Title:

Detection of Fingermarks by Colloidal Gold (MMD/SMD) – Beyond the pH 3 Limit

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Abstract

This work is part of a continuing goal to improve the multimetal deposition technique (MMD), as well as the single-metal deposition (SMD), to make them more robust, more user-friendly, and less labour-intensive. Indeed, two major limitations of the MMD/SMD were identified: (1) the synthesis of colloidal gold, which is quite labour-intensive, and (2) the sharp decrease in efficiency observed when the pH of the working solution is increased above pH 3. About the synthesis protocol, it has been simplified so that there is no more need to monitor the temperature during the synthesis. The efficiency has also been improved by adding aspartic acid, conjointly with sodium citrate, during the synthesis of colloidal gold. This extends the range of pH for which it is possible to detect fingermarks in the frame of the MMD/SMD. The operational range is now extended from 2 to 6.7, compared to 2 to 3 for the previous formulations. The increased robustness of the working solution may improve the ability of the technique to process substrates that tend to increase the pH of the solution after their immersion.

Keywords:

Fingermark detection; gold nanoparticle; colloidal solution; multimetal deposition; single-metal deposition; aspartic acid

Introduction

Multimetal deposition (MMD) is a well-known fingermark detection technique based on the use of metal nanoparticles in solution. First proposed in 1989 [1], MMD (currently known as "MMD-I") aims at detecting latent fingermarks on a wide range of substrates through a two-step procedure (Figure 1). First, the deposition of gold nanoparticles onto the latent secretion is promoted under specific experimental conditions. This is followed by a silver-based enhancement step allowing the visualization of the latent fingermarks. As a result, the detected fingermarks appear as dark-brown marks on a most likely unstained substrate and as light marks on dark substrates. An early publication referred to MMD as "The Universal Process" [2]. This denomination was not further retained, but actually emphasized a major strength of the MMD process: its ability to detect marks on a wide range of substrates, being porous, non-porous, or semi-porous. The latter is considered as difficult to process using conventional techniques which are generally limited to strictly porous or non-porous substrates. A good illustration of this versatility is the example given in Saunders' article: the processing of a computer floppy disk. Such an item is composed of three distinct surfaces (i.e., metal, paper, and plastic) and would have required at least two conventional techniques to be applied sequentially to detect fingermarks (one for the paper surface and another for the non-porous ones). The use of MMD-I allows the processing of these three different surfaces simultaneously. Despite this advantage, the success of MMD-I was limited and the technique was seldom applied in casework. Several drawbacks can explain this lack of success: (1) MMD-I is a labour-intensive technique, with several rinsing and immersion baths; (2) it is quite a time-consuming technique, requiring at least one hour to complete the process; (3) the deposition of the gold nanoparticles onto secretions occurs only if the pH of the colloidal gold solution is set to a precise and narrow range of values (ca. 3.0; outside this range, the efficiency of the method significantly drops, explaining the difficulties encountered with

some alkaline papers); (4) the original silver enhancement step takes place quickly, within 1 or 2 minutes, and could cause unwanted background darkening if the substrate is left too long in the enhancement bath; and, finally, (5) dark-brown marks are obtained, which can be problematic on dark or patterned substrates.

Since 1989, several research projects aimed at increasing the efficiency of MMD as well as its robustness towards experimental conditions (particularly, the pH), in addition to simplifying its experimental protocol (Figure 2). A major evolution was the development of the "MMD-II", ca. ten years after the MMD-I [3]. Modifications were brought on the colloidal gold synthesis and on the silver-enhancement step, but the overall detection mechanism remained unchanged (i.e., gold deposition followed by metal-based enhancement). Regarding the colloidal gold synthesis, gold nanoparticles of 14 nm (diameter) were preferred to the 30 nm nanoparticles used in the MMD-I, both being monodisperse. The silver enhancement process was also completely modified so that the risk of background darkening was consistently reduced (but not avoided completely). As a result, MMD-II proved to be more robust and more efficient compared to MMD-I, and was consequently proposed as a replacement for the original technique [4]. Nevertheless, MMD-II still suffered from major drawbacks: (1) the colloidal gold synthesis is more complex and time-consuming compared to MMD-I; (2) the protocol is still labour-intensive (even if the processing time has been reduced to ca. 40 minutes compared to MMD-I); and (3) the working solution still needs to be set within a narrow range of pH values, the authors recommending between 2.5 and 2.8. Deviating from these values would result in a drastic drop in the efficiency on MMD-II (especially when the pH is higher than required), with almost no result obtained when working at a pH of 4 or above.

A second major evolution of the technique was the development of the single-metal deposition (SMD) method, proposed as an alternative to MMD-II [5]. The modifications are related to the metal-enhancement step only, the colloidal gold deposition remaining unchanged compared to MMD-II. By replacing the "silver on gold" enhancement mechanism by a "gold on gold" one, it has been possible to offer the following advantages compared to MMD-II: cheaper technique, less reagents involved, less labour-intensive protocol, and almost no risk of unwanted background darkening. Moreover, it has been shown that SMD was overall equally effective (or more, but never less) compared to MMD-II [6]. From an historical perspective of modifications to MMD, SMD should have been called "MMD-II_b". However, since there is no need for two different metals to obtain visible marks, the name was changed from "multi-" to "single-metal deposition". Given that SMD affords only advantages compared to MMD-II, it was strongly recommended to replace MMD-II by SMD for those considering this technique for casework. Despite these improvements, the pH dependence remained (since the colloidal gold deposition step is identical to MMD-II) and difficulties were still encountered when dealing with some types of paper.

Other modifications to the original technique were also proposed but do not constitute alternatives to MMD/SMD, due to an "ongoing development" status or due to a restricted application range. The experimental protocol has, for example, been reduced to one immersion bath by the use of cyclodextrin-functionalized gold nanoparticles, but the technique requires heavy synthesis skills [7]. A promising development consisted in proposing a luminescent version of the MMD by reducing zinc oxide (ZnO) instead of silver during the metal-enhancement step, but the technique was limited to non-porous substrates [8]. A one-step MMD-like process was also recently proposed, using glucose-capped gold nanoparticles that are said to be operative over a wider range of pH (i.e., 2.5 to 5.0) [9]. On

the contrary to what is affirmed in the article, the mechanism looks more like a “gold-based SPR” (Small Particle Reagent), especially when it is said that the colloidal gold solution is blueish whereas it should be ruby-red. The blueish colour is a consequence of nanoparticle aggregation towards submicron-ranged aggregates, detrimental to the MMD mechanism.

Despite the successive attempts to improve MMD/SMD, the necessity to set the pH of the colloidal gold solution within a narrow range of values to observe the deposition of gold nanoparticles onto the latent secretion remains the weakest point of the technique. Indeed, a pH-meter has to be used and, more constraining, a slight deviation from these conditions could lead to poor quality marks or no result at all, especially on porous substrates. Whatever the quality or sensitivity of the subsequent enhancement/visualization step, if gold nanoparticles fail to be specifically entrapped in the latent secretions, the technique will fail to detect the latent fingermarks. To explain this strong dependency on pH, it is assumed that the mechanism of deposition of gold nanoparticles towards latent secretion is mostly due to electrostatic attraction between negatively-charged gold nanoparticles and positively-charged components of the latent secretion [10]. The negative charge of the gold nanoparticles is due to the use of sodium citrate during the colloidal gold synthesis. The obtained gold nanoparticles are surrounded by a layer of adsorbed citrate ions, bearing three carboxylic acid terminal functions (Figure 3a). Gold nanoparticles are consequently fully negatively charged at neutral pH, but their negative charge decreases as the pH of the solution is progressively brought towards acid values (due to the successive protonation of the carboxylate groups). On the contrary, it is assumed that the latent secretions bear a positive charge at low pH values due to the presence of amine groups (e.g., amino acids and proteins) or double bonds (e.g., unsaturated lipids) [11]. This positive charge decreases as the pH is increased, due to the deprotonation of carboxylic acid groups contained in some organic compounds. By setting the

pH to a narrow range (e.g., between 2.5 and 2.8 for MMD-II or SMD), it is assumed that the negative charge of the gold nanoparticles is still sufficient to create an attractive interaction with the positively-charged latent secretions. If the pH is too low, latent secretions become more positively-charged but gold nanoparticles are no longer negatively-charged (complete neutralization of the carboxylate groups). On the contrary, if the pH is too high, the latent secretions tend to become neutral while gold nanoparticles become more negatively-charged. Such a narrow pH range consequently constitutes what we could call "best-so-far" conditions, which are far from being optimal in terms of electrostatic attraction (Figure 4). It also explains why the efficiency of the MMD/SMD drastically drops when deviating from these values.

