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Diimide-Enhanced Fingerprint Detection with Photoluminescent CdS/Dendrimer Nanocomposites

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ABSTRACT: The chemical development of latent fingerprints by nanocomposites that involve photoluminescent cadmium sulfide nanoparticle aggregates with Starburst® dendrimer is demonstrated. The dendrimer bonds to fingerprint residue via its terminal functional groups. When these are amino groups (generation 4 dendrimer), the binding is enhanced by fingerprint pre-treatment with diimide. The diimide converts carboxylic acid moieties of the fingerprint residue to esters that then react with the dendrimer amino groups to form amide linkages. The cadmium sulfide/generation 4 dendrimer development of fingerprints is enhanced by elevated temperature also. Finally, fingerprint development with carboxylate-functionalized cadmium sulfide/generation 3.5 dendrimer nanocomposites is examined. Here, diimide treatment of the dendrimer itself aids the subsequent fingerprint labeling, which involves amino acid of the figerprint residue. Nanocomposite fingerprint detection is compatible with time-resolved imaging for background fluorescence elimination.

KEYWORDS: forensic science, fingerprints, photoluminescence, cadmium sulfide, dendrimer, nanocomposite, diimide, time-resolved imaging

As part of a comprehensive investigation of the application of photoluminescent semiconductor nanocrystals and nanocomposites to the detection of latent fingerprints, we recently studied cadmium sulfide (CdS) nanocrystals as well as CdS/dendrimer nanocomposites (1,2). The motivation for examining this application in general is that nanocrystals and nanocomposites are robust and flexible in terms of attachment of conjugating functionalities for selective tagging of fingerprints by both physical and chemical mechanisms, that there is tunability in terms of excitation and luminescence color, and that the luminescences are intense, with lifetimes eminently suited to time-resolved techniques for background fluorescence suppression. One thus has a potentially universal strategy for latent fingerprint detection with capabilities beyond those of the current fluorescence-based approaches, and, for that matter, the more traditional procedures (3–5).

In this article, we focus on the mechanism(s) involved in the selective tagging of fingerprints with CdS/dendrimer nanocomposites. Our earlier work (2) with such nanocomposites utilized generation 4 Starburst® (PAMAM) dendrimer, which is commercially available (Aldrich). The results obtained suggested that chemical bonding to carboxylic acid or ester moieties of the fingerprint residue via the amino terminal groups of the dendrimer (forming amide linkages) was taking place, but it was not clear to what extent such bonding occurred, as opposed to preferential adherence to fingerprints by physical mechanisms akin to those involved in fingerprint staining with fluorescent dyes. Thus, we aim here to determine whether chemical labeling of fingerprints is, in fact, taking place, as necessary if universal applicability, especially in connection with porous surfaces, is eventually to be achieved.

Basic Chemical Strategy for Fingerprint Tagging

The basic intent in selecting amino-group-terminated dendrimer was to target fingerprint lipids, namely fatty acids or triglycerides, i.e., carboxylic acids or esters. Ester moieties are also present when fingerprints are processed by cyanoacrylate fuming. The lipid focus was motivated by the fact that current chemical fingerprint detection methodology typically does not target lipids, but amino acids (or proteins) instead, thus mostly neglecting a class of compounds copiously present in fingerprint residue. Figure 1 depicts the general scheme of the desired chemistry, with the carboxylic acid representing the fingerprint and the amine the CdS/dendrimer nanocomposite. The dendrimer here serves the dual function of incorporating CdS nanoparticles and of bonding, via its terminal groups, to the fingerprint. We note immediately that one could equally envision a strategy in which the amine represents the fingerprint and the acid the dendrimer. This would be an analog to amino acid or protein-based procedures such as ninhydrin and DFO. Thus, we also take up in this article dendrimers with carboxylate functionality.

In the approach of bonding to lipids (fatty acids) of fingerprint material, it is to be recognized that OH is a poor leaving group, so that one has to anticipate that the reaction to form the amide linkage might not occur easily. Accordingly, we examine in the present study the utilization of diimides to convert the carboxylic acid to an ester that more readily reacts with amine to form the amide. Diimide use in concert with mercaptoacetic acid-functionalized CdSe

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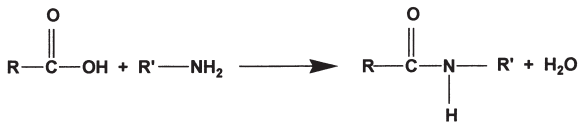

