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# TR-04-96
# Advanced Scientific Research For A New Europium Based Fluorescent Dye

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**TECHNICAL REPORT**
**March, 1996**

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NOTE: Further information about this report can be obtained by calling the CPRC information number (613) 998-6343

## EXECUTIVE SUMMARY

Dr. Della Wilkinson's work for the fiscal year 1995/1996 addressed a number of issues on behalf of the Forensic Identification community.

This particular report deals with the use of europium chelates for the visualization of fingerprints on porous and non-porous surfaces. The chelate detects the lipid component of the fingerpirnt by transferring into the mehanol that dissolves in the lipid. While the approach is feasible, todate comparisons of the current formulatin of TECTOPO have shown that the sensitivity is inferior to physical developer. Consequently, this technique is not recommended for casework.

Additionally, this report provides an update on the communication of her work to the police community.



## SOMMAIRE

Le présent rapport porte sur l'utilisation de chélates d'europium pour la révélation d'empreintes digitales sur des surfaces poreuses et non poreuses. Ces chélates détectent les constituants lipides de l'empreinte lorsqu'on plonge le support dans une solution de méthanol qui se dissout dans les lipides. Bien que le procédé soit réalisable, les comparaisons avec la formule TECTOPO actuelle ont démontré jusqu'à maintenant que sa sensibilité est inférieure à celle du révélateur physique. En conséquence, cette technique n'est pas recommandée pour les expertises.

Ce rapport fournit une mise à jour de l'information transmise à la collectivité policière par Della Wilkinson, Ph. D., concernant son travail. M$^{me}$ Wilkinson a examiné en 1995-1996 un certain nombre de questions pour la police judiciaire.

The research described in this report was conducted on a part-time basis from April 1995 to March 1996 under contract to the Canadian Police Research Centre (CPRC) and the Forensic Research and Review Section (FIRRS) of the Royal Canadian Mounted Police (RCMP). The information is presented under the two subject headings; fluorescent one-step lipid reagent; and, communication of information arising from the research to the police community.

**1) A Fluorescent One Step Lipid Reagent**

The first use of europium for the visualisation of fingerprints on paper was reported by Roland Menzel [1]. He first treated the fingerprint with ninhydrin and then with a solution containing europium ions. Ruhemann's Purple is produced when ninhydrin reacts with the amino acids present in a fingerprint. Further reaction of this compound occurs with europium, reportedly, forming a weakly fluorescent material as a result of energy transfer from the Ruhemann's Purple into the europium ion. Unfortunately the fluorescence is very weak and expensive time-resolved imaging equipment is required to obtain reasonable print detail.

The development of TEC (a fluorescent europium chelate produced by the reaction of europium trivalent cations with aryl-ß-diketones) for the visualisation of cyanoacrylate treated fingerprints [2], lead me to believe that the successful application of europium for porous exhibits was based in improving the chemistry not the imaging equipment.

In contrast to Menzel's work on paper which attempts to detect the amino acids (present in sweat), I was interested in visualising the lipids which are present in sebum. I felt that developing a fluorescent lipid reagent was important for several reasons. Firstly, amino acid reagents develop latents which tend to have very spotty, non-continuous ridge detail whereas ridges developed by lipid reagents are even and continuous. Secondly, the only available treatment for lipid prints on paper is physical developer which is non-fluorescent. A fluorescent technique would help remove unwanted background patterns. Finally, progress in this area could lead to a breakthrough in the detection of latents on human skin.

The spectral characteristics of europium chelates are ideal for visualising fingerprints. Excitation of the organic ligand occurs in the UV and europium narrow band emission (10 nm) is observed at 614 nm as a result of intramolecular energy transfer. The large Stokes shift of about 260 nm and the narrow emission band allow for much flexible filtering of unwanted backgrounds.

Two approaches to achieve fingerprint visualisation on porous surfaces by a europium chelate were identified; solvent transfer which had proven to be so successful with TEC and CA, and, surfactant carrier which is currently used for physical developer [3].

(i) Solvent Transfer

In a similar manner to the transfer of TEC into CA, the transfer of TEC into lipids by using methanol and water to

establish a two phase system, can also be achieved. The methanol partially dissolves in the lipid of the fingerprint, since TEC is insoluble in water and prefers the hydrophobic environment of the methanol inside the lipid, some TEC transfers into the lipid. Good quality prints on foil treated with TEC in a methanol water mixture can be successfully visualised without cyanoacrylate fuming. However, the TEC formulation was not successful for visualising good quality prints on paper.

At this point the chemical structure of TEC was modified to make the chelate even more insoluble in water and therefore increase its desire to migrate into the lipid. The TEC structure consists of the europium metal ion and three equivalents of the organic ligand, in this case thenoyl-trifluoroacetone. The modification was achieved by the addition of two equivalents of trioctylphosphine oxide which displaces the two coordinating water molecules to generate a large bulky hydrophobic shell surrounding the europium ion. One consequence of replacing the water molecules with synergic groups is to increase the quantum yield of the resulting chelate relative to TEC. This new formulation will be referred to as TECTOPO.

