TECHNICAL NOTE

CRIMINALISTICS; PATHOLOGY AND BIOLOGY

Andrzej Olejniczak,[1] *M.Sc.; Aleksandra W. Cyganiuk,*[1] *M.Sc.; and Jerzy P. Lukaszewicz,*[1] *Ph.D.*

1,2,4-Triazine-Based Chromogenic Reagents for the Detection of Microtraces of Various Metals Left on Human Skin*

ABSTRACT: This article extends the application of 1,2,4-triazine-based chromogenic reagents to the detection of nonferrous metal traces left on contact with canvas and human skin. The possibility of detection of iron traces resulting from contact with objects made of stainless steel was investigated as well. Additionally, the ability of triazines to form chromatic complexes with Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, and Al^{3+} ions was studied spectrophotometrically. Molar absorption coefficients, ranging from 8.8 to $29.9 \times 10^3/M/cm$, provide high sensitivity of 1,2,4-triazines toward nonferrous ions, thus, enabling the detection at concentrations as low as a few μM. The method was sensitive enough to detect traces resulting from a 1-min contact with a stainless steel made object, which is commonly considered as a corrosion-resistant material. The amounts of metal ions transferred to the skin after a 2-min contact with objects made of brass, zinc, and copper were sufficient to develop chromatic imprints.

KEYWORDS: forensic science, trace metal detection, firearm imprints, 1,2,4-triazines, chromogenic reagents, stainless steel

In crimes committed with the use of metal objects such as firearms or knives, some trace quantities of metals can be left on the perpetrator's skin or clothes. One of the tests used to find iron traces on the suspect's skin is a trace-metal-detection-test (TMDT) (1). In the test, iron traces are visualized by obtaining chromatic complexes of metal with a suitably matched chelating agent. The method makes it possible, in many cases, to reconstruct not only the outline of the metal object, but also some details of its surface topography. Historically, the first reagent used to develop traces was 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine (PDT) (2,3). The commercially available product, containing PDT as the active substance, is produced under the Ferroprint brand. It has been successfully used in a number of investigations, in particular in confirming suicide cases and finding the murderer in homicide–suicide cases (4).

The factors influencing the choice of a given compound as the ligand in TMDT are the reaction sensitivity (understood as the minimum concentration of metal ions required to trigger a visible chromatic effect) and the location of the absorption band in the region enabling easy observation of the chromatic effect. Systematic research in the field partially established the impact of the structure of 1,2,4-triazine derivatives on the spectral properties of the obtained complexes (5,6). Some of the new compounds resulted in improved sensitivity to Fe(II), when compared to PDT (5–7). The synthesis procedure was also modified to reduce the use of harmful chemicals (6,8).

The concentration of microtraces of metals remaining on skin or clothes depends on a number of factors. The following are believed to be the most crucial: the contact time with the object, the time elapsed between the contact with the object and the test, the grip strength, the moisture on the hand, the sweat composition, the temperature, the effect of washing the hands, and the chemical properties of the surface of the object material.

Direct contact of metal or alloy with the human skin triggers redox processes resulting in the release of metal ions. The potential corrosive properties (oxidation and dissolution) are demonstrated by both sweat and sebum (9–12). The composition and secretion level of these multicomponent biologic fluids can fluctuate considerably (13–15). It was shown, however, that the intensity of the developed marks is directly proportional to the perspiration level (16) and the concentration of chloride anions (17). The influence of the other skin excretion components was insignificant (17).

The released metal ions can migrate inside the skin and undergo complexation with amino acids and proteins. There are two important consequences involved. First, metal ions are immobilized on the skin. The process increases the chance of finding the traces after some time (up to 24 h from the contact time in laboratory tests [7,18,19]). What is more, washing the hands, if not carried out directly after the contact, does not exert a significant negative effect on the detection rate (20). Another effect of the diffusion and chelation is a reduction in the concentration of metal ions in the boundary layer. The process disturbs the electrochemical equilibrium and leads to an increased release of metal (21,22).

Despite the fact that the chemical composition of the material surface plays a crucial role in metal release, its influence on the scope and limitations of TMDT has not been systematically studied. The majority of tests have been conducted with the use of ingot iron, whereas the instruments of crime can also be made of

[1]Faculty of Chemistry, Nicolaus Copernicus University, 87-100 Toruń, Poland.

