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Atomic-level separation of thiolate-protected metal clusters

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Fine metal clusters have attracted much attention from the viewpoints of both basic and applied science for many years because of their unique physical/chemical properties and functions, which differ from those of bulk metals. Among these materials, thiolate (SR)-protected gold clusters (Auₙ(SR)m clusters) have been the most studied metal clusters since 2000 because of the ease of synthesis and handling. However, in the early 2000’s, it was not easy to isolate these metal clusters. Therefore, high-resolution separation methods were explored, and several atomic-level separation methods, including polyacrylamide gel electrophoresis (PAGE), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), were successively established. These techniques have made it possible to isolate a series of Auₙ(SR)m clusters, and much knowledge has been obtained on the correlation between the chemical composition and fundamental properties such as the stability, electronic structure, and physical properties of Auₙ(SR)m clusters. In addition, these high-resolution separation techniques are now also frequently used to evaluate the distribution of the product and to track the reaction process. In this way, high-resolution separation techniques have played an essential role in the study of Auₙ(SR)m clusters. However, only a few reviews have focused on this work. This review focuses on PAGE, HPLC, and TLC separation techniques, which offer high resolution and repeatability, and summarizes previous studies on high-resolution separation of Auₙ(SR)m and related clusters with the purpose of promoting a better understanding of the features and the utility of these techniques.

1. Introduction

Fine metal clusters smaller than ~2 nm (< ~100 atoms) exhibit different geometrical/electronic structures than those of bulk metals.1 Metal clusters often form a unique framework, such as an icosahedral structure, to suppress the surface energy instead of the close-packed structure often observed in bulk metal. In addition, metal clusters exhibit a discrete electronic structure unlike bulk metal. Because of these characteristic geometrical/electronic structures, fine metal clusters exhibit unique physical/chemical properties and functions that differ from those of bulk metals. In addition, the physical/chemical properties and functions of metal clusters vary significantly depending on the number of constituent atoms. For these reasons, fine metal clusters have attracted considerable attention for many years in the fields of both basic and applied science.

For these metal clusters, it is essential to conduct investigations on metal clusters controlled with atomic precision to understand the structures and physical/chemical properties. For this purpose, gas-phase beam experiments are superior 2−6 and they led researches of the metal clusters in the 1980’s and the 1990’s. Many unique characteristics of metal clusters were discovered during this period by observing the mass distributions and electronic structures in gas-phase beam experiments. In parallel with such physical experiments, the research on the synthesis of phosphine (PR₃)-protected metal clusters was also actively pursued during this period.7−15 The main purpose of these studies was to reveal the correlation between chemical composition and geometrical structure, and the geometrical structures of some PR₃-protected metal clusters were determined by single-crystal X-ray diffraction (SC-XRD) at that time. Regarding the thiolate (SR)-protected metal clusters described below, the geometrical structure was first determined by SC-XRD in 2007; thus, the development of the researches on PR₃-protected metal clusters at that time was remarkable. Since 2000, nanotechnology has been adopted as a national policy in many countries, and the main target metal clusters have shifted to SR-protected gold clusters (Auₙ(SR)m clusters).16−33 Auₙ(SR)m clusters can be synthesized by simply mixing reagents in a solvent in air. Furthermore, Auₙ(SR)m clusters are stable both in solution and in the solid state because the SR group forms a strong bond with Au. Such Auₙ(SR)m clusters have a low threshold even for researchers who have not worked on metal cluster synthesis and show great potential for material applications. It is presumed that these factors are largely related to the explosive increase in the number of studies on Auₙ(SR)m clusters since 2000.20 However, for Auₙ(SR)m clusters, single crystallization techniques could not be found, and thus, they could not be
isolated in the early stage. Then, high-resolution separation techniques were developed to isolate $\text{Au}_{n}(\text{SR})_m$ clusters. In earlier studies, separation techniques that relied on differences in solubility to solvents were mainly used.\textsuperscript{34-38} Then, atomic-level separation techniques, such as polyacrylamide gel electrophoresis (PAGE) and liquid chromatography (LC) were developed. These methods made it possible to isolate a series of $\text{Au}_{n}(\text{SR})_m$ clusters and obtain knowledge on the correlation between the chemical composition and the fundamental properties (e.g., stability,\textsuperscript{39,40} electronic structure,\textsuperscript{41} physical/chemical properties\textsuperscript{32,41}) of $\text{Au}_{n}(\text{SR})_m$ clusters. After these separation techniques were established, the experimental conditions for synthesizing only stable metal clusters were also determined by using the features of their optical absorption spectra. As a result, several methods were established for selectively synthesizing stable $\text{Au}_{n}(\text{SR})_m$ clusters with atomic precision.\textsuperscript{24,44} In addition, because crystallization was easier for isolated or selectively synthesized $\text{Au}_{n}(\text{SR})_m$ clusters, the geometrical structures were also determined by SC-XRD for several $\text{Au}_{n}(\text{SR})_m$ clusters.\textsuperscript{45} In parallel with these studies, studies on the application of these precisely controlled $\text{Au}_{n}(\text{SR})_m$ and related clusters for chemical sensors,\textsuperscript{46,47} photosensitization,\textsuperscript{48} catalysts,\textsuperscript{49,50} and solar cells\textsuperscript{51,52} were conducted.\textsuperscript{53}

Thus, high-resolution separation techniques have played an extremely important role in the study of $\text{Au}_{n}(\text{SR})_m$ clusters. Recently, some stable metal clusters can be synthesized with atomic precision.\textsuperscript{24,44} However, not all clusters can currently be synthesized size-selectively. Thus, the use of high-resolution separation techniques remains indispensable for understanding the correlation between the chemical composition and structure/physical properties of SR-protected metal clusters. Moreover, these separation techniques are also needed for purification of the main products and for isolation of metastable species. In addition to the use for fractionation as described above, these separation techniques are also often used to evaluate the distribution of a product and to track reaction. Despite their importance, few reviews have focused on these techniques in the study of $\text{Au}_{n}(\text{SR})_m$ clusters.\textsuperscript{54}

This review summarizes research on the high-resolution separation of $\text{Au}_{n}(\text{SR})_m$ and related clusters. In particular, we describe previous studies on PAGE, high-performance LC (HPLC), and thin-layer chromatography (TLC), which are separation techniques with high resolution and repeatability. The aim of this review is to provide a deep understanding of the features and usefulness of the three separation techniques through past research. The following section outlines the basic principles and experimental methods of PAGE, HPLC, and TLC. Then, in section 3, we briefly describe previous high-resolution separation methods that were used before the appearance of these three techniques. Next, sections 4 to 6 outline previous works on PAGE, HPLC, and TLC, respectively. Finally, section 7 summarizes the review and briefly describes future expectations.

