Separation of carbon nanotubes (CNTs) by the separation method for biomolecules

— Towards large-scale, low-cost separation of metallic and semiconducting CNTs —

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There are two types of carbon nanotubes (CNTs): metallic and semiconducting. To exploit their superior electric properties, mixtures of these two types of CNTs should be separated. For industrial applications, a large-scale, low-cost separation method is required. We successfully developed novel separation methods for CNTs by applying separation methods for biomolecules. We first applied agarose gel electrophoresis, and finally achieved large-scale, low-cost separation by the column method. Using this method, we provided separated CNT samples. A separation method for single structure semiconducting CNTs was also developed by overloading CNTs into tandemly arranged multi-columns. The point of timing of patent application, publication of research results, and budget application to carry out this research effectively is also presented in this paper.

Keywords : Carbon nanotubes, metallic/semiconducting, separation, gel, biomolecules

1 Introduction

1.1 Background – Expectation and issues for the application of CNTs to electronics

Carbon nanotubes (CNTs) have structures where a sheet composed of hexagonal arrangement of carbon atoms is rolled into a cylindrical shape. They are extremely thin and long materials with diameter of several nanometers to tens of nanometer (nanometer is one-millionth of millimeter), and with length over micrometer (Fig. 1). In 1991, the "multi-wall CNTs" in which the CNTs are stacked coaxially in multiple layers were observed under the electron microscope[1] by Iijima of the Fundamental Research Laboratories, NEC Corporation (Iijima currently works as the director of Nanotube Research Center, AIST).

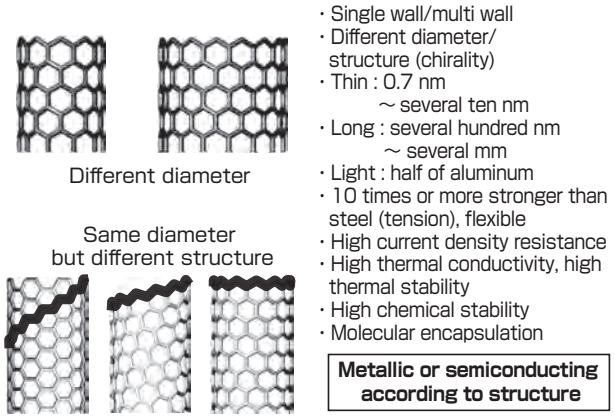

Fig. 1 Structure and characteristics of CNTs

Then, in 1993, "single-wall CNTs" were discovered.[2][3] In CNT research, the theoretical calculations were done actively due to their relatively simple molecular structure. From the calculations, it was predicted that, despite being a substance consisting solely of carbon atoms, CNTs would have extremely excellent properties such as lighter weight and lower density than aluminum, over 10 times the strength of steel, higher electroconductivity than copper, as well as diverse properties including high thermal conductivity, high thermal stability, and high chemical stability. Also, the theoretical calculation predicted that they would behave like metals or semiconductors due to their arrangement (structure) of the carbon atoms.[4] As mentioned above, since CNTs are nanomaterials with excellent potential, expectation is increasing for their new applications that cannot be achieved by other materials. Particularly looking at the electronics application, metallic CNTs, when made into thin film, do not require rare metal like the current transparent conductive film, and can be used as a transparent conductive film that can be bent due to their high mechanical strength. For semiconducting CNTs, high-speed transistor can be fabricated easily by coating, and a transparent and flexible film can be created in this case also. However, there are barriers in the electronic application of CNTs. That is, using the conventional CNT synthesis method, the selective synthesis of metallic CNTs or semiconducting CNTs is not possible, and the result obtained is a mixture of metallic and semiconducting products (normally, ratio of metallic:semiconducting is 1:2). Therefore, research for the separation of metallic and semiconducting CNTs is crucial.

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1.2 Objective – Supply of large-scale, low-cost separated CNTs

