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Departament d'Ensenyament
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Protocol
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Química

Electroforesi.

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ELECTROFORESI.

Introducció. Quan en una mescla hi ha substàncies ionitzables, es pot obtenir una certa separació d'aquelles per l'acció d'un camp elèctric. L'electroforesi és una forma incompleta d'electròlisi, en la que els productes no s'alliberen en els electrodes, sino que els seus moviments de separació queden parats en un punt intermig entre els electrodes.

En l'electroforesi de zona les substàncies test s'apliquen en forma de taca o ratlla sobre un suport relativament ampli. El medi usat com a suport és en general, paper de filtre, o membrana d'acetat de cel.lulosa, ...

La ionització dels compostos electrovalents serveix de base a l'electroforesi. Per efectuar una separació completa convé que les substàncies surtin d'un mateix punt i tinguin un camí llarg i lliure pel recorregut de separació. Aquesta és una altra diferència entre l'electroforesi de paper i l'electròlisi, ja que en aquesta última hi ha una distribució inicial uniforme dels soluts en tot el bany electrolític.

El paper de filtre a l'electroforesi actua com a receptor de les substàncies disoltes i proporciona el camí perquè es traslladin. Quan es para el corrent, si s'asseca el paper, les substàncies queden fixades "in situ", en la posició màxima que han arribat amb l'electroforesi.

Les caracterísitiques que determinen el comportament dels ions són dues : el tamany del ió hidratat en la sol.lució i la quantitat de càrrega. Aquestes caracterísitiques tenen efectes oposats : com mes gran és l'ió mes lent es desplaça, i com mes càrrega té es mou mes depressa. Les substàncies sense càrrega es queden en el punt d'origen.

El paper de filtre humit amb la sol.lució tampó fa de pont conductor entre els electrodes i el corrent elèctric circula establint un camp elèctric. Les sustàncies ionitzades de la mescla test són atretes cap a l'electrode de signe oposat a la càrrega de cadascuna d'elles i comencen a moure's a través del paper. Així s'obté una separació monodimensional.

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Material. Font d'alimentació
2 càpsules Petri
2 resistències d'estufa
paper de filtre 30x5 cm
indicadors : verd de bromcresol
ferroína
indicador Yamada
blau de metilè
Sol.lució Na_2CO_3 0,1 M

Mètode: S'humiteja un troç de paper de filtre amb una sol.lució buffer, per exemple Na_2CO_3, que permeti passar el corrent elèctric. Esposa el paper entre les dues càpsules de manera que els extrems quedin sumergits un en el bany de lànode i l'altre en el del càtode. Llavors s'aplica en el punt mig del paper amb un capilar una rattla de la sol.lució test de les substàncies o una gota de no més de 3-4 mm de diàmetre.

Es dona pas al corrent elèctric, 160-180 V durant 10-15 minuts, amb les dues resistències en contacte amb la so.lució test. Cal tenir precaució amb les enrampades.

Per millorar la visualització dels resultats de l'electroforesi, es pot tenir el resultat de l'electroforesi de cada un dels indicadors per separat.

Bibliografía :

Journal of Chemical Education. Vol 56 Number 5, maig 1979.Lavallee, Daugherty. The Electrophoresis of Indicators.

[242 (31)]

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The Electrophoresis of Indicators

An analogy to isoenzyme separation

Electrophoresis is a method of separation based on the principle that ions which differ in charge will migrate at different rates when placed in an electric field. In practice, the sample is applied to a cellulose acetate strip which subsequently is used as a salt bridge between the anode and cathode compartments of an electrolytic cell. The cell compartments contain a buffer of appropriate pH. When a current is applied to the cell, the differently charged ions in the sample migrate at different rates through the salt bridge and become separated. Electrophoresis is used routinely in clinical laboratories to perform serum protein and isoenzyme assays. The principles, techniques, and importance of isoenzyme assays can be easily understood and appreciated by General Chemistry students.[2]

A lecture demonstration involving the actual separation of isoenzymes is not practical. The reagents needed are unstable, moderately expensive, and not likely to be found as common items in most chemistry department stockrooms. Moreover, visualization of the separated isoenzymes requires an incubation period for the color development reaction. The lengthy incubation period makes the demonstration too time consuming to be done during one lecture period.

We have developed a lecture demonstration which illustrates the principles involved in the separation of isoenzymes but avoids the problems inherent in isoenzyme separations. The separation of a mixture of indicators utilizes inexpensive reagents which are available in most chemistry stockrooms. Because the indicators are colored materials, the separated components of the mixture are visible and no incubation period is necessary. Although many different mixtures of indicators can be separated, one which works particularly well is a solution containing ferroin, methyl orange, and bromocresol green. At a pH of 11.0 all of these indicators bear different charges. Their structures are shown in Figure 1.