FIG. 1—*General scheme of amide formation from acid and amine.*

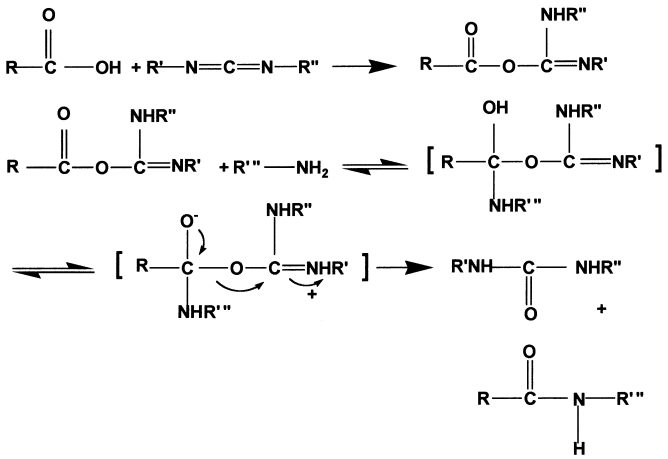

FIG. 2—*Diimide-mediated formation of amide from acid and amine.*

quantum dots for labeling protein has been reported (6). The general chemical scheme is shown in Fig. 2. In the shown reaction, RCOOH represents the fingerprint carboxylic acid, R'"-N=C=N-R" the diimide, R'"-NH_2 the dendrimer, and RCONHR'" the formed amide, namely the tagged fingerprint.

Diimide Pre-Treatment

The initial fingerprint treatment by diimide utilized 1,3-dicyclohexylcarbodiimide. As obtained commercially, this non-polar compound comes in 1M dichloromethane solution. For reasons of economy, we diluted this solution (with dichloromethane). However, reasonably high diimed concentration should be desirable to minimize the time needed for reaction, which is important to minimize bleeding of fingerprint detail. Somewhat arbitrarily, we settled on a 2.5% diimide by weight working solution. Both cyanoacrylate fumed and unfumed fingerprints (on aluminum foil, polyethylene, and paper in the unfumed case) were immersed in the diimide solution (at room temperature) for varying amounts of time. Since the cyanoacrylate polymer formed on fingerprint fuming already contains ester moieties, no effect should result from exposure to diimide. The immersion of fumed prints thus was intended to serve as a control experiment. Indeed, comparison of fumed fingerprints that were immersed in the diimide and fingerprints that were immersed for equal times in neat dichloromethane showed no differences in development after subsequent treatment with CdS/generation 4 dendrimer and luminescence examination. Unfumed fingerprints could be exposed to the diimide solution or dichloromethane solvent only for short times (on the order of minutes) because the fingerprints tended to be dissolved. The situation is reminiscent of the limit in the time an unfumed fingerprint can be exposed to methanol solution of CdS/dendrimer (2). With such short immersion times, no appreciable difference in the subsequent CdS/dendrimer development of fingerprints was found.

Because the CdS/dendrimer nanocomposite tends to adhere everywhere on polyethylene, not just to fingerprints, the results with this material were difficult to visualize. A similar background problem was found with paper as well. Thus, we focused in the work described below on fingerprints (fresh to 2-weeks-old) on aluminum foil. Because of dissolution of fingerprint constituents in the aggressive dichloromethane, we examined the use of a diimide soluble in water, namely 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Initially, we selected as the solvent system the 1:9 methanol:water mixture of our earlier study (2) of CdS/dendrimer development of unfumed fingerprints. Prolonged immersion in this diimide solution resulted in chemical attack on the aluminum foil itself, however. Thus, the methanol was deleted and the diimide fingerprint pre-treatment used a standard solution of 2.5% diimide (by weight) in water. Fingerprints immersed in water alone for the same time served as controls. Subsequent CdS/generation 4 dendrimer fingerprint processing utilized 2×10^{-4} M/8×10^{-5} M nanocomposite formulation and 1:9 methanol:water solvent system, as described in detail before (2).