This modified formulation resulted in much brighter fingerprint fluorescence, even weak latents on foil could be seen. Such fingerprints are as brightly fluorescent as prints that have been developed with CA prior to treatment with TEC.

In all of these experiments a variety of paper surfaces were used as well as a variety of fingerprint donors. Each donor deposited several fingerprints in sequence so that both good and

poor quality latents were visualised. When tested on paper, this formulation easily developed freshly deposited fingerprints. However, as is apparent in Figure 1, there is a significant amount of chelate that has adhered to the background which reduces the contrast between print and paper. This has turned out to be a serious difficulty. It is a consequence of the insoluble nature of the chelate.

Several strategies for the improvement of the background were identified and tested. These ranged from removal of the chelate by washing to measures designed to prevent the chelate from depositing on the background. Washing either involved immersion in a detergent solution so that the detergent would solubilise the chelate adhering to the background, or, washing with a chemical that would replace the organic ligand of the fluorescent chelate to form a non-fluorescent one.

Washing by simply dipping the paper into a solution of detergent immediately following immersion in the chelate solution proved very successful for fingerprints deposited on plastics but did not greatly improve the background on porous surfaces. Using a solution containing 5% Tergitol-7 left the background more uniform but its intensity remained unchanged.

Immersion in a solution of disodium EDTA ($8 \times 10^{-4}$M) following TECTOPO treatment again showed some improvement in background fluorescence. The EDTA replaces the organic ligand that envelopes the europium ion to form a europium chelate that does not fluoresce under UV radiation. However, if the porous samples are left for to long in this solution the fingerprint fades completely. Citric

acid solutions were also tested but they appeared to be too aggressive and destroyed the chelate in the fingerprint.

Several europium chelates were tested involving different synergic groups to see if changing the solubility or the reactivity of the chelate would result in less background contamination.

The background fluorescence observed in papers treated with TECTOPO could be due to a direct binding of the chelate with the phenolic compounds present on the surface of the paper. Attempts were made to block the available coordination sites on TEC with a phenolic chelate rather than TOPO which does not bind strongly enough. A europium-ß-diketone complex, involving a synergic agent with a phenolic functional group, was prepared using Chrysin. However, experiments using this complex to detect lipid fingerprints on paper, failed to visualize the prints, and, still showed significant background fluorescence.

Experiments were conducted to determine if using $Eu(TTA)_3Phen$ over $Eu(TTA)_32TOPO$ lead to greater contrast, since the former chelate exhibits improved solubility in solution which may reduce background fluorescence. However, the latter shows increased solubility in lipids which may enhance brightness of fingerprint fluorescence. TECTOPO proved to be the best formulation.

(ii) Surfactant carrier

As mentioned earlier physical developer utilises surfactants in it's formulation to prevent background contamination by excess silver deposition. Physical Developer is a silver based aqueous reagent for paper which reacts with components of sebaceous sweat

to form a grey silver deposit. The surfactants stabilize the metallic silver in the aqueous solution. Experiments were conducted to see if surfactants could be used in a similar manner to prevent TECTOPO deposition on the background surface. Two aqueous solutions were tested; a combination of n-dodecylamine acetate and synperonic-N, and, a solution of Triton X-100. In addition, introducing a surfactant into the methanol solution was also considered.

Neither of the aqueous surfactant/ dye solutions showed any transfer of TECTOPO into the fingerprint whereas incorporating the detergent (Tergitol-7) into the dye solution proved to be the most satisfactory method for reducing the formation of background fluorescence. The sample shown in Figure 2a has been treated with a TECTOPO formulation containing Tergitol-7 and then washed in water. The sample in Figure 2b has been treated with TECTOPO and left unwashed. The background of the former has been removed completely and the contrast is excellent.

(iii) Comparison of TECTOPO with Physical Developer

Currently, the formulation of this lipid reagent requires dissolving 23 mg of europium chloride hexahydrate in 300 ml of distilled water. 2 ml of Tergitol-7 are then added to the aqueous solution. In a separate vessel 42 mg of thenoyltrifluoroacetone and 50 mg of trioctylphosphine oxide are dissolved in 700 ml of methanol. These solutions are mixed using a magnetic stirrer for 30 minutes and the solution is ready for use. This was the formulation used in the following comparison study with physical

developer.

Fingerprints were deposited by a variety of donors on a variety of surfaces in sequence. Each donor deposited a cluster of three fingerprints at a time with the middle fingerprint being positioned on a central line. After deposition the samples were cut in half. The pre-mixed physical developer solution was used. Samples were initially soaked for five minutes in maleic acid and then agitated in physical developer for twenty minutes before washing for fifteen minutes in three separate water baths. The TECTOPO samples were dipped for five seconds in the solution and immediately immersed in a water wash for another five seconds.

