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For product information, contact:
UVP, LLC Upland, CA / (800) 452-6788 / (909) 946-3197
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LABORATORY APPLICATIONS FOR ULTRAVIOLET EQUIPMENT

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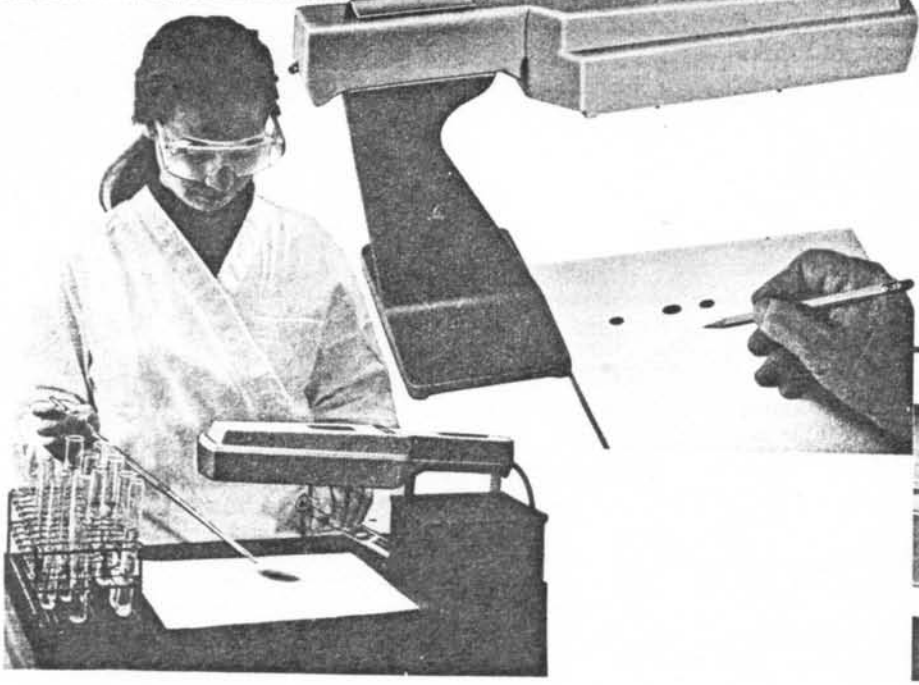

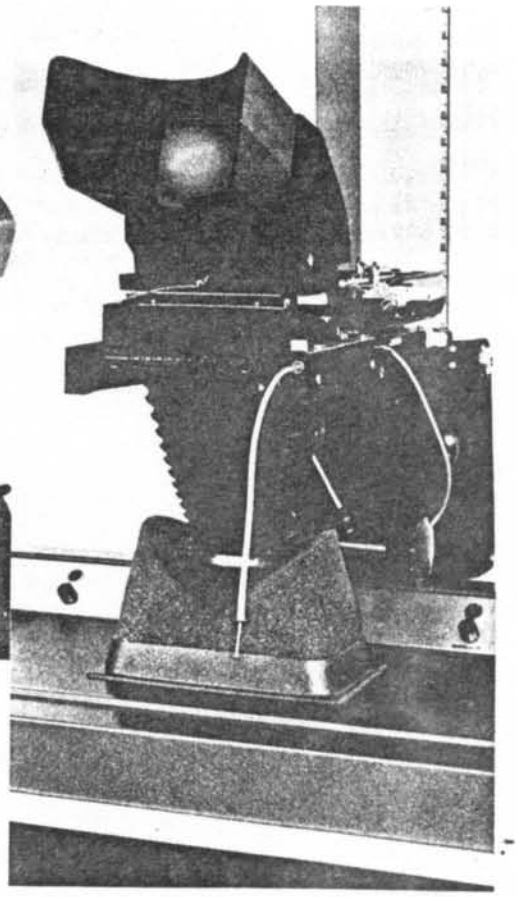

LABORATORY APPLICATIONS OF ULTRAVIOLET LAMPS

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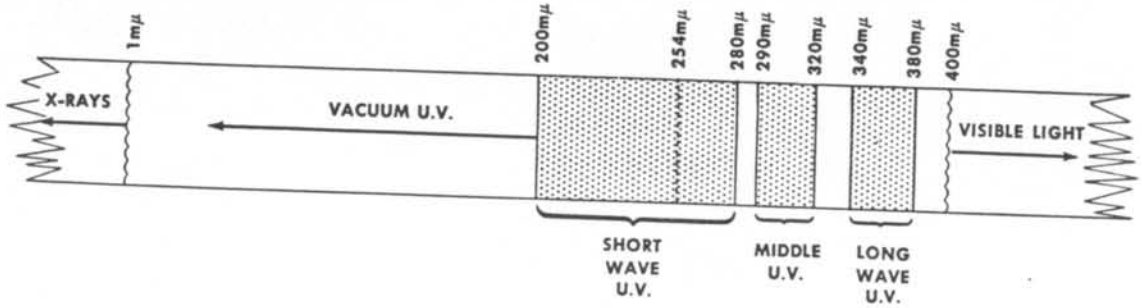
INTRODUCTION

This paper is intended to provide information on the applications of ultraviolet radiation that have significance in laboratory applications, wherever science finds the need for this modern, useful tool.

In order to illustrate the value of ultraviolet lamps several papers are mentioned and occasionally quoted. Other data is from personal communication and experience with the problems of the laboratory over the past 30 years.

For those who may have forgotten some of the basic principles of ultraviolet radiation and fluorescence, an explanation and discussion of terms is in order.



A portion of the Electromagnetic spectrum, emphasizing the Ultraviolet region.

ULTRAVIOLET RADIATION DEFINED

Ultraviolet light is a form of radiation which cannot be detected by the human eye. It merges into visible light on one boundary and into x-rays on the other. These limits are often arbitrarily set at approximately one millimicron for the X-ray to ultraviolet gradation and approximately 400 millimicrons for ultraviolet to visible light.

KINDS OF ULTRAVIOLET

In order to define the type of ultraviolet radiation under discussion it is necessary to define the radiation in terms of wave length. While some waves, as in radio, may be many feet long; waves in the ultraviolet and visible range are very short. In the latter regions one of two different units is normally used for measuring wave length. One of these is the angstrom unit, equal to 1×10^{-8} cm. The other unit is the millimicron, abbreviated $m\mu$, and is

the terminology used throughout this paper. For quick reference from one unit to the other, one mμ equals 10 angstrom units.

U.V. radiation is often divided into arbitrary classifications relative to specific character and activity. These classifications, used throughout this discussion, are as follows:

1. Long wave ultraviolet is radiation from approximately 340 mμ to 380 mμ, usually considered to peak at 365 mμ. This region is often called "black light," "Wood's Light," or near ultraviolet. BLAK-RAY® lamps referred to in this paper are, by definition, long wave U.V. emitting lamps. Long wave ultraviolet is harmless, does not cause erythema on normal skin or eyes.

