect heavy metals and changes in pH. However, the technical innovations in fluorescence microscopy have enabled to visualization of various biological molecules and protein moieties with high resolution. The aforementioned can be gained upon conjugation with certain organic dyes, fluorescent proteins, or quantum dots. But among these, proteins and organic dyes are not widely preferred because they suffer from photobleaching and generate inadequate data. Furthermore, the fluorescence estimation is quite useful in understanding the kinetics of energy transfer and excitation dynamics between hyperbranched macromolecules [13].

2.7 Dielectric spectroscopy

Dielectric relaxation spectroscopy (DRS) serves as a predominant tool to investigate the molecular dynamics of hyperbranched structures at a wide range of lengths and time scales that are not feasible with other spectroscopic techniques. The knowledge of molecular dynamics helps in understanding unresolved queries regarding the pathway, characteristic features, and architecture of dendrimers. It probes the molecular pathway and electrical characteristics of polymers generated through perturbations as a result of differential voltage and measures its response. The polarizations arise through various mechanisms such as atomic, electronic, dipolar arrangement, and interfacial properties which are used to enumerate the orientation of side groups detected beneath the glass transition temperature through

ISSN:1624-1940
http://magellanes.com/

the interaction of the applied external field to the dipole moment of the molecules [14].

2.8 X-ray photoelectron spectroscopy (XPS):

XPS is used for the analysis of chemical constituents at the surface. The basic principle involves irradiating the solid with soft X-rays and analyzing the energy of the emitted electrons. Each element has a unique spectrum and in case of a combination of elements, the obtained peaks are summed wand and the analysis is made. Since the electron path in solids is limited, the obtained atomic peaks are due to those that exist in the top layers, which differentiates the XPS from other analytical techniques. Furthermore, XPS is used to investigate the characteristics of cross-linked micelles with the dendritic shell that play a crucial role in drug delivery, entrapment of undesired substances, and isolation of metabolic products. XPS in conjugation with high-resolution electron microscopy is used to study the properties of terminal end molecules. XPS possesses specific advantages such as being efficacious in analyzing a wide range of inorganic and organic materials with less time. Apart from the above, the XPS suffers from a few limitations such as reproducibility, compatibility with vacuum, and optimized size [15].

2.9 Energy dispersive X-ray spectroscopy (EDXS):

EDXS is a valuable tool of microanalysis which is coupled with electron microscopy to detect the elemental composition of the sample where it generates a characteristic peak for every element in its spectrum by allowing the interaction of atoms with X-rays in the sample. EDXS is a sophisticated tool to detect the therapeutic performance of several therapeutic agents such as chemotherapeutic moieties etc. It has a large advantage in the detection of pathological calcium deposition which is used to characterize the composition of benign and malignant lesions and breast calcifications. The realization of the dream of personalized medicine for every unique human being can be possible using EDX microanalysis in biomedical research and diagnosis [16].

2.10 Near Edge X-ray absorption fine structure spectroscopy (NEXAFS)

Near-edge X-ray absorption fine structure spectroscopy (NEXAFS) **was used** for exploring chemical bonding, electronic structure, surface chemistry, and the degree of alignment of SWNTs (single-walled carbon nanotubes). NEXAFS is utilized to characterize the structural configuration and to detail the environment of dendritic nano-composites. NEXAFS spectroscopy can be utilized to decide: 1) the presence of imperfections and amorphous content in carbon nanotubes; 2) changing levels of bond hybridization in blended sp2/sp3-reinforced carbon materials; 3) the level of vertical arrangement in nanotube tests; and 4) the nature of oxygen-containing functional groups on nanotubes surfaces. Effective acknowledgment of the utilization of NEXAFS spectroscopy as a corresponding device in examining the electronic and structural properties of nanostructures. Further, NEXAFS needs to focus on the *in-situ* perception of the doping and chemical functionalization of carbon nanotubes as well as the determination of order in other aligned nanoscale frameworks [17].

