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Spectroscopy of Rare Earth-Ruhemann's Purple Complexes

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ABSTRACT: The complexes Ruhemann's Purples form with europium or terbium salts are suitable for fingerprint development on strongly fluorescent surfaces because they show enhancement of the lanthanide luminescence via intramolecular energy transfer and because the lifetime of the luminescence is much longer than that of the usual background fluorescence. They are therefore suitable for time-resolved luminescence imaging. The complexes are useful for fingerprint staining on smooth surfaces, since they are readily prepared in solution, as well as for fingerprint detection on porous surfaces, such as paper, where the procedure is similar to the by now routine ninhydrin/zinc chloride treatment, except that the nitrate or acetate are used in many instances, instead of the chloride, in the rare earth case, depending on paper type.

KEYWORDS: criminalistics, fingerprints, lasers, time-resolved imaging, europium, terbium, ninhydrin, ninhydrin analogs, Ruhemann's Purple

The detection of latent fingerprints by laser, or, more generally, fluorescence detection of fingerprints (since filtered arc lamps are widely used these days as well, albeit with lesser detectability) provides great sensitivity. Fingerprint treatments such as rhodamine 6G staining and ninhydrin/zinc chloride are now routine. What has kept laser fingerprint detection from becoming a truly universal technique is that many surfaces fluoresce very intensely under the laser illumination, overwhelming the fingerprint fluorescence. Such surfaces were until recently not amenable to the current routine procedures such as those mentioned above. To mitigate this deficiency we have been exploring time-resolved luminescence imaging [1-4]. The basic principle of the technique is as follows. The beam of an argon-ion laser (the still most widely used laser type for fingerprint work) is chopped by a mechanical light chopper or electro-optic modulator so that laser pulses with sharp cut-off illuminate the article under scrutiny. The article is viewed with an imaging device synchronized to the laser pulses such that it turns on shortly after laser cut-off and turns off shortly before onset of the next laser pulse. References 1 and 2 provide the details of our first prototype instrument. The imaging device is operated in this way because the offending background fluorescences have short lifetimes (of nanosecond order), that is, the background fluorescence decays very quickly after laser pulse cut-off. If fingerprint treatments can be developed such that much longer luminescence lifetimes result, then the imaging device will only detect this long-lived luminescence and will suppress the

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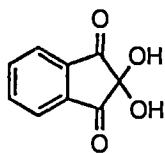
background. The crux of the practical implementation of time-resolved imaging is the formulation and optimization of such treatments, and the development of a more user-friendly second-generation imaging system. Our focus in this article is on the first issue. The second one is dealt with in a separate article [5].

In our earlier work, which was somewhat preliminary in scope, we explored two general chemical strategies. One involved the use of transition metal complexes that yield charge transfer phosphorescences with microsecond-order lifetimes. These complexes would be used as staining dyes for smooth surfaces much like rhodamine 6G, or be incorporated into dusting powders [3,4]. Alternatively, for porous surfaces such as paper, ninhydrin treatment followed by treatment with rare earth salts, involving the lanthanides Eu^{3+} or Tb^{3+} , an analog to the ninhydrin/zinc chloride treatment, was investigated [1,2]. The formed rare earth-Ruhemann's Purple complexes exhibit luminescence lifetimes of millisecond order. Some of the above transition metal complexes are amenable to excitation with blue-green light (the customary Ar-laser output), others respond to near-ultraviolet excitation (also obtainable with Ar-lasers). Such wavelength switching, although not difficult, is clumsy nonetheless. Worse, for time-resolved imaging of luminescence of microsecond order lifetime, the laser chopping requires devices such as electro-optic modulators. These are not only expensive, but demand delicate optical alignment and careful gain and bias adjustment. For luminescence of millisecond-order lifetimes the laser chopping is much easier since a cheap and easily usable mechanical light chopper (rotating wheel with holes in it) suffices.

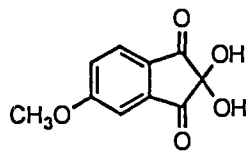
Rare earth-Ruhemann's Purple complexes, where Ruhemann's Purple (RP) is the reaction product of ninhydrin with an amino acid, are readily prepared in solution. If such solutions were effective for fingerprint staining, akin to rhodamine 6G, then one should be able to use this chemical strategy for fingerprints on smooth and porous surfaces alike. This could reduce instrumentation cost and also make for ease of instrumentation operation. With these advantageous feature in mind, we have undertaken a study of a range of rare earth-Ruhemann's Purple-type complexes. To place the aims of this study in perspective, we digress briefly to a review of the ninhydrin/zinc chloride treatment. There, the questions immediately arise whether zinc(2+) is the optimal ion and whether ninhydrin analogs could produce superior results. Zinc is indeed the best ion for the desired complexation [6]. Ninhydrin analogs including those of Fig. 1 (and others) have been investigated by us and other groups. 5-methoxyninhydrin is far superior to ninhydrin from the fluorescence perspective [7]. The sulfur-containing ninhydrin analogs are better still [8-10]. A similar optimization study is called for in terms of rare earth complexes, and we report on it here.