Diimide pre-treatment of fingerprint halves for times ranging from seconds to roughly one hour produced no enhancement over the corresponding water-immersed control-halves. For time spans from about 5 to 24 h, substantially enhanced CdS/dendrimer development of fingerprint detail was found, however, with the enhancement increase leveling off by 24 h. An example of thus diimide-enhanced (24 h) CdS/dendrimer fingerprint detection is shown in Fig. 3. Our observations are indicative of predominant chemical fingerprint development, although there is some contribution by preferential physical adherence of the nanocomposite, as indicated by results also with polyethylene, paper, and cyanoacrylate ester-fumed fingerprints (2). Quite generally, chemical reaction rates increase with temperature. Thus, we examined diimide pre-treatment at 60°C, as compared with room temperature. At the elevated temperature, however, chemical attack on the aluminum foil substrate itself occurred for heating times longer than about 1 h. With shorter heating times, no enhancement increase with heating was observed. If there is also chemical binding of the nanocomposite to the fingerprint residue in the absence of the diimide pre-treatment, then one might expect to also see some

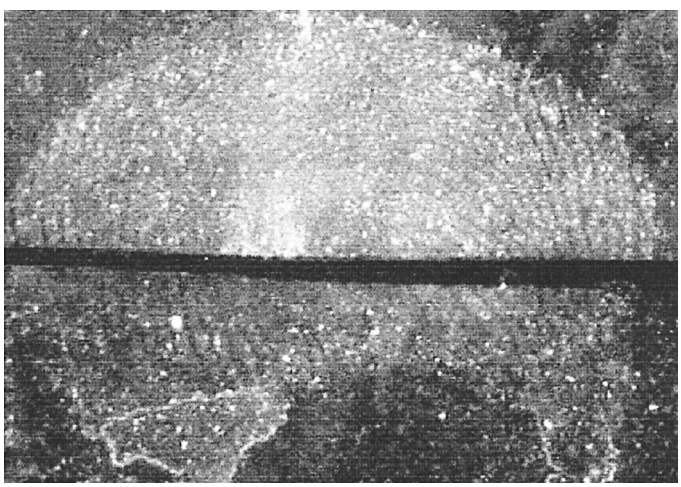

FIG. 3—*CdS/generation 4 dendrimer-developed fingerprint on aluminum foil with (top half) and without (bottom half) diimide pre-treatment. This and all other pictures of this article are hard-copy of photographs taken with a digital camera (Kodak DC 120 with close-up lens).*

FIG. 4—*CdS/generation 4 dendrimer-developed fingerprint on aluminum foil with (top half) and without (bottom half) heating.*

increase in fingerprint development directly by CdS/dendrimer solution upon elevation of the temperature. Indeed, such increase is found, as shown in Fig. 4, which compares the two halves of a fingerprint developed for 5 h at 60°C and at room temperature.

Because of the problem of adherence of unreacted nanocomposite to substrates holding fingerprints, we examined the prospect of sequential development in which the print is first subjected to immersion in diimide solution, then rinsed, then immersed in dendrimer solution (without CdS incorporated into the dendrimer), rinsed again, and finally subjected to in situ incorporation of nanoparticles (CdS in the present study) of the photoluminescent semiconductor material, followed by a final rinse. The rinsings after each chemical step would serve to remove, as much as possible, unreacted material. Preliminary results show that this sequential development is feasible. Gentle rinses with acetone appear to be promising. Optimization of chemical and rinsing solvents, concentrations, reaction temperature, pH, substrate effect, etc., will require protracted experimental work that is beyond the scope of the present account. However, the matter of acetone utilization is further considered below in connection with carboxylate-functionalized dendrimer.

