A QUICK TEST FOR SULFITES ON FOODS AND NITRATES IN DRINKING WATER

by

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B. S., Saint Mary's College, 1984

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

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INTRODUCTION

Approximately 20% of the population in the United States suffer from allergies (7). Their pathological reactions range anywhere from harmless sneezing and itching to possibly fatal respiratory arrest. A variety of antigens exist that can provoke these reactions in humans such as molds, foods, plants, animals, and chemicals. Among the 20% of the people who suffer from allergies in the United States, 10 million of them are asthmatic (5,7). Within this population of 10 million asthmatics, 5 to 10% of these people suffer from what doctor describe as a sensitivity syndrome to sulfites (a food additive)(5,7). It has been determined that non-asthmatic people can also be allergic to sulfite (6). Allergic reactions can include hives, nausea, diarrhea, shortness of breath, and fatal shock(see Table I). These sulfite sensitive people have reported reactions after ingesting foods and drinks that have been treated with sulfite (see Table II, III, IV)(2). Sulfite has been blamed for at least 12 deaths in the last three years and there have been approximately 500 reports of adverse reactions (21).

Sulfiting, the use of either sulfur dioxide or sulfites, has been dated to the ancient Egyptians and Romans. They used the fumes of burning sulfur as a sanitizing agent in their wine making process. Sulfites are not only used as sanitizing agents, but are also used to prevent microbial spoilage and as an antioxidant to slow oxidative discoloration and browning (1).

In an aqueous solution, the following reactions take place:

$$SO_2 + H_2O \leftrightarrows H_2SO_3$$

$$2H_2SO_3 \leftrightarrows H^+, HSO_3^- + 2H^+, SO_3^= \quad (11)$$

TABLE I

Allergic reactions in sulfite sensitive people in order of severity

Weakness
Flushing
Headaches
Dizziness
Nausea
Abdominal pains
Rapid Pulse
Hives
Chest Tightness
Dyspnea (Wheezing)
Cyanosis
Loss of Consciousness
Coma
Brain Damage

TABLE II

Partial list of foods containing sulfiting agents (2)

Weight Watchers Fruit Snacks
Good Seasons Salad Dressing Mix (various varieties)
Wines (most)
Sunsweet Selected Sun-Dried Apricots
Bonner Golden Raisins
ReaLemon Lemon Juice from concentrate
Paisley Farm Dilled Cauliflower
Old El Paso Pickled Hot Jalapeno Peppers
Sun Maid California Selected Dried Fruits and Raisins Fruit Bits
Trappey's Sulcito Peperoncini Salad Peppers
Liberty Colored Pineapple Wedges
Bell Fruit Cake Mix
Betty Crocker Hamburger Helper
French's Idaho Mashed Potatoes
Pillsbury Apricot Nut Quick Bread Mix
Uncle Ben's Brown & Wild Rice with Mushrooms (and other varieties)
Pillsbury Plus Carrot 'n Spice Cake Mix
Most shell fish and some fin fish
Betty Crocker Snackin' Cake Carrot Nut Cake Mix
Betty Crocker Hickory Smoke Cheese Flavored Potatoes
Prescription drugs, including Bronkosol, Decadron, Adrenalin chloride, Metoclopramide hydrochloride (Reglan injectable), microNEFRIN, Dopamine; dozens of other prescription drugs

TABLE III

Guide to sulfited foods

FOOD CATEGORY	TYPES OF FOOD
Baked Goods	Cookies
	Crackers
	Crepes
	Mixes with Dried Fruits and Dried Vegetables
	Pie Crust
	Pizza Crust
	Quiche Crust
	Soft Pretzels
	Tortillas
	Tortilla Shells
	Waffles
Alcoholic Beverages	Beer
	Cocktail Mixes
	Wine
Nonalcoholic Beverages and Beverage Bases	Cola Type
	Fruit Type
Coffee and Tea	Instant Tea
Condiments and Relishes	Horseradish Relish
	Onion Relish
	Pickle Relish
	Pickles
	Olives
	Salad Dressing Mixes
	Wine Vinegar
Confections and Frostings	Brown Sugar
	Raw Sugar
	Powdered Sugar
Dairy Product Analogs	Filled Milk
Fish Products (Processed--Frozen, Canned, and Dried)	Clams
	Shrimp
	Lobster
	Crab
	Scallops
	Dried Cod
Fresh Fish	Clams
	Shrimp
	Lobster
	Crab
	Scallops
Fresh Fruit	Fruit Salad Bars

TABLE III (cont.)

Guide to sulfited foods

FOOD CATEGORY	TYPES OF FOOD
Fresh Fruit	Fruit Salads (Deli) Grapes
Fresh Vegetables	Vegetable Salad Bars Mushrooms Avocado Salad (Guacamole) Shredded Cabbage (Cole Slaw)
Gelatins, Puddings, and Fillings	Fruit Fillings (including Apple) Flavored Gelatin Pectin Jelling Agents Unflavored Gelatin
Grain Products and Pastas	Corn Starch Modified Food Starch Spinach Pasta Breadings Batters Noodles/Rice Mixes
Gravies and Sauces	Gravies (including Milk-Based)
Hard Candy	Clear,Hard Candy
Jams and Jellies	Jams Jellies
Nuts and Nut Products	Shredded Coconut
Plant Protein Products	Soy Protein
Processed Fruits	Canned, Bottled, or Frozen Fruit Juices (including Lemon, Lime, Grape, Apple, and Orange) Dried Fruit (including Apples, Apricots, Pineapple, Peaches, Pears, Golden Raisins, and Prunes) Canned, Bottled, or Frozen Dietetic Fruit or Fruit Juices Maraschino Cherries Glaced Fruit
Processed Vegetables and Vegetables Juices	Vegetable Juices Canned Vegetables (including Potatoes and Hominy)

TABLE III (cont.)

Guide to sulfited foods

FOOD CATEGORY	TYPES OF FOOD
	Pickled Vegetables (including Sauerkraut, Cauliflower, and Peppers)
	Dried Vegetables
	Instant Mashed Potatoes
	Frozen Vegetables (including Potatoes, Spinach, Other Green Vegetables, and Avocado Mix)
	Potato Salad (Deli)
Snack Foods	Dried Fruit Snacks
	Trail Mixes
	Filled Crackers
	Tortilla Chips
	Potato Chips
Soups and Soup Mixes	Canned Seafood Soups
	Dried Soup Mixes
White Granulated Sugar	White Granulated Sugar
Sweet Sauces, Toppings, and Syrups	Corn Syrup
	Maple Syrup
	Fruit Toppings
	High Fructose Corn Syrup
	Pancake Syrup

TABLE IV

Recommended Levels of SO_2 in Dried and Dehydrated Fruits and Vegetables at Start of Storage*

Product	SO_2 ppm	Product	SO_2 ppm
Apricots	2000	Sulfur bleached raisins	1500
Peaches	2000	Apples	800
Nectarines	2000	Cabbages	750-1500
Pears	1000	Potatoes	200-250
Golden, bleached raisins	800	Carrots	200-250

*Source: Monsanto Technical Bulletin 1-250.

The growth of bacteria, yeasts, and mold are known to be inhibited by the presence of sulfurous acid. The mechanisms developed by Chichester and Tanner by which sulfurous acid inhibits these microorganisms are: the reaction of bisulfite with acetaldehyde in the cell, the reduction of essential disulfide linkages in enzymes, and the formation of bisulfite addition compounds which interfere with respiratory reactions involving nicotinamide dinucleotide (11).

The antibrowning mechanism can be explained by dividing it into two classes, nonenzymatic and enzymatic browning. The nonenzymatic browning is thought to be inhibited by an interaction between bisulfite and active carbonyl groups. This mechanism is thought to work in conjunction with the bleaching action of sulfur dioxide on the melanoidin pigments, thus producing an effective method to inhibit nonenzymatic browning (11).

Enzymatic browning is the result of phenolic compounds being enzyme-catalytically oxidized to pigments which produce the brown color. The mechanism by which this method is effective is the bleaching action of sulfur dioxide on anthobyanin pigments (11).

Limitations of sulfiting imposed by the first of the national food and drug laws in the U.S.A. occurred during the 19^{th} century when sulfite was irresponsibly applied to meat and fish in order to sell it over an extended period of time (1). Sulfiting today has been extended to fresh, canned, and dehydrated fruits and vegetables, shellfish, beer, wine, and salad bar ingredients; it has been discontinued for meats and fish.

With the health trends that exist today, the public is demanding that restaurants serve more fresh fruits and vegetables on their menus. This demand for healthy, appetizing fruits and vegetables at the salad bar is the source of the present dilema. Restaurants are currently spraying their

salad bars with solutions of potassium metabisulfite, sodium bisulfite, or sodium sulfite in order to keep their items from browning and to increase the time over which they can be served. It has been estimated that the average person consumes 2 to 3 mg of sulfite daily with an additional 5 to 10 mg of sulfite consumed in wine and beer (2). The average salad bar consumer ingests 25 to 100 mg of sulfite when eating salads, vegetables (especially potatoes), and avocado dips in just one meal (2). The consumer cannot detect sulfite on the fruits or vegetables by smell or taste. According to the FDA, sulfite cannot be washed off of the fruits and vegetables (20).

In September of 1984, 60 Minutes aired a program which addressed the sulfite problem. During this program 60 Minutes showed asthmatics being tested with plecebos and different levels of sulfite along with the reaction that occurred. Their reactions varied from no reaction with the plecebos to mild dizziness to difficulty in breathing and panic a few minutes after ingesting a sulfite tablet. Dr. Ronald Simon, a research scientist at the Scripps Clinic and Research Foundation in La Jolla, California feels that asthmatics react to sulfites by inhaling sulfur dioxide gas which is released in their mouths (20). If enough sulfur dioxide is released and inhaled, an asthmatic attack can be initiated. Once the asthma reaction is initiated, it prevents enough oxygen from getting through the lungs and into the blood. The person then begins to produce toxins in their blood, turn blue, and their blood pressure falls which causes them to become unconscious and go into shock. This can cause brain damage or be lethal. Dr. Simon has determined that the most sensitive person can detect a total amount of sulfite at a level as low as 5 mg (7). His research has also demonstrated that not only asthmatics are having reactions to sulfites, but other allergy

prone people are also reacting to sulfites. Dr. Simon feels that these people either lack the enzyme to metabolize the sulfite or are simply allergic to the sulfite. According to a report by the FDA, 30% of the sulfite sensitive people are nonasthmatic(6).

Recently sulfiting has come under fire by consumer advocate Dr. Michael Jacobson for being a health hazard (24). Jacobson, at the Center for Science in the Public Interest in Washington D.C., went to battle with the FDA who was about to affirm sulfiting agents as "members in good standing on the Generally Recognized as Safe (GRAS) list". The FDA has admitted that it may have slipped and is currently re-evaluating its stance on the issue. As of August 14th, 1985, the FDA had proposed a ban of sulfites on fresh fruit and vegetables (23). It was reported in 1982 that the Center for Science in the Public Interest wanted to limit the amount of sulfite that a person injested to be 350 mg/serving (4).

OBJECTIVE

The objective of this research was to provide a rapid and readily accessible method of detection for sulfites at salad bars, where the majority of allergic reactions have occurred. This test strip must be simple to use, provide an immediate response, produce colors that are easily distinguished even in dimly lighted rooms, be more sensitive than the most sensitive person, be stable for several months, not be effected by large temperature changes, and be inexpensive.

LITERATURE REVIEW

Changes in color to make qualitative and quantitative measurements are used almost everyday by analytical chemists; it is the principle of color change that the existing methods of sulfite detection are based upon. The majority of the work on sulfites was performed in the period from 1920 to 1930 with very little work being done currently. Those methods that appeared to be most applicable to the current problem will be discussed.

The first method involves a color change from white to red. This method was developed by Fritz Feigl and was published in 1954 (8). The test has a limit of detection of 3.2 µg and involves the combination of one drop of each of the following solutions (in order of addition):

1 N Potassium ferrocyanide ($K_4Fe(CN)_6$).

Cold saturated zinc sulfate solution.

1% Sodium nitroprusside ($Na_2Fe(CN)_5(NO)\cdot 2H_2O$).

A white precipitate of zinc ferrocyanide is formed when the solutions are combined and turns red when a neutral solution of sulfite is applied to the precipitate (Figure I). When this method was tried, it was found that the white precipitate actually only turned faint pink when sulfite was applied to the precipitate. It was also discovered that the solid must be used immediately after preparation or little color was formed and that the pH of the sulfite solution was crucial.

The second method, also developed by Feigl, involves the decolorization of a 2% aqueous solution of malachite green (Figure II)(8). The limit of detection for this method is 1 µg. The method of decolorization involves the formation of the leuco form of malachite green where the addition of a HSO_3^- group destroys the quinoidal structure (Figure II). The malachite

Figure I; Chemical reaction of the nitroprusside test

$K_4Fe(CN)_6$

+

$ZnSO_4$

+

$Na_2[Fe(CN)_5NO].H_2O$
(SODIUM NITROPRUSSIDE)

↓

$\underline{Zn_2[Fe(CN)_6]}$

+

SULFITE

↓

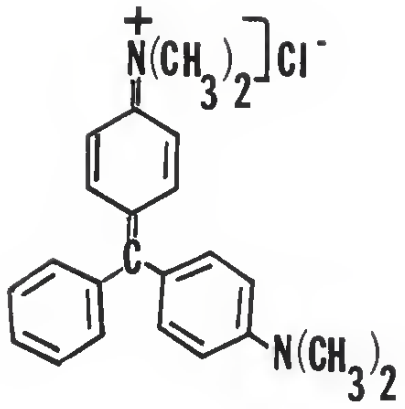
RED COLOR

Figure II; a. Malachite green

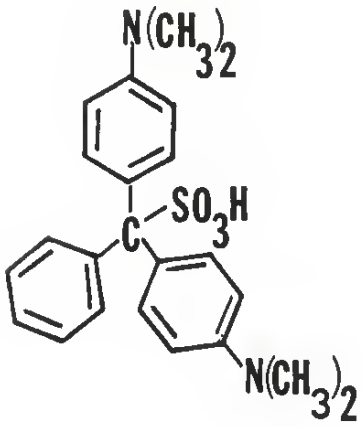
b. Leuco structure

Figure II

a.)



b.)



green reaction was found to work well, but the color change from light green to colorless was not always easy to visualize on paper.

The next method involves the determination of sulfur dioxide with rosaniline dyes in which similar procedures have been developed by Steigmann (16) and King & Pruden (19). Both of these procedures involve the reaction of p-rosaniline hydrochloride and formaldehyde with sulfite which forms the reddish-purple p-rosaniline methyl sulfanic acid. The product is then detected spectrophotometrically. This method is not entirely specific for sulfite and has interferences from thiosulfates, mercaptans, thioacids, and heavy metals. There exists another method that is similar to this method and is recognized by the Association of Official Analytical Chemist (AOAC)(9). This method combines 100 mg of p-rosaniline HCl with 200 mL of water which is then followed by the addition of 160 mL of 6 M HCl and then diluted to 1 L. The colorless solution is allowed to stand for 12 hours after which a red-violet color appears in the presence of sulfite. The limit of detection for this method is 10 ppm.

Another method that is used frequently by the food industry to measure sulfite is the modified Monier-Williams Technique (13,17). This technique involves the evolution of SO_2 gas from the food sample by distillation with alkali hydroxide. The distillate is then either treated as SO_2 or oxidized to H_2SO_4 which can then be treated gravimetrically as $BaSO_4$ or by titration. One of the main drawbacks with this method is that volatile acids and organic sulfur compounds give erronious results. The limit of detection is thought to be around 50 ppm.

A method developed by Axelrod et al.(12) involves the use of fluorimetry to detect and measure sulfite. This method is similar to a photometric method reported by West and Gaeke (26). SO_2 is bubbled into 0.1

M $HgCl_4^=$ and then is reacted with formaldehyde. The resulting formaldehyde-bisulfite complex ($HCHO\text{-}HSO_3^-$) is then reacted with 5-aminofluorescein which produces a nonfluorescent species. An indirect measurement of sulfite can then be made through the amount of suppression by the nonfluorescent species. The limit of detection is 0.02 ppm SO_2 in $HgCl_4^=$ solution. Axelrod has reported interferences with K, Ca, Mg, Cu, OAc^-, NO_2^-, I^-, and Fe(III).

