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Multiwell Spot-Test Technique

La Técnica Multiwell Spot-Test

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Abstract - The "mutiwell spot-test" technique is presented as a microscale teaching tool for basic chemistry. The multiwell plate allows students to simultaneously observe the results of up to 96 spot-tests. Six experiments related to solubility rules, acid-base indicators, corrosion, precipitation, redox reactions and complex ion formation are described. The technique is flexible as it can be adapted to many classes of reactions and it may use common and low cost materials. These kind of experiments have the inherent advantadge of microscale techniques and have a positive response from the students. It was found that the proper use of the mutiwell spot-test technique can facilitate the establishment of student's conceptual connections between various related reactions.

Keywords - Microscale Laboratory, Qualitative Analysis, Equilibrium, Metals, Inorganic Chemistry.

Resumen - La técnica "multiwell spot test" es presentada como una herramienta de enseñanza de química básica. La placa multiwell permite a los estudiantes la vizualización simultanea de resultados de hasta 96 spot-tests. Se describen seis experimentos, relacionados con reglas de solubilidad, indicadores ácido-base, corrosión, precipitación, reacciones redox y de formación de compuestos complejos. La técnica es flexible y puede ser adaptada a varias clases de reacciones, utilizando materiales ordinarios y de bajo costo. Este tipo de experimentos tienen la ventaja inherente de las técnicas en microescala y permiten una respuesta positiva por parte de los alumnos. Fue descubierto que el uso apropiado de la técnica multiwell spot-test puede ayudar a establecer conexiones conceptuales de los estudiantes entre varias reacciones relacionadas.

Palabras clave - Laboratorio em Microescala, Análisis cualitativo, Metales, Química Inorgánica.

Introduction

In undergraduate Inorganic and Analytical Chemistry courses the students are presented to a plethora of test-tube reactions. They are supposed to remember important reactions such as identification tests, precipitation, redox reactions and complex ion formation. The amount of information is so big that they generally rather memorize the reactions than systematically correlate them.

In order to aid the establishment of conceptual connections between reactions we devised experiments where the results of many related reactions (about 100) could be visualized simultaneously. The most successful idea was to make spot-tests inside each of the 96 wells of a multiwell plate (Epp, 1991) see Figure I. Multiwell plates are used for biochemical purposes such as microbiological cloning, immunological assays and cell cultures. There are also some chemistry kits commercially available. The present work also contributes to the general effort of implementing microscale in the undergraduate chemistry lab (Szafran, 1989).

Amongst the various types of multiwell plates we chose the non-sterile polystyrene, flat-bottom 96 spot-plate for the following reasons:

1) The wells are big enough to allow water drops to get in without capillarity problems;

2) The transparency of the plate and the well's flat bottom allow the spot-tests to be easily visualized against light or dark screens with or without magnifying lenses;

3) The plate is cheap - A package with 10 such plates costs less than U$ 20.00.

4) The plate is light and small - each plate weights some 52 g and measures around 8.2 x 12.5 cm;

5) The plate can be easily handled and can even be passed hand-to-hand amongst students in a classroom;

6) The plates are easy to clean;

7) The number of wells in the plate fits to the number of desired spot-tests to be observed per plate.

Some of the limitations of these plates are the low heat resistance and the organic solvent incompatibility of polystyrene. Glass plates are also commercially available but are considerably more expensive than the polystyrene ones.

Experimental

In the following paragraphs we describe details of one experiment to show how we have been using the multiwell spot-test plate technique and present a series of other experiments to demonstrate the flexibility of the technique. For each experiment a Table that will contain the results of the spot-tests performed in the various wells of the plate must be built (like Table I see below). The actual dimensions of the plate (Figure I see below) should coincide with those of the drawn Table in such a way that the plate could be placed above the Table allowing formulas and names of substances in cells 1 through 12 and A through H to be easily visualized. One column and one row for blank tests may also be used if necessary.

In the Experiment below, the sections: Pre-lab assignment, Materials, Procedures and In-lab assignment are described as they would be given to the students. The section Results and Comments is directed to the teacher and contain ideas on how to extend the experiment and how other concepts can be worked out.

Experiment 1 - Precipitation reactions and solubility rules - transition metal ions.