The metallic and semiconducting CNTs are both materials with uniform hydrophobic surface where the sheet consisting only of carbon atoms is rolled into a tube. Both are similar in appearance and are difficult to tell apart. As CNTs after synthesis form extremely strong bundles, and as they are difficult to separate into individual CNTs, this inhibited their separation research. After more than ten years since the prediction of the metallic and semiconducting CNTs by theoretical studies,[4] the research for the extraction and separation of the metallic and semiconducting CNTs started to be published.[5]-[10] For example, they included methods such as selective oxidation,[5] dielectrophoresis,[6] extraction using amines,[7] extraction using polymers,[8] DNA dispersion and chromatographic separation,[9] density gradient ultracentrifugation,[10] and others. These methods can be divided roughly as follows: (1) "selective destruction" where either the metallic or semiconducting CNTs are obtained by selectively destroying the other, (2) "selective extraction" where either the metallic or conducting CNTs are extracted selectively, and (3) "separation" where the metallic and semiconducting CNTs are separated and both CNTs are recovered. Among these methods, the density gradient ultracentrifugation published by Hersam *et al*. in 2006[10] was an innovative method where highly pure metallic and semiconducting CNTs could be obtained. However, there was some room for improvement such as the ultracentrifuge was extremely expensive and not suitable for mass processing, and it required a long time for separation (12 hours). Inexpensive separation of CNTs in large amounts is necessary for the industrial utilization of the metallic and semiconducting CNTs. Although it is possible to lower the cost by mass-production, it is first necessary to accomplish mass processing. While it is possible to conduct some researches with a small amount of samples, large amounts are needed in other cases. Therefore, we decided to conduct the CNT separation research with the objective of developing a method to supply large-scale, low-cost separated metallic and semiconducting CNTs.

2 Scenario for realizing the objective

2.1 Application of the biomolecular separation method to CNTs (fusion of different fields)

The aforementioned density gradient ultracentrifugation is a method used frequently in the field of life science to extract substances such as DNA. By processing the dispersed CNTs using DNA in column chromatography, relatively good results were obtained for separating the metallic and semiconducting CNTs.[9] For the separation of nanomaterial CNTs, good results were obtained by using the biomolecule-separation method or by using the biomolecules themselves. Tanaka, one of the authors, originally specialized in biochemistry and was knowledgeable in the separation and purification of biomolecules such as DNA and protein. When Tanaka joined AIST, he was assigned to a position to promote the fusion of different fields (biotechnology and nanotechnology), and was looking for a new research topic. He learned from Kataura, the other author, that CNTs were mixtures with various thickness, length, and electric properties, and that the separation was a major issue. Therefore, the two decided to conduct joint research on CNT separation. Kataura, who has a background in physics with a specialty in solid state spectroscopy and materials science, and Tanaka, who has a background in biology, started the collaborative research fusing different fields. When the fields differ, the terminologies and common knowledge differ greatly, and that often prevents smooth communication. However, totally different points of view may result in ideas never conceived before, and this becomes the driving force that propels the research toward a good direction. With the joint research by the researchers of different fields as the starting point, the research for the separation of metallic and semiconducting CNTs progressed from the agarose gel electrophoresis,[11] the separation using gel but no electric field (e.g. freeze-thaw-squeeze method, etc.),[12] the column method,[13] and further to the separation of single-structure semiconducting CNTs using the multicolumn method[14] (Fig. 2). These could be categorized as *Type 1 Basic Research*. The column method was suitable for low-cost, large-scale separation, and this was *Type 2 Basic Research* where massive amount of CNTs was actually separated. Currently, we are able to supply the samples of separated metallic and semiconducting CNTs. The details of the development of the separation method will be explained.

2.2 Discovery of the original separation method using gel (gel electrophoresis)

Before starting the research for CNT separation using gel, we tried to separate the CNTs using the molecular recognition ability of protein, a biomolecule. However, a long time was required before the result could be obtained in this method, and we experienced long periods without any good results. Then, the separation of metallic and semiconducting CNTs

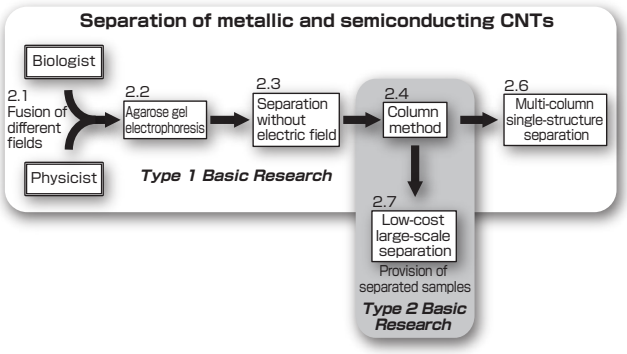

Fig. 2 Scenario for the development of large-scale, low-cost separation method for metallic and semiconducting CNTs