Lanthanide-Ruhemann's Purple Complexes

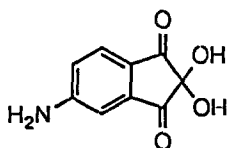
A number of lanthanide ions (normally in 3+ form) exhibit luminescence with reasonable high quantum efficiencies because the 4f valence electrons are shielded. Intensities are generally low, however, because molar extinction coefficients are very low (4f-4f transitions are parity-forbidden). Indeed, solutions of most lanthanide salts are colorless, i.e., do not absorb light appreciably, and without absorption photoluminescence cannot be produced. However, it has been known for quite some time [11] that the lanthanide luminescence can be enhanced via intramolecular energy transfer when the ion is complexed with a suitable organic ligand (good absorber). The lanthanides readily form complexes with Ruhemann's Purple. Since Eu^{3+} and Tb^{3+} are the most notorious luminescers among the lanthanides, we expected that they would be the best candidates for our purposes. Nonetheless, we also investigated Ce^{3+} , Pr^{3+} , Nd^{3+} , Sm^{3+} , Gd^{3+} , Dy^{3+} , Ho^{3+} , and Er^{3+} . Not surprisingly, none of these were found to be even remotely competitive in luminescence with Eu^{3+} and Tb^{3+} in terms of enhanced emission in Ruhemann's Purple complexes. We note here that strongly enhanced lanthanide emission is essential. Lan-



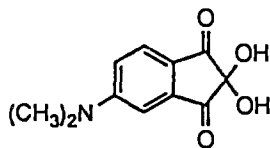
ninhydrin



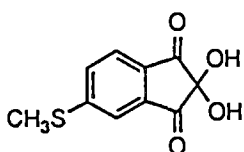
5-methoxyninhydrin



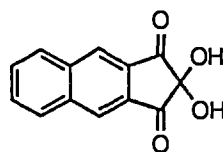
5-aminoninhydrin



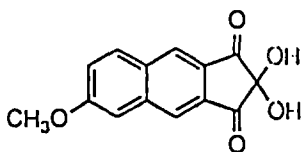
5-dimethylaminoninhydrin



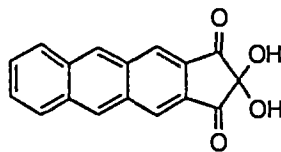
5-methylthioninhydrin



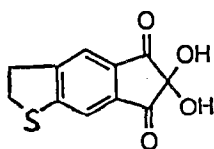
benzo[f]ninhydrin



7-methoxybenzo[f]ninhydrin



naphtho[f]ninhydrin



thieno(f)ninhydrin

FIG. 1—Ninhydrin and ninhydrin analog molecules.

thanide salts in solution are, after all, sprayed onto articles that have first been treated with ninhydrin, much like zinc chloride, if one deals with porous surfaces. Unreacted lanthanide ions thus cover the surface, and they may luminesce appreciably. Indeed Eu^{3+} and Tb^{3+} do just that. Tb^{3+} luminesces in the green while Eu^{3+} emits in the red. The spectroscopic details are given in Refs 2 and 1, respectively.

We found that the ratio of enhanced to unenhanced luminescence was higher for Eu^{3+} than for Tb^{3+} , and we therefore focus our attention on Eu^{3+} in the following sections of this article, where we examine complexes which involve the ninhydrin analogs of Fig. 1. We point out, though, that what holds for Eu^{3+} holds for Tb^{3+} . We report explicitly those ninhydrin analogs that yielded the best results, namely 5-methoxyninhydrin, benzo[f]ninhydrin, 5-methylthioninhydrin and thieno[f]ninhydrin. For comparison we also report on ninhydrin itself and 7-methoxybenzo[f]ninhydrin, which did not produce good results. The remaining ninhydrin analogs (see Fig. 1) which we do not report on explicitly, yielded intermediate results.

Optical Spectroscopic Properties of Eu-RP Complexes

Excitation Spectra

The sources of excitation in our laboratory are: continuous wave (CW) Argon-ion lasers (Coherent INNOVA 90; Spectra Physics 171; Spectra physics 164), two dye lasers (Coherent CR-599-OEM; Spectra physics 375-50), a UV lamp (UVG-54 mineral light) emitting between 254 nm, and 400 nm, and, a Xenon arc lamp (Ealing 1288) suitable for UV-Visible spectroscopy. To find the best possible wavelength to excite luminescence of our samples, excitation spectra are helpful. The Eu-RP complexes were spotted on TLC (thin layer chromatography) plates (Silica gel on glass, without UV indicator), and excitation spectra were recorded. The best possible wavelength to excite luminescence of the samples, in the range 300–550 nm, are reported in Table 1.