Excellent results can be obtained using cellulose acetate membranes and a commercial electrophoresis cell; however, equally good results can be obtained using much simpler (and cheaper) equipment. Our electrophoresis cell consists of two plastic freezer cartons (pint size) containing a loop of nichrome wire which serves as an electrode. A strip of filter paper can be used in place of the cellulose acetate membrane.

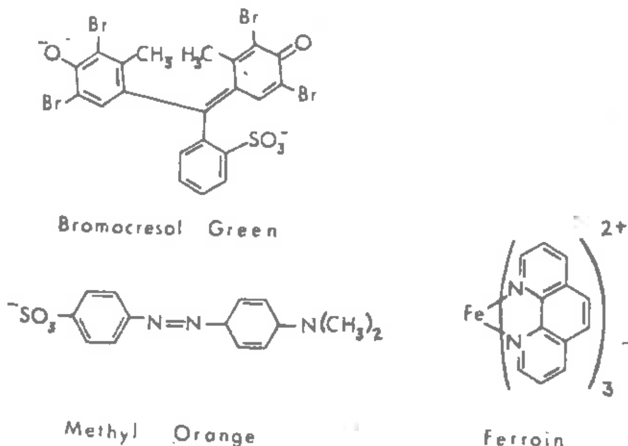

Figure 1. Structures of three indicators at pH 11.0. Note differences in charges.

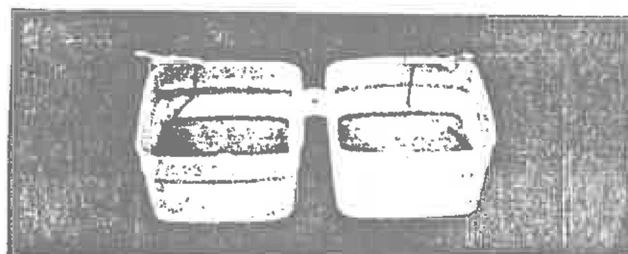

Figure 2. The electrophoresis cell in operation.

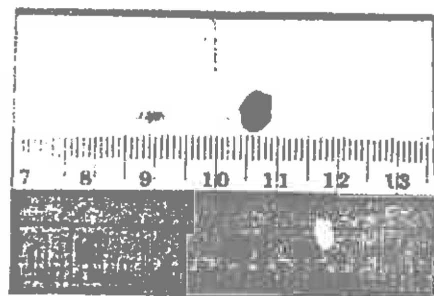

Figure 3. Separated indicators after electrophoresis. From right to left: (anode to the right) ferroin, methyl orange, bromocresol green. The vertical line marks the point of application.

Experimental

Materials and Supplies

D.C. power supply capable of supplying a potential of 200 volts is adequate.
Electrophoresis tank consisting of two freezer cartons containing a loop of 18 gauge nichrome wire (See Fig. 2).
Filter paper, Whatman 541, 12.5 cm, cut into 1-in. wide strips.
Indicators
0.025 *M* ferroin, G. F. Smith
methyl orange, Eastman #14330
bromocresol green, Eastman #1782
buffer, pH 11.0 buffer capsules, pHydrion buffer available from Micro Essential Laboratory, Brooklyn, NY

Method

Prepare a mixture of the indicators by dissolving an amount of bromocresol green about one half the size of a grain of rice in 10 ml of 95% ethanol. Add two to four times as much methyl orange as bromocresol green and 1 ml of ferroin solution. The concentration of this solution is of some importance. Solutions which are too concentrated do not separate well and leave a spot at the point of application.

Dissolve one capsule of the buffer powder in 100 ml of water and pour 50 ml of the solution into each of the freezer cartons.

Briefly soak a strip of filter paper in the buffer solution. Blot the filter paper nearly dry between two layers of paper towels. Apply a small amount of the indicator mixture to the center of the strip of filter paper. A wooden applicator stick works well for the application of the sample. The applied sample should produce a spot no more than 3–4 mm in diameter.

Drape the filter paper strip between the two cartons containing the buffer making certain that the ends of the strip are in contact with the buffer. Connect the nichrome electrodes to the power supply and carry out the electrophoresis at ~180 V for 10–15 min. **Precautions against electric shock should be observed.** Figure 2 shows the electrophoresis cell in operation.

[1] To whom correspondence should be addressed.
[2] N. A. Daugherty, J. CHEM. EDUC., 56, in press (1979).

