# Preparations of chemical sensors for simple formalin detection in contaminated food

(A model for final project works for pre-service teachers in chemistry subject)

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**Abstract.** Teaching chemistry must include some learning skills in scientific works. The students must have experience conducting small research in chemistry to train them the scientific methods. Some chemistry projects have been carried out by the students of teaching training college (FKIP) as called pre-service teachers in chemistry subject. The students were given research problem that is how to formulate a simple sensor for detection of formalin in contaminated food. The students were asked to review literature on the composition and analysis technique of formalin. The students wrote the research proposal and presented their proposal in front of four reviewers before they start conducting research. The project of formalin sensor preparation was started with studies of the active reagent composition in liquids phase, immobilizing the active reagent in soft media, and following with studies of the sensor sensitivity toward formalin contamination in food. The Schiff Fuchsine (SF) and NASH methods for aldehyde determination were chosen as the basic theory for formalin determination. Schiff Fuchsine (SF) in diluted sulfuric acid solution was kept overnight before use. The media for holding of the reagent was cotton coated with chitosan, then it was pasted in tacon foil, this sensor was called forpastrip (student project-1). It could detect formalin as low as 2% (v/v) of formalin or equivalent with 0,8 % of formaldehyde in contaminated food and the expired date was 10 weeks. The forpastrips was tested on food and the chemical interference was also studied (student project-2). Another project was also carried by replacing the cotton with synthetic felt and this formula was recorded as student project-3. The SF reagent immobilized in synthetic felt coated by chitosan was inserted into narrow-plastic straws. The sensitivity of this sensor increased and the detection limit as low as 0.25% (v/v) of formalin in contaminated food and the expired date was more than 12 weeks. The forth project was replacing SF with NASH reagent immobilized in cotton coated by chitosan and inserted in narrow-plastic straws. The detection limit was 0.015% (v/v) of formalin in contaminated food. All Sensors were still effective to detect formalin in sample although the sample contained of sugar, salt, fat and protein as the impurity. The research findings were written by students in their final report (script, like a thesis) after approved by the two supervisors. They students then presented their research in comprehensive exam in front of reviewers before they were graduated. Having interviews with students, they claimed that they have experience in conducting research, have followed all scientific methods. They said the research theme is very interesting, simple, contextual and it might be applied in high school.

**Key words:** formalin, schiff fuchsine, chitosan, NASH, student final project

## Introduction

Science especially chemistry has been considered as a difficult subject by young students since the chemistry has been taught on the abstract part at early stage of chemistry introduction (Ben-Zvi, Eylon, & Silberstein, 1988). The chemistry subject consist of three levels which is macroscopic, submicroscopic and symbolic which is only the macroscopic can be readily observed (Johnstone & Shuaili, 1991, Tsaparlis & Gorezi, 1997). The macroscopic represents some chemistry learning activities in laboratory. As part of science, chemistry are also developed with scientific methods by collecting and analyzing some empirical data. The scientific method comprised some activities such as formulating research problem, planning research procedure, conducting research, analyzing data, formulating the conclusion and raising another research question. However those skills are seldom practiced in regular laboratory practicum (e.g., Hofstein & Lunetta, 1982; Hofstein, 2004). Whereas, these skills are essential, allowing students to understand of the scientific method (Blosser, 1983).

The students are guided to conduct a small scale research to give experience to work as scientist. Through conducting research, student learned science development, broaden and comprehend their knowledge in solving the scientific problem. The research problem is selected with some consideration which are relatively simple, has urgency with community need and it might be applied in high school laboratory. Therefore in this case, formulation of formalin detector for contaminated food was chosen as the research project.

Food contaminated formalin has been an national issue in Indonesia. Some traditional food sellers used formalin as food preservative which is illegal according to Indoensian law and FAO regulation. The present of formalin in the food is not easily detected because food sample must analyze in chemical laboratory for sequence chemical analysis. Some research has been proved that 24 out of 91 food samples were positively contaminated by formalin (Nuryasin, 2006).

There have been several methods in formalin determination. Some of which are Nash method (Arifin et al., 2005; Kartadarma et al., 2005), Chromatropic acid & Huhner-Fulton test (Horwith & Latimer, 2005) and Schiff- Fuchsine and other methods (Kleeberg & Klinger, 1982; Chai et al., 2007; Dolaria & Manik, 2007). Those methods required several steps of laboratory procedure using some toxic and corrosive chemicals, thereby they are not simple and practical ways for community. Recently there is formalin kit test with commercial name "antilin". It was made violet solution that change to violet in formalin. This also will let to color ambiguous and still not a simple method. Therefore, students are guide to conduct research project to formulate a simple formalin sensor that can be used by everybody. The sensor will be formulated by immobilizing the active reagent in chitosan and sensor it is called formalin paper strip test (forpastrips).