The next method indirectly involves the use of atomic absorption spectroscopy. Jungreis and Anavi (15) developed a method where sulfite is added to mercury oxide (HgO). The pH of the solution is then adjusted to 11 and allowed to mix for 1 hour. During this mixing period, mercury is transformed from the solid state to the very stable $[Hg(SO_4)_2]^{-2}$ complex. The amount of sulfite present can then be determined from a plot of the absorbance of the sample minus the absorbance of the blank versus the concentration. Two of the drawbacks with this method are: 1) the amount of SO_3^{-2} must be between 11.9 - 83.3 ppm, and 2) Hg^{+2}, I^-, $S_2O_3^{-2}$, and SCN^- interfere.

Another method facilitates the Monier-Williams technique in combination with ion chromatography as a detection system (18). This method was first described by Padgett who modified the Monier-Williams purge-and-trap apparatus to allow the sulfite to distill directly and then used ion chromatography as the detection system. Padgett's improvements allowed for more specificity but was subject to interferences from co-eluting peaks and nonreproducable results. Further improvements have been made by Sullivan and Smith (11) which render this combination free from interferences and able to produce reproducable and acceptable recoveries. The method by Sullivan and Smith involves several modifications of the method developed by Padgett. These modifications are as follows: 1) in order to control the

flow of nitrogen better, the distillation apparatus was changed slightly. 2) it was discovered that 50 mL of 10 $\underline{N}$ H_3PO_4 was sufficient for digestion. 3) the pH of the trapping solution must remain constant. 4) the parameters for the detection system were optimized by using a column with improved separation characteristics and efficiency. It was also discovered that the concentration of the eluent was extremely critical. The limit of detection for this method is 1 ppm.

The last method is one that is more than 100 years old and is used as the official method for the examination of water and wastewater for determining sulfites (14,25). The procedure is as follows: 500 mL of the sample is added to 10 mL of standard iodine solution along with 1 g of potassium iodide. A back titration is then performed using standard thiosulfate solution and starch indicator. The amount of sulfite present in the water can then be determined. One of the major sources of error in this method is that sulfites are often oxidized by atmospheric oxygen. One of the drawbacks to this method is that it requires considerable time to perform and a certain amount of technique.

There exist two commercial methods for the detection for sulfite. The first is produced by CHEMetrics, INC. This test involves iodide - iodate chemistry and is available in five different levels of monitoring with the lowest level at 2 ppm. This method was found to be cumbersome to perform and required a large amount of sulfite solution for the test. The other test strip is manufactured by Anspec, INC. The test strip makes use of Feigl's nitroprusside reaction. The major drawback with this strip is that the color change reported by the company is from pink to brick red which for some individuals may be hard to visualize.

After examining the above methods, it was determined that none of the methods available were completely satisfactory. This led to the decision to test various dye combinations on paper strips and TLC plate sections. The following is a discussion of those investigations that led to the development of a satisfactory test strip - one that meets all of the requirements listed on page 11.

EXPERIMENTAL

CHEMICALS:

Alumina, W200 acid, ICN Pharmaceuticals Inc., Lot number 113.

Silica Gel 28-200 mesh grade 12, Fisher Scientific Co.,Lot number 710878.

Alumina TLC plates neutral, basic, acidic, Eastman Kodak, Lot numbers 70201203(EM), 6J77011, 2G77013EC.

Florisil 60-100 mesh, Fisher Scientific Co., Lot number 792676.

Chromosorb W DMCS treated and acid washed 60/80 mesh, Applied Science Lab., Lot number 906.

Whatman filter paper number 2.

Orange I, Allied Chemical Co., Lot number 113.

Brillant Green, Fisher Scientific Co., Lot number 714072.

Malachite Green, Fisher Scientific Co., Lot number 753025.

Fast Red S, Allied Chemical Co., Lot number 1167101.

Orange II, Fisher Scientific Co., Lot number 7213.

Sodium Bisulfate, Fisher Scientific Co., Lot number 796154.

Sodium hydrogen carbonate.

Ascorbic acid, Mallinckrodt Co., Lot number WCSR.

BHT, Eastman Chemical, Feb. 12, 1958.

BHA, Eastman Chemical, Feb. 12, 1958.

Citric acid 0616, Mallinckrodt Co., Lot number TSK.

Acetone.

"Fresh Spud", Commercial preparation, Diamond Crystal Salt Co., Wilmington, MA 01887.

"SpraMent" art and display adhesive, 3M Co.

All chemicals were reagent grade unless stated otherwise.

PROCEDURE FOR PREPARING THE TEST STRIP

Combine 1.34 g of unsieved alumina, 0.20 g of orange I and dissolve in about 15 mL of acetone. Allow to evaporate to dryness with stirring. Add 0.12 g of the brilliant green, 15 mL of acetone, and evaporate to dryness with gentle and occasional stirring. Sieve this mixture, keeping the 80 to 120 mesh fraction. Add 0.05 times the weight of the sieved product of $NaHCO_3$. Mix the solids. Spray a piece of white cardboard, 5 x 7.5 cm, with SpraMent and then sprinkle the particles onto the adhesive. The loose particles are then removed and the adhesive is given time to set, usually about 3 minutes. The cardboard is then cut into strips approximately 1.5 x 75 mm, which are then further reduced to 1.5 x 5 mm. The 1.5 x 5 mm pieces are then glued to one end of a 1 x 7.5 cm stip of cardboard (like an index card). The strip is ready for use.

PROCEDURE FOR USE

Moist fruits and vegetables

Place the dye side of the test strip onto a wet area of a piece of lettuce, apple, banana, or potatoe, etc. The black strip will turn red where the moisture is within 15 seconds if sulfite is present and a dark green if not. The green color forms slower than the red color. If the test strip is placed directly onto an apple slice a false positive sometimes occurs. This can be avoided by allowing a drop of water to fall from the apple to the test strip. After a few seconds a proper test is then observed.

Dried fruits and vegetables

Place one drop of water on the item to be tested and allow the drop of water to remain on the fruit for 5 seconds. Then allow the drop of water to fall on the strip. Again the color will be red if sulfites are present and green if not.

French fries

Bend a French fry until it breaks and exposes the inner portion. Place one drop of water on the exposed portion and lay the strip on the drop of water. A positive reaction is a red color as before, a negative reaction is an orange background with large green spots. The use of disodium hydrogen phosphate at the concentrations used on products sold for home use did not have any noticeable effect.

Wines

Place one drop of wine on the test strip. A positive test will turn green for approximately a half of a second and then will turn orange. A negative test, when tested with a blank of 12.5% ethanol, will turn green immediately.

RESULTS AND DISCUSSION

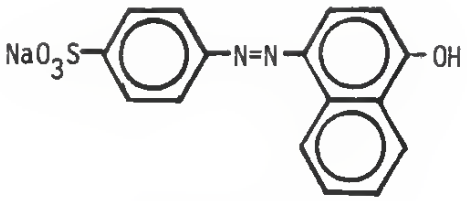
The methods described in the literature were examined to determine if they could be suitably modified. All proved to be unsatisfactory for one or more reasons. A discussion between Dr.'s Meloan and Lambert brought out the fact that Dr. Lambert had worked with orange I, orange II, fast red S, and brilliant green many years ago in an attempt to detect SO_2 in air by a piezoelectric method. The method was not sufficiently sensitive but the color changes desired for the current problem appeared to be suitable. Various combinations of these dyes on various supports were then examined. All reactions on papers of various types were too slow and supports such as silica gel, alumina, Chromosorb, and Florisil were examined.

The final dye mixture consists of orange I and brilliant green. Orange I is not effected by sulfite but sulfite reacts with brilliant green at the central carbon to remove the conjugation and produce a colorless compound. This reaction works best at a neutral pH and $NaHCO_3$ is added for pH adjustment (Figure III).

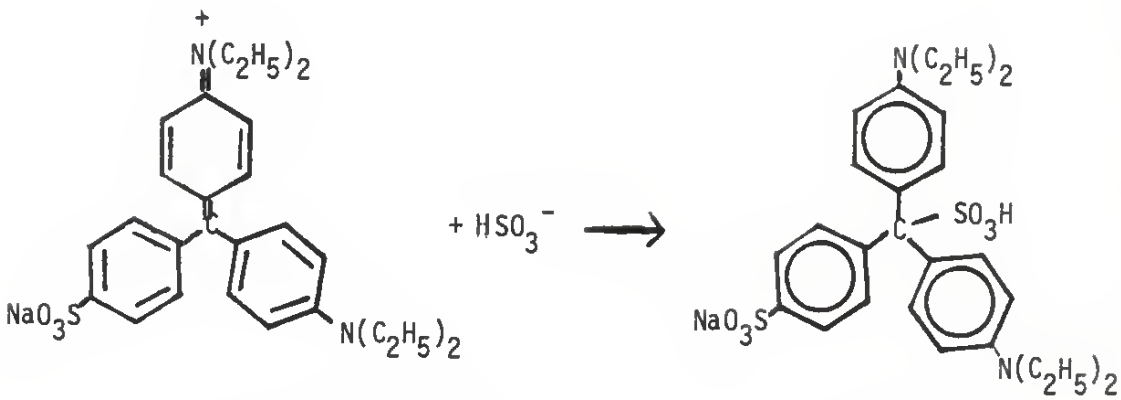
The mixture of the dyes is a dark green, almost black color. When the sulfite reacts with the brilliant green to make it colorless, the red color of the orange I becomes visible. If no sulfite is present then the unreacted brilliant green dissolves slightly in the water of the test drop producing a dark green color.

Other dyes tried were fast red S and orange II (Figure IV). These did not produce the deep red color desired although it is thought that they could be made to work. In both the fast red S and orange II the OH is in the ortho position while in orange I the OH is in the para position. This could be an area which might explain why fast red S and orange II do not

Figure III; Chemical reaction of orange I and brilliant green with sulfite



Orange I

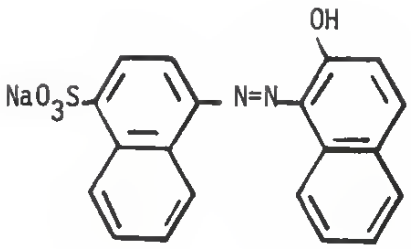


Brilliant green (green) (colorless)

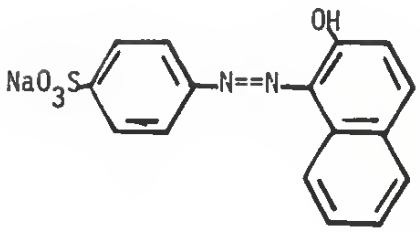
Figure IV; a). Fast red S

b). Orange II

a.)



b.)



react anologously to orange I. It is interesting to note that in the case of the orange II no orange color was seen at all while if two naphthyl groups are present and the OH in the ortho position (fast red S) the orange color reappears.

Malachite green was also tried (see Table V) on alumina and silica gel with orange I. The malachite green provided a weak color and took some time to react. In order to enhance the weak color provided by the malachite green, brilliant green was added to the test mixture (see Table V). Even with the brilliant green added, the green color was still weak. Also, a 0.1% acetone solution of brilliant green was tested and found not to react to sulfite. This particular test was modeled after the fact that a 0.1% aqueous solution of malachite green decolorizes when tested with sulfite.

EFFECT OF THE SUPPORT

The support was found to be quite critical. Initial studies on paper produced the desired color change but only after 20-30 minutes. This was believed to be due to the depth of penetration into the paper plus a surface adsorption that slowed down the reaction of the sulfite with the dye.

Silica gel and alumina were tried in an attempt to provide a support that would not allow deep penetration and possibly provide a different surface reaction. It was found that no reaction would take place on silica gel even up to 300 ppm sulfite (see Table V). Alumina powder stuck to double sided adhesive foam, provided a reaction at the desired rate but gave erratic results. Alumina TLC plates usually gave a positive result even with distilled-deionized water. Initial studies were done using acid alumina (see Table VI). Numerous tests were performed varying the ratios of the dyes. The mixes were tested unsieved and without the addition of sodium bicarbonate. While testing the mixes, it was noted that when various

TABLE V

Investigation of various dye and support ratios

Orange I	Malachite Green	Silica Gel	Mesh Size	Sulfite	H_2O
0.10g	0.02g	1.34g	-	orange	orange
0.10	0.04	1.34	-	orange	green-orange
0.10	0.06	1.34	-	orange with some green	faint green

Orange I	Malachite Green	Alumina	Mesh Size	Sulfite	H_2O
0.20g	0.094g	1.34g	100	blue green	blue green
			150	orange	orange
0.20	0.07	1.34	100	orange	orange
0.20	0.08	1.34	100	orange	orange with green spots
0.20	0.02*	1.34		orange	orange with green spots
0.20	0.04*	1.34		orange	orange
0.10	0.02	1.34		orange with some green	blue green
0.10	0.03	1.34		lt. orange	lt. blue green
0.10	0.04	1.34		lt. orange	lt. blue green
0.10	0.05	1.34		lt. orange	lt. blue green
0.10	0.06	1.34		lt. orange	lt. blue green
0.10	0.07	1.34		green	green
0.13	0.065	1.34		orange	orange
0.13	0.075	1.34		orange	orange
0.13	0.085	1.34		orange	orange

Orange I	Brilliant Green	Silica Gel	Mesh Size	Sulfite	H_2O
0.20g	0.11g	1.34g	65	orange	orange
0.20	0.13	1.34	65	orange	orange
0.20	0.15	1.34	65	orange	orange
0.20	0.18	1.34	80	orange	orange
0.20	0.18	1.34	150	orange	orange

* 0.10 g of brilliant green was added in addition to the shown amount of malachite green.

TABLE VI

Various trial ratios of Orange I to Brilliant Green to Alumina

Orange I	Brilliant Green	Al_2O_3	Sulfite	H_2O
0.11g	0.10g	4.94g	orange	orange
0.20	0.125	1.34	orange	blue green
0.10	0.10	1.34	light orange	blue green
0.17	0.08	1.34	orange with green spots	green
0.12*	0.13	1.34*	blue green	blue green
0.12	0.18	1.34	orange	red-orange
0.20	0.10	1.34	orange	orange
0.20	0.11	1.34	orange	orange with some green
0.20	0.14#	1.34#	no color	no color
0.20	0.125	1.34	orange→green (100 mesh particles)	blue green
			orange (150 mesh particles)	orange
0.20	0.125	1.34	orange with green spots (80 mesh particles)	blue green
0.20	0.100	1.34	orange with green spots	orange with green spots
0.170	0.110	1.34	orange with many green spots	
0.180	0.110	1.34	orange with green spots (80 mesh particles)	greenish

*Denotes that water was used to absorb the orange I onto the alumina.

#Denotes that ethanol was used to absorb the brilliant green onto the alumina and orange I (acetone was used for the absorption of orange I).

particle sizes were picked up with the tape, inconsistent results occured when tested with water and sulfite. This led to the decision to sieve the mix. The sieving helped to alleviate the inconsistent results, but then led to the discovery of another inconsistency. It was found that if the particles were greater than 80 mesh, then no reaction would occur with sulfite even up to 300 ppm. If the alumina was smaller than 150 mesh, then a false positive was obtained in every test (see Table VI). If the alumina particle size is smaller than 80 mesh and larger than 120 mesh then the desired reaction goes within 15 seconds down to 0.5 µg of sulfite. It was interesting to note that when the mix was dried in the oven for 1 hour at 100 °C only an orange color was obtained when tested with water or with sulfite.

Silica gel was tried next (see Table V). The result of using silica gel was that when tested with water a false positive was always obtained even at various particle sizes.

Additional supports tested included Chromosorb and Florisil (see Table VII). Both of these supports provided false positives when tested with water. The Chromosorb turned orange at all particle sizes, while the Florisil had some green spots at 60 mesh, but was very slow to react. Thus, alumina (80-120 mesh) was choosen as the best support for the above reasons.

DETECTION LIMITS

The limit of detection was determined to be 25 ppm which was obtained within 15 seconds with one drop of solution. This is equivalent to 0.5 µg of sulfite. Based on the amounts used in commercial applications, one drop of the sulfite spray solution would contain between 0.2-0.4 mg of sulfite and one drop of the dip solution would contain about 5 mg. Therefore the test strip is capable of detecting sulfite at concentrations many times

TABLE VII

Additional dyes studied

Fast Red S	Brilliant Green	Alumina	$NaCO_3$	Sulfite	Water
0.20g	0.12g	1.34g	yes	orange	orange

Orange II	Brilliant Green	Alumina	$NaCO_3$	Sulfite	Water
0.20g	0.12g	1.34g	yes	clear	green

Additional supports studied

Orange I	Brilliant Green	Chromosorb	$NaCO_3$	Sulfite	Water
0.20g	0.12	2.13g	no	orange	orange

Orange I	Brilliant Green	Florisil	$NaCO_3$	Sulfite	Water
0.20g	0.12g	1.53	no	lt.orange	orange with green spots (60 mesh; reacts very slow)
					orange with less green spots (80 mesh)
					orange (greater than 80 mesh)

Alumina treated with a saturated solution of $NaHCO_3$ in acetone

Orange I	Brilliant Green	Alumina	Mesh Size	Sulfite	Water
0.20	0.12	1.34	< 80	green	green (very intense)
			80-120	orange	army green
			>120	orange	orange
			60-120	orange	blue green

lower than might be expected to be encountered. The most sensitive person yet discovered requires at least 5 mg of sulfite to get a reaction. At 0.5 µg/drop this could be spread out over 10,000 drops (500 mL!) in one salad and still be detected, an unreasonable practical situation.