2. Principles and Procedure of Each Separation Technique

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{Schematic illustration of (A) PAGE, (B) HPLC, and (C) TLC separation techniques.}
\end{figure}

2.1. Polyacrylamide gel electrophoresis

When a direct current flows through a solution, the charged sample in the solution moves in the direction of the oppositely charged electrode. A method of separating a substance based on this principle is called electrophoresis. In PAGE, substances move in a three-dimensional network consisting of polyacrylamide gel. Each metal cluster can be separated by this method because the mobility of the clusters differs depending on the charge, particle size, and shape. PAGE is generally used for the separation of hydrophilic $\text{Au}_{n}(\text{SR})_m$ and its related clusters containing a ligand with a dissociable functional group.

In the experiment, first, a polyacrylamide-gel running layer is prepared between two plates using acrylamide and bis(acrylamide). Then, a polyacrylamide-gel stacking layer is prepared using acrylamide and bis(acrylamide) with a different concentration than that of the running layer. A sample solution containing several percent of glycerol is dropped on the upper end of the gel. Then, electrophoresis is performed by applying a voltage to the electrodes placed on the upper and lower sides of the gel. Each cluster is separated into several bands (Figure 1A). Bands containing each metal cluster are cut out using a utility knife, and the clusters are eluted from the gel by leaving them in water and collecting them from the solution.

The device for PAGE is simple and inexpensive (Figure 1A). In addition, when the cluster mixture is separated by PAGE, the separation of each component cluster can be visually confirmed. It is also possible to specify luminescent clusters by ultraviolet light irradiation. Moreover, PAGE is also useful for evaluating the product size and shape because the mobility of each metal...
cluster varies depending on the particle size and shape as well as the charge and the interaction with the solvent.

2.2. Liquid chromatography

Chromatography is a method used to separate a sample based on the difference in the extent of the interaction between the sample and the stationary phase. When the mobile phase is liquid, the technique is called LC.

2.2.1. Open column chromatography

For classical LC, the sample is loaded on the upper end of an open column with the stationary phase. Then, the mobile phase is passed through from the upper end of the column to separate the substances. Because of its simplicity, this method is often used for washing metal–SR complexes with greatly different sizes. However, there are almost no examples of Au\(_n\)(SR)\(_m\) and their related clusters being separated with atomic precision because it is difficult to achieve a high degree of precision using this method. Therefore, this review does not introduce separation using this method.

2.2.2. High-performance liquid chromatography

When a high pressure is applied in LC, the mobile phase passes through the column at a high flow rate. Consequently, the time during which the substance remains in the stationary phase is shortened, and the resolution and detection sensitivity are improved. This separation method is called HPLC to distinguish it from classical LC. The principle of separation used in HPLC is roughly divided into four types: adsorption, partition, ion exchange, and size exclusion. In addition, various other separation modes are used in actual HPLC analysis; however, these modes can be recognised as modifications or combinations of the above four separation principles in terms of the separation mechanism. The normal phase is the separation condition in which the polarity of the stationary phase is higher than that of the mobile phase. In contrast, reverse phase (RP) is the separation condition in which the polarity of the stationary phase is lower than that of the mobile phase (Figure 1B). In the separation of Au\(_n\)(SR)\(_m\) and their related clusters, partition, adsorption, and size exclusion have been used as the main separation principles.

The general HPLC system used for such separation consists of a mobile-phase solvent reservoir, a degasser device, a high-pressure liquid pump, an injector, a column, and a detector (Figure 1B). There are two types of mobile phases, one using a single mobile phase and the other using multiple types of mobile-phase solvents while arbitrarily changing their volume ratio. The first method is called isocratic mode, and the second method is called gradient mode. In general, a column is produced by filling a stainless-steel tube with a particulate stationary phase (column filler) at high pressure and sealing its upper and lower ends with a sintered porous stainless filter. Silica gel particles with surfaces modified by an organic layer are often used as the column filler (Figure 1B).

HPLC has advantages of resolution and repeatability. There is also the advantage that electrospray ionization (ESI) mass spectrometry (MS) can be used for the detection. As opposed to separation by PAGE, when the HPLC apparatus and ESI mass spectrometer are directly connected (LC/MS), all the generated clusters can be directly led to the MS section. Thus, it is possible to determine the chemical composition of very minor components that cannot be detected by PAGE.

2.2.3. Thin-layer chromatography

There have been some reports on the separation of Au\(_n\)(SR)\(_m\) clusters by TLC using a planar stationary phase in recent years. The TLC plates are prepared by coating a stationary phase on a flat glass, aluminium, or plastic plate. Currently, TLC plates coated with various types of stationary phases are commercially available. The separation principles of adsorption, partition, ion exchange, and size exclusion can also be used in TLC separation.
In the experiment, the solution containing the sample is spotted 15 to 25 mm above the lower end of the TLC plate using a micropipette or a capillary tube. After drying the sample, the plate is allowed to stand with the spot facing down in a sealed container containing a solvent (the mobile phase). The sample is separated by rising of the solvent from the lower side via capillary action (Figure 1C). The band containing each Au\(_n\)(SR)\(_m\) cluster is cut out with a spatula, and each Au\(_n\)(SR)\(_m\) cluster is collected from the plate.

With TLC, it is difficult to achieve the same degree of resolution as HPLC. However, TLC uses a less expensive and simpler experimental apparatus than HPLC. Therefore, when the distances that each Au\(_n\)(SR)\(_m\) cluster travel on the plate are significantly different, the target Au\(_n\)(SR)\(_m\) cluster can be easily and inexpensively isolated using this method. It is also one of the advantages of this method that the separation of Au\(_n\)(SR)\(_m\) clusters can be visually confirmed, as for PAGE.

### 3. Early Studies on Separation

Many attempts were made to separate Au\(_n\)(SR)\(_m\) clusters based on the number of constituent atoms before the use of the above three methods (PAGE, HPLC, and TLC).\(^{34-38}\) For example, Whetten et al. synthesized Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) clusters (SC\(_{2}H_{2}S_{2}I\) = alkanethiolate) as a ligand. They gradually added a poor solvent and precipitated clusters from large to small in order.\(^{35}\) In these studies, each separated Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) cluster was evaluated mainly using matrix-assisted laser desorption/ionization (MALDI)-MS. In the mass spectrum of each Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) cluster, ion peaks appeared in different mass regions (Figure 2A), which indicated that the Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) clusters were separated based on their size. Unfortunately, these mass spectra included only the fragmentation peaks of each Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) cluster.\(^{38}\) Therefore, the chemical composition of each Au\(_n\)(SC\(_{2}H_{2}S_{2}I\))\(_m\) cluster was not accurately determined at this stage. Murray et al. also separated Au\(_n\)(SR)\(_m\) clusters (SR = SC\(_{2}H_{2}S_{2}I\) or PET) using solvent-selective extraction.\(^{55}\) Although this method has been revealed to be useful for obtaining each stable Au\(_n\)(SR)\(_m\) clusters at atomic resolution in the later studies,\(^{56-58}\) and thereby often used for the isolation of Au\(_n\)(SR)\(_m\) clusters in a large quantities in recent studies,\(^{59-63}\) unfortunately, the separated Au\(_n\)(SR)\(_m\) clusters could not be confirmed to be isolated at atomic resolution at this stage. However, these studies provided considerable information on the correlation between the particle size and electronic structure in the Au\(_n\)(SR)\(_m\) clusters (Figure 2B).\(^{34-38}\) Since then, the separation technique for Au\(_n\)(SR)\(_m\) clusters has progressed rapidly with the introduction of separation methods offering high resolution and repeatability and the improvement of mass spectrometers.