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aluminum, different alloys (e.g., stainless steel), and coated steel. To the best of our knowledge, only one study has examined the possibility of detection of iron traces resulting from contact with different types of firearms (18). The authors used the different types of chromogenic reagents, including PDT. The results pointed out that imprints of good quality could be developed only in the case of steel firearms. Neither aluminum-framed firearms nor stainless steel firearms gave a positive reaction to the test.

Recently, another study has investigated the interference of nonferrous ions on iron (II)–ferrozine (PDT analogue) complex formation (23). The release of metals from alloys is not directly correlated with metal content in the alloy, but it is affected by the action of electromotive forces generated by the presence of other metals and the microenvironment of the alloy surface. Consequently, it is difficult to predict precisely the release rate. For this reason, the objective of the present article was to study the possibility of applying triazines in the detection of iron traces after contact with objects made of stainless steel and of traces of other metals left after contact with nonferrous alloys and metals. The study was conducted on two planes. On the one hand, spectrophotometric studies were aimed at finding those metal ions, which are capable of forming chromatic complexes with triazines, and determining the reaction sensitivity (on the basis of molar absorption coefficients). Practical tests, on the other hand, provided an opportunity to verify whether the release of metal from a given material on contact with hand skin (or its substitute) is sufficient to develop a chromatic effect.

Materials and Methods

Materials and Reagents

A series of triazine ligands: PDT, 5,6-bis-(4-methylphenyl)-3-(2-pyridyl)-1,2,4-triazine (PDMPT), 5,6-bis-(4-methoxyphenyl)-3-(2-pyridyl)-1,2,4-triazine (PBMPT), 5,6-bis-(2-furyl)-3-(2-pyridyl)-1,2,4-triazine (PBFT), and 3,5,6-tri-(2-pyridyl)-1,2,4-triazine (TPT) were synthesized as described in our previous work (8). Triazines solutions of 1 mM in ethanol (99.8%, spectroscopic grade) were obtained by continuous stirring for 48 h at room temperature. Solutions of 1.25 mM of Fe(II), Co(II), Cu(II), Zn(II), Cr(III), Ni(II) were prepared by dissolving $Ni(NO_3)_2.6H_2O$, $Al(NO_3)_3.9H_2O$, $FeSO_4.7H_2O$, $CuSO_4.5H_2O$, $CrCl_3.2H_2O$, and $Co(NO_3)_2.6H_2O$ in distilled water. The detection experiments of metal traces left by different metallic materials were performed with an aqueous solution of NaCl (100 mM) serving as artificial sweat. A water solution of $SnCl_2$ was used as a reducing agent for Fe^{3+}. Shapes made of stainless steel, copper, brass, zinc, chromium, nickel, and aluminum (c. 37 mm OD, 5 mm thick with a 20-mm hole) were used as test metal items. The chemical composition of each material is shown in Table 1. The metal shapes were washed with detergent, rinsed with distilled water, degreased with acetone, and dried. No visible rust spots were observed on the surface of those metals.

Spectrophotometric Studies

To achieve efficient metal–triazine complex formation, the spectral studies were carried out in a water–ethanol system (1:1) with at least 20-fold molar excess of ligand. The solutions of final concentration equal to 5, 10, 15, and 20 μM were prepared by transferring 5 mL of each ligand solution (triazine I–V) into a 10-mL volumetric flask, pipetting with different amounts of 5–20 μL of metal ions solution (Co, Cr, Ni, Al, Cu, respectively), and diluting to volume with distilled water. The absorption spectra of metal–triazine complexes were recorded in the 350–720 nm region with a standard spectrophotometer equipped with 1-cm quartz cells. The absorbance was measured against ligand blank (water–ethanol) solution prepared under the same conditions with the exception of adding metal ions.

Detection of Metal Traces Left by Different Materials

The preliminary experiments were carried out with the cellulose textile backgrounds used as a substitute of human skin and/or clothing. Each cellulose background was wetted with artificial sweat. The excess of sweat solution was removed by carefully squeezing the cellulose background, which remained slightly wet during the contact with metallic items. The shapes made of an appropriate metal were put on the surface of the cellulose background and left for different contact times of 10, 20, and 30 min, respectively. Next, after the designated contact time, the shapes were removed and the backgrounds were immediately treated with 50 μL of appropriate triazine solution. In the case of iron-containing test materials, before the triazine treatment, the backgrounds were spiked with 0.1 mL of 0.001 mM $SnCl_2$ solution.