2.11 Gel Electrophoresis

Characterization of dendrimers and their forms is basic for their fruitful synthesis and applications. Electrophoresis is a preferred strategy in biochemical examination. Electrophoresis equipment and capability are generally accessible. Likewise, simple modifications of analytical conditions permit one to isolate any charged, water-soluble dendrimers of differing shapes and dimensions (e.g., nucleic acid and polylysine dendrimers). The end signal is visual and result interpretation is relatively easy. Electrophoresis is likewise a modest strategy that doesn't need modern instrumentation and exceptionally skilled operators. A substantially less costly option is polyacrylamide gel electrophoresis (PAGE), which has been utilized to isolate PAMAM dendrimers of different generations. PAGE has numerous likely benefits for dendrimer characterization. It permits partition under physiological

ISSN:1624-1940
http://magellanes.com/

circumstances, requires a small sample size, yields a visual end signal, and is a gentle, non-destructive method that doesn't cause fragmentation of the dendrimer sample [18].

2.12 Circular dichroism

The degree of compaction, conformation, and binding accessibility of the dendrimer-DNA complexes is described by linear and circular dichroism (CD). Circular dichroism gives hints to the structure of the condensed DNA demonstrating long-range order between the helices, for example, in polymer-salt-induced cholesteric liquid crystalline domains, one possible shape being a toroidal design. The CD is utilized for insight regarding the geometrical configuration of chiral dendrimers. The CD estimations were performed on a Jasco J-810 spectropolarimeter at 25 °C utilizing a 1 cm quartz cell. Spectra were kept in 1 nm increased from 220 to 400 nm and baseline-corrected by subtracting appropriate blanks. The bandwidth was set to 1 nm, the reaction was 0.5 s, and the output speed was 50 nm/min [19].

2.13 High performance liquid chromatography (HPLC)

These HPLCs have exploited their significance in the characterization of dendrimers to detail their biological interactions, polydispersity, solubility, and heterogeneity. The experimental data is generated based on the effective interaction between the stationary phase and conjugates, which is useful for understanding the surface properties of hyperbranched structures. In HPLC analysis, acetonitrile is the solvent of choice and to counteract its shortage in recent years, researchers have focused on *ultra-performance liquid chromatography* (UPLC). The UPLC possesses peculiar columns that demand less eluent and the dimensions are well optimized than traditional HPLC columns. Furthermore, the UPLC can generate results in less course time, i.e., in 10 to 15 minutes than the HPLC, which requires a minimum of 35 minutes to complete the analysis. Furthermore, UPLC can be coupled with a mass spectrophotometer for product identification with a minimum time of analysis. Although the HPLC method is quite interesting, it is not opted for cationic dendrimers because of their elevated charge and polarity, and further they cannot be easily separated using a reverse phase HPLC column. The above confronts are overcome through alterations in mobile phase composition such as ion-pairing reagents or hydrophilic interaction chromatography. However, *ion pair reverse-phase HPLC* is opted for the retention of peptides and similar compounds through hydrophobic ions in the mobile phase. The above effect is produced through either the formation of an ion pair complex in the mobile phase or tuning the stationary phase that behaves as an ion pair complex [20]. The reverse phase HPLC suffers from numerous drawbacks such as the segregation of highly charged polymers and oligomers is enormous because they get adsorbed onto the stationary phase and generate peak broadening.

2.14 DSC studies

Differential examining calorimetry (DSC) was utilized to measure the glass transition temperature (Tg) of blends of dendritic and linear polymers to look for shifted single transitions. The miscibility of blends can be immediately screened by visual perception and DSC of cast films. For miscibility to be present, the blends should be optically clear and have a Tg moderate between those of the blend parts. The benefits are that the difference (neutron or x-beam) isn't required and molecular mass or size is not important. Limitations of this method include: 1. A solvent impact where phase separation happens during the drying process in any event, for a miscible blend. 2. Refractive indices of the polymers being excessively near one another to look overcast when phase separated. 3. Tg's that are too near to one another or too frail to be seen. 4. Blends made from compositions near 0 % or 100 % can make phase partition difficult to determine [21].

