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Activity

Chemistry through Tattoo Inks: A Multilevel Approach to a Practice on the Rise for Eliciting Interest in Chemical Education

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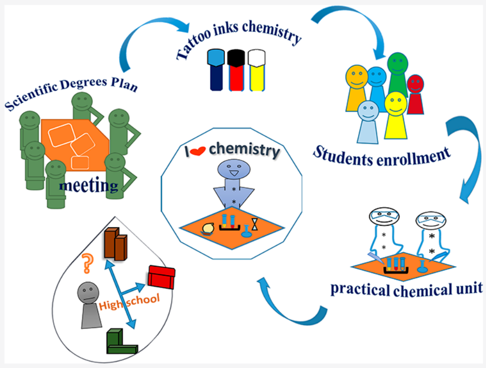
ABSTRACT: Within the framework of a nationwide project to boost students' enrollment in scientific disciplines in Italy, a multilevel science project was designed with a focus on the chemistry of tattoo inks, offering immediate connection with 16−18 year-old high school students. The approach takes into account time constraints, since all sessions have a maximum span of 8 h, and the heterogeneity of the audience, made up of students without background restrictions. Tattoos are perceived as a form of body art and can be conveniently used as the "A" in the STEAM (science, technology, engineering, art, and mathematics) methodology. The project involved active lectures and guided inquiry into the simple chemical concepts related to tattoo inks, addressed in practical units with multioutcome experiments and comparative instrumental analysis. The connections with correlated issues, such as norms regarding tattoo ink composition and verification, were also discussed. The efficacy of the tattoo ink Scientific Degree Plan experience was then evaluated through two types of surveys: one on the enjoyment of the plan and the other on mastery of the chemical concepts at the end of the experience.



KEYWORDS: *General Public, High School/Introductory Chemistry, Inquiry-Based/Discovery Learning, Student-Centered Learning, Dyes/Pigments, Chromatography, Safety/Hazards*

INTRODUCTION

The Italian Ministry of University, Research and Public Education implemented the Scientific Degrees Plan (SDP) in 2014, in cooperation with the National Manufacturers' Association and the National Conference of Deans of the Faculties of Science and Technologies.[1] The national SDP project was designed to promote enrollment in science degree courses, with the 2-fold aim of directing high school students toward studies with more promising potential employment perspectives, and allaying insufficient coverage of job openings in scientific fields.[2] Science education in general, of chemistry in particular, is often perceived as a complicated subject, irrelevant to daily life, in many countries. The associated image of being out-of-reality, or indeed dangerous,[3] has led to low enrollment levels,[4] especially compared with those of other countries.[5] The national SDP was conceived as a support platform to provide active orientation for students. It is aimed at teenagers, mostly 16−18 year-olds in their last years of high school, and is designed to provide them with a less scary, more friendly, and more appealing perception of science, particularly with regard to our department of chemistry. The task is made difficult by the heterogeneity of the target audience, since admission to chemistry degree courses in Italy is open to students of all backgrounds without restrictions. This implies, for instance, that the faculty of chemistry can be equally accessed by students from institutes of arts or of agriculture, or from gymnasiums. Thus, some may have lab experience, while others none at all; likewise, some may be familiar with instrumental analysis while others know little beyond the rudiments of chemistry.

Within this framework, each campus establishes its own educational plans, providing guidance activities for students and teacher training. The developed guidelines also aim to favor gender balance in degree enrollment to counteract the unequal gender distribution in STEM disciplines,[6] and to reduce the dropout rate during the first years of the degree course. It also aims to further innovative educational methodologies favoring motivated participation of the students in the activities in equipped laboratories. Furthermore, it aims at intensifying the relationship between the school and university systems by promoting laboratory activity, representing the characterizing factor of the entire project.

In seeking ways to elicit the students' interest in chemical science, a theoretical−practical experience course on "Tattoo inks chemistry" was drawn up, directed at high school students and designed to take place at a university campus under the

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supervision of university personnel, in cooperation with high school teachers.

In the STEAM methodology, arts integration into STEM is considered beneficial to engage minority and female students.[7] As jobs increasingly rely on technology and integrated STEM skills, all students need opportunities to develop their creative capacities along with their scientific skills.[8] In general, the project integrates and uses the arts to help express STEM concepts, thus offering contextual learning and utilizing subject overlap for greater understanding.[9] In a cross-disciplinary approach, it focuses on observing one discipline through the perspective of another one.[10] Overall, the integration of creativity into STEM disciplines may also help critical thinking regarding real problems, facilitating science learning[11] and increasing motivation toward STEM education.[12]

Tattooing is a body art of increasing popularity, especially among young people,[13] that is also growing in social acceptance.[14] Moreover, it is an excellent tool for implementing divergent thinking within the STEAM framework. It offers the opportunity for a cross-disciplinary approach, where the chemistry of inks is linked to the appeal of images expressed through drawings on the skin.

The choice of topic proved to be effective in gaining the attention of the students who took part in the SPD experience, since a majority admitted to have at least one tattoo or to be thinking of getting one. They thus showed interest in the several connected aspects that were presented, which they could experience in lab themselves.

Previously, Chamberlain and Rogers[15] introduced tattoos and their removal within a 4 week summer camp for high school students on the application of biochemistry.

A different approach was used by Stuckey and Eilks targeting ninth graders of a comprehensive school, hence, 14−15 year-old students,[16] and focusing on the relevance of science learning. In particular, their paper operationalizes a theoretical framework that reorganizes the different understandings and dimensions of the relevance of science education. Then, they drew a lesson focusing on "both the chemical aspects of tattoo inks and the societal and vocational issues surrounding the practice of tattooing".[16] The intervention was, then, evaluated in terms of motivation.

The SDP plan is directed toward students in grades 12 and 13, who have already selected the curriculum of their high school degree and are actually completing it, and who admittedly have or are planning to have a tattoo soon. Therefore, we opted for a different approach stemming out of their choice of the type of tattoo and what it can express, and connecting them to different aspects of chemistry. The inquiry-learning approach in the STEAM framework offers greater flexibility in adapting the lessons units to the specific output of the audience. Furthermore, we take the option to introduce them to university environment and make them familiar with laboratories and equipment for instrumental analysis.

We believe this could be a platform of more general interest, based on guided inquiry, multioutcome experiments and comparative instrumental analysis.

