Automated System for Determining Respiratory Gas Exchange of Plant Materials[1]

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Abstract. A continuous gas flow system is described which utilizes a paramagnetic O_2 analyzer and an infra-red CO_2 analyzer to automatically sample, analyze, and record respiratory gas exchange of plant material. The system monitors gas exchange of up to 96 plant samples sequentially, repeatedly, and over extended time periods.

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Introduction

DETERMINATIONS of the respiration rate of plants or harvested plant organs are essential to a great number of investigations. Knowledge of respiratory behavior facilitates the development of improved techniques for harvesting, handling, storing, and transporting perishable fresh fruits and vegetables. Many factors have been studied and are continually being evaluated in relation to their influence on respiration rate.

A comprehensive review of various procedures for measuring respiration rate of fruits and other plant organs has been prepared by Biale (1). There are many methods currently employed to measure respiration rate. All are based on measurement of O_2, CO_2 production, or both in either continuous flow or static systems. Continuous flow systems employ a metered gas stream at an appropriate rate to circumvent excessive depletion of O_2 or accumulation of CO_2, ethylene or other volatile gases which may alter respiration rates as determined by a static system. It is desirable to measure both O_2 consumption and CO_2 production since the respiratory quotient (CO_2/O_2) may change either as a result of treatment or naturally during the course of development, maturation, ripening, or senescence of the plant tissues. The change in RQ may indicate a fundamental change in respiratory metabolism of the tissue. This may arise from a change in metabolic