Emission Lifetime

Eu^{3+} emits at 579, 592, and 615 nm, the latter emission being the most intense one. Since in fingerprint work one deals with solid surfaces, $EuCl_3 \cdot 6H_2O$ in a 1×10^{-3} M methanol solution was spotted on a TLC plate and the fluorescence lifetime at 615 nm was measured to be about 0.4 ns. Solutions of 2×10^{-3} M of the different RPs were mixed with solutions of 1×10^{-3} M of $EuCl_3 \cdot 6H_2O$ (as we show later, the stoichiometry of Eu:RP is 1:2). The complexes were spotted on TLC plates, and the fluorescence lifetimes were measured as before at 615 nm. The lifetimes were essentially unchanged, as expected in line with the properties of the rare earths. Lifetimes of Eu-RP complexes were the same as for $EuCl_3 \cdot 6H_2O$, in solution. It is important to mention this because it proves that intramolecular energy transfer actually occurs. A strongly enhanced Eu-

TABLE 1—Excitations in the range 300–550 nm.

Compound	Excitation λ (nm)
Eu-RP(ninhydrin)	330–360
Eu-RP(5-methoxynin.)	340–365
Eu-RP(benzof[nin.])	340–360
Eu-RP(5-methylthionin.)	320–360
Eu-RP(thieno[f]nin.)	320–340
EU-RP(7-methoxybenzo[f]nin.)	340–360

TABLE 2—Absorption peaks of RPs in the 200–800 nm range.

Compound	Peaks (nm)
RP(ninhydrin)	210–286; 408; 580
RP(5-methoxynin.)	214–244; 278; 408; 584
RP(benzo[f]nin.)	214–236; 286; 362; 436; 634
RP(7-methoxybenzo[f]nin.)	210–252; 438; 636; 658
RP(5-methylthionin.)	212–218; 258; 318; 418; 594
RP(thieno[f]nin.)	210–238; 270; 420; 610

TABLE 3—Absorption peaks of the complexes in the range 200–800 nm.

Compound	Absorption peaks (nm)
Eu-RP(ninhydrin)	208–228; 364; 400; 506
Eu-RP(5-methoxynin.)	232; 364; 394; 416; 516
Eu-RP(benzo[f]nin.)	210; 234; 270; 314; 364; 446; 548
Eu-RP(7-methoxybenzo[f]nin.)	208; 256; 278; 330; 414; 562
Eu-RP(5-methylthionin.)	210–246; 314; 398; 534
Eu-RP(thieno[f]nin.)	208; 242; 272; 314; 422; 534

RP emission resulting from an increase of Eu^{3+} emission quantum yield instead of energy transfer would radically alter the lifetime.

Absorption Spectra

Ninhydrin and ninhydrin analogs were dissolved in methanol to make solutions of $6 \times 10^{-5} M$. They were reacted with an excess of the amino acid glycine to make RP solutions of $3 \times 10^{-5} M$. The glycine reaction with ninhydrin and analogs was at room temperature and it is very slow largely because glycine is not readily soluble in methanol. The samples were let stand about half a day with occasionally stirring to react completely and reach the maximum coloration. The absorption spectra of the Ruhemann's Purples were measured at room temperature using a HP 8451A Diode Array Spectrophotometer. Table 2 lists the peaks of absorptions of each compound.

Ninhydrin and ninhydrin analogs themselves have a broad band of peaks in the ultra-violet (UV) only. In addition to broad absorption in the UV, there are at least two more peaks in the visible in the RPs. The absorbing states in the near UV are of most interest for our purposes because they can transfer the energy absorbed to the rare earth ions and enhance their luminescence.

Solutions of $3 \times 10^{-5} M$ of RPs were titrated with solutions of $3 \cdot 10^{-5} M$ of $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$. The reactions take place instantly and the RPs (purple, blue, green or blue-green) turn red or red-orange depending on which compounds are mixed. All the reactions were at room temperature. The absorption spectra were taken in the range 200–800 nm, and the absorption peaks are reported in Table 3.

Emission Spectra of Eu(III)-RP Complexes

The ligand environment constitutes only a small perturbation of the atomic energy levels of the rare earth ions. Therefore, the emission spectra of the complexes show the same features as the rare earths themselves. To show the enhancement of the fluorescence, we plot in Fig. 2 an emission spectrum of a solution ($1 \times 10^{-3} M$) of $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ compared to that of the same solution of Eu mixed with an equal volume of a 2×10^{-3}

