STUDIES ON THE PROTECTION OF INSECT PATHOGENS FROM SUNLIGHT INACTIVATION I. PRELIMINARY LABORATORY TESTS

by

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ABSTRACT

Laboratory tests conducted to determine the spectral absorption of Bacillus thuringiensis spores and crystals and nuclear polyhedrosis virus, showed that the spores and virus absorbed wavelengths below 320 nm. Crystals absorbed the same wavelength range but fluoresced indicating that sunlight wavelengths have no detrimental effect on them. The germicidal sunlight wavelengths for spores was shown to be between 290 and 400 nm. Ten percent molasses was an effective absorber of germicidal sunlight radiation since it absorbed below 360 nm and transmitted above 380 nm where beneficial radiation is most effective. Exposure of spores mixed with sugars, dyes or synthetic UV absorbers indicated that 25 to 50% molasses, 1% Uvinul DS49 and 5% Blancophor were moderately to highly effective in protecting the bacteria spores from sunlight inactivation.

STUDIES ON THE PROTECTION OF INSECT PATHOGENS FROM SUNLIGHT INACTIVATION

I. PRELIMINARY LABORATORY TESTS

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The characteristically rapid decline in activity of insect pathogens exposed to sunlight constitutes a major problem in their success as microbial control agents. The germicidal wavelengths which penetrate atmospheric ozone lie between 290 and 380 nm (Kleczkowski 1957; Seliger and McElroy 1965; Webb and Tai 1969). The wavelengths within this range which are most germicidal to insect pathogen appear to be below 366 nm (Bullock et al 1970, Morris 1971). The most effective method reported for protecting insect pathogens from rapid inactivation is encapsulation of the infective particles (Ignoffo and Batzer 1971, Cantwell 1967, Martouret and Anglade 1971) but this is a difficult and expensive operation. The addition of some adjuvants to spray mixes appears to increase persistence of nuclear polyhedrosis viruses but very little work has been published on the protection of Bacillus thuringiensis (B.t.) spores (Yendol and Hamlen 1973, Jaques 1972).

That B.t. spores are highly sensitive to sunlight is well documented (Cantwell and Franklin 1966, Pinnock et al 1971, Jaques 1972, Ahmed et al 1973, Frye et al 1973, Ignoffo et al 1974, Morris and Hildebrand 1974). The toxic crystal component of B.t. does not appear to be affected by germicidal wavelengths (Cantwell 1967; Burgess et al 1975).

The objectives of the present studies were to:

(1) Determine the absorbance spectrum of nuclear polyhedrosis virus and B.t. spores and crystals and certain dyes using polychromatic and monochromatic illumination.

(2) Study the effect of certain UV absorbers on the viability of these pathogens following exposure to artificial and natural sunlight. "Ultra violet absorber" is used in the sense of any substance which is capable of absorbing ultra violet radiation, which can convert this radiation into a harmless form of energy, and is not altered or destroyed in the process.

MATERIALS AND METHODS

The first series of tests involved the exposure of known amounts of B.t. spores on millipore filter membrane to incident energy from a Vita-Lite lamp. Suspensions of pure B.t. spores and budworm NPV donated by Drs. P. Fast and J. C. Cunningham, respectively, Insect Pathology Research Institute, Sault Ste. Marie, Ontario, were deposited on the membrane surface and dried. The deposits were exposed at 15 cm below a Vita-Lite lamp for varying periods of time at the end of which they were placed on the surface of tryticase soy agar and incubated overnight at 29°C. The number of colonies developing on the millipore surface indicated the degree of viability. The lamps were measured for spectral irradiance in the ultra violet region (NRC contract). The objective was to determine how closely the lamp simulated sunlight in terms of the amount and range of radiation. According to the manufacturers (Duro Test, Montreal) it simulates indoors the color

rendition of sunlight and emits the same amount of ultraviolet in relation to foot candles as does the sun.

Next, reflectance and transmittance measurements were made on some dyes, sugars, spores, and polyhedral inclusion bodies on the filter membranes with and without the dyes and sugars as protectants. The objective was to determine behaviour of these materials in terms of their absorption properties. These tests were done under NRC contract using Zeiss and Cary Model 14 spectrophotometers with both monochromatic and polychromatic illumination (Table 4). The Uvitex, Erio Acid Red and Maxilon dyes used are moderately to highly water soluble or suspensible fluorescent substances and according to the manufacturers (Ciba-Geigy Canada Limited), have absorption roughly in the 290-400 nm range. Brilliant Sulfo Flavine (Chemical Developments of Canada Limited) is also highly fluorescent and has been used as a tracer dye in aerial application of B.t. and NPV.