■ DEVELOPMENT OF THE TATTOO INK EDUCATIONAL PLATFORM

The boundary condition of an SDP experience is the limited time span, since it is supposed to be bookended in a day dedicated to the purpose; hence, the time span is 8 full hours, with opportune breaks, where theoretical and practical units take place. In order to motivate participants toward chemical studies in 1 day, we tried to shape the experience as a meaningful blending of chemical inputs and experimental counterparts, within the topic of tattoos. In drawing up the framework for the SDP experience, we followed the classical approach of providing theoretical units first, because we needed to provide a common background to a heterogeneous group and because we needed to ensure all participants had proper safety instructions before entering the lab.

The structuring of the platform went through different stages, including the choice of the aspects related to the tattoo inks to present and how they would be implemented in the theoretical and in the practical units.

- Aspects to highlight: They have to be fundamental, easy to grasp, and strongly related to the properties of tattoo inks. They should be of immediate impact, be open to further development, have a good basis for discussion, and be verifiable in the lab right after the discussion. To this end, solubility/dispersibility is an optimal topic, since the basic concept is immediate. In general, it is essential that inks contain pigments; hence, they are coloring agents that are insoluble in any solvent, though with some modifications to ensure injectability. Ensuing experiments on solubility/dispersibility can easily be planned.
- Topics to choose: The topics related to tattoo inks allow intersections with other disciplines to feed interests in branches of chemistry with applications in various fields. Labeling issues are thus an important topic and are easy to verify, such as by reading and comparing tattoo ink labels.
- Modalities of the theoretical units: Active learning, where students are engaged in the learning process, was deemed as a proper approach, since it is often considered a way to maximize learning performance.[17] Furthermore, it reportedly favors the achievements of underrepresented students,[18] and a system to support STEM education.[19] In this case, the active lecture is an effective asset:[20] no matter what, everybody has on opinion on tattoos, and any opinion can be a starting point for a pathway leading to the inks' properties. The development of the units was designed to create intersecting topics, with the purpose of providing a common background, thus connecting chemistry to other fields. Thus, a guided inquiry[21,22] was set up to appraise the logic of the topics and approach the subsequent practice with appropriate conceptual tools.
- Modalities of the practical units: We opted for multi-outcome experiments (MOEs) for providing the participants with the ownership of their experience.[23] We find this to be a fundamental point, especially for experiments which need to be performed in a short time span, because it requires the participants to critically analyze and interpret their acquired individual results, thus gaining a more thorough understanding of the concepts illustrated through the theoretical units and boosting self-confidence with respect to lab activity. Furthermore, the type of activity is designed to address both algorithmic and conceptual thinking in problems solving.[24] To this end, combined lab and instrumental analyses were foreseen in metal determination, as part of the lab activity.

Finally, the teaching, learning, and training activities were evaluated through two types of surveys, one to assess the

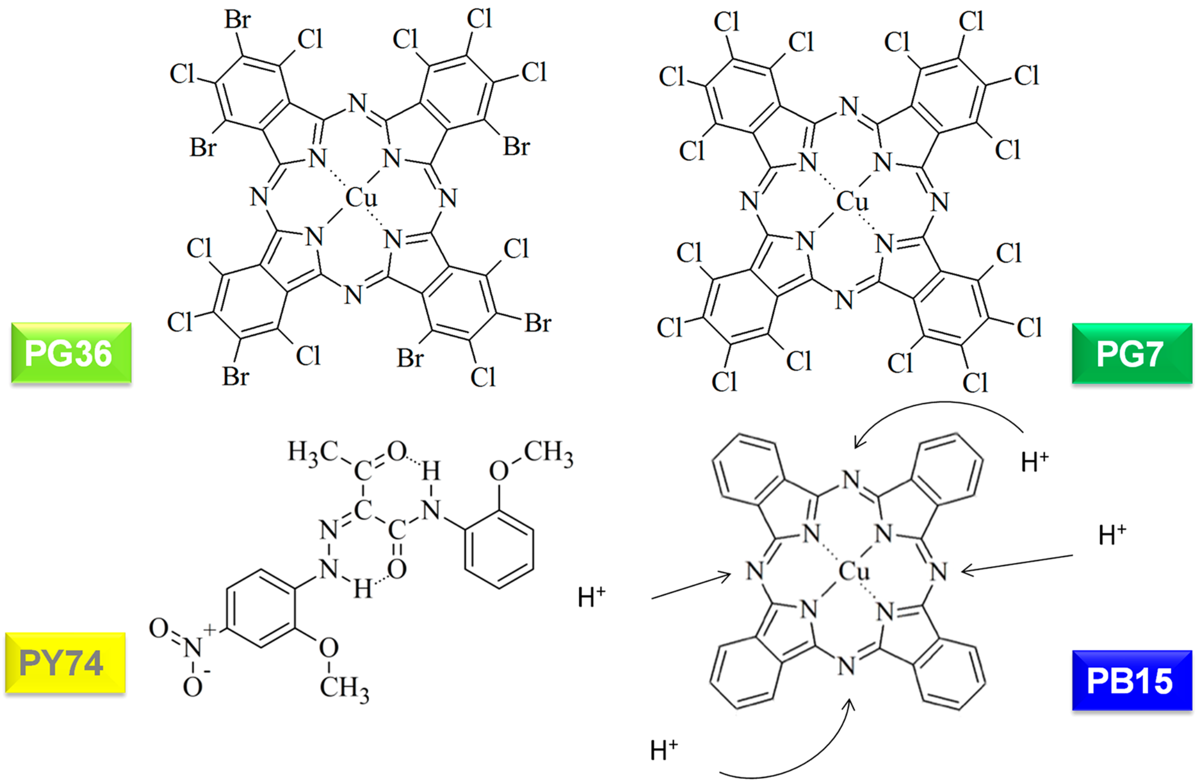

Figure 1. Sketch of the pigments used during the chemical tests. The arrows indicate the bridge position of CuPC (PB15) which can be protonated in an acidic environment.

enjoyment of the SDP experience, and the other to monitor how familiar the students became with the proposed chemical concepts.