ISSN:1624-1940
http://magellanes.com/

2.15 Scattering techniques

The Scattering strategies, for example, small angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) to determine the miscibility of blends of dendrimers, dendrigrafts, or hyper-branched linear polymers or interpenetrating polymer networks (IPN). Small-angle X-ray scattering (SAXS) provides information about their average radius of gyration (Rg) in solution. The structural properties of dendrimers to be investigated by SANS include intermolecular structure, intramolecular cavity, radius-of-gyration (RG), effective charge number of a single dendrimer molecule, and water penetration into the interior of the dendrimers. The structural information of SANS experiments is obtained by the comparison between the experimentally detected spectra and the theoretical results of the system. Small angle neutron scattering (SANS) gives access to the radius of gyration, but may also disclose more accurate information than SAXS. The location of the ending groups has also been determined by SANS experiments conducted with PAMAM dendrimers and PPI dendrimers. Laser light scattering (LLS) decides the hydrodynamic radius of dendrimers. Dynamic LLS is generally utilized for the detection of aggregates [22].

2.16 Electron paramagnetic resonance

Electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy is a method for studying chemical species that have at least one unpaired electron, for example, organic and inorganic free radicals or inorganic complexes having a transition metal ion. EPR is viewed as valuable for dendrimer characterization, explicitly, for deciding the number, distribution of numbers, and spatial distribution of the molecule. Utilizing spin-label and spin-probe techniques, electron paramagnetic resonance (EPR) has been demonstrated to be an exceptionally valuable device in researching the properties of polymeric and macromolecular systems that are of biochemical or industrial relevance [23].

2.17 Electrochemistry

Electrochemistry might bear the cost of principally three sorts of data concerning the structure of dendrimers. Exhaustive coulometry has been utilized to measure the number of electroactive groups, in most cases ferrocenes, connected to the outer layer of PPI, poly (aryl ether), or PMMH dendrimers, and furthermore for naphthalene groups connected to PAMAM dendrimers. The degree of burying of electroactive groups inside the dendrimers can be identified by cyclic voltammetry. At last, electrochemistry gives data about the possibility of interaction (or not, most generally) of electroactive end groups between them, traducing a close proximity [24].

2.18 Electrophoresis

Gel electrophoresis is generally utilized in biology for the routine analysis and partition of biopolymers like proteins and nucleic acids. This strategy was utilized for the assessment of purity and homogeneity of a few sorts of water-soluble dendrimers, for example, PAMAM dendrimers having NH3 + or CO2- end groups, PLy dendrimers, PPI dendrimers, nucleic acid dendrimers, or phenylacetylene dendrimers. Gel electrophoresis was likewise utilized for studying the interaction between positively charged dendrimers and DNA, considering transfection experiments. It was found that complex formation depends both upon the generation (size), and the charge ratio of PAMAM dendrimers and poly (ethylene glycol)- block-poly(l-lysine) dendrimers [25].

2.19 X-ray Diffraction studies (XRD):

The XRD is used for assessing the structure of materials where the atoms are aligned in short interspaces

ISSN:1624-1940
http://magellanes.com/

and upon irradiation they generate exceptionally diffusible XRD patterns and recommend a special analytical technique such as atomic pair distribution function for interpreting the experimental data. In spite, the current analytical technique exerts a few drawbacks such as its inefficacy to determine the molecular structure of solid and amorphous dendrimers. Although single-crystal XRD is widely preferred for the structural characterization of dendrimers, it is quite difficult to generate crystals of optimized size for analysis. In such critical situations, powder XRD serves as a potent source for the estimation of structural parameters for organic solids and hyperbranched polymers. The powder XRD is efficient for products having moderate complexity and might be challenging for products consisting of numerous independent molecules in the hyperbranched structure. In this technique, a trial crystalline structure is generated whose critical parameters are calculated and compared with the experimental data of powder XRD [26]. SAXS is a matured analytical technique for generating precise information regarding particle size (from 1-100nm) and shape. In SAXS, the high-frequency X-rays impinge on the sample whose interaction with the corresponding electrons generates elastically scattered waves with fluctuations in its electron density that can be recorded and analyzed. The technical advancements in SAXS have led to the emergence of ultra-small-angle X-ray scattering techniques (USNXS) that can probe dendritic inhomogeneities in the range of 1-1000nm [27]. Similarly, Wide wide-angle X-ray scattering technique (WAXS) is useful for furnishing structural information up to 50Å with less signal-to-noise ratio than SAXS. WAXS is highly recommended to characterize the structural dissimilarities of macromolecular architectures and holds potentiality in the identification and characterization of the structural framework of protein molecules. However, the small angle neutron scattering technique (SANS) is a potent technique that functions on the principle of contrast variation between solvent and solute that differs due to a blend of deuterated and protonated solvents. Due to the interaction of neutrons with the atomic nucleus, it produces a well-defined scattering length that differs from hydrogen and deuterium. The intensity of scattering relies on the difference in density between the scatter and the solvent which forms a basis for the estimation of the density profile that defines the physiochemical properties of dendrimers. [28]

