concentration. The ratio of antibody binding sites to conjugate in the complex is adjusted to give a suitable dose response curve. The use of the immunoassay in the reversible complex format allows all reagents to be combined and stored in one compartment prior to use. This lends itself readily to constructing dry reagent analytical strips.

In preparing strips for drug detection by a competitive protein binding reaction (SLFIA), it was essential to utilize procedures that would prevent premature interaction of constituents. It was necessary to prevent conjugate binding to the antibody or conjugate hydrolysis by β-galactosidase prior to sample addition. This was accomplished by introducing the conjugate into the strip with a nonaqueous solvent. In this procedure, it was also important that the solvent did not adversely affect the antibody or the enzyme. This procedure was used to produce analytical strips for the quantitative detection of antiasthmatic drugs (theophylline), antibiotics (gentamicin, amikacin, and tobramycin), and antiepileptic drugs (primidone, phenytoin, and carbamazepine).

Of the two types of assay strips described, the competitive binding assay is the more rapid. Only 3 min are required per assay.

As described in the Results section, the use of the linear portion of the dose response curve allows calibration of a strip with two standards. A two-point standard curve was constructed for the theophylline strip and used for the correlation study. This eliminates the necessity for multiple calibrators required by many immunoassays. The availability of all assay constituents in one strip eliminates the requirement for reconstituting reagents, diluting of stock reagents, separating steps, and multiple additions to the assay medium. Only a single dilution of the sample is required to initiate the assay. This technology, therefore, has the potential to bring a level of convenience to immunochemistry not available with current methodology.

In summary, it was demonstrated that paper matrices are suitable for conducting immunoassays. Dry reagent strips containing all assay constituents can be prepared for the specific detection of therapeutic drugs. These assays are rapid and require minimal technical skill.

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Registry No. Theophylline, 58-55-9; tobramycin, 32986-56-4; gentamicin, 1403-66-3; amikacin, 37517-28-5; carbamazepine, 298-46-4; primidone, 125-33-7; phenytoin, 57-41-0.

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Automated Instrumentation for Fluorescence Assays on Reagent Strips

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An automated instrument has been constructed for measurement of fluorescence and reflectance from reagent strips. Examples of front face fluorescence measurements for the detection of fluorescent products on reagent strips are described. These reagent strips can be automatically loaded, samples automatically sequenced and dispensed, and reflectance and fluorescence measurements automatically made and stored on magnetic media for subsequent analyses.

Traditional wet chemistry has generally been performed by using some sort of glass or quartz container within which solutions are placed, both for separate reaction steps and also for a possible analysis which may be spectroscopic in nature. Recently, new blood chemistry products (*1*) have been introduced in the field of clinical chemistry which incorporate all reagents within a single solid matrix such as paper or thin films. The format which uses the paper matrix will be denoted a reagent strip. For these strips, the sample is applied to the top of the paper and spectroscopic measurements are made from the top.

After a sample has been applied to a reagent strip, a reaction will occur. If it is to be followed spectroscopically, the reagent strip may be directly placed into an instrument made for that purpose. In the case of diffuse reflectance spectroscopy, a

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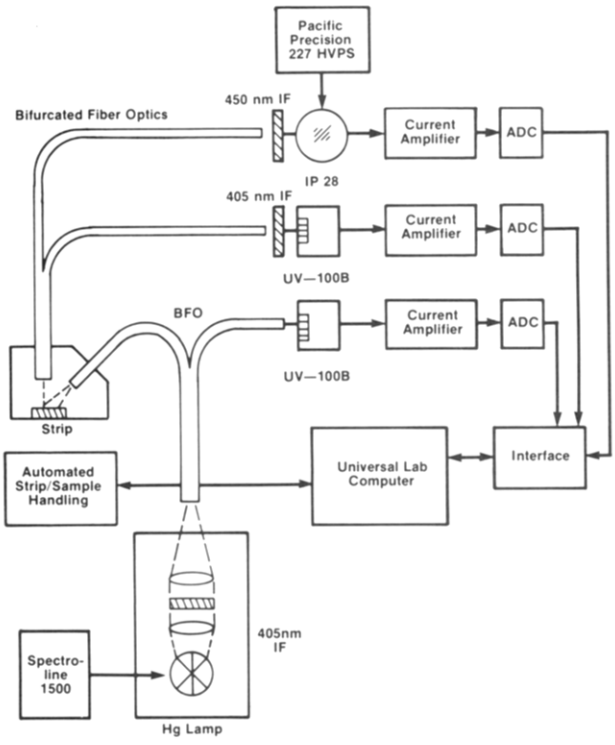

