SIMULTANEOUS DETERMINATION OF pH AND REDOX POTENTIAL: APPLICATION TO CIGARETTE SMOKE

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A method is presented for the simultaneous determination of the redox potential and the pH of the tobacco smoke during smoking. With both values available, this method allows for the continuous determination of the redox potential during smoking, corrected for alterations in the pH of the solution. Since tobacco smoke shows a time dependent alteration in the reducing ability within the first few puffs, this method is more relevant to the actual physiological conditions than previous methods. The determination of the pH reflects the contribution of both phases of the smoke. This method allows the monitoring of two parameters which have major effects upon smoke composition, quality, and physiological systems.

INTRODUCTION

This report describes the development of an instrument for the simultaneous determination of the pH and redox potential of aqueous solutions, and the application of this apparatus for the monitoring of cigarette smoke on a puff-by-puff basis. The pH of the smoke has a major effect upon its flavor and aroma and recent work has indicated that it also contributes greatly to its physiological effect (13,16). Because of the aerosol nature of cigarette smoke, pH cannot be determined by the usual methods since there can be no continuous aqueous phase. Most workers, consequently, define the pH of tobacco smoke as that pH of an aqueous solution of the smoke under certain defined conditions. Such a value is naturally dependent upon the aqueous solubility, the ionization, and the relative concentrations of the acids and bases in the tobacco smoke. This value might be expected to be also influenced by the parameters of tobacco variety, curing conditions, and smoking conditions (5,13). As an example, an extreme variation in smoke pH is noted between cigar and cigarette smoke. Armitage and Turner (1) have reported that nicotine exists as the unionized free base in a greater concentration in cigar smoke (pH 8.5) than in cigarette smoke (pH 5.3), thus allowing a greater absorption of the nicotine through the oral mucous membranes. Lakritz et al. (10,11,14) have further shown that artificial alteration of smoke pH by use of additives changes smoke composition and filtration efficiency. Most experiments have employed the method of Grob (5) for the determination of the pH. This method involves water extraction of the smoke which is passed through and absorbed on a cotton plug, which would preferentially favor the contribution to the pH of the particulate matter phase. Sensabaugh and Cundiff (12) have attempted to alleviate this deficiency through measurement of the acid-base effect of whole smoke on an aqueous film upon a combination pH electrode. This method, however, does have an error of unknown magnitude from the aerosol effect mentioned above.

More recently, the redox potential of tobacco smoke solutions has been examined. Wickham et al. (15) employed an alkaline ferricyanide-ferrocyanide redox system and reported a reducing capacity of 1.2-4.5 mg glucose/g tobacco burned. Kobashi and Sakaguchi (8) found slightly lower values, employing the Smogyi method. Hagopian and Rosenkrantz (6,7) used the quantitative reduction of the redox dye, Blue Tetrazolium, by cigarette smoke to estimate the degree of retention of particulate matter in the human respiratory tract during smoking. We have reported on the redox characteristics of cigarette smoke, as determined both colorimetrically with a redox indicator, 2,6-dichlorophenol-indophenol, and electrometrically with a platinum electrode and silver-silver chloride half cell (2). The reducing capacity of the whole smoke or of the phases was time dependent, and indicated a rapid reduction of the test medium after the addition of the smoke solutions.

Because of the interrelationship of pH and redox potentials and the importance of both factors in the composition, quality, and physiological effects of the smoke, we have developed a system by which these values could be determined simultaneously during the smoking of the cigarette showing the contribution from each successive puff. This method also has applicability in the determination of filtration efficiency, and has been employed by us for studies on additives both to tobacco and filters (3,9). Essentially, the system consists of a glass vessel containing a known volume of dilute aqueous buffer and two combination electrodes (**Figure 1**). The apparatus is constructed easily from 30 mm i.d. glass tubing, and holds a working volume of from 5 to 15 mls.