The triazines, which tested positive in the above-mentioned experiments, were evaluated as potential developers of metallic imprints left on human hands. The shapes made of different materials were pressed against the inner surface of a human palm for a period of 1–2 min. Then, the shape was removed, and the contact spot was covered with the cellulose tampon saturated with triazine solution and, additionally, in the case of iron-containing materials, with a reducing agent. After 10 sec, the cellulose background was removed and the presence of colored marks was checked visually.

Results and Discussion

To demonstrate the possibility of developing chromatic complexes of trazines and metals contained in common nonferrous alloys and metals, spectral studies were conducted with the use of solutions of the following ions: Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, Al^{3+}. Among the tested metals, no absorption bands were observed in

TABLE 1—Chemical composition of metals and alloys (% w/w).

Sample	C	Mg	Al	Cr	Mn	Fe	Ni	Cu	Zn
Iron	0.015	–	–	–	0.04	99.32	–	–	–
Stainless steel	0.05	–	–	17.59	2.40	72.03	7.52	–	–
Zinc	–	–	–	–	–	3.49	–	–	96.51
Brass	–	–	–	–	–	–	–	62.19	37.81
Copper	–	–	–	–	–	–	–	99.90	–
Aluminum	–	1.47	98.53	–	–	–	–	–	–
Chromo-nickel	–	–	–	60.79	2.44	–	36.77	–	–

the UV–Vis region for Al^{3+} and Cr^{3+} ions involving complexes. The absence of chromatic reaction excludes the use of triazines in the detection of traces of those metals. Absorption spectra of triazine complexes with the 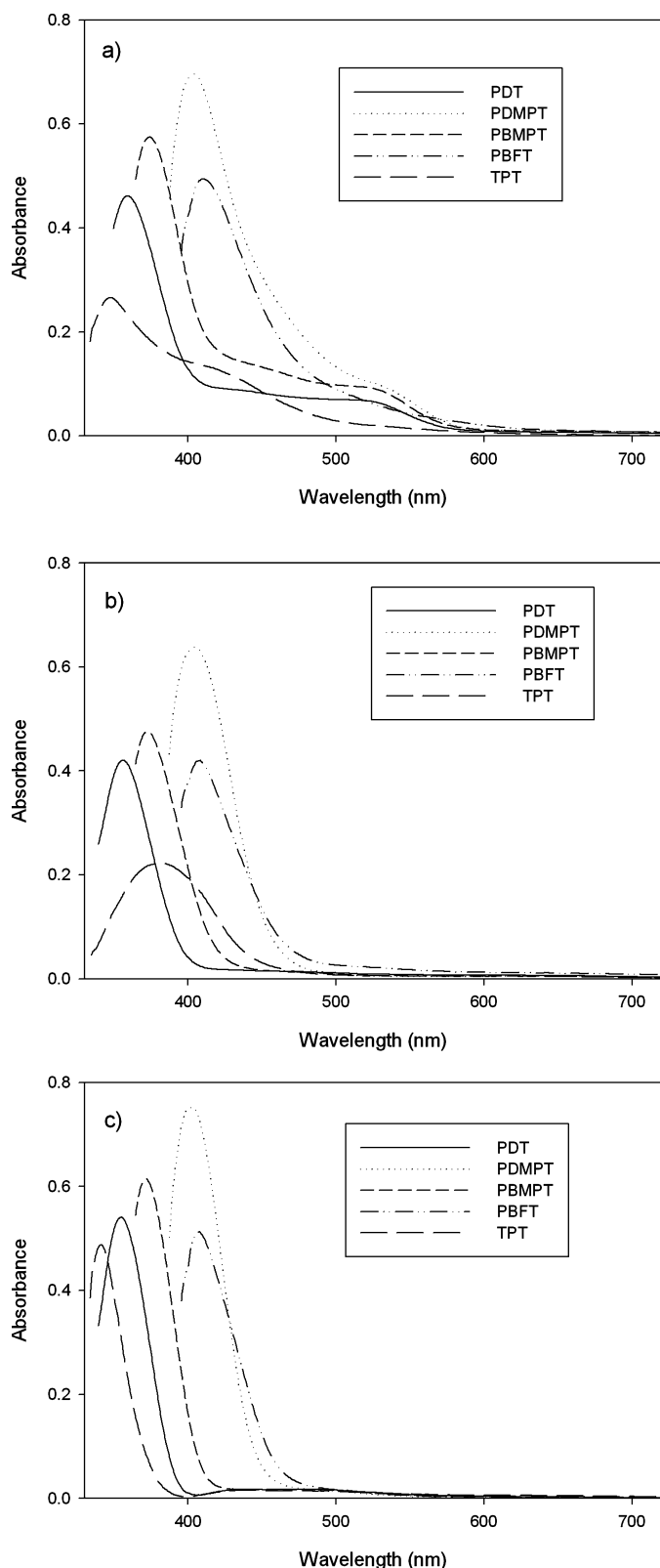other metals, shown in the region of 360–720 nm in Fig. 1, confirm the formation of chromatic chelates. Characteristic spectral data such as the wavelength at maximum absorption, molar absorption coefficient, and complex color have been presented in Table 2. At *c.* 20-fold excess of ligand, the observed increase in absorbance is directly proportional to the concentration of metal ions and obeys Beer's law in the studied region of 5–25 μM. The color of the obtained complexes is different from the analogous iron–triazine complexes, which should facilitate distinguishing iron traces from the traces left after contact with objects made of nonferrous metals. However, the fact that most of the nonferrous metal–triazine complexes exhibited nearly identical color is undoubtedly a drawback.