2.20 Microscopic studies

2.20.1 Atomic force microscopy

Atomic force microscopy was enlightened in the 1990s and became much more familiar because it enables to generate of high-resolution images at the molecular level in a physiological environment. The AFM is designed on the collaborative principles of stylus profilometer and scanning tunneling microscopy. The AFM uses an exclusive probe that does not cause surface abrasion and is effective in imaging inorganic substances at the atomic level. It can generate prominent images without sample preparation and serves as a potent tool in imaging cell surfaces. Although the AFM has evinced its significance in cellular biology, there are a few drawbacks such as onerous because of the probe tips that can instigate deformations and soft and sticky interactions between the cellular surface and probe tips. The time factor is also one of the critical parameters because the imaging process is relatively slow, which triggers advanced technologies for sample preparation for cellular imaging [29].

2.20.2 Confocal Laser scanning microscopy (CLSM):

Confocal laser scanning microscopy (CLSM) is a classical microscopic technique used to provide the morphological information of cells and tissues with the aid of an optical platform. The CLSM provides regeneration of images based on point-to-point scanning and provides high-resolution images due to distinct laser sectioning that does not allow superimposition of the exterior focus on the interior focus field. Other microscopic techniques cause sample distortion due to prolonged exposure to the laser light and during sample sectioning and its fixation. Whereas, with CLSM the above drawback is overcome through proper storage of the sample that enables it to image multiple times, and the conclusions are

ISSN:1624-1940
http://magellanes.com/

drawn out. In recent times, reflectance CLSM has been used in dermatology to image abnormal skin conditions and the effect of dendritic formulations in treating skin abnormalities [30].

2.20.3 Scanning tunneling microscopy (STM)

In dendrimers, the knowledge of binding sites and their orientation is crucial as they show a profound effect on the release characteristics and entrapment efficacy. Although extensive analytical studies are accessible there exists a dearth at the molecular level that lacks investigators to predict drug interactions either at the terminal end or at the dendritic interior. The above challenges are due to impediments in envisaging the dendritic architecture which can be resolved with the aid of STM due to its enhanced resolution characteristics. Furthermore, the microscopic tip exerts a predefined force on the substrate atoms. The force exerts both electrostatic and Vander Waals contributions whose calibration of the tip position and its corresponding voltage may lead to an alteration in the magnitude and direction of the force. Furthermore, the microscope tip pulls off the atom from its surface while it is surface-bound. STM generates high-resolution images and is widely preferred in semi-conductive and conductive systems. The STM is preferred for imaging of higher-generation dendrimers because it can diminish the tunneling issues of insulators and can generate high-resolution images [31].

2.20.4Transmittance electron microscopy (TEM)