In an attempt to tackle the problem of the pH dependence of MMD/SMD, we modified the gold nanoparticles so that their negative charge should be strengthened at low pH. To reach this goal, we chose to use aspartic acid, conjointly with sodium citrate, during the synthesis of the gold nanoparticles (Figure 3b). The efficiency of this new colloidal gold formulation (called "Au-ASP") was compared with the reference solution (called "Au-Schnetz"), in the frame of the SMD protocol. We observed that using "Au-ASP" allowed extending the range of pH for which SMD is able to detect fingermarks to a range from pH 2.0 to ca. 6.7 (which is the unmodified pH of the colloidal gold, right after its synthesis). We also confirmed the active role played by aspartic acid by synthesizing a third colloidal gold solution, called "Au-noASP", whose synthesis is identical to Au-ASP except that aspartic acid is not added.

It should be noted that in the rest of the article, only the term "SMD" will be considered. This is simply due to the fact that this study was performed by following a gold enhancement protocol. However, it should be kept in mind that these results are also valid for MMD-II

since they share the same initial step (i.e., the gold nanoparticle deposition protocol), and differ only by the second step of the detection (i.e., the metal-based enhancement).

Material and methods

Tetrachloroauric (III) acid trihydrate (Merck) and all other chemicals (Sigma-Aldrich) were of high purity grade and were used without further purification. RO/DI or bidistilled water can be used during the synthesis of colloidal gold.

As mentioned above, three kinds of colloidal gold solution were synthesized: (1) "Au-Schnetz", synthesized according to the recommended publication [3]; (2) "Au-ASP", synthesized according to a published protocol [12] which has been modified, as described below; and (3) "Au-noASP" synthesized according to the same modified protocol but using sodium citrate only (no aspartic acid). For each colloidal gold solution, the size of the nanoparticles and their zeta potential were measured, at different pH values, using a Zetasizer Nano ZS (Malvern Instruments Ltd.). Simply stated, the zeta potential is a measure of the electric potential at the interface between a nanoparticle and the surrounding solution. It is widely accepted that a zeta potential above +30 mV or below -30 mV is required for a colloidal solution to remain stable as a result of the electrostatic repulsion between the nanoparticles in solution [13].

"Au-Schnetz" is synthesized as follows: in a first flask, 500 µL $HAuCl_4$ (10 %, w/v) are added to 400 mL water. In a second flask, 20 mL of a trisodium citrate dihydrate solution (1 %, w/v) and 100 µl of a tannic acid (1 %, w/v) are added to 75 mL water. Both solutions are heated separately to 60°C. When this temperature is reached, the citrate solution is quickly poured in the first flask under strong stirring and the mixture is heated to boiling. The colour of the

solution successively turns from yellow to colourless, then purple, and finally ruby-red. At this moment, heat is removed and the colloidal gold solution is allowed to cool down to room temperature. The volume is finally adjusted to 500 mL with water.

"Au-ASP" is synthesized as follows: in a flask, 570 µL $HAuCl_4$ (10 %, w/v) are added to 275 mL water. The solution is heated to boiling. At this moment, 25 mL of a trisodium citrate dihydrate solution (2 %, w/v) containing aspartic acid ($1.45\ 10^{-5}$ mol/L) are quickly added to the boiling solution (note: aspartic acid was first dissolved in another beaker, using equimolar NaOH to help its solubilisation). The mixture is kept under boiling until the ruby-red colour is obtained, after which the heat is removed and the colloidal gold solution allowed to cool down to room temperature. The volume is finally adjusted to 300 mL with water. Before use or storage, the solution is diluted by a factor of 2.4x using water (final volume: 720 mL). This dilution step was performed to ensure that the resulting Au-ASP solution is at the same nanoparticle concentration as for Au-Schnetz, avoiding any effect of the concentration on the quality of the results.

"Au-noASP" is synthesized as follows: in a flask, 570 µL $HAuCl_4$ (10 %, w/v) are added to 275 mL water. The solution is heated to boiling. Then, 25 mL of a trisodium citrate dihydrate solution (2 %, w/v) is quickly added to the boiling solution. The rest of the procedure is identical to that of Au-ASP.

Fingermark samples were collected by considering six different porous substrates, four non-porous substrates, five different donors, and ages ranging from one week to six years for some marks. The combination of all these parameters gave us a total of 14 different samples to be processed (see Table 1 for details). It should be noted that, for each sample, mixed marks

were deposited (i.e., sebum- and sweat-containing marks). The donors were asked to touch their forehead, then homogenize the secretions by rubbing their hands together before touching the substrates. To limit the effect of the secretion enrichment, two successive marks were left for each sample without reloading the finger.

A correct way to compare two techniques (or two sets of parameters) consists of cutting each sample bearing fingermark(s) in half along the vertical axis, so that the left and the right fingermark halves are processed separately, following two distinct protocols. Two runs of experiments were conducted in this study. The first run involves the comparison of Au-ASP (challenger – left half) with Au-Schnetz (reference – right half), and more particularly the evolution of their efficiency according to the pH. To reach this goal, five different pH values were tested: 2, 2.65, 3, 4, and 5. The value of 2.65 was chosen to meet the recommended optimal pH range as specified by Schnetz for MMD-II, which was from 2.5 to 2.8. To test the effect of aspartic acid on the detection, a second run consisted of comparing Au-noASP (challenger – left half) with Au-ASP (reference – right half), at five different pH values: 2, 3, 4, 5, and unmodified pH. The "unmodified pH" parameter consisted of using the colloidal gold without modifying its pH. A pH value of ca. 6.7 was measured for both Au-ASP and Au-noASP after their synthesis. A total of 280 fingermarks were cut in half and processed (i.e., 14 samples, two marks per sample, two runs of experiments, five pH conditions per run of experiment). More importantly, it should be emphasized that Au-ASP was applied on either the left halves (first run of experiments) or the right halves (second run of experiments), ensuring that there was no left-half versus right-half bias on the observed efficiency.

The colloidal gold deposition (common to Au-Schnetz, Au-ASP, and Au-noASP) was performed as follows: (1) a surfactant (Tween 20) is added to colloidal gold (0.1 %, v/v) and

the solution is stirred for 10 minutes; (2) the pH is set to the desired value using citric acid 0.1M (note: from pH 2.65, HCl 1M was used to reach pH 2); and (3) the working solution is poured into a flat dish and the samples immersed under orbital agitation (ca. 50-70 $r.min^{-1}$) for 20 minutes. The metal enhancement was performed according to the protocol described in [5].

After being rinsed and air-dried, all the fingermarks were reassembled from their halves and scanned using a Canoscan 8400 F (Canon) at a resolution of 1200 dpi, without any digital enhancement (at this point of the digitalization). To provide an objective means of judging relative efficiency according to pH, all the fingermarks from a set of experiment (same substrate, donor and age, but different pH values) were collected on a Photoshop layer and post-processed simultaneously using the same digital enhancement (i.e., conversion to gray scale and level adjustment). This way, the only two parameters that could influence the rendering of a mark compared to another from the same set was the pH value and the two compared protocols (left and right halves). Both the original scanned images and the digital enhanced ones were presented to the evaluators during the evaluation process.

The quality evaluation process was established following recommendations from Kent [14]:

1. Five different persons (further referred as the "evaluators") accustomed to fingermark detection and fingerprint identification were asked to individually participate in the evaluation process.

2. Each evaluator was first informed that the aim was to evaluate the ridge quality information (whatever the number of minutiae visible in the observed area). To reach this goal, four quality levels were defined and were associated with four numerical values (i.e., 0, 1, 2, and 4). These levels were qualitatively described to each evaluator (see Table 2) with no additional information provided that would otherwise influence the evaluator.

3. Each half-fingermark was scored independently from its complementary half ("blind" evaluation), to ensure that the evaluator scores the quality of the mark itself without being influenced by the corresponding half. A whole set of left-half-marks was first presented to the evaluator before presenting him/her the right-half-marks, to avoid a memory effect that could otherwise influence the scores given to two successive and corresponding half-marks.

4. Once all the five evaluators had completed the scoring process, each half-mark was characterized by five scores that were finally averaged so that a unique score remained. These average scores were used to further compute various trends, by considering specific pH ranges, or by grouping the samples according to the surface type or the substrate nature (see Table 1 for the different classes of substrates).

Results

1. Characterization of the three colloidal gold solutions

Schnetz's synthesis produced monodisperse gold nanoparticles characterized by a hydrodynamic diameter of 17.8 nm (± 0.4) and a zeta potential of -47.3 mV (± 0.7), with an initial pH of ca. 6.2. By considering the density of metallic gold (19.3 g/cm³) and assuming perfect spheres are obtained, it is possible to estimate the concentration of gold nanoparticles in the colloidal gold solution, that is, 1.45 10^{-9} mol/L for Au-Schnetz (if it is considered that a nanoparticle is a molecular entity). Upon the addition of Tween 20, a slight increase in the nanoparticle size was observed (19.2 nm ± 1.3), which is logically due to the chemical structure of Tween 20, which is thought to adsorb around the nanoparticles, as well as a reduction in the zeta potential by almost half (-22.8 mV ± 0.5), mostly due to a screening effect.