Pre-lab assignment

P1- Using the solubility rules for simple salts in water predict which salts will precipitate when each nitrate solution of chromium (III), manganese (II), iron (III), cobalt (II) , nickel (II) , copper (II), silver (I), zinc (II), cadmium (II) and mercury (II) is mixed with each sodium solution of phosphate, hydroxide, carbonate, chloride, thiocyanate, iodide and sulfide.

P2- Predict which of the precipitates formed on P1 would dissolve if 6M nitric acid were added to the cell.

Materials - One 96 flat-bottom well plate, one thin glass rod and one washing bottle with water. Ten dropping bottles containing 0.1 mol/L solutions of nitrate salts of the following cations: chromium (III), manganese (II), iron (III), cobalt (II), nickel (II), copper (II), silver (I), zinc (II), cadmium (II) and mercury (II). These solutions will be referred to as cation solutions. Seven dropping bottles containing 0.1 mol/L solutions of the sodium salts of the following anions: phosphate, hydroxide, carbonate, chloride, thiocyanate, iodide and sulfide. These solutions will be referred to as anion solutions. One dropping bottle containing nitric acid 6 mol/L should also be available. (Nitric acid was suggested for this test because all nitrate salts are water soluble.)

Table of Results - In Table I the formula of each of the ten cations are written in each of the cells numbered 1 to 10 and cell 11 has the word "blank". The formula of each of the seven anions are written on each of the cells labelled A through G and cell H has the word "blank".

Procedure-1) Mixing salt solutions - Place one drop of chromium (III) solution on each of the eight wells of column 1 of the plate. Repeat the procedure for each of the remaining nine cation solutions on their corresponding columns. Place one drop of the phosphate solution on each of the eleven wells of row A of the plate. Observe the interaction between the phosphate solution and the ten cation solutions. Repeat the procedure for each of the remaining six anion solutions on their corresponding rows.

In-lab assignment

L1- When a reaction occurs mark the corresponding cell of Table I with: a **P** If a solid precipitate was formed, a **C** if a color change occurred, a **G** if gas was produced, an **N** if no reaction could be seen. Take the blank tests into account .

L2- For each observed reaction write down its equation and record the colors and appearance of the reagents and products (Be careful to distinguish between what you observe and what you think is happening – sometimes the actual reaction is not what you expected.)

L3- Were your predictions based on P2 confirmed ?

L4- Imagine you were given one of the 7 anion solution bottles without its label and were asked to answer if the anion is thiocyanate using solely its interaction with one of the 10 cation solutions. Which cation solution would you choose ?

L5- Same as L4 but now you want to find out if the anion phosphate is present ?

L6- Same as L4 but you need to know if the anion is iodide ?

Procedure-2) <u>Adding acid to the precipitates</u> - Place one drop of nitric acid 6 mol/L on each well of the Multiwell plate containing solid precipitates prepared in procedure-1 and stir the well contents with a clean thin glass rod.

In-lab assignment

L7- If a precipitate dissolves in acid mark the corresponding cell of Table I with **DA**, if it does not dissolve write **NA.** For each dissolution reaction write down the corresponding equation.

L8- Which solid precipitates did not dissolve in acid ? Interpret why some salts dissolve and why some don't based on the acid-base characteristics of the anions.

Procedure-3) <u>Discarding the residues</u> - Pour the contents of the plate into a large beaker (2000 mL) and neutralize the solution acidity with sodium bicarbonate. Ask your lab instructor for further directions.

Results and Comments In Table I one can see the typical results obtained. All phosphate, carbonate and sulfide salts of the 10 cations will precipitate. All silver salts of the 7 anions will precipitate. All hydroxide salts will precipitate except those of zinc(II), cadmium(II) and mercury(II). Copper(II) thiocyanate will precipitate. In the chromium(III) sulfide and carbonate wells the precipitate is chromium(III) hydroxide. In the iron(III) sulfide well the precipitate is actually iron(II) sulfide due to the reduction of iron(III) by the sulfide. In the silver hydroxide well the precipitate is silver oxide. The tan manganese(II) hydroxide will get oxidized by air to the brown manganese dioxide, the former is soluble in acid but the latter is not. Under the experimental conditions above mercury(II) iodide, mercury(II) thiocyanate and cadmium(II) thiocyanate do not precipitate. In the iron(III) thiocyanate well the soluble red complex $[Fe(SCN)(H_2O)_5]^{2+}$ is formed. Iodide ions reduce iron(III) forming some solid iodine and the anion triiodide. Iodide also reduces copper(II) to copper (I) forming solid copper(I) iodide and iodine. In the chromium (III) and iron (III) carbonate wells, some carbon dioxide gas is formed due to the reaction between the carbonate and the acid present in the metal solution.

