Chromatography of Nanoparticles

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Science that deals with the matter at nanometer scale (10^{-9}m = 1nm) is termed as Nanotechnology. Nanotechnology helps to weild matter at the molecular and atomic dimensions to yield nanomaterials. Nanomaterials exist in the range of 1 to 100 nm and possess distinct chemical and physical properties. Mechanical strengths, melting points, optical properties, surface area and magnetizations are few of the notable physical properties of nanomaterials. Quantum effects and number of atoms in the surfaces of the nanomaterials influences its chemical properties and stability. Surface of nanoparticles consists of more atoms and large surface area in comparison to microparticles. Nanomaterials are increasingly used for various applications and encountered frequently by Scientists, Medical Professionals, Engineers and general public. Medicinal values of colloidal gold[1] were discovered by medical doctors in the middle ages. Apart from the medicinal values of colloidal gold, gold particles along with tin oxide called as purple of cassius[2] has been used as a colorant in glasses during ancient times.

Soluble gold was used to treat heart diseases, tumors, epilepsy, syphilis and dysentery[1]. Michael Faraday's experiment to reduce gold auric chloride using phosphorus in the presence of carbon disulfide resulted in red colloidal gold solution and gave birth to modern nanotechnology[3]. This breakthrough experiment by Michael Faraday inspired many Scientists to synthesis, modify and probe the properties of metal nanoparticles using various substrates and solvents.

Characterization of Nanoparticles: Nanomaterial's has its impact on various sectors such as health, engineering, agriculture, textile, food packaging and so on. As the need for nanomaterials grows day by day, it is necessary to have appropriate analytical technologies[4, 5] for characterization.

Several technologies are available for the qualitative analysis of nanomaterials, but not many techniques are there for purification of nanomaterials. Transmission electron microscopes[6], Dynamic Light Scattering[7], Powder X-ray diffraction[8], UV-Vis spectroscopy[9], Infrared Spectroscopy[10] etc., were regularly used to understand different properties of nanomaterials. All the above techniques are qualitative and cannot be used to separate a mixture of nanomaterials of various sizes and shapes. Nanomaterials are defined by shape, size, physical and chemical properties. Most of the techniques are utilized to measure the size of the nanoparticles. Size of the nanoparticles plays a major role in describing its physical and chemical properties. As the particle size decreases, its property changes drastically with respect to bulk materials. Nanomaterials and bulk materials exhibits extremely different