When considering the use of aspartic acid, in addition to citrate ions, it can be seen that the resulting gold nanoparticles are very similar to the Schnetz particles in both size and zeta potential (Table 3). Au-ASP nanoparticles are monodisperse and characterized by a diameter of 16.5 nm (± 0.7) and a zeta potential of -42.7 mV (± 2.3), with an initial pH of ca. 6.7. For the Au-ASP solution, the estimation of the concentration of gold nanoparticles was 3.47 10^{-9} mol/L. Given that Au-ASP is initially more concentrated than for Au-Schnetz, we decided to dilute the solution by 2.4x before use, to obtain a final concentration (in nanoparticles) equal to that of Schnetz. Upon the addition of Tween 20, a slight increase in the nanoparticle size was observed (18.1 nm ± 0.4), combined with a diminution of the zeta potential by almost half (-24.5 mV ± 1.1), as expected.

Finally, when considering Au-noASP, it can be seen that the zeta potential is initially similar to that of Au-ASP (-45.6 mV ± 0.5), as well as the size of the nanoparticles (13.5 nm ± 0.3), although slightly smaller. The addition of Tween 20 slightly increased the size of the nanoparticles (14.7 nm ± 0.7), as expected, but mainly reduced significantly the zeta potential (-11.7 mV ± 0.6). The reduction in zeta potential (one fourth of the original value) was higher than what was observed for Au-Schnetz and Au-ASP (half of the original value).

Besides these physico-chemical characteristics, the three colloidal gold solutions were ruby-red in colour, and were stable over the tested range of pH values (i.e., from original pH down to 2.0). They were consequently suitable for the detection of fingermarks according to the SMD protocol.

2. Quality of the fingermarks detected using Au-Schnetz

A first observation was that porous substrates were characterized by lower average scores (i.e., 1.0) compared to non-porous surfaces (i.e., 2.1) over the whole pH range (i.e., from 2 to 5) – see Table 4. The non-bleached papers and the polypropylene (PP) sleeves gave the best results, with average quality scores of 2.1 and 2.5, respectively. All other substrates gave quality scores equal to or below 1 over this extended pH range, which can be seen as unsuccessful development if we refer to Table 2.

A more interesting exploitation of the results is seen when distinguishing two ranges of pH: (1) the "lower range" (i.e., pH 2–3 / acidic, as recommended by Schnetz and Margot [3]); and (2) the "higher range" (i.e., pH 4–5 / towards neutrality). When the pH is increased from the lower range of pH to the higher one, the average scores dropped from 1.6 to 0.3 for porous substrates, and from 2.5 to 1.7 for the non-porous ones. The severe drop in quality for porous substrates is even more pronounced when looking at the substrate which gave the highest scores – non-bleached papers – whose average score was 3.0 for the lower pH range, but drops to 0.7 for the higher pH range. This can be explained by two phenomena: (1) no sign of fingermark development (no detection); or (2) a significant rise in background staining when the detection is performed at higher pH values.

From these observations, it was confirmed that the pH range is critical for the detection of fingermarks; Au-Schnetz is quite successful for a pH ranging from 2 to 3, but a significant loss of efficiency is observed above pH 3, especially for porous substrates (Figures 5 and 6). Moreover, some porous substrates were shown to be problematic along the whole pH range. For example, bleached paper and the brown paper envelope were characterized by very low average scores (i.e., 0.2 and 0.5, respectively) in the lower pH range, which is, however, the

recommended range. These results will be further discussed, but it is already possible to say that they are consistent with Schnetz's conclusions concerning MMD-II.

3. Quality of the fingermarks detected using Au-ASP

Contrary to Au-Schnetz, Au-ASP behaved extremely well over the whole range of pH values that were monitored in this first run of experiment, i.e., from pH 2 to 5. Porous substrates were characterized by an average score of 1.9, and non-porous ones by a score of 2.4 (Table 4). One reason explaining this trend is that the background staining remains almost unchanged across the whole range of pH, with no excessive rise at higher pH values (in contrast to what was observed for Au-Schnetz). Non-bleached papers and polypropylene (PP) sleeves remained the samples giving the best results, with an identical average quality score of 2.9 for the pH 2–5 range. Bleached paper and LDPE bags were still problematic, with average scores of 0.7 and 0.8, respectively, even if the bleached paper score is significantly higher than the one observed with Au-Schnetz (i.e., 0.1).

If the lower pH range (i.e., pH 2–3) is distinguished from the higher one (i.e., pH 4–5), the average scores for the porous substrates slightly dropped from 2.0 to 1.7 when considering higher pH values, while the scores for non-porous substrates dropped from 2.7 to 2.0. The decrease in scores observed when increasing the pH is substantially lower for Au-ASP (i.e., -15 % for porous substrates) than when using Au-Schnetz (i.e., -81 % for the same class of substrates). When illustrating the difference in scores for each of the 14 substrates according to the lower and higher pH ranges, it is possible to see that both colloidal gold solutions behave quite similarly in terms of fingermark quality, in the lower pH range (Figure 5-top). Nevertheless, the main difference appears in the higher pH range (Figure 5-bottom), for which Au-ASP clearly outperforms Au-Schnetz, with the latter unable to detect marks on most of the

substrates at these pH values (average score of zero). This trend is visually illustrated in Figure 6, for non-bleached paper (sample #2 from Table 1), grey envelope paper (sample #6), and polypropylene sleeve (sample #13).

With scores superior to 1 for most of the samples in the higher range of pH, Au-ASP is consequently able to detect fingermarks with good ridge details and sufficient contrast even at pH 4 and 5. Another interesting observation is that the quality of the marks on the non-bleached papers remained unchanged (i.e., 2.9), even at higher pH values (Figures 5 and 6). For non-porous substrates, the drop in quality is similar for both colloidal gold solutions (i.e., -32 % for Au-Schnetz and -26 % for Au-ASP) when the pH is raised up to 4–5. An extensive comparison between Au-ASP and Au-Schnetz shows that almost all the samples are characterized by higher scores when Au-ASP is used. The highest increases in score were observed for the porous substrates, with an average gain of +25 % (pH 2–3) and +467 % (pH 4–5) when compared with Au-Schnetz, whereas the gain with non-porous substrates is lower: +8 % (pH 2–3) and +18 % (pH 4–5).

4. Quality of the fingermarks detected using Au-noASP

When comparing both synthetic protocols, the only difference between Au-ASP and Au-noASP is the presence of aspartic acid (ASP), conjointly with sodium citrate in a molar ratio of 1/100 (ASP : sodium citrate) for Au-ASP, and its absence for Au-noASP. Despite this slight difference in formulation, the behaviour of these two colloidal gold solutions differs significantly when used in the SMD method (Figure 7), similar to what was observed when comparing Au-ASP with Au-Schnetz. This clearly illustrates the active role played by aspartic acid with respect to the increased performance observed for Au-ASP compared to Au-Schnetz.

Both Au-ASP and Au-noASP behaved similarly up to pH 3, but a serious drop in the scores was observed for Au-noASP above this value, especially on porous substrates. Finally, it should be emphasized that, at pH 6.7, Au-ASP still succeeded in detecting marks (with a relatively good quality for some substrates) with an average value of 1.1 for porous substrates and 2.4 for non-porous ones. Average scores of 0.1 and 0.5 were obtained with Au-noASP under the same conditions.

When comparing the scores obtained with Au-Schnetz to the ones obtained with Au-noASP, it appears that both techniques are characterized by almost equal scores, with somewhat slightly higher values for Au-noASP. For example, in the lower pH range, porous substrates are characterized by average scores of 1.6 (Au-Schnetz) and 1.7 (Au-noASP), and non-porous substrates scores of 2.5 (Au-Schnetz) and 2.7 (Au-noASP). When increasing the pH towards the higher pH range, scores dropped to 0.3 (Au-Schnetz) and 0.6 (Au-noASP) for the porous substrates, and to 1.7 (Au-Schnetz) and 1.5 (Au-noASP) for the non-porous ones. The highest gain was observed for the brown envelope in the pH 2–3 range, with scores of 1.5 (Au-noASP) compared with 0.5 (Au-Schnetz).

Stricto sensu, it appears that the trend in terms of quality of development is the following: Au-ASP >> Au-noASP ≥ Au-Schnetz.