All precipitates will dissolve in 6 mol/L nitric acid except , manganese dioxide, copper(II) thiocyanate, copper (I) iodide, the silver salts of chloride, thiocyanate, iodide, and sulfide, and the sulfide salts of cobalt(II), nickel(II), copper(II), and mercury (II).

Nitrate may oxidize iodide and any yellowish colour arising in the iodide wells might be due to the triiodide anion.

Besides the concepts worked in the above questions students may be asked to : 1) Identify a given unknown cation or anion solution, 2) Devise a flow chart using the presented spot-tests to identify unknown cation (or anion) solutions. 3) Search in the literature what would happen if less common transition metal cation solutions like ruthenium (III), platinum (II) or (IV), gold (I) or (III) were tested against the anion solutions, 4) Find out what would happen if less air stable cations such as iron (II) or tin (II) were tested against the anions, 5) Find out what would happen if anions like hexacyanoferrate, chromate, dichromate, acetate and oxalate were tested against the cation solutions, 6) describe the morfology of the precipitates using a microscope.

Further Experiments

In the Experiments below we list the substances to be placed in columns and rows of the multiwell plate, comment on the results and give ideas on how each experiment can be used to discuss a number of chemical concepts and theories. Experiments A though D have already been given to students whereas Experiment E hasn't .

Experiment A - Indicators and acid base equilibria

In this experiment one should use twelve buffer solutions with pH values of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 in columns 1 through 12 (each buffer solution must have its pH value within ±0.1 pH units). In rows A through H the following eight 0.05% indicator solutions in water or water-ethanol must be used : crystal violet, thymol blue, bromophenol blue, methyl orange, methyl red, bromocresol purple, bromothymol blue and phenolphthalein.

Results and Comments - A beautifull color display will be the result of the experiment where the student will be able to visualize the pH range of color change for each indicator. A similar effect can be seen in the multiwell plate in the cover picture of J.Chem. Educ. 1989 (66). The student can easily visualize the pH range of color change of each indicator. The instructor may work the concepts of

1) pH and acid-base equilibria,

2) pH buffers,

3) indicators for acid-base titrations,

4) color mixtures,

5) pKa and the color change,

6) structural and electronic rearrangements in the acid and basic forms.

This experiment can be modified or extended to :

1) record visible spectrum of an indicator solution at same concentration at different pH values.

2) include a pH insensitive dye (or other colored substance) to contrast with the sensitive ones.

Experiment B - Reactivity of metals - corrosion and deposition

In this experiment one could use the following solutions in columns 1 through 12 : distilled water, hydrochloric acid 6 and 0.1 mol/L, nitric acid 6 and 0.1 mol/L, sulfuric acid 6 and 0.1 mol/L, sodium hydroxide 6 mol/L, copper(II) nitrate 0.1 mol/L, silver nitrate 0.1 mol/L, zinc nitrate 0.1 mol/L, and mercury (II) nitrate 0.1 mol/L. In the rows A through H one must place a piece of each of the following metals magnesium, aluminum, tin, lead, iron, copper, zinc and platinum. These metals should be in the form of wires, small scrapes, turnings or grains (powdered metals should be avoided). It is important that the surface of the metal is clean.

Results and Comments - A number of interesting reactions such as metal dissolution, gas formation, and metal deposition may occur. The following concepts can be tackled : 1) effect of acids on metals, 2) redox displacement reactions, 3) corrosion, 4) passivation, 5) electrochemical series, 6) hydrogen production, 7) Acid corrosion and concentration effects, 8) Metallic materials for reactors and chemical transportation tanks, 9) surface reactions. This experiment can be modified or extended to : 1) include other metals or alloys, 2) observe the metal surface morfology using microscope or stereoscope, 3) include other solutions, 4) observe the concentration effect over a wider range.