■ BACKGROUND INFORMATION ON TATTOO INKS: COMPOSITION AND REGULATIONS

Tattoo inks are typically made of two components: the pigment and the vehicle. The pigment imparts the color, and it is by definition insoluble in all solvents; the vehicle is a mixture of compounds that has the function of guaranteeing viscosity and hence injectability under the skin while ensuring suitable antimicrobial properties. The use of pigments instead of colorants is an essential issue when tattooing, because the coloring agent under the skin must not be either water- or fat-soluble; otherwise, it does not remain localized to produce an underskin pattern. Nonetheless, some additional treatment of the pigments is necessary to allow a small degree of solubility for blending with the vehicle and avoiding separation of the ink into its components. This treatment is known as a finishing procedure, and it is typically undisclosed by manufacturers. The materials used as pigments are extremely varied and have changed enormously over the years, partly because of the pursuit of more brilliant colors, partly because of the availability of pigments, and ultimately because of the full-blown toxicity of some of them, such as chromium compounds. On the other hand, tattooing is a very ancient practice, with the first tattooed body being Ötzi in the late Neolithic[20] age. Since then, there has been considerable variability in the composition of the inks used. An initial broad categorization of pigments distinguishes between the inorganic and organic, with the former being mostly metal oxides and sulfides with the addition of carbon black, and the latter being macrocycles such as phthalocyanines, azide compounds, and quinophthalone,[25] though such a distinction is somewhat labile. Common tattoo inks in western markets are mostly metal sulfide and oxide free, with TiO_2 being one of the few accepted ones as part of white inks, or as a lightening agent of other colors. However, the real composition of inks is quite often an unknown, because very few countries have clear legislation on permitted and restricted pigments and other components. First, Switzerland[26] and Germany,[27] followed by Sweden,[28] The Netherlands,[29] France,[30] and Spain,[31] issued regulations on tattoo inks and pigments. Because of such regulations, it is quite common to find tattoo inks with composition labels in the European market (though it is compulsory only in a few states). Also, in parallel, numerous regulation violations were revealed.[32,33] Italy is one of the European countries in which such legislation has not yet been implemented. In 2016, a law was proposed on safety conditions for tattooing, but it has still to be passed. The United States lies in a blurry zone, since tattoo inks and pigments fall under regulation of the Food and Drug Administration (FDA)[34,35] and are itemized in the Code of Federal Regulations.[36] Within this framework, no color additives have been approved by the FDA for use in injections, including tattooing. The practice of tattooing is regulated by single states and local jurisdictions, with the FDA monitoring color additives and adverse events. No composition labeling is required for inks in the US market. The Asian market is wide, complex, and hard to categorize. On top of this, it is difficult to gather information on existing regulations. In spite of this, the goods flow is remarkable, and the composition of products from and for eastern markets is an issue, as indeed is the labeling of inks. A clear issue related to the inks and their safe usage is the metal content. Some metals such as Ti or Cu are necessary to impart the color and are considered harmless;[37,38] others are allergenic or toxic, such as Ni,[39] Cr,[40] or Co.[41]

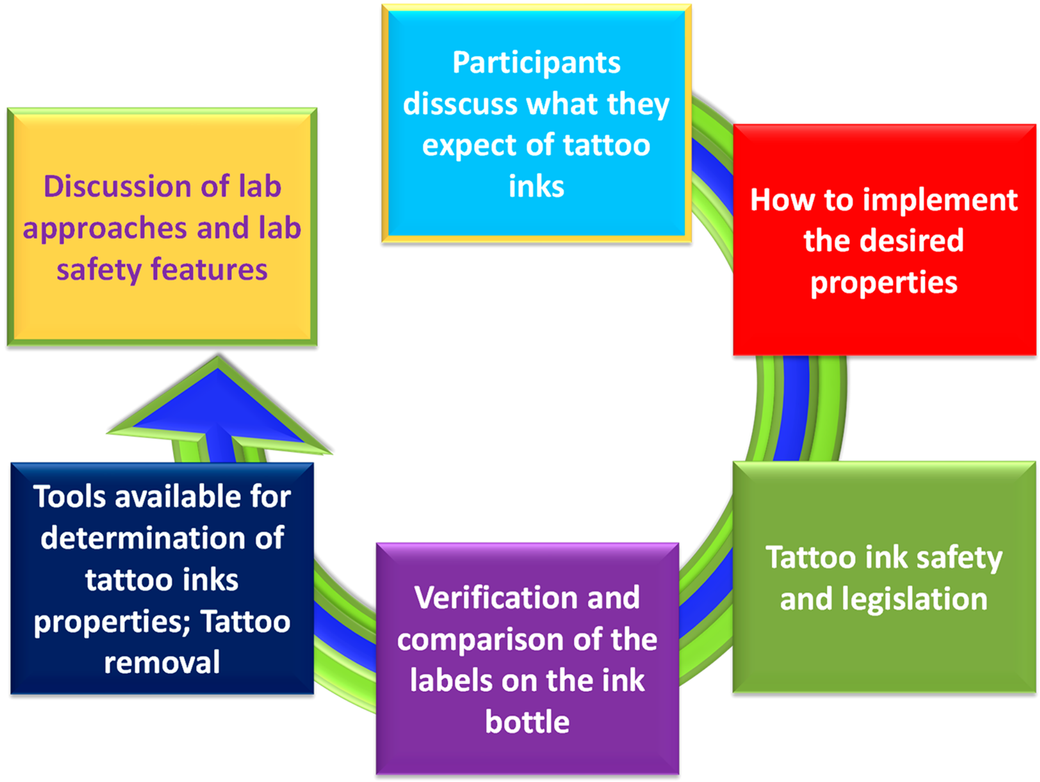

Figure 2. Guided inquiry of the tattoo ink theoretical units within the SDP. The inquiry starts at the light blue box, with the yellow frame, and it moves clockwise, toward the yellow box, with the green frame.

■ MATERIALS, HAZARDS, AND INSTRUMENTAL ANALYSES

The materials used in the SDP laboratories are either inks or pigments, which are supposed to be injected under the skin. Some of the pigments used are purchased in local stores which specialize in raw materials for arts and conservation for artists, hence not requiring further precaution. The chemical tests require the use of routine solvents, such as water, acetone, and a few acids and bases. However, for good lab practice, the chemical units were carried out according to the safety regulations by using personal protective equipment. The handling of hazardous materials used for the tests was done in safe conditions under a tutor's supervision.