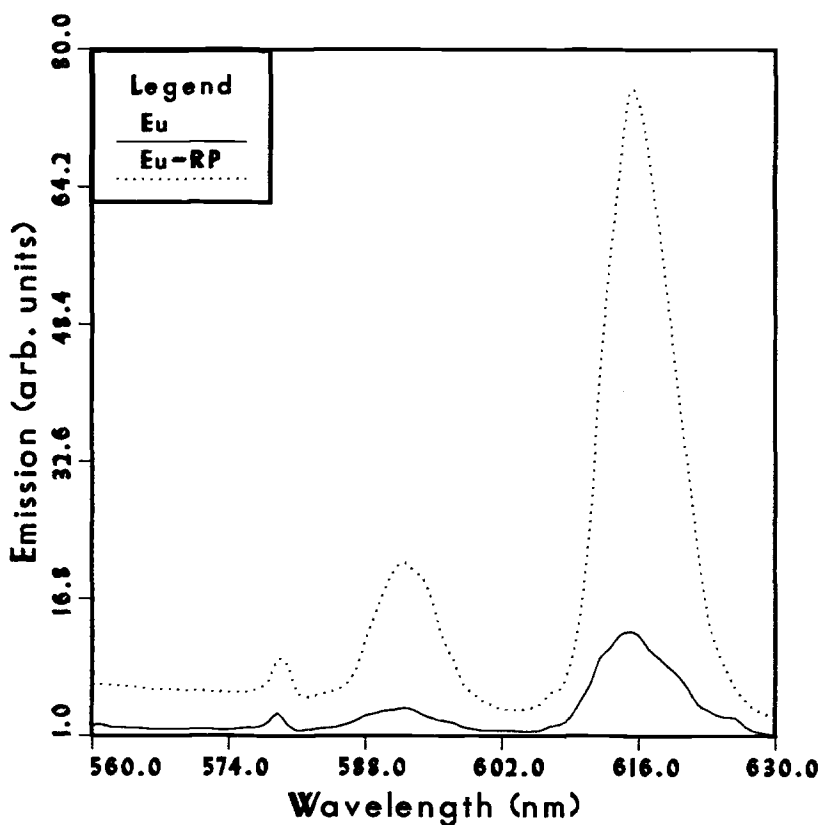


FIG. 2—Emission spectra of *Eu* and *Eu-RP*.

M solution (because of the earlier-mentioned stoichiometry) of RP(5-methoxyninhydrin), both spotted on a TLC plate. The concentration of Eu^{3+} in the TLC spot corresponding to the complex is half that in the TLC spot of $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$. The peak at 615 nm is seven times higher in the *Eu-RP* complex than in *Eu* by itself. Figure 3 shows the enhancement in pictorial form. (Photographs of solution as well as TLC plate spots). To obtain the TLC photograph, RP and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ were spotted on the plate such that the two spots overlapped partially. The enhanced Eu^{3+} emission is seen in the overlap region.

Job's Method Analysis of the *Eu*(III)-RP Complexes

We had originally assumed that the structure of the *Eu*-RP complexes would resemble that of the *Zn*-RP complex which contains one Zn^{2+} ion and one RP molecule [12]. RP acts as a tridentate ligand in *Zn*-RP. One chloride anion and two waters of hydration complete the (distorted) octahedral coordination of Zn^{2+} . The trivalent lanthanides are well-known to preferentially form compounds of octahedral coordination as well. Indeed the presence of 6 waters of hydration in $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ is an indicator of this. Several observations, however, made us suspect that the stoichiometry of the *Eu*-RP complexes might not be 1:1 as in *Zn*-RP. When RP and ZnCl_2 are spotted onto a TLC plate such that the two spots overlap partially, akin to Fig. 3(b), the *Zn*-RP complex forms in the overlap region, as is readily seen by the characteristic yellow fluorescence which develops nearly instantaneously (under blue-green excitation). We note here that this fluorescence

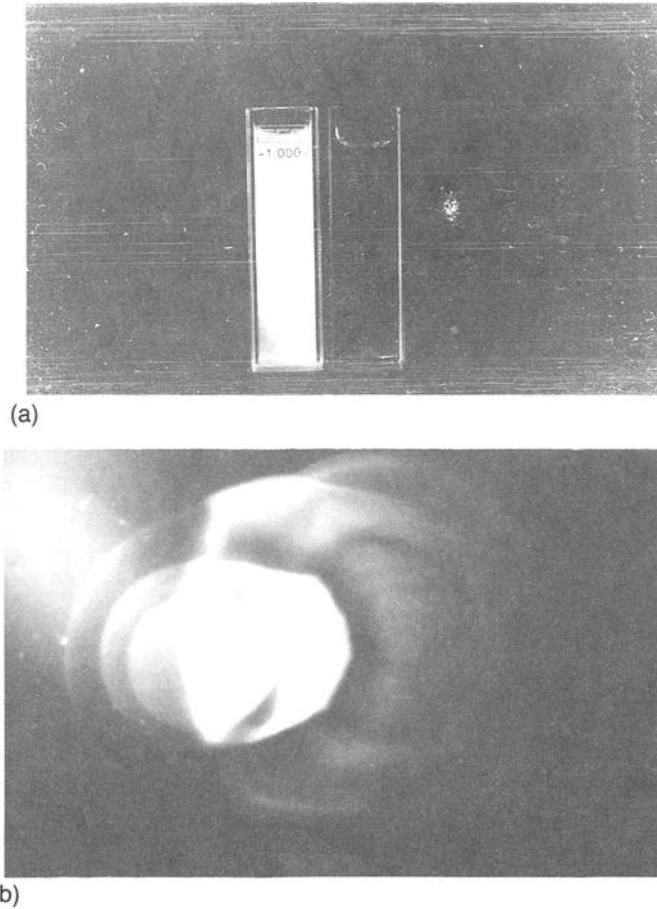


FIG. 3—Emission of Eu, right, compared to Eu-RP(5-methoxynin.), left, in solution (a) and on TLC plate (b). See text for discussion.