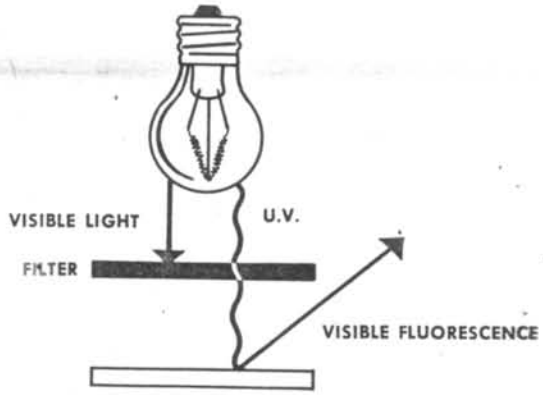
   Long wave ultraviolet causes fluorescence in many natural and manufactured substances. It is the most important wave length in clinical diagnosis, quality control procedures, and fluorescent titration. There are many important uses for long wave ultraviolet in chromatography for location of spots on paper and thin layer chromatograms.

Ultraviolet important in chromatography...

2. Middle ultraviolet is radiation from approximately 290 to 320 mμ, the shortest radiation reaching the earth from the sun. This band of ultraviolet rays is best known for its sun tanning effect on human skin and blistering sunburn on those who remain in the sun too long. This radiation has very little scientific value in the laboratory.

3. Short wave ultraviolet is radiation from 200 mμ to approximately 280 mμ. Usually considered to peak at 254 mμ as this is the strong resonance radiation line of a mercury glow discharge lamp. This radiation may also be called far ultraviolet as it is at a greater distance from visible light than the classifications previously mentioned. MINERALIGHT® lamps referred to in this paper are, by definition, short wave ultraviolet sources. Shortwave ultraviolet may cause sunburn of the unprotected eyes or skin. Short wave ultraviolet has long been known for its germicidal effects and its ability, when properly filtered, to cause fluorescence in hundreds of minerals. It is now extensively used in the laboratory to observe spots on chromatograms through quenching of background fluorescence and to observe substances not fluorescent with long wave ultraviolet.

4. Vacuum ultraviolet is radiation shorter in wave length than 200 mμ. This radiation is not propagated through air, hence the name "vacuum" ultraviolet. With the advent of the space age this has become a more important tool although it still is not a laboratory tool within the limits of this discussion.



HOW ULTRAVIOLET RADIATION IS PRODUCED

Ultraviolet radiation may be produced in several different ways. Two basic differences are found in lamps known as low pressure and high pressure. The low pressure lamp is ordinarily operated at an internal pressure of less than 40 millimeters of mercury.

In the case of the low pressure ultraviolet lamp, production of the ultraviolet in the 254 mμ range is caused by discharging electricity through a carrier gas such as argon which ionizes enough to cause a glow discharge and develop a slight amount of heat causing the mercury in the tube to vaporize. As the mercury vaporizes it is ionized by the electric discharge and gives off certain wave lengths in the visible region, especially in the green and blue. At the same time it emits some long wave ultraviolet. However, the maximum emission from such a tube is at the 254 mμ line, 80 to 95% of the radiation produced being at this wave length. This is frequently termed the mercury resonance line. As short wave ultraviolet does not penetrate glass, a tube made of quartz or silica material must be used to transmit this radiation. As visible light is also produced it is necessary to use a filter which will transmit the short wave ultraviolet but absorb the visible light. This filter is used only when looking for fluorescence or quenching effects and is not used for photo-chemical applications.

When a high percentage of long wave ultraviolet is desired from a low pressure ultraviolet lamp it is produced by changing the short wave ultraviolet to long wave ultraviolet with a conversion system. The interior of the tube is coated with a special phosphor which absorbs short wave ultraviolet and emits long wave ultraviolet plus some visible light. A filter is again used to eliminate most of the visible light and allow the long wave ultraviolet light to be transmitted.

High pressure ultraviolet sources have much higher operating pressure than the low pressure type. The pressures may be in the order of one to several atmospheres. These lamps require a great deal of current and while their actual output of ultraviolet may be high, efficiency is very low due to heat loss. High pressure lamps are frequently used because of the amount of long wave ultraviolet that is produced. However, their production of short wave ultraviolet is minimal.

LUMINESCENCE, FLUORESCENCE, AND PHOSPHORESCENCE

Luminescence is a general term for the "cold light" emitted by a substance. It is different from incandescence as no heat is required to produce the light. Luminescence may be the result of the ability of a substance to transform ultraviolet energy to visible light and includes the phenomena of fluorescence and phosphorescence.

Fluorescence is the ability of certain material to absorb ultraviolet energy and transform the energy from invisible radiation to visible light. Fluorescence occurs only while the material is being activated by the ultraviolet radiation.

Phosphorescence is the ability of the material to retain some of the energy which it receives and to release this energy in the form of visible light; not only during the period of activation, when it is called fluorescence, but also to continue emitting light after the activating source has been removed.

Chromatography most important application...

ULTRAVIOLET APPLICATIONS FOR PAPER AND THIN LAYER CHROMATOGRAPHY

Chromatography is one of the most important applications of ultraviolet radiation. There is frequent mention in the literature that thin layer and paper chromatograms be inspected under long wave and short wave ultraviolet for fluorescent or absorbent spots prior to any type of reagent application. In addition, the literature abounds in references to ultraviolet light sources for detecting or differentiating materials on chromatograms. Some of these have been selected to give a broad idea of the importance of this tool. The references are categorized according to specific areas of interest.

CHROMATOGRAPHY IN MEDICAL RESEARCH AND LABORATORY

The U. S. Pharmacopoeia XVI lists a number of references to chromatography. Among these is an assay procedure for Cortisone acetate,[1] also indicating that the principal spots of Prednisolone, Prednisolone acetate and Prednisone show a brilliant blue fluorescence with a MINERALIGHT lamp. Cortisone and Hydrocortisone and their acetates do not fluoresce under these conditions. In this same reference it is pointed out that the identification of Ethinyl Estradiol[2] is accomplished due to the fact that this compound gives a yellow spot on paper chromatograms which appears green under ultraviolet light.

The analysis of conjugated Estrogen preparations was studied by Carol et al[3] who used a CHROMATO-VUE® long and short wave viewing cabinet. Kunze and Markham[4] have also made paper chromatographic tests for identity and purity of Adrenal Cortex hormones.

Separation of some alkaloids, steroids and synthetic compounds by thin layer chromatography was discussed by Korzun, Dorfman

Separation of alkaloids and synthetic compounds...

and Brody.[5] In their paper they mentioned that an ultraviolet lamp is used to locate materials that fluoresce or quench ultraviolet light.