In more detail, four pigments were used in the lab: PG36, i.e., hexabromodecachloro copper phthalocyanine; PG7, i.e., hexadecachloro copper phthalocyanine; PB15, i.e., α-copper phthalocyanine (α-CuPc); and PY74, i.e., 2-((2-methoxy-4-nitrophenyl)azo)-*o*-acetoacetanisidide. A sketch of the used pigments is presented in Figure 1

Among them, PG7 is a restricted green pigment in tattoo inks in the countries where regulations were issued, whereas PG36 is a permitted green. As far as PB15 is concerned, it is a blue pigment and comes in several "flavors", i.e., in different phases or polymorphs.[42] In particular, 5 polymorphs are listed among the pigments commonly used for painting and coloring purposes: unstabilized α-CuPc (PB15:0), noncrystallizing α-CuPc (PB15:1), β-CuPc (PB15:3), nonflocculating β-CuPc (PB15:4), and ε-CuPc (PB15:6).[43,44] Only PB15:0 is considered safe.[31]

PY74 is a yellow azo compound; its color strength is higher than that of normal azo pigment, and it is a major pigment in several tattoo inks.

Solvents and reagents used in the SDP laboratories are propan-2-ol, acetone, THF, 1 M HCl, 1 M NaOH, and deionized water. Propan-2-ol is a customary component of the tattoo inks as a preserving agent with some biocide properties.

A total of 20 different inks of different colors were examined. Eight of them were branded Eternal Inks, Inc., Munich, and officially imported: white, black, yellow, red, blue, green (three types). Since this group of inks was destined for the European market, the composition was featured on the bottle labels. A second group of 7 inks of different colors was branded Solong. In spite of the different colors, all bottles had the same composition listed on the label. Five edible natural inks were included in the study. Edible inks have recently been introduced in the markets as more brilliant and attractive color variants. No composition was declared on the labels despite the proclaimed naturalness and edibility.

The EDX analyses were made by coupling the field emission scanning electron microscope (SUPRA 35, Carl Zeiss SMT, Oberkochen, Germany) with energy dispersive microanalysis (EDS/EDX, INCAx-sight, Model: 7426, Oxford Instruments, Abingdon, Oxfordshire, UK), operating at 20 kV.

■ METHOLOLOGICAL APPROACHES AND EXPERIMENTAL DESIGN

The methodological approaches used in the SDP program were MOEs designed to provide tools both for people with a more pronounced algorithmic attitude in problem solving as well as conceptual thinking. The chemistry concepts used in the SDP program are solubility/dispersibility, metal content, and separation by chromatography, addressed both by chemical tests and by instrumental analysis. Due to the heterogeneity of the groups, theoretical units always preceded the experimental

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J. Chem. Educ. XXXX, XXX, XXX−XXX

ones, to make sure that proper safety instructions were enacted. Inks can be complex and also a big unknown, without background information. Guided inquiry and active learning were embraced for gathering attention and stimulating interest.

■ THEORETICAL UNITS

The theoretical units open with a discussion on the properties of tattoo inks as opposed to the participants' expectations for how tattoo inks should be. This is a key point of the whole program, because it is the contact point between the students' perceptions and chemistry. A well-defined tattooed drawing, with a colorful, brilliant appearance, along with nice 3D effects, appeals to the audience and engages its attention. The next step in the development of the theoretical units is how to implement these characteristics, beyond the skills of the tattooist, who cannot do much with poor quality inks. At this point, basic concepts of chemistry applied to tattoo inks can be introduced by addressing the questions of solubility/dispersibility, molecular properties that guarantee the colors and their stability through time. The guided inquiry follows the scheme of Figure 2, and it is designed to draw the attention of the audience toward the chemical aspects of the tattoos, simultaneously addressing participants with different interests and different ways of thinking. The legislative aspects were introduced for those who are more interested in science regulations and are ultimately more oriented toward the forensic aspects of science, whereas composition and associated techniques are more suited to those attracted by the analytical aspects. Properties shaping tends to suit synthesis-oriented audiences. Dealing with legislative aspects of the tattoo inks was also introduced by Stuckey and Eilks in their intervention.[16] In more detail, once the essential chemical properties are discussed, the audience is again active in examining the labels of the 20 different ink bottles. Different types of labeling, clear labeling mistakes, or no labeling at all are all possible options which are verified and discussed. Furthermore, mismatch between declared and actual content is considered, and detection and verification tools are discussed. Among detection tools, spectroscopic and biochemical techniques are illustrated, and their complementarity is stressed. Additional aspects for discussion that relate to daily experience with tattoos are the possible interference of the tattoos with image diagnostics, such as MRI and ultrasounds, and methods, risks, and effectiveness of tattoo removal, for whatever reason. Potential risks associated with the sterile conditions of tattooing and allergic reactions[45−50] are part of the discussion.

Once the framework is complete, the lab activities are addressed along with related safety instructions.

Finally, the theoretical units were delivered to a group of 4 different sessions, 3 of which were delivered by under 32 year-olds, to limit the age gap with the audience. The sessions were held with material available for handling and checking, such as the ink bottles and the pigments, and slides of two types, "variable" and "fixed" ones. The variable slides deal with different aspects related to the inks, i.e., legislation, spectra, solubility, removal techniques, analyses of the removed fragments, enzymatic reactions for metal detection, etc. The fixed ones are instructions for the lab activities during the practical units, and safe behaviors in a chemical lab. This kind of structuring allows for inquiry-based learning, which can be adapted to the response of the audience. Depending on the background of the group of the day and on their affinity for the different aspects related to tattoo inks, different approaches can be proposed. Therefore, in some cases, the spectroscopic analysis can be emphasized; in other cases, it can be the legislation. In all cases, the students need to be informed on the safety measures, the experiences they will make in the lab on an individual basis, and the relationship of these with the tattoo ink chemistry. This type of intervention can be directed more specifically on different aspects, depending on the audience. One issue can be the correspondence between the real and declared composition of inks and possible false declarations.[33] In this case, the issue can be developed regarding the methods to determine the ink composition.[51] In other cases, if the legislation becomes the main topic, it can be assessed whether to find the regulations,[27−32] and how to compare them to the labels.

