SPECTROPHOTOMETER

1.0.0 INRODUCTION

Spectrophotometry is a process where we measured absorption and transmittance of monochromatic light in terms of ratio or a function of the ratio, of the radiant power of the two beams as a functional of spectral wave length. These two beams may be separated in time, space or both

1.1.0 The basic introduction of electromagnetic spectrum

The absorption and the emission of energy in the electromagnetic spectrum occur in discrete packets or photons. The various regions in the electromagnetic spectrum are shown in figure-1 along with the nature of the changes brought about by the radiation

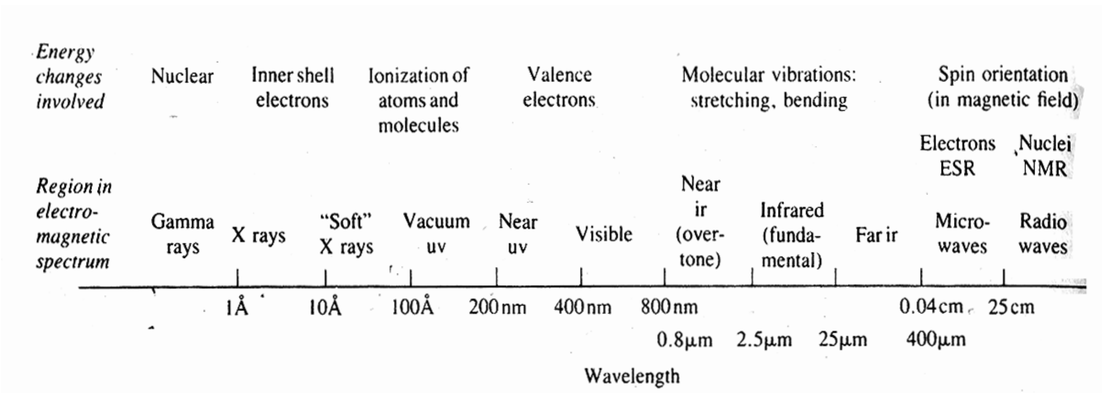


Figure-1
Schematic diagram of electromagnetic spectrum. Wavelength scale is nonlinear.

WAVELENGTH REGION, mμ	TRANSMITTED COLOR	COMPLEMENTARY HUE
<380	Ultraviolet	
380–435	Violet	Yellowish green
435–480	Blue	Yellow
480–490	Greenish blue	Orange
490–500	Bluish green	Red
500–560	Green	Purple
560–580	Yellowish green	Violet
580–595	Yellow	Blue
595–650	Orange	Greenish blue
650–780	Red	Bluish green
>780	Near infrared	

Figure-2
Relation between absorption of light and color

Figure-2 shows an enlargement of the visible region, with the transmitted colors that corresponds to various wavelengths. The ultraviolet region extends from 185nm to the visible; shorter wavelength is considered to be the fair (or vacuum) ultraviolet region. The extreme ultraviolet overlaps the soft X-ray portion the spectrum.

1.2.0 THEORY

It is possible for a ray of light to be absorbed by some material and simply pass through others without being affected. When a molecule absorbs light, energy is transferred from the ray of light to the molecule. If the frequency of the electronic and magnetic fields of a ray of light match the frequency at which molecules will vibrate, then light will be absorbed, if the frequency does not match, then the light will pass straight through unaltered. Inert molecules whether solid or liquid appear colored due to the way they modify light illuminating the object. Thus different objects absorb some wavelengths and reflect others. For example, if a white light passes through a yellow solution, it absorbs all colors except yellow.

1.2.1 BEER'S LAW

The light transmitted through a solution changes as an inverse logarithmic relationship to the sample concentration.

Transmission $T = (It / Io)$

Optical density $O.D = \log (Io / It)$

Where It = Intensity of light passing through the sample
Io = Intensity of light falling on the sample

Absorbance $A = a\ b\ c$

Where a = absorbity constant of the specimen
b = light path length
c = concentration

If the percentage transmission is converted to optical density, the relation of optical density to concentration is shown below:

Concentration	% T	OD
0	100.0	0.0
1	50.0	0.3
2	25.0	0.6
3	12.5	0.9

1.3.0 DEFINATION OF VISIBLE SPECTROPHOTOMETER

The visible spectrophotometer is single – beam ratio-indicating instrument with a wavelength range of 340-600nm (Nanometer). The basic wavelength range may be converted to 340-950 nm by using accessory phototube and filters.

1.3.1 MAIN PARTS

Visible spectrophotometer essentially consists of following basic components as shown in figure-3

1.3.2 Radiation Source (Light)

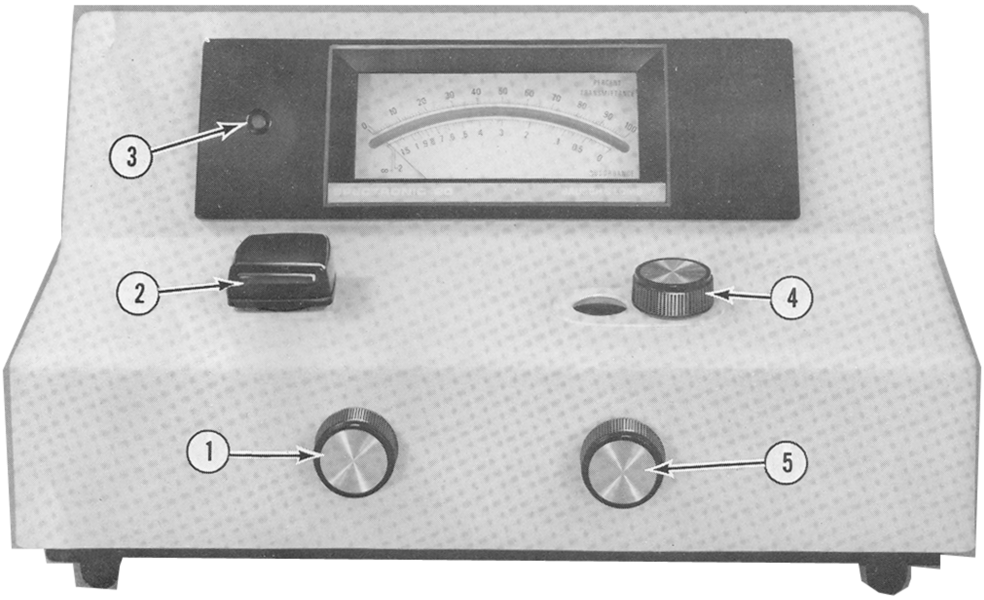
1.3.3 Optical System (Monochromatic)