## Materials and Methods

Four students were given the assignment for their final projects that were how to fabricate a simple formalin sensor for contaminated food. The students were write proposal and collecting several theory and research on formalin determination. Their proposal was reviewed by two advisors and asked them to do some necessary correction before they presented in a forum where four lectures acted as the reviewers. If all reviewers agreed to proceed the project, then students started working in laboratories conducting the small scale research with the following procedure:

### *Student Research Project-1: Formulation of formalin paper trip test*

The main materials were chitosan isolated from tiger shrimps (*Penaeus monodon*) shell, Schiff- Fuchsine, formalin and food samples. Chitosan was isolated from dried shrimps shell by several steps starting with washing, sun dring and powdering the shells. The following steps were demineralization by soaking the shell powder in dilute hydrochloric acid, deprotenation by addition of diluted NaOH and deacetylation with refluxing the shell in NaOH 50% and characterized using FT-IR technique as described in previous methods (Adlim, 2003; Poeloengasih et al., 2008). .

*Synthesis of formalin test paper strips (forpastrips)*

Amount of 0,5 g of chitosan flakes was diluted in 100 ml of 5% aqueous acetic acid and mixed until all chitosan dissolved. Each 20 ml of the chitosan solution was pipetted and transferred into 5 beaker glasses. Each beaker glass was added Schiff Fuchsine (*4-rosaline hydrochloride*) in various volumes; 5 ml, 4 ml, 3 ml & 2 ml. To each of these mixtures, 3 ml of 2,5 M sulfuric acid was added and stirred. The solution of 1 ml of commercial formalin, which had been previously diluted with water ten times, was added each mixture. The color change was recorded with digital camera. The similar experiments were repeated by changing the concentration of sulfuric acid and substituting sulfuric acid with hydrochloric acid, sodium sulfate, sodium thiocyanate and sodium nitrite in equivalent concentration and volumes and experiment of excluded chitosan.

*Immobilization of SF reagent*
Having found the best formula of SF-chitosan mixture and the mixture was immobilized in several soft materials. The materials tested were cotton, tissue paper, filter papers. Each peach of materials was attached in several harder supported such as glassy photograph papers, cart papers and takon (mica) foil which then called as forpastrips. The study aimed to obtain the best immobilized and supported material for SF. The forpastrips were dipped in SF-chitosan mixture before drying in a oven at 50$^{o}$C for 3 hours. The dried forpastrips was soaked in 10% of commercial formalin solution for 2 minutes. The 10% formalin was made by diluting ten times of commercial formalin with water. The color changes were observed and recorded with digital camera.
*Chitosan role*
The SF mixture formula was changed, in which chitosan was excluded in mixture and but used chitosan as "coat" for forpastrips. The forpastrips were soaked in chitosan solution before drying process. Another experiment the forpastrips were previously dried and subsequently soaked into chitosan solution. The drying time and technique were also study to obtain the drying condition optimum.

*Detection limit*
Several forpastrips were prepared and coated with chitosan before drying. Each forpastrips were tested by dipping the forpastrips in separated beaker containing various concentration of formalin solution starting from 10%, 10%, 8%, 6%, 4%, 2%, 1,2%, 0,8% up to 0,4%. Should color change observable at certain concentration of formalin, then the test was continued for more diluted solution. The confirmation was also done by using the SF solution. The color changes were recorded by using digital photo camera.

*Chemical interference*
Several amount of 10% formalin solutions were prepared in separated beaker glasses. A solution was used as the control, whereas the others were added a few drops of salt, sugar, amylum solution, egg york or chicken fat. The sensitivity of forpastrips toward formalin with chemical interference was tested by dipping the forpastrips in these solution compared with the control.

***Student Project-2: Study the effectivity forpastrips on formalin determination of food samples***

*Test on simulated samples*
Simulated samples were prepared by using fresh chicken, fish meats, tofu and cooked noodles as pure sample. Those samples were cut in several pieces and took some as sample control. Whereas, other were dipped in formalin in various concentration such 10%, 10%, 8%, 6%, 4%, 2%, 1,2%, 0,8% up to 0,4%. The forpastrips was sticked on the wet sample or dipped in the sample extract then compare with control. The experiments were repeated to study the effect chemical interference; the present of salt, sugar, carbohydrate, protein and lipid as described previously.