■ PRACTICAL UNITS

The practical units were designed for addressing heterogeneous groups from different backgrounds, to achieve quick impact MOEs suited to different ways of thinking. This was achieved by implementing tests in the lab or with instrumental techniques, on the solubility/dispersibility of pigments, water content, the metal content in the inks, and the paper chromatography. Stuckey and Eilks reported a solubility test on inks.[52] Here, we focus on pigments, because they are the coloring agents that remain under the skin, and it is fundamental that they are insoluble in any solvent, because this gives indications of the stability upon injection, even when coming in contact with layers of the skin with different polarities.[53] Inks, i.e., the sum of the pigment and the vehicle, are made water-soluble by the producers to ensure their fluidity when being injected. Because of this, we proposed to verify the water content, i.e., that the ink is water-soluble, despite the coloring agents typically being insoluble. Metal contents are verified with two tests, i.e., an enzymatic assay with the potato slices, followed by instrumental analysis by EDX, to verify which metals are present. The test with the potato slice can also be used as a kinetic test, since the rate of the gas evolution depends on whether the metal is complexed or not. Finally, paper chromatography is used as a simple test to verify, if possible, the number of pigments present. Of course, the efficacy of this test depends on the mobility of the pigments in the mobile phase (solvents of mixture of solvents). The lab experiments are made individually; therefore, each student has a station and has the option to choose the specific types of solubility tests, and metal and water content tests, as well as chromatography tests.

Solubility/Dispersibility

The solubility/dispersibility test is quick and low-risk, and it can be used for MOEs. In the practical experience, the students have 4 different pigments available and 6 different solvents, and they are invited to verify the solubility/dispersibility of 3 pigment/solvent combinations of choice. The chosen solvents have different polarities and the capability of forming hydrogen bonds. The pigments are insoluble in water, propan-2-ol, acetone, THF, and NaOH.[54] In HCl, PG7, PG36, and PY74 are still insoluble, but PB15 can be partially dissolved, depending on the pH. This occurs because of the capability of strong acids to protonate the nitrogen of the phthalocyanine in bridge positions (see Figure 1), which, then, gives hydrogen bonds in water.[55] At a pH value of 1, some dissolution can already be observed.

From the practical point of view, while in most cases the pigment powder simply floats on top of the liquid solvent, in certain conditions additional scenarios are also possible, depending on the specific pigment/solvent of choice. The

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J. Chem. Educ. XXXX, XXX, XXX−XXX

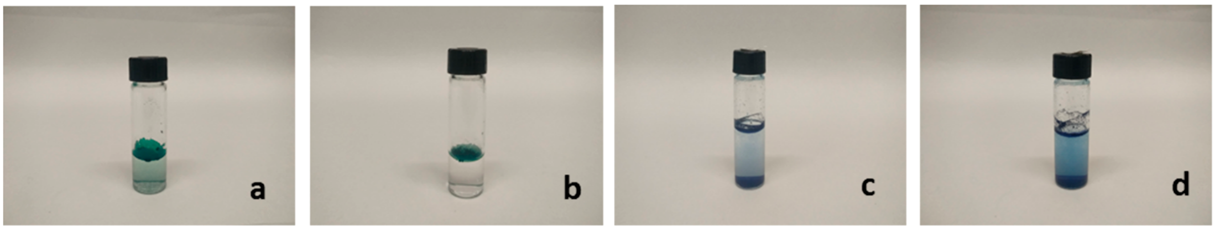

Figure 3. Vials with pigments in different solvent: (a) PG36 in propan-2-ol after mixing and (b) after a few minutes, and (c) PB15 in HCl 2 M after mixing and (d) after a few minutes.

Figure 4. Slices of potatoes before (Bef.) and after (Aft.) pouring H_2O_2. The top left panels refer to a potato without any ink. All the other panels correspond to potato slices dipped into some of the different inks used during the SPD. The red, violet, and green colored potato slices manifest gas evolution upon dripping H_2O_2; the blue and the black ones (3rd row from the top) do not, thus indicating the presence of metals. A version of the violet potato slice has been made where only a portion is dipped into the ink, and the noncolored portion is an immediate reference. The potato slices of the bottom row are drenched in "Mint Green" ink (left panels) and "Green Concentrate" ink (right panels), with the former manifesting a more intense gas evolution due to the presence of TiO_2, which is absent in the latter.

dispersions of PG36 and PG7 in propan-2-ol are fairly stable, and after initial mixing and shaking, they do give the impression of being a solution. However, after a few minutes, the powder floats back to the top of the vial (Figure 3a,b). PB15 in an acidic environment mostly floats on top of the solvent, but it also imparts a pale blue color (Figure 3c), which persists also after a few minutes, thus implying limited solubility (Figure 3d). Therefore, although the test is simple, it does have open answers which require the students to trust their observations and avoid coming to hasty conclusions (a good dispersion may be misleading). Finally, the students are invited to make their own considerations on the usefulness of a good dispersion in the application of tattoo inks.

Water Content

This is a quick test to verify that inks do contain water, in spite of the pigments' insolubility. In other words, it shows that compounds with different characteristics can be combined to give a blend ensuring the tattoo ink has both the color requirements and the injectability. In more detail, pigments for tattoo inks are made partially water-soluble by an undisclosed finishing procedure. The verification of water content in the inks is an indirect test of the pigment modification prior to the inks' production. The students are invited again to pick any of the tattoo inks available, pour a few drops in a crucible, cover it with a convex glass plate, and warm it on a heating plate. After a while, the glass plate is full of condensation and can be probed by dehydrated salts such as $CuSO_4$ which change color upon rehydration. The color turning from white to blue indicates the presence of water.

Metal Content in Tattoo Inks

The metal content of tattoo inks is verified in two different tests: in a lab and with instruments. The test is based on the enzymatic reaction of the catalase and peroxidase.[56] Catalase is a common enzyme which can be found in nearly any living organism and selectively oxidizes hydrogen peroxide to O_2 according to the reaction $2H_2O_2 \rightleftarrows O_2 + H_2O$. It can be inhibited by metals in