1.3.4 Sample Section

1.3.5 Detector

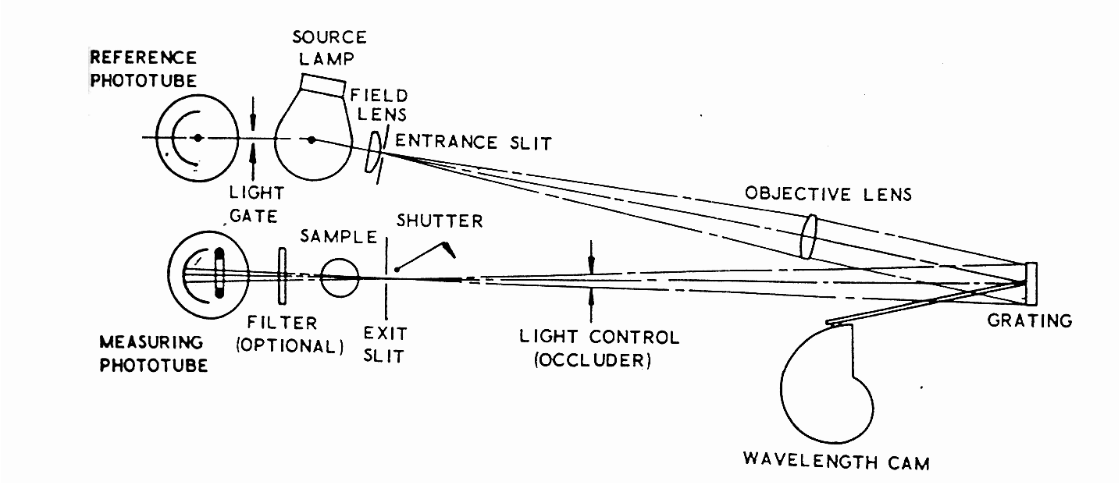
1.3.6 Filters

1.3.7 Read Out



1. Power Switch / Zero Control	3. Pilot Lamp
2. Sample Holder	4. Wavelength

Figure-3(a) view of instrument



(b) optical diagram of Bausch & Lomb – 20 Colorimeter

1.4.0 RADIATION SOURCE

1.4.1 TUNGSTEN LAMP

Most of the spectrometers use a tungsten lamp as the radiation source. These lamps provide a bright low-cost, broad band source of visible radiation. The figure-4 shows the advantage of continues output throughout the visible spectrum. For these lamps output decreases towards the ultra-violet, but there is more than sufficient output throughout the visible spectrum to energize the photo detector

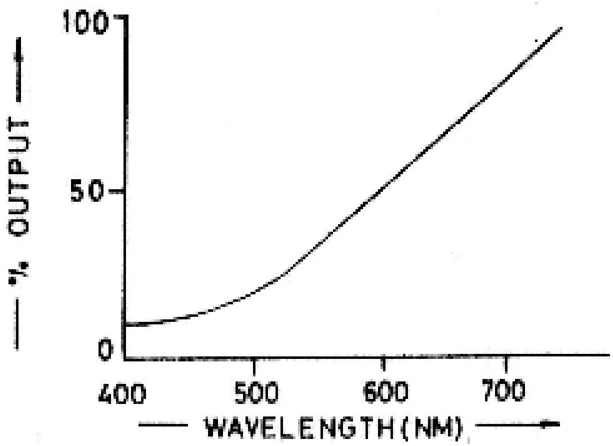


Figure-4
% output vs .wavelength curve in visible range of tungsten lamp

Now a day's many manufacturers use Tungsten - Halogen lamps ; are a special class with iodine added to normal filling gases. The envelope is fabricated of Quartz to tolerate a higher lamp operating temperature. These lamps maintain over 90% of their initial light output throughout life.

1.4.2 OPTICAL SYSTEM (Monochromator)

Much versatile optical instruments is designed around a monochromator, the primary function of a monochromator is to provide a beam of radiant energy of a given nominal wavelength and spectral bandwidth. A secondary function of the monochromator is the adjustment of the energy throughout.

A Monochromator generally consists of following components

1.4.3 Lenses ; are used to collect radiation from a source and direct it to the monochromator entrance slit

1.4.4 Entrance Slit ; provides a narrow optical images of the radiation Source

1.4.5 Collimator Lens ; renders the light spreading from the entrance slit Parallel.

1.4.6 Exit Slit ; to isolate the desired spectral band by blocking all of the dispersed radiation excepts that within a given resolution element.

1.4.7 Discursive Device ; a grating or prism can be used as a dispersive device for dispersing the incident radiation.

1.4.8 PRISMS ; The action of a prism depends upon the refraction of light by the prism material.Quartz or fused silica prism is mandatory for inclusion of the ultra-violet spect-rum below 350nm.Cornu-type mounting and Littrow-type mounting is shown in Fig-5

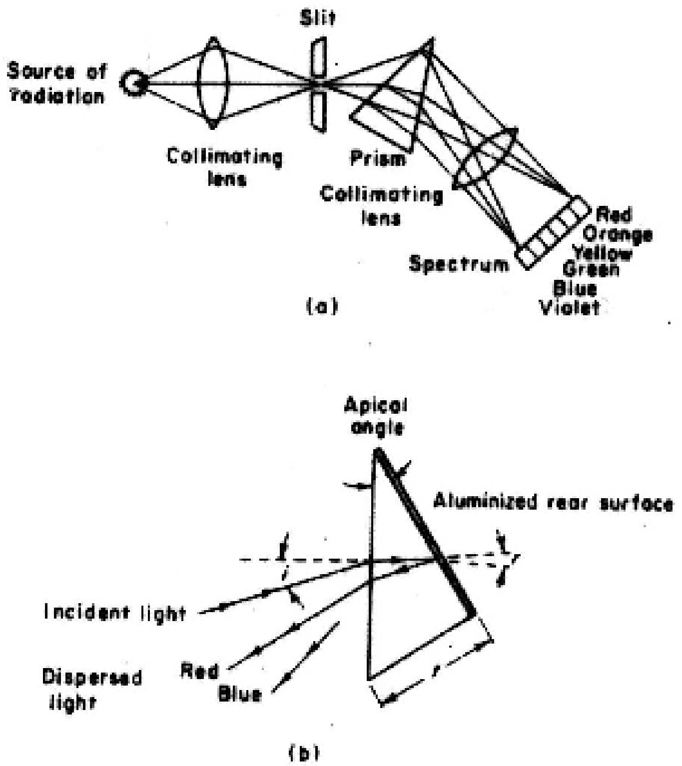


Figure-5
(a) cornue-type mounting (b) littrow-type mounting. i. is the angle of incidence, r is the angle of refraction, t is the base width of the prism,and optical angle is 30^0

1.4.9 GRATING ; Dispersal of light may also be obtained by means of a diffraction grating. In single beam spectrophotometers generally grating is used as a discursive device. A grating consists of a large number of parallel lines (actually grooves) ruled at extremely close intervals, perhaps 15000 or 30000 lines per inch,on a highly polished surface e.g. Aluminum. Ruling numbers from 20 grooves/mm in far infrared too as many as 3600 or more grooves/mm for the visible and ultraviolet regions. Grating offer better discrimination than prism,and and wave length calibration curve is linear. View of master grating and interference of rays from grating are shown in figure-6 and figure-7

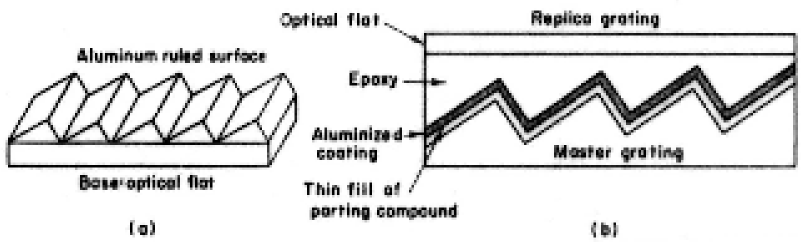


Figure-6
(a) view of master grating (b) cutaway view of steps in making a replica grating