For visualization of steroids Waldi[6] states, "We find that in TLC, spraying with a 30-40% o-phosphoric acid is advantageous. — After heating for 7-15 minutes at 110-120°C, the steroids fluoresce strongly in UV light (365 mμ). — It is necessary to observe the intensification of the colors during heating. — Cholesterol and its esters fluoresce pink-red, later rust-red. Estrogens are first a brilliant yellow, then orange and finally orange-red. Pregnenolone and dehydro-androsterone show a brilliant violet. — Aldosterone shows as a brilliant green."

Paper chromatography of Flavins and Flavinnucleotides was reported by Kilgour, Felton and Huennekens.[7] In this work they used the MINERALIGHT V-41 lamp now replaced by UVS-54, for the quenched Nucleotides as well as the B-100A BLAK-RAY lamp for the fluorescent portions.

In a study of nucleotides, Randerath[8] observed that the chromatography can be observed in progress with short wave ultraviolet as a covered jar is not necessary. He also traces his chromatograms on cellophane under short wave ultraviolet.

The chromatographic behavior of 38 authentic Indole compounds is discussed in a paper on the Indole acids of human urine by Armstrong, Shaw, Gortatowski and Singer.[9] In the behavior table the reaction of the various compounds to ultraviolet light are listed. Stahl[10] gives tables of fluorescent response of "simple" indole derivatives as seen with 365 mμ radiation after treatment with formaldehyde reagent. Limits of detection are as low as .005 μg. Armstrong, Shaw and Wall[11] have written a paper on the Phenolic acid of human urine and the chromatographic behavior of 49 simple Phenolic acids is described. Again, a chromatographic behavior table is given listing the reaction to ultraviolet radiation of each of the compounds.

The synthesis of some model Pyrimidine Nucleosides was discussed by Miles[12] who used a MINERALIGHT lamp to detect the substances by quenching of the fluorescence of the background paper.

Shaw[13] used a special MINERALIGHT lamp adapted for his needs, to produce radiation from 280 to 340 mμ. He found this wavelength very useful on paper chromatograms for detecting Imidazole derivatives related to Histidine and Imidazolepyruvic acid. While the latter material tends to be unstable the special lamp discloses impurities associated with Imidazolepyruvic acid and is therefore better able to define its purity.

Smith,[14] in discussing the long wave "Wood's Light" mentioned that it is of particular use in the study of Indoles and Iodo-Amino Acids located with the Ceric-Arsenite reagents. Smith[15] recommends short wave ultraviolet radiation for detection of the Phenylthiohydantion Aminoacids. These compounds show

Chromatographic behavior of Indole compounds...

as dark spots quenching the fluorescence of the paper. A method for eliminating interfering Pyridine is given.

Brenner et al[16] find that phosphor impregnated silica gel TLC plates with short wave ultraviolet radiation is a very sensitive detection method for DNP-Amino acids. " — even O-DNP-tyrosine is seen in quantities as small as .06 μg." This quenching method of location with ultraviolet was also found applicable to PTH-Amino acids with sensitivities to .1 μg.

Smith[17] states, "DNP-Hydrazones absorb in the ultraviolet region and so appear as dark spots on the chromatogram. This is a highly sensitive method of location and amounts of less than 1 microgram can be detected."

He also suggests that all chromatograms involving Phenols[18] should be examined in ultraviolet light and that chromatograms which have been exposed to ammonia should be examined under these conditions since the fluorescence of some Phenols undergoes a change. He gives a table relative to the fluorescence of the various Phenols before and after ammonia exposure.

Applewhite[19] et al discuss methods of thin layer chromatography and uses of ultraviolet radiation. They include data on quenching with inorganic phosphors and fluorescein spray methods for fatty acid detection.

Ruggieri[20] obtained complete separation of the methylesters from non-saponifiable contaminants. He used dichlorofluorescein spray to detect spots in ultraviolet light.

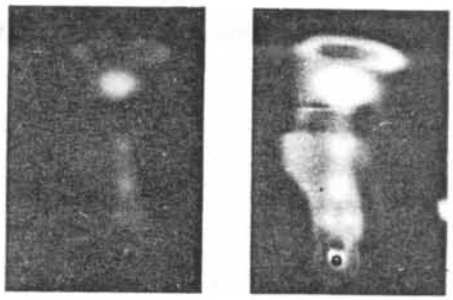
Almost every lipid can be recognized...

Mangold[21] states, "Almost every lipid can be recognized, after spraying the chromatogram (TLC) with an ethanolic 0.2% solution of 2,7-dichlorofluorescein in U.V. light (270 mμ) as light green fluorescent spots on a dark violet background. With this reagent 1-5 μg of a compound can be detected." Mangold also refers to a Rhodamine B solution for the same purpose. Lipids containing conjugated double bonds are best seen on phosphor impregnated silica gel plates.

CHROMATOGRAPHY IN THE PHARMACEUTICAL AND CHEMICAL INDUSTRY

The identification and determination of origin of Cannabis by means of chromatography have been reported by Davis, Farmilo and Osadchuk.[22] The now obsolete model SL-2537 MINERALIGHT lamp was used, presently replaced by the UVS-54 MINERALIGHT lamp.

The analysis of Digoxin preparations was reported on by Houk, Alexander, and Banes.[23] They noted the following fluorescence in order of decreasing Rf values: Digitoxin gives a yellow orange fluorescent spot, Gitoxin a yellow to blue spot, Digoxin a blue spot, Diginatin and Lanatoside C blue spots near the starting line.



Left: TLC under normal ultraviolet radiation. Right: Same TLC has brighter, higher contrast with Transilluminator

The fluorescent color with 365 mμ radiation of 48 alkaloids is listed in a table by Waldi.[24] The various classes of alkaloids are discussed with numerous references to their fluorescent colors.

A study of the chromatography and electrophoresis of Phenothiazine drugs was made by Mellinger and Keeler[25] who observed that ultraviolet radiation of the shorter wave lengths was very satisfactory for locating the spots since all Phenothiazine derivatives studies showed a visible fluorescence of varying intensities. A table is given in their paper of the fluorescent colors of the various Phenothiazine compounds studied.

Klein and Kho[26] have described a chromatographic identification method for sulfonamides. Identification, in part, is by fluorescein impregnated plates and short wave ultraviolet. Maienthal, Carol, and Kunze[27] used a CHROMATO-VUE Cabinet successfully in their work on the analysis of mixed Sulfonamides by quantitative paper chromatography.

The Glycosidal variations of Ornithogallum umbellatum during growth was studied by Locock and Paterson.[28] Their extracts were found to give many fluorescent spots and help determine the nature of the separated compounds.

An excellent paper was written by Fischl and Segal[29] on the identification of Barbiturates by chromatography. They sprayed the chromatograms with a Salicylate reagent which provides an internal screen for the visualization of the Barbiturates under ultraviolet light. They used the MINERALIGHT Lamp Model R-52. Grieg[30] did similar work in this same field but he attached the chromatogram to a fluorescent screen instead of providing a built-in fluorescent viewing screen.