Only cobalt(II)- and iron(II)-containing chelates exhibited complex spectra and, as a result, complex color. This makes the visual distinguishing of the nonferrous metals traces virtually impossible (without resorting to instrumental methods).

Molar absorption coefficients (calculated on the basis of the slope of $A = f[C]$ line) demonstrate that PDMPT has the highest sensitivity to nonferrous metal ions. The molar absorption coefficients of nonferrous metal–PDMPT complexes are similar to the values obtained for iron complexes and higher than for the iron–PDT complex (Table 2). Moreover, the absorption maximum of the PDMPT complexes is located in the visible region, and when compared to other triazines, shifted toward longer wavelengths.

To simulate similar experimental conditions (i.e., temperature, thrust exerted by the tested object, concentration of chloride anions in the artificial sweat, and moisture level), a number of preliminary tests were conducted with a substitute of the human skin and artificial sweat. The experimental results for contact times of 10, 20, 30 min with materials made of copper, zinc, high-grade stainless

FIG. 1—*Absorption spectra of 1,2,4-triazine complexes with (a) Co^{2+}, (b) Cu^{2+}, and (c) Ni^{2+} in a region of 330–720 nm.*

TABLE 2—*Spectroscopic characteristics of metal–triazine complexes in water–ethanol mixtures.*

Ion	Triazine	λ_{max} (nm)	ε (10^3 dm³/mol/cm)	Color
Co^{2+}	PDT	358.5	18.5	Tea
	PDMPT	403.7	28.1	Orange
	PBMPT	373.4	22.9	Tea
	PBFT	410.3	19.0	Tea
	TPT	347.5	10.6	Tea
Cu^{2+}	PDT	356.0*	17.0*	Colorless
	PDMPT	405.0	25.5	Lemon
	PBMPT	372.1	19.4	Yellowish
	PBFT	407.6	16.1	Yellow
	TPT	380.4	8.8	Lemon
Ni^{2+}	PDT	354.5*	21.7*	Colorless
	PDMPT	402.4	29.9	Yellow
	PBMPT	371.5	24.4	Colorless
	PBFT	407.6	20.4	Yellow
	TPT	341.3	20.3	Colorless
Zn^{2+}	PDT	356.5	ND	Colorless
	PDMPT	400.0	ND	Lemon
	PBMPT	368.0	ND	Colorless
	PBFT	382.0	ND	Yellow
	TPT	342.5	ND	Colorless
Fe^{2+}	PDT	553[†], 555[‡]	24.0[†], 24.3[‡]	Pink
	PDMPT	561.0	27.5[†]	Pink
	PBMPT	562.0	32.5[†]	Pink
	PBFT	576.0	41.3[†]	Blue
	TPT	563.5	ND	Blue