The smoke from a test cigarette is bubbled through the solution under standardized conditions, and the resultant alterations in the pH and the redox potential of the solution are measured by the two electrodes and recorded graphically. Variations with puffs and with time can be visualized, and their effect upon other systems determined.

EXPERIMENTAL

Redox potentials were measured by a combination platinum electrode and silver-silver chloride half cell (Beckman #39186)[2]. The electrode was standardized with saturated quinhydrone in 0.1 N HCl, as suggested by Clark (4).

[1]*Agricultural Research Service, U.S. Department of Agriculture. Contribution received July 16, 1971. Tob. Sci. XIX: 1-3, 1975.*

[2]*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

Because of certain characteristics of tobacco smoke solutions, the electrodes had to be cleaned by special techniques between uses. Water-insoluble material of the tobacco smoke collects on the glass surfaces of the electrodes and interferes with the subsequent determinations unless removed. Normal lipid solvents, e.g., acetone, while removing this deposit, also precipitate the potassium chloride salt solution within the wick of the silver-silver chloride half cell. Our procedure for cleaning the electrodes consisted of a washing between determinations with warm distilled water followed by a 5 minute storage in dimethyl sulfoxide (DMSO) solvent. The electrodes were rinsed with distilled water, and if peroxide and peroxidase oxidation had not been employed in the experiment (14), the electrodes were stored for 1 minute in a dilute 3% hydrogen peroxide—.01% peroxidase solution. This treatment converted the oxidizable materials remaining to a more easily removed form, which was then removed by a second round of DMSO-distilled water washes and rinses. The effectiveness of the washings was determined by monitoring the buffer solutions. The control buffer had a value of 225 ± 15 mvs.

The pH was measured by a combination electrode (Beckman #39183). This electrode was standardized with buffer, pH 6.86, before each experiment, and was also cleaned as above.

Each electrode was connected to a separate potentiometer for amplification and readout, and then to a two-pen recorder (**Figure 1**). The amplifier for the pH electrode employed the expanded scale (Beckman Expandomatic) for the measurement of small values. The redox potential, conversely, has a large variation necessitating a double pole, double throw (DPDT) knife switch with a choice of a 10 or 10 ohm resistor in the circuit. This allowed the redox potential values to be reflected within the limits of the recorder.

The two electrodes were inserted through a Neoprene stopper into the glass vessel through which the smoke was bubbled. An optional hypodermic needle through the stopper allows samples of the solution or the contained atmosphere to be removed for analysis during operation (2). The cigarette was placed within the filter holder, with or without a Cambridge filter, and smoked mechanically under standard conditions (puff rate = one per minute; puff volume = 35 mls; and puff duration = 2 seconds). Following the smoking of the cigarette, the reaction vessel was connected to nitrogen gas or to the atmosphere and observations continued on reoxidation of the solutions (2,9).

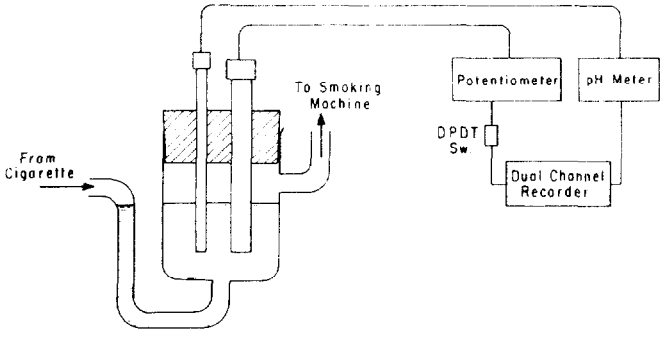

Figure 1. Diagram of system used to measure and record changes in EMF and pH simultaneously. Smoking vessel is shown enlarged to illustrate details. Vessel in diameter of 30 mm and height of 52 mm. Combination pH and platinum electrodes are inserted through Neoprene stopper into glass vessel.