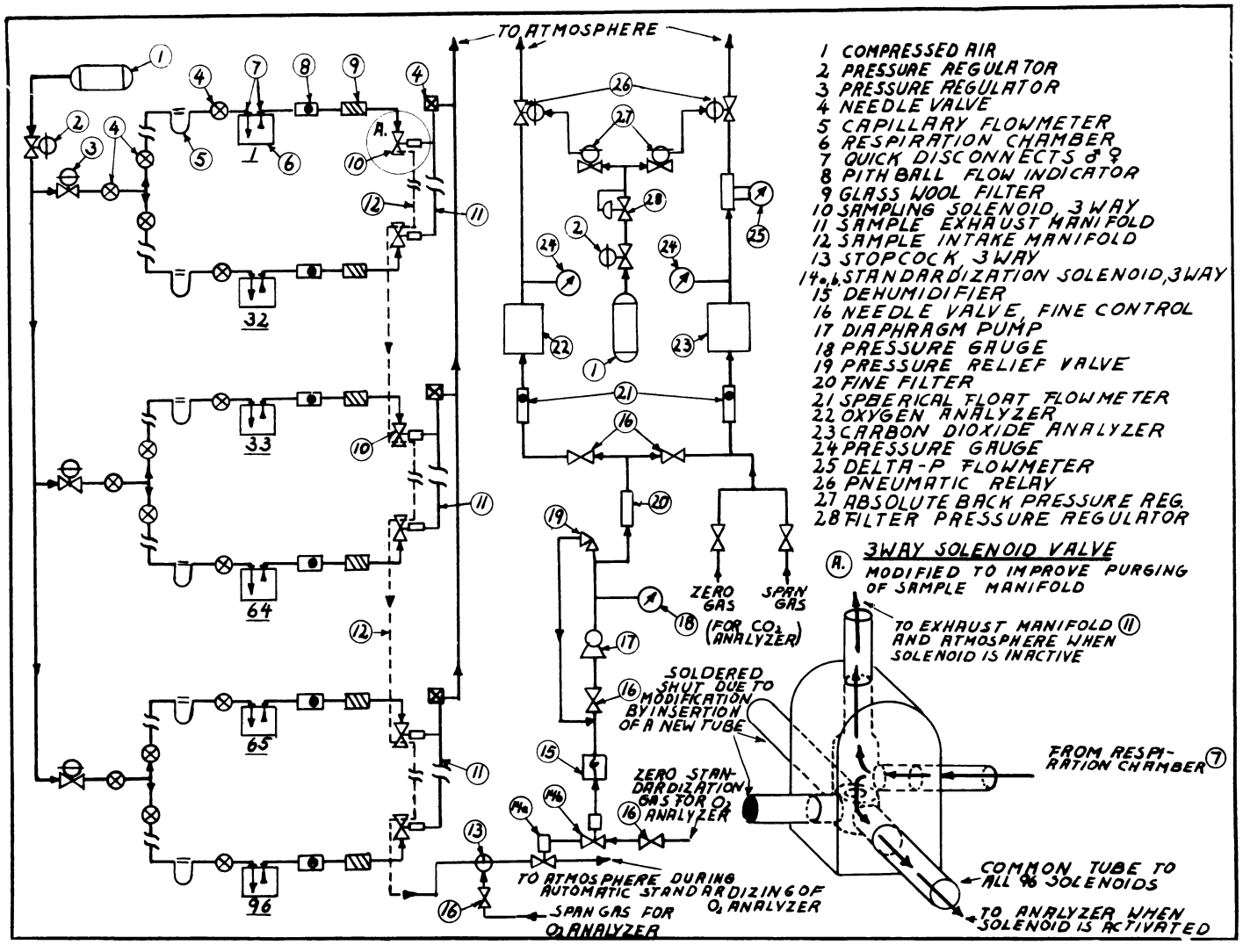

Fig. 1. Flow diagram for gas sample handling in the APRIL system.

substrate or a change in relative contribution of glycolysis and the citric acid cycle to respiratory CO_2.

An automated system of O_2 and CO_2 analysis is described which has proven to be an accurate and dependable research tool over several years of continuous operation. Construction details and operating principles of this system including heretofore (2) undisclosed modifications are given, which contribute to dependability and accuracy of the system.

Experimental

The APRIL system. Oxygen and CO_2 analyses in the "APRIL" system (Automated Photosynthesis and Respiration Integrating Laboratory) were performed with a Model G-2 paramagnetic O_2 analyzer and a Model IR-115 infra-red CO_2 analyzer, both of Beckman Instruments, Inc. Young and Biale (4) and Lougheed and Franklin (3) have already reported the use of this type of instrumentation for O_2 and CO_2 analyses, respectively. The systems employed by these workers, however, did not assess net changes in both gases concomitantly.

A flow diagram of the sample handling system is shown in Fig. 1 and will be described in some detail since it is of vital importance for the successful operation of the system. There are 32 respiration chambers (6′) located in each of 3 constant temperature rooms giving a total of 96 sample points for analysis. Each room is equipped with a capillary flow meter assembly which supplies individual chambers a measured flow rate of air. A fixed flow rate of air at 300 ml per minute is routinely employed for all samples to simplify calculations. Sample weights are varied according to tissue type, temperature, or other experimental variables in order to achieve measurable O_2 and CO_2 levels in the flowing gas stream. The O_2 and CO_2 content of the incoming air is monitored in 2 or 3 of the 32 chambers in each room under exactly the same conditions as for the remaining sample-containing chambers. As air passes over the respiring tissue the O_2 content becomes slightly depleted and the CO_2 content slightly enriched from its initial value. The effluent gas from each chamber flows to a sampling manifold of 3-way solenoid valves located behind the analyzer console. Thus, a representative sample of the effluent gas from any one of the 96 chambers is available on command to enter the sample intake manifold (12′) in sequence and be carried to the analyzers. When not being analyzed the effluent gas is vented to the atmosphere through an exhaust manifold (11′). An expanded view (A) of the 3-way sampling solenoid (10′) is shown in the lower right corner of Fig. 1. The valve ports were modified to eliminate dead space and provide positive and rapid purging of the sample intake manifold. The common tube connecting all 96 solenoids is less than 50 ml in volume.