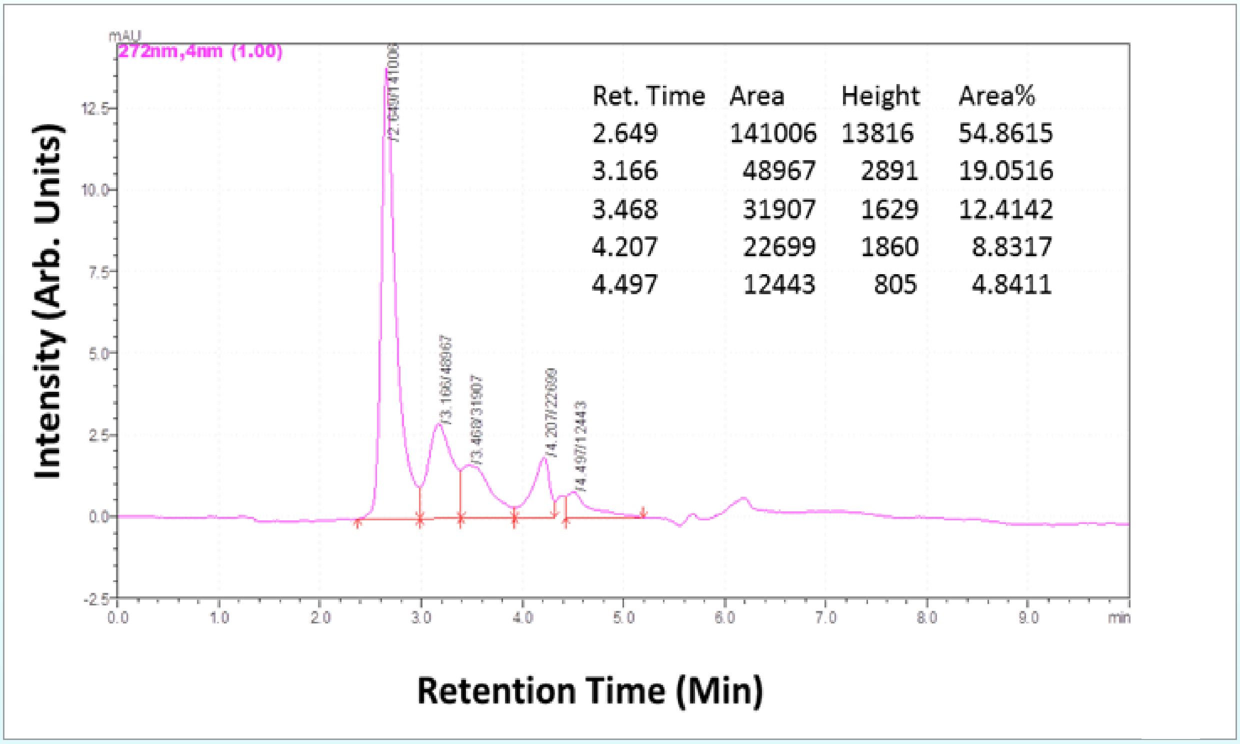

Fig. 1: LC-Chromatogram of Gold Nanoparticle. Co-eluting peaks could be buffer components or residues.

characteristics. For example gold is a solid yellow metal but nanomaterial of gold is soluble in solvents or buffer and is purple in color.

Many technologies are employed by Scientists to differentiate bulk materials from nanomaterials. Transmission electron microscopy[6] and Dynamic light scattering methods[7] are used to measure the size of the nanoparticles in nanometer scale. Scanning electron microscope provides the morphology of materials. Powder X-ray diffraction measurements[8] of nanoparticles reveal the crystalline nature and size of the nanomaterials. UV-Vis spectroscopy[9] provides the wavelength of nanoparticles with respect to size. All the above technologies confirm the size of the nanoparticles. Infrared spectroscopy[10] helps to detect the functional groups coated on nanoparticles, the presence of metal-oxygen bonds etc. Thus Infrared spectroscopy[10] confirms the presence of organic, organo-metallic functional groups in the nanomaterial. Size exclusion chromatography helps to separate the nanoparticles based on its size. Energy dispersive X-Ray (EDX) composition analysis is performed to estimate the composition and stoichiometry of nanomaterials. All these above technologies complement one another to probe and understand the physical and chemical properties of nanomaterials.

Difficulties in Characterization of Nanoparticles

Most of the existing technologies barring UV-Vis spectroscopy, Dynamic light scattering and Chromatography are used to analyze the nanomaterials in the solid state. Nanomaterials in the liquid form need to undergo special sample preparation techniques for characterization. Nanomaterials derived from metals such as zinc, copper, iron, titanium, magnesium etc., are solid in nature that can be dispersed in solvents but do not dissolve in any solvents. Nanomaterials derived from gold and silver are soluble in solvents and exhibits different colors. Similarly selective polymeric nanomaterials also dissolve in solvents. Nanomaterials that are soluble in solvents can be analyzed by chromatographic