In discussing Barbiturates, Smith[31] says as follows, "the Barbiturate spots may be identified on dried papers by illuminating with an ultraviolet lamp of maximum 254 mμ after the paper has been exposed to the saturated atmosphere of ammonia. They appear as dark spots on fluorescent paper and can be marked with a pencil. This method is very sensitive and well defined spots can be obtained with 10 to 25 micrograms of Barbiturate. With light of 360 mμ maximum intensity only the Thiobarbiturates can be clearly observed. This provides a useful means of locating either the Thio-barbiturtaes or all Barbiturates without chemical treatment of the paper."

Barbiturates appear as dark spots...

In the chemical field, Mitchell[32] in his paper on separation and identification of acids by paper chromatography mentions that smaller quantities of Salicylic acid are detectable under long wave ultraviolet light than the method that is generally used. He also points out that the intensity of fluorescence is increased if the paper is sprayed with acid. Mitchell[33] also describes the separation and identification of six Arseno-organic compounds where he uses a BLAK-RAY long wave lamp and a MINERALIGHT short wave lamp. His process is logically stepped to separate each of the compounds.

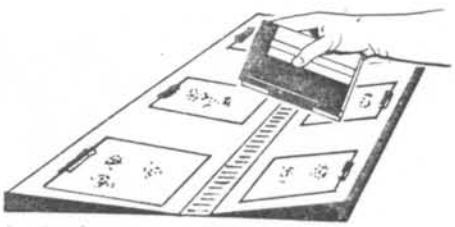
Lyman et al[34] describe an ultraviolet method for following and maintaining control of the methylation of a polyhydroxybenzoic acid. It is suggested that the method could be used to follow reactions of other aromatic compounds.

A method using Pyridine as a reagent for detection of acid on paper chromatograms has been described by Cerbulis and Taylor.[35] The paper in part states "Organic acids generally have been detected on paper chromatograms by use of pH indicators and some specific coloring agents. Ultraviolet light has been used only for the detection of certain aromatic acids.

"It was observed in this laboratory that Krebs cycle acids, separated after using a Pyridine containing solvent system, gave dark blue spots on a light blue background under ultraviolet light. This phenomenon was investigated further and was useful in the detection of acids on paper chromatograms.

"Malic, Citric, Isocitric, Malonic, Maleic, Cis-aconitic, Aconitic anhydride, Oxalic, A-Ketoglutaric, Succinic, Lactic, Fumaric, Gluceronic, Mandelic, P-hydroxy-benzoic, Phosphoric and Sulfuric acid gave fairly visible spots. Fatty acids like Butyric, Caproic, Caprylic, Capric, Lauric, Oleic, and Stearic gave more diffuse spots with no separation. Only the Amino acids and sugars did not show up. The acids could be detected in crude extracts containing acids, amino acids, and sugars."

The differential analysis of Phosphate mixtures by use of paper chromatography and long wave ultraviolet is discussed by Karl-Kroupa.[36] In this work the ultraviolet light is used for irradiation and color development not for fluorescent or quenched spots.



Color development on chromatograms often requires ultraviolet radiation

CHROMATOGRAPHY IN THE FOOD AND AGRICULTURE INDUSTRIES

Chlorogenic acid in coffee and coffee substitutes was reported on in two different papers by Gnagy[37] who used the short wave and long wave CHROMATO-VUE portable dark room.

One hundred fourteen Organic Pesticides...

One hundred fourteen Chlorinated Organic Pesticides were studied by Mitchell[38] in a massive report with a view to their separation and identification. He later reported on two herbicides.[39] Mitchell recommended the use of a long wave black light to view all chromatograms in a dark room for fluorescence or quenched areas before and after application of the chromogenic agent. In addition, germicidal fixtures were used, similar to the BLAK-RAY XX-15 equipped with germicidal tubes, to expose the chromogenic agent. Morley and Chiba[40] have published on "Thin Layer Chromatography for Chlorinated Pesticide Residue Analysis Without Cleanup." In their work they used the MINERALIGHT UVS-54 without the filter for the development of the Chromogenic reagent.

Location methods for insecticides...

A review of location methods for insecticides is given by Ganshirt[41] including development by exposure, fluorescence and quenching. A new method for detection of hexachlorocyclohexane, sensitive to .02 μg, by impregnating plates with sodium fluorescein is described.

Five carbamate insecticides were studied by Eberle and Gunther.[42] Fluorescence quenching and iodine vapor was used to determine the lower limits of detection of four of the carbamates on thin layer chromatograms. The fifth carbamate was made visible by forming a rhodamine dyestuff using ultraviolet exposure and ammonia vapor.

In addition, the same paper reports on photodecomposition of five carbamates and their enols. 254 mμ radiation from a CHROMATO-VUE Cabinet was utilized.

The chromatographic evaluation of Vanilla extracts, was reported on in a paper by Jorysch[43] who pointed out that some of the materials fluoresce when the chromatograms are exposed to ultraviolet light and the patterns that are observed are distinctive enough to furnish identification. Along with the paper are presented pictures of fluorescent chromatograms. In this particular application long wave ultraviolet light at 365 mμ is ordinarily used. The B-100A BLAK-RAY lamp or the CHROMATO-VUE viewing cabinet is recommended.

Stahl[44] et al used two dimensional chromatograms with ultraviolet to define pure and adulterated vanilla extracts. A series of colored pictures graphically demonstrates results.

Mitchell[45] has also reported on a chromatographic procedure for the separation and identification of Coumarin, Dihydrocoumarin and Methylcoumarin. In this work he used a long wave ultraviolet light similar to the UVL-56 BLAK-RAY Lamp. Bernhard[46] used short-wave ultraviolet and thin layer chromatography to observe eight coumarin analogues in lemon juice.

Information on a procedure for detecting and quantifying pollen Flavonoids by Fluorescence from paper for discrimination of allergenic pollens has been discussed by Inglett, Miller and Lodge.[47]

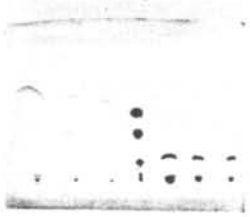
"TLC plates of A vitamins can best be evaluated by inspection with ultraviolet light" according to Bolliger.[48] Several Vitamin A derivatives fluoresce yellow-green at 365 mμ. With radiation at 254 mμ absorbing spots are seen if a fluorescent additive is contained in the absorbent. Under the same conditions 254 mμ radiation shows D vitamins as absorbent spots. Tocopherol (Vitamin E) has the same reaction although a sodium fluorescein treatment gives more sensitive detection.

K vitamins are also detectable through quenching of fluorescent background. The spots fluoresce yellow after an exposure of 10 minutes to a"quartz lamp."