Discussion

1. Synthetic protocols

When analyzing Au-ASP and Au-Schnetz in terms of nanoparticle size and zeta potential, it appeared that they were almost identical, with no significant differences. Indeed, when

looking at the size distributions, both formulations can be considered as monodisperse with a single, narrow peak (not illustrated). However, when comparing the synthetic protocols, it is clear that Au-ASP affords noteworthy advantages. For Au-Schnetz, it is recommended to bring two different solutions to 60°C before mixing them and allowing them to boil. This monitoring of the temperature requires significant time and attention from the experimenter. For Au-ASP, one solution has to be brought to the boil before a second one is quickly added at once. The protocol is simpler and less labour-intensive for Au-ASP (and for Au-noASP, in the same way). This information is highly valuable for people considering this technique in their laboratory since the labour-intensive synthetic protocol is currently one of the weak points of the MMD/SMD methodologies.

2. Evaluation protocol and score grades

The aim, with this evaluation protocol, was to promote an objective evaluation of the intrinsic quality of each detected half-fingermark (i.e., ridge detail and contrast), without the influence induced by the direct comparison of one technique with another (which is the case when both corresponding half-fingermarks are presented side-by-side to an examiner). A first decision was thus to ask examiners to score half-fingermarks, taken separately, with no information concerning the detection procedure or about the corresponding half. Moreover, two corresponding halves were not presented successively to the examiners. The participation of people having no link with the experimental work was also seen as a guarantee of a non-oriented evaluation of the results.

The examiners had to assess the quality of each half-fingermark according to four grades, which were defined by following recommendations made by Kent [14]. As described in the Material and methods section, the choice for the 4 quality grades (i.e., 0, 1, 2, and 4), without

an intermediate "3" (see Table 2), was directly inspired from Kent's article. We saw this as a good way to prompt people to make a decision, especially when the fingermarks were of a good or very good quality (i.e., it was seen as a good way to avoid the use of an intermediate value, which people would choose by easiness). However, in discussion with Kent, it appeared that an error had been made in the cited article, and that there should have been a "3" score. Given that we had already asked our evaluators to grade the half-fingermarks by considering only four possibilities (i.e., 0, 1, 2, and 4), we couldn't simply replace all the "4s" by "3s" in the score tables (in terms of integrity of the results). This explains why the results presented in this article are still based on the "0,1,2,4" grades. It should be noted that we temporarily replaced the "4s" by the "3s" to ensure that this operation doesn't modify in some way or another the trends reported in this manuscript. It logically did not change anything in terms of the improved performance observed for Au-ASP compared to Au-Schnetz (and Au-noASP). Finally, despite the fact that each examiner had to score the half-fingermarks with, for sole indication, the definitions reported in Table 2, a strong consistency was observed across the five participants. Moreover, no significant difference was observed when comparing the scores given for Au-ASP-processed marks from the first set of experiments (left-oriented halves vs Au-Schnetz) and the second set (right-oriented halves vs Au-noASP). This illustrates that there was no left-half versus right-hald bias effect on the perceived efficiency of each technique.

In order to improve the scoring protocol for forthcoming studies, we are currently working on a way to propose to the examiners only "one-sided" fingermark images. This means, for example, that all right-half marks would be horizontally flipped to look like left-half marks. This transformation would have no effect on the intrinsic quality of the marks (the only effect would be on an identification process, which is out of the scope of such research). By doing

this, we would guarantee that the examiners will not be influenced by the orientation (left or right) of the halved marks. We will certainly also review the scoring grades, and adopt a conventional "0,1,2,3" grade. These modifications will be applied in our forthcoming works.

3. Efficiency of each colloidal gold solution to detect fingermarks

If, from an analytical point of view, Au-Schnetz, Au-ASP, and Au-noASP share similar nanoparticle sizes and zeta potential values, it is interesting to note that the behaviour of these three colloidal gold solutions was shown to be extremely different when used in the SMD method. The results obtained with Au-Schnetz were logically consistent with Schnetz's conclusions concerning MMD-II. Recall that he recommended setting the pH of the colloidal gold solution between 2.5 and 2.8 for optimal results. Additionally, he also concluded that no detection occurred at a pH above 3.5, which is confirmed for all the porous substrates we tested (with the exception of sample #1), if it is considered that an average score superior to 1.0 is required to qualify the detection as being successful (meaning that some ridges could be used in an identification/exclusion process). The sharp decrease in efficiency observed for Au-Schnetz above pH 3.5 logically led to the consideration of two pH ranges: a "lower range" (covering pH 2 to 3) and a "higher range" (covering pH 4 to 5 or 6.7). In this way, the interpretation of the quality scores was much more meaningful than considering the efficiency of the techniques over the whole range of pH values.

As expected, Au-noASP behaved like Au-Schnetz, with a serious drop in fingermark quality above pH 3, since both solutions involved citrate-capped gold nanoparticles only. It should be noted that Au-noASP gave slightly better results than Au-Schnetz, which may be explained by the higher quantity of sodium citrate used in Au-noASP compared with Au-Schnetz –

offering perhaps a better surface covering, leading to better interactions with the secretion residue.

When introducing Au-ASP to replace Au-Schnetz, we observed a global increase in the quality of the detected marks, especially on porous substrates. More interestingly, Au-ASP appeared to be more robust towards increases in the pH value, with marks successfully detected at pH values above 3, even up to pH 6.7. This increased efficiency at pH > 3 when using Au-ASP does not mean that we would recommend setting the pH of the colloidal gold solution at a pH of 4 or 5 before processing the samples of interest. The pH still needs to be lowered below pH 3 to give optimal results. Nonetheless, this ability to detect marks at pH > 3 means that the solution remains able to detect fingermarks even if a sample strongly modifies the pH of the working solution after its immersion (which could happen with some porous substrates), which is not always the case with Au-Schnetz.

4. The mechanistic role played by aspartic acid

The active role played by aspartic acid was demonstrated by comparing the efficiency of Au-ASP with Au-noASP. In this case, only Au-ASP was able to detect marks with good quality above pH 3. It is possible to explain the higher quality of the marks processed by Au-ASP at low pH by a more resistant negative charge below pH 3 (aspartic acid being characterized by a lower pKa value) resulting in increased electrostatic interactions. However, the exact role played by aspartic acid is still unknown for higher pH values. In this case, the negative charge of the gold nanoparticles is no more a problem (as it increases progressively), but the charge on the secretion residue decreases to reach neutrality. This means that, under such conditions, the electrostatic attraction theory is no longer able to fully explain why Au-ASP is still able to detect marks. Further experiments are consequently required to fully understand which

chemical process could explain the unique efficiency of Au-ASP at pH > 3. Since it is expected that some adsorbed citrate ions are replaced by covalently-bound aspartic acid molecules, the amino acid nature of aspartic acid could consequently play a role when interacting with the secretion residue. For example, amide bonds may be created, as well as a network of H-bonds as has been observed with cysteine-capped gold nanoparticles [15]. Moreover, it also seems clear that the presence of aspartic acid has an effect on the interaction of Tween 20 with the gold nanoparticles. Indeed, it has been observed that the zeta potential decreases much more for Au-noASP (from -45.6 mV to -11.7 mV) compared to Au-ASP (from -42.7 mV to -24.5 mV) once Tween 20 is added in the working solution. One explanation for this phenomenon could be that Tween 20 interacts with gold nanoparticles through a ligand-exchange process, replacing some adsorbed citrate ions. This process may be hampered in the case of covalently-bound aspartic acid molecules, resulting in a reduced Tween 20 covering which means less screening effect (higher zeta potential).

Conclusions

This work is part of a continuing goal to improve the multimetal deposition technique (MMD), to make it more robust, more user-friendly, and less labour-intensive. Among the successive developments that were published, it is possible to identify two major evolutions of the techniques: (1) MMD-II, for which both the colloidal gold and enhancement step were optimized compared to MMD-I; and (2) single metal deposition (SMD), for which the enhancement step was optimized compared to MMD-II, while keeping the colloidal gold step unchanged. Despite the latter developments, the technique still suffered from limitations, particularly in relation with the colloidal gold: (1) a labour-intensive synthesis (for MMD-II and SMD); and (2) a protocol highly sensitive towards experimental parameters, such as the pH of the solution.

Concerning the synthetic protocol, the alternative procedure proposed in this study consists in first bringing to the boil a solution of tetrachloroauric acid ($HAuCl_4$, precursor of the gold nanoparticles) in an Erlenmeyer flask, before quickly adding the solution containing the reducers (i.e., sodium citrate and aspartic acid). After 5–10 minutes, the solution is characterized by a ruby-red color, characteristic of nanoparticles of ca. 15 nm in diameter. Through this alternative synthetic protocol, there is no longer a need for someone to monitor the temperature of two beakers using two thermometers.

