# SPOT TEST ANALYSIS

## Clinical, Environmental, Forensic, and Geochemical Applications

Ervin Jungreis

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*To Nancy*

# PREFACE

In the past several decades chemical analysis has undergone a formidable process of sophistication. Advanced instrumental tools have improved the generation of the signal given by the analyte and transduced it to a more conveniently measured one, and with the tremendous developments in the field of electronics, enormous amplification of the resulting signal has become feasible.

Parallel with this trend toward instrumental sophistication, a trend toward simplification was seen in some marginal selected areas in the form of simple, rapid, and inexpensive spot and screening tests. A screening test is defined as a simple method that provides a sufficient answer to the analytical question with a minimum expenditure of time and money. Rapid methods in various areas of application register gross deviation from a norm, and although positive results generally require confirmation, the negative findings are conclusive. It would be wasteful to devote resources to attaining precision or accuracy several magnitudes beyond that necessary in the clinical urine analysis, for example, when pathological changes in the composition of urine are evidenced by a simple spot test.

It must be emphasized that the applicability of spot test methodology is limited even in the areas discussed in this book, and that for the exact determination of most chemical substances, complex analytical procedures are unavoidable. Although the use of screening tests is a marginal one; the margin, however, is quite significant.

This book deals with the contemporary uses of spot and screening tests in clinical, forensic, geochemical, soil, and water testing and in occupational safety protection. It is intended to be a laboratory reference book providing detailed procedural information.

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Jerusalem, Israel
November 1984

Ervin Jungreis

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# INTRODUCTION

In the last three decades the classical spot reactions have been successfully applied in large-scale analytical problems in clinical analysis, in control tests of air quality, in food and water analysis, in geochemical prospecting, in crime laboratories, in soil testing, and in juridical chemical studies.

Commercial companies in both the United States and Europe are selling vast quantities of compact spot test systems for the qualitative analysis of urine and water, in which the goal is the rapid establishment of the presence or absence of certain materials. The extreme simplicity of these tests, their time- and money-saving nature, and their ultimate reliability make these tests very useful, and the analytical problems solved using simple reagent strips and preprepared reagent pillows or tablets constitute a significant part of the total spectrum of analytical problems.

Aquatic ecology tests are based on spot test analysis. Water quality, whether the criteria are based on human consumption, irrigation, or feed lot or farmland run off, can be examined easily and reliably and soil characteristics can be established by unsophisticated spot tests.

Around the middle of the last century single-chemical tests were already being performed on filter paper matrices. Runge was one of the important pioneers in those days in the use of absorbent papers as the seat of the chemical reaction (1). In a sense, he can be regarded as the creator of paper chromatography and spot test analysis. For testing for free chlorine, he impregnated paper with starch and potassium iodide, as described in Volume II of his "Farbenchemie." When the paper was spotted with bleaching solution a blue fleck appeared because of the liberation of iodine and its reaction with starch. This was probably the earliest scientifically checked authentic spot reaction with impregnated paper; even more amazing, this reagent paper has been sold commercially ever since. This test antedated Schiff's test for uric acid in urine on silver carbonate by 25 years.

The modern development of spot test methods of analysis occupied Fritz Feigl for half a century. Even before their systematic development began, spot tests were used in isolated organic and inorganic applications.

According to the writings of Pliny the Elder, early Romans detected iron in vinegar by means of a reagent paper prepared by soaking papyrus in an extract obtained from gall nuts.

Modern instrumental analytical chemistry, the application of microprocessors, and the rapid advances in spectral, chromatographic, nuclear, mass spectroscopic, and electroanalytic research changed analytical chemistry spectacularly, dramatically reducing the complexity of the wet analytical procedures. Nearly any physical property characteristic of a particular element or compound was made the basis of an analytical method. Together with this rapid sophistication took place another development in certain distinct areas of application, in exactly the opposite direction. This was the search for simple, compact, and inexpensive analytical devices for semiquantitative evaluation of certain elements or compounds where such an approximate evaluation has diagnostic value in at least the first stage of the examination. A new look was taken at the old spot tests; sometimes they were refined, and sometimes new ones were elaborated. In many of the rapid analytical procedures the human eye was used as a detector to measure color changes; others employed simple reflectometric instruments. Major manufacturers of chemical reagents concentrated their efforts mainly on three applicative areas, namely, clinical analysis, water quality control tests, and air quality control tests, because of the vast number of screening tests needed in these areas.

Urine analysis test strips for such preliminary screening are offered by several reagent firms. The several simultaneous spot reactions carried out by just dipping the strip into the urine provide reliable evidence of pathological changes in the composition of the urine. This testing for complete chemical urinalysis in both hospital and private practice is the first step toward establishing a diagnosis that can then be corroborated by clinical examination and quantitative laboratory tests.