*Expired date determination*
Hundred peace of forpastrips were prepared and stored into a plastic pack. Every week a forpastrip was taken and used to determination of formalin in simulated sample. The work was done every week until the forpastrip considered expired or no longer sensitive for formalin determination.

*Test on suspected food sample*
The students have gone to 5 locations of traditional markets to take some sample that suspected contaminating with formalin. The samples were taken from pasar Lampulo Banda Aceh, pasar Simpang Limun Medan, Pasar Besi Medan & Pasar Sukaraman Medan. The samples were fresh chicken, fish meat, tofu and cook noodle. The forpastrips were used to

determination formalin in this sample and as confirmation, Schiff- Fuchsine reagent was also used to present of formalin.

***Student Project 3: Using synthetic wool as the soft media in formulating the forpastrip paper test***

The similar experiment procedure on forpastrip formulation was repeated by another students by replacing cotton with more strong soft media including synthetic wool, fiberglass, synthetic rubber foam etc to find more enduring media. The study also included the heating technique and the hard support was replaced with hollow-narrow-plastic-strews in which the soft media was inserted.

***Student Project 4: Using NASH reagent in formulating the forpastrip paper test***

Another student repeated the experiment procedure and changed the SF with Nash reagent. He studied the sensitivity, the detection limit etc on simulated sample and food samples. The support material of forpastrip was also modified using shield transparent-plastic-hollow-narrow-pipe in which soft media was inserted.

**Results and Discussion**

Amount of 37.31 g chitin has been isolated from 87 gram dried shrimp shells (*Penaeus monodon*). After deacethylation process, there were 28 g chitosan has been converted from chitin. The chitosan were analyzed using FT-IR methods at chemistry ITB lab and found carbonyl and amine absorption at 1656.85 $cm^{-1}$ and 3448.72 $cm^{-1}$ then the deacetylation degree was calculated as 54,95%.

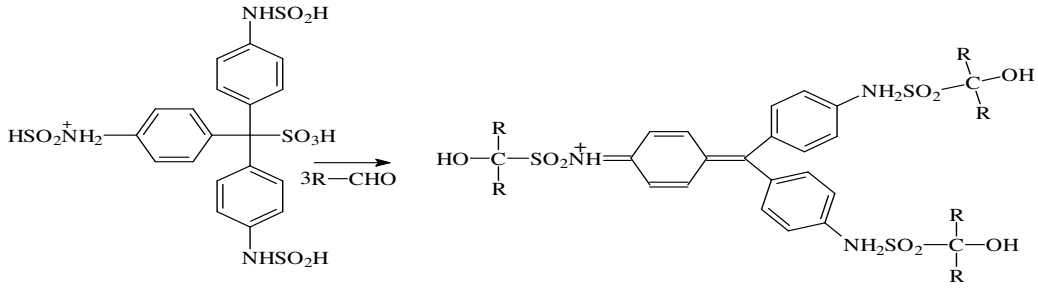
***The forpastrips***

The mixture formula of SF-chitosan, sulfuric acid must present and it could not be substituted with other chemical tested. The color of mixture is shown in the Table 1.

Table 1. The composition of mixture before immobilization

| Mixture | Color change in the solution | | |
|---|---|---|---|
| | Cotton | Synthetic wool | Filter papers |
| SF + formalin | amaranth pink 248 | amaranth pink 248 | amaranth pink 248 |
| Chi + HOAc + SF | amaranth pink 248 | amaranth pink 248 | amaranth pink 248 |
| Chi + HOAc + SF + sulfuric acid | Colorless | Colorless | Colorless |
| Chi + HOAc + SF + sulfuric acid + formalin | Blueis violet 205 | Blueis violet 205 | Blueis violet 205 |

When SF reacted with formalin, the solution turned to amaranth pink and change to the dipper color in higher concentration of formalin. Reaction SF with sulfuric acid or sulfite gives sulfonasi quinoit that is colorless and change to bluis violet 205 after reacted with aldehyde in this case was formalin as shown in this mechanism.




SF reacted with $H_2SO_4$ formed sulfonate quinoit (colorless)

Sulfonate quinoit after reacted with aldehyde (violet)

Figure 1. Reaction Mechanism of SF-aldehyde (Anonymous, 2010[b])

The composition of mixture or called formula had been studied using various amount of chtosan and SF as shown in Table 2. Chitosan has no effect on the color changed of the mixture reaction SF-formalin. The higher concentration of SF is the darker color of the reaction product. From this data we found the optimum proportion of SF and sulfuric acid to prepare the formula. The mixture then was immobilized in soft media and supported by mica (tacon) foil or transparent plastic strews.