### 4. PAGE Separation

#### 4.1. PAGE separation for isolation

PAGE was first applied to separate Au\(_n\)(SR)\(_m\) clusters in 1998. Schaa and Whetten et al. separated Au\(_n\)(SG)\(_m\) clusters (SG = glutathioninate) into multiple bands using PAGE (Figure 3A).\(^{54}\) For the separation, they made gels in higher concentration (~40%) than the gels used in protein separations. This optimization of the gel concentration led to the high-resolution separation of Au\(_n\)(SG)\(_m\) clusters (Figure 3B). They also measured the Au\(_n\)(SG)\(_m\)....

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**Figure. 3** (A) PAGE separation of Au\(_n\)(SG)\(_m\) clusters and (B) optical absorption spectra of the main product in the sample in (A) from an early study.\(^{64}\) (C) ESI source constructed in the study of ref. 41. (D) ESI mass spectrum of each fraction separated by PAGE.\(^{64}\) Reproduced with permission from ref. 64 and 41. Copyright 1998 American Chemical Society and Copyright 2005 American Chemical Society.
homemade apparatus, an ionized solvent containing Au using their homemade ESI mass spectrometer. Using their method could be used to separate a mixture into each Au clusters were assigned as Au cluster with atomic precision. achieved in the mass spectra, and the separated Au compositions of the Au clusters could be efficiently introduced into the mass spectrometer (Figure 3C).

Later, Negishi and Tsukuda et al. demonstrated that this isolation of Au clusters is especially stable. Stable clusters with similar absorption spectra had previously been reported by Whetten et al. and Murray et al. This study revealed that the exact chemical composition of these clusters was Au$_{32}$(SR)$_{18}$. For the Au$_{32}$(SR)$_{18}$ clusters, Kimura et al. and Dass et al. separated larger-sized clusters up to Au$_{56}$(SG)$_{29}$ and Au$_{92}$(SG)$_{26}$, respectively. In Dass’s study, the chemical composition of each cluster was determined using a commercially available mass spectrometer. The detection sensitivity of commercial apparatuses have improved, and in research since 2008, the chemical composition of separated clusters has been evaluated using commercial rather than homemade apparatuses.

The isolation of Au$_{n}$(SG)$_{m}$ clusters with atomic precision enabled a deep understanding of the stability, structure, and other properties of Au$_{n}$(SG)$_{m}$ clusters to be obtained. Tsukuda and Teranishi et al. showed that a series of Au$_{n}$(SG)$_{m}$ clusters exhibited especially high stability against etching by excess thiol (Figure 4A) and that Au$_{32}$(SG)$_{18}$ could be selectively synthesized in a solution where etching is likely to occur. Kojima and Tsukuda et al. experimentally demonstrated using Au$_{39}$(SG)$_{24}$, respectively (Figure 3D). This study also clarified that Au$_{32}$(SG)$_{18}$ is especially stable. Stable clusters with similar absorption spectra had previously been reported by Whetten et al. and Murray et al. This study revealed that the exact chemical composition of these clusters was Au$_{32}$(SR)$_{18}$.

Figure 4 (A) Photographs of a series of Au$_{n}$(SG)$_{m}$ cluster aequous solutions (n = 10, 15, 18, 22, 25, 29, 33, and 39) before and after a 3-h etching reaction. (B) Mössbauer spectrum of Au$_{32}$(SG)$_{18}$. (C) X-ray absorption spectra (top) and XMCD (bottom) spectra of Au$_{32}$(SG)$_{18}$ at the (a) Au L$_{2}$ and (b) Au L$_{3}$ edges with an applied magnetic field of 10 T at 2.7 K. Reprinted with permission from ref. 39, 70, and 42. Copyright 2007 Wiley-VCH, Copyright 2007 American Chemical Society, and Copyright 2006 American Chemical Society.

Figure 5 (A) PAGE separation of (a) Au$_{4}$(SG)$_{m}$, (b) Au$_{4}$(SPG)$_{m}$, and (c) Au$_{4}$(S(PG)$_{m}$ clusters. (B) Comparison of optical absorption spectra of Au$_{4}$(SR)$_{14}$, Au$_{4}$(SR)$_{16}$, and Au$_{4}$(SR)$_{18}$ with different ligand structures. (C) PAGE separation of Au$_{8}$(SG)$_{m}$ clusters. Reproduced with permission from ref. 73 and 79. Copyright 2006 American Chemical Society and Copyright 2010 American Chemical Society.
Tsukuda and Yokoyama et al. showed that fine Au\(_{m}\) clusters could exhibit magnetism using X-ray magnetic circular dichroism (XMCD) spectrometry (Figure 4C). \(^{42}\) Unfortunately, the interpretations in these studies were not necessarily accurate. The exact geometrical structure of Au\(_{25}\)(SC\(_2\)H\(_4\)Ph)\(_8\) (SC\(_2\)H\(_4\)Ph = phenylethanethiolate) was later determined using SC-XRD by Murray et al. and Jin et al., independently. \(^{56,71}\) Jin et al. clarified the exact origin of the magnetism using electron spin resonance spectroscopy. \(^{72}\) However, studies on a series of isolated Au\(_{m}\)(SG)\(_m\) clusters by Tsukuda et al. have provided many new insights into the stability, electronic/geometrical structures, and other properties of Au\(_{m}\)(SR)\(_m\) clusters. These findings further demonstrate that it is essential to conduct research on fine metal clusters with controlled chemical composition to elucidate their fundamental properties.

Separation using PAGE has also been applied for the isolation of Au\(_{m}\)(SR)\(_m\) clusters protected with other hydrophilic ligands. Negishi and Tsukuda et al. separated tiopronin (S(PG))- or mercaptosuccinic acid (S(SA))-protected Au clusters using PAGE (Figure 5A). \(^{73}\) They showed that the chemical compositions of the isolated Au\(_{m}\)(SR)\(_m\) clusters differed depending on the ligand structure by comparing the chemical compositions of Au\(_{m}\)(S(PG))\(_m\), Au\(_{m}\)(S(SA))\(_m\), and Au\(_{m}\)(SG)\(_m\) clusters. \(^{73}\) Comparison of the optical absorption spectra of these clusters with the same chemical composition revealed that the electronic structure of Au\(_{m}\)(SR)\(_m\) clusters also changes depending on the ligand structure (Figure 5B). \(^{73}\) Tsukuda et al. isolated Au\(_{m}\)(S(PG))\(_m\) at high purity and obtained information on its geometrical structure in a later study. \(^{74}\) In addition, other hydrophilic Au\(_{m}\)(SR)\(_m\) clusters were separated using PAGE by Bürgi et al. \((\text{SR} = \text{N-isobutyl-cysteine})^{75,76}\) and Yao et al. \((\text{SR} = \text{penicillamine (Pen)})^{77}\) and 3-mercaptophenyl-boronic acid. \(^{78}\)

Separation using PAGE has also been applied for the isolation of other metal clusters. Bigioni et al. separated Ag\(_n\)(SG)\(_m\) clusters using PAGE (Figure 5C) and suggested that Ag\(_n\)(SG)\(_m\) clusters could have a different chemical composition than Au\(_n\)(SG)\(_m\) clusters by comparing each gel. \(^{79}\) The main product of Ag\(_n\)(SG)\(_m\) clusters was later determined to be Ag\(_{32}\)(SG)\(_{19}\) by Griffith and Bigioni et al. \(^{80}\) and this chemical composition actually cannot be observed for Au\(_n\)(SG)\(_m\) clusters.