using the density gradient ultracentrifugation was published.[10] Thinking that if the metallic and semiconducting CNTs could be separated by slight density difference, then the separation could be also done easily by charge difference, we stopped the research for separation using protein and started the research for separation by gel electrophoresis. In biological experiments, agarose gel electrophoresis is used for DNA separation while acrylamide gel electrophoresis is used frequently for protein separation. Since the thickness and length of CNTs resembled DNA, we applied the agarose electrophoresis to CNT separation. The agarose gel electrophoresis is a simple and frequently used separation technique used in undergraduate lab courses. For DNA separation, the ordinary method used was the submarine gel electrophoresis (method where gel with thickness 5~10 mm is submerged in the electrophoresis buffer solution, and the 10~20 μl sample is injected in the small wells made in the gel). However, for CNT separation, we used the electrophoresis using the agarose gel inside a glass tube, and this was an uncommon method for DNA separation. This method allows over ten times more sample supply compared to the submarine gel electrophoresis, and even slight differences can be detected. By preparing the CNT dispersion using sodium dodecyl sulfate (SDS) that is a general surfactant used in toothpaste and shampoo, and then applying this to the agarose gel electrophoresis using a glass tube, we were able to separate the metallic and semiconducting CNTs (Fig. 3a).[11] The metallic and semiconducting CNTs were separated at the forefront and tail end of electrophoresis, respectively. This method can be done in about one hour using extremely inexpensive equipment. However, the yield was not high, and about 80 % of CNTs remained unseparated in the center.

The agarose electrophoresis was originally a method for separating DNA according to the length difference, and it was thought that the length-distribution of the CNTs worked negatively in the separation of metallic and semiconducting CNTs in the above gel electrophoresis method. To solve this problem, we applied the two-dimensional electrophoresis in which the electrophoresis was conducted by combining different separating conditions. The two-dimensional electrophoresis is a method used in the proteome analysis where the total proteins of cells or tissues are separated. By conducting the second run perpendicularly to the direction of the first run, it is possible to separate the proteins that could not be separated on the first run of gel electrophoresis. In the CNT separation, the first separation by length was done by agarose gel electrophoresis using sodium cholate as the dispersant, and the second electrophoresis (for separation of metallic and semiconducting) was done by replacing the surfactant to SDS in the gel. Then, a strange phenomenon occurred where the semiconducting CNTs did not move from the first gel and only the metallic CNTs moved and separated. The same phenomenon was confirmed when the "CNT-containing gel," where the CNT/SDS dispersion and melted agarose gel were mixed and gelated, was used from the beginning as the sample of electrophoresis, instead of replacing with SDS in the gel (Fig. 3b). Compared to the situation where the CNT dispersant was used as the sample, in the electrophoresis using the "CNT-containing gel," the yield improved dramatically to almost 100 %, the separation purity improved, the separation time was shortened to within 30 min, and this turned out to be an extremely efficient separation method. This was the first report in which the metallic and semiconducting CNTs were separated efficiently by gel electrophoresis.[11] The above separation is a specific phenomenon observed only in the combination of a specific gel and a surfactant, i.e. agarose and SDS. This combination is important in the separation method shown in subchapters 2.3 and 2.4.

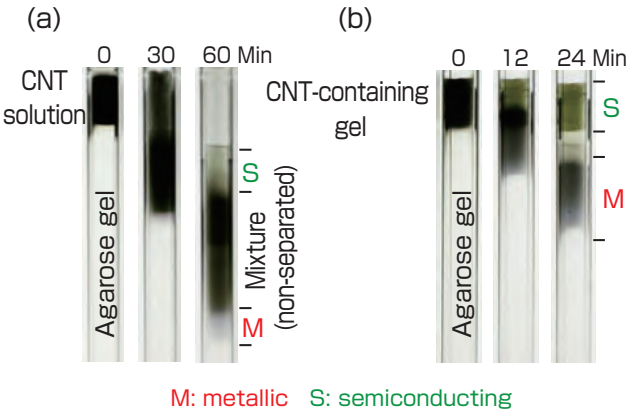

Fig. 3 Separation of the metallic and semiconducting CNTs by agarose gel electrophoresis (a: solution sample, b: gel sample)
The CNT mixture before separation is black in color, but the CNTs assume different colors upon separation because the metallic and semiconducting CNTs have different optical absorption wavelengths.

2.3 Improvement for simpler, larger-scale, and lower-cost separation method (separation without electric field)

In the electrophoresis using the CNT-containing gel, a strange phenomenon occurred where the semiconducting CNTs did not move at all and only the metallic CNTs moved. Since it involved the metallic and semiconducting CNTs that had different electric properties, it was thought that the electric field played an important role. Therefore, to investigate the necessity of the electric field, we conducted an experiment to see whether the separation occurred without an electric field. As a result, it was found that the electric field was not necessary. For example, the metallic CNTs eluted from the gel simply by immersing the CNT-containing gel in the SDS aqueous solution, and were separated from the semiconducting CNTs remaining in the gel. Also, it was possible to separate by directly centrifuging the CNT-containing gel and obtaining the solution containing the metallic CNTs. As a result of analyzing the transistor fabricated by the separated semiconducting CNTs, the

separation was confirmed from the observation of electrical properties.[15]