Table 2. The effect formula composition on the color of reaction SF-formalin

| No | Chitosan (ml) | SF (ml) | Sulfuric acid 2,5 M (ml) | Color change with nomenclature |
|---|---|---|---|---|
| A | 20 | 5,0 | 3 | Blueis violet 205 |
| B | 20 | 4,0 | 3 | bluish lavender 210 |
| C | 20 | 3,0 | 3 | Burn blue 199 |
| D | 20 | 2,5 | 3 | Dark corinthian purple 259 |
| E | 0 | 5,0 | 3 | Blueis violet 205 |
| F | 0 | 4,0 | 3 | bluish lavender 210 |
| G | 0 | 3,0 | 3 | Burn blue 199 |
| H | 0 | 2,5 | 3 | Dark corinthian purple 259 |

The sulfuric acid was essential to control color before SF react with formalin. However sulfuric acid precipitated out chitosan. Some chemical had been used to substitute the sulfuric acid and reacted as sulfuric acid but they also precipitated out chitosan and this led to not homogenous color change in forpastrip.

Table 3. The effect of chemicals sulfuric acid substitute on the color of Chi-SF solution

| Mixtures | Chemicals, Sulfuric acid substitute | Color change in Chi-SF solution |
|---|---|---|
| Chi + SF | HCl 2,5 M | No color change & no precipitation |
| Chi + SF | $Na_2SO_4$ 2,5 M | *Change to colorless and precipitation* |
| Chi + SF | $Na_2SCN$ 2,5 M | No color change & no precipitation |
| Chi + SF | $Na_2SO_3$ 2,5 M | *Change to colorless and precipitation* |

*Immobilization of SF reagent*
Cotton could hold SF solution better compared with filter papers and synthetic wool but cotton has low durability in sulfuric acid. The best holder for this media was either tacon (mica) foil or transparent plastic strew.

*The Chitosan role*
Chitosan has no effect on the color change during SF-reaction as shown in Table 2. Whereas, the higher concentration of SF the darker color of the reaction product. In present of chitosan however, made the forpastrip stable and without chitosan the SF leached out from forpastrips.

Coating forpastrip was necessary to slow down the evaporation of the SF which affected the expired time. Coating forpastrips with chitosan should be done after forpastrips air dried. Further drying at low temperature was also necessary before packing process of forpastrips. Otherwise the color change in forpastrips would not homogeneous.

***Minimum Detection limit***
Detection limit of the forpastrips was carried out starting from 10% diluted commercial formalin. The commercial formalin was considered as a concentrated solution (100%). The concentrated formalin solution was diluted with water to make 10% up 0,4% of formalin solution as described in Table 4. The detection was determined at minimum concentration of formalin that changes the color of forpastrips. As shown in Table 4, the forpastrips can

only detected formalin at low as 2% of formalin or equivalent to 0,8 % of formaldehyde, by assumption that formalin contained 40% volume of formaldehyde.

Table 4. Minimum detection limit of forpastrip

| Dilute commercial formalin (% v/v) | Equivalent to formaldehyde (% v/v) | Color change |
|---|---|---|
| 1 | 0,4 | No color change |
| 2 | 0,8 | Shell pink 31 |
| 3 | 1,2 | Shrimp pink 28 |
| 5 | 2,0 | pale persian lilac 250 |
| 10 | 4,0 | phlox pink 222 |
| 15 | 6,0 | Pleroma violet 207 |
| 20 | 8,0 | Phlox purple 237 |
| 25 | 10,0 | Petunia violet 219 |

***Chemical interference***

The sensitvity of forpastrips did not interfere by chemical in food on the chemicals such as salt, sugar, protein and fat. The color of forpastrips changed to purple with or without those chemical in food.

***Forpastrips responded in simulated sample and food samples***

Forpastrips were used to test the present of formalin in simulated samples as shown in Table 5. Then, the forpastrips were used for determination of formalin in food sold in traditional market. Investigation was carried out in four traditional markets in Medan is Lemonade Market, Market Bakti, Iron Market and Market Sukaraman. Sample was randomly purchased from those markets, they were fresh fish, fresh chicken meat, yellow cook noodles, noodles tiaw (spagetti), meatballs and Tofu as shown in Table 6. Each sample extract was taken tested to determine the present of formalin in two ways: using forpastrips and use SF solution as the confirmation. The results showed that samples of foodstuffs from all locations.