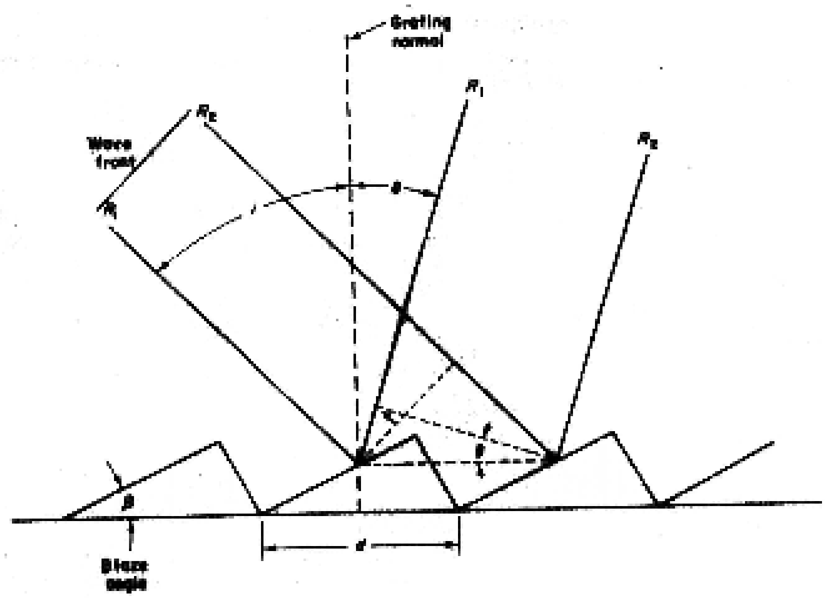


Figure-7
Interference of rays from successive grooves of a reflection grating 'i' is angle of incidence, 'r' is angle of reflectance, 'd' is grating constant.

1.4.10 SAMPLE SECTION ;

Provision is made for inserting the sample solution usually is located immediately in front of the detector and after the dispersing device. Cuvette (Glass tube) will vary from a set of test tubes, matched and tested for uniform thickness and optical path length. High quality rectangular corvettes, constructed of glass or quartz with uniformly clear and polished plane parallel faces. In sample holder section a provision is provided to block radiant energy or stray light through gravity control shutter in front of detector. This shutter also helps for dark current or zero adjustment setting.

1.4.11 DETECTOR;

The following light detectors are used in spectrophotometers ;

1.4.12 PHOTOEMISSIVE TUBE
1.4.13 PHOTODIODE
1.4.14 PHOTOMULTIPLIER TUBE

1.5.0 PHOTOEMMISIVE TUBES;

The typical single stage vacuum phototube contains a light sensitive cathode in the form of a half cylinder of metal, coated on its receiving surface with a light sensitive layer. When radiation strikes the photocathode, photoelectrons are ejected and are drawn to the positive anode, constituting the current. Maintaining the anode at about +90 Volt relative to the cathode collects all the electrons. Three types of photo emissive tubes are available in market with their wavelength range.

1.5.1	Blue - Sensitive Phototube	385 -600nm
1.5.2	Red - Sensitive Phototube	600-950nm
1.5.3	Broad Range Sensitive Phototube	380-950nm

Photo emissive tube and its accessory circuit is shown in figure-8

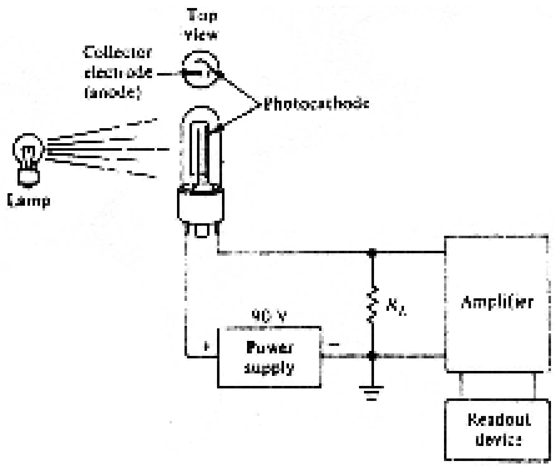
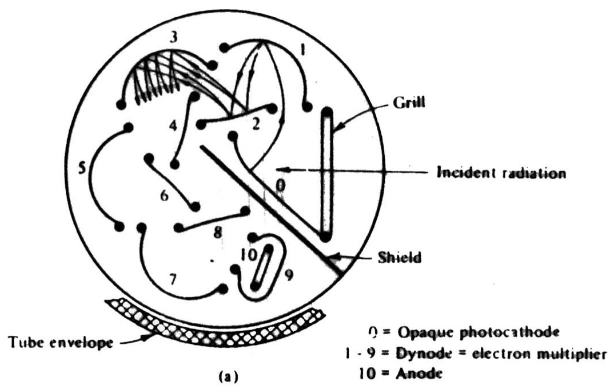


Figure-8

1.5.4 PHOTOMULTIPLIER TUBE;

Photomultiplier tube is a combination of a photo emissive cathode and an internal electron multiplying chain of dynodes. Incident radiation ejects photoelectrons from the cathode. The emitted photoelectrons are focused by an electrostatic field and accelerated towards the first dynode to successive dynodes by keeping potential difference of about 75-100 Volt exists between adjacent dynodes and finally impinges on the anode. The voltage between the final dynode and the anode is restricted to 50 volt or less. Diagram of Photomultiplier is shown in figure-9



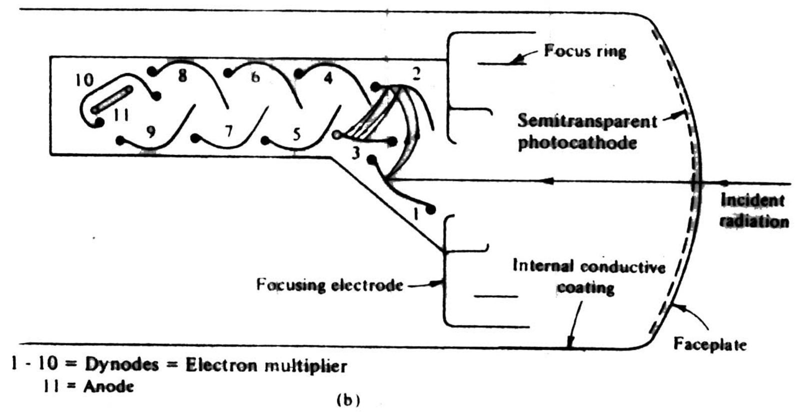


Figure-9
Diagram of a Photomultiplier tubes. Dashed lines are the paths traveled by the secondary electrons as they are focused by each succeeding dynode field in turn.

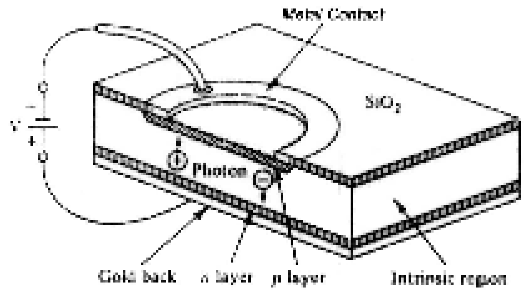


Figure-10

1.5.5 PHOTODIODES ;

Photodiodes operate on a completely different principle from the previous detectors. The process starts with a very high-resistivity active silicon material. Very shallow p and n diffusion are made in the top and bottom surface and the top surface is covered with a protective SiO_2 layer. A photon must reach the active area (intrinsic) to produce current flow in the external circuit. A p-n semiconductor junction is reverse biased so that no current flows when photons interact with diode, electrons are promoted to the conduction band where they can act as
change carriers. Thus the generated current is proportional to the incident light intensity. Figure-10 shows the construction of a planar-diffused p-n junction photodiode.