is due to the RP ligand, not Zn^{2+} . The reason the Zn-RP complex fluoresces, whereas RP does not, is discussed in *Ref 6*. When the same thing is done with RP and the europium salt and the TLC plate is illuminated by near-UV light no enhanced europium luminescence is observed in the overlap region at first. It takes one to several hours for the enhanced luminescence in the overlap region to reach full strength. If Eu-RP is prepared in (methanol) solution and scrapings of silica gel from a TLC plate are added to the solution, the solution color (orange-red) changes to the characteristic purplish blue of RP, indicating decomposition of the complex. Clearly, the europium complex is rather less robust than the zinc complex. After the methanol solvent is left to evaporate, the europium complex re-forms over time. When the Eu-RP complex is prepared in methanol and water is added, the complex again decomposes as indicated by the color change. This also happens for the zinc complex, but more slowly. Given these rather substantial differences in behavior of the zinc and europium complexes, we investigated the stoichiometry of Eu-RP complexes via a titration technique reported by Job [13].

Equimolar solutions of RP and $EuCl_3 \cdot 6H_2O$ ($1 \times 10^{-4} M$) in methanol were prepared and mixed in varying proportions. The absorbance of each solution was measured at the peak absorption in the visible of the Eu-RP complex solution (504 nm) as shown in Table

TABLE 4—Maximum characteristic absorption for *Eu-RP(nin.)* at 504 nm, and *Eu-RP(5-methoxynin.)* at 516 nm.

X(mL)	Eu-RP(nin.)	Eu-RP(5-methoxynin.)
0.0	0.080	0.061
3.5	0.455	0.381
7.0	0.802	0.584
10.0	0.668	0.477
13.5	0.452	0.348
17.0	0.200	0.160
20.0	0.031	0.031

4. Figure 4 is the plot of the absorbance against the volume $X(\text{mL})$ of $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ added to $(20 - X)\text{mL}$ of RP solution. The curve in Fig. 4 indicates that the complex formed in solution between RP and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ has a ratio of $2\text{RP}:1\text{Eu}$. Similar work has been done by Lennard et al. [12] for RP - (*Zn*, *Cd*, and *Hg*) to determine that the RP - (*Zn*, *Cd*, and *Hg*) ratio was 1:1. When a similar titration study was done by us on Zn-RP, the maximum characteristic absorption (at 490 nm) occurred for $x = 10$, indicative of 1:1 stoichiometry, in agreement with the results in Ref 12.

Given the proclivity of Eu^{3+} to form compounds with octahedral coordination and the 1:2 Eu:RP stoichiometry, and given that RP acts as a tridentate ligand in ZnRP, we propose that one RP molecule bonds to positions 1, 2, 3 in Fig. 5 and another to positions 1', 2', 3', with Eu^{3+} at the center of the octahedron. Since Eu^{3+} is a trivalent cation while RP is a monovalent anion, the proposed structure of the europium complex thus is $[\text{EuRP}_2]^+ \text{X}^-$, where X^- denotes the anion in the $\text{EuX}_3 \cdot 6\text{H}_2\text{O}$ salt used to form the complex. The soluble and readily available europium salts are chloride, nitrate, and

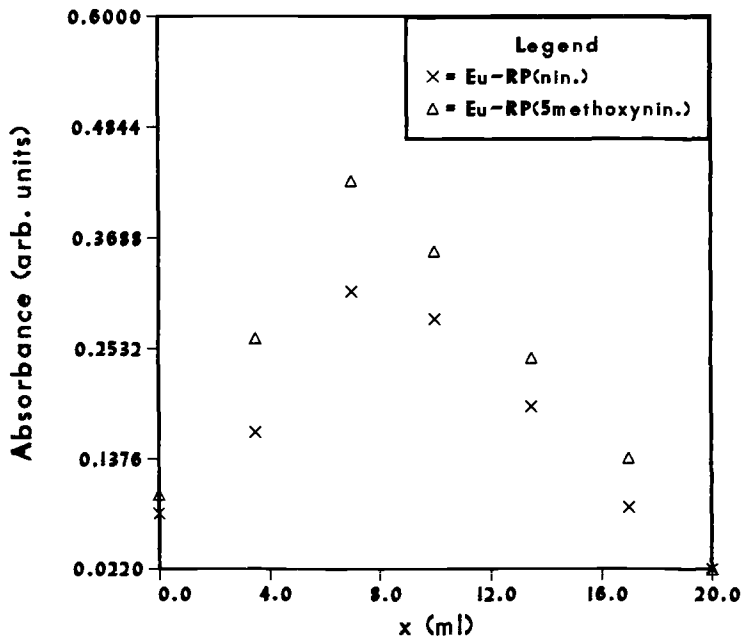


FIG. 4—Results of the titration method.