In section 4.1, we introduced the isolation of metal clusters by PAGE. Meanwhile, fine metal clusters often exhibit a colour that depends on the number of constituent atoms. Thus, when they are separated by PAGE, the distribution of their chemical compositions can be confirmed visually. For this reason, PAGE is also often used to examine the distribution of chemical compositions of a product, optimize reaction conditions, elucidate the degraded processes, and track reactions. \(^{102-110}\)

For example, Tsukuda and Teranishi et al. observed that Au\(_{25}\)(SG)\(_{19}\) was a major product when a triphenylphosphine (PPh\(_3\))-protected Au cluster was mixed with glutathione (GSH). They attempted to optimize the reaction conditions to selectively synthesize such a stable cluster with atomic
the pH of the solution to near neutral or by adding Ag(NO₃). The results indicated that high-purity Au clusters synthesized under an argon atmosphere by adding a radical initiator to the reaction vessel. Bigioni et al. observed the degradation process of Au₃₅(SG)₀₈ and Au₃₆(SG)₀₉ using PAGE and discussed why Au clusters were selectively synthesized in the reaction between the PPh₃-protected Au cluster and GSH.

Ackerson et al. synthesized Au₁₀₂(p-MBA)₄₄ (p-MBA = p-mercaptobenzoic acid) under different gas atmospheres and examined the distribution of products using PAGE. They observed that O₂ was required as a gas for radical generation when using an organic solvent during the synthesis (Figure 7A). They also showed that Au₁₀₂(p-MBA)₄₄ could be synthesized under an argon atmosphere by adding a radical initiator to the reaction vessel. Bigioni et al. observed the degradation process of Au₃₅(SG)₀₈ under various conditions (time, pH, presence or absence of salt, etc.) using PAGE. These studies revealed that the degradation of the Au₃₅(SG)₀₈ clusters can be relatively suppressed by adjusting the pH of the solution to near neutral or by adding Ag⁺ ions into the solution. Bakr et al. demonstrated using PAGE that the structural changes between Ag₃₅(SG)₀₈ and Ag₄₆(4-FTP)₃₀ (4-FTP = 4-fluorothiophenolate) induced by ligand exchange are reversible (Figure 7B). Xie et al. revealed that Au₃₅(SG)₀₈ could be produced as an intermediate in the synthesis of Au₃₂(SG)₀₈ with carbon monoxide (CO) reduction by monitoring the reaction using PAGE.

In this way, the use of PAGE enables the chemical composition distribution of the product to be clarified. Through the use of such PAGE separation, much knowledge has been obtained on appropriate synthesis conditions, stable conditions, and reaction mechanisms.

4.3. PAGE separation to identify luminescent cluster

Fine noble metal clusters often exhibit PL. Au and Ag are harmless elements, unlike cadmium and arsenic, which form compound semiconductors with strong emission. However, it is not easy to identify a metal cluster that emits PL with a high quantum yield from a mixture. However, if the mixture is separated by PAGE and then the gel is irradiated with ultraviolet light, it is possible to visually identify the metal cluster exhibiting PL. Therefore, PAGE has often been utilized to identify luminescent metal clusters.

Au₃₂(SG)₀₈, which emits red light with high quantum yield (~8%), was identified by such PAGE separation. Xie et al. synthesized Au₃₂(SG)₀₈ clusters using CO as the reduction agent and separated the mixture into each band using PAGE. The products were separated into four different Au₃₂(SG)₀₈ clusters. Irradiation of the obtained gel with ultraviolet light revealed that Au₃₂(SG)₀₈, which has the lowest mobility, emits strong red PL (Figure 8A). Based on the results of DFT calculation and X-ray absorption spectroscopy, they suggested that Au₃₂(SG)₀₈ has a geometrical structure in which the Au core is covered by a mercaptobenzoic acid (SA) ligand and reduced to form a Au₃₂ (SG)₀₈ cluster. Pradeep et al. succeeded in finding Ag₃₅(SA)₀₇ that emits red PL and Ag₃₆(SG)₀₉ that emits blue PL using PAGE separation (Figure 8B).

4.4. PAGE separation for structural estimation

In PAGE separation, the mobility of each metal cluster depends on the particle size and shape as well as its charge and interaction with the solvent. Thus, there are several examples of the use of PAGE for structural and shape evaluation of Au₃₅(SG)₀₈ clusters.

Ackerson et al. synthesized Au₃₅(p-MBA)₀₄ and Au₃₆(p-MBA)₀₄ with known chemical composition through a gel and estimated the correlation between the mobility and chemical composition. Then, they ran Au₃₅(p-MBA)₀₄ clusters with unknown size were run through a gel at the same concentration, and the chemical composition was estimated to be Au₁₀₂(p-MBA)₀₄. Lehtovaara, Hämäkinen, and Pettersson et al. demonstrated by PAGE separation that mixing Au₁₀₂(p-MBA)₀₄ or Au₁₀₂(p-MBA)₀₄ with biphenyl-4,4’-dithiol causes ligand
exchange, resulting in monomerization of Au\(_{102}\) (p-MBA)\(_{44}\) or Au\(_{\sim 250}\) (p-MBA)\(_n\) (Figure 9).\(^{119}\)

4.5. Sodium dodecyl sulfate-PAGE separation

In the PAGE separation described thus far, not only the molecular weight of the cluster but also the charge state was reflected in the mobility. Therefore, to more accurately estimate the correlation between the chemical composition and mobility, it is necessary to exclude the effect of the charge state. Adding sodium dodecyl sulfate (SDS) to the sample solution and electrophoresis solution results in an almost constant charge-to-size ratio of the cluster, eliminating the effect of charge state on the mobility. Such a SDS-PAGE solution is used in protein separation.\(^{120}\)

Cheng and Häkkinen et al. used SDS-PAGE for separation of Au\(_n\) (SR)\(_m\) clusters.\(^{121}\) They replaced some ligands of Au\(_{102}\) (p-MBA)\(_{44}\) with maleimide (C\(_6\)MI) to bind Au\(_{102}\) (p-MBA)\(_{44}\) efficiently to nanocapsid. They confirmed using SDS-PAGE that the Au\(_{102}\) (p-MBA)\(_{44}\) (C\(_6\)MI)\(_x\) bound to nanocapsid with higher efficiency than the unreacted Au\(_{102}\) (p-MBA)\(_{44}\).