Experiment C - Precipitation reactions and solubility rules - representative metal ions

This experiment is analogous to that described above in Experiment 1. One should use in columns 1 through 11 the following 0.1 mol/L solutions of nitrate salts of the cations : lithium, potassium, magnesium, calcium, strontium, barium, aluminum, tin(IV), lead(II) and bismuth(III). In rows A through G one should use the following 0.1 mol/L solutions of the sodium salts : phosphate, hydroxide, carbonate, chloride, sulfate, oxalate and sulfide. The acid dissolution step should also be performed.

Results and Comments - As in Experiment 1 a number of precipitates will form and most will dissolve in acid. Most of the concepts to be worked will be similar. Using the results of the two experiments the teacher can make a comparison between transition and representative metal ions showing the differences in the solubility and in the color of the salts. The experiment can be modified or extended along the same lines mentioned in Experiment 1.

Experiment D - Redox Reactions

In this experiment the solutions have to be stored in a fridge, protected from light , and should preferably be used no more than five weeks after their preparation. In columns 1 through 7 the following oxidizing agents are to be used : 0.1 mol/L solutions of : potassium dichromate, potassium permanganate, potassium iodate, mercury (II) chloride, bismuth (III) chloride, solid sodium bismuthate and solid lead dioxide. Each drop of the oxidizing agents has to be acidified with one drop of H_2SO_4 1 mol/L before the adition of the reducing agents. In rows A through H the following reagents should be used : 0.1 mol/L solutions of sodium thiosulfate, potassium iodide, sodium sulfite, manganese (II) chloride, sodium oxalate, iron (II) sulfate, tin (II) chloride and hydrogen peroxide 10%.

Results and Comments - A number of colorful redox reactions will occur including some traditional identification spot-tests such as the manganese(II)-bismuthate and the tin(II)-mercury(II) reactions (Lagowski, 1991). The teacher may work : 1) with methods of balancing redox reactions, 2) with Latimer diagrams, 3) with relative oxidizing and reducing power, 4) thermodynamic versus kinetic effects. The experiment can be modified to 1) perform reactions in basic media, 2) replace or include other oxidizing or reducing agents.

Experiment E - The Iron(III)-thiocyanate complex and the concentration effect

In this Experiment, in columns 1 through 7, one should use $FeCl_3$ solutions with the following concentrations 0.1, $5 \cdot 10^{-2}$, $1 \cdot 10^{-2}$, $5 \cdot 10^{-3}$, $1 \cdot 10^{-3}$, $5 \cdot 10^{-4}$, and $1 \cdot 10^{-4}$ mol/L (All with 1% HNO_3) . In rows A through G the the KSCN solutions with the following concentrations should be used : 0.1, $5 \cdot 10^{-2}$, $1 \cdot 10^{-2}$, $5 \cdot 10^{-3}$, $1 \cdot 10^{-3}$, $5 \cdot 10^{-4}$, and $1 \cdot 10^{-4}$ mol/L.

Results and Comments - This experiment was tested by the lab instructors but has not yet been given to students. Nevertheles we expect it to have a good student response since it has an interesting visual impact displaying a bidimensional color gradient where the blood red color of the iron(II)-thiocyanate complex in well A-1 fades from left to right and from top to bottom in the plate reaching a colorless solution in well G-7. The concepts of complex formation, stability constants and chemical equilibria can be worked out. The experiment can be extended to determine the solution concentration with colorimetry or spectrophotometry.

Discussion

The experiments described gave an example of how one can manipulate the multiwell spot test technique. We may call Experiment 1 "transition metal cations versus anions" and Experiment C "representative metal cations versus anions". We may call Experiment A "pH buffers versus pH indicators", whereas Experiment B may be viewed as "metals versus solutions". Experiment D may be regarded as "oxidizing versus reducing agents" and Experiment E can be considered an "iron(III) versus thiocyanate concentration gradient map".

The Experiments 1, A, B, C, and D adapted to the conditions of our laboratories and courses - were included into a first year chemistry major lab course in 1996, 1997 and 1998 and into a third year Geology major lab course in 1996. Each group of students was supplied with an instruction manual and with a kit consisting of a multiwell plate, dropping bottles with reagent solutions and vials with solid reagents.