1.6.0 FILTERS:

The colorimeter is partly judged by the quality of the color filters or monochromatic used.

Filters are of two types:

1.6.1 Wide Band Filter.

Wide band filter, no matter how concentrated the solution is, some radiation will remain in it will give a non-linear calibration.

1.6.2 Narrow Band Filter.

With the narrow band filter, the optical density will be linear with respect to concentration. Therefore the bandwidth of the filter should be narrower then the absorption bandwidth of the solution.

Three types of filters are generally manufactured:

1.6.3 Glass Filters:

The glass filters consists of a solid sheet of glass that has been colored by a paint/dye which is either dissolved or dispersed in the glass, are wide band pass filters.

1.6.4 Gelatin Filters:

This type of filters consists of a dyed gelatin film sandwiched between glass sheets. Gelatin filters have a narrow band pass than glass filters.

1.6.5 Interference Filters:

This type has a narrow bandwidth then the above filters and may be obtained over a wide range of peak wavelength. This type consists of an evaporated coating of a transparent dielectric material of low refractive index between semitransparent silver films. Magnesium fluoride is commonly used a dielectric due to its hardness.
Interference filters can have any peak wavelength between 200-800nm and bandwidth between 7-40nm.

1.7.0 READ - OUT:

1.7.1 Analogue
1.7.2 Digital
1.7.3 Recorder

As be are concerned with the subject of spectrophotometer. Monochromator is generally used in single beam and double beam spectrophotometers.

1.8.0 MONOCHROMATOR:

It is the most accurate, selective and optically efficient way of defining the wavelength. Light from source illuminates a prism or diffraction grating, which diffracts the various wavelengths of white light at different angles. The dispersed beam is then refocused on a exit slit. Wavelength can be selected by rotating the prism or grating so that required wavelength pass through the exit slit. Diffraction grating offer better discrimination then prism, and wavelength calibration curve is linear.

1.9.0 Intensity Control:

There may be provision for intensity control of light through following provisions:

1.9.1 Variable slit between grating/prism and exit slit.
1.9.2 Variable electronic voltage control circuit for source lamp supply.

2.0.0 OPERATION:

It requires three basic solutions.

2.0.1 Blank solution (Distilled Water)

2.0.2 Standard/Reference solution.

2.0.3 Unknown solution.

2.1.0 Measurement :

Having prepared the solution, we first select the appropriate wavelength. Usually the operator and mode of measurement (Transmittance/Absorption or Concentration) will know this in case of digital read out system.
In analogue read out system, meter scale is calibrated reciprocal for transmittance and absorption.

2.1.1 Turn power switch ON allow five minutes (Warm up type instruments)
2.1.2 Turn the wavelength control to desired wavelength setting.
2.1.3 Adjust Zero control until read out indicate zero (There should be no test tube in the instrument and the sample holder cover must be closed.
2.1.4 Fill a test tube one-half full of Blank solution.
2.1.5 Insert test tube into sample holder and close cover (Align the etched mark on test tubes with line on sample holder).
2.1.6 Adjust 100 % transmittance or sensitivity control knob to read 100 or 000 in case of absorbance mode.
2.1.7 Fill another test tube half full of unknown sample liquid.

2.1.8 Remove the blank solution test tube and insert sample test tube and close the sample holders cover (Align the etched mark on test tube with line on sample holder).

2.1.9 Observe % transmittance or absorbance reading.

2.2.0 ACCURACY CHECK:

Two methods for wavelength accuracy check:

2.2.1 The Didymium Filter.

2.2.2 Stock Cobalt Solution.

2.2.3 Didymium Filter

2.2.4 Turn instrument ON

2.2.5 Sample holder empty, set wavelength scale to 485nm.

2.2.6 Set read to 0% transmittance.

2.2.7 Set 100% transmittance with clean distilled water.

2.2.8 Remove distilled water and place the didymium filter (In case if there is spacein sample holder for installing didymium filter then leave the distilled water tube in the sample holder) and record the % transmittance.

2.2.9 Repeat above steps until indications have been recorded for wavelength scale setting of 490,495,500 and 505nm.

2.2.9 The wave length scale is properly calibrated when the maximum % transmittance occurs between 492 and 502nm.

2.2.10 An additional check may be made by using wavelength scale setting of 575,580,585,590 and 595nm. The wavelength scale is properly calibrated when a minimum % transmittance occurs between 582 & 588nm.

2.3.0 Stock Cobalt Solution:

Preparation of stock cobalt solution

Into a 1-liter volumetric flask, place approximately 1000ml distilled water, add slowly and with caution, 10ml concentrated Hydrochloric Acid and mix it.

Place 22-23gm cobalt chloride and Dissolve it in the 1% hydrochloric acid.

2.3.1 Procedure:

2.3.2 Turn instrument ON.

2.3.3 Set wavelength scale to 500nm.

2.3.4 Set 0% transmittance.

2.3.5 Insert clean distilled water in a test tube and place into the sample holder. **2.3.6** Set 100% transmittance.

2.3.7 Replace the distilled water with a sample of Stock Cobalt Solution and record the % transmittance.

2.3.8 Repeat the steps above for wavelength scale setting of 505,510,515 and 520nm and record.

2.3.9 The wavelength scale is properly caliberated when the minimum % transmittance occurs between 505 & 515nm.

2.4.0 LINERITY CHECK:

The linearity of the read out system can be checked by setting wavelength scale between 420 to 520nm. After doing the previous steps and setting of 100% transmittance of any sample for known wavelength. Then dilute this sample accurately with an equal volume of clean distilled water and read the transmittance. The indication should be one half of the indication compare to 100% previous setting. Further dilution this recorded sample accurately with an equal volume of clean distilled water. The indication should be one fourth of the indication determined in first step i.e.(100% transmittance).

Note : Common causes of non-linearity are stray light or a defective photodetector.

2.5.0 PRECAUTIONS:

For getting accurate results and to prolong the life of spectrophotometer, the following steps may be observed.

2.5.1 Before putting the instrument into operation the mains power cord should be checked for proper connections. The instrument should be properly grounded. The mains voltage should also be checked.

2.5.2 Do not keep the instrument in a place where it is hot and humid.

2.5.3 Do not spill reagents or chemicals on the instruments. If spilling occurs, then wipe off the surface as soon as possible before it infiltrates inside the instrument.

2.5.4 Do not give violent jerks to the instrument in transit and avoid abrupt motion.

2.5.5 Do not touch the glass bulb of tungsten lamp with bare hands. Any finger points formed on envelope must be removed with alcohol before switching on the lamp.

If the lamp is switched on before cleaning with alcohol, then the finger point will form a black coating, which will be very difficult to remove.

2.5.6 No air bubble should be present in the solution especially, when the liquid is transferred from one test tube to another or otherwise, it will cause inaccuracy in the result.

2.5.7 Do not overload the meter. Ensure that the meter needle does not go beyond the full scale.

2.5.8 Avoid exposure to direct sun light.

2.5.9 For accurate measurements, avoid use of the instrument in the vicinity of powerful noise generator.

2.5.10 Strictly not permissible to touch with bare fingers or hard tools the surface of optical mirrors and grating/prism.

2.6.0 GENERAL SAFETY:

2.6.1 Be extremely careful if instrument is to be operated without cover.

2.6.2 Disconnect the power from instrument while removing, repairing or replacing components/PCB.

2.6.3 Allow enough time for discharge of capacitors after instrument is switched OFF for servicing.

2.6.4 Do not use steel wool, emery, sandpaper or files for cleaning of the instrument.

2.6.5 Use only properly rated fuses.

2.6.6 When servicing has been completed, thoroughly inspect the instrument for loose boards, loose cables, cold solder joints, loose hardware, wire bits, etc.