INTERFERENCES

The test strip was tested for interferences and it was found that none of the common preservatives used on these items of food produce a false positive. Ascorbic acid and citric acid were tested at 10% and 1% concentrations and produced an olive green color. They produced no noticeable interference when present in the commercial preparation "Fresh Spud" when it was tested. Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and propyl gallate were too insoluble in water to interfere.

BUFFERING

Once the dye-alumina ratios were established, it was noted that various intensities and colors of green were obtained depending on the type of water that was used for the test. Distilled water (pH 5.6) gave a grass green color while tap water (pH 8.5-9) gave a very intense blue-green color. From the research that had been done while working with malachite green, which must be done at a neutral pH, it was decided to buffer the system. The first buffer system was formed by the addition of sodium bicarbonate (see Table VIII). Various amounts were tested on many of the test mixes. It was finally discovered that a factor of 0.05 times the weight of the mix of sodium bicarbonate would produce the best color. While working out the factor of 0.05, it was noted that if an excess of sodium bicarbonate was added to the mix, a false positive was obtained. In this method; dry, powdered, sodium bicarbonate was added to the final mix after the dyes were

TABLE VIII

Various dye and support ratios in chronological order

Orange I	Brilliant Green	Al_2O_3	Mesh Size	Mix	$NaHCO_3$	Sulfite	H_2O
0.20g	0.11g	1.34g				orange with green spots	
			80	1.12g*	0.21g	orange	blue green
0.18	0.110	1.34	80			green	green
			100			orange with green spots	sl. green
				1.34*	0.25	green	green
			80			orange	green
0.17	0.110	1.34	80			orange with many green spots	
			100			orange with few green spots	
			80	1.08*	0.20	orange	green
0.20	**0.12**	**1.34**	**80**	**0.85**	**0.166**	**orange**	**green**
			80-150	0.93	0.157	orange with green spots	green
0.20	0.115	1.34	100	0.46	0.04	orange with green spots	green
0.20	0.125	1.34	100	0.72	0.13	lt.orange	green
0.20	0.11	1.34	100	0.90	0.08	orange	orange
					0.08	orange	orange with green spots
					0.01	orange	orange with green spots
					0.01	orange	orange
0.15	0.115	1.34	120	0.60	0.11	green	green
0.20	0.115	1.34	120	0.77	0.14	orange	green
			150			orange	orange
0.20	0.10	1.34	120	0.71	0.07	orange	green
			-200			orange	green
			150-200			orange	orange
0.20	0.09	1.34	120	0.77	0.07	orange	orange
			100	0.23	0.02	orange with green spots	
					0.09	orange	orange
0.20	0.11	1.34	120	1.10	0.10	green	green
					0.10	orange	orange
0.20	0.12	1.34	120-150	0.10	0.01	orange	orange
			80-120		**0.01**	**orange**	**green**
			150		0.02	orange	orange
			80		0.01	green	green

---

* Denotes that these trials were done at a later date, after the discovery that $NaHCO_3$ must be added.

applied and the mix had been sieved. Another method with sodium bicarbonate was tried (see Table VII). Before the dyes were applied to the alumina, it was pretreated with a saturated solution of sodium bicarbonate in acetone. Acetone was used instead of water so that the surface water on the alumina was not driven off in the evaporation of the solvent. After the pretreatment of the alumina, the dyes were applied in the normal manner. The only difference that was noted in this part of the procedure was that the orange I absorbed onto the alumina much faster than onto the untreated alumina. When this mix was tested the only difference that was noted was in the particle size. By using this method, 60-120 mesh particles can be used, where as in the method where the sodium bicarbonate is added as a powder, only 80-120 mesh particles can be used.

Two additional buffer systems, acetate and borate were tried. An acetate buffer (pH = 5) was chosen to be on the acid side of sodium bicarbonate and borate (pH = 9) was chosen to be on the basic side of sodium bicarbonate. The sodium acetate was added to the mix using the 0.05 factor to calculate the amount. The results, when tested with water and sulfite, were that particles ranging from less than 80 mesh to greater than 120 mesh turned green when tested with water, while the same range turned orange when tested with sulfite with the strongest orange color from particles greater than 120 mesh.

The borate buffer system was formed using sodium borate and was added in the same manner as the sodium acetate. The results from the borate buffer were that all particles turned red when tested with water and orange when tested with sulfite. This result could be expected since the borate buffer system buffers at a higher pH than do the acetate and bicarbonate

system and since brilliant green is similar to malachite green which is buffered at a neutral pH.

SOLVENTS

All of the mixes were made using acetone as a solvent except for two cases where water and ethanol were used (see Table VI). Acetone was initially choosen for its ease of evaporation. The two cases from Table VI where the solvent deviates from acetone when performed to, (1) determine if more orange I could be absorbed on the alumina since orange I is more soluble in water than acetone, and (2) see if more brilliant green could be absorbed on the alumina since it is slightly more soluble in ethanol than in acetone. Water was not used again for a solvent for the reason stated in the discussion of supports, and ethanol was not used again because when it was used the mix did not produce any color change when either water or sulfite were added. It was noted that during the absorption of brilliant green onto the alumina in acetone, that if the solution was stirred too frequently, the mix (when the acetone was evaporated) was not dark green to black in color but light brown. This is thought to possibly be due to a slight change in the crystal packing arrangement. This brown mix gave only false positives.

Additional solvents that were studied include DMF, chloroform, and methylene chloride (see Table IX). Results from the study done with DMF indicate that correct results can be obtained from particles less than 80 mesh to particles greater than 120 mesh. The results when methylene chloride was used were not as good as in the DMF solvent study. This solvent allowed correct test colors to be produced, but the green color was often not a pure green, but a mixture of green spots and orange spots. The worst solvent results came from the study using chloroform. The test colors

TABLE IX

Effect of different solvents on the best dye ratio

Orange I	Brilliant Green	Alumina	Mesh Size	Sulfite	Water	Solvent
0.20	0.12	1.34	< 80	orange	green	DMF[a]
			80-120	orange	lt.green	DMF
			>120	orange	green	DMF
0.20	0.12	1.34	< 80	lt.orange	orange	$CHCl_3$[b]
			80-120	lt.orange	orange	$CHCl_3$
			>120	lt.orange	orange	$CHCl_3$
0.20	0.12	1.34	< 80	lt.orange	greenish	CH_2Cl_2[c]
			80-120	lt.orange	greenish	CH_2Cl_2
			>120	lt.orange	green→red	CH_2Cl_2
0.20	0.12	1.34	80-120	orange	green	C_3H_6O[d]

[a] dipole constant = 3.82

[b] dipole constant = 1.01

[c] dipole constant = 1.60

[d] dipole constant = 2.88

that were produced were always orange, no green color was ever obtained at any particle size. From the solvent study it can be concluded that the solvent is a key factor and that a polar solvent is necessary. One possible explanation as to why a polar solvent is necessary is that it allows the two dyes to orient and lie on the surface of the alumina in such a way as to produce the desired test colors.

SURFACE EFFECTS

In order to investigate the effect of surface charge a three layer mixture was tried. This involved absorbing 0.10g of orange I onto 1.34g of alumina using acetone as the solvent which was followed by 0.12g of brilliant green. The final layer consisted of 0.10g of orange I. This in effect should give no surface charge since the alumina has a positive charge, the orange I has a negative charge, and the brilliant green has a positive charge. Thus the charges according to the layers are as follows: (+) from the alumina, (-) from the orange I, (+) from the brilliant green, and (-) from the final layer of orange I. The result when tested with sulfite was that the mix turned red at all particle sizes while when tested with water all particles turned red with green spots. These results indicate that surface charge is not a major factor in obtaining a mix that gives correct test results.

Since the surface of the alumina has a positive charge it was thought that the amount of the two dyes could be in a 1:1 ratio. This in fact was not the case. Several mixes were prepared in which the ratio of the two dyes was varied from under a 1:1 ratio to over a 1:1 ratio (see Table X). The mix that gives the correct test results has a dye ratio that is not a 1:1 ratio, but a 1:0.44 (orange I:brilliant green) ratio.

In order to investigate the phenomenona of large particles giving false

TABLE X

DYE RATIOS

Orange I	Brilliant Green
1	0.45
1	0.73
1	0.34
1	0.78
1	1.20
1	1.20
1	0.363
1	**0.44**
1	0.25
	0.29
	0.308
	0.345
	0.38
1	0.399
1	0.444
1	0.470
1	0.417
1	0.556
1	0.326
1	0.471

---------Bold numbers indicate the best dye ratio.

negative results and small particles giving false positives, scanning electron micrographs were taken of the alumina particles. Scanning electron micrographs were also taken of silica, Chromosorb, and Florisil particles. The particles of alumina coated with both dyes that were less than 80 mesh (always negative results) showed a large net of thin needle-like crystals on the surface (Figure V). The particles that were greater than 150 mesh (always positive results) showed only a few thin needle-like crystals and did not show a net of crystals on the surface as the less than 80 mesh did (Figure VI).

In order to identify the crystals in the scanning electron micrographs, micrographs were taken of (1) acid alumina, (2) alumina coated with orange I, (3) alumina coated with brilliant green, (4) alumina coated with both dyes (at the proper mesh size), and (5) alumina coated with both dyes (brown in color). The acid alumina surface showed areas with small particles on the surface, but no crystal-like structures (Figure VII). The surface of the alumina coated with orange I showed some areas with flat crystals on both 80 and 150 mesh particles (Figure VIII). The surface of the alumina coated with brilliant green did not show any crystals, but showed a thin film coating the surface (Figure IX). The scanning electron micrographs of alumina coated with both dyes showed both crystals, needle-like and flat, on the surface (Figure X). The surface of the mix that was brown in color appeared to be coated with many little particles and some fine needle-like crystals (Figure XI). The appearance on the surface between the mix that was dark green to black in color and the mix that was brown in color was significantly different. This difference is a possible explanation for the brown mix giving false positives although it is not understood why.

Figure V; Scanning electron micrograph of alumina coated with orange I and brilliant green - 80 mesh. False negatives.

Figure V

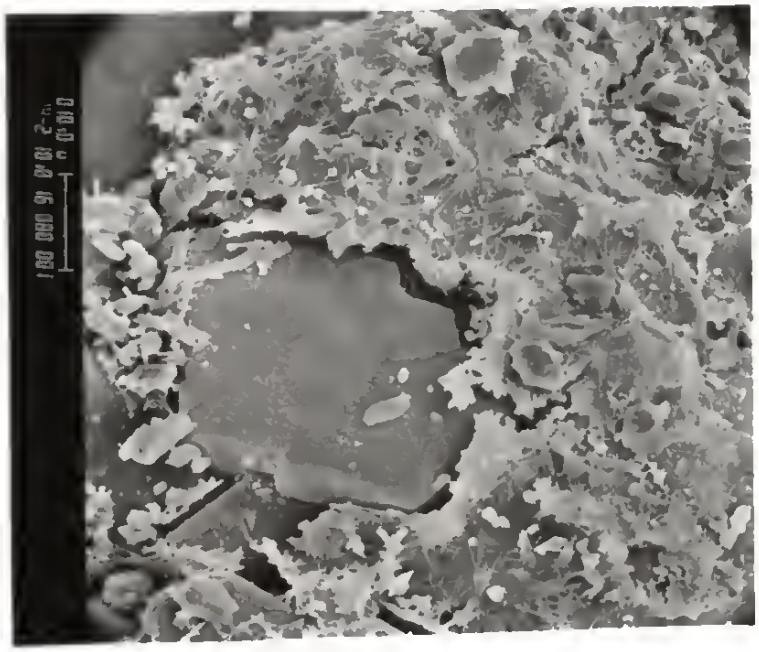


Figure VI; Scanning electron micrograph of alumina coated with orange I and brilliant green - 150 mesh. False positives.

Figure VI

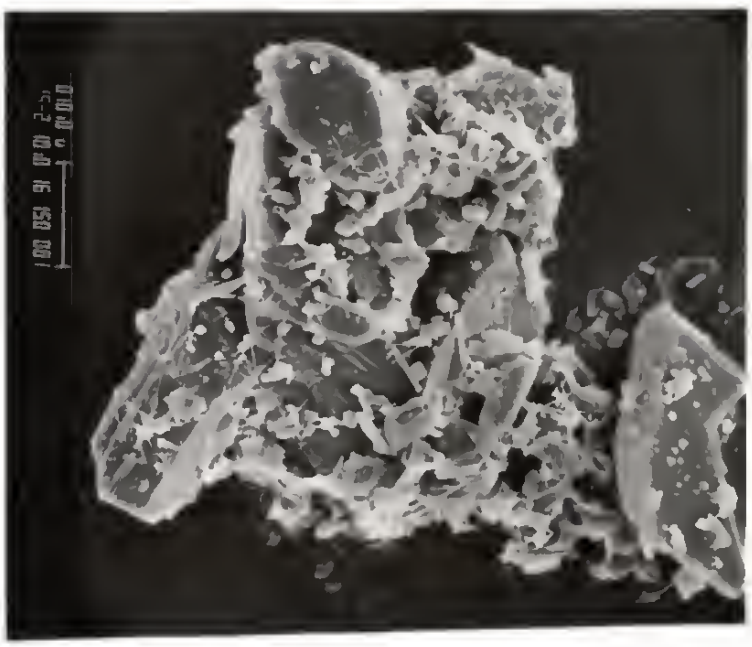
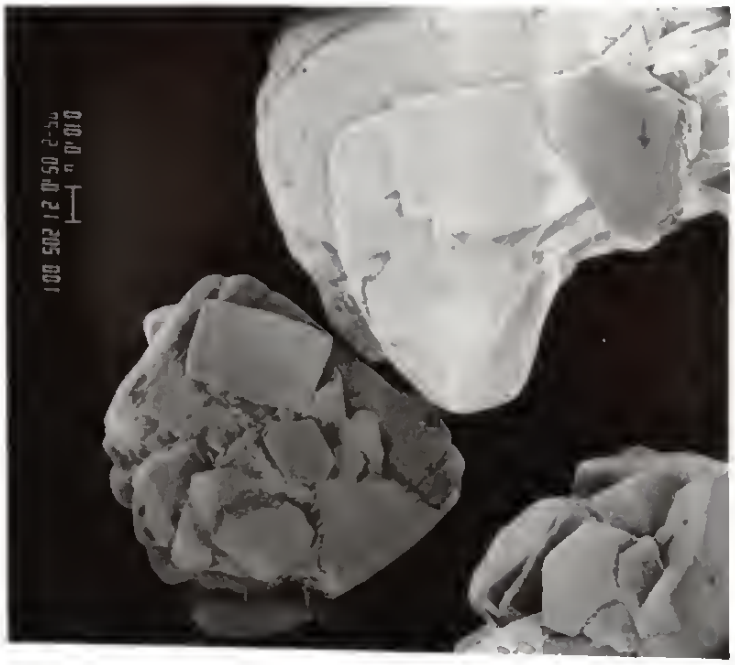


Figure VII; Scanning electron micrograph of acid alumina



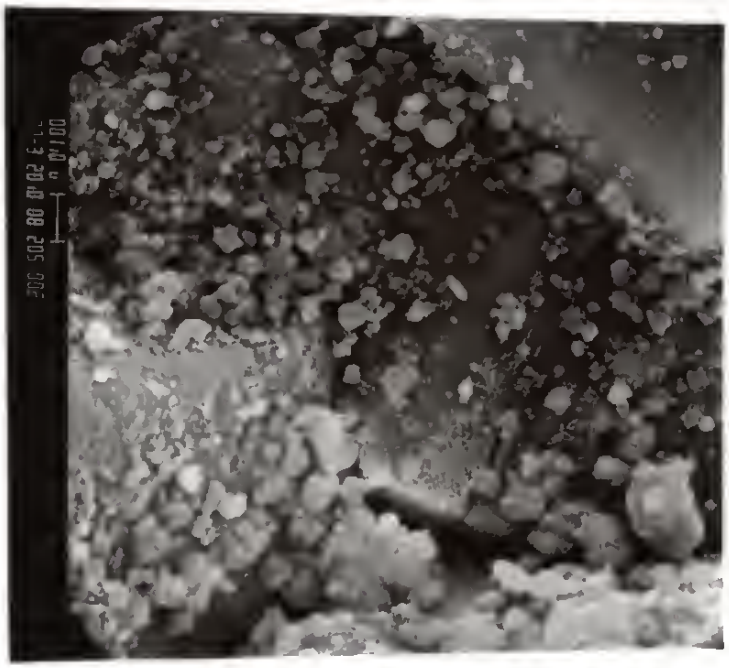
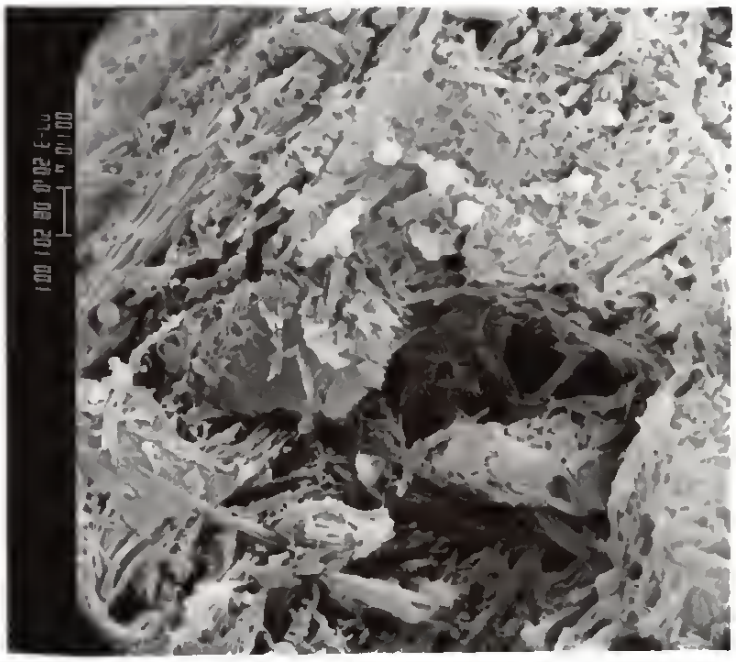


Figure VIII; Scanning electron micrograph of alumina coated with orange I

a) 80 mesh

b) 150 mesh

a.)



b.)