Water soluble vitamins of the B group and C may be located with ultraviolet radiation. Riboflavin has a yellow fluorescence with long wave ultraviolet, sensitive to .01 μg. Vitamins B_1, C, and nicotinamide give absorbing spots with 254 mμ radiation on a fluorescent background. B_1 is also fluorescent violet in U.V. (254 mμ). However, potassium ferricyanide reagent develops a bright blue fluorescence in long wave (365 mμ), sensitive to .03 μg. Vitamin B_6 fluoresces dark blue with long or short wave ultraviolet.

A study of the bacterial degradation products of Riboflavin with quenching of background fluorescence was studied by Miles, Symrniotis and Stadtman.[49]

Stanley[50] in his article "Chromatostrips Show Citrus Flavor Relations" makes an interesting statement about the value of the paper chromatogram. "A valuable feature of the exposed surface is that the strip can be viewed directly under ultraviolet light for locating fluorescent compounds. If fluorescent mineral phosphors are incorporated in the coating, compounds absorbing light in the excitation region for the phosphors (around 260 mμ) can be located under a short wave ultraviolet lamp. Compounds exerting this quenching effect appear as purple spots on a yellow background." Stanley further mentions a method for detecting adulteration of flavorings as follows: "The Grapefruit product, 7-Hydroxycoumarin fluoresced an intense pale blue under ultraviolet illumination whereas the lemon oil product 5-Hydroxy-7-Methyoxycoumarin, exhibited no fluorescence. In the method of analysis the oil sample is spotted on a chromatostrip, developed with a suitable solvent, the selected spot (in this instance the fastest moving spot that is fluorescent under ultraviolet light) is cut out, taken into dilute hydrochloric acid, warmed, extracted into chloroform, the chloroform removed by evaporation and the residue taken up in alkaline 75% alcohol. If grapefruit oil was originally present the final solution will fluoresce in ultraviolet light. At little as 1% grapefruit oil is detectable by visual comparison."



Short wave (254mμ) is often absorbed to give dark spots

Essential oils have been characterized by Reitsema[51] in their response to ultraviolet and other location methods. Thin layer chromatography was used in establishing the extensive list given.

In discussing visualization of Pyrone derivatives Stahl[52] states, "It is essential before applying spray reagents, to examine the chromatogram in long- and short-wave U.V.-light —." He gives a table of several derivatives indicating fluorescence reactions before and after treating with alkali.

It is essential to examine chromatograms in long- and short-wave U.V. ...

Carcinogenic aflatoxins in various food products have been studied in TLC plates. Pons and Goldblatt[53] published on the determination of Aflatoxins in cottonseed products. Robertson et al[54] published an assay method for Aflatoxins in peanuts. The Southern Utilization Research and Development Division of the Agricultural Research Service in New Orleans has mimeographed a procedure for determination of Aflatoxins in agricultural products.[55] These substances fluoresce a dim blue under long wave ultraviolet at 365 mμ. The ARS procedure recommends the C-6 CHROMATO-VUE and BLAK-RAY C-50 TRANSILLUMINATOR with Contrast Filter for best visualization of the dim fluorescence.

Ultraviolet light valuable as physical location reagent...

CHROMATOGRAPHIC METHODS

Several helpful methods in observing Chromatograms have been mentioned in the literature or have been developed to clarify observations.

Smith[56] has several references to the use of ultraviolet in Chromatography. In discussing long wave ultraviolet which he calls "Wood's" light he states "attention is again drawn to the value of ultraviolet light as a physical location reagent, and to its use before the application of chemical location agents, between the application of reagents in a multiple dipping sequence, and after the final application of such chemical reagents."

In an article entitled "Thin Layer Chromatography, Recent Developments in Equipment and Application," Wollish, Schmall, and Hawrylyshyn[57] mention as follows: "for the visualization of compounds that do not react to chemical spray reagents, Kirchner's procedure of incorporating fluorescent compounds such as Zinc-Cadmium Sulfide and Zinc, Silicate, originally proposed by Sease, has proved very useful. In such cases the compound will appear as a dark absorbent spot on a fluorescent background under short wave ultraviolet light.

"Fluorescent plates can also be prepared by using a .04% solution of Sodium-Fluorescein instead of water. After inspection of the developed plates, the fluorescence can be extinguished by exposure to Bromine vapor, which will cause certain unsaturated compounds, particularly those with Ethylenic-type double bonds, to fluoresce under ultraviolet. Other useful fluorescent sprays are 5% Quinine Sulfate in alcohol and 1% Sulfosalicylic acid in acetone." At the present time several manufacturers include fluorescent phosphors, activated by 254 mμ ultraviolet, to their adsorbent. The phosphors have become more sophisticated with better color and finer grain. A number of manufacturers make prepared TLC plates with and without the phosphor in the adsorbent.

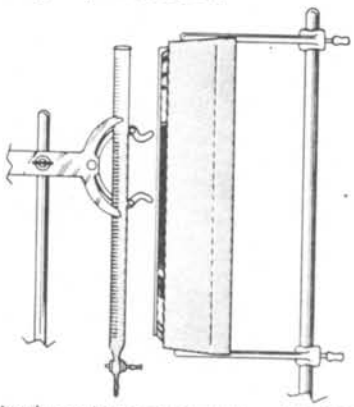
Best to view paper with ultraviolet lamp...

Smith[58] has also stated, "Unquestionably the best method of locating Purine and Pyrimidine derivatives on paper chromatograms or Electrophoretic strips is to view the paper under an ultraviolet lamp which has a high emission in the wave length range 250-280 mμ and in which visible light is filtered out. Under such illumination Purine and Pyrimidine derivatives generally appear as dark spots against a rather light faint blue fluorescence of the paper. A notable exception to this rule is that after chromatography in an acid solvent for exposure to the fumes of hydrochloric acid, Guanine and Xanthine and their compounds fluoresce quite strongly. They are, therefore, easy to distinguish from other naturally occuring Purine and Pyrimidine derivatives."

COLUMN CHROMATOGRAPHY

The field of column chromatography has in recent years become much more sophisticated but it is often advisable to use an ultraviolet light source to follow the flow of certain fluorescent compounds. A 365 mμ BLAK-RAY lamp may be used with any ordinary column but in the case of a compound which reacts only with the 254 mμ wave lengths, it will be necessary to use either quartz or vycor glass as the shorter wave lengths will not penetrate ordinary glass. In the petroleum industry, column chromatography has been most importantly used for the determination and separation of Aliphatic and Aromatic Hydrocarbons in petroleum crude oils. While most researchers are more familiar with column chromatography in the organic processes there are a number of applications with this method in the inorganic area. Smith[58a] has written a very good book on this subject complete with detailed procedure.