Transmittance electron microscopy is exclusively preferred for the visualization of intracellular constituents. The enhanced resolution characteristics of TEM enabled it to investigate the insights of cellular dynamics. In recent years, high-resolution transmission electron microscopy (HRTEM) has been preferred for the characterization of macromolecules because it can produce the structural configuration of solid substances about 0.08nm. In conventional transmittance electron microscopy, the specimen gets illuminated with a high-frequency electronic beam, and the image gets generated through a pattern of the lens similar to an optical microscope. The HRTEM can perform the structural analysis with minute sample quantities and play a significant role in drug delivery. Despite the miscellaneous advantages, it suffers from a few drawbacks such as staining, aberrations that create interference to the image quality, and dehydration of samples or cryogenicity that provokes uncertainty in biocompatibility. Scanning tunneling electron microscopy (STEM) is preferred for the characterization of nano-architectures at various modes because it can furnish ultimate knowledge on the shape and elemental composition of dendrimers concerning an individual atom. Furthermore, cryogenic transmission electron microscopy (cryo-TEM) enables one to gain knowledge on the size and shape of macromolecules under investigation and serves as a complementary technique to depict the assembly of biological and particulate matter such as bacteria, viruses, etc. The cryo-TEM enables to characterize of the intermediate structures and plays a crucial role in monitoring the synthetic pathways and is preferred to study the cell membrane integrity that might alter due to the conjugation of synthetic or natural molecules [32].

2.21 Size exclusion chromatography (SEC)

Gel permeation chromatography (GPC), otherwise called size exclusion chromatography (SEC), is a profoundly significant instrument that isolates molecules given their hydrodynamic volume or size. With advanced detection systems coupled to GPC, data about polymers, like molecular weight (Mw) distribution, average molecular mass, and degree of branching, can be procured. Naden BJ and Colleagues described the adsorption of poly (hydroxystearic corrosive) on TiO2 NPs utilizing GPC. The latter technique has the option to determine and measure the adsorbed molecules by size. Most sorts of dendrimers were described by SEC, even self-assembled dendrimers. SEC was additionally used to screen size changes of arborols (ARB) dendrimers with pH variance [33].

Conclusion

The distinctive and skillfully crafted dendrimers are hyperbranched structures. Despite the development

ISSN:1624-1940
http://magellanes.com/

of numerous synthetic methods and dendrimer varieties, there is still a knowledge gap regarding the physiochemical characteristics of dendrimers. Computer simulations have greatly improved our understanding of the underlying theories that explain the unique properties of dendrimers, but they have also raised expectations for molecular orientation and its effects. As drug delivery advances, other factors such as dendrimer growth, the ability to entrap free volume, and back folding of chains all depend on it. Understanding the intermolecular and intermolecular attractions in great detail is necessary to list the applications for dendrimers. As a result, the theoretical perspective on dendrimer characterization provides a basis for exploring various interactions in well-defined polymer families.

Acknowledgements:
The authors would like to thank the management of Annamacharya College of Pharmacy, Rajampet, Andhra Pradesh, for providing the facilities and encouraging them throughout the study.

Funding sources:
This research did not receive any specific grant from the funding agencies in the public, commercial, or not-for-profit sectors.

References:

[1] P. Patel, V. Patel, P.M. Patel, "Synthetic Strategy of Dendrimers: A review", Journal og Indian Chemical Society, 99(2022)100514, Doi: 10.1016/j.jics.2022.100514

[2] F. Nazafi, M. Salami-Kalajahi, H. Roghani-Mamaqani, "Janus-type dendrimers: Synthesis, properties, and applications", Journal of molecular liquids, 347(2022)118396. doi.10.1016/j.molliq.2021.118396.

[3] V.L. Furer, J.P. Major, A.M. Caminade, V.I. Kovalenko, Elementoorganic dendrimer characterization by Raman spectroscopy, Polymer. 45 (2004) 5889–5895.

[4] D. Stöckigt, G. Lohmer, D. Belder, Separation and Identification of Basic Dendrimers Using Capillary Electrophoresis On-line Coupled to a Sector Mass Spectrometer. Rapid Communications in Mass Spectrometry, 10 (1996) 521–526, DOI: 10.1002/(sici)1097-0231(19960331)10:5<521::aid-rcm518>3.0.co;2-g

[5] M. Przybylski, M.O. Glocker, Electrospray Mass Spectrometry of Biomacromolecular Complexes with Noncovalent Interactions—New Analytical Perspectives for Supramolecular Chemistry and Molecular Recognition Processes. Angewandte Chemie International Edition in English, 35(1996) 806–826, DOI:10.1002/anie.199608061

[6] N.K. Kaiser, G.E. Skulason, C.R. Weisbrod, J.E. Bruce, A novel fourier transform ion cyclotron resonance mass spectrometer with improved ion trapping and detection capabilities. Journal of the American Society for Mass Spectrometry, 20 (2009) 755–762, DOI: 10.1016/j.jasms.2008.12.022