5. HPLC Separation

As with PAGE, there have been many reports on the separation of Au\(_n\) (SR)\(_m\) and related clusters using HPLC.\(^{122-145}\) The main separation methods include RP partition chromatography, ion-pair chromatography, hydrophilic interaction liquid chromatography, size exclusion chromatography, and chiral chromatography (Figure 10). In the following, research examples are introduced.

5.1. Reverse phase partition chromatography

RP partition chromatography provides ease of handling and has many stationary phases with a high number of theoretical plates. Furthermore, this method also facilitates the use of LC/MS because the mobile phase used in this chromatography technique is suitable for the ionization of the cluster in ESI-MS. Because of these advantages, more than 80% of HPLC analysis uses an RP column.

In RP partition chromatography, the polarity of the stationary phase is lower than that of the mobile phase (Figure 18). Under this condition, the solute is in partition equilibrium between the stationary and mobile phases, and each solute is separated by a difference in the partition coefficient. In this chromatography method, silica gel supports chemically modified with an octadecyl group (C18), an octyl group (C8), a
phenyl group (Ph), etc. are used as the stationary phase (Figure 11A).

5.1.1. Separation of clusters by cluster size

Murray et al. first attempted to separate Au(m) clusters using RP partition chromatography. In 2003, they synthesized a mixture of Au(n)(SC6H5)2 clusters (SC6H5 = hexanethiolate) and attempted to separate each cluster using a C8 column.146 The Au(n)(SR)m clusters with metal cores of approximately 1.5 nm were separated into multiple peaks (Figure 12A). They estimated the size of Au(n)(SC6H5)m clusters contained in each fraction using methods such as optical absorption spectroscopy and electrochemical measurements and showed that smaller clusters eluted at shorter retention time.147,148 The cluster included in the earliest eluting peak was assigned to Au38, and that included in the second eluting peak was assigned to Au140. The researchers also observed that connecting a C8 column to a Ph column improved the resolution. They succeeded in separating Au140 and Au225, which has a larger metal core, using such a connected column.149

Negishi et al. succeeded in separating a series of Au(m)(SC6H5)3 clusters (SC6H5 = dodecanethiolate) depending on the number of constituent atoms using such a connected column. Four distinct peaks were observed in the chromatogram, corresponding to Au102(SH)24, Au130(SH)25, Au144(SH)26, and Au157(SH)28 clusters, respectively.150 Although isolation of Au102(SR)44 and Au144(SR)60 had been previously reported,101,151 Au130(SR)30 and Au157(SR)38 clusters were successfully isolated for the first time in this experiment.152,153 They also succeeded in finding an experimental condition for separating Au((m)SC6H5)3 clusters in a wide range from Au246(SR)18 to Au320(SC6H5)30 at the same time (Figure 12B).154 In cooperation with Hakkinen and Tsukuda et al., they clarified the electronic and geometrical structures of a series of isolated Au(m)(SC6H5)3 clusters and concluded that the transition from bulk to non-bulk occurs in the region from Au237(SC6H5)28 to Au248(SC6H5)26 in the Au(m)(SC6H5)3 clusters (Figure 12C).154 Regarding this transition, the recent studies by Jin et al.155,156 and Dass et al.157,158 revealed that the transition from bulk to non-bulk occurs between Au272(SR)18 to Au246(SR)18 in the case of Au(m)(SR)3 clusters including benzenthioate in the functional group of the ligand. Knope et al. also studied the separation of these Au(m)(SC6H5)3 clusters. They found the conditions for separating Au25(SC6H5)18 and Au34(SC6H5)24 with high resolution and revealed the correlation between alkyl chain length and retention time through experiments conducted under such a condition.159

Black and Whetten et al. separated Au(m)(SH)3 clusters using a capillary C18 column. Their use of a capillary column was unique, enabling suppression of the quantity of the mobile phase and thereby direct evaluation of the chemical composition of the eluted cluster with a directly connected ESI
easily achieved using RP partition chromatography. The physical and chemical properties of Au\(_{n}\)(SR)\(_m\) clusters vary depending on the ligand type in addition to the core size. For example, the solubility in a solvent and the quantum yield of PL vary depending on the functional group of the ligand. When a SR with a specific function is used as the ligand, it is possible to provide a specific function, such as molecular recognition ability or catalytic ability, to the Au\(_{n}\)(SR)\(_m\) clusters. Thus, controlling the ligand composition is an extremely effective way to control the function of the cluster. If it were possible to substitute only a limited number of ligands with other SRs, strict control of the function of metal clusters might be possible as well as regular arrangement of metal clusters on the substrate. However, when Au\(_{n}\)(SR)\(_m\) clusters are synthesized using multiple types of SRs, the product typically has a distribution in the ligand combination. Therefore, to realize strict control of functions and precise arrangement of clusters, it is necessary to separate the obtained mixture at high resolution depending on the ligand combination. Using RP partition chromatography, it is also possible to separate Au\(_{n}\)(SR)\(_m\)-..(SR)\(_n\) clusters with two types of ligands (SR\(_1\) and SR\(_2\)) depending on the ligand combination.

Negishi and Pradeep et al. reported that multiple ligands of Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\) can be exchanged to SBB (4-tert-butylenzeneathiolate) via the reaction of Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\) with 4-tert-butylenzeneathiol (BBSH) in solution. However, the obtained cluster had a distribution in the number of exchanged ligands. They succeeded in separating such a mixture depending on ligand composition using RP partition chromatography. In this experiment, first, all the Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\)-..(SBB) was adsorbed on the stationary phase. Then, Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\)-..(SBB), with higher polarity was eluted sequentially by decreasing the polarity of the mobile phase using a gradient program (Figure 14A). It is assumed that the difference in the adsorption ability to the stationary phase was also involved in this separation in addition to the difference in the partition coefficient. Niihori et al. demonstrated that such a method is applicable to the separation of various Au\(_{24}\)Pd(SR)\(_{18}\)-..(SR)\(_{2}\) clusters with two types of SRs (Figure 14B). In later studies, Niihori et al. also succeeded in further improving the resolution, thereby enabling the separation of Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\)-..(SC\(_{25}\))\(_{18}\) (x = 1, 2) depending on the coordination isomers (Figure 14C). Such separation revealed a preferred site for ligand exchange.

5.1.3. Separation of clusters by ligand combination

As described above, in RP partition chromatography, each solute can be separated by the difference in the partition coefficient in the column. Because the charge state of the solute greatly affects the partition coefficient, separating Au\(_n\)(SC\(_2\)H\(_4\)Ph)\(_m\) clusters with different charge states can be easily achieved using RP partition chromatography.