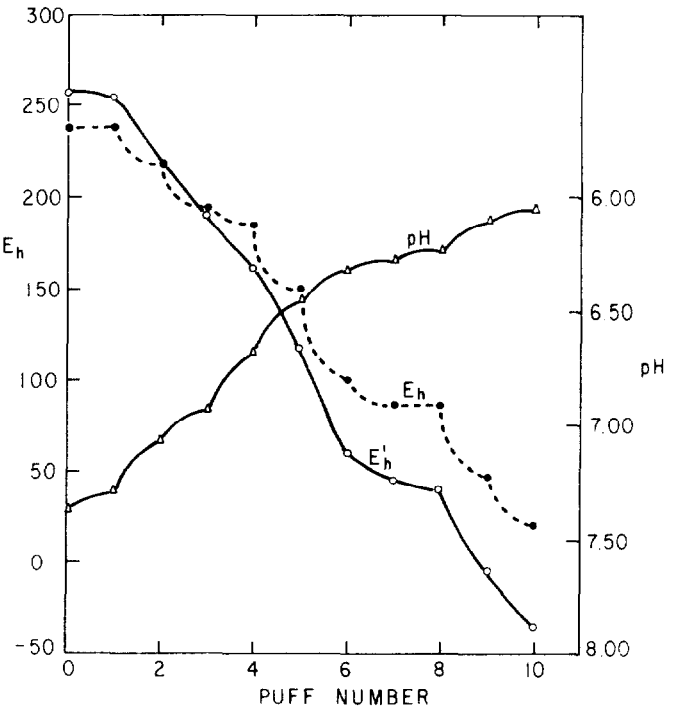

Figure 2. Plot of changes observed on a puff-by-puff basis on pH and redox potential (E_h). Redox potential corrected for effect of pH is shown as E_h'.

The aqueous solutions employed within the smoking vessel can be selected for specific types of experiments. Biological fluids, as saliva, blood plasma or citrated whole blood may be used if desired. We have used buffered isotonic saline solutions (0.001 to 0.005 M phosphate buffers, pH 7.4) and distilled water for most of our studies. Volumes between 5 and 15 mls may be used, with smaller volumes recommended whenever the possibility of foaming exists. If desired by the investigator, a modification in the apparatus by the addition of two three-way valves can direct the smoke either to or around the vessel, so that the effect of individual puffs can be measured. Although not all of the smoke components are trapped by the aqueous solutions, the components which may be involved in biological systems will be, and can give an indication of possible activity.

RESULTS AND DISCUSSION

A representative recorder chart of the simultaneous change in pH and redox potential of an unbuffered solution is shown in **Figure 2.** Since the redox potential as determined is influenced by changes in pH, readings were corrected to a standard pH of 7.0.

The Nernst equation: $E_h = E_0 - \frac{RT}{nF} \ln \frac{(oxid)}{(red)}$ = .059 pH for oxidation-reduction reactions involving proton shifts may be rearranged with the experimentally determined values on one side of the equation to give:

$$E_h - .059\ pH = E_0 - \frac{RT}{nF} \ln \frac{(oxid)}{(red)}$$

where E_h is the observed redox potential in volts, E_0 standard potential in volts of a particular redox system and the pH is that of the solution. For a given redox system, each one unit change in pH produces a corresponding change of 59 millivolts in redox potential. Although such corrections on an unknown

system might be tenuous, experimental evidence comparing the corrected redox values of unbuffered solutions to those of similar cigarettes in highly buffered solutions which prevented pH shifts give credence to this supposition.