In connection with organic column chromatography, it is interesting to note a paper by Eichholz[59] on the fluorescent control of ion exchange. The introduction to this paper is as follows: "Changes in fluorescence associated with the loading of an ion exchange resin indicate that measurement of fluorescence can lead to a practical method of control for ion exchange processes. This is the preliminary conclusion drawn from experiments conducted by the Mines Branch, Department of Mines and Technical Surveys, Ottawa, Canada. In studies that are taken so far, changes in fluorescence were sufficiently great in some cases to indicate that resin elution has been completed, and later, after loading was started, that the resin had reached the condition of maximum loading." Flint and Eichholz[60] also wrote a paper on the same subject at a later date. In both cases a PEN-RAY lamp was used as the ultraviolet source to illuminate the samples so the fluorescent emission could be measured by a phototube.



Laboratory stand may be used to hold long BLAK-RAY lamps in column chromatography

ELECTROCHROMATOGRAPHY

For electrophoresis and electrochromatography, sometimes called curtain electro-phoresis, the ultraviolet light has been found to be of great value also. Karler[61] has stated that one should use the long wave ultraviolet lamp to follow blue fluorescent Albumin bands in blood serum or plasma fractionation. Further that the betalipoprotein, when too faint to be seen in ordinary light, can be seen as a blue fluorescence under 365 mμ radiation. Further, he has remarked that one should always use both long wave and short wave ultraviolet sources in running electrophoretic strips in curtains to trace the progress. Indicators are commonly found in most complex substances as urines and other untreated biological or clinical fluids.

Once the curtain is dried it should be subjected to careful scrutiny with both types of ultraviolet sources in total darkness. Fluorescence is often more apparent in the dried paper over fluorescence in the wet state. Karler[62] has found that there are few preparations except extremely pure compounds, which do not in themselves have one or more compounds which fluoresce or absorb in the ultraviolet or at least modify the slight background fluorescence of the paper.

Many qualitative analyses... require ultraviolet...

SPOT TEST ANALYSIS

Feigl[63] in his excellent book on "Spot Tests" gives a number of qualitative analyses which require ultraviolet sources. He makes an astute observation that is applicable to all types of fluorescent analyses. "It is essential that fluorescent compounds be formed or quenched by the reaction of non-fluorescent compounds or that a characteristic change in fluorescence be seen." He uses this principle throughout his book wherever fluorescence is mentioned. Separate tests for Beryllium and Aluminum are given that are accurate and non-interfering; the reactions depending upon Morin. Since the time of Feigl's test for Beryllium a more sensitive test has been developed by the Bureau of Mines,[64] using Quinizarin and long wave or short wave ultraviolet. Feigl[65] also has a test for Ammonia and one for Salicylaldehyde or Hydrazine[66] utilizing ultraviolet at 254 mμ.

Among other fluorescence tests in Feigl's volumes are a test for O-Hydroxyaldehydes[67] by the formation of Aldazines, for Glycerol[68] by the formation of 8-Hydroxyquinoline, for Citric acid[69] by conversion into Ammonium Citrazinate, and for 8-Hydroxyquinoline.[70]

TITRATION

In the clinical laboratory the titration of various compounds has been aided greatly by using a fluorescent indicator as an end point for extremely precise work. There are a great number of possible indicators that could be used but the following list gives a number of the more useful indicators:

INDICATOR	FLUORESCENCE CHANGE	pH RANGE
Acridine	green to violet	4.9 - 5.1
Benzoflavin	yellow to green	0.3 - 1.7
Dichlorofluorescein	colorless to green	4.0 - 6.0
Beta-Naphthol	colorless to blue	6 - 8
Resorufin	yellow to orange	4.4 - 6.4
Salicylic Acid	colorless to dark blue	2.5 - 3.5
Thioflavin	colorless to greenish	6.5 - 7.6

A great deal of work has been done on the micro-titration of Calcium in blood serum. Ashby and Roberts[71] reported on a micro determination of Calcium in blood serum in 1957, while Diehl and Ellingboe[72] reported on an indicator for titration of Calcium in the presence of Magnesium in 1956. Beckman Instruments[73] published a procedure book on an ultramicro analytical system in 1960. Klass[74] refined the method of the use of Calcein indicator for serum Calcium utilizing the 365 mμ radiation from an Ultra-Violet Products Inc. "Fluorescence Analysis Cabinet." Unfortunately, the "Fluorescence Analysis Cabinet," used by Klass, had insufficient ultraviolet energy for the best end point determination. Since that time others have utilized ultraviolet hand-held lamps and, even in darkened rooms, have not achieved great success for accurate end points. Finally, it was discovered that the BLAK-RAY B-100A was extremely successful in determining end points and that it could be used by average technicians for titration in the *fully lighted laboratory*. The BLAK-RAY B-100A lamp has made the Calcium titration much more accurate as well as faster and easier.

BLAK-RAY B-100A was successful in determining end points...

Powerful B-100A BLAK-RAY Lamp desirable for fluorescence titration.

In the titration of other metals, Dolezal, Patrovsky, Sulcek and Svasta[75] published data on the analytical chemistry of Gallium in 1959. Since that time a great deal of work has been done on chelometric titration with metalfluorechromic indicators. Several papers by Wilkins,[76] and by Hibbs and Wilkins,[77] describe the use of the metalfluorechromic indicators. Fisher Scientific Co.[78] has published a technical data sheet on the method. The method for titrating metals is very good and procedures are outlined for the determination of aluminum, chromium, cobalt, copper, iron, nickel, titanium, and zinc. Excellent results can be obtained with a B-100A BLAK-RAY fixture, which is powerful enough to titrate in *standard laboratory room illumination.* It is obvious intensity is very important when one considers that many titrators have only two 4 watt fluorescent tubes with an intensity of approximately 150 microwatts per square centimeter at 18 inches, while at the same distance the B-100A's intensity is approximately 8,000 microwatts per square centimeter.

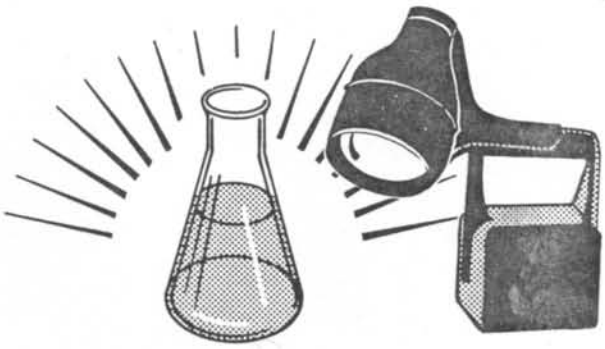
Porphyrins... fluoresce a pink to reddish color in urine...