Freeze-thaw-squeeze method is a method for recovering the DNA that was separated by agarose gel electrophoresis.[16] While this method is extremely simple, the recovery rate of DNA is not very high, and it has become obsolete in the biological experiments. However, from the viewpoint of the researchers of the physics field, the separation "just by squeezing with hands" was amazing beyond imagination, and the freeze-thaw-squeeze method was applied to the separation of metallic and semiconducting CNTs. When the CNT-containing gel after freezing and thawing was squeezed by hand, the solution containing the metallic CNTs was squeezed out of the gel, and could be separated easily from the gel residue containing the semiconducting CNTs (Fig. 4). This was an extremely simple separation method of metallic and semiconducting CNTs that could be done using a home refrigerator. The paper featuring the result of this "simple separation without electric field" was published in a major international journal.[12] This is a good example of fusion of different fields, where a method that is commonplace in a certain field is taken up from a different viewpoint in a different field.

When the CNT/SDS dispersion and agarose gel beads were mixed, the semiconducting CNTs were adsorbed selectively by the gel and could be separated from the metallic CNTs remaining in the solution (batch separation). While this is simple, it shows an important point in considering the separation principle: the selective adsorption of semiconducting CNTs to the gel. Initially, we selected the agarose gel due to the size similarity of CNTs and DNA, and we found there was not much significance in the size of the mesh structure of the agarose gel, but the most important point was the selective adsorption when the SDS was used. The separation of metallic and semiconducting CNTs was accomplished by the combination of agarose gel and SDS that was discovered by coincidence. In gel electrophoresis, the separation efficiency was greatly improved by using the CNT-containing gel as the sample, but the CNT-containing gel was not necessary for separation. We believe the separation was improved as a result of increased area where the agarose and CNTs could interact, as the agarose was already dissolved when the CNT-containing gel was prepared. In the batch separation using the CNT dispersion, when the surface area is increased by decreasing the size of gel beads, the separation time shortens, the binding capacity increases greatly, and the separation is improved greatly.

2.4 Column separation method

The final form of the separation of metallic and semiconducting CNTs using agarose gel is the column separation (Fig. 5). This stemmed from the thinking that if the batch separation where the aforementioned CNT dispersion and gel beads are mixed could be done, then a continuous separation using a column should be possible. The agarose gel beads used for the column separation were originally made for the purpose of separation of the biomolecule protein. Here again, Tanaka's biochemistry background became useful. When the CNT/SDS dispersion was poured in the column filled with agarose gel beads, the semiconducting CNTs were adsorbed on the gel while the metallic CNTs were recovered in the non-adsorbed fraction. The semiconducting CNTs adsorbed on the gel were recovered as a solution after washing with an elution solution containing different types of surfactants. The separation purity improved, the gel did not have to be removed from the semiconducting CNTs, and the gel could be used repeatedly. The chromatography technique is applicable to scale-up and automation, and it is an appropriate method for low-cost mass-production of the metallic and semiconducting CNTs.[13]

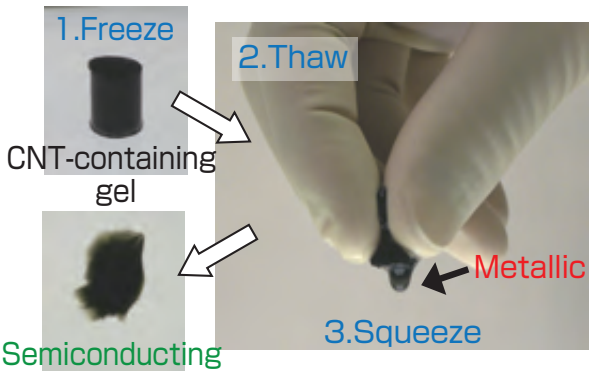

Fig. 4 Separation of the metallic and semiconducting CNTs by freeze-thaw-squeeze method using the CNT-containing gel

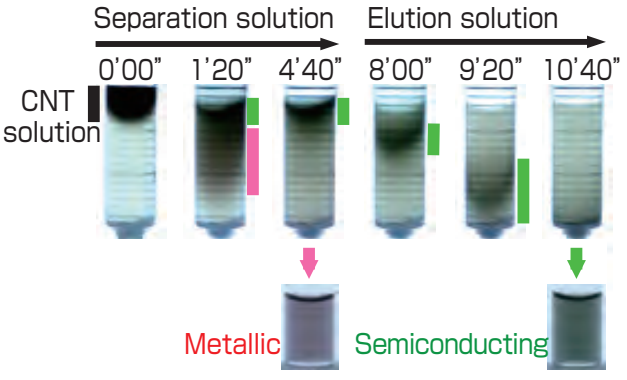

Fig. 5 Separation of the metallic and semiconducting CNTs by column using agarose gel beads
When the diameters of CNTs differ, the absorption wavelength range changes in metallic (or semiconducting) CNTs, and the colors become different from the colors of CNTs in Figs. 3 and 4.

