

Short Communication

Protein Bands Detection by Nanoparticles after Paper Chromatography

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Abstract:

Paper chromatography is an analytical technique for separating and identifying mixtures of materials. It is a useful technique since it is relatively quick, and requires small quantities of the material. By emergence of nanotechnology, paper chromatography has found many applications in biology and biotechnology. In this study, we employed gold and silver nanoparticles to detect protein bands after the paper chromatography method. We first performed paper chromatography on a solution containing Bovine Serum Albumin (BSA) protein. Then, the gold and silver nanoparticles were exploited for paper coloration. As a result, it was noticed that location of the protein bands was clearly distinct and detectable with this technique.

Keywords: chromatography, detection, nanoparticle, protein.

1. INTRODUCTION

An analytical method for mixture separation and identification is paper chromatography. Paper chromatography is a useful technique since it is relatively quick and requires small quantities of the material. In paper chromatography method, substances are distributed between a stationary phase and a mobile phase. The stationary phase is usually a piece of high quality filter paper. On the other hand, the mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it [1, 2].

The use of nanomaterials in biotechnology merges the fields of material science and biology. Nanoparticles provide a particularly useful platform, demonstrating unique properties with potentially wide-ranging applications [3].

Nanotechnology in biology and biotechnology has also found many applications [4]. Some applications of nanomaterials in biology or medicine include fluorescent biological labels [5,6], detection of pathogens [7], drug and gene delivery [8,9,10], detection of proteins [11,12], hyperthermia [13,14], tissue engineering [15,16], imaging contrast enhancement [17], separation and purification of biological molecules and cells [18].

In this study we introduce a new approach for detecting proteins after paper chromatography.

2. MATERIALS AND METHODS

2.1. Nanoparticles synthesis

Gold nanoparticles were synthesized according

to the description of Adam. D [19]. 20 ml of 1.0 mM HAuCl_4 was added to a tub on a stirring hot plate. Then a magnetic stir bar was placed in solution and it was brought to a rolling boil. When the solution started to boil, 2 ml of a 1% solution of trisodium citrate dehydrate $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ was quickly added. In this process, gold nanoparticles gradually form as the citrate reduces the gold (III), which turns the solution to a deep red color.

Silver nanoparticles were also produced via recovery of silver nitrate using a sodium citrate salt, according to the description of Lee and Meisel [20].

Solutions of reacting materials were all prepared in distilled water. In a typical experiment, 50 ml of 10–3 M AgNO_3 was heated to the boiling temperature. Meanwhile, 5 ml of 1 % trisodium citrate was added drop by drop to this solution. During the process, solution was mixed vigorously. Solution was heated until a change in color was observed. Then, it was removed from the heating element, and stirred until cooled down to the room temperature [21].

2.2. Protein paper chromatography

First the chromatography strip was cut out of the filter paper. Next, the solution of protein

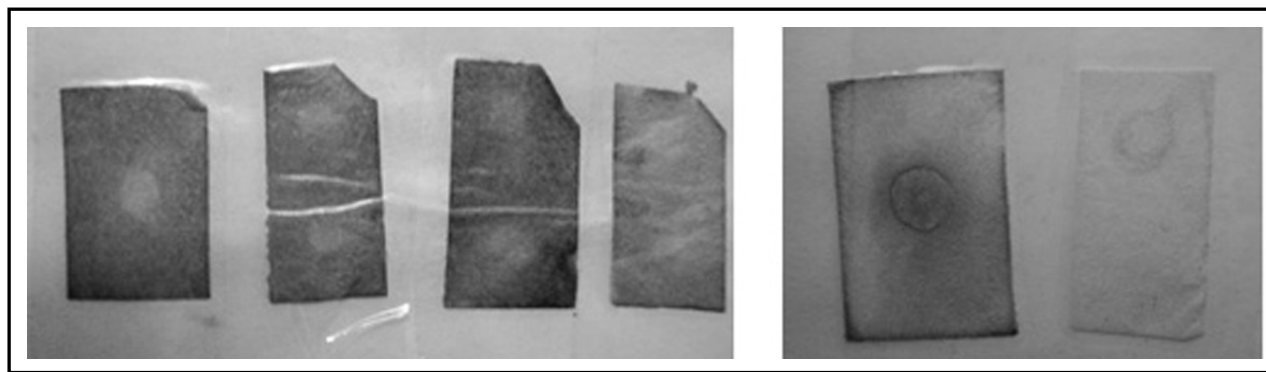
was first mixed with Sodium dodecyl sulphate (SDS), and then the mixture was put on the start line with a toothpick. Buffer was poured into the jar just to cover the bottom. The strip of the chromatography paper with sample(s) was put in the chromatography chamber, so that bottom of the strip touched the solvent. Solvent climbed up the strip, while dragged the sample with it. Paper was removed from the chamber either when the solvent front was a bit away from the top, or if it didn't move up any more. As the solvent came up, proteins stopped moving at different points, and were separated.