Upon energizing a solenoid, the sample is carried through a dehumidifier (15′) which lowers the gas to a constant dew point. It is essential that the standardizing gas and span gas for the O_2 analyzer are brought to the same dew point as the samples prior to analysis. It has a suppressed range of 21.0 to 20.5% O_2 and slight dilution of the O_2 content by water vapor is greatly magnified within this narrow range. After leaving the dehumidifier, the samples pass through a diaphragm pump (17′) which elevates the gas pressure to 50″ water column necessary for the operation of the pneumatic relays (26′) and absolute back pressure regulators (27′) which maintain a pressure of 760 mm Hg as the gases leave the O_2 analyzer. Absolute sample exit pressure is required to isolate the suppressed range O_2 analyzer from baro-

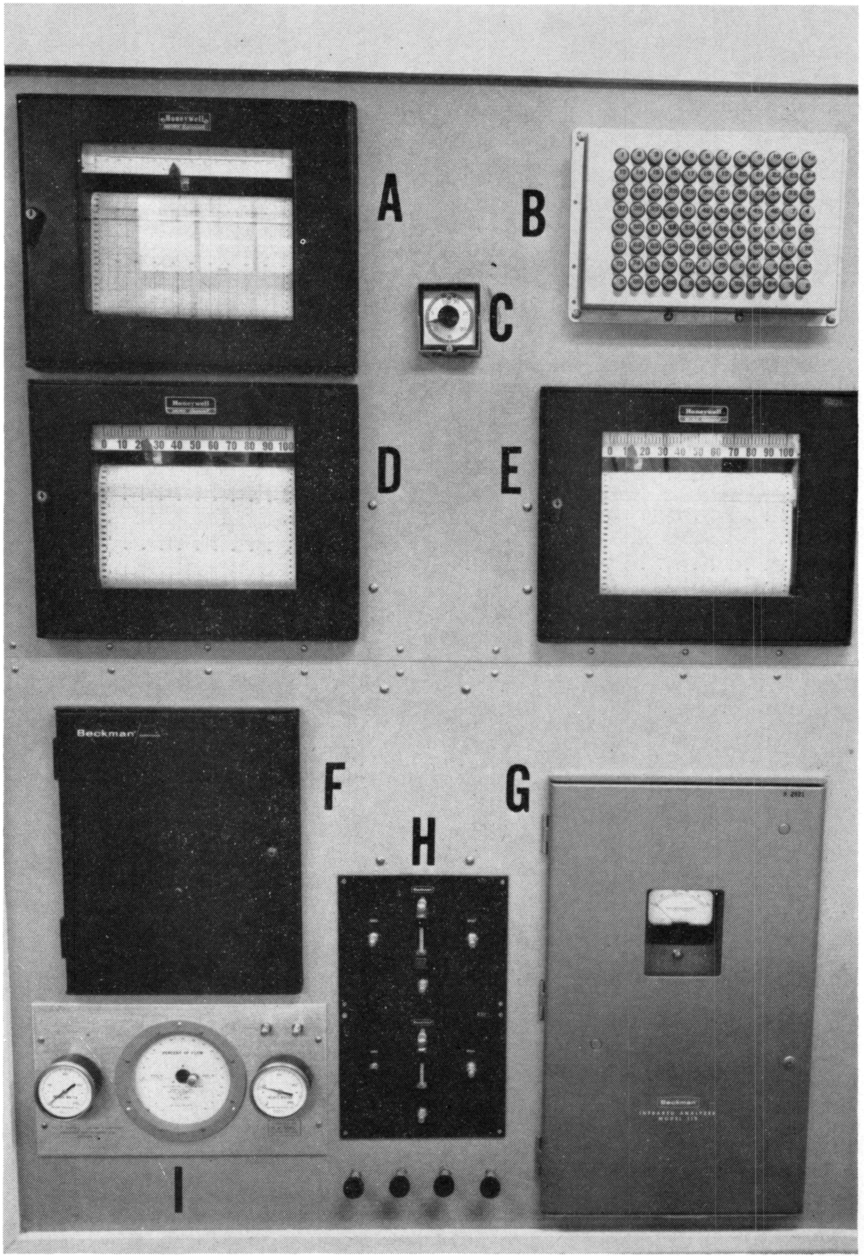

Fig. 2. Gas analyzer instrument console. A) temperature recorder for respirometer rooms, B) sample indicator panel, C) automatic reset timer, D) O_2 analyzer recorder, E) CO_2 analyzer recorder, F) O_2 analyzer, G) CO_2 analyzer, H) gas selector and flow control, and I) laminar flow meter and sample pressure gauge.