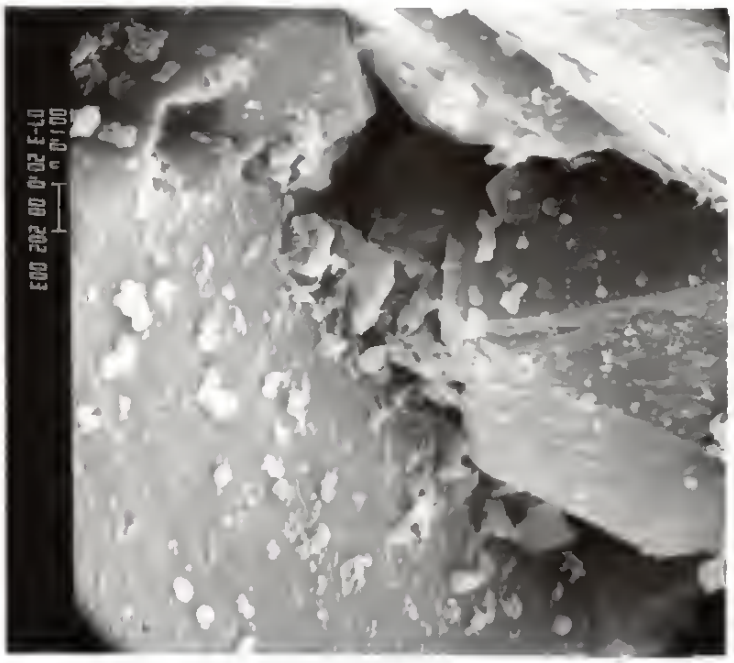
Figure IX; Scanning electron micrograph of alumina coated with brilliant green

a) 80 mesh

b) 150 mesh

Figure IX

a.)



b.)

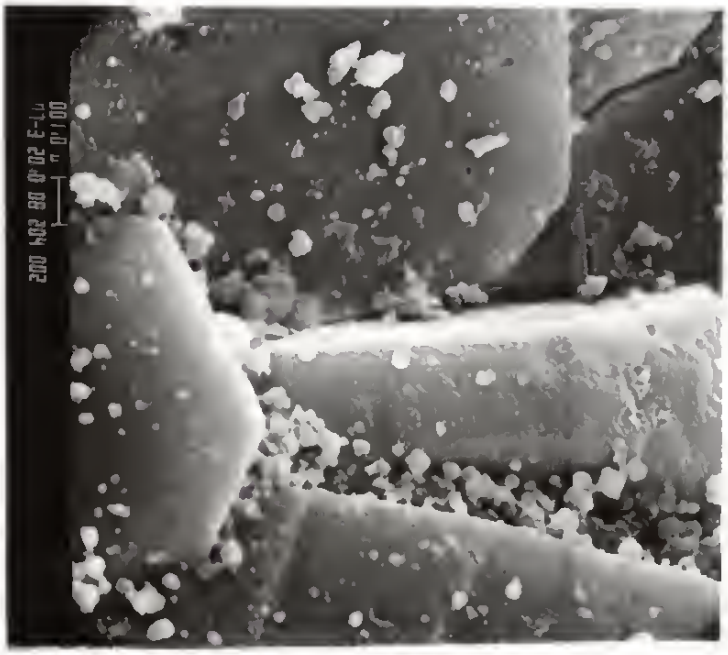


Figure X; Scanning electron micrograph of alumina coated with orange I and brilliant green - 80 to 120 mesh. Correct test colors.

Figure X

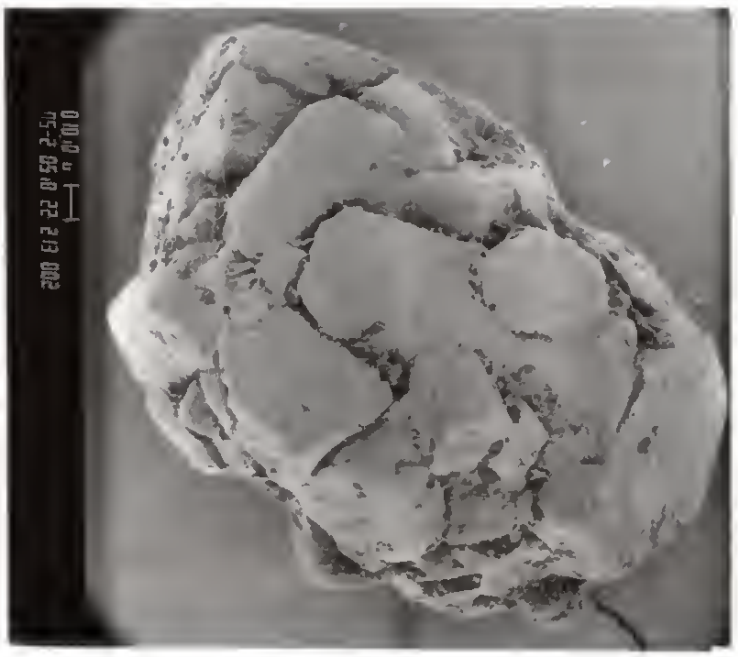


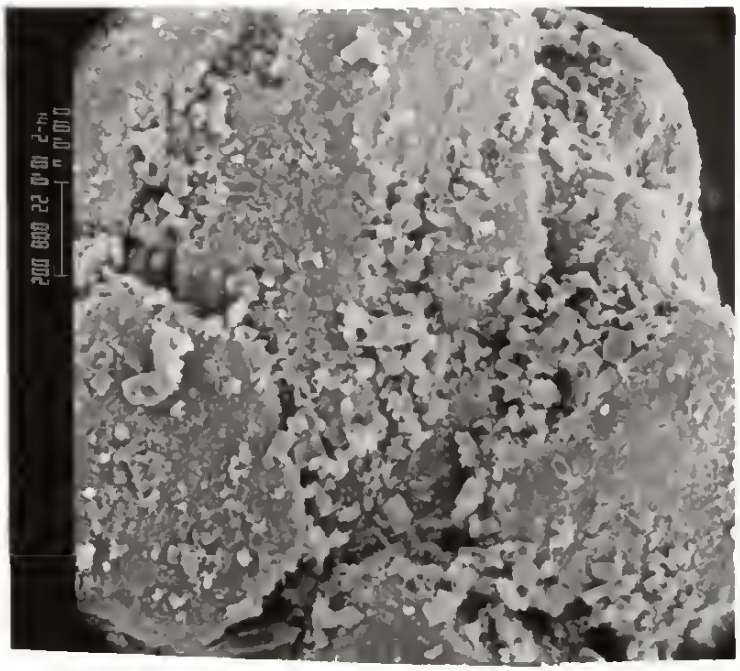
Figure XI; Scanning electron micrograph of alumina coated with orange I and brilliant green - brown mix. Inconsistent test colors.

a) 80 mesh

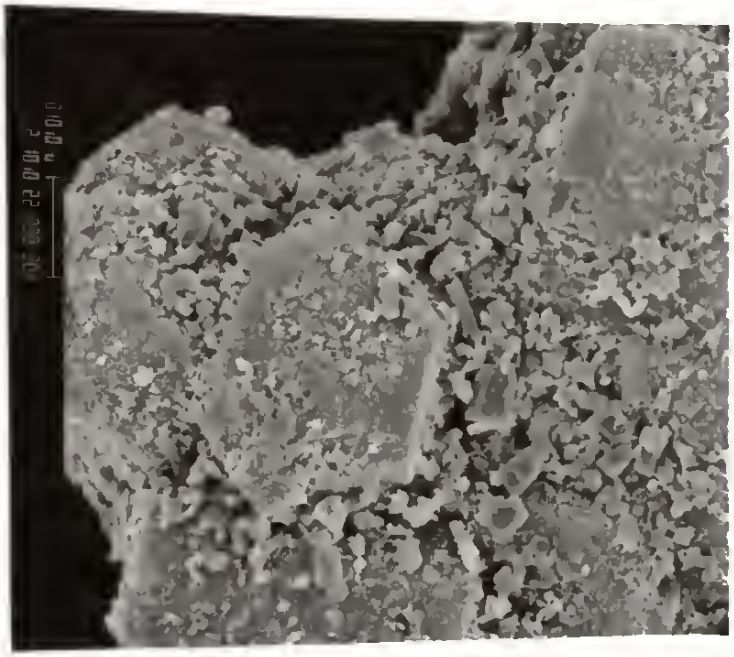
b) 150 mesh

Figure XI

a.)



b.)



SILICA GEL

No tests with silica gel produced satisfactory results, regardless of the particles size. The next series of scanning electron micrographs were taken of (1) silica gel, (2) silica gel with orange I (< 80 mesh, 80, and 150 mesh), (3) silica gel with brilliant green (< 80 mesh, 80, and 150 mesh), and (4) silica gel with orange I and brilliant green (80 and 150 mesh). The surface of the silica gel appeared to be covered with many small pieces (Figure XII), while the surface of the silica gel particles coated with orange I (80 and 150 mesh) appeared to have a network of flat crystals on the surface (Figure XIII). The silica gel particles coated with orange I (< 80 mesh) exhibited areas where the network of crystals had attatched themselves to the surface and areas where the crystals were in a very loose arrangement and appeared not to be attached to the surface. The surface of the silica gel which had been coated with brilliant green did not have any crystals (Figure XIV). The particles at all mesh sizes showed a surface to which many small particles had been attached to. Finally, the surface of the silica gel that was coated with both dyes appeared different at each of the mesh sizes (Figure XV). The particles at 80 mesh showed some areas with thin needle-like crystals and many smaller particles in the surrounding areas, while other particles were smooth and showed no crystal formation. Particles at 150 mesh exhibited a very thick network of flat crystals.

The micrographs of the Chromosorb particles coated with both dyes showed no signs of crystals at 60 and 80 mesh size, although they did appear to be coated with something (Figure XVI).

The micrographs of the Florisil particles coated with both dyes exhibited signs of crystals at 60, 80, and 150 mesh sizes, but the surface appeared to be covered with many small particles (Figure XVII).

Figure XII; Scanning electron micrograph of silica gel

Figure XII

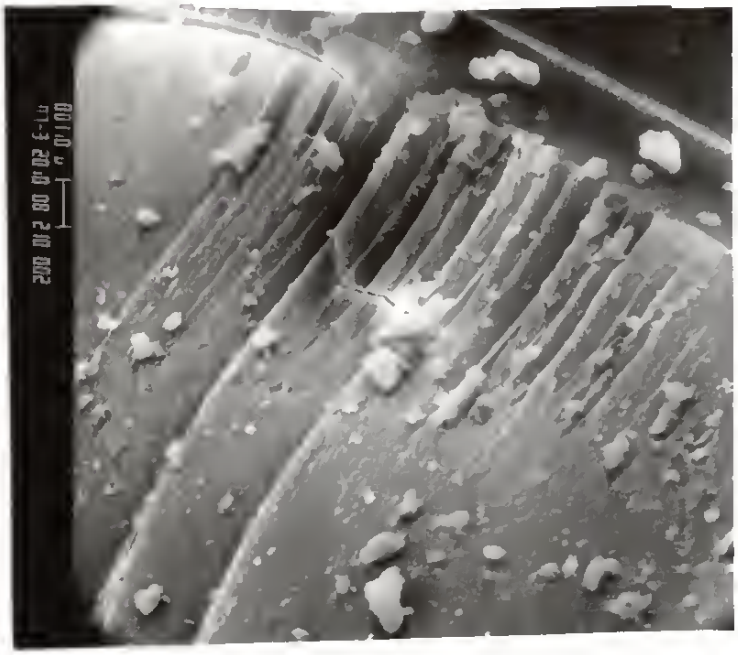


Figure XIII; Scanning electron micrograph of silica gel coated with orange I

a) less than 80 mesh

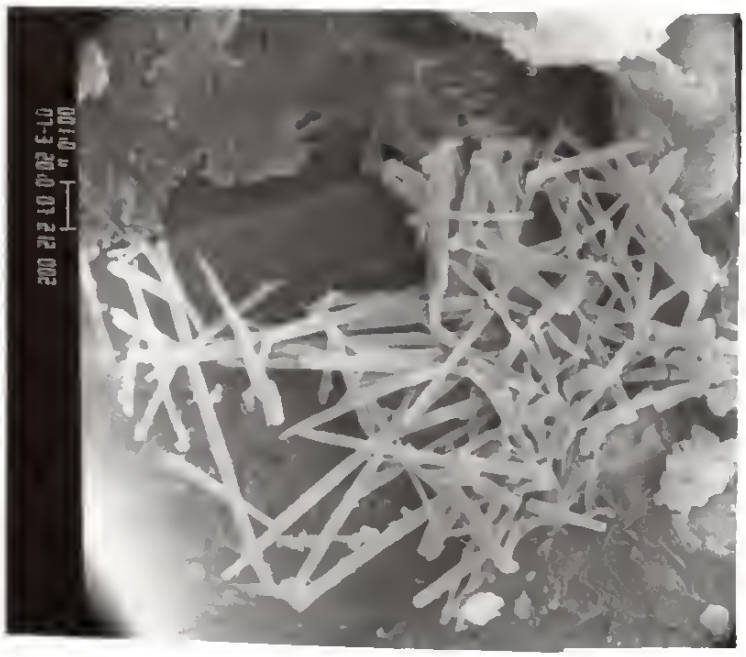
b) 80 mesh

c) 150 mesh

a.)

Figure XIII

a.)



a.)