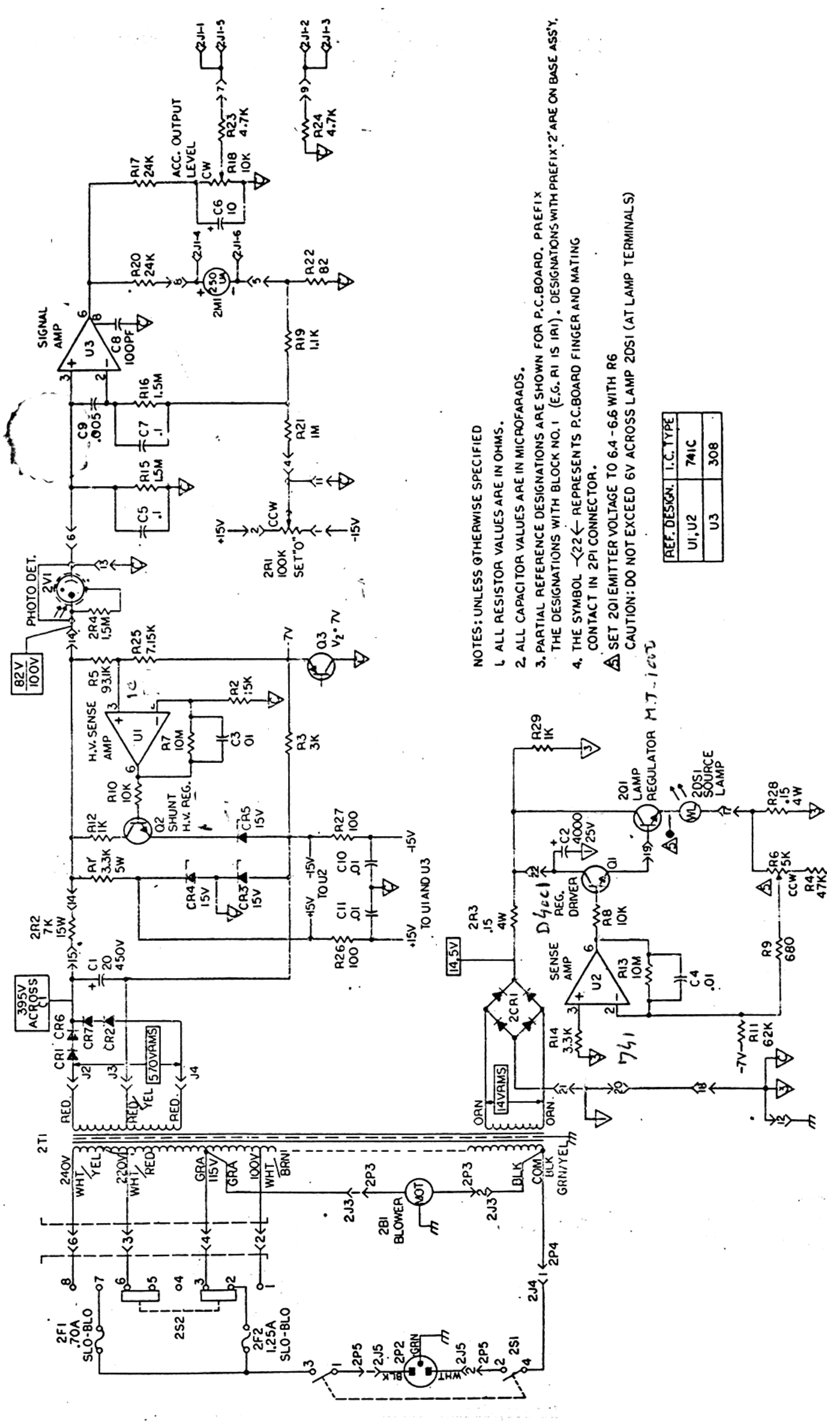

NOTES: UNLESS OTHERWISE SPECIFIED
1. ALL RESISTOR VALUES ARE IN OHMS.
2. ALL CAPACITOR VALUES ARE IN MICROFARADS.
3. PARTIAL REFERENCE DESIGNATIONS ARE SHOWN FOR P.C.BOARD. PREFIX THE DESIGNATIONS WITH BLOCK NO. 1 (E.G. R1 IS 1R1). DESIGNATIONS WITH PREFIX "2" ARE ON BASE ASS'Y.
4. THE SYMBOL ⤙22⟵ REPRESENTS P.C.BOARD FINGER AND MATING CONTACT IN 2P1 CONNECTOR.
5. SET 2Q1 EMITTER VOLTAGE TO 6.4 - 6.6 WITH R6
CAUTION: DO NOT EXCEED 6V ACROSS LAMP 2DS1 (AT LAMP TERMINALS)
REF. DESIGN. | I.C. TYPE
U1, U2 | 741C
U3 | 308
SIGNAL AMP
H.V. SENSE AMP
SHUNT H.V. REG.
PHOTO DET.
ACC. OUTPUT LEVEL
SENSE AMP
REG. DRIVER
2Q1 LAMP REGULATOR
2DS1 SOURCE LAMP
2B1 BLOWER
TO U1 AND U3
M.T. 1001

figure-11

3.0.0 TROUBLE SHOOTING CHART

PROCEDURE	NORMAL INDICATION	MALFUNCTION	CORRECTIVE ACTION
TURN ON INSTRUMENT	Source lamp lights (indicated externally by the reflectance of the source lamp light through a red prism mounted on the bezel)	Source lamp does not light.	1.Check source lamp wiring connections. 2.Check source lamp voltage. Emitter voltage for transistor 2Q1 should be between 6.4 and 6.6 VDC. If necessary adjust R6 (caution: do not exceed 6.0 VDC across lamp). 3.Replace source lamp. 2S2-line SW. setting.
	Fan operates	Fan does not operate	1.Check fan motor wiring connections. 2.Check for evidence of over-heating of fan motor. 3.Check for motor voltage. Should be between 100-130VAC. 4.Replace fan motor.
BLOCK OUT SAMPLE COMPARTMENT AND SET FOR ZERO METER INDICATION.	Meter can be set to zero. er indication	Meter cannot be set to zero	1.Check $\pm$15volt power supply circuit. 2.Troubleshoot switch/Pot (2S1/2R1) & cable harness assay. 3.Check measuring phototube. 4.Check for light leaks.
	0% meter indication is stable.	0% meter indication is not stable.	1.Check $\pm$15Volt DC . 2.Check measuring phototube power supply. 3. Remove phototube to check amplifier U3 stability 4. Check phototube.
SET WAVELENGTH AT 340nm AND SET METER FOR 100% T.	Meter can be set to 100% T.	Meter cannot be set to 100% T.	1.Check power supply circuit. 2.Check measuring phototube. 3.Check for low lamp voltage. 4.Check cleanliness of grating.