metric pressure fluctuations as a 1% change in barometric pressure is equivalent to a change in O_2 concentration of approximately 0.2%. This is equivalent to 40% of the analyzer range. The capacity of the diaphragm pump is adjusted closely to the normal sample flow rate maintaining the same flow over the samples during analysis and between analyses. This is achieved through the use of a fine control needle valve (16′) and pressure relief valve (19′). The pressurized sample is then split and enters the O_2 analyzer and CO_2 analyzer in parallel, each at the rate of 150 ml per minute. Analysis of both CO_2 and O_2 is performed at the end of the purge period at which time the values are recorded. The next sample in sequence is then introduced.

Sample selection is controlled by two 48-point stepping switches wired in 8 banks of 12 (96 sample points) together with a controller which provides recycling of any or all of the 8 banks. Samples are switched automatically and sequentially within each bank on command from an external reset timer which in addition initiates recording of the O_2 and CO_2 analyses.

The time required to purge the sample handling system from the sampling manifold through the analyzer cells dictates the minimum frequency of analysis. This is about 7 min for the flow rate we employ. Recycling the 96 sample points with an analysis dwell period of 7½ min cessive determinations on a particular sample. Each sample is monitored every 4 hr when 24 samples are being recycled with an analysis dwell period of 10 min.

O_2 and CO_2 analyzers. The infrared CO_2 analyzer employs a 1.25 inch absorption cell. The instrument is calibrated over a range of 0 to 4000 ppm CO_2 with specially prepared gas mixtures. The output from the analyzer is slightly nonlinear with increasing CO_2 concentrations. A quadratic equation of CO_2 concentration as a function of recorder values from 0 to 100 was obtained from which two-way tables were prepared to facilitate respiratory rate calculations. The calibration equation was used directly as part of CDC–3600 computer program for data processing. The IR–115 Analyzer does not have an automatic standardization feature, but drift from zero or span reference points is extremely slight over periods of days or weeks at a time.

The oxygen analyzer, because of its range, will measure very precisely the O_2 content of gas mixtures containing between 20.5 and 21.0% O_2. In effect, the analyzer measuring circuit utilizes 20.5% O_2 as the zero point on the recorder and expands the increment from 20.5 to 21.0 over the 100 point scale of the recorder, making each scale division equivalent to 50 ppm O_2. The O_2 analyzer is equipped with an automatic standardization feature which in our application is programmed to occur after a given number of samples. The frequency of standardization is dependent upon the dwell time per sample and the number of samples being recycled. Four hours is the maximum standardizing interval and the process is completed during the normal sample dwell time. The standardization gas is dry air (20.93%) admitted in the same manner as the samples. The span of the instrument is set by a gas of an accurately known O_2 content between 20.5 and 20.7%.

Calibration of the O_2 analyzer. The absolute content of O_2 is critical to set the span of O_2 analyzers in this suppressed range, and routine analytical procedures for O_2 such as gas chromatography, mass spectrometry, or micro-Scholander do not provide a sufficiently accurate analysis giving, at best, values of ±0.05%. An accurate O_2 analysis of our span gas was attained employing a biological procedure based on the assumption that the certified analysis of dry air was 20.93% and adjusting the recorder of the O_2 analyzer accordingly. The respiratory quotient of several samples of dormant potato tubers was determined by a manometric procedure. The effluent gas from samples of the same tubers was then analyzed for CO_2 with high accuracy in the infra-red analyzer while the same gas was passing through the O_2 analyzer. Knowing the respiratory quotient and the rate of CO_2 production, the O_2 content of the effluent gas was calculated and employed to set the span point of the O_2 analyzer. The O_2 content of commercially prepared span gas was then determined, and subsequent cylinders of span gas have been analyzed with reference to the original cylinder. Respiration rate is routinely expressed as ml of O_2 produced per kilogram of tissue per hour at standard conditions. The calculation for O_2 follows:

$$\text{ml } O_2/\text{kg/hr} = \frac{(\text{flow in ml/hr})\ (273)\ (P)}{(\text{weight in kg})\ (T)\ (760)\ (\%\ O_2 - \%\ O_2 \text{ sample})}$$

and for CO_2:

$$\text{ml } CO_2/\text{kg/hr} = \frac{(\text{flow in ml/hr})\ (273)\ (P)}{(\text{weight in kg})\ (T)\ (760)\ \text{corrected \% } CO_2}$$