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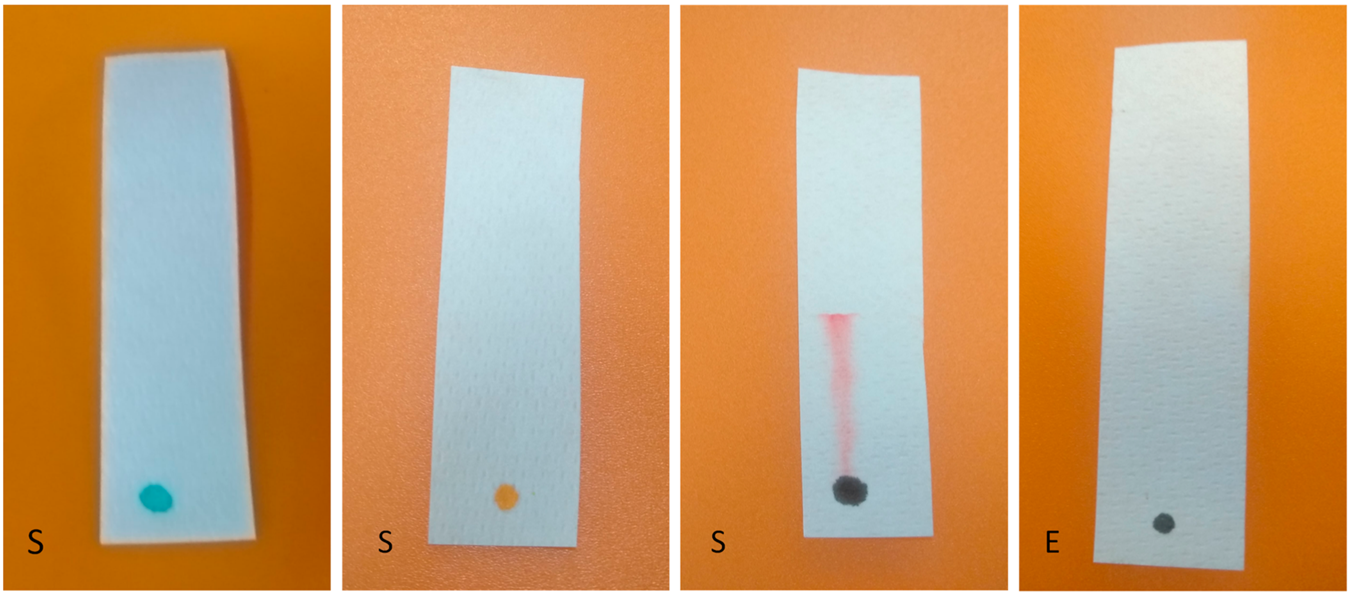

Figure 5. Paper chromatography of the green, orange, and black inks by Solong (S) and black ink by Eternal (E).

any kind of environment.[57,58] Since it is also present in tubers such as potatoes, a straightforward test can be devised to detect metals in tattoo inks. The version of the catalase assay on a potato slice was subsequently implemented at Purdue University[59] and by Stuckey and Eilks.[52] Visually, this corresponds to the observation of gas evolution when H_2O_2 is poured onto a potato slice (Figure 4, see the top left panels Bef. and Aft.). Catalase is inhibited by metals and so is the gas evolution. This SDP experiment is based on the comparative observation of O_2 development from a sheer potato slice and potatoes slices immersed separately for 15 min in 3 inks of choice, out of the 20 available, upon pouring H_2O_2. The presence of metals in the inks inhibits the catalase, and as a consequence, there is no gas evolution. It must be noted that this test works well on "free" metals, metal oxides, and sulfides, but it works poorly on complexed metals such as in CuPc. An example of the different reactivity can be appreciated in the videos numbered 8 and 9 of the Supporting Information, referring to the tests with Green Concentrate and Mint Green of the brand Eternal. They both contain the pigment PG36 (according the label, though PG7, according to the subsequent analyses), in any case a halogenated CuPc. In addition, Mint Green contains TiO_2 as a whitening agent to lighten the hue. When tested with H_2O_2, the potato slide embedded in Mint Green reacts more promptly and with a more efficient gas evolution as compared to Green Concentrate, due to the additional action of TiO_2. Furthermore, the variety of inks proposed also allows for a few "unexpected" cases, such as the catalase inhibition by one of the black inks which is supposed to contain carbon black as pigment and, hence, to be metal-free (Figure 4, see bottom right panels Bef. and Aft.).

The visual effectiveness of the metal determination in tattoo inks through the potatoes slices is highlighted in the short videos shot for different ink colors, in the Supporting Information (videos 1−7). At the end of the test, the participants are invited to compare their observations and make hypotheses (conceptual thinking).

The metal content of "critical" inks, i.e., those with an unexpected answer is, then, verified by EDX analysis, alongside others with an expected response. This type of analysis is suitable for people more prone to algorithmic thinking, since the assignments are based on the one-to-one correspondence between X-ray lines and composition. Among other things, we found Ti in the white ink, and Cu in the black ink inhibiting the catalase (see Supporting Information, Figures SI1 and SI2), and the Solong inks typically also had a non-negligible content of Ca and Mg. Once the type of metal present in a given ink has been established, it is possible to make further hypotheses and assessments through conceptual thinking, such as the association of Ca and Mg with the use of tap water.

Paper Chromatography

A further MOE consisted of a paper chromatography quality test performed to discern whether an ink of choice contained one or more pigments. The students prepared strips of absorbent paper and deposited a drop of an ink of choice. A mixture of toluene and THF was used as the mobile phase. In some of the inks with more than one pigment, one proves to be more mobile in the given mixture of solvents. The best separation was achieved with a toluene/THF mixture 20:80 and the violet ink by Solong. At this point, the students were invited to check the number of pigments on the label, where possible, and evaluate whether the separation or lack of separation on the paper chromatography is consistent with the content declared on the label, or if there are technical reasons why the separation is not possible. Usually, inks based on CuPc do not separate because of the very limited mobility of the phthalocyanines in general (chromatography of phthalocyanines is very difficult and can be achieved with toxic solvents, such as pyridine). In Figure 5, the chromatography on paper is reported for the green, orange, and black inks by Solong (marked with an S), compared to the black ink by Eternal (marked with an E). The black ink by Solong reveals the presence of an additional red component.

■ INTERVENTION EVALUATION

The enrollment of students in the SDP is made on a voluntary basis, after a meeting with potentially interested high school teachers, during which the educational plan and the activities, methods, and goals involved are illustrated. Part of the intervention is devoted to the teachers themselves to help