PROCEDURE	NORMAL INDICATION	MALFUNCTION	CORRECTIVE ACTION
SET WAVELENGTH AT 340nm AND SET METER FOR 100% T.	100% T meter indication is stable.	100% T meter indication is not stable.	1.Check location of the instrument (magnetic fields,voltage variation, etc. 2.Check source lamp circuit. 3.Check measuring phototube.
SET WAVELENGTH AT 600nm AND SET METER FOR 100% T.	Meter can be set to 100%T.	Meter cannot be set to 100%T.	1.Check measuring phototube. 2.Replace /Check printed circuit board.
	100%T meter indication is stable.	100%T meter indication is not stable.	Same as above

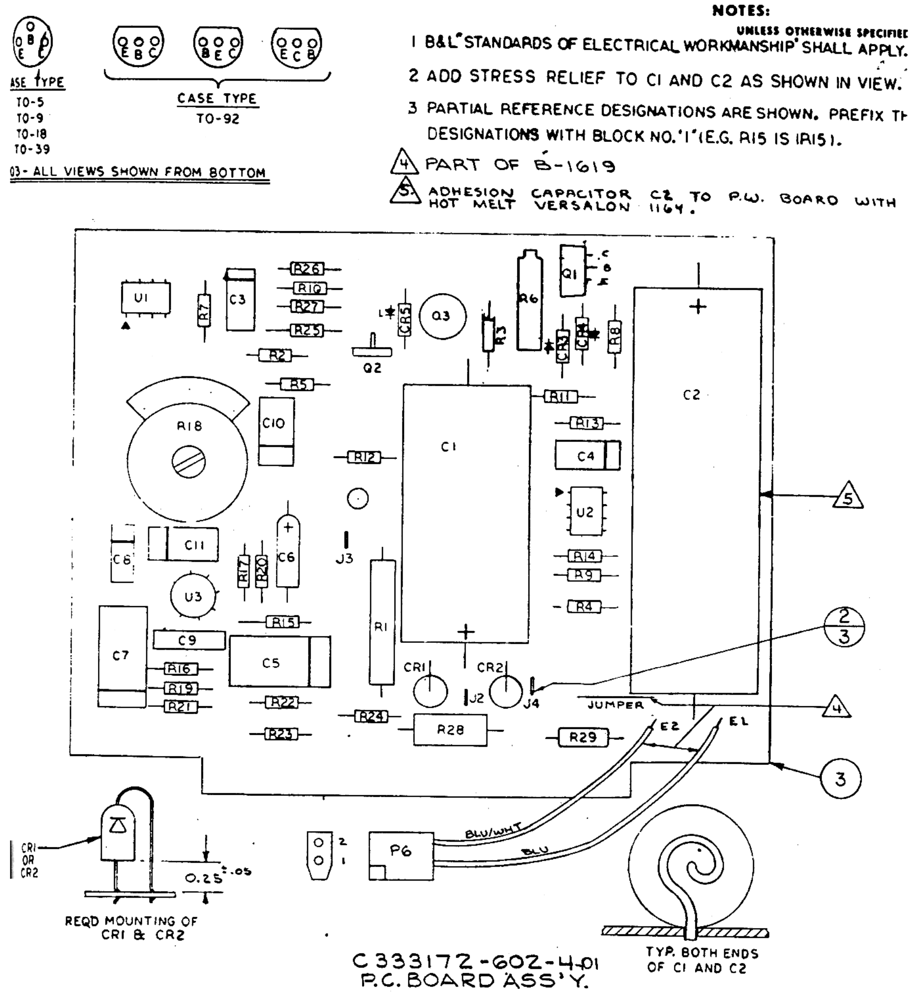
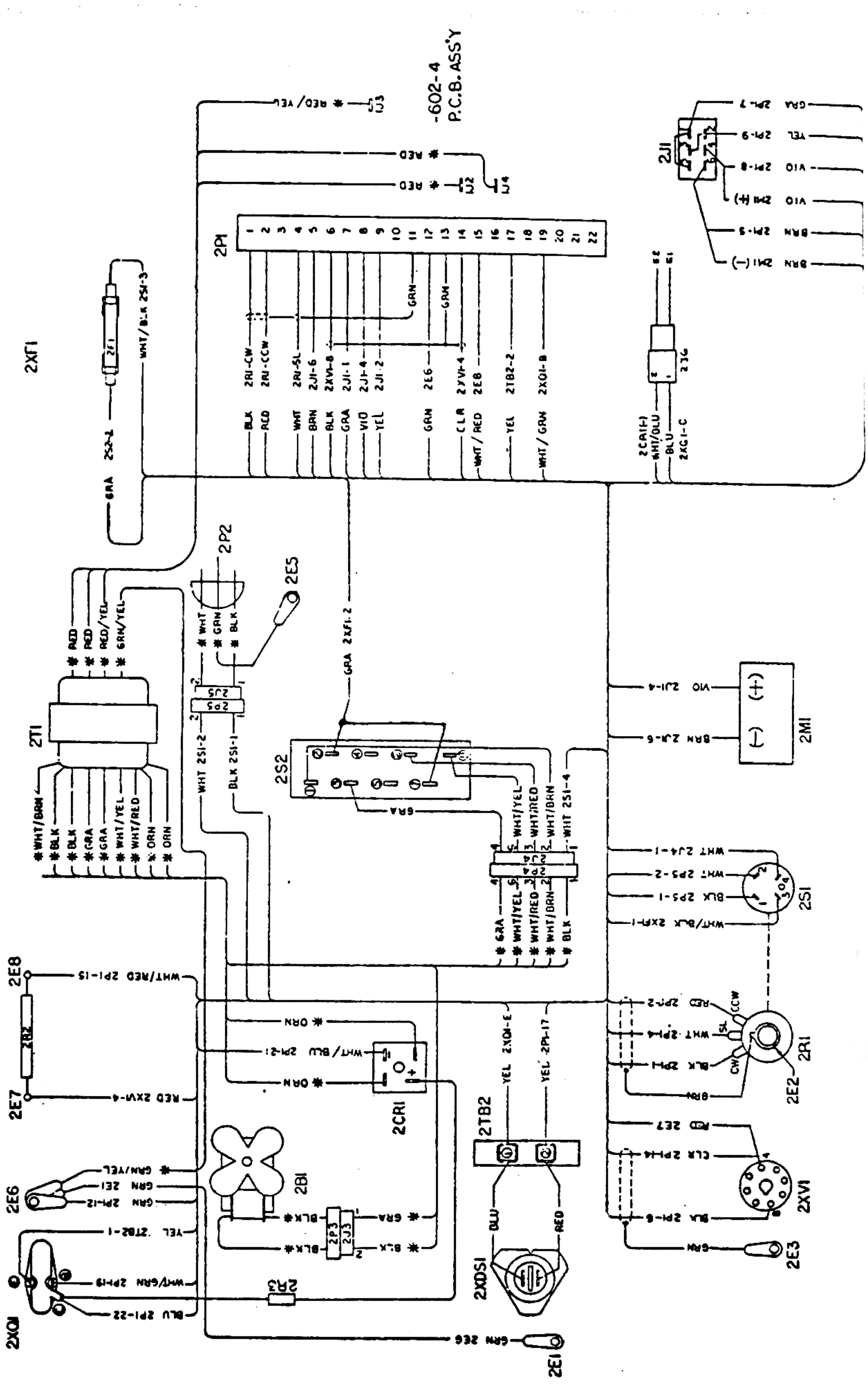


Figure-12



602-4
P.C.B. ASS'Y
2XF1
2F1
2P1
2J1
2P2
2E5
2T1
2P5
2J5
2S2
2J4
2P4
2M1
2S1
2E8
2R2
2E7
2CR1
2R1
2E2
2TB2
2E6
2B1
2P3
2J3
2XV1
2E3
2XO1
2R3
2XDS1
2E1