OTHER CLINICAL APPLICATIONS

In addition to chromatography and titration methods mentioned above which may be used in the clinical laboratory, there are several applications that are clinical or semi-diagnostic in nature. Tetracycline is being used for investigation of gastric-carcinoma,[79] as this drug appears to be specific for certain cancer cells causing them to fluoresce under ultraviolet at 365 mμ. Other tests such as the viability of organs and skin[80] through the injection of fluorescent compounds are widely known in medical circles. Such applications as these are not necessarily done in the laboratory but may be occasionally performed there under the supervision of medical personnel.

In the clinical laboratory there has been an increasing interest in the study of Porphyrins excreted in the urine, and a mass endeavor towards locating victims of Porphyria is now being undertaken. These compounds fluoresce a pink to reddish color in the urine.[81] A B-100A BLAK-RAY lamp is specific for this analysis.

A new method of diagnosis of Lead Poisoning by the fluorescence of the erythrocytes has been discussed by Schwartz and Wikoff.[82] Schwartz has prepared a table indicating erythrocyte Protoporphyrin levels observed in various diseases. In cases of lead poisoning the Protoporphyrin increases markedly, i.e. 2000 micrograms of Protoporphyrin per 100 ml. of cells. The normal quantity is 20 to 50 micrograms. It is possible, however, that iron deficiencies may increase the Protoporphyrin level to approximately 200 to 800 micrograms per 100 ml. of cells. As Protoporphyrin is fluorescent, a microscopic examination of the cells is an excellent aid to the diagnosis of lead poisoning.



Urine Porphyrin visualization requires B-100A BLAK-RAY lamp

Ultraviolet microscopy has been widely used with fluorescent tagged antibody tests for certain specific diseases. However, ultraviolet substage illuminators are expensive, and sometimes difficult to operate. At the present time, there is a method being developed for macroscopic antibody determination.[83] These spot tests are accomplished on ordinary glass microscope slides and the mixture of the tagged antibody with the serum will develop fluorescence under long wave ultraviolet if there is a positive reaction. The instrument used is the UVL-56 BLAK-RAY lamp. The slide may be held edgewise against the face of the lamp filter in order to see the greenish fluorescence. So far the tests have been developed for Lupus and Syphilis and there has been some discussion about the difficulty of seeing the low brightness fluorescence. Ultra-violet Products, Inc. has developed a special Contrast Filter[84] which allows the fluorescence to be discerned much more easily. The glass slide with the spots on it is put directly on the face of the filter of the UVL-56 BLAK-RAY lamp and the observer looks through the hand held Contrast Filter at the slide in a darkened area. In this manner, all background interfering radiation

New contrast filter sharpens fluorescence viewing

is eliminated and only the fluorescence is seen. The Contrast Filter is regularly supplied with CHROMATO-VUE accessory TRANSILLUMINATORS to eliminate background and promote clear vision of very dim fluorescent substances.

WIDE FIELD ULTRAVIOLET MICROSCOPY[85]

A unique method of microscopic examination has been developed through the use of PEN-RAY® lamps, specifically adapted to wide field stereoscopic microscopes. The lamps are used in pairs operated from one power supply and fitted with 254 mμ filter assemblies; then placed on each side of the object to be examined. While the PEN-RAY lamps are not considered powerful lamps, nevertheless, their proximity to the subject being examined produces a very high intensity of short wave ultraviolet, difficult to achieve in any other manner.

It is also possible to use this equipment with the biological microscope if the distance from the microscope objective to the object being examined is not too small. When using the short wave PEN-RAY lamps above the stage with a biological microscope it will be necessary to use a quartz cover slip.

The PEN-RAY microscopy system may also be used for 365 mμ illumination by substituting 365 mμ filter assemblies for the 254 mμ filter assemblies. While there are powerful sources of this radiation available on the market for sub-stage illumination, the principle again holds that the closer the source to the object the higher the brightness of fluorescence of that object.

The short wave PEN-RAY lamps are frequently used for the study of minerals, phosphors and inorganic chemicals, while the long wave is frequently used for biological and organic compounds. By using air sampling devices it is quite frequently possible to study air pollution from factory exhausts and through the use of airborne synthetic phosphors to make motion studies. The equipment lends itself to organic and inorganic impurity studies as well as structural variations in composition. It is thus possible to study alteration of minerals, such as Pitchblende, changing to its fluorescent alteration product.

Photochemical cross-linking and mutation ...

PHOTOCHEMISTRY

Photochemical reactions are often initiated by ultraviolet radiation. These reactions may be constructive or destructive and consist of such broad categories as Halogenation of Hydrocarbons, Polymerization, and Decomposition. Various types of equipment are available for this application, among them a recently announced immersible PCQ quartz lamp with 24/40 ground joint stopper for use in reaction flasks.[86] Emission from this equipment peaks at 254 mμ. Also available for photochemical activation are a series of grid lamps, emitting at 254 or 365 mμ, of various sizes plus coiled and flow-through models.

One of the methods that has been used is a conversion of 7-Dehydrocholestrol to Vitamin D.* Also the manufacture of Cyclohexane Oxime in the production of nylon.

Some very interesting work has been done by Ponnamperuma[87] where he was able to synthesize the ATP molecule (Adenosine Tri-Phosphate) from inorganic materials. A PEN-RAY lamps was used as part of the energy in this reaction.

PCQ-9G-1 Photochemical lamp may be immersed in solutions

Bencze, Burckhardt and Yost[88] have reported on a photochemical preparation, rearrangement, and dehydration of symmetrical methyl and phenyl Pyridyl Glycols. A standard PEN-RAY lamp was used in these studies.

Photochemical reactions may also be used for cross linking Polymers such as certain plastics, for life testing products through radiation and for mutating yeasts, bacteria, pollens, and other cells. In the latter connection, Friedman and Ceponis[89] reported on the effect of ultraviolet light on pectolytic enzyme production and pathogenicity of Pseudomonas. They were able to determine loss of pathogenicity of the soft rot bacterium, Pseudomonas marginalis. Germicidal lamps fitted in a fixture such as the BLAK-RAY XX-15 were used. Azuma, Newton and Witter[90] have reported on the production of psychrophilic mutants from mesophilic bacteria by ultraviolet irradiation. They also used germicidal fixtures of wave length 254 mμ. Lunden and Wallace[91] in their report on some effects of ultraviolet light on barley and oat embryos state in part, "Ultraviolet light, a non-ionizing radiation, appears to have certain advantages as a mutagen because the induced genetic changes with it are accompanied by fewer radical derangements in the genetic material."

The production of ozone may be considered a photochemical reaction. This is easily accomplished with low pressure quartz lamps. It is the 185 mμ region, emitted from low pressure quartz lamps, which accomplishes the

production of ozone from oxygen. The PCQ-9G-1 Photochemical Quartz Lamp is especially efficient in the production of ozone. This equipment may be inserted into a three hole reaction flask and oxygen may be introduced into one side and a rich mixture of ozone pulled out of the other side. This method gives the highest proportion of ozone. Air may be irradiated also if a lower concentration is desired. PCQ continuous flow lamps are also excellent for ozone production, especially with oxygen input. (See Ultra-Violet Products Inc. data sheet No. 125 on Photochemical lamps.)