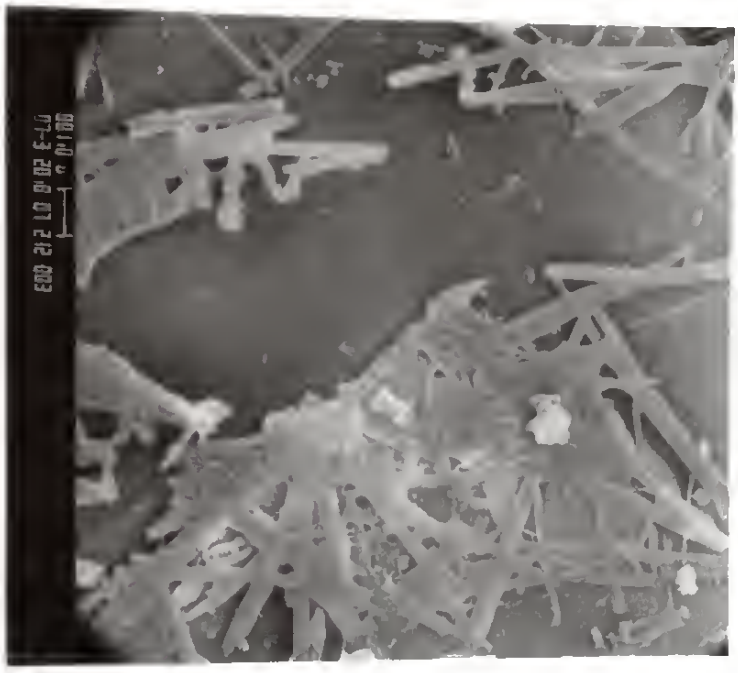
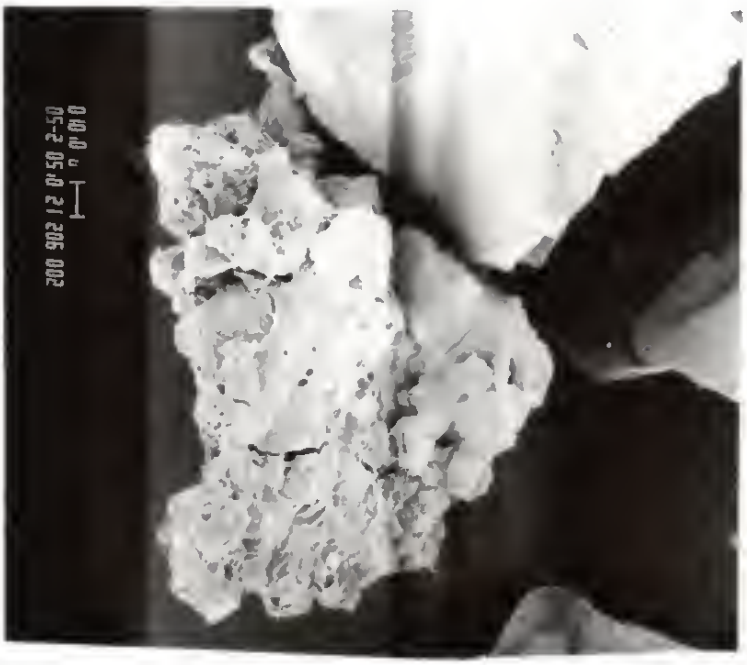


Figure XIII

b.)



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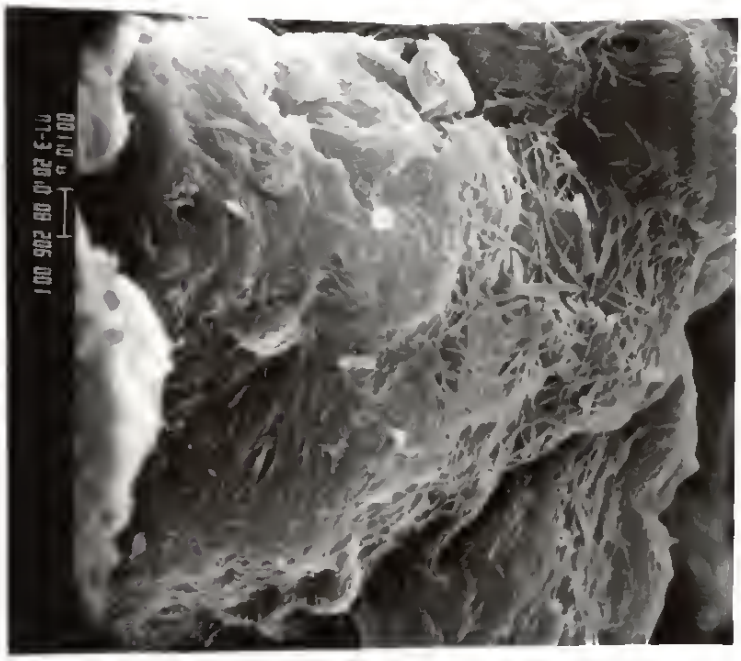
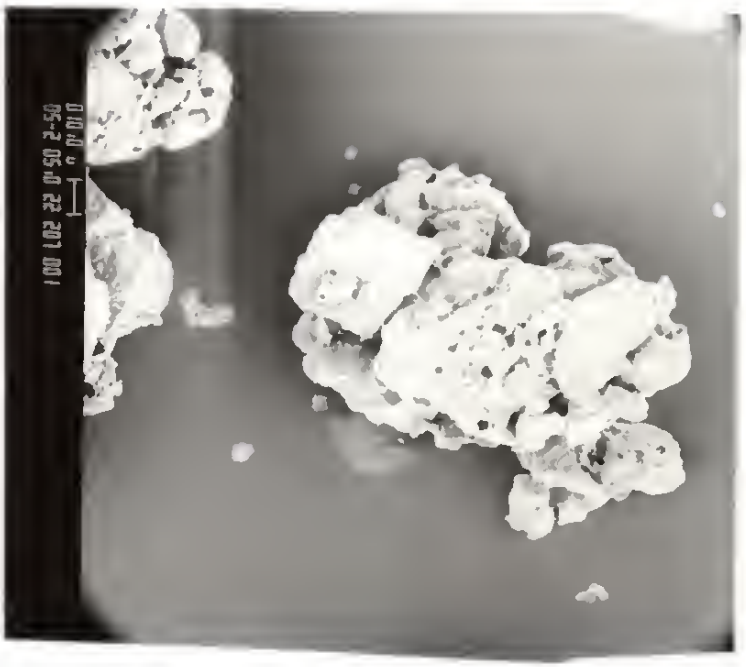


c.)



c.)

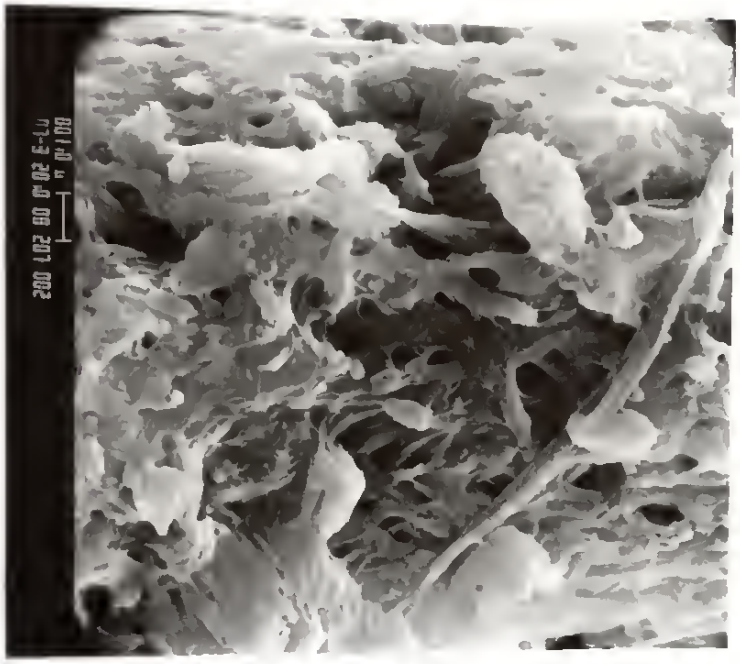


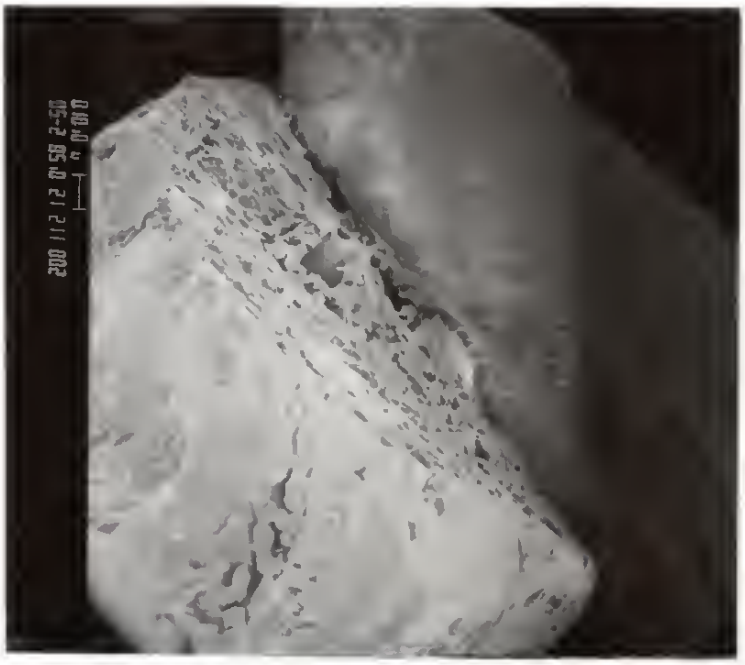
Figure XIV; Scanning electron micrograph of silica gel coated with brilliant green

a) < 80 mesh

b) 80 mesh

c) 150 mesh

a.)



a.)

Figure XIV

b.)

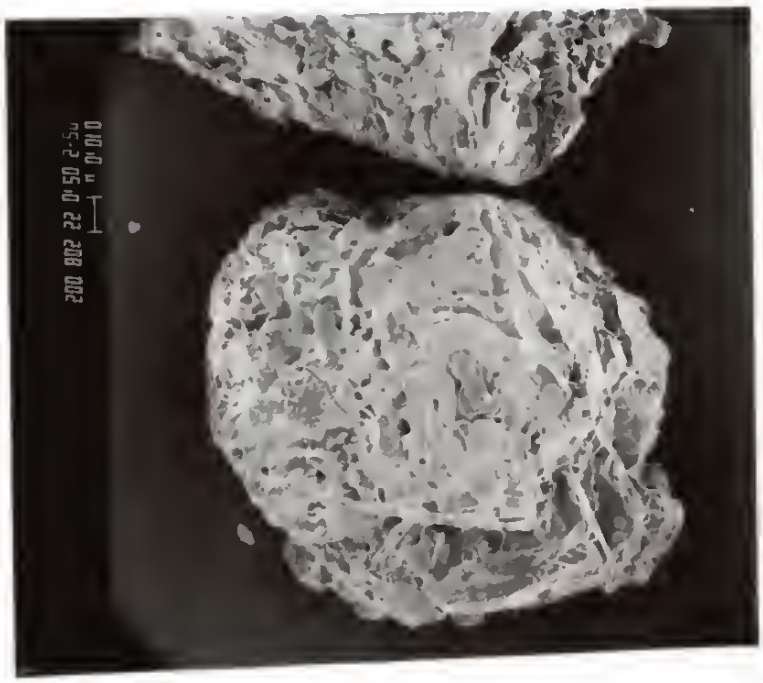
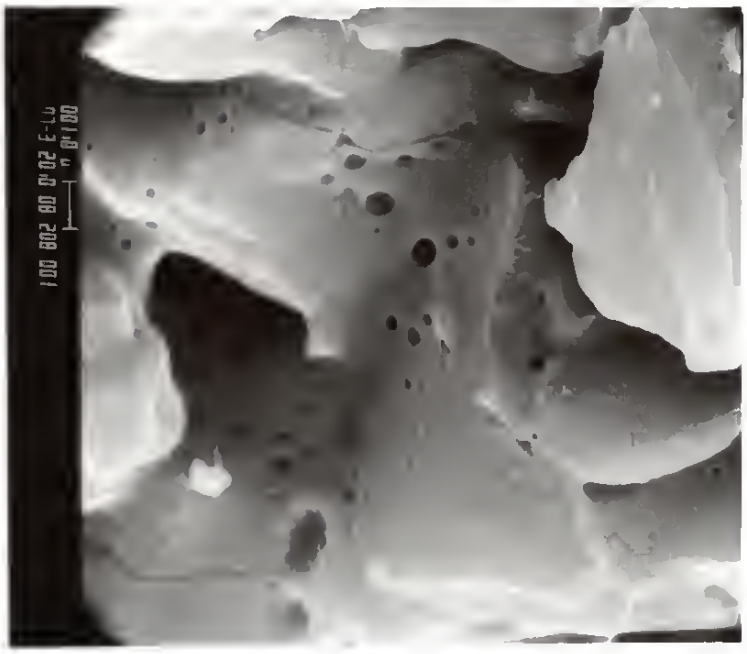


Figure XIV

b.)



b.)

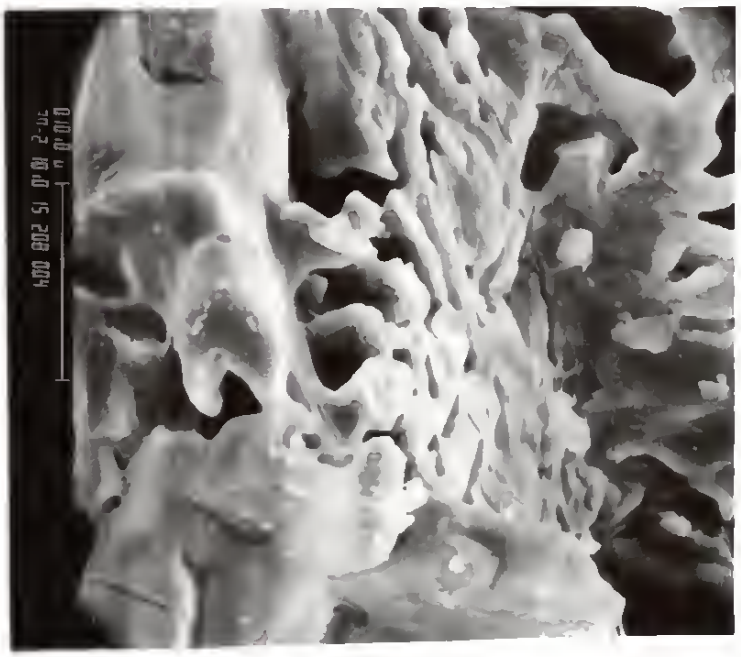
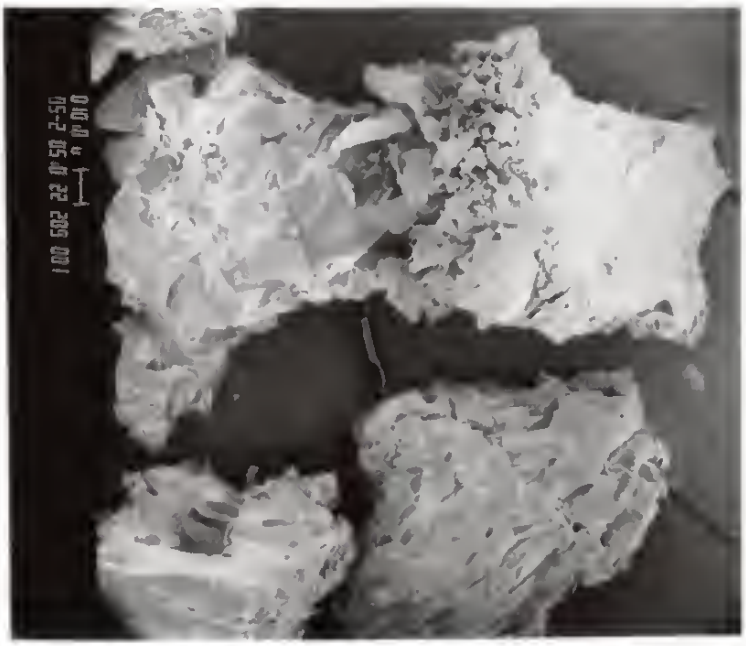


Figure XIV

c.)



c.)

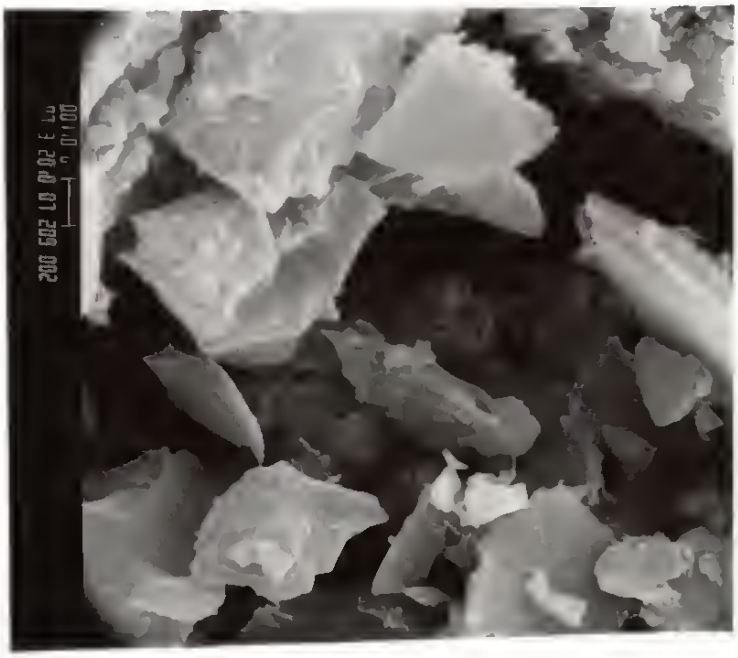


Figure XV; Scanning electron micrograph of silica gel coated with orange I and brilliant green. False positives.

a) 80 mesh

b) 150 mesh

a.)