Figure 1. Block diagram of the instrument.

commercial instrument, the Ames Seralyzer, is available. In this work, an instrument is described which has been constructed to measure the fluorescence as well as the reflectance spectra of reagent strips.

The method of substrate labeled fluorescence immunoassay (SLFIA) (*2–4*) has been applied to the detection of therapeutic drugs in serum. More recently, an assay for theophylline using SLFIA in a dry reagent format has been described (*5*). The general application of SLFIA to dry reagent analytical elements is described in the companion paper (*6*). Although initially constructed for measurements using this method, the instrument is adaptable for measurement of fluorescence on strips from any method. In order to demonstrate further the capabilities of this instrument, examples of fluorescence assays on reagent strips are presented in this work which do not use the SLFIA method. Previous studies by Guilbault and coworkers (*7–9*) have demonstrated the feasibility of preparing solid-phase devices for the determination of analytes by fluorescent methods. However, the instrumentation in those studies was not designed for routine measurements of fluorescence on horizontally mounted strips.

EXPERIMENTAL SECTION

Instrumentation. A block diagram of the instrument is shown in Figure 1. The instrument uses a continuous light source and selects excitation and detection wavelengths with interference filters. These filters are appropriately chosen for the particular fluorescent product being detected. Fiber optics are used throughout, from the lamp to the detector. Samples are held in a carousel, and reagent strips are contained in a special cassette. Since these two functions are automated, it is only necessary for the operator to load the samples and the strips. Computer control of the device motions and data collection maximizes instrument throughput, frees the operators time for other uses, and provides reproducible positioning of the strip in the readhead.

Reagent Strip Handling. A picture of the strip handling part of the instrument is shown in Figure 2. Strips are placed in a cassette (A) which has a maximum capacity of ca. 50. Loading is done either by manual methods or with a specially designed loading device. Strips are loaded into the cassette such that the paper pads are oriented face up (see Figure 3). When installed into the instrument, both front and back walls of the cassette are

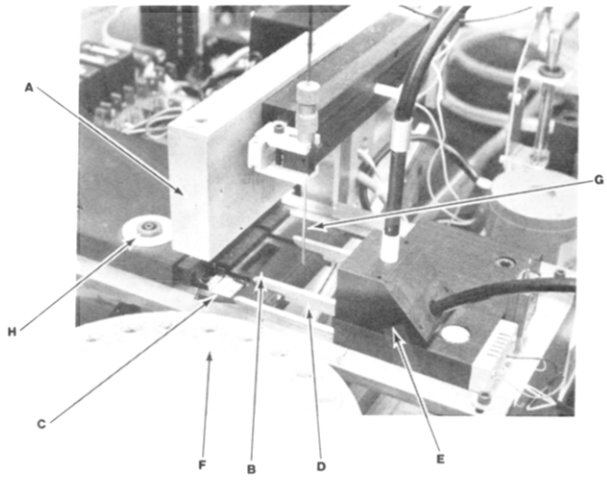

Figure 2. Automated instrument strip handling section: (A) strip cassette; (B) opening for strip loader; (C) loaded strip position; (D) hold-down bars; (E) readhead; (F) vials on carousel; (G) pipet tip; (H) rinse well.