STERILIZATION

Sterilization in the biological laboratory is often of great importance. Bacteriological handling hoods where pathogenic bacteria are handled may require sterilization lamps mounted inside the hood. BGN-18 STERIL-AIRE® lamps are helpful in reducing the bacteria count.

Room sterilization is also desirable, especially in bacteriological laboratories. In this case it is recommended that high intensity ultraviolet radiation be used with a number of BGN-18 or BGN-36 STERILAIRE lamps mounted around the room. Automatic switching equipment should be placed on the entry door so that the lamps will be on, only when no personnel is in the laboratory.

Sterilization of small quantities of liquid materials may be rapidly accomplished with the PCQ-9G-1 photochemical immersion lamp in a reaction flask. Sterilization may often be accomplished in only a few minutes. Surface irradiation is performed with the powerful grid type PCQ lamps.

Incipient contamination may be detected...

CONTAMINATION

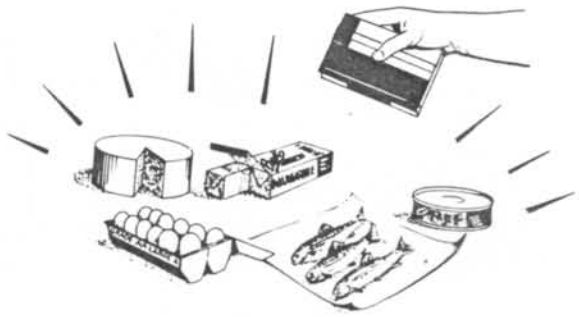
Contamination may be of various types which will require analysis in the laboratory either as to the nature of the contaminant or the quantity present. Many of the contamination problems are in relation to food and include such subjects as the age and quality of olive oils, mixtures of foreign fats with butter and analysis of fat content of margarine. Incipient contamination may also be detected as in the case of the rot forming bacteria Pseudomonas aeruginosa which causes rot in eggs, meat and fish. As this particular bacterium develops a fluorescent compound, fluorescin, as a product of its metabolism, the presence of the bacteria may be easily determined by its brilliant yellow-green fluorescence under 365 mμ ultraviolet radiation. Mold formation on many foods such as cheese, may also be determined prior to heavy growth of the mold.

Rodent contamination is one of the oldest known uses of ultraviolet in the public health field. The urine of the rodent is highly fluorescent and is easily seen on bags containing flour, grain, beans, and other foods. Rodent excreta in flour may also be determined by the non-fluorescence of the excreta against the fluorescent background of the flour.

Many, many other applications of fluorescence are used in the food industry such as the determination of borers in shelled pecans and the laboratory identification of bacterial ring-rot in potato tubers[92] or in the dairy industry where cleanliness is a considerable problem, due to the deposition of fluorescent "milk stone" in holding tanks and pipelines. The laboratories of milk organizations are often called upon to test for normally invisible milk stone in order to prevent the build-up of high bacteria count in the milk.

Mercury detected in small quantities...

Other than in the food industry, contamination may be a problem in many areas where one substance is accidentally mixed with another substance causing impurity. One of the problems is in submarines and in many industrial areas for the detection of mercury which can be accomplished easily by 254 mμ radiation. Mercury vapor may be detected in small quantities by use of a fluorescent screen which fluoresces with 254 mμ radiation but exhibits a dark shadow on the screen due to ultraviolet absorption by mercury. Automatic equipment may be easily made with a short-wave sensitive photo-tube which, in the presence of mercury, will interrupt radiation from a PEN-RAY lamp and sound an alarm.



Portable lamps for contamination inspection in laboratory, plant, or field

TRACER TECHNOLOGY

Tagging with tracers and additives of a fluorescent nature has become a familiar application. The effluent from factory smokestacks are frequently checked by public health officials. The motion of air may be studied by fluorescent particles of light weight in the air stream, while virtually any liquid may be marked so that motion may be followed.

Tracers may be, and often are, invisible to the unaided eye unless an ultraviolet source is used. Hundreds of special purpose dyes, phosphors and other formulated materials are available for specific laboratory tracing techniques.

MINERAL IDENTIFICATION

Ultraviolet light sources, particularly the 254 mμ MINERALIGHT type of equipment, have long been used for mineral identification in the field and laboratory. A large number of minerals respond to this wavelength and some to the 365 mμ radiation. Many of the minerals have a specific response or perhaps different response to each of the wave lengths. Gleason,[93] in his book, "An Ultraviolet Guide to Minerals," gives a great many helpful aids in fluorescent mineral identification.

A geological laboratory will often find fluorescent non-economic minerals valuable for tracing of ores in faulted off veins. Many minerals, such as Calcite, are fluorescent and in the vein structure associated with ore. Offsets by faulting can often be traced whether through macroscopic or microscopic examinations of the ore materials.

In this same connection, petroleum laboratories require the use of the 365 mμ BLAK-RAY type of equipment in order to determine when their drilling operation is entering oil bearing zones. All of the crude oils fluoresce and the cuttings or cores are consistently examined with long wave ultraviolet light.

PEN-RAY LAMP standard for calibration...

WAVE LENGTH CALIBRATION

In the laboratory it is frequently necessary to calibrate various types of spectro-photometric equipment. The PEN-RAY lamp has been widely used for this purpose. Due to the fact that it has sharp, well separated lines in both the visible and ultraviolet regions this lamp has become a standard for calibration purposes. It may also be used to calibrate fluorometers and flame photometers, as well as other photometric devices.

Of recent development are a series of rare gas lamps, which are used to calibrate spectrograms through the use of specific lines given off by the rare gases. These gases may be either Helium, Krypton, Neon, Argon or Xenon and are of the standard PEN-RAY Style. (See Ultra-Violet Products, Inc. PEN-RAY Data Sheet No. 100.)

PEN-RAY LAMP source of monochromatic radiation...

OPTICAL APPLICATIONS

The PEN-RAY lamp through the means of its discrete spectral lines, as mentioned above, is particularly adaptable as a source of monochromatic radiation for the optical laboratory. Visible wave lengths at 546 and 436 mμ are easily obtained through the use of simple filters or monochromators may be used for these and other wave lengths. The small PEN-RAY lamp is widely used as a source of monochromatic radiation for the alignment of optical instruments and as a light source for a number of various optical instruments. These may consist of collimators and auto-collimators, plus interferometers and similar instruments.

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ULTRA-VIOLET PRODUCTS, INC.

UVP, Inc. Upland, CA / (800) 452-6788 / (909) 946-3197 / uvp@uvp.com
Ultra-Violet Products Ltd, Cambridge UK / +44(0)1223-420022 / uvp@uvp.co.uk

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