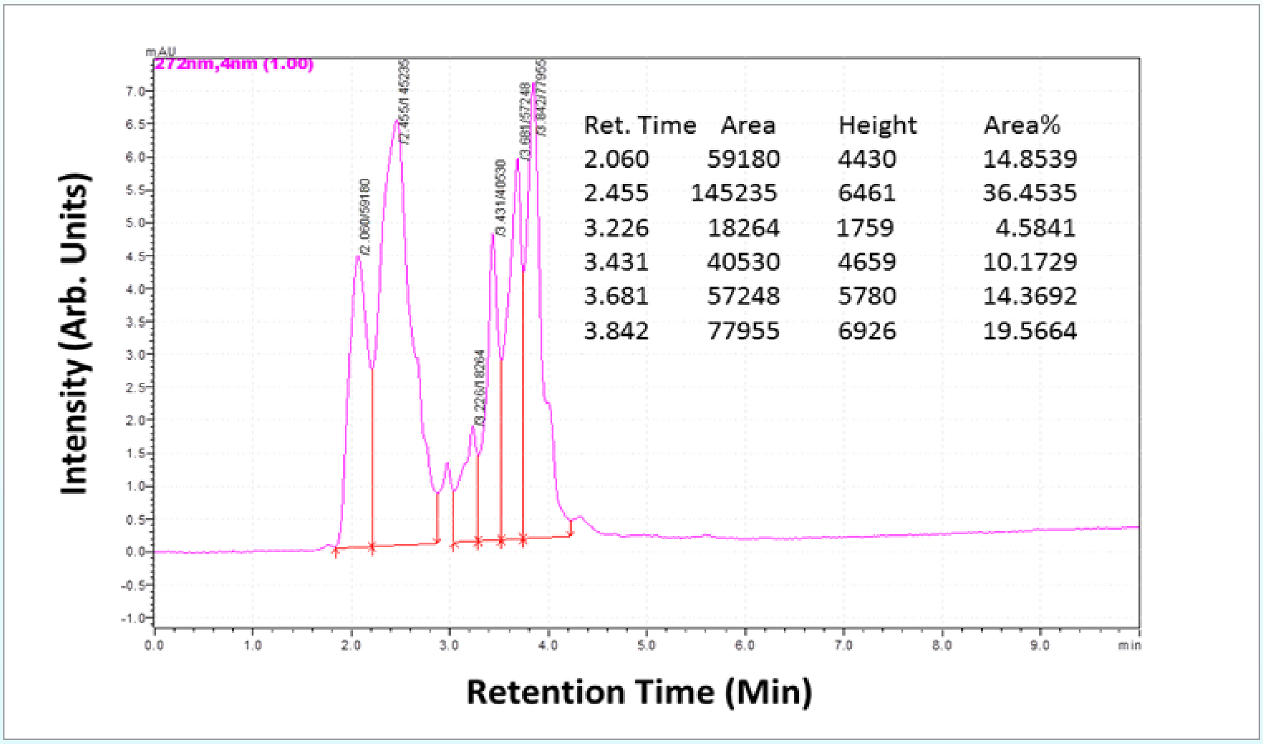

Fig. 2: LC-Chromatogram of Gold Nanoparticle obtained from biological route. Two clusters of peaks were identified. First group of peak was observed between 1 to 3 Min and Second group of peaks were detected between 3 to 4 min.

methods whereas nanomaterials in the solid forms cannot be analyzed by chromatographic methods. Therefore chromatography is restricted only to the analysis of soluble nanoparticles. New *in vitro* methodologies have to be developed to dissolve the insoluble nanomaterials in solvents.

Synthesis and Purification of Soluble Nanoparticles Synthesis of nanoparticles by biological routes[11], chemical methods and physical methods results in nanoparticles of various sizes and shapes. Therefore there is a need to purify the nanoparticles to segregate them according to their size and shape. Chromatography is the answer to separate and purify nanoparticles of various size and shapes. Size exclusion chromatography has been shown as a tool to separate nanoparticles of various sizes. Recently in our laboratory we have used liquid chromatography with C18 column to purify the nanoparticles[12]. Nanoparticles are synthesized from both chemical and biological routes for different applications.

Most often, Biosynthesis of nanoparticles results in mixture of nanoparticles with bye products compared to that of chemical synthesis. Nanoparticle synthesis by chemical routes also results in nanoparticles with fewer amounts of bye products. For example synthesis of Gold Nanoparticle using chemical method will result in both gold nanoparticles and gold auric chloride that was not completely reduced. During the biosynthesis, reducing agent is derived from biomolecules and therefore mixture of bye products is always expected.

Size Exclusion Chromatography for Characterization of Nanoparticles

Size exclusion chromatography[13, 14] consists of a column with a solid support packed with microscopic beads or spheres. These microscopic beads are column beads that play a crucial role in separating the molecules of various sizes. Nanoparticles of various sizes dissolved in a liquid are generally applied through this column bed. For example if a solution consisting of a mixture of 10nm, 50nm and 100 nm sized nanoparticles is applied through the column bed; nanoparticle with a size of 100nm elutes first followed by 50 nm sized nanoparticles and later 10 nm sized nanoparticles. The column beads serves as a sieves or traps and filters the 10nm sized nanoparticles, thus nanoparticles with small diameters stay in the column for a longer period of time. Nanoparticles with large diameters such as 100 nm do not stay in the column bed for a longer time and elutes fast. Nanoparticles with intermediate size have intermediate elution time. Since size exclusion chromatography[13,14] is specifically used to filter molecule based on its size, it cannot separate molecules based on its polarity. Therefore in the present study, we have used High Performance Liquid Chromatography to purify the nanoparticles of a particular size from other buffer impurities and organic moieties.

Chromatography of Nanoparticles for purification

High Performance Liquid Chromatography from Shimadzu with C18 column is used in our laboratory[12] to understand the constituents of synthesized nanoparticles. Under the constant mobile phase conditions i.e mobile phase with a mixture of 70% acetonitrile and 30% water we could separate nanoparticles from bye products. In one of our recent publication in American Journal of Analytical Chemistry we have demonstrated how to purify the gold nanoparticles that are obtained from chemical routes where gold auric chloride is the starting material. Starting material and expected gold nanoparticle were identified at two different retention time's i.e gold auric chloride elutes at the retention time of 2 min and gold nanoparticle elutes at the retention time of 2.649 min. Thus, we have proved that Ultra Fast Liquid Chromatography, Shimadzu with appropriate C18 column can be effectively utilized to purify the nanoparticles. Figure 1 shows the gold nanoparticle that elutes at the retention time of 2.649 min. Insert of Figure 1 captures the relative area of the gold nanoparticle to that of other constituents. Shimadzu's High Performance Liquid Chromatography is accurate enough to purify and characterize the nanoparticles.