2.3. Staining of paper

The paper containing protein was put in the nanoparticles solution. Due to the size of the chromatography paper, the solution should be poured on the paper until the paper is completely covered. In this work, about 5 mL of nanoparticle solution was proved to be adequate.

3. RESULTS AND DISCUSSION

Silver and gold nanoparticles synthesized via reduction reactions are shown in Figure 1.



a.

b.

Figure 2: Paper staining by nanoparticles. Staining with gold nanoparticles makes paper dingy (Figure 2.a), while staining with silver nanoparticles, results in a yellow to brown color (Figure 2.b). In both cases, repulsion due to the negative charges of citrate and SDS distinguishes the proteins as the bright spots.

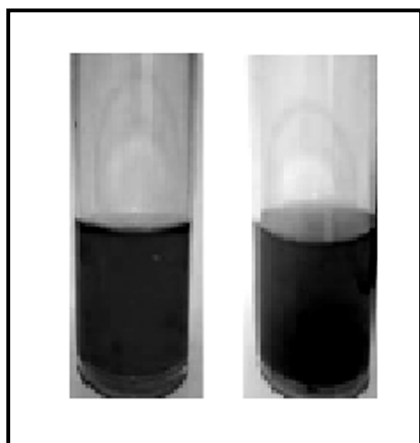


Figure 1: Gold nanoparticles are red (right) and silver nanoparticles are yellow to brown (left).

As can be observed in Figure 2, after paper staining by gold (Figure 2a) and silver (Figure 2b) nanoparticles, the protein is distinguished by its place.

The nanoparticles were made with sodium citrate, which acts as a reducing and capping agent and results in synthesized nanoparticles conjugated to citrate.

Prior to being placed on the paper, protein solution was mixed with SDS, which causes all proteins to gain a negative charge. Therefore, when the paper is put in the nanoparticle solution, negative charges repel each other, and make the paper wholly colorful, except for the area that is covered by the protein. Then protein bands are observed with a good resolution.

4. CONCLUSIONS

Paper chromatography is an inexpensive and powerful analytical tool that requires very small quantities of the material. It is rapid and easy to handle, while chromatograms can be stored for the future references. In this article, a new approach based on nanotechnology was introduced, which is used for detection of protein bands on paper after chromatography.

The results demonstrated the possibility of using gold and silver nanoparticles instead of fluorescent

and chemical dyes for staining the protein bands. This test can be an introduction to exploitation of gold and silver nanoparticles for staining the proteins after western blot as well. By binding a specific antibody to nanoparticles, the complex can be used for special staining of the protein bands.

In future work, we intend to study the effect of nanoparticle size on the staining quality of the paper chromatography.