The effect of the dyes on germination of B.t. spores were studied by incorporating 0.1% concentration w/v of each in trypticase soy broth with a known concentration of commercial B. thuringiensis. Calibrated loopfuls (0.01 ml) of the culture broth were smeared on trypticase soy agar at intervals of 1, 2, 4 and 6 hours and the number of colonies counted at 24 hour incubation at 29°C.

The germicidal effect of Vita-Lite radiation on B.t. (Thuricide, Sandoz-Warner, Homestead, Florida) spores mixed with the various dyes and sugars, with benzyl cinnamate in emulsion or with the commercially available sunlight protectant, IMC (Sandoz Werner, Homestead, Florida) was determined. Known amounts of commercial B.t. plus protectants were placed on millipore filter membranes and these were placed on agar surface following the appropriate exposure to radiation. Dipel spores were, in addition

treated with peptone milk and peptone-charcoal combinations (Tables 4 & 5).

To check the sunlight wavelength range that is germicidal for B.t. spores, millipore filters bearing the spores were covered with plastic filters into which were incorporated substances which absorb wavelengths of 400-700 nm or transmits wavelength of 300-700 nm. Exposure lasted a full day in bright sunlight during April when the average daily sunlight radiation rate is about 390 cal/cm^2. Spores mixed with dyes and sugars were also exposed to direct sunlight.

The protective effect of various synthetic ultraviolet absorbers were next studied. Dipel 36B, a highly concentrated commercial formulation of naked spores and crystals of B.t. was diluted 1/100 and various concentrations of the various adjuvants added. Clean microscope slides were dipped in the suspensions during stirring on a magnetic stirrer, drained onto absorbent paper and dried. Sunlight exposed and unexposed slides were washed with 100 ml of distilled water and calibrated loopfuls of the wash were smeared onto brain heart infusion agar surface and incubated overnight at 29^{o}C. Percent inactivation of spores was calculated as a percentage of the unexposed. Uvinul DS49, MS40 and Blancophor SV (Chemical Developments of Canada) are water soluble absorbers used commercially in cosmetics and water-based paint formulations. Cyasorb $^{(R)}$ 284 (American Cyanamid, N.J.) is a highly water soluble experimental ultraviolet absorber. PVP (polyvinylpyrrolidone, a water soluble polymer) and CMC (sodium carboxymethylcellulose, a water soluble cellulose ether) included in the test suspensions are effective film formers (supplied by Chemical Developments) and are used primarily in cosmetic, textile, pharmaceutical and food industries. Spectral transmittance of 2.5 mg of DS49/100 ml, 2.5 mg of MS 40/100 ml

and 10 mg of Cysorb/100 ml are 45%, 18% and 50% respectively, at wavelength 300 mm acording to the manufacturers.

Lastly, the ultraviolet absorptive efficiencies of a large variety of substances or mixtures of substances were compared by measuring their spectral transmittance on a Beckman Acta C III model UV spectrophotometer at 7 wavelengths between 250 and 380 um. It was necessary to dilute some of the original solutions in order to record some measure of transmittance.

RESULTS AND DISCUSSIONS

Data on the spectral irradiance from the sunlight lamps (Table 1) indicated that they do cover the range of sunlight wavelengths but, as expected, with only a fraction of sunlight intensity. For example, at the sunlight germicidal wavelength of 315 nm, the lamp intensity measured as $\mu W/cm^2$ was only 0.18% of sunlight intensity. At the sunlight germicidal wavelength of 300 nm spectral irradiance from the lamp was zero. The usual bactericidal efficiency of UV radiation is given in Table 2 for comparison. Results of the lamp exposure tests would, therefore, be very rough indicators of what can be expected from sunlight exposure.