The values were corrected by the formula: $E_h' = E_h - 0.059\ (7.0 - pH)$ to give a value E_h', which values are also plotted in **Figure 2.**

Smoking of additional cigarettes through the same solutions will continue the decrease in pH and redox potential. These values appear to level off asymtotically from saturation of the solutions by the smoke substances. **Table 1** lists some representative values from whole smoke and the vapor phase of both a regular unfiltered 85 mm cigarette and a cigarette with commercial cellulose acetate-charcoal filter. A comparison of the values indicates that the vapor phase of the smoke contributes the majority of the acidic components, e.g., formic, acetic and other volatile acids. Carbon dioxide produced in the combustion process could contribute to the pH also by the formation of carbonic acid. However, as this acid disassociates at low pH's, room temperatures, and under mechanical agitation, all of which can be present in the vessel, the contribution to the pH by carbonic acid is probably minor or limited. The weak acids and their salts contribute more to this measurement. The similarity of the pH values between the regular and the filtered cigarette indicates that only a limited removal of these acidic substances occurs with the complete smoking of the cigarette. In the whole smoke, the particulate matter interacts with these acidic substances and buffers further the decline in pH. The smoke of the commercially filtered cigarette having a lower content of particulate matter shows a somewhat lower buffering effect.

The various smokes also show differing effects on the redox potential. The whole smoke from the regular cigarette shows the greatest reducing effect (+238 to +178 mvs uncorrected or +258 to +256 mvs corrected for pH). The differential effect on particulate phase filtration of the combination charcoal-cellulose acetate filter is shown in comparison with the regular cigarette. Simple mechanical filtration of the smoke as by a Cambridge filter leads to values shown in the vapor phase of the regular cigarette, indicating a selective filtration of certain reducing compounds as yet unidentified from the smoke of the combination filter. Such smoke substances as the polyphenols can act both as oxidants or reductants and may contribute to the redox potential (3,13). This method may also be applicable for measuring filter efficiencies under standard conditions (2,3).

Colorimetric redox measurements do not reflect the contribution of the vapor phase to the redox potential alteration, because of the "poise" or "potential buffering" effect of the oxidation-eduction dye (2), nor do the colorimetric methods reflect large changes in redox potential. A hundred-fold alteration in the ratio of oxidized and reduced forms of the indicator dye would only indicate a change of 2. x 59 or 118 millivolts. The colorimetric dye is dependent upon the quantities of the reductant(s) whereas the electrometric method as employed here is more sensitive to the redox potentials of the various components. The wide range in the redox potential found (> 200 mv) implies that participation of a series of coupled oxidation-reduction pairs rather than a single substance may be responsible.

TABLE 1

Table 1: Effect of filtration (combination charcoal-cellulose acetate) and successive smoking (1 to 3 cigarettes) on the redox potential, pH, and corrected redox potentials of a 15 ml unbuffered solution.

	# Cigarettes smoked	Whole smoke E(mv)	pH	Eh'	Vapor phase E(mv)	pH	Eh'
Initial		+238	7.33	+258	+238	7.33	+258
Regular Cigarette	1	+020	6.06	−035	+088	5.45	−003
	2	−125	5.95	−187	−010	5.43	−103
	3	−178	5.68	−256	−042	5.40	−136
Filter* Cigarette.	1	−120	5.65	+040	+205	5.62	+124
	2	+020	5.55	−066	+060	5.43	−033
	3	+008	5.55	−078	+030	5.43	−063

Combination charcoal-cellulose acetate. The cigarettes were smoked using standard smoking conditions, 8 puffs to the cigarette through 10 mls of dibasic phosphate buffer (0.01M, pH 7.2) in the apparatus shown in Figure 1. For vapor phase studies, a Cambridge filter was inserted between the cigarette and the apparatus.

SUMMARY

A method is presented for the simultaneous determination of the redox potential and the pH of the tobacco smoke during smoking. With both values available, this method allows for the continuous determination of the redox potential during smoking, corrected for alterations in the pH of the solution. Since tobacco smoke shows a time dependent alteration in the reducing ability within the first few puffs, this method is more relevant to the actual physiological conditions than previous methods: The determination of the pH reflects the contribution of both phases of the smoke. This method allows the monitoring of two parameters which have major effects upon smoke composition, quality, and physiological systems.

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