The use of aspartic acid (at a molar ratio of 1 % with sodium citrate) during the synthesis of the colloidal gold was also proposed. The obtained gold nanoparticles afforded an interesting and valuable behaviour, especially by being able to detect fingermarks at high pH values (at least up to pH 6.7) when used in the SMD method. Recall that multimetal deposition (MMD-I) and its alternatives (i.e., MMD-II and SMD) are limited to an upper pH limit of 3, above which a sharp decrease in efficiency is observed, with almost no fingermarks detected on most substrates. The addition of aspartic acid has for effect an extension to the range of pH for which fingermarks (and more particularly, ridge detail) can be observed. Through the use of Au-ASP, the dependence towards a pH-meter is greatly reduced since it is no longer required to accurately set the pH of the colloidal gold solution inside a narrow range of values. It is still recommended to set the pH between 2 and 3 for optimal results, but we have shown that if the pH is marginally too low or too high then this will not hamper the detection of fingermarks. It also means that if a substrate raises the pH of the colloidal gold solution then it will not necessarily mean that no fingermarks will be detected (which can be the case with the current multimetal deposition techniques).

Further research is on-going, more particularly to optimize the use of Au-ASP (and, consequently, to propose an optimized "SMD-II" protocol) and to try to understand the role played by aspartic acid in the extended effectiveness of the method.

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Figure captions

Figure 1

Schematic illustration of the two steps characterizing the multimetal deposition technique (MMD): gold nanoparticle deposition followed by metal enhancement. This chart is valid for MMD-I, MMD-II, and SMD, which differ in regards with the colloidal gold synthesis protocols or the metal used for the enhancement. (Image source: [16])

Figure 2

Evolution of the multimetal deposition technique, from MMD-I to "Au-ASP" (as a premise of a forthcoming "SMD-II"). Each major modification between a technique and its evolution is illustrated by a green arrow.

Figure 3

Illustration of the acid/base forms of (a) sodium citrate and (b) aspartic acid, in regards with the evolution of pH. On both sides of a pKa value, the specie which predominates in solution is illustrated. It is also believed that aspartic acid is covalently bound to the gold nanoparticles through its amine group.

Figure 4

Hypothesized evolution of the charge of the secretion residue (+) and the citrate-capped gold nanoparticles (-) in regards with the evolution of pH. The narrow pH range, considered as being optimal for the multimetal deposition technique, is illustrated in green.

Figure 5

Chart comparing the efficiency of Au-ASP and Au-Schnetz in terms of fingermark quality (ridge details and contrast). The illustrated scores are the averaged values (taking into account

the scores of all five examiners) associated with each sample for the pH range 2-3 (up) and 4-5 (below). Au-ASP is depicted in blue, and Au-Schnetz in orange.

Figure 6

Evolution of the efficiency of the SMD to detect fingermarks by using Au-ASP (left halves) and Au-Schnetz (right halves) at different pH values, for (a) recycled paper - sample #2 from Table 1, (b) grey paper envelope - sample #6, and (c) polypropylene sleeve - sample #13.

Figure 7

Chart comparing the efficiency of Au-ASP and Au-noASP in terms of fingermark quality (ridge details and contrast). The illustrated scores are the averaged values (taking into account the scores of all the examiners) associated with each sample for the pH range 2-3 (up) and 4-6.7 (below). Au-ASP is depicted in blue, and Au-noASP in violet.

Table caption

Table 1

Details of the 14 samples that were considered during this study, in terms of substrate nature, donor, and fingermark age. For each sample, two fingermarks in depletion were considered, which were further cut in half, vertically, to compare two techniques or two sets of parameters.

Table 2

Table describing the four quality grades aiming at assessing the quality of the questioned half-fingermarks (the same table was given to each examiner).

Table 3

Physico-chemical characteristics of the three colloidal gold solutions (i.e., Au-Schnetz, Au-ASP, and Au-noASP) in terms of nanoparticle diameter and zeta potential, after synthesis ("no Tween 20") and at different pH values after the addition of Tween 20.

Table 4

Average quality values of the fingermarks detected using either Au-ASP or Au-Schnetz. The values reported in the table were calculated on the basis of the average quality scores given by the examiners, further grouped according to the substrate nature or the pH range ("full" – from 2 to 5, "lower" – from 2 to 3, and "higher" – from 4 to 5).

Figure 1
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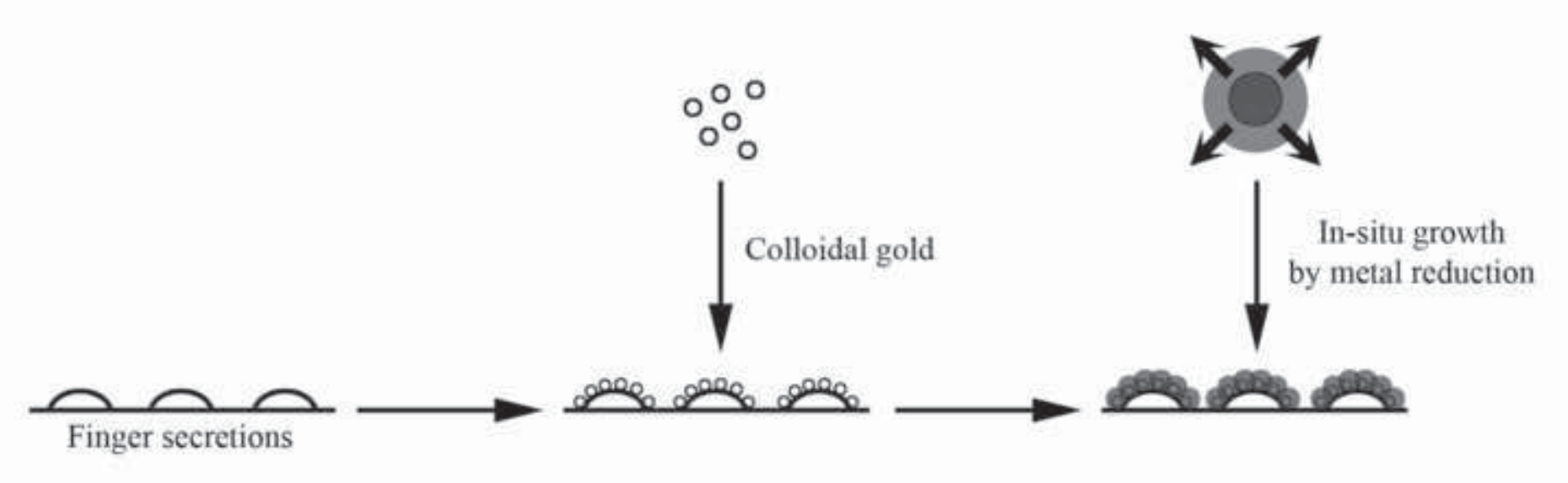

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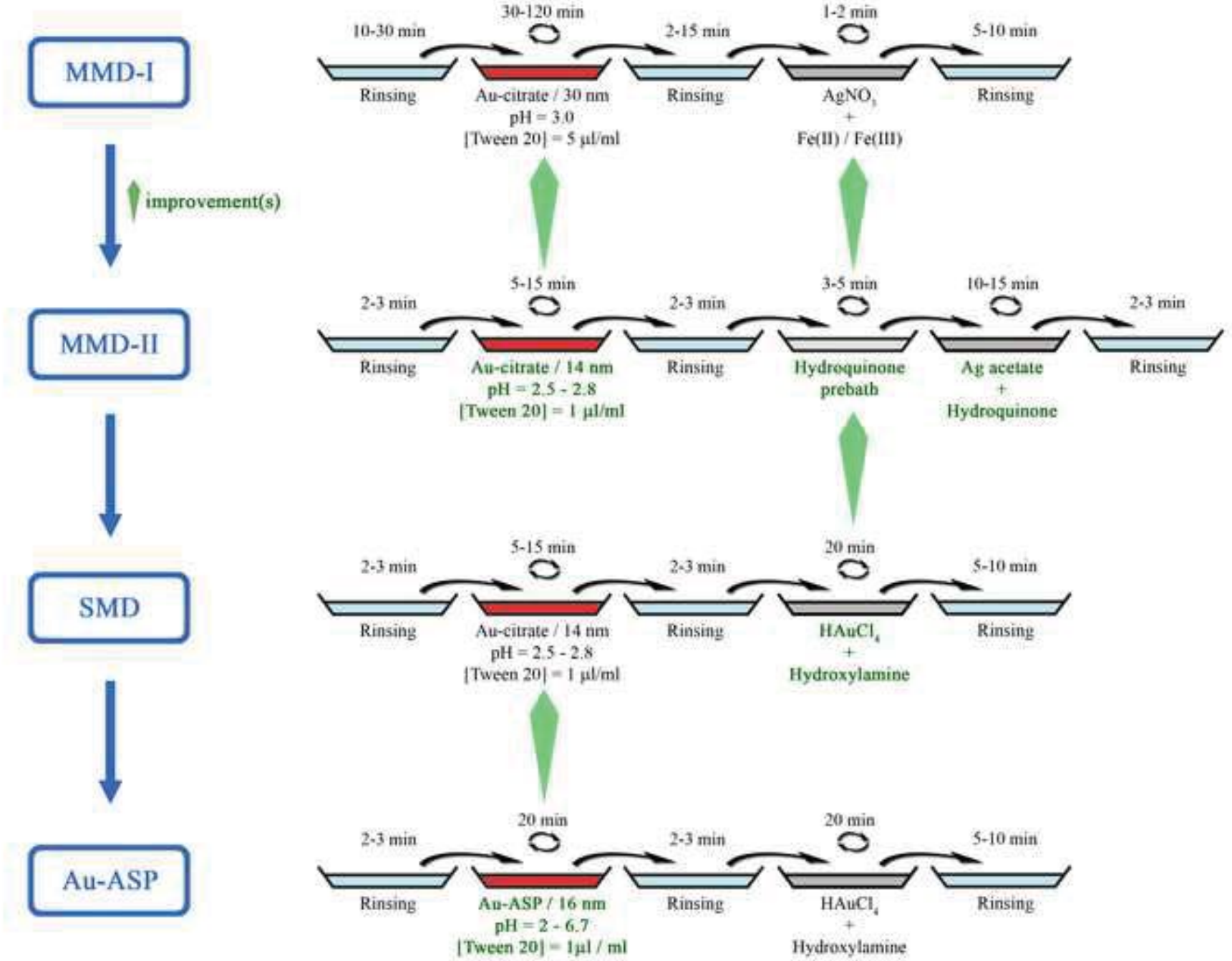