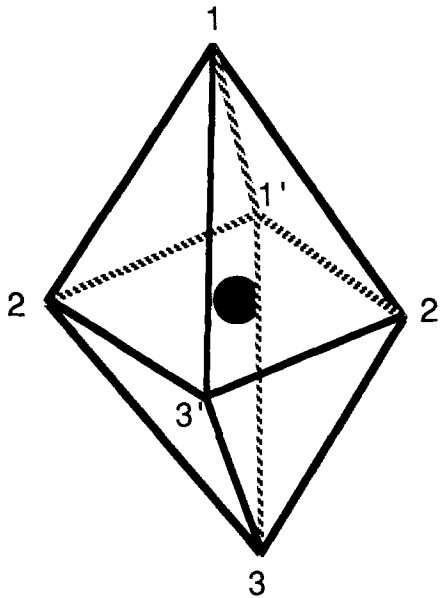


FIG. 5—Proposed structure for Eu-RP Complex.

acetate (all hexahydrates). To see if the anion has any effect on the energy transfer, the Eu-RP complexes were prepared in the solution using the three salts. The nitrate yielded the strongest enhanced Eu^{3+} luminescence.

Energy Transfer from the Ligand Compounds to Eu(III)

Optically excited atoms and molecules are capable of transferring their electronic excitation energy to other atoms or molecules. In some cases, this is accompanied by the transfer of an electron. However, in other cases, this energy transfer takes place without such charge transfer. The phenomenon of intramolecular energy transfer in rare earth chelate was first reported by Weissman [11], who studied chelates of Eu, Sm, and Tb with various ligands. He concluded that the excitation light is absorbed by the organic part of the lanthanide complex and emitted as the line spectrum of the lanthanide ion. A quantum mechanical description of intermolecular energy transfer between neighboring molecules via a dipole-dipole mechanism was given by Förster [14]. The mechanism involves the absorption spectrum of the energy receiver, the emission spectrum of the energy donor, the excitation time of the molecules, and the intermolecular distance.

A mechanism of energy transfer in rare earth chelates was published by Crosby et al. [15–17]. It involves the participation of the first excited singlet (S_1) and triplet (T_1) states of the ligand. After excitation of the chelate to the first excited singlet state ($S_0 \rightarrow S_1$), the molecule may be deactivated to S_0 (ground state), resulting in molecular fluorescence, or the molecule may undergo non-radiative intersystem crossing to the triplet state ($S_1 \rightsquigarrow T_1$). From this state it can decay radiatively ($T_1 \rightarrow S_0$), giving rise to molecular phosphorescence, or undergo a non radiative transition to a low-lying rare earth ion state ($T_1 \rightsquigarrow RE$). After this indirect excitation by energy transfer, the ion may undergo a radiative transition resulting in characteristic line emission if a resonance level of the ion is excited, or it may be deactivated via non radiative processes. This energy transfer scheme was based on the observation that sensitized lanthanide emission occurs for chelates

whose triplet states lie above the emitting lanthanide level, but not for chelates whose triplet states are below this level. Kleinerman [18,19], pointed out that Crosby et al.'s model is not conclusively supported by the evidence, and that in some chelate systems the energy transfer process seems to proceed through an inductive resonance mechanism connecting a ligand-excited singlet level and a rare-earth-ion level higher than the emissive one.

Indeed there are a variety of mechanism by which energy transfer can take place, even from higher excited states, including charge transfer, dipole-quadrupole interactions, and exchange mechanisms. A dipole-dipole mechanism, such as the Förster process, is not likely to be operative, being parity-forbidden. A salient conclusion by Kleinerman was that ligand-rare earth energy transfer is efficient provided that the ligand lowest excited singlet level lies above the emitting rare earth level. Neither this scheme nor that of Crosby hold for our complexes.

Tb-RP(benzo[f]ninyhydrin) shows enhanced Tb luminescence under near-ultraviolet excitation, much like Eu-RP. In this complex the absorption band corresponding to the $S_0 \rightarrow S_1$ transition lies at about 550 nm, corresponding to an energy of about $18\,200\text{ cm}^{-1}$. The 7F_6 (ground state)- 5D_4 (emitting state) energy gap for Tb^{3+} is $20\,400\text{ cm}^{-1}$. The ligand first excited singlet state (S_1) and therefore also the lowest triplet state (T_1) both lie below the emitting 5D_4 level, and yet sensitized emission takes place. Clearly, a higher excited ligand state is involved in the energy transfer process. Indeed, our results indicate that in the rare earth-RP complexes the positions of S_1 and T_1 are of no relevance to speak of. For instance, Tb-RP(ninyhydrin), which shows sensitized Tb luminescence under near-ultraviolet excitation, has the $S_0 \rightarrow S_1$ absorption band at about 510 nm. The absorption band is broad, having considerable overlap with the 7F_6 - 5D_4 transition. If a resonance-type mechanism involving S_1 were operative, then we would expect to observe sensitized Tb luminescence under excitation by 488 nm Ar-laser light. This was not found. None of the Eu-RP or Tb-RP complexes showed any enhanced emission under blue-green excitation, which rules out $S_1 \rightsquigarrow T_1 \rightsquigarrow$ rare earth energy transfer. If $S_1 \rightsquigarrow$ rare earth energy transfer via dipole-dipole or dipole-quadrupole coupling were operative, then enhanced emission of Eu-RP(7-methoxybenzo[f]ninyhydrin or benzo[f]ninyhydrin), where the overlap between the Stokes-shifted S_1 level and the emissive 5D_1 level of Eu^{3+} (at 579 nm) is good, should be obtained under dye laser excitation in the 550-580 nm range. Again, no enhanced emission of any Eu-RP complex was found under such excitation. In the Tb, Eu-RP complexes the energy transfer could proceed by $S_n \rightsquigarrow RE$, or $T_n \rightsquigarrow RE$, where S_n and T_n denote a higher excited ligand singlet or triplet state. Cascading from the higher rare earth states could then take place down to the emitting level.