Uvitex and Erio Acid dyes at 0.1% had no detrimental effect on spore germination (Table 3). Spectral reflectance measurements of B. thuringiensis spores and nuclear polyhedrosis virus and the dyes (Table 4; Figs. A1-A3; B1-B12) show increasing absorption with polychromatic light and monochromatic viewing by the crystals below 320 nm (Figs. A1, A2, A3). The spore-crystal complex reflectance curve decreased uniformly from 400 nm down. The reflectance of plain millipore filter

paper decreased slowly at lower wavelengths. Reflectance measurements using monochromatic illumination and broad band viewing showed that crystals absorbed at wavelengths below 320 and then fluoresced indicating lack of inactivating effect at that wavelength. The spores and crystals when combined showed less fluorescence suggesting that the spores absorb below 320 nm. It should be noted, however, that the photomultiplier used in the monochromatic broad band measurements was limited in sensitivity. Visual observation of the fluorescence of the spores and crystals showed bluish white fluorescence of the spores at all wavelengths from 290 nm to 390 nm with strong absorption below 310 nm.

The measurements with polychromatic illumination and monochromatic viewing are the most useful since they show that radiation is available to affect the spores (Fig. A3). The higher values of reflectance with monochromatic illumination and broad band viewing indicate that radiation is being reflected or emitted as can be seen at or above the wavelength indicated on the horizontal scales. The transmittance measurements on the solutions in cells 1 cm thick (Table 3) are not as useful because the solutions absorb some of the fluorescent light. Measurement of fluorescent radiation at the front of the cell was not done but should be equivalent to the reflectance of the materials on paper. According to Setlow (1966) the harmful radiation is below 315 nm for germicidal effect and from 450-550 nm for photodynamic action.

The spectral measurements of the dyes with polychromatic illumination indicate that Uvitex EBF and ERN-P absorbed radiation below 450 nm (Figs. B2, B4). Erio Acid Red, Erio Yellow and Brilliant Sulfo Flavine absorbed this wavelength as well but not lower ones (Figs. B6, B12). Molasses was potentially the most useful since it absorbed

below 360 nm and transmitted above 380 nm where beneficial energy is most effective. Tea (Fig. B9) might be helpful but is probably not as good as molasses since tea absorbs also the beneficial radiation from 380 to 400 nm. The best fluorescent material would absorb below 320 nm, from 440 to 600, and emit above 600 nm without absorbing between 320 and 410 nm.

Erio Yellow passes relatively more far UV than near UV compared to BSF. In the visible, the absorption spectrum is very similar for the two materials. Erio Yellow and BSF both kill the spores, an effect which would continue in the light. Similarly the poor behaviour in light of cells in Maxilon Brilliant Flavine and Maxilon Brilliant Pink B is probably a carry-over of the killing effect in the dark.

Erio Acid Red passes radiation up to 500 nm and absorbs from 500 to 600 nm whereas BSF absorbs from 370 to 500 nm. It looks as though the characteristics of Erio Acid Red should be better than BSF's characteristics for protecting the spores. When the fluorescent dye is absorbed on the crystal or spores, the spectral absorption characteristics may change, thus the measurements on the dye alone do not necessarily indicate the protection properties of the dye in practice. Because the crystals and spores may change the spectral properties of the dye (when the dye is absorbed on them) the spectrophotometric measurements on the dye solutions are not necessarily indicative of the spectral radiation present in practice. Similarly the addition of molasses may change the spectral properties in an unpredictable manner. Thus the measurements can only be indicative of what may happen and no certainty can be attached. The biological survival tests under the lamps or in daylight are a better indicator of the survival of the cells. Even that is not certain because

when the mixture is on the spruce needles the spectral radiation incident on the cells will be modified by the spectral absorption of the needles.

It appears from the present data that if a fluorescent dye is to be used for protective action, it should produce a red fluorescence and should absorb radiation below 320 nm or be combined with a material which does absorb below that wavelength. It should not absorb from 320-420 nm and it should not harm the spores in the dark.

Studies on the effect of incident light from the Vita-Lite lamps on spores with or without protective sugars or dyes showed that only molasses was consistently effective in reducing inactivation (Tables 5 and 6). This agrees with the data on spectral absorption of these substances. Spores on filter membrances covered with plastic filters which absorb 400-700 nm wavelength and exposed to one day of sunlight were not inactivated (Table 7). Those covered with filters which transmit 300-700 nm were all killed. This is proof that for B.t. spores the germicidal sunlight band lies between 290 and 400 nm, i.e. mid UV to far UV.

Preliminary tests with dyes and sugars showed that after 12 hours of direct exposure to sunlight, spores applied with 25% and 50% molasses retained 13 and 36%, respectively, of their original activity. None of the other substances gave protection (Table 8). Untreated spores were totally inactivated. Preliminary tests with DS49 plus a film former, showed some promise (Table 9) and 5% Blancophor, an optical brightener was moderately effective (Table 10).