[7] T.F. Magnera, D.E. David, D. Stulik, R.G. Orth, H.T. Jonkman, J. Michl, Production of hydrated metal ions by fast ion or atom beam sputtering. Collision-induced dissociation and successive hydration energies of gaseous copper+ with 1-4 water molecules, Journal of the American Chemical Society, 111 (1989) 5036–5043, DOI: 10.1021/ja00196a003

[8] J.F. Mahoney, J. Perel, S.A. Ruatta, P.A. Martino, S. Husain, K. Cook, T.D. Lee, Massive cluster

ISSN:1624-1940
http://magellanes.com/

impact mass spectrometry: A new desorption method for the analysis of large biomolecules, Rapid Communications in Mass Spectrometry, 5 (1991) 441–445, DOI:10.1002/rcm.1290051004

[9] Y. Ju, Applications of Matrix-Assisted Laser Desorption Ionization In-Source Decay Mass Spectrometry, Advancements of Mass Spectrometry in Biomedical Research, (2019) 45–54, DOI: 10.1007/978-3-030-15950-4_3

[10] I. Noda, Two-Dimensional Correlation Analysis Useful for Spectroscopy, Chromatography, and Other Analytical Measurements, Analytical Sciences, 23 (2007) 139–146, DOI: 10.2116/analsci.23.139

[11] L. M. Bronstein, Z. B. Shifrina, Dendrimers as Encapsulating, Stabilizing, or Directing Agents for Inorganic Nanoparticles. Chemical Reviews, 111 (2011) 5301–5344, DOI: 10.1021/cr2000724

[12] K. Kim, J. Lee, G. Jo, S. Shin, J. B. Kim, J. H. Jang, Dendrimer-Capped Gold Nanoparticles for Highly Reliable and Robust Surface Enhanced Raman Scattering, ACS Applied Materials & Interfaces, 8 (2016) 20379–20384, DOI: 10.1021/acsami.6b05710

[13] E. Sorsak, J. V. Valh, S. K. Urek, A. Lobnik, Application of PAMAM dendrimers in optical sensing, The Analyst, 140 (2015) 976–989, DOI: 10.1039/c4an00825a

[14] B. Trahasch, B. Stühn, H. Frey, K. Lorenz, Dielectric Relaxation in Carbosilane Dendrimers with Perfluorinated End Groups, Macromolecules, 32 (1999) 1962–1966, DOI: 10.1021/ma981075e

[15] S. Liu, Y. Ma, S. P. Armes, C. Perruchot, J. F. Watts, Direct Verification of the Core−Shell Structure of Shell Cross-Linked Micelles in the Solid State Using X-ray Photoelectron Spectroscopy, Langmuir, 18 (2002), 7780–7784, DOI: 10.1021/la020517j

[16] Manuel Scimeca, Simone Bischetti, Harpreet Kaur Lamsira, Rita Bonfiglio, Elena Bonanno. Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and Diagnosis. European Journal of Histochemistry. 2018; volume 62:2841

[17] Tirandai Hemraj-Benny, Sarbajit Banerjee, Sharadha Sambasivan, Mahalingam Balasubramanian, Daniel A. Fischer, Gyula Eres, Alexander A. Puretzky, David B. Geohegan, Douglas H. Lowndes, Weiqiang Han, James A. Misewich, and Stanislaus S. Wong. Near-Edge X-ray Absorption Fine Structure Spectroscopy as a Tool for Investigating Nanomaterials. Willey Inter Science, 2006, 2, No. 1, 26 – 35.

[18] J. Peterson, V. Allikmaa, J. Subbi, T. Pehk, and M. Lopp, “Structural deviations in poly(amidoamine) dendrimers: a MALDI-TOF MS analysis,” *European Polymer Journal*, vol. 39, no. 1, pp. 33–42, 2003.

[19] Bengt Norden, Kristina Fant, Elin K. Esbjo¨rner, Per Lincoln. DNA Condensation by PAMAM Dendrimers: Self-Assembly Characteristics and Effect on Transcription. *Biochemistry* **2008,** *47,* 1732-1740.