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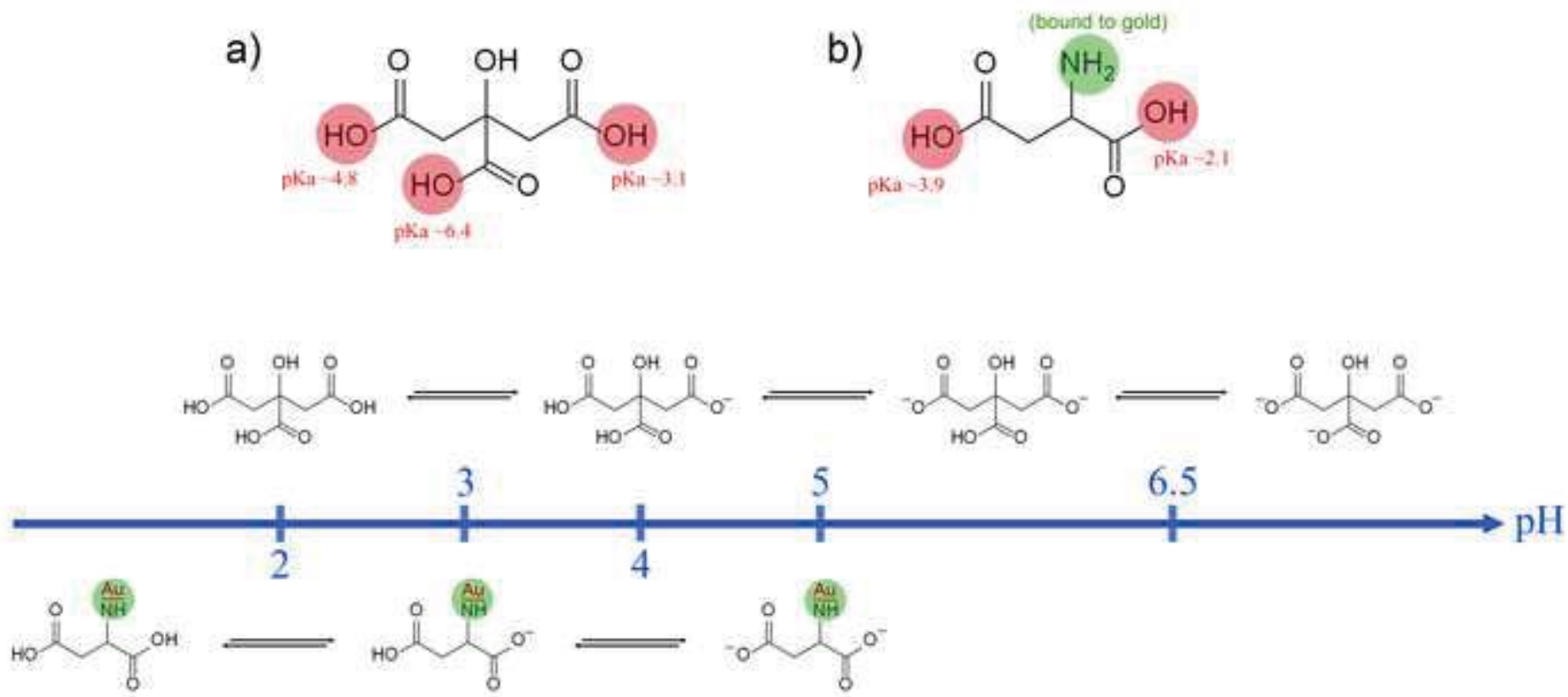

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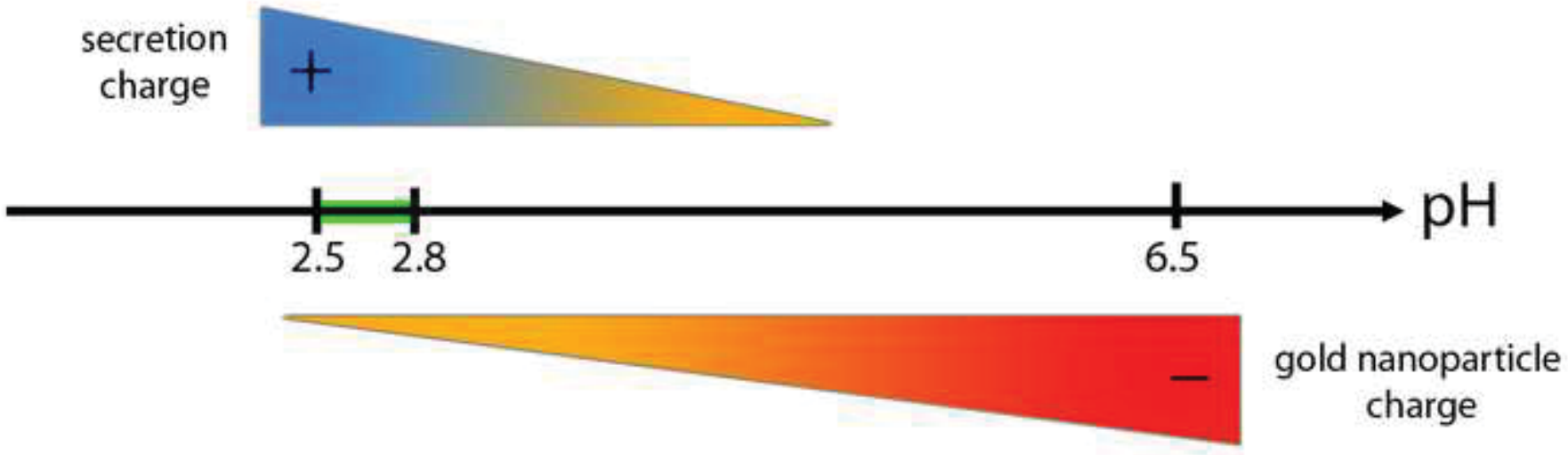