The last series of exposure tests involved mixtures of various synthetic UV absorbers and the two film formers (CMC and PVP) in various concentrations. With total sunlight radiation of 227 cal/cm^2

which is 50% of the daily radiation to which the spores would be exposed when aerially applied in late May, 1% Cyasorb + PVP, 1% DS49 + PVP, 5% Blancophor + CMC, 25% Molasses + CMC, 50% Molasses + CMC and 50% Molasses + PVP, appeared to be highly effective in maintaining spore activity (Table 11). The film formers themselves do not contribute directly to radiation protection (Table 9) but in combination with B.t. suspensions for aerial application, they would perform important functions. PVP acts as a suspension stabilizer and forms hard films. Moisture taken up or retained from the air by PVP acts as a stabilizer for the films which become tacky at 70% RH and at 50% RH they contain 18% moisture. It is compatible with many natural and synthetic resins, many chemicals and inorganic salts and has an acute oral toxicity for white rats and guinea pigs of LD_{50} < 100 g/kg of body weight. It is inexpensive ($1.36/lb). CMC has film forming, suspending, stabilizing, emulsifying, thickening and adhesive properties. In general, it is slower dissolving than PVP but "Fast Dissolving Grades" are available.

Results of the tests on the comparative spectral transmittance of various substances (Table 12) indicated the 0.1% Erio Acid Red, 0.25% molasses, 1% peptone milk, 0.01% DS49, 0.01% MS40 and 0.5 ppm Blancophor were the most effective absorbers within the sunlight germicidal waveband (280-360 μm). These substances warrant further testing.

SUMMARY AND CONCLUSIONS

Experiments were designed to determine the spectral reflectance and transmittance of pure Bacillus thuringiensis (B.t.) spores and crystals, a nuclear polyhedrosis virus and various dyes in solution. The spectral irradiance of a Vita-Lite sunlamp used for

exposing the pathogens to irradiation was also measured. B.t. spores mixed with the fluorescent dyes, various sugars and synthetic ultraviolet absorbers were exposed for various time periods to radiation from the lamp and from direct sunlight and changes in viability of the spores determined. The sunlight germicidal wavelengths for B.t. spores were checked by exposing them to sunlight covered with plastic plates in which 300 to 700 nm ultraviolet wavelengths were incorporated.

The results indicated that the sunlamp covered the range of sunlight wavelengths but with only a fraction of sunlight intensity. With polychromatic light and monochromatic viewing, the crystals showed increasing absorption below 320 nm and then fluoresced indicating a lack of inactivating effect. The spores absorbed radiation below 320 nm and fluoresced at all wavelengths from 290 to 390 nm. Of the UV absorbers tested, molasses was the most effective since it absorbed radiation below 360 nm and transmitted above 380 nm where beneficial energy is most effective. It was apparent that if fluorescent dyes are to be used for protective action, they should produce a red fluorescence and should absorb radiation below 320 nm or be combined with a material which does. They should not absorb from 320 to 420 nm and should not harm the spores in the dark.

Exposure of B.t. spores covered with the plastic UV-absorber plates showed that the germicidal wavelengths of natural sunlight for B.t. spores lie between 290 nm and 400 nm. Exposure of the spores mixed with sugar, dyes or synthetic UV absorbers, indicated that 25 to 50% molasses, 1% Univul DS49 and 5% Blancophor were moderately to highly effective in protecting the spores from sunlight inactivation.

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Table 1

Measured Spectral Irradiance of Continuum and Lines from Two 40-W Vitalite Lamps in Fixture at 15.2 cm from Lamps. Daylight Irradiance for Comparison

Wavelength nm	Daylight* Spectral Irradiance μW per cm^2 per 5 nm	Vitalite Lamps Spectral Irradiance μW per cm^2 per 5 nm
295	0.0	0.0
300	2.35	0.000
305	10.0	0.003
310	23	0.019
315	45.5	0.085
320	75.5	0.245
325	89.5	0.58
330	129	1.15
335	142	2.05
340	143	2.85
345	144	3.80
350	150	4.50
355	172	4.85
360	159	4.8
365	182	4.25
370	200	4.25
375	172	4.10
380	196	4.15
385	162	4.40
390	200	6.15
395	178	6.05
400	270	7.60
SPECTRAL LINE POWER		μW cm^{-2}
296.7		0.0067
302.4		0.0055
313.0		2.13
334.2		0.68
365.0		14.6

* Air Mass 2 at Pretoria, South Africa.