ISSN:1624-1940
http://magellanes.com/

[20] M. Shibue, C.T. Mant, R.S. Hodges, Effect of anionic ion-pairing reagent hydrophobicity on selectivity of peptide separations by reversed-phase liquid chromatography. Journal of Chromatography A, 1080 (2005) 68–75, DOI: 10.1016/j.chroma.2005.03.035

[21] Eric J. Amis, Barry J. Bauer, Andreas Topp, Ty J. Prosa, Dawei Liu, Catheryn L. Jackson, Alamgir Karim, Jack Douglas. Preparation and Characterization of Polymer/Dendrimer Blends Progress Report 2/24/97

[22] Tariq Baig, Jyoti Nayak, Vandana Dwivedi, Akanksha Singh, Ankur Srivastava and Pushpendra Kumar Tripathi. A Review about Dendrimers: Synthesis, Types, Characterization and Applications. International Journal of advances in Pharmacy, Biology and Chemistry.2015; 4(1): 44-59.

[23] Walter ED, Sebby KB, Usselman RJ, Singel DJ, Cloninger MJ. Characterization of heterogeneously functionalized dendrimers by mass spectrometry and EPR spectroscopy. J Phys Chem B. 2005; 109: 21532-8.

[24] Anne-Marie Caminade, Regis Laurent, Jean-Pierre Majoral. Characterization of dendrimers. Advanced Drug Delivery Reviews 57 (2005) 2130– 2146.

[25] J.S. Choi, E.J. Lee, Y.H. Choi, Y.J. Jeong, J.S. Park, Poly(ethylene glycol)-block-poly(l-lysine) dendrimer: novel linear polymer/dendrimer block copolymer forming a spherical water-soluble polyionic complex with DNA, Bioconjug. Chem. 10 (1999) 62– 65.

[26] R.Cerny, Crystal Structure of Non-molecular Compounds from Powder Diffraction, Croatia Chemica Acta, 79 (2006) 319-326.

[27] J.M. Carpenter, M. Agamalian, K.C. Littrell, P. Thiyagarajan, C. Rehm, Time-of-flight implementation of an ultra-small-angle neutron scattering instrument, Journal of Applied Crystallography, 36 (2003) 763–768, DOI: 10.1107/s0021889803003960

[28] T. Narayanan, High brilliance small-angle X-ray scattering applied to soft matter, Current Opinion in Colloid & Interface Science, 14 (2009) 409–415, DOI: 10.1016/j.cocis.2009.05.005

[29] D. Shcharbin, E. Pedziwiatr, J. Blasiak, M. Bryszewska, How to study dendriplexes II: Transfection and cytotoxicity, Journal of Controlled Release, 141 (2010) 110–127, DOI: 10.1016/j.jconrel.2009.09.030

[30] L. W. Zhang, N. A. Monteiro-Riviere, Use of confocal microscopy for nanoparticle drug delivery through skin, Journal of Biomedical Optics, 18 (2012) 061214, DOI: 10.1117/1.jbo.18.6.061214

[31] C. J. Fleming, N. N. Yin, S. L. Riechers, G. Chu, and G. Y. Liu, “High-resolution imaging of the intramolecular structure of indomethacin-carrying dendrimers by scanning tunneling microscopy,” ACS Nano, vol. 5, no. 3, pp. 1685–1692, 2011.

[32] J. Kuntsche, J.C. Horst, H. Bunjes, Cryogenic transmission electron microscopy (cryo-TEM) for studying the morphology of colloidal drug delivery systems, International Journal of

ISSN:1624-1940
http://magellanes.com/

Pharmaceutics, 417 (2011) 120–137, DOI: 10.1016/j.ijpharm.2011.02.001

[33] Y.-J. Kim, J.-W. Kim, J.-E. Lee, J.-H. Ryu, J. Kim, I.-S. Chang and K.-D. Suh, Synthesis and adsorption properties of gold nanoparticles within pores of surface-functional porous polymer microspheres. J Polym Sci Pol Chem, 2004, 42(22): 5627–5635.