Figure-13

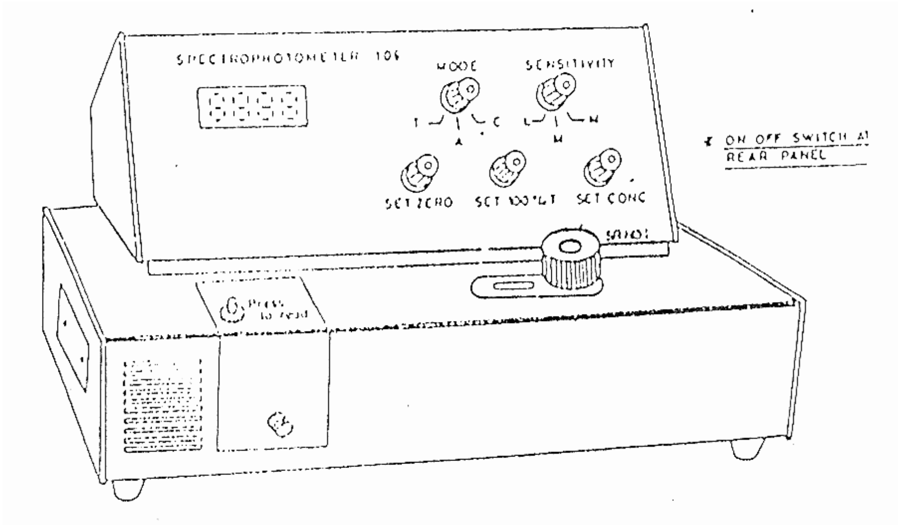


Figure-14

View of instrument with description of controls SYSTRONIC Spectrophotometer Model 106.

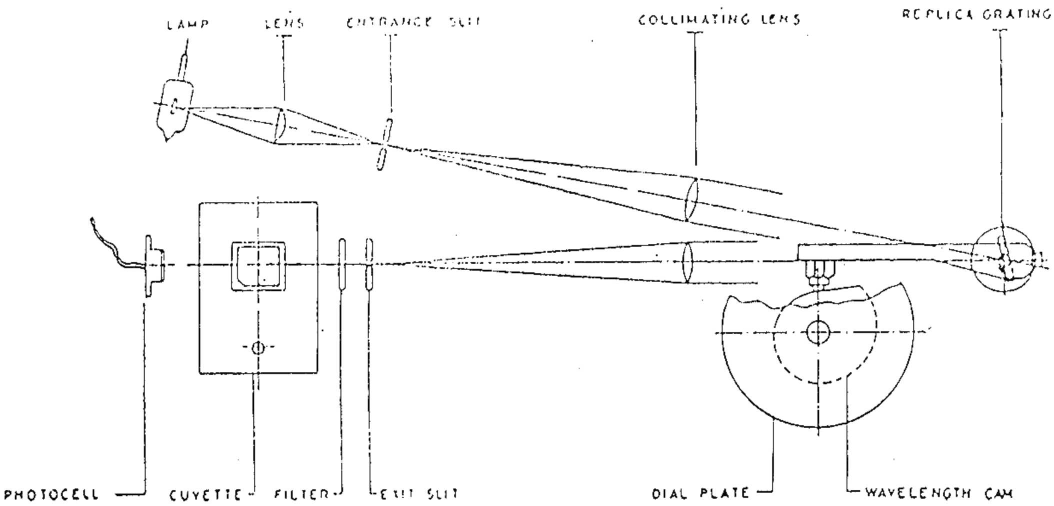


Figure-15
Optical diagram SYSTRONIC Spectrophotometer Model 105/106.

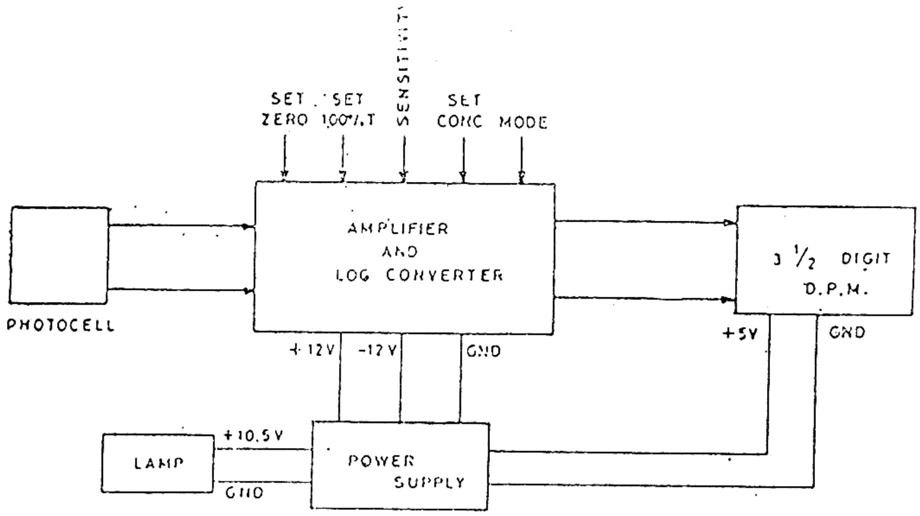


Figure-16
Block diagram SYSTRONIC Spectrophotometer Model 106.