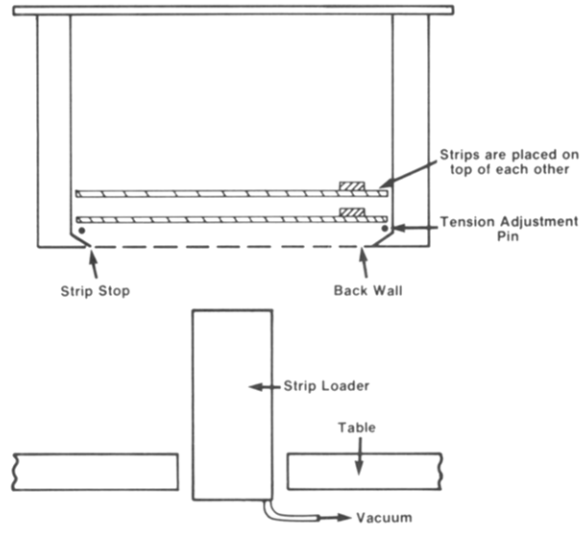

Figure 3. Automated strip loader and cassette.

closed, with the only opening at the bottom.

The strip loader (opening at B) contains a vacuum attachment. In the most extended position, the strip loader moves inside the cassette and comes in contact with the lowest strip. A vacuum seal is thus made. When the strip loader moves down and out of the cassette, the lowest strip is removed. The loader then moves further down through the table opening and positions the strip on the table at (C).

The strip is held firmly against the table by three hold-down bars (D). It is also aligned squarely against two edges which provide positive, reproducible positioning. One of these edges is connected to a stepping motor which can move the strip on the table to the dispense and then to the read position in the readhead (E). At the end of the read cycle, the table returns to the home position. Simultaneously, the strip is deposited into a waste container.

Sample Handling. The samples are placed in plastic vials (F) on a carousel which has a maximum capacity of 24 (see Figure 2). Each time the strip table moves from the home position to the readhead and back, the sample carousel is indexed by one. This is subject to a mechanical override. If replicates are to be measured, the carousel is automatically sequenced to the initial vial position.

Sample Pipetting. After the strip is loaded and moved to the dispense position of the table, the sample in the vial is dispensed onto the strip. The pipet tip (G) is constructed of a syringe needle attached to a stepping motor controlled syringe. The needle is mounted on a movable arm and is connected with flexible tubing

to the syringe. Through two ac motors, the arm can position the needle in two horizontal positions—above the strip and above the sample—and at two vertical positions—a height which clears all barriers between sample and strip and a height which is directly at the reagent strip surface and which also corresponds to immersion in the rinse well.

Thus, by appropriate coordination of the motor motions, a sample is pipetted onto the strip. After sample pipetting, the arm moves up and the strip is moved into the readhead. At this time, the needle travels down into a rinse well (H) in preparation for the next sample.

Optical. A continuous 40-W ac mercury lamp is used as the light source with a Spectroline 1500 power supply (Figure 1). The lamp is mounted in an Oriel lamp housing, Model 6385/6327, with fiber optic adapter, Model 6450. A special motor driven interference filter holder is installed to allow selection of the excitation wavelength by software. The most common wavelengths used are 405 nm for SLFIA and 340 nm for systems measuring NADH fluorescence.

The common leg of a UV transmitting bifurcated fiber optic bundle is attached to the lamp. One fiber bundle is directed toward a reference photodiode (PD) which operates in transconductance mode. The signal from this detector is thus proportional to the lamp intensity at the excitation frequency. The other bundle is mounted on the readhead (D) (Figure 2) at a 45° angle to the normal. A second bifurcated fiber optic is mounted with the common leg normal to the strip. This fiber bundle is used to collect the light leaving the strip (reflectance and fluorescence). Both fiber optics are ca. 0.5 cm from the surface of the 0.5 × 1.0 cm reagent strip. The entire readhead is light tight while the strip is in the read position.

From the collection fiber optic, one bundle is directed toward a PD with a 405-nm interference filter in place. This gives a measure of the amount of excitation light reflected from the strip. The other bundle is directed toward a 450-nm filter and an RCA 1P28 photomultiplier. The signal from the 1P28 measures fluorescence from the strip. The voltage divider used is the Pacific Precision Model 3150.

Electronics and Computer Control. All of the signals from the photodetectors are processed through current-to-voltage converters. All ranges are adjusted to give a signal of ca. 1 V for the maximum expected photodetector current. These three signals are then passed to three ADC's, which convert the analog voltage to a 12-bit digital word using the Intersil ICL-7109 ADC. The digital words are then input from parallel data ports.