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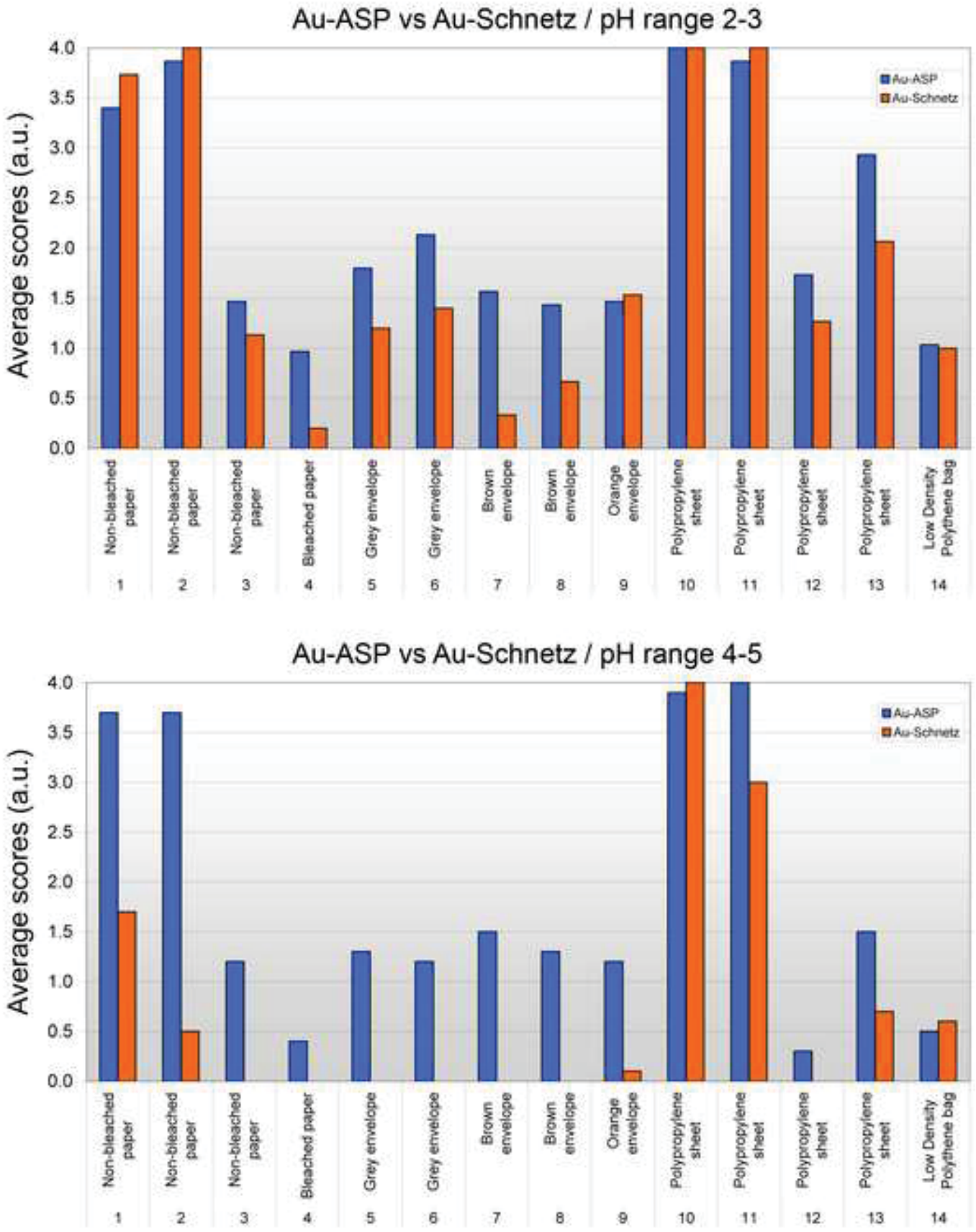

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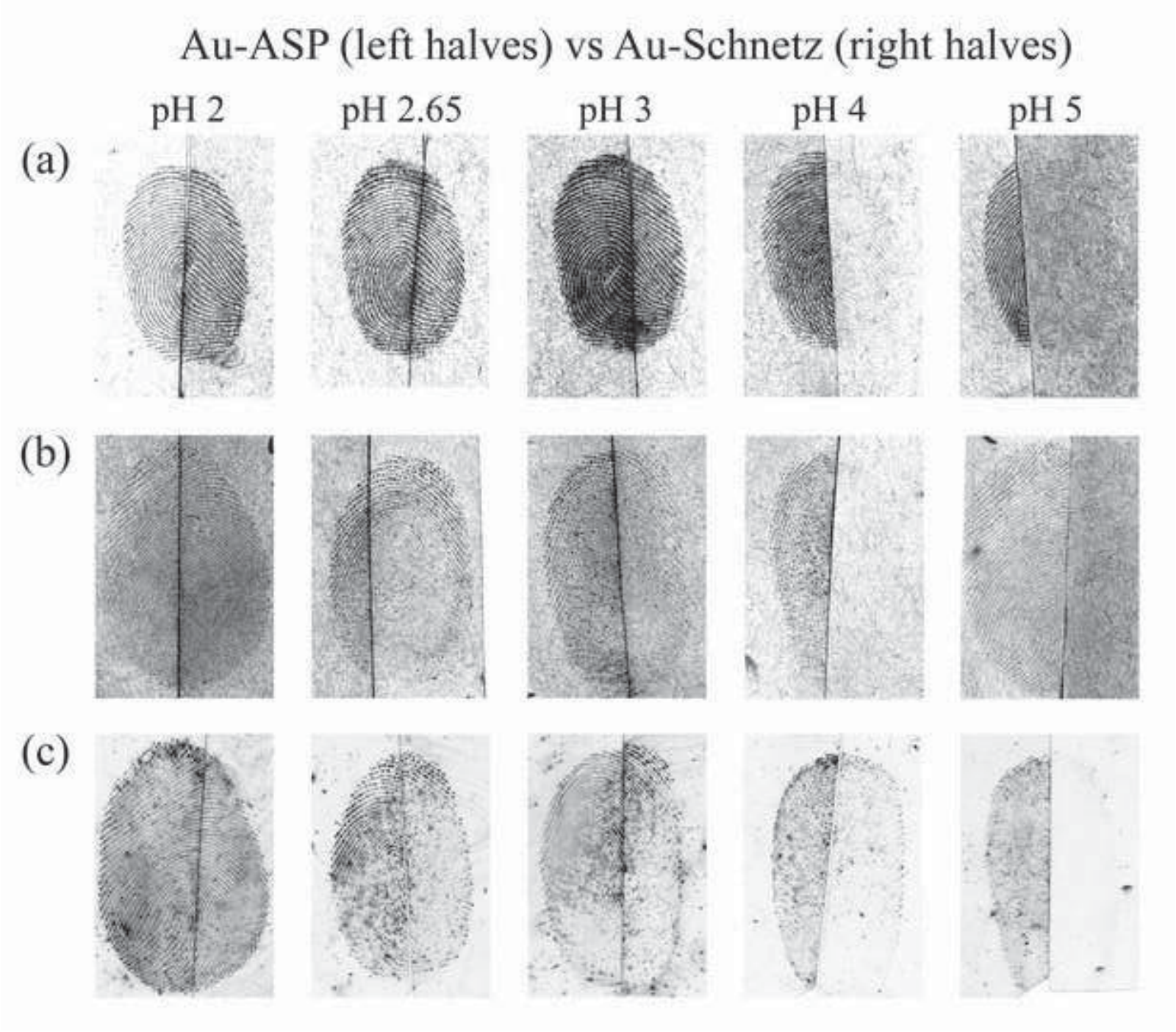

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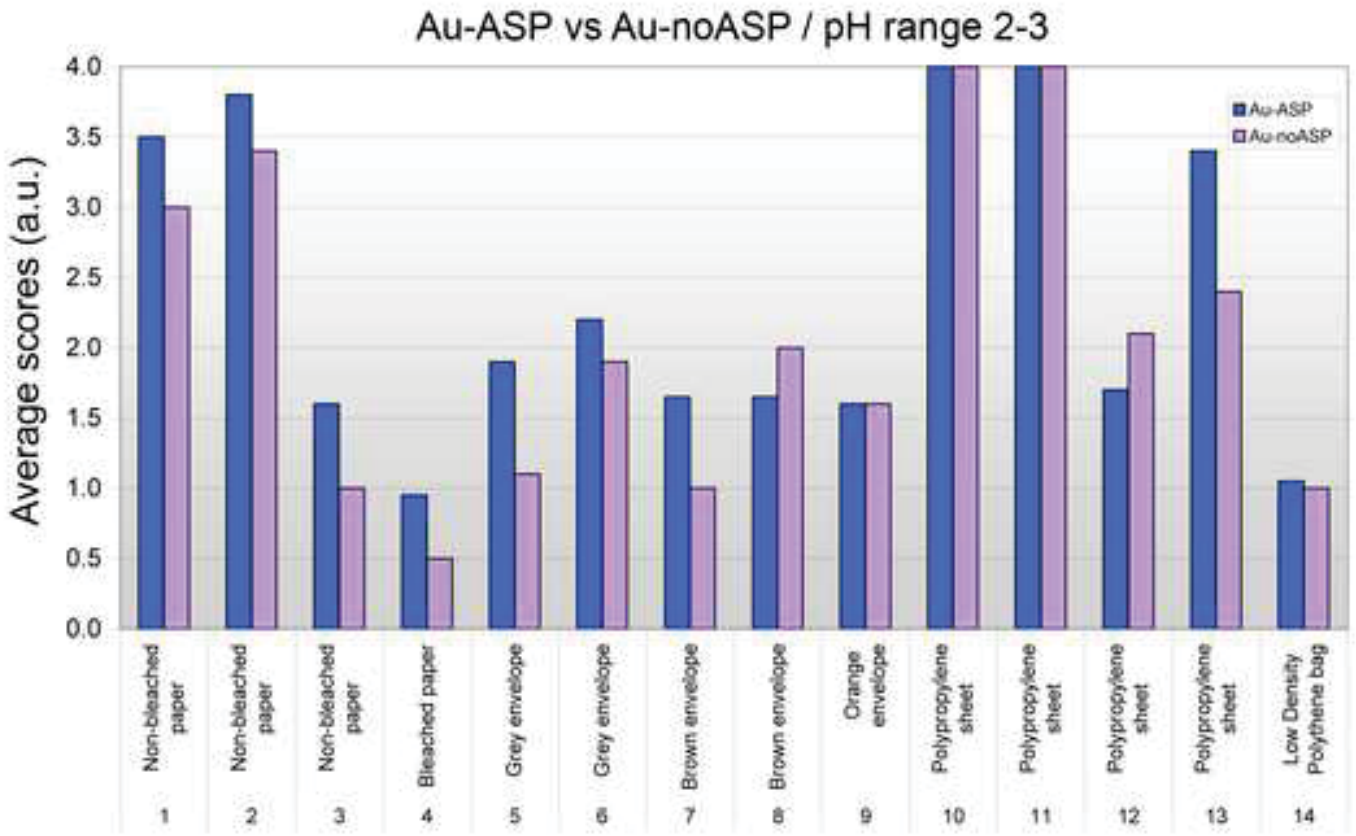