Negishi et al. reported that [Au\(_{25}\)(SC\(_2\)H\(_2\))\(_{18}\)]\(^+\) and [Au\(_{25}\)(SC\(_2\)H\(_2\))\(_{18}\)]\(^-\) elute at significantly different retention times in RP partition chromatography (Figure 13). In this study, they estimated the charge state of Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\) with unknown charge state by comparing the retention time of Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\) with that of [Au\(_{25}\)(SC\(_2\)H\(_2\))\(_{18}\)]\(^+\) and [Au\(_{25}\)(SC\(_2\)H\(_2\))\(_{18}\)]\(^-\). This comparison elucidated that Au\(_{24}\)Pd(SC\(_{12}\)H\(_{25}\))\(_{18}\) could be synthesized in the neutral form ([Au\(_{24}\)Pd(SR)\(_{18}\)]\(^0\)). They also demonstrated by a similar comparison that Au\(_{25}\)Ag(SC\(_{25}\))\(_{18}\) could be synthesized in the negative form ([Au\(_{25}\)Ag(SR)\(_{18}\)]\(^-\)). Their interpretation was later confirmed by SC-XRD analysis of Au\(_{24}\)Pd(SR)\(_{18}\) and Au\(_{25}\)Ag(SR)\(_{18}\) (SR = SC\(_2\)H\(_4\)Ph).

5.1.4. Separation of alloy clusters depending on the chemical composition of the metal core

Replacing Au atoms in Au\(_n\)(SR)\(_m\) clusters with another metal is expected to produce new electronic structures, physical/chemical properties, and functions different from...
those of the monometallic Au\textsubscript{n}(SR)\textsubscript{m} clusters. Therefore, research on alloy clusters has recently become a hot topic\textsuperscript{23,53,162−165,172,174−213}. To deeply understand the correlation between structure and physical/chemical properties in such an alloy cluster, it is necessary to control the chemical composition of the alloy cluster. Synthesis methods with atomic precision have already been established for several alloy clusters. However, many alloy clusters cannot yet be synthesized with atomic precision. The representative clusters are 25-atom and 38-atom clusters composed of Au and Ag (Au\textsubscript{25−x}Ag\textsubscript{x}(SR)\textsubscript{18} and Au\textsubscript{38−x}Ag\textsubscript{x}(SR)\textsubscript{24})\textsuperscript{163,164,210,214}. Using RP partition chromatography, it is also possible to separate these mixtures depending on the chemical composition of the metal core.

Niihori et al. prepared a mixture of Au\textsubscript{25−x}Ag\textsubscript{x}(SC\textsubscript{4}H\textsubscript{9})\textsubscript{18}(x = 1–4) and separated it using RP partition chromatography. In this study, they used a column with a core−shell-type stationary phase. As a result, Au\textsubscript{25−x}Ag\textsubscript{x}(SC\textsubscript{4}H\textsubscript{9})\textsubscript{18}(x = 1–4) was separated at high resolution (Figure 15A).\textsuperscript{212} The optical absorption spectrum of the separated Au\textsubscript{25−x}Ag\textsubscript{x}(SC\textsubscript{4}H\textsubscript{9})\textsubscript{18}(x = 1–4) revealed the correlation between the chemical composition and electronic structure in Au\textsubscript{25−x}Ag\textsubscript{x}(SC\textsubscript{4}H\textsubscript{9})\textsubscript{18}(x = 1–4) with atomic accuracy (Figure 15B). This method also enabled the separation of structural isomers in Au\textsubscript{25−x}Ag\textsubscript{x}(SC\textsubscript{4}H\textsubscript{9})\textsubscript{18}(x = 2,3) (Figure 15A).\textsuperscript{212} The researchers...
and tetrabutylammonium fluoride ([C₄H₉]₄N⁺F⁻) for the ion-pair reagent.²¹⁶ Au₉(NALC)ₘ and Au₉(S(PG))ₘ clusters were separated depending on the core size because [C₄H₉]₄N⁺ ions formed ion pairs with hydrophilic clusters, and therefore, the cluster surface caused a hydrophobic interaction with the stationary phase. Later, Choi et al. attempted to separate Au₉(NALC)ₘ clusters under similar experimental conditions. In the study by Murray et al., the separated Au₉(NALC)ₘ clusters were characterized by optical absorption spectroscopy.²¹⁶ However, Choi et al. separated each fraction and obtained their MALDI mass spectra (Figure 16B). They confirmed that the Au₉(NALC)ₘ clusters could be separated depending on the chemical composition using this method.²¹⁷ They also attempted to separate Au₉(NALC)ₘ clusters using an ultra HPLC (UHPLC) instrument. The results indicated that the separation could be performed in approximately 10% of the retention time with sharper peaks appearing in the chromatogram compared with the results obtained using the normal HPLC apparatus.²¹⁸ Dong and Choi et al. conducted similar experiments on Pd₉(NALC)ₘ clusters and succeeded in separating them depending on the chemical composition.²¹⁹ Niihori et al. performed separation of Au₉(SG)ₘ clusters including the most commonly used hydrophilic ligand, SG, using ion-pair chromatography and succeeded in achieving higher-resolution separation than PAGE separation.²¹⁵

In these studies, ion-pair chromatography was used for the separation; however, the chemical composition of the separated clusters was not determined by LC/MS. Because (C₄H₉)₄N⁺ easily forms an ion pair with the dissociable functional group of the ligand and easily interacts with the stationary phase, a relatively high-resolution separation could be achieved when (C₄H₉)₄N⁺ is used as the ion-pair reagent. However, (C₄H₉)₄N⁺ is non-volatile and cannot be used in LC/MS because the salt precipitates during the ionization process. Whetten et al. overcame these challenges by using volatile triethylamine as the ion-pair reagent and succeeded in both the separation and determination of the chemical composition of the separated clusters by LC/MS for Au₉(p-MBA)ₘ (n = 5–102, m = 5–44) and Au₉(m-MBA)ₘ clusters (m-MBA = m-mercaptobenzoic acid; n = 48–67, m = 26–30; Figure 16C).²²⁰,²²¹ Whetten et al. also succeeded in identifying Au₉(p-MBA)ₘ and [Ag₂₅(LA)₁₃]³⁻ (LA = (R)-α-lipoic acid) clusters and performing structural analysis of [Ag₂₅(LA)₁₃]³⁻.²²²,²²³

5.3 Hydrophilic interaction liquid chromatography

In this way, by using an ion-pair reagent, it is possible to separate the hydrophilic clusters depending on the core size using RP partition chromatography. In addition, when a volatile ion-pair reagent is used, the chemical composition of the separated clusters can be determined using an ESI mass spectrometer directly connected with RP partition chromatography. However, if a hydrophilic interaction liquid chromatography (HILIC) column²²⁴ were used for the separation of the hydrophilic clusters, even ion-pair reagents become unnecessary, and the separation and evaluation of hydrophilic clusters would be facilitated.
Niihori et al. attempted the separation and evaluation of $\text{Au}_n(\text{SG})_m$ and $\text{Au}_n^{-x}\text{M}_x(\text{SG})_m$ alloy clusters ($\text{M} = \text{Ag}, \text{Cu}$ or $\text{Pd}$) by LC/MS using an HILIC column (Figure 17A). They used two columns (a ZIC-cHILIC and Amido-80 column; Figure 11B). The $\text{Au}_n(\text{SG})_m$ clusters were separated with high resolution.