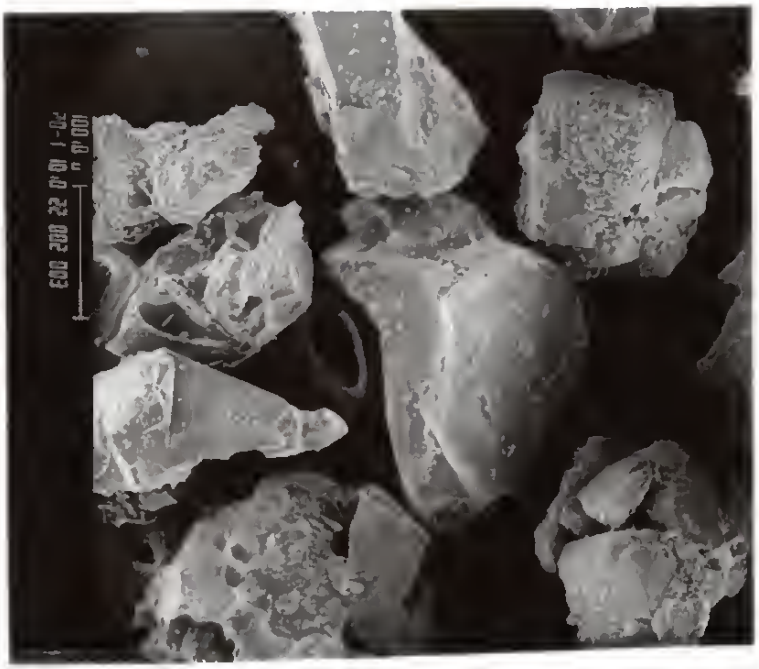
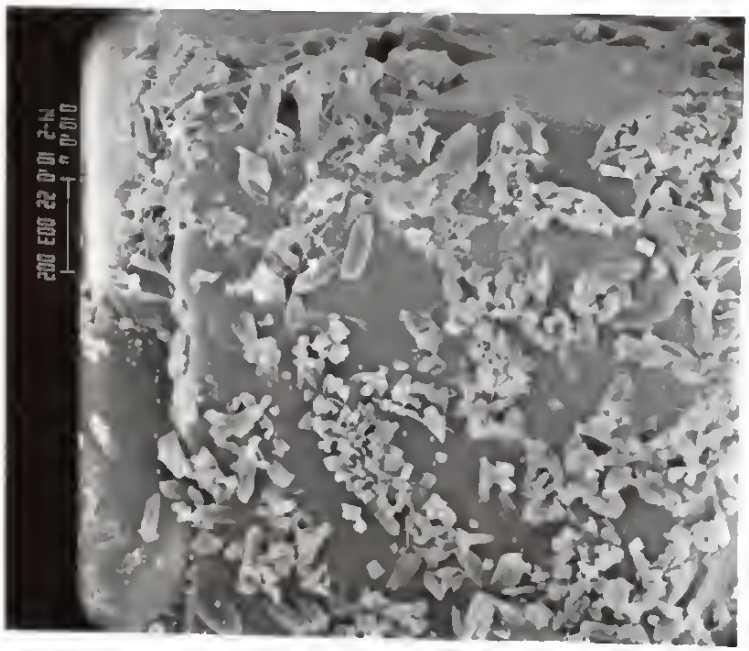
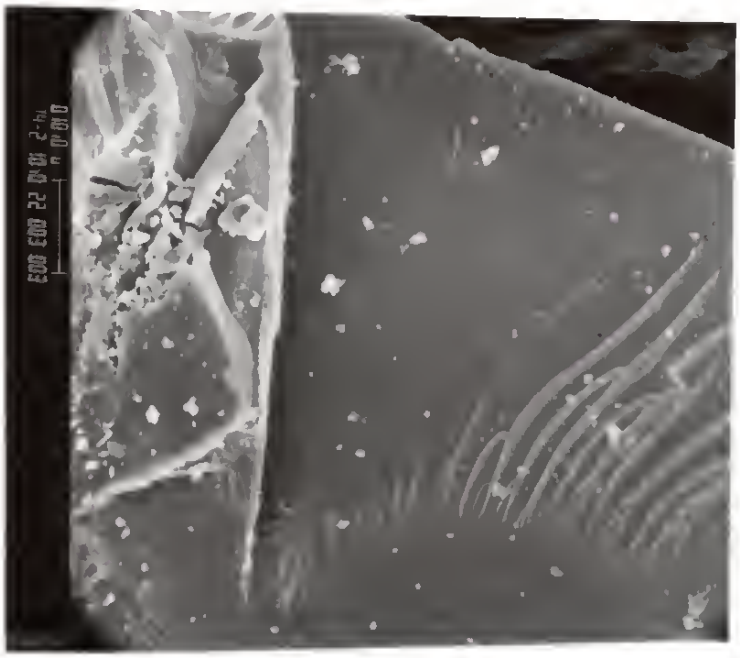


Figure XV

a.)



a.)



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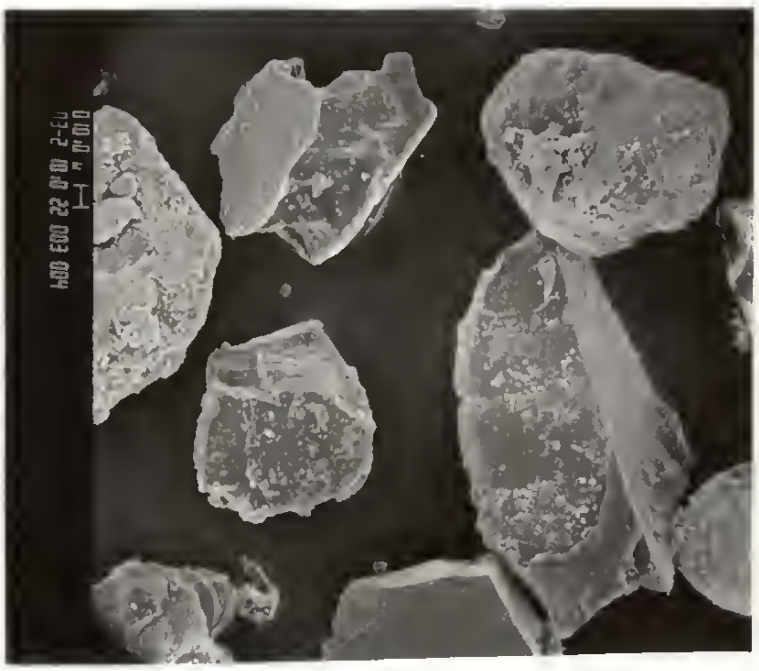

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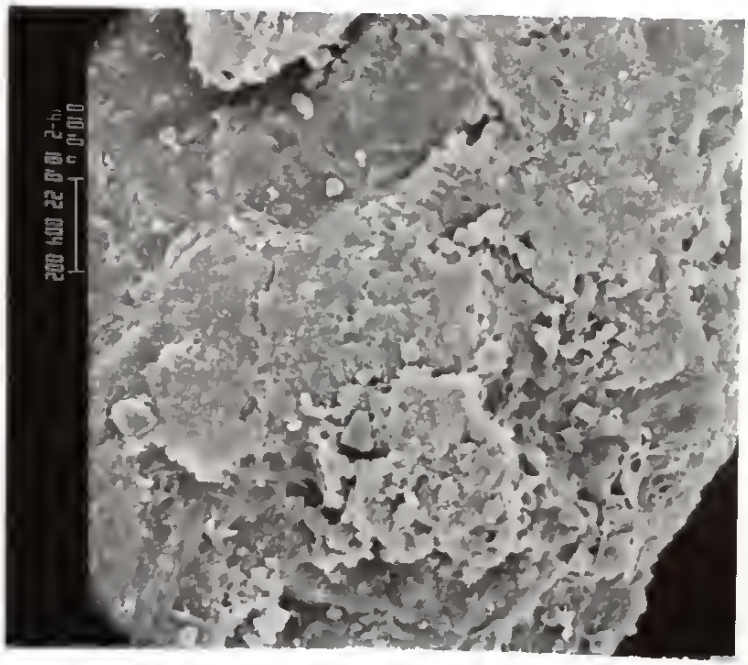

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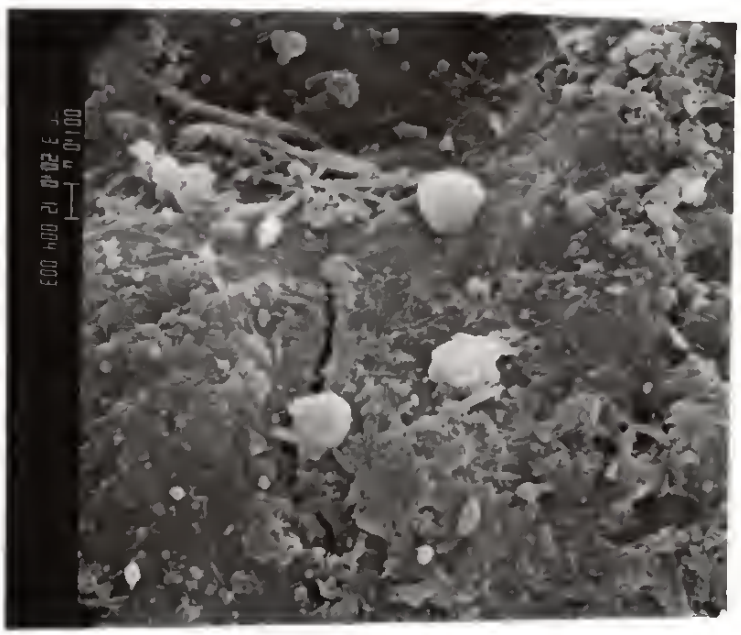


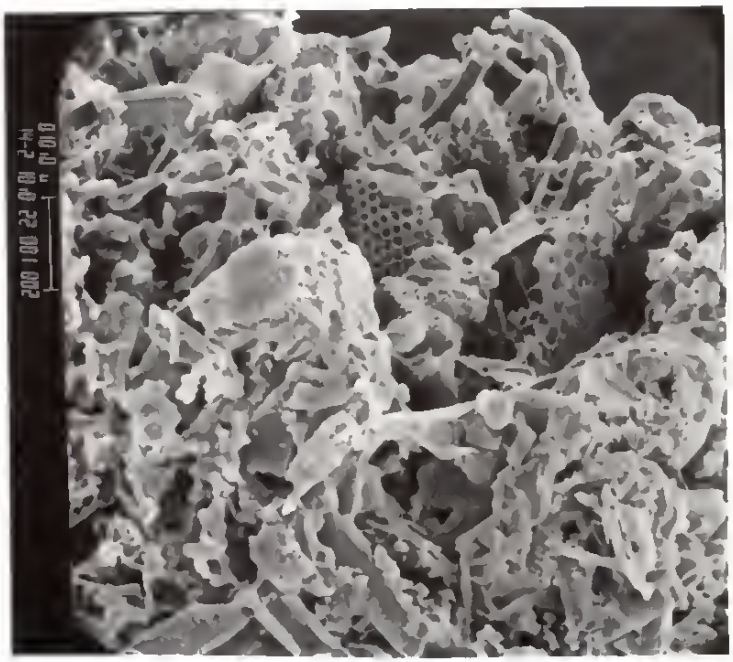
Figure XVI; Scanning electron micrograph of Chromosorb coated with orange I and brilliant green. False positives.

a) 60 mesh

b) 80 mesh

Figure XVI

a.)



b.)

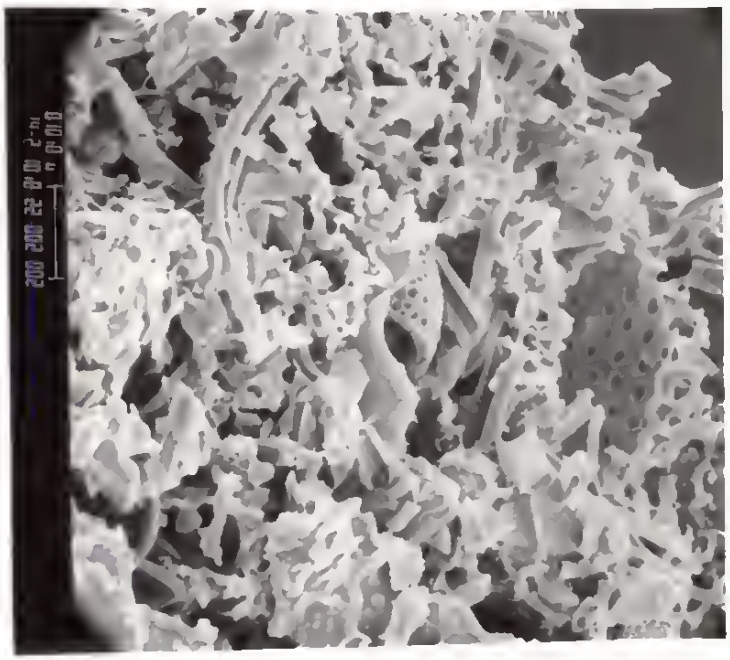


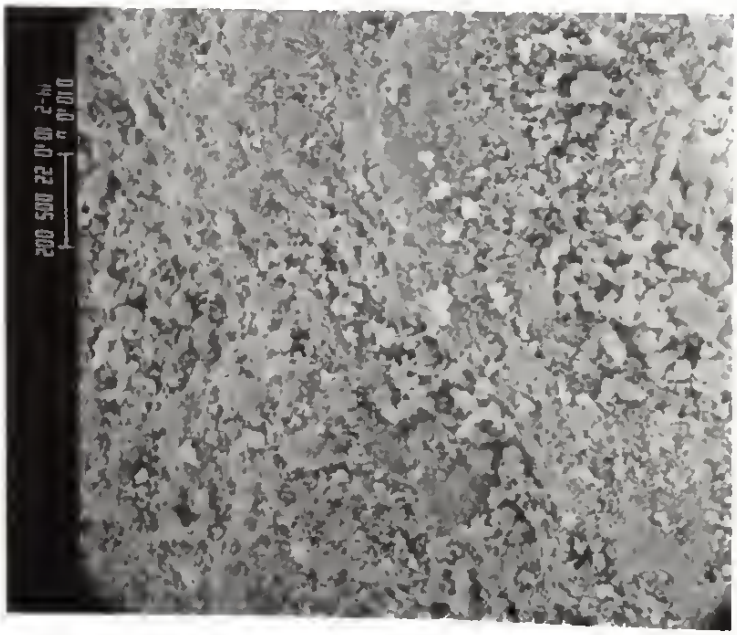
Figure XVII; Scanning electron micrograph of Florisil coated with orange I and brilliant green. False positives.

a) 60 mesh

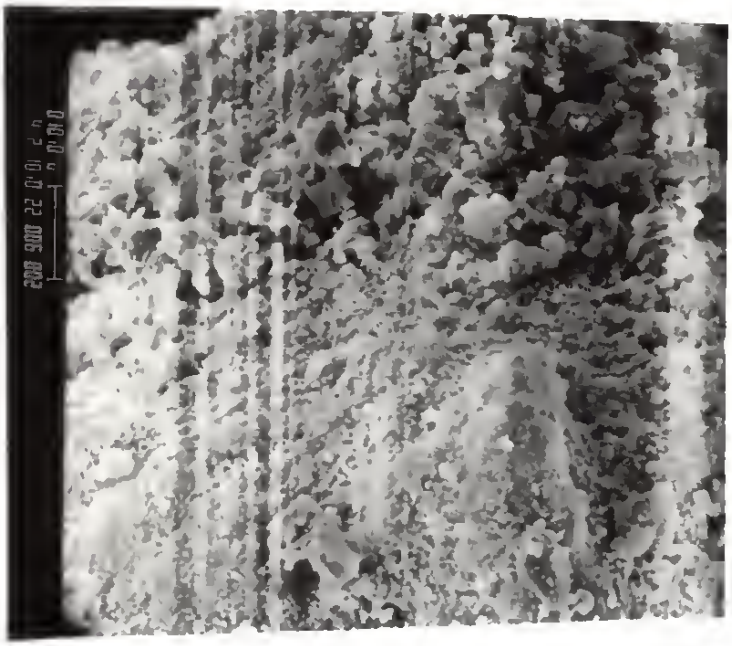
b) 80 mesh

c) 150 mesh

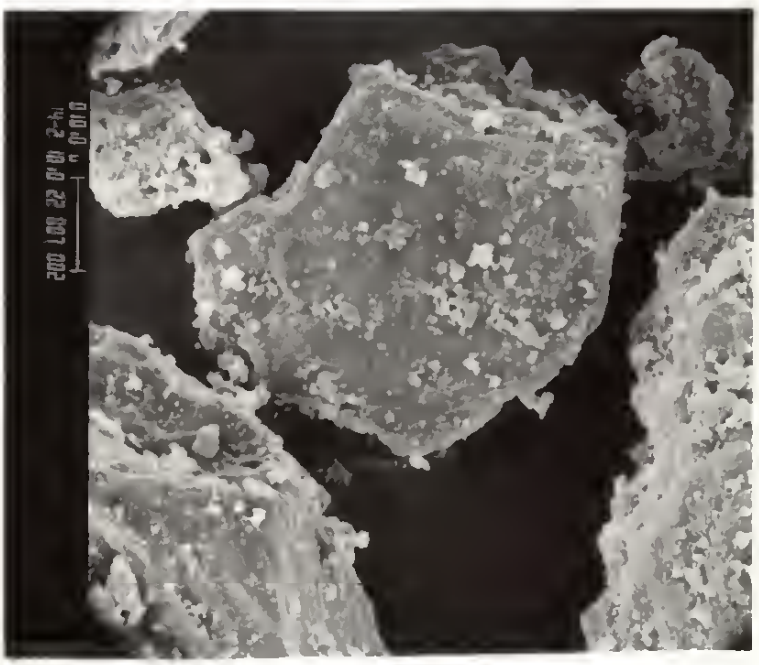
a.)



b.)



c.)