Generation 3.5 Starburst® Dendrimer

The reaction scheme of Fig. 1 is in principle applicable to use of photoluminescent semiconductor nanocrystals or nanocomposites functionalized with carboxylic acid. This prospect was suggested already some time ago (3). Its feasibility is taken up here in connection with generation 3.5 Starburst® dendrimer, which has carboxylate (sodium salt) terminal groups. The selection of generation 3.5 was made because, like generation 4, the dendrimer has 64 terminal groups. Thus, direct comparison with generation 4 dendrimer can be made. The CdS/dendrimer nanocomposite preparation was performed in a manner identical to what we have reported already (2), with methanol as well as 1:9 methanol:water solvent systems and 2×10^{-4} M/8×10^{-5} M CdS/dendrimer concentration. As with generation 4, the methanol solution formulation was ineffective with unfumed fingerprint samples. With fumed fingerprints, the results were roughly comparable to what we have seen before (2) with generation 4, with no advantage presented by generation 3.5. The 1:9 methanol:water formulation of CdS/generation 3.5 dendrimer produced no fingerprint development at all for unfumed fingerprints on aluminum foil (or polyethylene), in contrast to what is obtained with the generation 4 counterpart. This might not be entirely surprising because different ingredients of the fingerprint residue are targeted. Lipid-rich fingerprint residue pertains to our study (e.g., from fingers rubbed on the forehead). Thus, the protein or amino acid of interest (to CdS/generation 3.5) might be expected to be buried among or beneath the lipids present. The situation would be aggravated by the fact that the polar reagent solvent system is inherently incompatible with the non-polar lipids. This incompatibility is reminiscent of what is encountered in lipid fingerprint development with lanthanide chelates (7,8). There, the solution to the difficulty involved addition to the solvent system of a small amount of acetone for the purpose of lipid solubilization. The same approach was employed here, with acentone concentrations up to 20% by volume added to the 1:9 methanol:water solution. The acetone additions produced only sporadic fingerprint development with generation 3.5 dendrimer upon sample (fingerprint on aluminum foil) immersions for as many as four days. In contrast, crisp luminescent detail was consistently found with generation 4 dendrimer after such immersions. This suggested to us that the solubilization issue was not the cause for failure. It occurred to us that instead the poor suitability of the dendrimer's carboxylate functionality for attack on the amino acids of fingerprint residue was the culprit. Thus, we examined the treatment of generation 3.5 dendrimer with diimide prior to fingerprint treatment. The successful sequence (in 1:9 methanol:water) was: generation 3.5 dendrimer + stoichiometric amount of diimide (keeping in mind that 64 functional groups are present in each dendrimer molecule) + heating at 60°C over night + CdS incorporation + fingerprint immersion. Figure 5 shows an example of a thus-developed fingerprint. The stoichiometric addition of diimide (about 2.4×10^{-4} M dendrimer:1.5×10^{-2} M diimide) was intended to minimize formation of ester in the fingerprint residue itself upon subsequent sample immersion, which would tie up amino acid that otherwise could react with dendrimer. The heating step was essential. Without it fingerprint development was very faint only. The 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride attacks alumium foil to give it a tarnished appearance much like tarnished silver. In absence of the heating step, the subsequently immersed aluminum foil fingerprint samples acquire this tarnishing, and there thus is concomitant counterproductive reaction of the diimide with

FIG. 5—*Diimide-mediated development of fingerprint (on aluminum foil) with CdS/generation 3.5 dendrimer. See text.*

carboxylic acid of the fingerprint residue. In presence of the heating step, no aluminum foil tarnishing is seen, indicating that the diimide is indeed reacting with the dendrimer, as is desired. CdS/dendrimer nanocomposite formation followed by diimide addition was not a successful sequence, perhaps because of excessive aggregation preceding the addition of the diimide. Some indication of this is provided by the substantial red shift in solution luminescence for dendrimer + CdS + diimide compared to dendrimer + diimide + CdS. The luminescence of Fig. 5 (intensity quite comparable to what one gets with generation 4) is orange, in contrast to the yellow-green one pertinent to Figs. 3 and 4. It is nicely excitable by near-ultraviolet, as well as blue (albeit rather more weakly). Both are obtainable from Ar-lasers, a convenience for the necessarily laser-based time-resolved imaging instrumentation.

We believe that we have achieved a reasonable grasp of the chemistry of fingerprint labeling with dendrimer. Thus, we are now initiating investigation of a broad range of surfaces and are extending our studies to functionalized photoluminescent nanocrystals and composites beyond CdS. Along similar lines, we are examining the incorporation into dendrimer of highly luminescent lanthanide complexes, which have luminescences of millisecond-order lifetimes, suitable for the gated imaging to suppress background fluorescence that has been mature for some time already from the instrumentation perspective (3).

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References

1. Menzel ER, Savoy SM, Ulvick SJ, Cheng KH, Murdock RH, Sudduth MR. Photoluminescent semiconductor nanocrystals for fingerprint detection. J Forensic Sci 2000;45(3):545–51.
2. Menzel ER, Takatsu M, Murdock RH, Bouldin KK, Cheng KH. Photoluminescent CdS/dendrimer nanocomposites for fingerprint detection. J Forensic Sci, 2000;45(4):770–3.
3. Menzel ER. Fingerprint detection with lasers. 2nd ed., revised and expanded. New York: Marcel Dekker, 1999.
4. Menzel ER. Photoluminescent semiconductor nanocrystals for latent fingerprint detection. Provisional patent application, filed June 9, 1999.
5. Menzel ER. Fluorescence in forensic science. In: Meyers RA, editor-in-chief. Encyclopedia of analytical chemistry. Chichester, UK: John Wiley & Sons, 2000, in press.
6. Chan WCW, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 1998;281:2016–8.
7. Allred CE, Menzel ER. A novel europium-bioconjugate method for latent fingerprint detection. Forensic Sci Int 1997;85:83–94.
8. Allred CE, Murdock RH, Menzel ER. New lipid-specific, rare earth-based chemical fingerprint detection method. J Forensic Ident 1997;47(5): 542–56.

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