In this study, they detected the $\text{Au}_n(\text{SG})_m$ clusters that had never been reported (Figure 17B). All the synthesized clusters were introduced to the mass spectrometer, unlike the case using PAGE (Figure 17A). Thus, small species that have almost never been observed before were also detected in addition to the previously reported stable species. The use of the HILIC column also enabled the separation of $\text{Au}_n^{-x}\text{M}_x(\text{SG})_m$ alloy clusters depending on the number of constituent atoms. The chemical composition evaluation by LC/MS resulted in the discovery of new $\text{Au}_n^{-x}\text{Pd}_x(\text{SG})_m$ clusters, such as $\text{Au}_{14}\text{Pd}(\text{SG})_{13}$, $\text{Au}_{24}\text{Pd}(\text{SG})_{18}$, and $\text{Au}_{28}\text{Pd}(\text{SG})_{20}$, which had not been reported until then.

Stamplecoskie et al. also separated $\text{Ag}_n(\text{SG})_m$ clusters using an amide column and revealed the size dependence of the optical properties of $\text{Ag}_n(\text{SG})_m$ clusters (although the technique is not described as HILIC in the original paper, it is speculated that each $\text{Ag}_n(\text{SG})_m$ cluster was separated in HILIC mode from the experimental conditions).

5.4. Size exclusion chromatography

In the RP partition chromatography and the HILIC described above, a chemical interaction (partitioning and/or adsorption) occurs between the stationary phase and clusters, and accordingly, the difference in the mobility of each cluster results in separation of the clusters. However, in size exclusion chromatography (SEC), each cluster is separated by physical size. Therefore, when separating the clusters using the size exclusion mode, a stationary phase that does not interact with the clusters must be selected. SEC is divided into gel permeation chromatography (GPC) and gel filtration chromatography (GFC). In GPC, the mobile phase is an organic solvent, and in GFC, the mobile phase is an aqueous solvent. In these separations, $\text{Au}_n(\text{SR})_m$ clusters elute from the column in the order from largest to smallest.

5.4.1. Gel permeation chromatography

It is reasonable to first consider using SEC for the separation of $\text{Au}_n(\text{SR})_m$ clusters depending on the core size. Wilcoxon et al. attempted to separate $\text{Au}_n(\text{SR})_m$ clusters using GPC in 2000. In this study, porous organics were used as the stationary phase (Figure 11C). When this experiment was conducted, it was not yet possible to determine the chemical composition of $\text{Au}_n(\text{SR})_m$ clusters.
clusters with atomic precision. Therefore, in their earlier work, they synthesized Au$_n$(SC$_{2n+1}$)$_m$ clusters with similar metal core sizes (~2 nm) using several SC$_{2n+1}$ with different alkyl chain lengths ($l = 6$–18). Then, the thickness of the ligand layer in each Au$_n$(SC$_{2n+1}$)$_m$ cluster ($l = 6$–18) was studied by comparing their retention times. Regarding the thickness of the ligand layer, Tsukuda et al. also later studied Pd$_n$(SC$_{2n+1}$)$_m$ clusters ($l = 10$, 12, 14, 16, or 18).227

Wilcoxon et al. then performed size separation of Au$_n$(SC$_{2n+1}$)$_m$ and Ag$_n$(SC$_{2n+2}$)$_m$ clusters with a metal core size of 1.3–8.0 nm. They obtained knowledge on the correlation between the particle size and electronic structure from their findings.228,229 Tsukuda et al. succeeded in separating Au$_n$(SC$_{18}$)$_m$ clusters with higher resolution by recycling (Figure 18A),230 and successfully isolated Au$_n$(SC$_{2n+1}$)$_m$ clusters ($l = 12, 18$) having Au$_{155}$ as the metal core with high purity.231 In a later study, Jin et al. revealed that this cluster has the chemical composition of Au$_{155}$(SR)$_{31}$ using ESI-MS (Figure 18B).232 Jin et al. also succeeded in separating Au$_n$(SC$_{2n}$H$_m$)$_m$ and Au$_n$(SC$_{2n}$H$_m$)$_{2m}$, whose chemical compositions are very similar, using GFC.233

GFC is also utilized for tracking reactions. Negishi et al. traced the growth of Au$_n$(SBB)$_m$ clusters using GFC. The clusters were observed to settle in a stable cluster at 96 h under their reaction conditions (Figure 18C).234 Jin et al. and Zhu et al. also used GFC to track several reactions and discovered a method to size-selectively synthesize Au$_n$(SR)$_m$ clusters, such as Au$_{13}$(SC$_2$H$_{12}$)$_2$H$_2$, Au$_{25}$(SC$_2$H$_{10}$)$_2$H$_6$, and Au$_{34}$(SC$_2$H$_{12}$)$_2$H$_{20}$, with atomic precision.235,236

5.4.2. Gel filtration chromatography

For the separation of hydrophilic clusters, there have only been a few reports on the separation by GFC because PAGE is frequently used for these clusters. Thus, the purpose of GFC separation is limited to cluster purification (Figure 18D).237 Inouye et al. used GFC to purify mercaptoacetic acid (MAA)-protected Pt clusters (Pt$_n$(MAA)$_m$). In this experiment, they used a stationary phase consisting of porous silica (Figure 11D). They succeeded in isolating Pt$_n$(MAA)$_m$, which emits blue light, and Pt$_n$(C$_2$H$_5$O$_2$)$_m$, which emits green light, with high purity using GFC.238

5.5. Chiral chromatography

In chiral chromatography, a compound is separated into optical isomers by utilizing the difference in interaction between the compound and a stationary phase with chirality. The stationary phase is composed of silica gel chemically modified with a chiral compound, such as cellulose. (Figure 11E). A “three-point bond model” has been proposed for the separation mechanism of the optical isomers.239 According to this mechanism, a compound and a chiral stationary phase form a diastereometric aggregate because of interaction at three binding sites. Because the binding energy of such an aggregate differs depending on the optical isomer (Figure 11E), a difference in the retention time of each optical isomer is observed. Interactions forming aggregates include hydrogen-bond formation, charge-transfer complex formation, ion-pair formation, coordination bonding to metals, and dipole–dipole interaction. In the actual separation, these interactions contribute singly or in combination.