Machine control is accomplished through parallel port input/output. Sets of four bits each are used to sequence stepping motors clockwise or counterclockwise. Ac motor control is done via solid-state relays which only require one bit each to turn a motor on or off. Position sensing is obtained through optoelectronic beam interrupters.

The computer consists of an 8080 based system with dual 8-in. floppy disk drives, 64K RAM, and the CP/M operating system. All machine control and data collection are accomplished with two programs. The first is written in assembly language and contains all I/O subroutines. The second is in BASIC and handles all motion coordination, data collection and manipulation, and file storage. Data analysis is performed primarily with the STATPAK BASIC program library routines, with ANOVA for variance analysis and KIN for plotting.

RESULTS AND DISCUSSION

Instrument Precision. For fluorescence calibration, the Ames Range Adjustment Solution (R) (7-hydroxycoumarin-3-[*N*-(2-hydroxyethyl)]carboximide or RAS) has been used. For this calibration solution, the instrument exhibits a precision in fluorescence of 0.075% coefficient of variation (coefficient of variation = standard deviation divided by mean times 100) for 1 μM RAS and of <0.1% coefficient of variation for 0.1 μM RAS. The concentration normalized standard deviation always gives a resolution < 1/1000 throughout this RAS interval.

For reflectance, a precision of 0.022% relative to 100% R has been achieved at a reflectance level of 30% R with 100 replicates. This implies that the measured precision at 30% R was limited by the 12-bit analog to digital converter resolution which has its theoretical limit at 0.024% R for 1-bit resolution. Other experiments using white filter paper such as Whatman 31ET or gray paper standards from Munsell also show that the reflectance precision is limited by the ADC within the range of 10% to 30% R and is no higher than 0.035% relative to 100% R at any level above 10% R.

Table I. Linearity Test with RAS Solutions

[RAS], μM	measd voltage (coeff of variation)	best fit voltage
0.0	0.0461 (2.1%)	0.0453
10.0	0.0544 (3.2%)	0.0537
50.0	0.0879 (2.5%)	0.0874
100.0	0.1286 (1.5%)	0.1295
200.0	0.211 (2.3%)	0.2136
400.0	0.383 (1.9%)	0.3819
std error of estimate:		0.00159
corr coeff:		0.99994

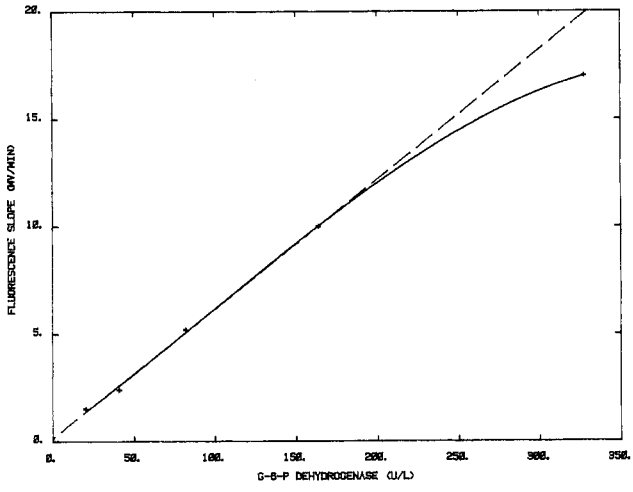

Figure 4. G-6-P dehydrogenase fluorescence assay on reagent strips. The slope of the fluorescence (mV/min) is plotted as a function of concentration.

Linearity and Detection Limit. On Table I, the results of a linearity test using various concentrations of RAS are shown. These data are the result of 15 replicates (15 different reagent strips). A linear regression analysis has given a correlation coefficient of better than 0.9999. From the standard error estimate of 0.001 59 on Table I, a total error figure of 0.682% has been calculated. There is thus no indication of nonlinearity in this system. The limit of detection for RAS on Whatman 54 paper is 2.5 nM.