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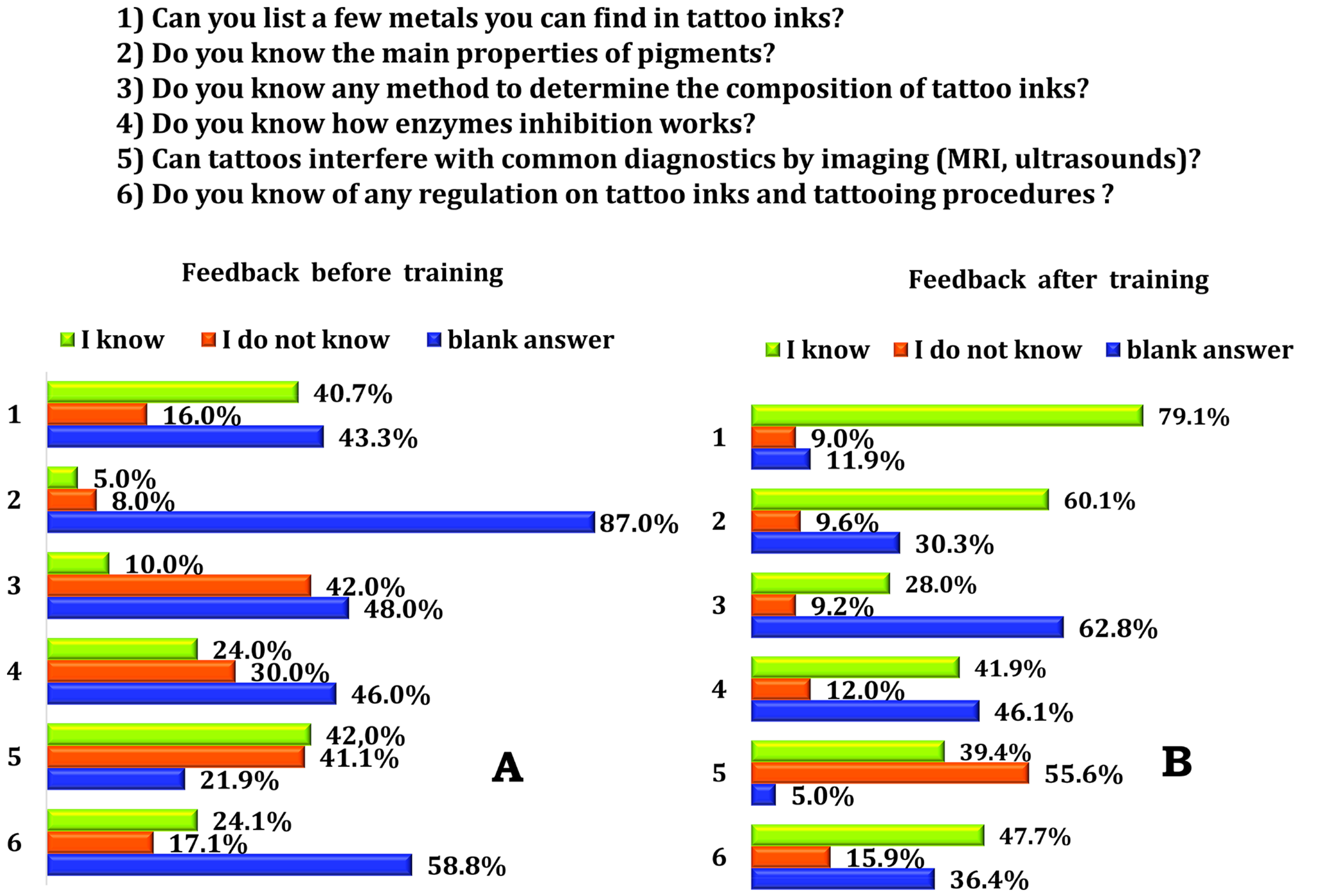

Figure 6. Feedback on the proposed chemical concepts, before and after the SDP experience.

them promote a seamless transition of the students from high school to university studies. The upper limit of the adherence to the plan is the availability of the university structure where the SDP takes place.

The efficacy of the SDP on tattoo inks was evaluated with two types of surveys, on how familiar the students became with the chemistry concept presented and on the enjoyment of the platform. The first survey was collected twice the same day, i.e., immediately before entering the theoretical units, and soon after collecting the last spectrum of the day. The survey on enjoyment was proposed to two groups of students, one which did and the other one which did not participate in the SDP. In the latter case, the test was slightly modified in terms of expression of interest toward the plan.

Student Enrollment Sample

During two school year periods, 180 students attending different high schools were freely enrolled for the first sample group (FSG) involved in the educational platform. The age group was 16−18 year-old, both male and female. Students involved in the educational platform participated in a survey to test knowledge and perception about the potential risks of tattooing practice through an anonymous, nongender specific questionnaire.

A control sample group (CS) of 172 students of public secondary schools not involved in the educational practical framework freely participated in the survey. Age range and gender distribution were the same as the FSG. In this case, the interest in attending such a lesson plan was investigated as a way of promoting the SDP framework.

Surveys Outcomes

Acquisition of Chemistry Concepts. The efficacy of this SPD experience in terms of acquisition of chemistry concepts as applied to a tangible example was assessed by feedback collected before and after the units were delivered. The outcomes of these surveys are reported in Figure 6A,B. The way this survey was collected calls for some elucidation, since it is a summary of open questions, in the form "I know, I do not know, blank"; i.e., the students were asked open questions, and if the answers were correct they were put in the group "I know". If they were wrong in the group "I do not know", or if there was no answer, it went into the group "blank".

It must be added that the participation in the last survey was lower than the first one, due to practical reasons, i.e., commuting schedule of the students, which was, sometimes, incompatible with the ending of the platform session. The type of questions followed three criteria; i.e., they referred to concepts the students would verify or test in the practical units (solubility/dispersibility, metal content), to concepts delivered in the theoretical units only, with "paper" examples, i.e., spectra of pigments, already collected and to interpret on the spot, and completely open questions, i.e., on legislation and interference with image diagnostics, which do not envisage a single answer. The best before/after impact is favorable for any concept verified in the lab and moderately effective if the concepts were examined only on paper. The issues on possible interference were well-perceived, since only old tattoos very rich in Fe can interfere with MRI, but that is a rare event (only one case is reported in the literature out of 134 cases studied[49]), and radiologists routinely working with MRI do not report any

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J. Chem. Educ. XXXX, XXX, XXX−XXX