Kornberg et al. determined the geometrical structure of the Au$_n$(SR)$_m$ cluster for the first time in 2007, revealing that the Au$_n$(SR)$_m$ cluster consisted of a Au core covered with multiple Au(I)-SR staple and that Au$_{100}$(p-MBA)$_{6}$ has optical isomers.240 Since then, many Au$_n$(SR)$_m$ clusters have been revealed to have optical isomers by SC-XRD analysis.61,241–243

Bürgi et al. succeeded for the first time in separating Au$_n$(SC$_2$H$_m$)$_m$ (Figure 19A) into chiral isomers using chiral chromatography in 2010 (Figure 19B).244 In this study, a stationary phase in which cellulose was chemically modified on the silica surface was used. The separation resulted in obtaining...
6. Thin-Layer Chromatography Separation

TLC uses an inexpensive and simple device different from that used for HPLC. Furthermore, in TLC, the separation can be visually confirmed similarly to PAGE. The use of preparative thin-layer chromatography (PTLC) using a thicker silica gel than that used in TLC makes it possible to isolate each cluster on a relatively large scale. Therefore, TLC is often used to confirm the purity of a product. When Wu and Jin et al. elucidated the geometrical structure of Au_{144} (SR)_{30} by SC-XRD, PTLC was used to increase its purity.\(^{263}\) For a mixture of Au_{m} (SR)_{m} and related clusters composed of clusters with great difference in size and charge state, this method can be used for the separation of clusters depending on the size and charge state.

Pradeep et al. succeeded in clearly separating Au_{252} (SC_{4}H_{6}Ph)_{24} and Au_{144} (SC_{4}H_{6}Ph)_{60} contained in the product using TLC in 2014.\(^{264}\) They also succeeded in separating Au_{252} (SC_{4}H_{6}Ph)_{24} and Au_{252} (SC_{4}H_{6}Ph)_{25} with different ligands, Au_{252} (Calix)_{2} (SC_{4}H_{6}Ph)_{25} (Calix = tetraphenyl calix[4]arene, 25,26,27,28-tetrasakis (4-mercapto-n-butoxy)calix[4]arene; Figure 20A) with different ligand combination, and [Au_{252} (SC_{4}H_{6}Ph)_{25}]^{+} and [Au_{252} (SC_{4}H_{6}Ph)_{25}]^{-} with different charge states using TLC. Wu et al. clearly separated Au_{252} (SC_{4}H_{6}Ph)_{24} and Au_{252} Ag_{4} (SC_{4}H_{6}Ph)_{18} using TLC (Figure 20B).\(^{265}\) Jin et al. succeeded in separating the structural isomers (T- and Q-isomer) of Au_{38} (SC_{4}H_{6}Ph)_{24} using TLC.\(^{266}\)

TLC is also frequently used in identifying optimal reaction conditions because the distribution of the product can be visually confirmed. Wu et al. reacted [Au_{252} (SC_{4}H_{6}Ph)_{25}]^{+} with Ag(I) ions and evaluated the distribution of the product. The analyses revealed that the anti-galvanic reaction occurring between [Au_{252} (SC_{4}H_{6}Ph)_{25}]^{+} with Ag(I) ions varied depending on the type and concentration of Ag(I) ions.\(^{267}\) Wu et al. also used PTLC to monitor the size conversion from Au_{44} (TBBT)_{28} (TBBT = 4-tert-butylbenzenethiolate) to Au_{38} (TBBT)_{24}. As a result, they succeeded in finding effective reaction conditions for such exchange reactions.\(^{268}\)

7. Summary and Outlook

This review summarized previous research on the high-resolution separation of Au_{m} (SR)_{m} and related clusters by focusing on PAGE, HPLC, and TLC. From this summary, the following characteristics of each separation method were revealed.

**PAGE:**

- **Main target:** hydrophilic clusters
- **Advantages:** i) inexpensive and simple, ii) possible to separate clusters containing 40 or less atoms with atomic precision, iii) possible to confirm separation visibly by the naked eye.

In addition to the separation of gold clusters, we explored the properties of clusters using techniques such as polarized light emission under understanding of the asymmetric catalytic activity and circularly polarized light emission\(^{269}\) of each optical isomer in the future.
**Main applications**: i) isolation, ii) reaction tracking, iii) search for/identification of photoluminescent clusters, iv) structural analysis, v) washing/purification

**HPLC**:

**Main target**: both hydrophilic and hydrophobic clusters

**Advantages**: i) high resolution, ii) high repeatability, iii) variety of separation modes, iv) chiral separation, v) possible to connect with ESI mass spectrometer, vi) possible to analyse minor species, vii) short separation time (UHPLC)

**Main applications**: i) isolation (but small amount), ii) reaction tracking, iii) structural analysis, iv) chiral separation, v) washing/purification

**TLC**:

**Main target**: hydrophobic clusters

**Advantages**: i) inexpensive and simple, ii) possible to confirm separation visibly by the naked eye

**Main applications**: i) isolation, ii) reaction tracking, iii) structural analysis, iv) washing/purification

Because the precise synthesis techniques of Au_{n} (SR)_{m} and related clusters have progressed rapidly in the last 20 years, many studies on their separation have been reported. We hope that this review will provide guidelines to understand the characteristics of each separation method and enable the selection of an appropriate separation method for the target experiment.

In addition, it is hoped that the established techniques will be used more widely in the future for the separation of metal clusters. The chemical properties of Au_{n} (SR)_{m} and related clusters have still not been clarified completely. For example, although it has been revealed that many Au_{n} (SR)_{m} and related clusters have a chiral structure, the asymmetric catalytic activity and circularly polarized luminescence of each optical isomer have not yet been revealed. It is expected that these physical/chemical properties can be elucidated by studying each optical isomer isolated by chiral chromatography. In addition, metal exchange between Au_{n−x} Ag_{x} (SR)_{m} clusters has been shown to occur in solution. Therefore, there are still many unclear points about the mechanism of the metal exchange reaction. A deep understanding of this mechanism could be obtained if the reaction proceeded using Au_{n−x} Ag_{x} (SR)_{m} clusters isolated with atomic precision and the reaction process was tracked using LC/MS. In future studies, the effective use of established separation techniques is expected to provide a deep understanding of the functions and properties of Au_{n} (SR)_{m} and related clusters.

Furthermore, there are some helpful separation/analysis techniques that should be actively incorporated in future research on metal clusters. For example, MS/MS in LC/MS is useful not only for determining the chemical composition of a product but also for analysing the structure of the product. An introduction of two-dimensional HPLC is also interesting for analysis. If such a method is introduced, comprehensive two-dimensional separation could be realised using two different separation modes. For instance, an optical isomer generated based on the position of an exchange ligand is expected to be isolated by this method.

As introduced in this review, separation/analysis techniques for metal clusters have been developing with the improvement of separation and analysis equipment. HPLC columns and mass spectrometers are still being improved day after day. We hope that the separation and analysis techniques for metal clusters will continue to evolve in the future with researchers being conscious of the latest techniques available for each device and continuing to actively incorporate such techniques into research on metal clusters.

**Conflicts of interest**

There are no conflicts to declare.

**Authorship contributions**

Y. Negishi constructed the structure of this review. T. Kawawaki wrote sections 1 and 7. K. Hamada wrote sections 2 and 3. S. Hashimoto and A. Ebina wrote sections 4–6. Y. Negishi and S. Hossain revised the entire draft before submission, and all the authors have approved the final version of the manuscript.

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This review summarizes previous studies on high-resolution separation of Au\(_n\)(SR)\(_m\) clusters for better understanding the utility of each separation technique.