2.5 Research promotion strategy (intellectual property, timing of publication, obtainment of research funds)

While we have discussed the research development, we shall discuss this research from a different angle or the ways of conducting research in terms of intellectual property, timing of publication, and obtainment of research funds (Fig. 6). Although, as researchers, we wanted to publish our discovery of this simple and efficient separation method of the metallic and semiconducting CNTs by gel electrophoresis, we could not publish immediately. Since the separation by gel electrophoresis was so simple and did not require expensive equipment, we feared that late-coming researchers might take the lead once this was published. Therefore, for some time after the discovery of the gel electrophoresis separation, we continued the research discreetly without publication. At the point we obtained full experimental data on the gel electrophoresis separation, we first applied for patent, and then confirmed that the separation without electric field was possible. To accelerate the research it was necessary to obtain research funds, but it was difficult for a researcher who only had experience in the biology field but no results in the CNT research to be granted funding. Although not shown in Fig. 6, I actually applied for grant immediately after the discovery of gel electrophoresis separation but failed to receive any grants. Although there was over half a year until the patent publication, we publicized the results widely through a press release at the same time we were presenting the result of gel electrophoresis in the academic society. The responses to the press release were positive, and it was covered by several newspapers including major papers, and we also were consulted by many companies. The timing of the press release was set to match the screening for external funds to which I applied earlier, and perhaps due to this strategy, relatively large funding was granted to me despite being a young researcher. The important point was that at that point, the key conditions of the experiment were not publicized in the press release, academic society presentation, or funding application. In the funding application at the beginning of 2008, the large-scale, low-cost column separation, which was the final form of the separation without electric field, was our research topic. In the summer and autumn of the same year, we received the poster award and gave our first presentation of this research topic at the academic society. As a result, we succeeded in signing a contract for joint research with funding, as well as obtained new external funding to clarify the separation principle. These further accelerated the speed of research. The first paper that described the details of gel electrophoresis[11] was published at the same time as the patent publication that was a year and half after the patent application. This series of development was done under extremely useful advice from Kataura who had abundant experience in intellectual property and press releases.

2.6 Separation of the single-structure semiconducting CNTs

We discussed the separation of metallic and semiconducting CNTs. However, strictly categorized, there are many structures among the semiconducting CNTs, and they are mixtures of different electrical properties called band gaps depending on the structure of the individual CNT. Completely uniform band gaps may be required in certain applications of CNTs using its semiconducting property, and

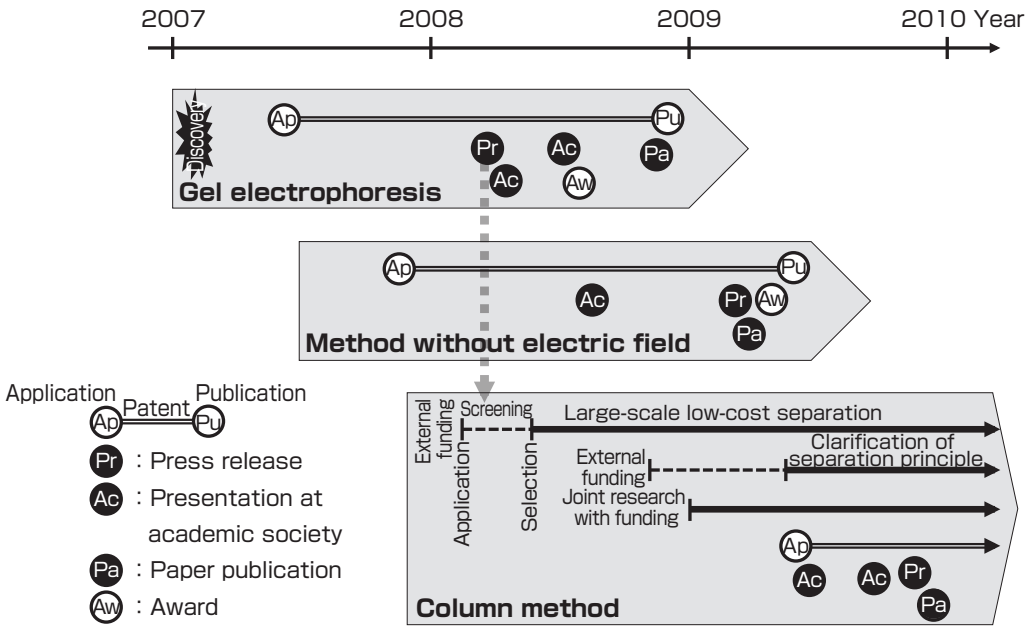

Fig. 6 Intellectual property strategy, timing of publication, and funding to effectively conduct the research, starting with the discovery of the separation of metallic and semiconducting CNTs using gel