The constant factors for a given experimental sample are grouped into a single constant to simplify calculation. In order to maintain a fixed flow rate of 300 ml of air passing over the tissue samples, the sample size is selected to bring the O_2 and CO_2 concentration in the effluent gas within the range of the analyzers. The following relationship is employed to estimate the effluent CO_2 concentration:

$$\%\ CO_2 = \frac{(\text{Respiration rate})\ (\text{weight}) \times 100}{\text{Flow rate} \times 60}$$

Where respiration rate is expressed as ml CO_2/kg/hr at a given temperature,

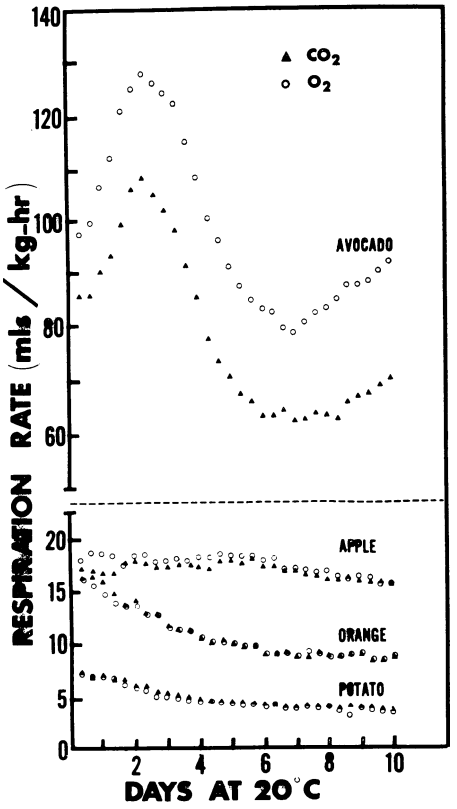

Fig. 3. O_2 consumption and CO_2 evolution of Fuerte avocados, Red Delicious apples, Washington Navel oranges, and Idaho potatoes at 20°C. These commodities were obtained from a local produce supplier in February, 1965.

weight is in kilograms, and the flow rate is 300 ml per minute.

In addition to measuring the gaseous exchange of CO_2 and O_2 during respiration, the system can also measure O_2 and CO_2 exchange during photosynthesis since the temperature controlled rooms containing the samples are equipped with adequate illumination for most plants. However, since its initial use in the fall of 1964 it has been used solely for respiratory analysis. The respiratory activity of a wide range of fruits and vegetables has been determined at various temperatures in relation to nutritional, cultural and environmental conditions during growth, maturation, ripening, and senescence; and as affected by pre- and post-harvest chemical treatments. Examples of the time-course change in O_2 consumption or CO_2 production rate for several tissues are depicted in Fig. 3 and 4.

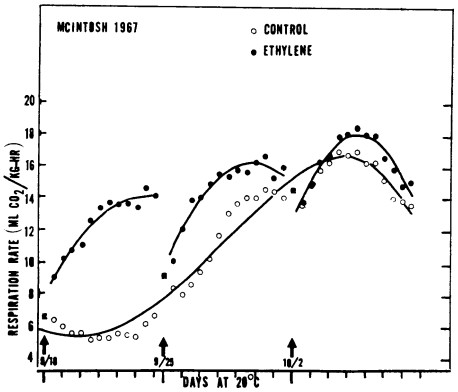

Fig. 4. CO_2 evolution of McIntosh apples at 20°C of fruits at 3 successive harvests and the response to exogenous ethylene. Ethylene treatment was 1000 ppm for 12 hours on the day of harvest.