To investigate whether T_n is involved in the energy transfer, we conducted a dual excitation experiment. The first excitation source is chosen to produce T_1 via $S_1 \rightsquigarrow T_1$ intersystem crossing. In our ligands, intersystem crossing is expected to be efficient since no appreciable ligand fluorescence is observed, unlike in the corresponding zinc complexes. The second excitation source is chosen to produce T_n via $T_1 - T_n$ triplet-triplet absorption. The first excitation was blue-green Ar-laser excitation, with high power. The second excitation utilized either an Ar-laser operating in the blue-green or a dye laser operating from green (about 560 nm) to red (about 620 nm). Samples were Eu-RPs spotted on TLC plates. Although we do not know the location of the T_1 level in these samples, we can safely assume that it must be somewhere between 600 and 1000 nm. We therefore have ample photon energies via the second excitation to reach T_n (at about 350 nm) from T_1 , or a higher triplet level from which T_n is reached via internal conversion. No europium emission was observed in any of our samples, suggesting that T_n is not involved in the sensitized europium emission process.

The mechanism of energy transfer in the RP-Eu complexes (not understood yet in detail), is quite different than the proposed energy transfer mechanism in the previous europium chelates studied in the literature. Our experiments suggest that it proceeds via

an upper excited state, most likely a singlet, of the chelate, most probably via a dipole-multipole interaction. A complete understanding of this mechanism will help solve the remaining unresolved problems in the use of these complexes in the fingerprint detection and, perhaps, in other utilizations.

Comparison of the Different Eu-RP Complexes

Solutions of about $2 \times 10^{-3} M$ of the different $EuCl_3 \cdot 6H_2O$ -RP complexes were spotted on TLC plates. Emission spectra were taken under Xenon lamp illumination through a filter monochromator. The excitation was around 370 nm with a bandpass of about 60 nm. Under the same experimental conditions the emission intensities of the peak at 615 nm are shown in Table 5.

The emission intensities of the different complexes show that the best combinations on TLC plates are those involving 5-methoxyninhydrin, and 5-methylthioninhydrin.

Applications

Fingerprint Development

Fingerprints deposited on several samples of porous surfaces (paper, card board) were then treated with ninhydrin, 5-methoxyninhydrin and benzo[f]ninhydrin. Some of the samples were left at ambient humidity and temperature, and some of the samples were incubated at about 50°C and 60% relative humidity. The samples left at ambient conditions were slower to develop. The samples were then sprayed with solutions of $EuCl_3 \cdot 6H_2O$. Under UV light, the red fluorescence from the fingerprint was generally comparable to that of the rest of the surface with only slight enhancement of luminescence. The usage of the time-resolved luminescence imaging system to picture the fingerprints was not often possible, because something quenched the energy transfer from the RP to Eu or possibly enhanced the emission of unreacted Eu (paper absorbs strongly in the near-UV and fluoresces as well as a result). $EuCl_3 \cdot 6H_2O$ was spotted on a TLC plate and onto paper. No substantial intensity differences were observed, indicating that enhancement of unreacted Eu emission is not the case. At first, we suspected that calcium or sodium salts in the fingerprint residue might quench the enhanced Eu emission by preferentially complexing with the RPs. To check on this eventuality, RP was prepared as before, and we added to it an excess of sodium chloride or calcium chloride and then spotted on TLC plates. Europium solution was spotted to partly overlap with the RP spot. Under UV light the overlap region fluoresces as without calcium or sodium salts. We conclude that neither sodium nor calcium are preventing the energy transfer. To check whether other constituents of fingerprint residue might be quenching the energy transfer, fingerprint residue was taken from fingers using cotton swabs wetted with methanol. Ninhydrin was added to form the RP. The RP from the fingerprint material and europium were

TABLE 5—Emission intensity of the 615 nm peak.