Table 2

Bactericidal Efficiency of U.V. Radiation*

λ	Relative Values
296.7	0.27
302.2	0.13
313.0	0.01

* From J.K. Setlow, "Photoreactivation", in Radiation Research Supplement Vol. 6: 141-155 (1966).

Table 3

Effect of Dyes on the Germination of *Bacillus thuringiensis* (Thuricide 16B) [1]

Dye Concn. in B.t. Suspension	No. of Replicates	Percent of Spores Inactivated at Indicated Hrs of Incubation Time[2]			
		1	2	4	6
Uvitex ERN-P, 0.1%	20	0	0	0	0
Uvitex E B F, 0.1%	20	4	0	0	0
Erio Acid Red x B, 0.1%	20	4	0	0	0
Erio Acid Red x B, 1%	10	46	51	88	99
Brilliant Sulfo Flavine 0.1%	30	11	30	97	99
Erio Yellow FF, 0.1%	20	0	17	97	99
Maxilon Brilliant Flavine FF 0.1%	20	14	42	84	94
Maxilon Brilliant Pink 0.1%	10	-	98	93	83

[1] pH of cultures 7.0 - 7.3

[2] Expressed as a percentage of untreated checks.

Table 4

Spectral Reflectance Measurements of B. thuringiensis and NPV with a Zeiss Spectrophotometer and Cary Model 14

Figure A3: Polychromatic Illumination

Monochromatic viewing spectral reflectance measured on Cary Model 14, deuterium source.

Curve 1 - Spores
" 2 - 100%

Figure B1: Monochromatic Illumination

Curve 1 - Plain filter
" 2 - NPV
" 3 - Erio Acid Red XB .01% and spores
" 4 - Maxilon Brilliant Pink B .001% and spores
" 5 - Maxilon Brilliant Pink B .0001% and spores
" 6 - 100%

Figure B2: Monochromatic Illumination

All these are spores plus the following:

Curve 1 - Maxilon Brilliant Flavine .01%
" 2 - Maxilon Brilliant Flavine .001%
" 3 - Uvitex ERN P 0.1%
" 4 - Uvitex EBF 0.1%
" 5 - Erio Yellow FF 0.1%
" 6 - BSF 0.1%
" 7 - 100%

Figure B3: Polychromatic Illumination

Samples plus spores.

Curve 1 - Plain filter
" 2 - NPV
" 3 - Erio Acid Red .01%
" 4 - Maxilon Brilliant Pink .001%
" 5 - Maxilon Brilliant Pink .0001%
" 6 - 100%

Figure B4: Polychromatic Illumination

Sample plus spores.

Curve 1 - Maxilon Brilliant Flavine .01%
" 2 - Maxilon Brilliant Flavine .001%
" 3 - Uvitex ERN P 0.1%
" 4 - Uvitex EBF 0.1%
" 5 - 100%

Table 4 Cont'd

Figure B5: Polychromatic Illumination

Samples plus spores.

Curve 1 - Erio Yellow FF 0.1%
" 2 - BSF 0.1%
" 3 - 100%

Figure B6: Polychromatic Illumination

Samples with no spores.

Curve 1 - Erio Acid Red .1%
" 2 - Erio Yellow .1%
" 3 - 100%

Figure B7: Polychromatic Illumination

Spores only.

Curve 1 - No filter)
" 2 - 400 nm cutoff filter) Cutoff filters
" 3 - 460 nm cutoff filter) have no effect.
" 4 - 100%)

Figure B8: Polychromatic Illumination

BSF only, 0.1%

Curve 1 - Xenon arc, no filter
" 2 - 400 nm cutoff filter plus arc
" 3 - 460 nm cutoff filter plus arc
" 4 - 100%

Figure B9: Polychromatic Illumination

Samples on paper.