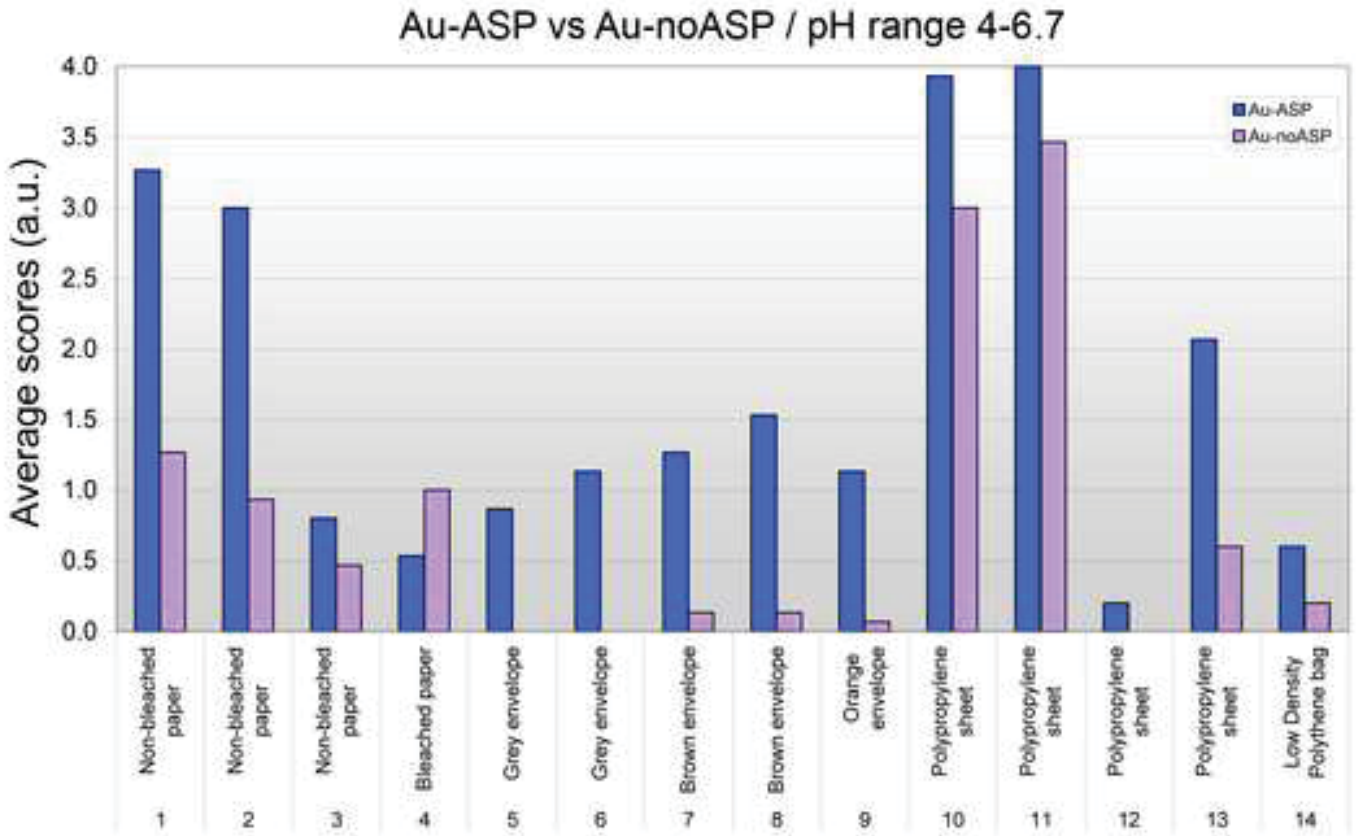

Table 1

Surface type	Sample #	Substrate	Brand/Supplier	Donor	Age of the marks
Porous	1	Non-bleached paper	Schneidersöhne Recyconomic	D/1	1 week
	2		Schneidersöhne Recyconomic	D/1	5 months
	3		M-Office (CH)	D/2	6 years
	4	Bleached paper	Xerox Business	D/2	6 years
	5	Paper envelope / grey	Goessler (CH)	D/3	1 month
	6		Goessler (CH)	D/4	1 month
	7	Paper envelope / brown	Goessler (CH)	D/3	1 month
	8		Goessler (CH)	D/4	1 month
	9	Paper envelope / orange	Goessler (CH)	D/3	1 month
Non-porous	10	Polypropylene sleeves	Office world (CH)	D/1	1 week
	11		Office world (CH)	D/1	5 months
	12		Migros (CH)	D/5	6 years
	13		Coop (CH)	D/4	1 month
	14	Low Density Polythene bag	Migros (CH)	D/5	6 years

Table 2

Score	Description
0	No ridges are visible at all, no sign of fingermark.
1	Ridges are visible over a small area of the mark or over the whole mark, but it is extremely difficult to retrieve second level characteristics (such as minutiae) due to extremely poor ridge details.
2	Ridges are visible on almost the whole area of the mark, and second level characteristics can be retrieved. Nevertheless, the quality is not optimal due to a low contrast (strong background staining or faint ridges).
4	Ridges are very well defined on the whole mark. Second level characteristics can easily be retrieved. The contrast is optimal with no (or extremely faint) background staining.

Table 3

Diameter size (nm)				
	pH	**Au-Schnetz**	**Au-ASP**	**Au-noASP**
No Tween 20	Initial	17.8 ± 0.4	16.5 ± 0.7	13.5 ± 0.3
With Tween 20	Initial	19.2 ± 1.3	18.1 ± 0.4	14.7 ± 0.7
	5	20.0 ± 0.5	17.2 ± 2.2	13.3 ± 0.7
	4	20.0 ± 0.6	17.4 ± 0.5	11.4 ± 1.9
	3	17.7 ± 0.9	18.2 ± 0.5	12.5 ± 0.9
	2.65	17.9 ± 0.8	17.0 ± 1.0	(not measured)
	2	17.2 ± 0.8	17.3 ± 1.4	12.9 ± 0.5

Zeta potential (mV)				
	pH	**Au-Schnetz**	**Au-ASP**	**Au-noASP**
No Tween 20	Initial	-47.3 ± 0.7	-42.7 ± 2.3	-45.6 ± 0.5
With Tween 20	Initial	-22.8 ± 0.5	-24.5 ± 1.1	-11.7 ± 0.6
	5	-19.6 ± 1.3	-21.2 ± 1.6	-13.1 ± 1.8
	4	-19.7 ± 1.6	-21.5 ± 1.5	-11.2 ± 0.8
	3	-15.7 ± 0.8	-17.0 ± 0.7	-8.3 ± 2.3
	2.65	-14.0 ± 0.5	-15.0 ± 1.4	(not measured)
	2	-6.7 ± 0.4	-8.9 ± 0.8	-4.2 ± 0.5

Table 4

Substrate nature	pH range: 2 – 5		pH range: 2 – 3		pH range: 4 – 5	
	Au-Schnetz	Au-ASP	Au-Schnetz	Au-ASP	Au-Schnetz	Au-ASP
Non-bleached paper	2.1	2.9	3.0	2.9	0.7	2.9
Bleached paper	0.1	0.7	0.2	1.0	0.0	0.4
Paper envelope / grey	0.8	1.7	1.3	2.0	0.0	1.3
Paper envelope / brown	0.3	1.5	0.5	1.5	0.0	1.4
Paper envelope / orange	1.0	1.4	1.5	1.5	0.1	1.2
Porous surfaces	**1.0**	**1.9**	**1.6**	**2.0**	**0.3**	**1.7**
PP sleeve	2.5	2.9	2.8	3.1	1.9	2.4
LDPE bag	0.8	0.8	1.0	1.0	0.6	0.5
Non-porous surfaces	**2.1**	**2.4**	**2.5**	**2.7**	**1.7**	**2.0**