RESULTS

In the companion paper (*6*), experimental results have been presented on measurements incorporating the SLFIA method. In this work, two assays based on other methods are presented. These assays are for aspartate amino transferase (AST) and glucose 6-phosphate (G-6-P) dehydrogenase. For both, all reagents are present in dry form on 0.5 cm by 1.0 cm Whatman 54 filter paper. The diluted serum sample is applied to the reagent strip, and the results are obtained by measurement on the automated instrument.

The assay for G-6-P dehydrogenase is based on the reaction

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-phosphogluconate} + \text{NADPH}$$

with excitation at 340 nm and detection at 450 nm. The dry strip contains the reagents G-6-P, nicotinamide adenine dinucleotide phosphate ($NADP^+$) and piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES). When diluted serum containing

any given concentration of G-6-P dehydrogenase is added to the strip, fluorescence of NADPH increases with a characteristic rate. Plotting the slope (in millivolts per minute) as a function of G-6-P dehydrogenase concentration in units per liter gives the dose response curve shown in Figure 4. The dose response curve shows that this assay is useful in the concentration range from 25 to 325 U/L.

The second assay to be presented here is for AST determination on paper. This assay is based on the reactions

$$\text{aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{glutamate}$$

$$\text{oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{malate} + \text{NAD}^+$$

The 0.5 cm by 1.0 cm reagent strip contains the dry reagents aspartate, α-ketoglutarate, malate dehydrogenase (MDH), NADH, and Tris (pH 8.0). The reaction is followed by observing the fluorescence of NADH at 450 nm after excitation at 340 nm. The reaction shows a decrease of fluorescence with time.

This dose response curve is linear in analyte concentration from 10 U/L to 260 U/L. It is characterized by a slope of −0.12 (mV/min)/(U/L).

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Determination of 51 Priority Organic Compounds after Extraction from Standard Reference Materials

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An extraction technique, involving homogenization of a sediment sample with dichloromethane at dual pH and phase separation by centrifugation, was used in the determination of 51 organic priority pollutants as identified in a standard reference sediment sample. These compounds were spiked into the sediment and equilibrated for a defined period of time. The extraction was performed first at pH >11 to isolate the base/neutral compounds; acidic compounds were extracted at pH <2. The extract containing the base/neutral compounds was fractionated by silica gel chromatography. The extract containing the acidic compounds was fractionated by gel permeation chromatography (GPC). Separation and identification of individual compounds in the silica gel fractions were accomplished by gas chromatography/mass spectrometry (GC/MS). Method precision and accuracy are discussed. Tentative identifications of other organic compounds found in the sediment are given.

Several extraction techniques including Soxhlet extraction (*1*), shaking (*2*), sonication (*3*), use of high-frequency mechanical dispersion (*4*), and steam distillation (*5*) have been used to isolate organic compounds from sediments. Mixtures of polar and nonpolar solvents are usually employed with the polar solvent added first to remove water from the sediment. Examples of such solvent pairs are acetone and dichloromethane (*6*), acetone and hexane (*7*), methanol and benzene (*8*), methanol and toluene (*9*), 2-propanol (*10*), etc. Soxhlet procedures were reported to give significantly greater recoveries than the sonication or steam distillation procedures (*11*); however, the evaluation was limited to PCB's and pesticides, and conclusions about the effectiveness of this technique when applied to a wider range or organic compounds could not be drawn.

While efficient extraction techniques are needed to isolate organic compounds from sediments, sample fractionation prior to gas chromatographic (GC) analysis and high-efficiency capillary columns are required to resolve the numerous components of a sediment extract. Common sample fractionation procedures prior to GC analysis involve: adsorption chromatography on silica gel (*12*), Florisil (*13*), cesium silicate (*14*), and/or gel permeation chromatography (*15*). Liquid–liquid partitioning procedures (*16*) have also been used.

With the recent development of the fused silica capillary columns that offer higher efficiency, improved peak shape, greater resolution, and inertness over the regular capillary columns, analysis of complex samples has been significantly improved. Furthermore, acidic and basic compounds can be analyzed on the same chromatographic column, either as separate fractions to avoid any possible chemical reactions or combined, in those cases when the combined extracts are shown to be chemically stable (*17*).

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