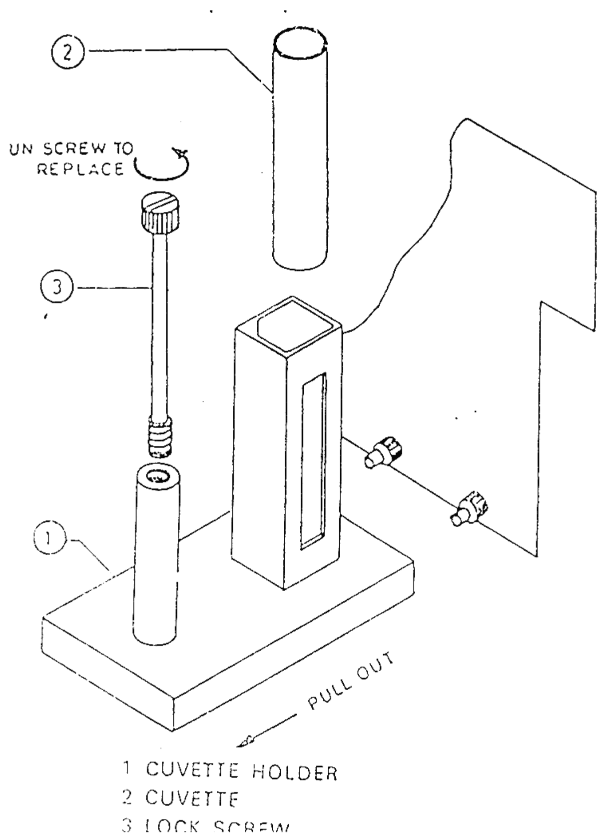


Figure-17
Replacement of cell holder SYSTRONIC Spectrophotometer

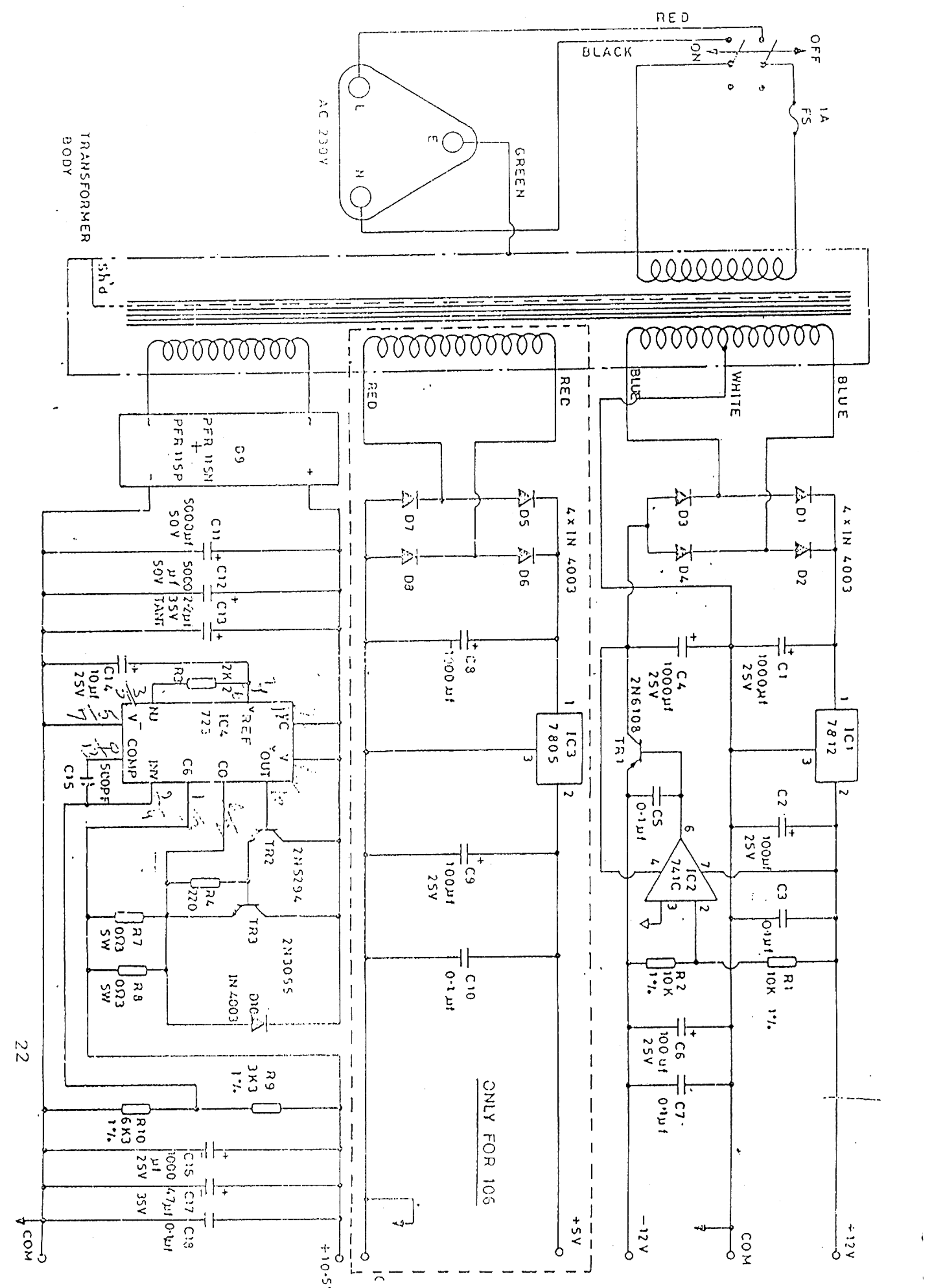


Figure-18
Circuit diagram of power supply SYSTRONIC Spectrophotometer Model 106/10

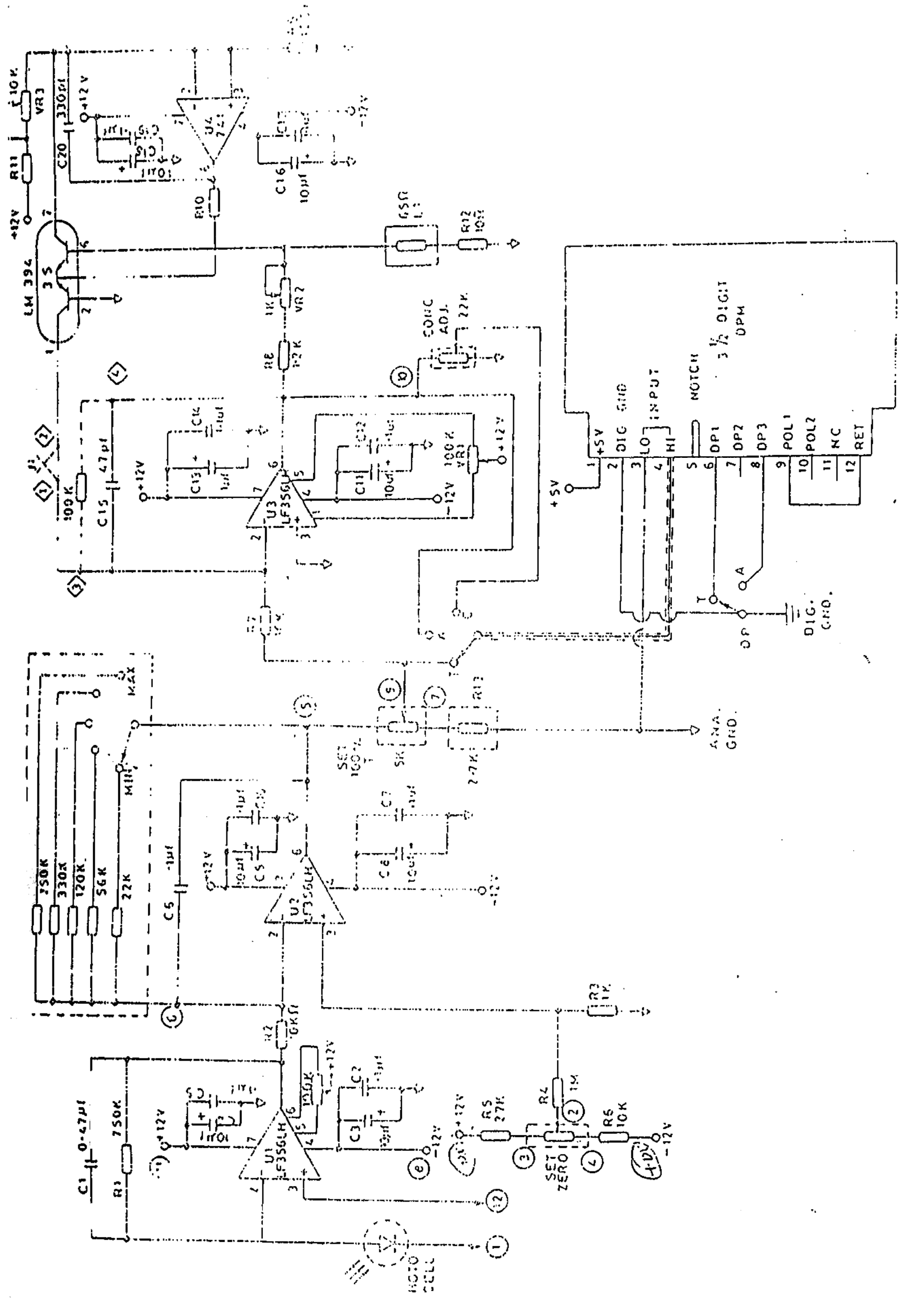


Figure-19
Circuit diagram of amplifier and log converter SYSTRONIC Spectrophotometer Model 106.
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