We percieved the growing motivation and understanding of reaction types by the students compared to those of previous years but we did not collect statistical data. It becomes obvious to the students why chemists use certain identification tests after inspection of the plates in Experiments 1, C or D. It is easier to teach the concept of pKa after experiment A. The ability of permanganate to act as an oxidizing agent is easily percieved after a quick look at the plate of experiment D. The multiwell spot-test technique also has the inherent advantadges of teaching with microscale (Szafran, 1989) in Chemistry such as the small amount of residues to treat, the minimization of reagent contamination due to the use of dropping bottles and the small size and weight of the materials.

Conclusions and Future Work

The multiwell spot-test technique allows the simultaneous visualization of the results of up to 96 chemical spot-tests. The technique, originally developed for microbiological studies, was adapted as a teaching tool for basic chemistry. Its flexibility was demonstrated by a number of experiments dealing with solubility rules, acid-base indicators, corrosion, precipitation, redox reactions and complex ion formation. This kind of experiment also has a conceptual link with the fast devoloping technique of combinatorial chemistry. We are currently devising an experiment "metal ions versus ligands" to demonstrate complex formation and gradient maps applied to precipitation reactions and one experiment with a precipitation-redissolution system such as copper(II) versus ammonia and mercury(II) versus iodide.

The materials required have low cost, are light and small and can be easily transported in the form of kits. These kind of experiments can be fitted to short or long lab sessions and can even be used in institutions with limited resources. They can either be performed in regular lab classes or as demonstrations where the plate is prepared by the instructor and then passed hand-to-hand amongst students. We hope that the use of this tool may help students of different academic institutions as it helped ours in making connections between the great variety of chemical interactions.

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Table I -Summary of spot-test results performed in a Multiwell plate of Experiment 1.

		1 Cr (III)	2 Mn (II)	3 Fe (III)	4 Co (II)	5 Ni (II)	6 Cu (II)	7 Ag (I)	8 Zn (II)	9 Cd (II)	10 Hg (II)	11 blank	12
A	PO_4^{3-}	P da	P da	P da	P da	P da	P da	P da	P da	P da	P da		
B	OH^-	P da	P na	P da	P da	P da	P da	P da	n	n	n		
C	CO_3^{2-}	P da	P da	P da	P da	P da	P da	P da	P da	P da	P da		
D	Cl^-	n	n	n	n	n	n	P na	n	n	n		
E	SCN^-	n	n	C	n	n	P na	P na	n	n	n		
F	I^-	n	n	P na	n	n	P na	P na	n	n	n		
G	S^{2-}	P da	P da	P da	P na	P na	P na	P na	P da	P da	P na		
H	blank												

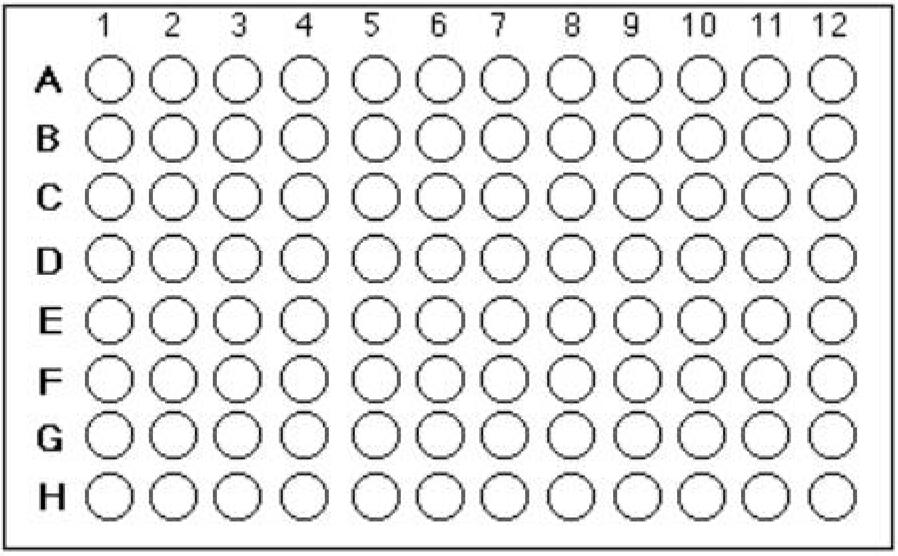

Figure I - Schematic representation of a 96 multiwell plate.