CONCLUSION

According to the results obtained from the tests of the moist fruits and vegetables, dried fruits and vegetables, french fries, and wines, the test strip was found to be simple to use and gave an immediate response. It also produced colors that were easily distinguished and was more sensitive than the most sensitive person, The test strip was found to be stable to temperature changes and when produced by hand, estimated to cost $0.52/500 strips.

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ANALYTICAL LETTERS, 19(1&2), 37-46 (1986)

TEST STRIP FOR THE RAPID IDENTIFICATION OF SULFITE ON FOODS

Key words: Sulfite, Food, Brilliant Green, Orange I

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ABSTRACT

A rapid, sensitive, test strip has been developed for those people allergic to sulfite on foods. The strip is composed of a mixture of orange I, brilliant green, and sodium hydrogen carbonate deposited on 80-120 mesh alumina. Within 15 seconds the black strip turns red in the presence of as little as 0.5 ug of sulfite or green in the absence of sulfite.

INTRODUCTION

Sulfur dioxide and sulfite ion have been used for decades as preservatives for foods [1]. The sulfite, in the form of aqueous solutions of sodium sulfite, hydrogen sulfite, or the metabisulfite, is sprayed on foods such as lettuce, apples, and bananas that turn brown on exposure to air, or the foods are dipped into the solutions[1]. In addition, sulfite is added to several dried foods such

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as apricots, peaches, and pears. Sulfite also is often added during the processing of french fried and hash brown potatoes before they are frozen and shipped to restaurants [2]. The dip solutions are commonly 10% and a typical spray solution contains one tablespoon of a mixture of sulfite, citric acid, ascorbic acid, and sodium carbonate per gallon of water. Sulfites also are added as a preservative in several drug preparations [3].

Within the past few years, evidence has been obtained that about 6 out of every 100 people have some degree of allergy to sulfite ion [4,5,6]. Usually, the reaction is shown by a difficulty in breathing within 10-15 minutes after exposure and the reaction persists from several minutes to a few days depending on the person's sensitivity. In moderate cases, convulsions have occurred; in extreme cases, people have lost consciousness; and in rare cases, death has resulted (12 in the past 3 years). This has been more noticeable recently because of the addition in most restaurants of elaborate salad bars in which the fruits and vegetables may be exposed to the air for several hours before they are eaten and may be treated with sulfite to prevent browning.

According to Simon [7], more sensitive people get a reaction from 10 mg of sulfite, but the most sensitive person yet found got a reaction from only 5 mg. The mechanism of the reaction is not yet known, although it is thought to be enzyme- related because of the rapidity of onset.

What is needed is a method that sensitive people can use to detect whether sulfite has been added to the food they want to eat.

The method must be rapid so it can be used by people in salad bar lines, it must be simple so anyone can use it, and the chemicals must be stable so that if the test is used only occasionally, it will still work properly. If color reactions are to be used, then the color change must be easily discernible in darkened rooms. To protect the credibility of the restaurants, false positives should be negligible.

Previously known reactions of sulfite or sulfur dioxide involve malachite green [8], sodium nitroprusside and rosaniline [9]. The malachite green reaction works well, but the color change from green to colorless is not always easy to visualize on paper. The nitroprusside reaction works when the solid is freshly prepared but not if it becomes dry, even if the solid is re-wetted. A commercial strip changes from light red to dark red, a color change hard to detect in dimly lit rooms. The rosaniline is primarily a colorimetric test for solutions. A recent test kit based on iodine-iodate [10] reactions is not practical as a consumer test because it requires several minutes, and several milliliters of liquid which would have to be carried in a purse or wallet.

A test strip, like a piece of litmus paper, that is easy to carry in a purse or wallet, that turns red (danger) well below the level of the most sensitive person, and turns green (safe) if only water is present is described.

EXPERIMENTAL

CHEMICALS:

Alumina, W200 acid, ICN Pharmaceuticals Inc.

Orange I, Allied Chemical Co.

Brilliant Green, Fisher Scientific Co.

Sodium hydrogen carbonate

Acetone

"Fresh Spud", Commercial preparation, Diamond Crystal Salt Co., Wilmington, MA 01887

"SpraMent" art and display adhesive, 3M Co.

PROCEDURE FOR PREPARING THE TEST STRIP

Combine 1.34 g of unsieved alumina and 0.20 g of orange I and dissolve in about 15 mL of acetone. Allow to evaporate to dryness with gentle and occasional stirring. Add 0.12 g of brilliant green, 15 mL of acetone, and evaporate to dryness. Sieve this mixture, keeping the 80-120 mesh fraction. Add $NaHCO_3$ at 0.05 times the weight of the sieved product. Mix the solid particles. Cut a 2 mm wide by 4 cm long slit in a 7.5 x 12.5 cm file card and place this stencil length wise over a 1 cm x 5 cm strip of white cardboard. Spray the slot with SpraMent, an adhesive, and then gently place the sticky side onto the particles. Remove the strip, tap gently to remove loose particles, and the strip is ready for use.

PROCEDURE FOR USE

Moist fruits and vegetables

Place the dye side of the test strip onto a wet area of a piece of lettuce, apple, etc. The black strip will turn red where the

moisture touches it within 15 seconds if sulfite is present and a dark green if not. These colors are easy to detect against the white background. The green color forms slower than the red color. If the test strip is placed directly onto an apple slice a false positive sometimes occurs. This can be avoided by allowing a drop of water to fall from the apple to the test strip and after a few seconds a proper test will be observed.

Dried fruits and vegetables

Place one drop of water on the item to be tested and then place the test strip on top of the drop of water. Hold the strip in place for about 5 seconds, then remove it. Again the color will be red if sulfites are present and green if not.

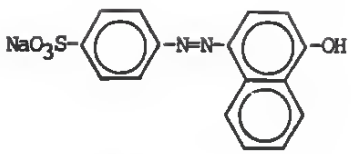
French fries

Bend a french fry until it breaks and exposes the inner portion. Place one drop of water on the exposed portion and lay the strip on the drop of water. A positive reaction is a red color as before, a negative reaction is an orange background with large green spots.

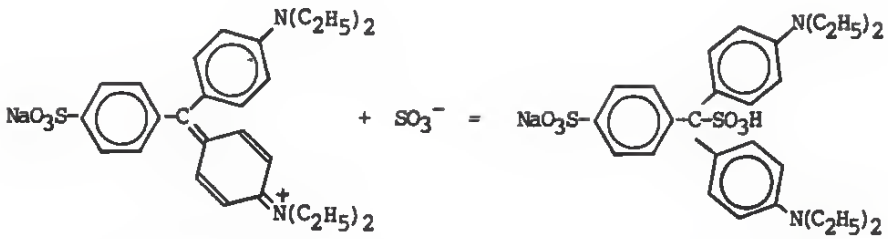
RESULTS AND DISCUSSION

General background

The final dye mixture consists of orange I and brilliant green. Orange I is not affected by sulfite but sulfite reacts with brilliant green at the central carbon to remove the conjugation and produce a colorless compound. This reaction works best at a pH around neutral, so $NaHCO_3$ is added for pH adjustment.



Orange I



Brilliant green (green) (colorless)

The mixture of the dyes is a dark green, almost blsck color. When the aulfite reacts with the brilliant green to make it colorless, the red color of the orange I becomes visible. If no sulfite is present, then the unreacted brilliant green dissolves slightly in the wster of the test drop producing a dark green color.

Dyes

Other dyes tried were Fast Red S and Orange II. These did not produce the deep red color desired, although they could be made to work. Brilliant green is more sensitive to sulfite thsn malachite green and appears to react faster.

The chemistry of the reaction of brilliant green with sulfite has not been reported previously. It was discovered several years ago by one of the suthors when attempting to develop a piezoelectric test for SO_2 in air. The test was not sufficiently aensitive and the

results not reported.

Support

This was found to be important. Initial studies on paper produced the desired color change but only after 20-30 minutes. This was believed to be due to the depth of penetration into the paper plus a surface adsorption that slowed down the reaction of the sulfite with the dye.

Silica gel and alumina were tried in an attempt to provide a support that would not allow deep penetration and possibly provide a different surface reaction. It was found that no reaction would take place on silica gel even up to 300 ppm sulfite. Alumina powder stuck to adhesive tape provided a reaction at the desired rate but gave erratic results. Alumina TLC plates usually gave a positive result even with distilled-deionized water. It was found that if the alumina was greater than 80 mesh then no reaction would occur with sulfite even up to 300 ppm. If the alumina was smaller than 150 mesh then a false positive was obtained in every test. If the alumina particle size is smaller than 80 mesh and larger than 120 mesh, then the reaction occurs within 15 seconds with as little as 0.5 ug of sulfite. This is not considered to be a problem, since it is easy to obtain a 80-120 mesh fraction.

Electronmicroscopic examination of the various particle sizes indicated that the large particles were covered with a thick layer of needle shaped crystals of brilliant green while the small particles had very few needle crystals on their surface. It is believed that the large crystals always produce a negative response because there

is not sufficient sulfite to bleach all of the brilliant green, the remaining brilliant green dissolves in the water present to produce the green solution. The very small crystals produce false positives because there is very little brilliant green on them and the orange I readily dissolves to produce a red solution.

Detection limits

It was found that a positive test could be obtained within 15 seconds with one drop of 25 ppm solution. This is equivalent to 0.5 ug of sulfite. One drop of a typical commercial sulfite spray solution would contain between 0.2-0.4 mg of sulfite and one drop of a dip solution would contain about 5 mg. Therefore, the test strip is capable of detecting sulfite at concentrations many times lower than might be expected to be encountered. The most sensitive person yet discovered requires at least 5 mg of sulfite to get a reaction. At 0.5 ug/drop, this could be spread out over 10,000 drops (500 mL!) in one salad and still be detected.

Effect of temperature

In a practical situation, a person may come into a restaurant or store from either very cold or very hot outside temperatures. The test strips would initially be at those temperatures and because of their lack of bulk would be expected to very rapidly reach ambient temperatures. However, the strips were both cooled to 12°C and heated to 40°C and tested immediately. No difference was noticed at the hotter temperature but a slightly more intense color was observed with the colder strip. For practical purposes, temperature has no effect.

Interferences

None of the other common preservatives used on the foods tested produce a false positive. The use of disodium hydrogen phosphate at the concentrations used on products sold for home use did not have any noticeable effect. Ascorbic acid and citric acid at 10% and 1% concentrations produced an olive green color. They caused no noticeable interference in tests of the commercial preparation "Fresh Spud". Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and propyl gallate are too insoluble in water to interfere.

ACKNOWLEDGEMENT

The financial assistance by the Kansas Agricultural Experiment Station for the purchase of the chemicals is appreciated. This is contribution No. 86-134-J.

The assistance of Eileen Schofield in the preparation of the manuscript is most appreciated.

The assistance of Wendy's in the preparation of test french fries was most helpful.

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Received November 13, 1985
Accepted November 19, 1985

PART B: A QUICK AND SIMPLE TEST FOR NITRATES IN DRINKING WATER

INTRODUCTION

The public has become increasingly concerned with the environmental damage and impact that technology has forced upon us. One of the issues that has been plaguing America is water contamination. A specific case that has surfaced is the danger of nitrate salts contaminating water (1). The source of the tainted water arises from excessive use of fertilizers, municipal and industrial waters, septic tanks, refuse dumps, and animal feedlots including run off and leaching. As the number and size of feedlots increase, this source of nitrate increases significantly. For example, an average steer of 450 kg excretes about 43 kg of nitrogen per year, a 16,000 head feedlot would produce about 700 metric tons of nitrogen per year -- an amount equivalent to a city with a population of 130,000 (1).

The population has a right to be concerned about the nitrate contamination of their water since excessive amounts of nitrate are known to cause detrimental effects on those exposed (1). In fact there are an average of 70 human illnesses and deaths due to nitrate poisoning each year in this country (2000 world wide), with additional losses of cattle, hogs, turkeys, and sheep (1). The first reporting of nitrate poisoning in humans was in 1944 and was reported to be most harmful to infants under six months of age (1). When infants injest water or food with high nitrate concentrations their bodies produce a situation known as infant methemoglobinemia. Infant methemoglobinemia is believed to occur as follows. The upper intestinal gastric juice of infants below 6 months of age is usually at pH 4 or higher. This pH level allows a nitrate reducing bacteria (B. subtilis) to grow in their intestines. This bacteria reduces any nitrate present to nitrite which can then readily pass into the blood

stream. Once the nitrite is in the blood stream, it reacts with the hemoglobin. Hemoglobin is a protein that carries the oxygen for the blood. At the center of every hemoglobin group is an iron atom (Fe^{+2}) which when the hemoglobin is oxidized to methemoglobin by nitrite, the iron is transfered from the ferrous to the ferric (Fe^{+3}) state. It is this transition to the ferric state that causes the hemoglobin to lose its ability to carry oxygen for the blood and once the blood cells are deprived of oxygen, the child turns blue from a lack of oxygen. If methemoglobinemia occurs for a short period of time, mental retardation can occur, but if it is not treated death can result (7). There are three other factors that exits making infants more prone to high levels of methemoglobin. They are (1) fetal hemoglobin, which is still present in newborns, and is more easily oxidized to methemoglobin than adult hemoglobin, (2) infants are deficient in two enzymes in their red blood cells, methemoglobin reductase and diaphorase, which convert methemoglobin to hemoglobin (1), and (3) infants drink more water on a per Kg basis.

The acceptable limit for nitrate appears to vary with the individual. However, the limit for infants has been set at 10 ppm and for adults at 45 ppm.(4) For cattle the range seems to be between 75 - 150 ppm which is exceeded by many partially dried up creeks. City water usually contains 3 - 5 ppm nitrate, but many rural wells may exceed 100 ppm.

Many people may wonder what are the consequences of continuous exposure to low levels of nitrate. To provide an answer to this question a toxicological study of nitrate was performed to investigate the possible physiological significance of low levels of methemoglobinemia such as might exist in cases of chronic subclinical intoxication (7). This particular study worked directly with nitrite rather than try to regulate the

conversion of nitrate to nitrite in experimental animals. The initial study was to determine the lethal doses and the kinetics of methemoglobin formation in albino sabra rats. Lethal doses were determined to be approximately 200 mg/kg. It was also determined that the methemoglobin level peaked after about 45 minutes to one hour and returned to normal in 3 to 4 hours after a single sublethal dose of nitrite was administered by oral intubation. It was interesting to note that as the levels of methemoglobin increased, the rats' body temperature decreased and then returned to normal during the 3 to 4 hour period. The return to normal during the 3 to 4 hour period indicated the effectiveness of the methemoglobin reductase system.