Curve 1 - Soy sauce
" 2 - Molasses 10%
" 3 - Tea
" 4 - 100%

Figure B10: Polychromatic Illumination

BSF and spores

Curve 1 - Xenon arc, no filter
" 2 - 400 nm cutoff, filter plus arc
" 3 - 460 nm cutoff, filter plus arc
" 4 - 100%

Table 4 Cont'd

Figure B11: Transmittance 1 cm cell

Curve 1 - Uvitex EBF .01%
" 2 - Uvitex ERN .01%
" 3 - Erio Acid Red .1%
" 4 - BSF .01%
" 5 - H_2O

Figure B12: Curve 1 - Polychromatic Illumination, BSF alone on paper
" 2 - Monochromatic Illumination, BSF alone
" 3 - 100%

Table 5

Effect of Incident Light from a Vita-Lite Lamp on Germination of *Bacillus thuringiensis* Spores (Thuricide 16B)

Protectant Concn. in B.t. Suspension	No. of Replicates	Percent of Original Activity Following Indicated Exposure Time (Hr) [1]				
		1	5	10	40	160
Control (no additive)	15	91	92	98	44	1
1% Benzyl Cinnamate	10	100	100	91	47	5
3% Benzyl Cinnamate	10	100	100	82	30	8
5% Benzyl Cinnamate	10	100	91	98	25	1
10% Molasses	10	100	100	100	74	-
25% Molasses	10	100	100	100	77	-
2.5% IMC	5	-	-	-	46	-
3% Inositol	5	-	-	-	28	-
5% Inositol	5	-	-	-	37	-
7% Inositol	5	-	-	-	36	-
10% Inositol	5	-	-	-	62	-
0.1% Uvitex ERN-P	5	-	-	-	43	1
0.1% Uvitex EBF	5	-	-	-	33	1
0.1% Erio Acid Red XB	5	-	-	-	57	4
0.1% Erio Yellow FF	5	-	-	-	56	2
0.1% Maxilon Brilliant Flavine 10 GFF	5	-	-	-	11	1
0.1% Maxilon Brilliant Pink B	5	-	-	-	37	2
0.1% Brilliant Sulfo Flavine	5	-	-	-	55	2

[1] Express as a percentage of untreated unexposed controls.

Table 6

Effect of Incident Light from a Vita-Lite Lamp on Germination of Bacillus thuringiensis Spores (Dipel WP)

Protectant Concn. in B.t. Suspension	No. of Replicates	Percent of Original Activity Following Indicated Exposure time (hr) [1]				
		1	5	10	40	160
Control (no additive)	20	100	100	83	29	1
10% Molasses	10	93	84	98	76	3
25% Molasses	10	81	71	92	88	6
3% Inositol	15	100	100	60	27	1
5% Inositol	15	96	90	84	48	1
7% Inositol	10	100	91	100	30	4
10% Inositol	10	83	68	59	25	1
2.5% IMC	10	100	88	88	52	2
10% Molasses + 1% Charcoal	5	-	-	-	61	-
10% Molasses + 1% Charcoal 1% Yeast Extract	5	-	-	-	20	-
1% Peptone Milk	5	-	-	-	27	-
3% Peptone Milk	5	-	-	-	40	-
5% Peptone Milk	5	-	-	-	44	-
1% Peptone Milk + 1% Charcoal	5	-	-	-	39	-
3% Peptone Milk + 1% Charcoal	5	-	-	-	35	-
5% Peptone Milk + 1% Charcoal	5	-	-	-	54	-
0.1% Uvitex ERN-P	5	-	-	-	53	-
0.1% Uvitex E B F	5	-	-	-	60	-
0.1% Erio Acid Red XB	5	-	-	-	50	-
0.1% Erio Yellow	5	-	-	-	61	-
0.1% Maxilon Brilliant Flavine 10 GFF	5	-	-	-	66	-
0.1% Maxilon Brilliant Pink B	5	-	-	-	56	-
0.1% Brilliant Sulfo Flavine	5	-	-	-	48	-

[1] Expresses as a percentage of untreated unexposed controls.

Table 7

Protection of *Bacillus thuringiensis* (Dipel 36B) Spores from Sunlight Inactivation by Ciba Geigy Plastic UV Filters

UV Filter Type	Mean Number of Viable Spores/ Millipore Membrane[1]
- UV exposed - filters 400-700 nm wavelengths	512
+ UV exposed - transmits 300-700 nm wavelengths	0
Unexposed Controls	415

[1] Four replicates per test.