The system can be employed for simultaneous analysis of O_2 and CO_2, or of either gas independently. The automatic sampling, analyzing, and recording features provide capacity to monitor respiration frequently and over extended time periods. Digital print out and tape punch equipment has been installed to facilitate computerized processing of the data.

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Influence of Fat-Sugar Derived Surfactants on Phosphorus Absorption Through Leaf Surfaces[1]

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Abstract. Fat-sugar derived surfactants were examined as to their effectiveness in the promotion of absorption of nutrient ions through leaf surfaces and subsequent translocation of these ions through the plant. The fat-sugar derived surfactants and several other commercial surfactants were added to solutions of P to test the effectiveness of the surfactants in promoting ion absorption and translocation.

The surfactants in the nutrient sprays at a 0.5% concentration were generally more effective than at 0.1% or 1.0% concentration with the exception of Polyoxyethylenated Tallow Sucroglyceride (PTS) which had its greatest effect at a 1.0% concentration. Sucro sperses and Sucro sols with 3 fatty acid molecules per sucrose molecule were more effective in increasing P uptake and transport than formulations with 1.5 or 4.5 fatty acid molecules per sucrose molecule. A 40:1 ratio of ethylene oxide to sucrose was most effective for increase of P absorption. Combining PTS and Sucroglyceride T-110 (T-110) increased P absorption more than if either surfactants were used alone. Ratios of 1:3 or 3:1 respectively, were most effective. T-110 and Sucrose Monotallowate (SMT) did not significantly increase absorption of P at any concentration tested. PTS was effective in increasing nutrient ion uptake at a 1% concentration, but it was found to be somewhat phytotoxic at this level. The fat-sugar complexes used as surfactants increased ion uptake in some instances more than 20 times compared to using no surfactant. When these same surfactants were compared to commercially used surfactants, they generally proved to be superior for use with the foliar applied nutrients.

Introduction

WHEN soil conditions favor nutrient fixation or insolubility of compounds of the nutrient then foliar application produces the most efficient plant response to these ions, especially those required in low total amounts by the plant. Various spray additives have been used to increase the rate and amount of nutrient ion absorption (see literature citations). Among these, the use of wetting agents such as the Tweens, X-77, Tergitol surfactants, etc., are probably most common. Surfactants reduce surface tension of solutions and allow a greater contact area to be maintained. The surface tension can be reduced enough to overcome adverse effects of air pockets caused by leaf surface hairs and wax and to facilitate penetration into the stomatal aperatures. Sugars have also been added to various spray solutions to regulate uptake more closely and to facilitate translocation of the substance once inside the plant (4, 10).

Fat-sugar derived surfactants were examined as to their effectiveness in the promotion of absorption of P ions through leaf surfaces, and subsequent translocation of these ions through the plant.

Materials and Methods

Snap bean '83 Tendercrop' seedlings were grown in soil, one plant per 4 inch pot in the greenhouse. The soil was mixed with peat moss at a 1:1 ratio and adjusted to pH 6.5 with lime. Nitrogen, P, and K were added at an equivalent of 150, 150 and 300 lb./acre (A), respectively.

The plants were allowed to grow for 21 days after emergence at which time they were treated. The treating solution contained the desired surfactant plus a 500 ppm P solution of radioactive Na H_2 $^{32}PO_4$ with an activity of 0.5 uc/ml as P^{32}. This solution was adjusted to pH 2.5 with 1 *N* HCl.

The following materials were tested as surfactant adjuvants at 0.1%, 0.5%, and 1.0% concentrations: Sucro-sperse 30-13, Sucro-sperse 30-115, Sucro-sperse 30-145, Sucro-sol 20-115, Sucro-sol 40-13, Sucro-sol 40-115, Sucro-sol 60-13, Sucro-sol 60-115, Sucro-sol 60-145, Sucrose Monotollowate (SMT), Polyoxyethylenated Tallow Sucroglyceride (PTS), Sucroglyceride T-110 (T-110), Tween 20 (polyoxyethylene sorbitan monolaurate) a nonionic surfactant, Tween 80 (polyoxyethylene sorbitan monolaurate-ester) a non-

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