REFERENCES:

1. Morgan E. D. and Wilson I. D. An Early Description of Paper Chromatography. *Chromatographia*, Vol. 60, (2004), pp.135-136.
2. Chatterji A. C. and Hari Bhagwan. Studies on paper chromatography. *Journal of analytical chemistry*, Vol. 156, (1957), pp. 7-15.
3. Mrinmoy De, Partha S. Ghosh, and Vincent M. Rotello. Applications of Nanoparticles in Biology, Vol. 20, (2008), pp. 4225-4241.
4. Upendra Kumar Parashar, P.S. Saxena. Role of Nanomaterials in biotechnology. *Digest Journal of Nanomaterials and Biostructures*, Vol. 3, (2008), pp. 81-87.
5. Wenwan Zhong. Nanomaterials in fluorescence-based biosensing. *Anal Bioanal Chem* Vol. 394, (2009), pp. 47-59.
6. Feng Wang, Wee Beng Tan, Yong Zhang, Xianping Fan and Minquan Wang. Luminescent nanomaterials for biological labeling. *Nanotechnolog*, Vol. 17, (2006), R1-R13.
7. Hua Yang, Huaping Li and Xiuping Jiang. Detection of foodborne pathogens using bioconjugated nanomaterials. *Microfluidics and nanofluidics*, Vol. 5, (2008), pp. 571-583.
8. Gang Liu, Magdalena Swierczewska, Seulki Lee, Xiaoyuan Chen. Functional nanoparticles for molecular imaging guided gene delivery. *Nano Today*, Vol. 5, (2010), pp. 524-539.
9. María Vallet-Regí, Eduardo Ruiz-Hernández, Blanca González, and Alejandro Baeza. Design of Smart Nanomaterials for Drug and Gene Delivery. *Journal of Biomaterials and Tissue Engineering*, Vol. 1, (2011), pp. 6-29.

10. Nelson A Ochekepe, Patrick O Olorunfemi and Ndidi C Ngwuluka. Nanotechnology and Drug Delivery Part 1: Background and Applications. *Tropical Journal of Pharmaceutical Research*, Vol. 8, No. 3, (2009), pp. 265-274.
11. Jian-Ru Gong. Label-Free Attomolar Detection of Proteins Using Integrated Nanoelectronic and Electrokinetic Devices. *Small*, Vol. 6, (2010), pp. 967-973.
12. Songjun Li, Jagdish Singh, He Li, Ipsita A. Banerjee. Lipid Nanoparticle-Mediated Detection of Proteins. *Biosensor Nanomaterials*, Vol. 5, (2011), pp. 678-684.
13. Terry B Huff, Ling Tong. Hypertermic effect of gold nanorods on tumor cells. *Nanomedicine*, Vol. 2, No. 1, (2007), pp. 125-132.
14. Challa S.S.R. Kumar, Faruq Mohammad. Magnetic nanomaterials for hyperthermia-based therapy and controlled drug delivery. *Advanced Drug Delivery Reviews*, Vol. 63, (2011), pp. 789-808.
15. Wei Jie, Li Yubao. Tissue engineering sca-old material of nano-apatite crystals and polyamide composite. *European Polymer Journal* Vol. 40, (2004), pp. 509-515.
16. Benjamin S. Harrison, Anthony Atala. Carbon nanotube applications for tissue engineering. *Biomaterials*, Vol. 28, (2007), pp. 344-353.
17. Megan A. Hahn, Amit K. Singh, Parvesh Sharma, Scott C. Brown and Brij M. Moudgil. Nanoparticles as contrast agents for in-vivo bioimaging: current status and future perspectives. *Analytical and Bioanalytical Chemistry*, Vo. 399, (2011), pp. 3-27.
18. Molday RS, MacKenzie D. Immunospecific ferromagnetic iron dextran reagents for the labeling and magnetic separation of cells. *J Immunol Methods*, Vo. 52, (1982), pp. 353-367.
19. Adam D. McFarland, Christy L. Haynes, Chad A. Mirkin, Richard P. Van Duyne and Hilary A. Godwin. Color My Nanoworld. *J. Chem. Educ.* Vol. 81, No. 4, (2004), 544A.
20. Fang, J., Zhong, C., Mu, R. The Study of Deposited Silver Particulate Films by Simple Method for Efficient SERS *Chemical Physics Letters*, Vol. 401, (2005), pp. 271-275.
21. Asta Šileikaitė, Igoris Prosyčėvas, Judita Puišo, Algimantas Juraitis, Asta Guobienė. Analysis of Silver Nanoparticles Produced by Chemical Reduction of Silver Salt Solution. *Materials science (Medžiagotyra)*, Vol. 12, (2006), pp. 287-291.