Table 8

Protective Effect of Various Substances against Sunlight Inactivation of *Bacillus thuringiensis* on Millipore Filter Membranes

Concn. of Protectants in B.t. Suspension	Number of Viable Spores per Membrane at Indicated Exposure Period (Hr) of Direct Sunlight[1]			
	0 (dark)	12	24	48
Control (Dipel alone)	12	0	0	0
10% Inositol	31	1	1	0
10% Molasses	16	2	1	2
25% Molasses	63	8	3	1
50% Molasses	55	20	21	2
0.1% Erio Acid Red XB	28	2	3	1
0.1% Uvitex ERN-P	27	2	2	0
1.0% Uvitex ERN-P	14	2	3	1
0.1% Uvitex EBF	36	1	0	1
1.0% Uvitex EBF	12	2	1	1
Control (Thuricide 16B alone)	51	0	0	0
10% Inositol	138	3	0	2
10% Molasses	95	5	1	1
25% Molasses	85	6	3	1
0.1% Erio Acid Red XB	116	4	3	1
0.1% Uvitex ERN-P	84	2	3	0
1.0% Uvitex ERN-P	107	2	3	1
0.1% Uvitex EBF	98	3	1	1
1.0% Uvitex EBF	79	4	1	1

[1] Two replicates each. Spores were exposed at Ottawa, May 28 to June 4, 1974. B.t. suspensions were sprayed onto filter membrane in a Potter's tower.

Table 9

Protective Effects of Various Chemical Agents Against Sunlight Inactivation of *Bacillus thuringiensis* Spores

Chemical Agent, Concn.	Percent of Original Activity of *B. thuringiensis* after 1 Day Exposure to Sunlight*
1% DS 49	17.3
1% DS 49 + 1% PVP	19.9
1% DS 49 + 1% CMC	19.2
0.5% MS 40	1.4
0.5% MS 40 + 1% PVP	10.7
0.5% MS 40 + 1% CMC	8.4
1% PVP	5.6
1% CMC	5.7
1% Cyasorb	3.4
1% Cyasorb + 1% PVP	11.6
1% Cyasorb + 1% CMC	9.2
B.t. - alone - exposed	0.8
B.t. - alone - dark	100.0

* Means of 4 replicates each. Total sunlight radiation, not recorded.

Table 10

Protection of *Bacillus thuringiensis* (Dipel 36B) Spores from Sunlight Inactivation by Blancophor, an Optical Brightener[1]

Concn. of Blancophor	Spores Germination	No. Viable Spores/ml of Suspension, Unexposed Slides		No. Viable Spores/ml of Suspension, Exposed Slides		Percent of Original Viability after 420 cal/cm^2 Exposure to Sunlight Radiation[2]	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
0 (Dipel 36B alone)	+ + + +	91,900	67,900	50	100	0.1	0.1
0.5%	+ + + +	112,800	306,300	5,080	8,600	4.5	2.8
1.0%	+ + + +	117,400	335,800	9,830	12,100	8.4	3.6
2.5%	+ + + +	120,000	253,200	31,400	35,400	26.2	14.0
5.0%	+ + + +	120,000	268,800	78,780	168,000	65.7	62.5

[1] A film former (polyvinylpyrrolidone) at 1.0% was added to all suspensions before dipping slides. Two slides dipped per concn. and 2 plates streaked per slide. Slides washed with 10 ml distilled water and 0.01 ml streaked on brain heart infusion agar.

[2] Exposure date was May 14, 1975.

Table 11

Protective Effect of Various Additives against Sunlight Inactivation of *Bacillus thuringiensis* (Dipel 36B)

Additives, Concn.	Specific Gravity	Settling Characteristics after 17 hr Standing	No. Viable Spores per ml of Suspension, Unexposed Slides	No. Viable Spores per ml of Suspension, Exposed Slides	Percent of Original Activity After 227 cal/cm^2 Sunlight Radiation
0 (Dipel 36B alone)	1.02	+ + + +	387,000	3,400	0.9
0.5% MS 40 + 0.5% CMC	1.00	+ +	69,500	6,000	8.6
0.5% MS 40 + 1.0% PVP	1.00	+ + + +	61,300	36,700	59.9
0.5% Cyasorb + 0.5% CMC	1.00	+ +	73,600	5,000	6.8
1.0% Cyasorb + 0.5% CMC	1.01	+ +	27,300	9,200	33.7
0.5% Cyasorb + 1% PVP	1.00	+ + + +	64,100	34,900	54.4
1.0% Cyasorb + 1% PVP	1.01	+ + + +	48,100	45,300	91.2
1.0% DS49 + 0.5% CMC	1.01	+ +	99,100	101,900	100.0
1.0% DS49 + 1% PVP	1.01	+ +	92,400	40,900	44.3
5.0% Blancophor + 0.5% CMC	1.01	+	70,300	81,100	100.0
25% CIB + 75% of 0.5% CMC	1.18	+	74,100	111,100	100.0
25% CIB + 1% PVP	1.09	+ +	45,200	30,900	68.4
50% CIB + 50% of 0.5% CMC	1.16	+	86,000	104,500	100.0
50% CIB + 1% PVP	1.16	+ +	59,100	71,900	100.0

Numbers are means of 6 replicates. Slides washed with 100 ml distilled water and 0.01 ml streaked on brain heart infusion agar. Exposure done during the month of November under cool temperatures.