PDT, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine; PDMPT, 5,6-bis-(4-methylphenyl)-3-(2-pyridyl)-1,2,4-triazine; PBMPT, 5,6-bis-(4-methoxyphenyl)-3-(2-pyridyl)-1,2,4-triazine; PBFT, 5,6-bis-(2-furyl)-3-(2-pyridyl)-1,2,4-triazine; TPT, 3,5,6-tri-(2-pyridyl)-1,2,4-triazine; ND, not determined.

*Determined for $[L] = 0.25 \times 10^{-3}$ mol/dm³

[†]From ref. (5)

[‡]From ref. (6)

TABLE 3—*Results of preliminary detection tests of metal traces left by different materials. The numbers indicate contact time (of 10, 20, 30 min) required to develop observable chromatic effect.*

Triazine	Stainless Steel	Copper	Zinc	Brass	Chrome-Nickel	Aluminum
PDT	10 weak	NO	NO	NO	NO	NO
PDMPT	10	10	10	10	30 weak	NO
PBMPT	10	NO	NO	NO	NO	NO
PBFT	10	10	10	10	NO	NO
TPT	20 weak	20 weak	30 weak	30 weak	NO	NO

PDT, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine; PDMPT, 5,6-bis-(4-methylphenyl)-3-(2-pyridyl)-1,2,4-triazine; PBMPT, 5,6-bis-(4-methoxyphenyl)-3-(2-pyridyl)-1,2,4-triazine; PBFT, 5,6-bis-(2-furyl)-3-(2-pyridyl)-1,2,4-triazine; TPT, 3,5,6-tri-(2-pyridyl)-1,2,4-triazine; NO, not observed.
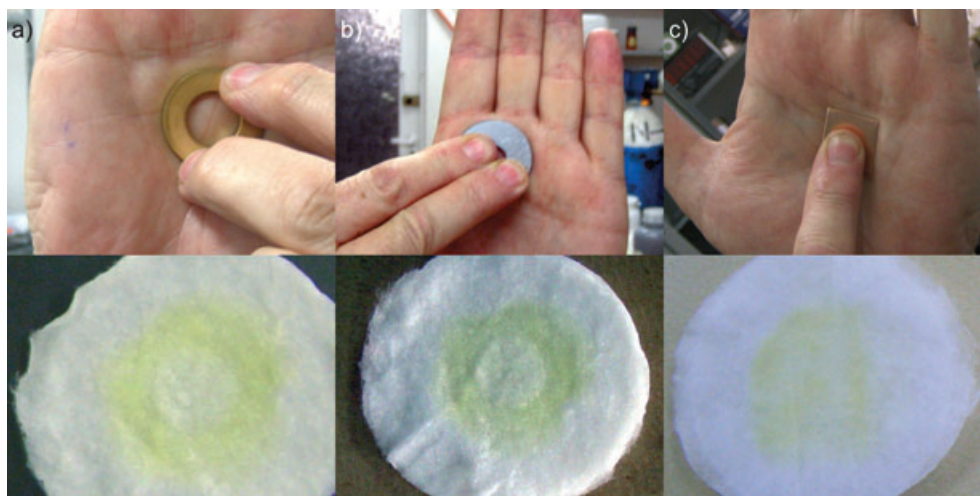

FIG. 2—*A set of backgrounds with colored traces transferred from: (a) brass, (b) zinc, (c) copper. Developing reagent: 5,6-bis-(4-methylphenyl)-3-(2-pyridyl)-1,2,4-triazine, room temperature, contact time: 30 min.*

FIG. 3—*Comparison of imprints intensities (developing reagent: triazine II) resulting from a 1-min contact with: (a) stainless steel ring, (b) uncoated iron ring.*

steel, brass, and chromo-nickel are shown in Table 3. They prove that in the case of the majority of tested materials, including high-grade stainless steel, the release of metal ions is sufficient to observe chromatic reaction with triazines. Only in the case of chromo-nickel alloy, the release of metal ions was too low and a low-intensity color was developed after a 30-min contact (PDMPT).