Results

After ~15 min of electrolysis, turn off the power supply and examine the filter paper strip. The three indicators should be well separated with the most negatively charged one (bromocresol green) migrating furthest toward the anode. As with all demonstrations, it is a good idea to do a few practice runs before classroom use. With some care it should be possible to obtain results similar to those shown in Figure 3.

A number of refinements may be incorporated into the demonstration. For example, solutions containing only one of the indicators can be electrophoresed along with the mixture and serve as "control sera." Solutions containing different numbers of indicators and having a similar appearance can be used as unknowns and illustrate well the utility of electrophoresis as an analytical tool.

letters

How Sweet it is!

To the Editor:

Professor H. A. Bent ["Energy & Exercise" pt. III, J. CHEM. EDUC., 55 586 (1978)] has a good case for berating the excessive consumption of sugar in the U.S.; he surely does not need to overstate his case.

I refer to the claim that U.S. sugar consumption, at 120 lb/person/year, constitutes approximately 25% of an average American's needed caloric intake. This claim does not allow for metabolic efficiency (maximally 25%, see part I, p. 458), wastage, or even use of sucrose as a raw material in industry (unless the latter is already allowed for in his basic figures). Thus, it seems to me that sugar's caloric equivalent in the diet is a maximum of 5%, and probably much less.

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To the Editor:

Several correspondents, as well as Professor Glasser, have misinterpreted some of the calculations cited in Parts I and III of the series on "Energy and Exercise." Perhaps the following comments will help set things straight.

U.S. "sugar consumption," 120 lb/person/year (Part III), is pounds of sugar swallowed per person per year. What sugar goes into a person must, of course, come out or pile up (Dalton's Law). Sugar piles up in our bodies chiefly as fat, flows chiefly as carbon dioxide and water—also as small amounts of (other) "wastage": chiefly sugar in (i) the bowels (molecules not absorbed by the intestines) and in (ii) the urine (particularly in untreated diabetics).

In considering the bio-thermodynamic significance of 120 lb of swallowed sugar per person per year, one may distinguish at least three "metabolic efficiencies." First is the body's "*bio-chemical*" efficiency for sugar—its efficiency in incorporating swallowed sugar into its metabolic machinery. That efficiency, as indicated above, is usually nearly 100%. Sugar is "highly digestible." Second is the body's *First-Law* efficiency—its efficiency in transforming a fuel's ΔH-of-combustion into heat and work. That efficiency is at all times 100%. Energy is always conserved. Third is the body's *Second-Law* efficiency—its efficiency in transforming a fuel's ΔG-of-combustion (for most purposes in biochemistry, practically equal to the fuel's ΔH-of-combustion) to useful work. That efficiency is at the very best about 25% (Part I). People produce more heat than work—and thermodynamicists, editors feel, perhaps more heat than en*lightenment*. For this third efficiency has little to do with the "caloric equivalent" of sugar.

To shed additional light on the caloric-equivalent-of-sugar calculation in Part III, and further doubt on Professor Glasser's final remark, we conclude (tongue-in-cheek) with the thermodynamicist's usual argument-by-contradiction. Suppose, with Professor Glasser, there is some biochemical bypass for sugar in the body; only one-fifth of the sugar swallowed is biochemically active. Then, with a yearly consumption of merely 24 lb per person of biochemically active—if nutritionally empty—sugar, it seems unlikely that America would be a nation with an alarming number of under-nourished, calorically over-stuffed citizens, contrary to observation.

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Organic Hazard for Safety Goggles

To the Editor:

One of the laboratory exercises done by my organic chemistry class this past quarter was the preparation and the separation of the diastereomeric 1,2,3,4-tetrabromobutanes. The products were placed in the student's lab drawers to air dry. My students wear soft plastic goggles for eye protection. As is commonly done, these were stored in the student's drawers.

Shortly after the beginning of the lab period following the preparation of the tetrabromides, some of my students complained of intense eye irritation and tear formation. When these students removed their goggles, the irritation subsided, and after some minutes, ceased. Their eyes were somewhat red at first and returned to normal as the irritation lessened. Some of my students said that the amount of their product had visibly diminished from the first to the second lab period.

My interpretation of these events is that one or both of the 1,2,3,4-tetrabromobutanes has a significant vapor pressure at room temperature. In some cases, the student's goggles were placed close enough to the product to dissolve the subliming molecules in the plastic. When the students put their goggles on, the molecules escaping from the goggles caused the eye irritation. Not every student experienced the irritation. This may be due to the fact that each student has two drawers in the lab or that some goggles were oriented in the drawers with their exterior parts toward the product.

I think that anyone using plastic eye protection, especially the soft goggle type, should be careful about storage near volatile organic materials. Goggles are a particular problem because they effectively enclose the eye area.

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