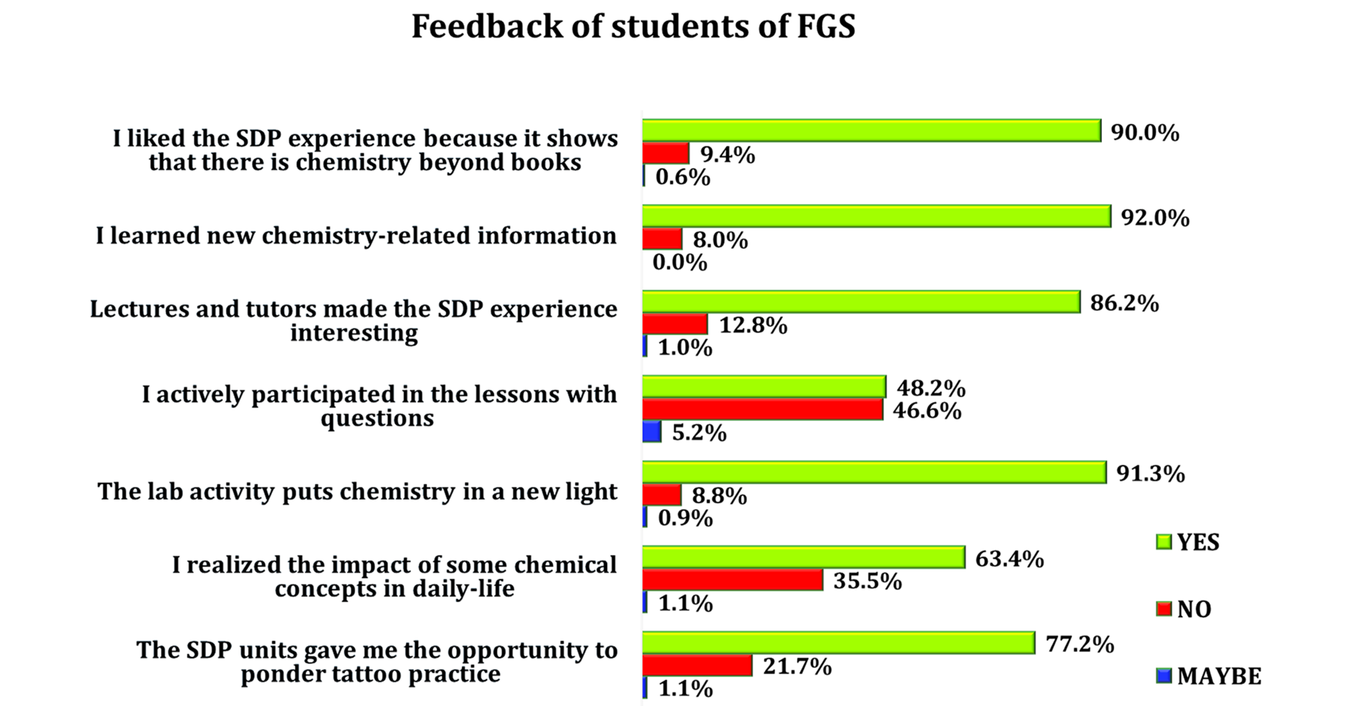

Figure 7. Feedback of the FSG students who participated to the SDP experience.

precaution for tattooed people.[50] Questions on regulations are not straightforward, since they are country-dependent and some degree of interpretation is inevitable. Therefore, the moderate improvement in the before/after comparison is to be expected. In this specific case, the "I know" group after training includes all answers, where reference to local legislation was explicitly mentioned.

Depending on the types of responses, different approaches are being planned to strengthen the SDP experience, either focusing on single aspects, for instance, regulations, including hygiene and health safety features, or dealing with different correlated aspects, such as tattoo removal.

On average, the type of multilevel approach adopted in this plan on tattoo inks seemed to be effective in raising the interest toward chemistry and induced improved chemical skills, especially when the chemical concepts could be immediately applied in the lab or through instrumental analysis.

Enjoyment of the Platform. As pointed out by Bodner et al.,[60] a quantitative assessment of the "performance of individual students or group of students" does not really give the measure of the efficacy of the innovation, since several reasons come together to shadow the impact of a content change or a new approach or innovation case. In a summative assessment, for instance, the choice of least effort[61] from the side of the students may hinder the outcome. However, an evaluation is important to get an indication of how this type of plan can and should be improved. Therefore, to get an insight into possible future adjustments, an evaluation was made about the enjoyment of the SPD experience, through a set of questions on the overall plan, the associated practical experience, the perception of the chemical knowledge related to the daily life, and chemistry in general. The efficacy of the lecturers and tutors was also the object of the survey. Since the whole SDP was taking place in 1 day, including theoretical units, practical units, and surveys both at the beginning and at the end of day, the second survey was also planned as a set of questions foreseeing yes/no/maybe types of answers. This allows feedback on the interest in this type of chemistry experience and an indication regarding whether the plan can proceed in the same format, or if it needs adjustment. Different or more detailed types of evaluations would be difficult to implement, to the episodic characteristic of the SDP experience, and would rather require more sessions or a planned pathway along one or more school terms.

The outcome of the experience was very positive, with a total of 90% of the students claiming to be satisfied and 92% declaring to have learned new information through this program (Figure 7).

A large appreciation was shown for the lab activity (including spectroscopy), which we found particularly rewarding, due to the heterogeneous group we addressed and because it is useful for the goal of enrolling students; it creates a connection between what students can expect in chemistry study and their perception of an enjoyable/useful experience. Perception, interest, and motivational opinions on tattooing practice were collected too by dedicated question formulation as for the other group. The CS was selected to collect feedback on their potential interest in attending to an experience of practical chemical activity (Figure SI3).

Some additional questions were added for the sample group to gather information regarding potential interest in more detailed aspects of removal, as an approach from a different perspective to the chemistry of tattooing.

CONCLUSIONS

In pursuit of enhancing the enrollment in scientific disciplines, an educational plan centered on tattoo ink chemistry was put in place, designed to address 16−18 year-old high school students with varied backgrounds. The ratio of this intervention was designed within the STEAM methodology, to elicit interest in chemistry through several pathways, including different systems of thinking. Students' feedback and classroom and lab observations during the practical activity indicated a highly

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J. Chem. Educ. XXXX, XXX, XXX−XXX

motivating plan. Relating chemistry to personal experiences helps with appreciation of the topic as well as several related aspects such as legislation. Tattoo ink proved to be a versatile topic, which allows for specific discussions depending on the input of the audience, thus helping to reach students with different interests and different backgrounds. Chemical tests and comparative instrumental activities, drawn up as MOEs addressing both conceptual and algorithmic thinking, promoted self-confidence and reliance on observation. Surveys on the enjoyment of the plan and mastering of the chemical concepts addressed during the plan were both positive.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c01205.

EDX spectra of the white ink and of the black ink that inhibit the catalase in the potato slice and surveys of SDP enjoyment (PDF, DOCX)

Videos of potato slices drenched or half drenched in tattoo inks of different colors and then treated with hydrogen peroxide, including a video of a pristine slice of potato for comparison purposes (ZIP)

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Notes

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