Samples were photographed individually using the appropriate light source. The TECTOPO samples were recorded on Technical Pan film using a hand held UV lamp. The physical developer samples were also photographed with technical pan film using halogen lights. Exposure times for the TECTOPO samples varied dramatically. For fresh samples the exposure time averaged approximately 15 seconds whereas for the aged samples times of 3 minutes were recorded.

The results were extremely mixed, for fresh samples, frequently the TECTOPO treated half showed more ridge detail. For four week old samples, the physical developer treated section appeared to be superior. In yet other samples the two techniques appeared equivalent. These conflicting results are fairly typical of this type of study and in the end it is the overall picture that tells the story. For this comparison physical developer proved to be the most consistent and sensitive technique.

*N.B. As a result of this comparison study it became clear that the physical developer solution was not functioning properly. Although the pre-mixed physical developer was in an unopened container the solution was over one year old. A comparison between recently purchased physical developer and the unopened aged solution showed significantly improved fingerprint development for the new solution. Obviously it is essential to date new physical developer to ensure that out-of-date solutions are not used on casework (see FIRRS Bulletin #039).*

(iv) Sequential use of TECTOPO with existing techniques

A surprising limitation of TECTOPO is the failure to develop latents following treatment of the sample with DFO. Figure 3 shows a sample that has been divided into two halves. One half has first been treated with DFO (including the twenty minutes of heating at 100°C) and then visualised with TECTOPO. The other half, which shows ridge detail, was just heated for 20 minutes at 100°C and then visualised with TECTOPO. Since ridge detail is visible on the right-hand-side after heating it would appear that the freon is interfering in some way with the TECTOPO treatment.

To confirm that this was the case a sample was prepared and half was treated with DFO without heating and then visualised with TECTOPO. The other half which showed identifiable ridge detail was simply treated with TECTOPO. That no ridge detail is visible on the side treated with DFO indicates that the solvent, freon, reduces the amount of lipid present in the fingerprint to an extent that means it is difficult to detect with TECTOPO. This

observations also confirms that TECTOPO is not as sensitive as physical developer at detecting lipids because physical developer still develops prints after DFO treatment.

(v) Alternative Applications of TECTOPO

The results are not all negative. The use of TECTOPO to visualize excellent quality prints on plastics without requiring cyanoacrylate fuming are encouraging. Plastic samples immersed in the TECTOPO solution for five seconds, washed in water and then hung to dry showed excellent quality prints as seen in Figure 4, which was photographed under UV illumination using the 614 nm narrow band filter.

Field tests on cigarette cartons both plastic and foil have indicated that TECTOPO is not as sensitive as CA/ dye stain. However, further tests are warranted on materials that do not accept CA well such as vinyls, cement, leather, etc.

(vi) Conclusion

In conclusion this research has successfully demonstrated the use of europium chelates for the visualisation of fingerprints on porous and non-porous surfaces. The chelate detects the lipid component of the fingerprint by transferring into the methanol that dissolves in the lipid. The approach is feasible, however, to date comparisons of the current formulation of TECTOPO have shown that the sensitivity is inferior to physical developer. As a result this technique is not recommended for casework.

(vii) References

[1] E. R. Menzel and K. E. Mitchell, "Intramolecular Energy Transfer in the Europium-Ruhemann's Purple Complex: Application to Latent Fingerprint Detection", *J. Forensic Sci.*, 35(1), **1990**, pp 35-45.

[2] Wilkinson, D. A., Watkin, J. E., "Europium Aryl-ß-ketone Complexes as Fluorescent Dyes for the Detection of Cyanoacrylate Developed Fingerprints on Human Skin", *Forensic Science International*, 60, **1993**, pp 67-79.

[3] Kent, T, Hardwick, S. A., *Manual of Fingerprint Development Techniques, Home Office*, Home Office Publication, UK, **1986**.

**2) Communication of information arising from research to police**

Two presentations entitled "One Step Fluorescent Detection Method for Lipid Fingerprints" and "Latent Fingerprint Detection on Human Skin using Iodine and α-naphthoflavone" were made to delegates at the International Fingerprint Symposium hosted by the Israeli State Police, in June 1995.

Several FIRRS Bulletins have been published and disseminated to both RCMP and non-RCMP Identification Sections throughout Canada. The bulletins provided information on the following subjects; FIRRS Bulletin 035, warned of the limited shelf-life of Physical Developer; FIRRS Bulletin 036, described the practical aspects of recovering fingerprints from human skin using iodine and α-naphthoflavone; FIRRS Bulletin 038, provided information with regard to improved results from cyanoacrylate treatment of cocaine-contaminated exhibits; and, FIRRS Bulletin 039, presented a more effective formulation for the cyanoacrylate dye, Brilliant Yellow.

Lectures were given for two Fluorescent Techniques Courses (intensive three day course) in January and February 1996. A morning seminar was also provided for the Canadian Police College to candidates on the senior forensic identification course, in August 1995.

Figures 1-4: TR-04-96-(2)