the separation of single-structure semiconducting CNTs was an important topic. We were successful in roughly separating the different types of semiconducting CNTs by gradually changing the elution condition for the semiconducting CNTs in the column separation using agarose gel.[17][18] However, there was a limit to the separation precision, and a separation method with higher precision was in demand. Zheng *et al.* developed a method of separating the CNTs dispersed using DNA by chromatography, and succeeded in separating the single-structure semiconducting CNTs by using DNA with specific sequences.[19] This paper was extremely innovative because there had been no previous report of separating 12 types of highly pure single-structure CNTs. However, the method required expensive synthesized DNA, the efficiency was not good since only one type of CNT could be extracted from the CNT mixture, and there was room for improvement. Then, we found that the single-structure CNT could be separated by column separation using the commercially available gel called Sephacryl, a polysaccharide like agarose (this gel was originally developed for biomolecule separation).[14] When we were investigating the amount of CNT dispersant that should be used for the gel, we added a large amount of the sample to the column, and found that only certain types of CNTs adsorbed on the gel and were separated. Normally, to improve the purity in column separation, the amount of sample is reduced or the column is elongated, but in this case, a totally opposite way of thinking was employed, and we obtained highly pure CNT structural separation by adding excessive amount of the sample to a small amount of gel. When a large amount of CNT dispersant was added to the column, competitive adsorption occurred among different types of CNTs, and as a result, only a few CNTs with the strongest adsorption were adsorbed to the gel. Using multiple columns arranged in line, we were able to separate several semiconducting CNTs with different adsorbability at one time (Fig. 7). The important point is this method does not require expensive reagent, and several CNTs of different structures can be obtained from the CNT mixture. The column method is appropriate for the large-scale, low-cost separation. As shown in Fig. 7, we succeeded in separating 13 types of single-structure semiconducting CNTs with different bright colors.[14]

2.7 Scaling-up the separation of metallic and semiconducting CNTs

The researches up to now were the *Type 1 Basic Research* conducted to gain fundamental knowledge such as the discovery of a new phenomenon or the development of a new separation method. In the *Type 2 Basic Research*, the research focuses on the ways to scale-up and to lower the cost of separation to enable the large-scale, low-cost separation of metallic and semiconducting CNTs. It is one step before the *Product Realization Research*. Although the metallic and semiconducting CNTs separated by density gradient ultracentrifugation method were already commercially available, we aimed to boost the processing amount to 10 or 100 times using the gel column separation method. We succeeded in dramatically lowering the cost of separation by replacing the reagent with an inexpensive one and developing a new gel. We also scaled-up the separation by connecting the column that had several thousand times more capacity than the one shown in Fig. 5 to the large chromatography device. We also engaged in the research on the large-scale preparation of the CNT dispersant used for separation, concurrently with the separation scaling-up. The scaling-up and high throughput were obtained for separation with no problem, and the system to separate the metallic and semiconducting CNTs at a daily production of 1 g was established. The separated CNTs are now distributed as samples through the Technology Research Association for Single Wall Carbon Nanotubes (TASC), a consortium of companies and AIST. Hence, we were able to accomplish the initial goal of "sending the separated metallic and semiconducting CNTs into society."