Table 5. Physical change of food sample contaminated formalin and forpastrip color change in simulated sample

| Food samples | Sample treatment | Formalin (%) | Physic appearance of sample & Forpastrips color change |
|---|---|---|---|
| Fresh chicken meat | Dipped into water | 0% | Sample : light pink<br>Forpastrips : light yellow (no color change) |
| | Dipped into formalin | 2% | Sample : more white<br>Forpastrips : light purple |
| | Dipped into formalin | 5% | Sample : more white<br>Forpastrips : Purple |
| | Dipped into formalin & washed with ice | 5% | Sample : more white<br>Forpastrips : Purple |
| Tofu | Dipped into water | 0% | Sample : white, soft<br>Forpastrips : light yellow (no color change) |
| | Dipped into formalin | 2% | Sample : white, chewy<br>Forpastrips : light purple |
| | Dipped into formalin | 5% | Sample : white, chewy<br>Forpastrips : purple |
| Cook noodles | Dipped into water air | 0% | Sample : yellow & soft<br>Forpastrips : light yellow (no color change) |
| | Dipped into formalin | 2% | Sample : light yellow, chewy<br>Forpastrips : light purple |
| | Dipped into formalin | 5% | Sample : light yellow<br>Forpastrips : purple |
| Fresh fish meat | Dipped into water air | 0% | Sample : brown, soft<br>Forpastrips : light yellow (no color change) |
| | Dipped into formalin | 2% | Sample : light purple, chewy<br>Forpastrips : light purple |

***The expired date of forpastrips***

Forpastrips are not reusable so that 100 pieces of forpastrips have been prepared and every week was taken and used to determine formalin in sample simulation for three months. The expired date for forpastrips was ten weeks or 2.5 months. After the period was soft media of forpastrips was decomposed due to the presence of sulfuric acid in the media.

***Replacing cotton as soft media with synthetic wool and plastic-transparent strew in forpastrip formula***

Replacing cotton with synthetic wool as the soft media made the forpastrips stronger and extended the expired date. Inserting the soft media in transparent plastic strew could protect the active reagent from evaporation.

***Replacing SF with NASH reagent in formulating the forpastrip paper test***

Replacing SF with NASH reagent increased high sensitivity of the forpastrip up to 0,015%. However NASH reagent is very volatile, it is required shield system for forpastrips.

Table 6. Formalin determination on food samples collecting from several traditional markets

| Sample locations | Samples | Method of formalin detemination | |
|---|---|---|---|
| | | SF solution (confirmation) | Forpastrips |
| Pasar Limun Medan | Fresh fish | negative | Negative |
| | Fresh chicken meat | negative | Negative |
| | Cook noodle | negative | Negative |
| | Cook noodle (spageti) | negative | Negative |
| | Bakso (cook meat ball) | negative | Negative |
| | Tofu | negative | Negative |
| Pasar Bakti Medan | Fresh fish | negative | Negative |
| | Fresh chicken meat | negative | Negative |
| | Cook noodle | negative | negative |
| | Cook noodle (spageti) | negative | negative |
| | Bakso (cook meat ball) | negative | negative |
| | Tofu | negative | negative |
| Pasar Besi Medan | Fresh fish | negative | negative |
| | Fresh chicken meat | negative | negative |
| | Cook noodle | negative | negative |
| | Cook noodle (spageti) | negative | negative |
| | Bakso (cook meat ball) | negative | negative |
| | Tofu | negative | negative |
| Pasar Sukaraman Medan | Fresh fish | negative | negative |
| | Fresh chicken meat | negative | negative |
| | Cook noodle | negative | negative |
| | Cook noodle (spageti) | negative | negative |
| | Bakso (cook meat ball) | negative | negative |
| | Tofu | negative | negative |

***Students' responds on their project***

Students said that they learn how to conduct research in chemistry with interesting topics. "we learned how to write research proposal, conducting research, reporting the research finding". We have practiced the sequence scientific methods.

**Conclusions**

Forpastrips prepared using various soft media and supports are sensitive to determine formalin in contaminated food. The synthetic wool was better than cotton in forpastrips formula. Transparent plastic strew gave better protection for the active reagent of the forpastrips. Using NASH reagent into soft media increased the sensitivity of forpastrip to detect as low as 0,015% (v/v) formalin in solution and contaminated food. Students who conducted this research project claimed that they learned and experienced how to conduct research in chemistry and experienced how to solve problem in community with chemistry research.

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