Compound	Emission (arb. units.)
Eu-RP(ninhydrin)	2.8
Eu-RP(5-methoxynin.)	7.3
Eu-RP(benzo[f]nin.)	7.2
Eu-RP(5-methylthionin.)	8.6
Eu-RP(thieno[f]nin.)	6.7
Eu-RP(7-methoxybenzo[f]nin.)	3.1

spotted as before. The overlapping region still showed energy transfer. We concluded that either the paper itself presents a problem, or that the enhancement of the europium emission, using the chloride salt, is simply insufficient in many instances. Since in solution the nitrate salt yielded the strongest enhancement in europium emission, we switched to this salt, and also the acetate, after 5-methoxyninhydrin treatment of fingerprints on various paper types. Quite acceptable fingerprint emission was obtained in many instances where the chloride was not effective. Figure 6 shows a photograph of a fingerprint on paper developed by 5-methoxyninhydrin/europium nitrate under near-UV excitation. The salient point here is that one not only has to optimize the ninhydrin analog and rare earth ion, but also the anion of the utilized rare earth salt. We expect that the sulfur-containing ninhydrin analogs may perform better still, but unfortunately we did not have on hand enough quantities of these reagents to do fingerprint work. They are as yet not commercially available. We are currently initiating a more comprehensive optimization study involving investigation of effects of alkalinity or acidity of the substrate, presence of fluorescent brighteners, temperature, humidity, etc.

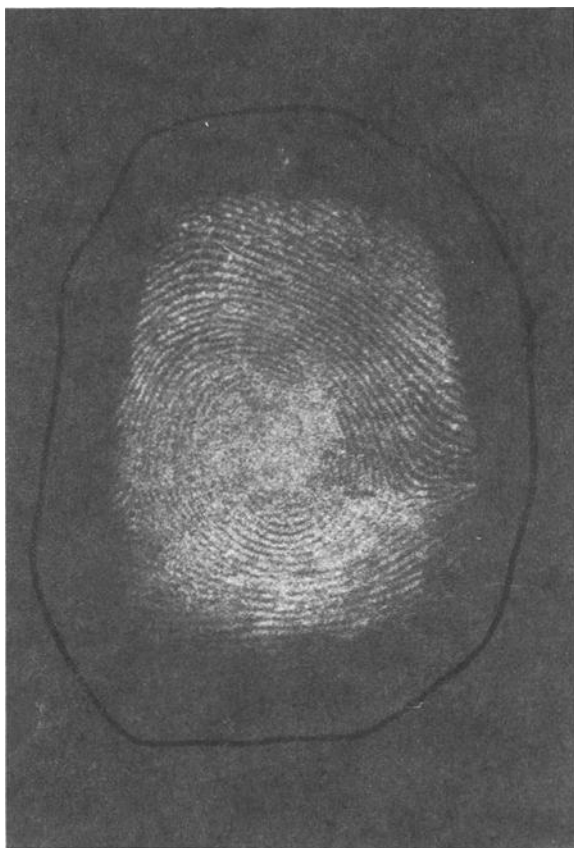
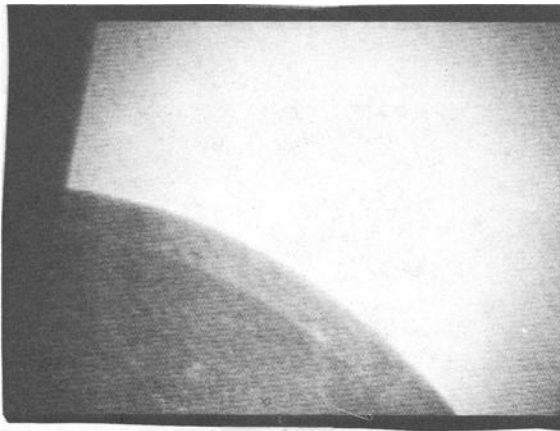


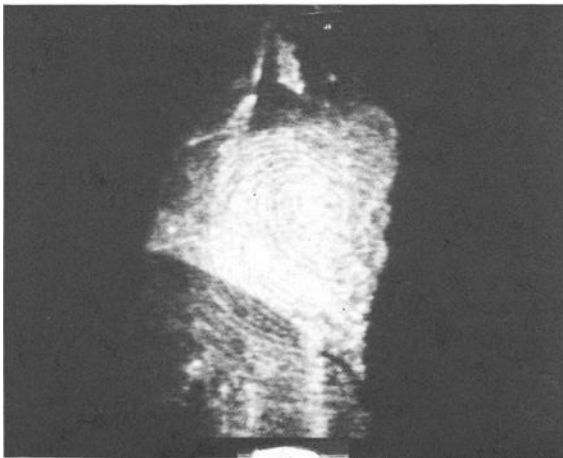
FIG. 6—Fingerprint on paper developed by 5-methoxyninhydrin/europium nitrate and photographed under near UV excitation.

Fingerprint Staining

Fingerprints were deposited on surfaces that can be stained (Aluminum cans, plastics, tapes, . . .) and the different Eu-RP complexes were used to stain these surfaces. The surfaces were chosen to show high background fluorescence, and our time-resolved luminescence imaging system was used to suppress the background. The results are promising [5] as seen in Fig. 7. The fingerprint shown was from a soft drink can stained with europium nitrate-RP(5-methoxyninhydrin). The reader should note that the pictures in Fig. 7 (from a thermoprinter) will likely have suffered loss of detail in reproduction. The bright luminescence band in Fig. 7b arises from greater adherence of the europium complex to the surface; it is not residual background fluorescence.



a



b

FIG. 7—Fingerprint stained with Eu-RP complex and developed by time-resolved imaging. Ungated (a), and gated camera (b).

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