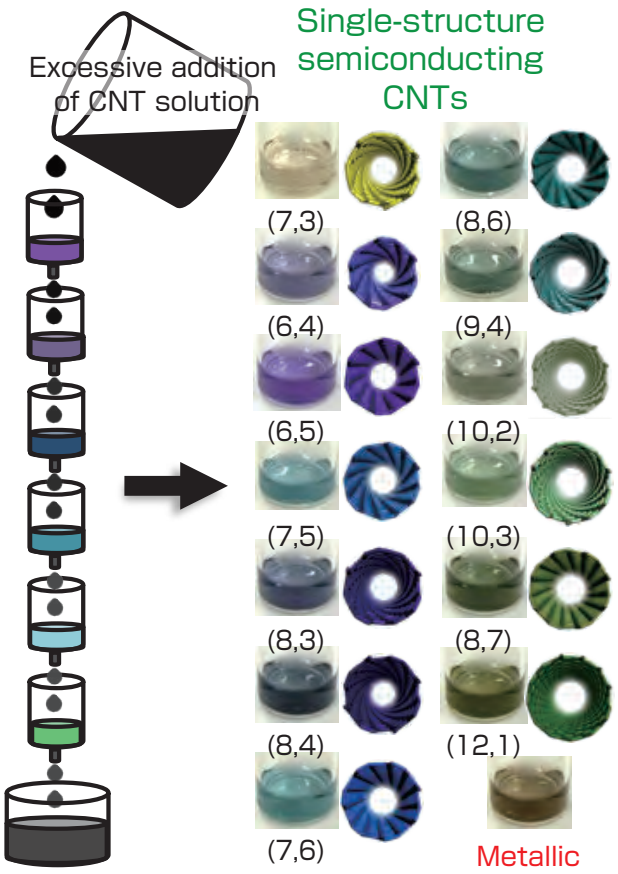

Fig. 7 Schematic diagram of the separation of single-structure semiconducting CNTs by multi-column method (left) and obtained dispersions (right)
The numbers (n, m) in the diagram are indices that represent the CNT structure. Brilliant colors are assumed when the separation is done to the single-structure semiconducting CNTs.

3 Future issues and prospects

3.1 Large-scale, long-length, and low-defect CNT dispersion method

In the column separation, the throughput of a daily production of 1 g has been realized, and currently, the daily production of 100 g is possible if the separation procedure is scaled-up. The issue is no longer "separation," but the bottleneck of the throughput is the preparation of the "CNT dispersion." There is also a major issue of loss of excellent properties of CNTs due to defects and breakages in the dispersion process. How to avoid defects in the CNTs, how not to cut them (to maintain length), and how to prepare the dispersion in large amounts are the major issues, and the researches are now being done to solve those issues.

3.2 Development of devices that maximize the property of CNTs

The initial objective "to send the separated CNTs into society" that we set at the beginning of the research was achieved in the form of sample distribution by TASC. We achieved the throughput of a daily production of 1 g, and the daily production of 10 g or 100 g is becoming possible. While we are able to contribute to both the basic and application researches of CNTs, separating large amounts of CNTs will not be useful unless there is a prospect of their use. It does not follow that once a new material is developed, some new application will be found instantly, and it is difficult to proceed to the next step unless we can show excellence of the property of our new material over the existing materials. Currently, we have set a new goal of developing a device that maximizes the excellent property of the separated CNTs, without stopping at "sending the separated CNTs into society."

3.3 Clarification of the separation principle

The separation of metallic and semiconducting CNTs using gel occurs when the semiconducting CNTs are selectively adsorbed to the gel when SDS is used as the dispersant in the agarose or Sephacryl gel. Separation was not confirmed in starch or gellan gum gels that are polysaccharides as in agarose, other than acrylamide gels.[11][12] It is thought that selective adsorption occurs due to the fine balance of the interaction of the four factors including metallic CNTs, semiconducting CNTs, gel, and SDS, when combining the specific gel and the dispersant. Recently, it was indicated that surfactants that could be used for the separation, other than SDS, could be found by large-scale screening, and the surfactants with appropriate dispersion property could be found based on the common structure.[20] That is, surfactant with high dispersibility (such as cholate or deoxycholate) disperses both the CNTs regardless of metallic or semiconducting, and do not cause separation. On the other hand, surfactant with moderate dispersibility (does not have high dispersibility) recognizes the slight difference between the metallic and semiconducting CNTs, enhances selective adsorption of the semiconducting CNTs to the gel, and causes the separation. However, the essential principle of the exact differences that lead to the separation of metallic and semiconducting CNTs is unclear, and this is a subject for future study.

3.4 Safety

Nanomaterials have been newly synthesized recently, and many have inadequate safety evaluation due to their short history. Today, there is a requirement for safety confirmation before starting to use a new material, and CNTs, which are representative nanomaterials, are no exception. Currently, many research institutions are gathering data on safety, but time is required for complete understanding. It is necessary to conduct the development of application and safety evaluation concurrently.

4 Summary

We succeeded in developing the low-cost and mass-producible separation method for metallic and semiconducting CNTs using agarose gel. We also developed a mass separation method for single-structure semiconducting CNTs using the Sephacryl gel. The mass separation of metallic and semiconducting CNTs was started and the samples are being supplied. The separation method was originally devised by AIST, and was born from the results of joint research among researchers of different fields. In conducting research effectively, it is necessary to make integrated decisions for funding, intellectual property strategy, and timing of academic publication. Development of application is essential for the industrial application of CNTs. We hope to contribute to the industrial application of CNTs by accelerating the development of application by using large-scale, low-cost separated metallic and semiconducting CNTs.