An important development is the application of spot test procedures for specific, visual determination of important components such as glucose and urea in whole blood. A semipermeable membrane on the impregnated reagent area serves as a barrier, and quantitation is established by giving the soluble component a predetermined time period to diffuse to the chromogenic reagent zone. The simplicity of this procedure allows the rapid, semiquantitative bedside measurement of blood urea nitrogen and blood glucose. Because no deproteinization or exact volume measurement of the blood is necessary, self-monitoring by diabetics or patients having an elevated or depressed renal threshold is feasible. Such "instant" blood sugar and blood urea nitrogen measurements can be carried out by simple visual comparison with a color chart or, for more exact

measurement, by a portable, battery-operated reflectance meter that measures the light reflected from the reagent strip and converts this to a reading.

The very same principle of rapid separation of low- and high-molecular-weight components in blood through semipermeable membranes in a spot reaction combined with automated colorimetric and potentiometric assays is used by the Eastman-Kodak Company in a very significant system that considerably simplifies and speeds up the routine analysis of substances in human body fluids. Thin-film coating technology, which is basic to modern color photography, was elaborated to create a system consisting of a spreading layer, a semipermeable layer, and a layer containing the pigment precursors. The potential uses of this thin-film, multilayer approach to rapid clinical colorimetric assays seem extensive. A variety of operations can be performed on serum components as they diffuse through the multilayer structure. These layers may contain enzymes, buffers, ion-exchange materials, semipermeable membranes, and mordant and masking layers (to separate dyed layers optically).

Besides using exact analytical methods, geochemical prospecting uses spot colorimetry, qualitative tests for pathfinders, and simple field methods for rough differentiation of ore bodies.

The testing of tap water, lake and stream limnology studies, and the determination of seasonal changes in water supplies are also feasible with simple tests. Semiquantitative measurement is achieved either by visual comparison with color discs or with battery-operated portable photometers. The cooling water used in industry and the quality of water used in agriculture, of boiler feed water, and of swimming pool water can all be monitored with spot-test-based chemical reactions.

In police investigations, detection of traces of metallic lead in certain portions of substrates indicates firearm discharge. Blood, sperm, explosive, and drug tests are a very important part of crime detection.

Air quality standards are often monitored by direct reading of colorimetric indicators. Reagent papers can be used to detect gases present in the atmosphere. A classical example is the Gutzeit method, in which arsine turns a mercuric-bromide-impregnated paper strip black. Semiquantitative screening is achieved by using a simple sampling device that passes a measured volume of air through a preprepared detector tube. The first indicating tubes containing solid chemicals for quick, direct reading were detection tubes for carbon monoxide, hydrogen sulfide, and benzene. A sample of the workroom atmosphere being tested is drawn through a glass tube containing the reagent system. The appearance of a color change indicates the presence of the particular contaminant, and

comparison of the hue produced against a set of standard colors, or measurement of the length of a stain, indicates the approximate level of the airborne contaminant.

In the last decade interest in methods for checking air quality in working areas has grown tremendously. Several commercial companies (Bacharach Industrial Instrument Co.; Davis Engineering Equipment Co. Inc.; Union Industrial Equipment Corp.; Mine Safety Appliances Co.; Acme Protection Equipment Corp.; Drägerwerk AG) have made impressive progress in constructing indicating tubes for rapid, convenient, and inexpensive analysis of toxic gases in air. These tests can be performed easily by semiskilled operators.

## REFERENCE

1. B. Anft, *J. Chem. Educ.* **32,** 566 (1953).

CHAPTER

2

# TECHNIQUES

The simplest methods of spot test analysis involve the mere mixing of a drop of the unknown substance and a drop of the reagent solution. The reaction medium is either a porous or a nonporous supporting surface such as filter paper, glass, or porcelain. Use of a porous medium (e.g., filter paper, asbestos, or gelatin) impregnated with water-soluble or water-insoluble reagents raises the sensitivity of the test. Applied spot test analyses in clinical chemistry and in compact water analysis systems use strips of absorbent cellulose, one end of which is impregnated with the reagent system. The impregnated reagent area is sometimes covered with a semipermeable membrane to prevent staining by a colored matrix (e.g., red blood cells).

Another type of spot test employs one of the reactants in the solid form. In many clinical and technical appications a stable reagent tablet is simply dropped into the test solution and the extent of precipitation or color formation established.

The equipment required for spot test analysis is made of glass, porcelain, plastic, and metal. A classical spot test laboratory setup consists of assorted sizes of beakers, volumetric flasks, Erlenmeyer flasks, suction flasks, round-bottom flasks, distillation heads, Conway cells, crystallizing dishes, evaporating dishes, filter sticks, separating funnels, extraction pipets, fritted glass crucibles, graduated cylinders, pipets, burets, weighing bottles, storage bottles, vials, test tubes, centrifuge tubes, microscope slides, cover glasses, and spot plates.

White and black porcelain crucibles, dishes, and spot plates should be on hand. Platinum equipment, including dishes, foils, crucibles, and boats, is standard for many spot test operations. Crucibles made of nickel and beakers of stainless steel are also valuable. The use of aluminum pans and dishes is very practical, since, owing to their low cost, they are discardable. Tweezers and spatulas made of nickel-plated steel are essential too. A good assortment of microburners and blast lamps should be on hand. Other essential metalware includes sand baths, water baths, tripods, ring stands, clamps, rings, and buret holders.

The following devices and equipment are essential in the classical spot test laboratory: hand balance, microanalytical balance, pH meter, ultravi-