A chronic test of rats was performed on three groups of rats with water containing nitrite (7). Group A was given water containing 4.5 g/L of $NaNO_2$, Group B was given 3.0 g/L $NaNO_2$, and Group C was not given any nitrite and was used as a control. The rats initially weighed approximately 110 g and were monitored for 56 days. The concentration of nitrite was calculated based on the concentration of nitrite in the water and the average daily intake of water. Group A's intake ranged from 610 - 1,066 mg/kg/day while Group B ranged from 450 - 831 mg/kg/day. All blood samples were taken at night during the rats' greatest period of activity.

The results after the 56 days showed a significant difference in the body weights of the rats. Group A increased to 150 g, Group B increased to 235 g, and Group C, the control, showed the greatest increased in weight to 270 g. To test the effect of methemoglobin on the behavior of animals a study was conducted in a barrier activity box using mice treated with nitrites. The mice showed a significant reduction in activity patterns when compared to a control group.

The toxicological study also investigated the effect of nitrites on newborn and suckling rats (7). The purpose of this section of the study was to determine if it might be possible to transfer nitrite by direct transplacental transfer from the mother to the fetus and if nitrite might transfer in the milk from the mother to the suckling rat. The study showed that there was transplacental transfer of nitrite from the mother to the fetus, but that there was no transfer of nitrite in milk from the mother to the suckling rat. The newborn rats suffering from transplacental transfer of nitrite showed very poor development at birth and a high death rate.

OBJECTIVE

The objective of this research was to provide a rapid and readily accessible method of detection for nitrates in well water, where the majority of contamination exists. The method is in the form of a colorimetric test using sulfanilic acid, zinc, and N-(1-Napthyl)-ethylenediamine dihydrochloride (NED). The chemical reactions are not new. What is new is the arrangement and amounts of reagents so that a very simple and rapid test can be made that covers the toxic range from infants to animals. The test apparatus must be disposible, not contaminate the water supply, the reagents must be stable, and not be a health hazard when disposed of. The method must be so simple that anyone can obtain correct results without having a knowlage of chemistry.

LITERATURE REVIEW

There exists several methods for the determination of nitrate. Most of the methods that have been developed are quite tedious and require a spectrophotometer which limits them in their practical applicability.

The first method for the determination of nitrate is the cadmium reduction method by Strickland and Parsons (6). This method involves the quantitative (almost) reduction of nitrate to nitrite through the use of a column containing amalgamated cadmium filings. The nitrite is diazotized with sulfanilamide and then detected colorimetrically as the colored azo dye which results from the coupling of N-(1-naphthyl)-ethylenediamine after the reaction of the nitrite with the sulfanilamide. The limit of detection is 2 μg NO_3-N /L. This method is manually tedious, requires large sample volumes (80-90 mL), and uses large amounts of cadmiun (a known carcinogen).

Another method is one of the more practical of the existing methods for nitrate determination. This method was developed by Gaughush and is a rapid manual method using a modification of the cadmium reduction method (5). The modification of the cadmium reduction method is the scaling down of reagent quantities to accommodate 5 mL samples and using reaction tubes rather than large columns. Otherwise the reaction is basically the same as the cadmium reduction developed by Strickland and Parsons. Nitrate concentrations of 2 - 100 μg NO_3-N/L can be detected in samples as small as 5 mL without loss of sensitivity.

The third method for the determination of nitrate was developed by Armstrong and is slightly more involved than the above method (4). The method involves enhancing the spectrophotometric determination of nitrate through the addition of equal volumes of H_2SO_4 and a chloride concentration

of 0.025 M or greater. This produces a shift from 210 mμ to 230 mμ. The purpose of the H_2SO_4 is to increase the reactivity of the nitrate ion which will make the destruction by hydrazine sulfate, a reducing agent, much easier. The sensitivity of this method is 100 μg NO_3^- N /L.

Another colorimetric method involves the use of brucine to produce a yellow color and was developed by Greenberg et al. (6). The method involves a series of reactions starting first with a reaction between the sample, NaCl, and H_2SO_4. The next reaction involves the addition of brucine-sulfanilic acid reagent to the solution above. This mixture is then placed in a boiling water bath for 20 minutes and the absorbance determined with a spectrophotometer. Two drawbacks with this method (1) are that the sample must be pretreated with a solution of sodium arsenite, which along with brucine is very toxic and (2) the limit of detection ranges from 0.1 - 1 mg NO_3^- N /L - a narrow range.

The use of chromotropic acid to determine nitrate was developed by West and Ramachandran (6). The principle of the method is that two moles of nitrate react with one mole of chromotropic acid. The result of this reaction is a yellow colored species which is then measured with a spectrophotometer. One of the advantages to this method is that the color that is produced is stable for up to 24 hours. Unfortunately the disadvantages to this method out number the advantages. Interferences include residual chlorine, certain oxidants, nitrite, ferric ion, barium, lead, strontium, iodide, iodate, selenite, and selenate ions. The problem with residual chlorine and oxidizing agents can be eliminated by the addition of sulfite. The problem with nitrite and ferric ion can be eliminated by the addition of urea and antimony respectively. The limit of detection for this procedure is from 0.1 - 5.0 mg NO_3^- N /L.

A method that faciliates the use of a noncolorimetric approach is Devarda's alloy reduction method (6). The procedure that is involved is similar to the procedure used in the distillation procedure of the Kjeldahl method. Nitrate is reduced by Devarda's alloy (50% copper, 45% aluminum, and 5% zinc) to ammonia and trapped in a solution containing boric acid. The determination of the ammonia can then be carried out as usual. One major drawback with this method is that ammonia and nitrite must be determined before the determination of nitrate. If not, the nitrite will be reduced to ammonia and the ammonia will distill over and interfere with the determination of nitrate. This method is good for samples containing more than 2 mg NO_3^- N /L.

One of the simplier methods involves the use of the nitrate ion selective electrode (6). The method simply involves using a commercially available electrode to detect the nitrate and then reading the results in millivolts off of a meter. Common interferences include chloride and bicarbonate ion. The chloride can be removed by the addition of Ag_2SO_4, while the bicarbonate is removed by adjusting the pH so that it is between 4 to 4.5. The limit of detection with this method is from 0.2 to 1,400 mg /L NO_3^- N. The cost, about $1,000, is not practical for intermittant use on the farm.

Two commercial products that were tested are (1) a product manufactured by Anspec called Nitrate Test, and (2) a product called Nitra Ver 5 manufactured by the Hach company. The Nitra Ver 5 test comes in the form of a reagent pillow which is cut open and added to 5 or 25 mL samples. The solution then turns a golden yellow color. The drawbacks with this method are that there are (1) no instructions with the test, (2) no color chart to match the sample color against, (3) the reaction takes 2-2.5 minutes, and

(4) a farmer does not know how to measure out 5 or 25 mL. There is no specified time on the test. The other test, by Anspec, is a test strip which can quantitate nitrate and qualitate nitrite. The strip is easy to use and fast. There is also a color chart to match sample colors against. The only drawbacks with this strip are that it is not readily available in the United States since it is produced in Germany and the range is too narrow.

EXPERIMENTAL

CHEMICALS:

Zinc metal(technical-powder-dust), Fisher Scientific Company.

Sulfanilic acid, Fisher Scientific Company, Lot number 736417.

N-(1-Napthyl)-ethylenediamine Dihydrochloride, Fisher Scientific Company, Lot number 786495.

Sodium nitrate, Mallinckrodt, Lot number 7808 BRZ.

Phenyl mercuric acetate, Eastman Kodak, 702-1.

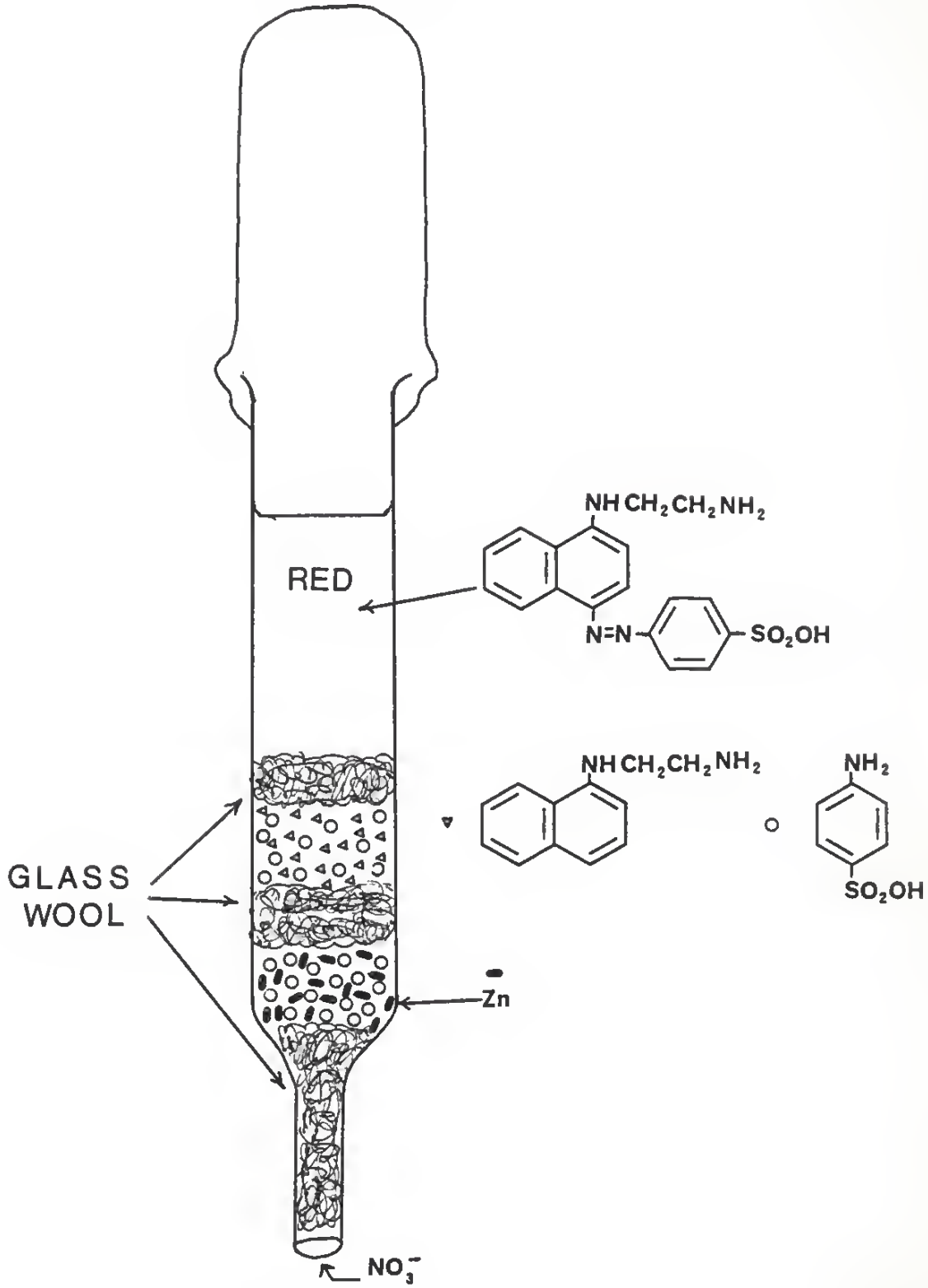
Glass wool.

All chemicals were reagent grade unless otherwise stated.

PROCEDURE FOR PREPARING THE TEST DROPPER

A small plug of glass wool is inserted into an eye dropper and placed at the tapered end of the dropper (Figure XVIII). On top of the glass wool plug is placed 0.1175 g of sulfanilic acid which is followed by 0.0038 g of zinc dust. On top of this combination is placed another plug of glass wool which is then followed by 0.0578 g of N-(1-Napthyl)-ethylenediamine dihydrochloride and sulfanilic acid in a 2 to 1 ratio. The N-(1-Napthyl)-ethylenediamine dihydrochloride is then topped off with a final plug of glass wool. The test dropper is then ready for use. In order to create a color chart, the test dropper is tested with solutions of sodium nitrate (containing phenyl mercuric acetate as a preservative) at various levels. If this is made available commercially, the color standards would be printed on the side of the container.

Figure XVIII; Diagram of the test dropper


RED
NH CH2CH2NH2
N=N
SO2OH
GLASS
WOOL
NH CH2CH2NH2
NH2
SO2OH
Zn
NO3-

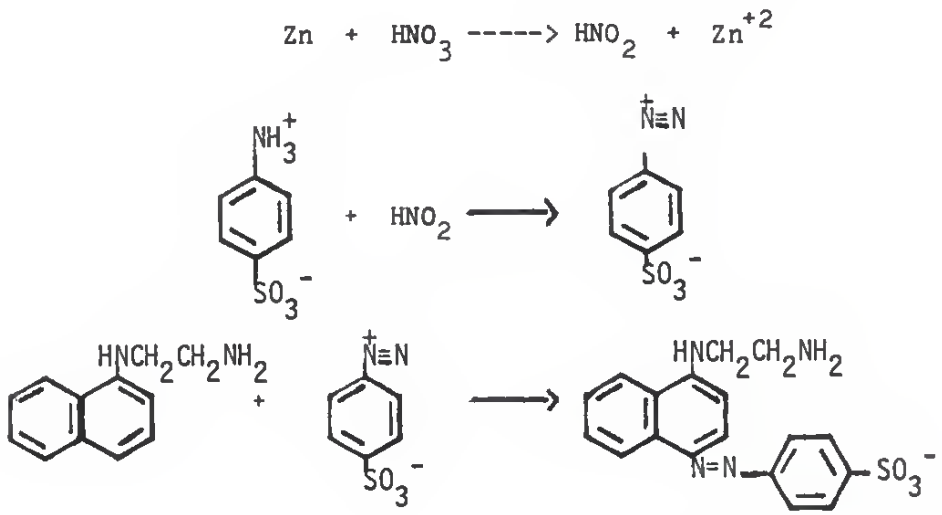
PROCEDURE FOR USE

The solution to be tested is suctioned up into the test dropper and allowed to react for 30 seconds. After the reaction period, the test dropper can then be matched against the reaction colors of known amounts of nitrate.

RESULTS AND DISCUSSION

Reaction

The reactions that take place in the test dropper are as follows:



red purple

Metals used for reduction

Another metal for the reduction, Mg, was tried in place of the powdered Zn. The Mg metal was not used in the final test dropper because it produced

large amounts of hydrogen gas. Granular Zn was also tried, but it failed to produce the reaction in a short period of time (it often took up to 24 hours to develop the color). Thus zinc powder was choosen because it provided a rapid reaction and did not produce a vigorous reaction as did the magnesium.

Effect of Layering the Reagents

This was found to be an extremely important fact. Initial studies combined the zinc powder with the N-(1-Napthyl)-ethylenediamine dihydrochloride. This caused the purple reaction color to rapidly turn brown in color. This is thought to be a chemical reaction between the zinc powder and the purple colored species. This can be supported by the fact that this phenomenon did not occur when the zinc powder was separated from the N-(1-Napthyl)-ethylenediamine dihydrochloride. Further studies indicated that the reaction time could be decreased by placing the zinc on top of the sulfanilic acid layer rather than in a separate layer. It is thought that when the solution of sulfanilic acid passed over the zinc, the surface of the zinc is cleaned by the sulfanilic acid. The effect of layering the reagents, especially the sulfanilic acid and the N-(1-Napthyl)-ethylenediamine dihydrochloride, also provided a small mixing chamber so that the color produced in the solution was uniform and without streaks.

CONCLUSION

According to the results obtained from the tests that were performed, the test dropper was found to be an accessible method of detection for nitrate in well water. It was also found to work well in the range from infants to adults to animals. The test dropper is simple to use, has reagents that are stable over an extended period of time, and are not a health hazard.

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A QUICK TEST FOR SULFITES ON FOODS AND NITRATES IN DRINKING WATER

by

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B. S., Saint Mary's College, 1984

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

ABSTRACT

A rapid, sensitive, test strip has been developed for those people allergic to sulfite on foods. The strip is composed of a mixture of orange I, brilliant green, and sodium hydrogen carbonate deposited on 80-120 mesh alumina. Within 15 seconds the black strip turns red in the presence of as little as 0.5 μg of sulfite or green in the absence of sulfite.

A quick and simple test for the detection of nitrates in drinking water has been developed. The test is composed of zinc dust, sulfanilic acid, and N-(1-naphthyl)-ethylenediamine dihydrochloride layered in a eye dropper. Within 30 seconds the dropper turns red-purple in the presence of as little as 10 ppm NO_3^- N or colorless in the absence of nitrate.