The highest intensity of coloring of microtraces left by the tested metals was obtained for PDMPT and PBFT. As far as PDT and PBMPT are concerned, which showed similar or higher

values of the molar absorption coefficients, no color was observed in the case of the majority of materials. Application of TPT, despite its low sensitivity to all tested cations, made it possible to obtain, after a prolonged contact time, a coloring of moderate intensity. The observed effects indicate that the shifting of the band toward the visible region plays a crucial role in trace detection. For those metal–triazine complexes for which the maximum absorption was situated in the near ultraviolet, the chromatic effect is developed only by the fragment of the band stretching in the visible region. The effect has an impact on detection sensitivity and also explains the low capability of PDT and PBMPT to develop traces.

The preliminary tests reveal interesting information with regard to stainless steel. Although the chemical composition of stainless steel was modified to improve the corrosion resistance (Table 1), a 10-min contact time was enough to develop an intensive coloring with the use of PDMPT, PBMPT, and PBFT, as well. PDT and TPT developed coloring of a lower intensity (hardly visible), and a prolonged contact time did not improve the intensity of the developed imprints significantly. The results can be explained by taking into account lower sensitivity of those two reagents (Table 2), and the fact that the metal dissolution rate reached a plateau (17). The findings are in agreement with the previous observations suggesting that the application of the well-established PDT reagent does not always produce a positive result while examining stainless steel objects.

Moreover, in all cases, apart from typical colors of iron(II) complexes, a yellow coloring was also observed. It was most likely a result of a competitive complexation of the reducing agent (Sn^{2+}) by triazines, which may negatively affect the detection threshold at a low concentration of iron. Therefore, a reduction in iron with the use of ascorbic acid, as suggested by other authors, could help avoid these interferences (23). However, other interferences are not excluded.

The possibility of detection of traces left by the above-mentioned materials was tested in a series of experiments with the human hand. A procedure enabling the transfer of traces to a neutral background was applied. What is more, the contact time was reduced to 1 min for stainless steel, and to 2 min for the other materials, expecting that a higher temperature of the hand (by c. 17°C when compared to the ambient temperature) would accelerate the process of metal release. In the course of experiments, as the authors subjectively believe, the level of perspiration was normal, which means the hand was not excessively wet. The conducted tests confirmed the results obtained for the substitutes of skin and sweat. Only in the case of chromo-nickel, no chromatic imprints were observed. In other cases (i.e., for high-grade stainless steel, zinc coated iron, copper, and brass), some chromatic imprints were observed, as shown in Figs. 2 and 3. The developed chromatic imprints provide information on both the size and shape of the object on contact with the hand.

Conclusions

Spectrophotometric studies proved the ability of triazines to create chromatic complexes not only with Fe^{2+} ions, but also with the Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} ions, which can be released on contact with commonly used nonferrous alloys and metals. Al^{3+} and Cr^{3+} ions do not form absorbing complexes in UV–Vis region.

The intensity of color and reaction sensitivity is clearly dependent on the type of metal and triazine. The highest intensity of coloring of microtraces left by the tested materials was obtained for PDMPT and PBFT. In the case of PDMPT, the molar absorption coefficients are higher for the complexes of all tested metals than the values obtained for the complexes of iron with PDT, which is indicative of a high sensitivity of this reagent. The other triazines, even though they do form chromatic complexes with the tested metals, as spectrophotometric studies have shown, their color is hardly visible. The observation confirms the important role the location of the absorption band plays in detection of traces.

Despite the short contact time with the human skin, the concentration of metal ions transferred to the skin is sufficient to develop chromatic imprints for materials made of high-grade stainless steel, brass, zinc, and zinc coatings, as well as copper.

Further works on the synthesis of new triazine-based chromogenic reagents are recommended. They should lead to new compounds of higher sensitivity and improved spectral characteristic (i.e., colors which contrast more with the human skin and which are diversified for different ions).

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Additional information and reprint requests:
Jerzy P. Łukaszewicz, Ph.D.
Associate Professor
Faculty of Chemistry
Research Group for Modeling and Synthesis of Novel Materials
Nicolaus Copernicus University
Gagarina 7, 87-100 Toruń
Poland
E-mail: lukaszju@chem.uni.torun.pl