Nanoparticles derived from biomolecules such as aloe vera and curcumin were also characterized using Shimadzu's Ultra Performance Liquid Chromatography. Liquid chromatography of nanoparticles coated with biomolecules exhibits many peaks compared to that of chemically derived nanoparticles. Figure 2 exhibits the contents of gold nanoparticles derived by biological method. Gold auric chloride was treated with aloe vera and the resulting content of the solution was analyzed. Gold nanoparticles were observed at the retention time of 2.455 min along with another peak at 2.06 min. Peak eluted at 2.06 min could be Gold nanoparticle of different size. Apart from this three more major peaks were observed between 3 to 4 min. These peaks could be gold nanoparticles coated with organic compounds from aloe vera or unreacted aloe vera. More studies are currently in progress to understand each peak of this chromatogram.

Chromatography Conditions in Shimadzu Prominence Liquid Chromatography to Study Nanoparticles

The HPLC system[12] used for the study was Shimadzu Prominence Liquid Chromatography (Shimadzu, Kyoto, Japan) assembled with DGU-20AS degasser for mobile phases, SIL-20AC injector valve with sample holder, LC-20AD pump, C10-10AS column holder with oven to control temperature, LC-20AD pump and SPD-M20A photo diode array detector to detect compounds at different wavelengths. The HPLC column used was C18 column with 5 micron particles of C18 packing material and 150 mm x 5 mm i.d. LC-Quant software associated with Shimadzu HPLC instrument was used for characterization of peaks. This highly sophisticated software is good to integrate and obtain the accurate area of the peaks. Gradient mobile phase conditions was employed during method development using different mobile phase combinations such as methanol/water; acetonitrile/water and acetonitrile/methanol systems. Ideal retention time for the elution of nanomaterial is thus confirmed and appropriate solvent system was selected for isocratic system. Acetonitrile/Water system with 70:30 ratio was identified as the ideal mobile phase condition to analyze the nanoparticle samples. Once the mobile phase ratio is confirmed gold nanoparticles, gold auric chloride and gold nanoparticles coated with biomolecules were analyzed. Peak areas help to confirm the relative percentage of formed nanomaterials.

Summary

New method using Shimadzu Liquid Chromatography has been developed to purify the nanoparticles. In the present study nanoparticles and nanoparticles coated with biomolecules were analyzed. Standard nanoparticle obtained from Sigma Aldrich was used as a standard and compared with nanoparticles synthesized by biological methods. This newly developed method is applicable to analyze gold auric chloride which is astarting material for synthesis, gold nanoparticle derived from gold auric chloride and gold nanoparticles coated with biomolecules. Gold auric chloride, gold nanoparticles, nanoparticles coated with biomolecules and buffer impurities elutes at different retention times. Thus this method is valuable and effective to purify the nanoparticles derived from biological methods and chemical methods.

Future Directions

Nanomaterials are increasingly used in healthcare, food industry, engineering, cosmetic products and agricultural sectors. Therefore food and drug administration guidelines are essential for safe usage of nanomaterials. If nanoparticles are injested in humans its pharmacokinetics, metabolism, distribution and permeability has to be clearly studied in detail[15]. Nanomedicine is an emerging field of interest, therefore purification and characterization of nanomedicines from blood and urine samples will be very important. Nanoparticles soluble in solvents or water can be well characterized using liquid chromatography. Liquid chromatography will be an effective tool to purify the nanoparticles. Once the method is developed utilizing liquid chromatography, developed method can be transferred to preparative HPLC to scale up the product. Similar to the present study using liquid chromatography to purify the gold nanoparticles, methods to purify and characterize other soluble nanoparticles need to be attempted. For example soluble nanoparticles derived from silver, polymers, biomolecules etc., can be purified using liquid chromatography. Apart from this integration of liquid chromatography with mass spectrometry will confirm the molecular weight and helps to characterize the nanomaterials and associated bye products derived during synthesis from chemical and biological methods. Thus the purified nanomaterials using liquid chromatography and further characterization with mass spectrometry will assist engineers and biomedical scientists to develop safe and quality nanoproducts.

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