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Discussions with Reviewers

1 Thought process for the fusion of different fields

Question (Toshimi Shimizu: Nanotechnology, Materials and Manufacturing, AIST; Yasushi Mitsuishi: AIST Tsukuba)

When the researchers of totally different fields engage in trial experiments that are considered lacking in common sense (though often unperceived) in their community, they may run into serendipitous innovative findings. I think the greatest factor that led to the success of this research is the thought process that transpired in the fusion of different fields, where the separation and purification methods of biomolecules such as DNA that are used regularly in biochemistry were applied to carbon nanotubes (CNTs). Therefore, for the scenario of this paper, I would like you to organize and describe thoroughly the thought processes of

how you evaluated the various elemental technologies to solve the problems and how they were selected and assembled.

Answer (Takeshi Tanaka)

The reason we selected electrophoresis was because we thought if the separation could be accomplished by density gradient centrifugation due to slight density differences, then the separation must also be possible by charge differences. Because the sizes of DNA and CNTs are similar, we selected the agarose electrophoresis used frequently in DNA separation. However, we found that rather than the size of gel mesh, the combination of the randomly selected agarose and SDS was important for separation. This means that the course was different from what we were thinking in the beginning, but we were able to obtain excellent results. As you indicated, I attempted to describe in detail the thought process and how the research policy was built around it.

2 Elemental technology

Question (Toshimi Shimizu)

In relation to the integration of elemental technologies, I don't think the progression from agarose electrophoresis → column method → multicolumn method is particularly an upgrade in life science research. However, when you used CNTs as the samples, I imagine that you ran into unique difficulties that you did not encounter in DNA. If you have any specific examples, please describe them. In some cases, it may become new elemental technologies. As a result, you will be able to appeal to the readers that it is not simply a biochemistry research method added to the elemental technology integration in the latter half.

Answer (Takeshi Tanaka)

The breakthroughs in the separation research for metallic and semiconducting CNTs using gel include the discovery of the combination of agarose gel and SDS, the improvement of yield by using the CNT-containing gel, the discovery of structure separation by excessive addition of sample, and others.

3 Side development of the gel material

Question (Toshimi Shimizu)

In the biomolecule separation, other than agarose gels, the polyacrylamide gel is widely used. In optimizing the gel material, were you engaged in any side development of the gel material?

Answer (Takeshi Tanaka)

The characteristic of the agarose gel is that the mesh structure is extremely large. This large mesh structure allows the separation of DNA, which is a giant biomolecule, by gel electrophoresis. Because CNT is similar to DNA in thickness and length, we used the agarose gel for CNT separation. On the other hand, the mesh of the acrylamide gel used frequently in protein separation is relatively small, and something extremely big like CNT is not likely to pass through the gel mesh. In fact, we conducted electrophoresis using the acrylamide gel, but failed to obtain separation. However, although we focused on the size of the agarose gel mesh at first, we found that the mesh size was not the essence of the separation, but rather, what was important was the specific interaction between the semiconducting CNTs and the gel when the combination of a certain gel and a dispersant was used. In fact, in column separation, the CNTs are separated by adsorbing on to the gel beads surface, even if we used the gel beads with high agarose concentration and therefore have dense mesh structure. However, in such a high concentration gel, adsorption to the gel decreases since the adsorption area is limited to the surface.

4 Separation mechanism

Question (Toshimi Shimizu)

When an unexpected result with good reproducibility is obtained, there must be some scientific basis. I think the value of this paper will be enhanced if you add the scientific explanation for the mechanism of separation.

Answer (Takeshi Tanaka)

Detailed explanations were added to subchapters 2.3 and 3.3 for the separation mechanism. As a phenomenon, we know that a selective adsorption of the gel and semiconducting CNTs occurs in the combination of a certain gel and a dispersant; however, the basic principle that causes the separation is not clear. This is an important research topic in the future.

5 Scaling-up

Question (Toshimi Shimizu)

The daily production of CNTs differs greatly depending on the types, from multiple to single wall. It is now on the order of several hundred grams to tons, but the order of kilograms or more will be necessary in the current separation and purification process. In that sense, is the scaling-up of over 1000 times the current state possible? Or, can the industrial demand be satisfied with the one-gram order of purified metallic or semiconducting carbon nanotubes? Please describe the social and industrial demands of scaling-up.

Answer (Takeshi Tanaka)

As described in subchapter 3.1, there is no scale-up limit for column separation itself, and I think the separation of 1,000 times more than the current state is possible. The bottleneck of throughput is the preparation of the dispersant, and we are engaging in research to improve this. However, as described in subchapter 3.2, without the development of application there will be no use, even if it can be separated in large volumes. Since the volume demand changes according to how the CNTs are used, it is still unclear how much throughput is necessary. Yet I feel that the abundant supply of large-scale, low-cost separated metallic and semiconducting CNTs will accelerate the development of applications. I added the relevant description in chapter 4 "Summary."