+ + + + indicates heavy sediment; + light sediment

Table 12

SPECTRAL TRANSMITTANCE OF EXPERIMENTAL UV ABSORBERS
FOR B.T. AT THE FOLLOWING WAVELENGTHS (nm)

ABSORBERS	DILUTION FACTOR	% TRANSMITTANCE						
		250	280	290	300	320	360	380
Uvitex ERN-P 0.1%	0	1.9	28.8	4.4	0.6	0.0	0.0	0.0
Uvitex EPF 0.1%	0	3.8	13.1	5.0	1.9	0.0	0.0	0.0
Erio Acid Red 0.1%	0	0.0	0.0	0.0	0.0	0.0	0.6	20.0
Brilliant Sulfo Flavine 0.1%	10	7.5	3.1	3.8	38.1	65.0	52.5	16.9
Erio Yellow FF 0.1%	10	7.5	3.8	3.8	34.4	65.6	52.5	17.5
Maxilon Brilliant Pink 0.1%	0	15.0	1.3	1.9	4.4	5.0	24.4	45.6
Maxilon Brilliant Flavine 0.1%	0	0.0	0.6	0.0	0.0	1.3	26.3	1.3
Benzyl Cinnamate 1%	10	4.4	0.0	0.0	0.0	9.4	28.8	34.4
CIB Molasses 10%	100	0.6	2.5	1.9	1.3	1.9	10.0	18.8
CIB Molasses 25%	100	0.0	0.0	0.0	0.0	0.0	2.5	7.5
CIB Molasses 50%	1,000	13.1	46.9	25.6	15.6	14.4	34.4	46.9
IMC Protectant 2.5%	100	0.0	1.3	0.6	2.5	6.9	28.1	36.9

Table 12 Cont'd

SPECTRAL TRANSMITTANCE OF EXPERIMENTAL UV ABSORBERS FOR B.T. AT THE FOLLOWING WAVELENGTHS (nm)

ABSORBERS	DILUTION FACTOR	% TRANSMITTANCE						
		250	280	290	300	320	360	380
Inosital 3%	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Inosital 5%	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Inosital 7%	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Inosital 10%	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Molasses 10% + Charcoal 1%	100	0.6	3.1	2.5	1.9	3.1	13.8	25.6
Molasses 10% + 1% Charcoal + Yeast Extract 1%	0	0.6	3.1	2.5	2.5	3.1	14.4	25.6
Peptone Milk 1%	0	0.0	0.0	0.0	0.0	0.0	5.0	17.5
Peptone Milk 3%	10	0.6	0.0	0.6	3.8	5.6	41.9	59.4
Peptone Milk 5%	10	0.0	0.0	0.0	0.6	3.1	20.0	38.1
Peptone Milk 1% + Charcoal 1%	0	0.0	0.0	0.0	0.0	1.3	8.8	21.3
Peptone Milk 5% + Charcoal 1%	10	0.0	0.0	0.0	1.3	5.0	21.3	40.6
Uvinul DS 49N 1%	100	0.6	1.3	0.6	1.9	1.9	8.8	53.8

Table 12 Cont'd

SPECTRAL TRANSMITTANCE OF EXPERIMENTAL UV ABSORBERS
FOR B.T. AT THE FOLLOWING WAVELENGTHS (nm)

ABSORBERS	DILUTION FACTOR	% TRANSMITTANCE						
		250	280	290	300	320	360	380
Uvinul DS49 1% + PVP 1%	100	5.0	1.9	2.5	5.6	3.8	20.6	73.1
	100	6.3	1.9	1.3	3.1	2.5	10.6	60.0
Uvinul DS49 1% + CMC 0.5%	100	0.6	1.3	0.6	2.5	2.5	9.4	57.5
Uvinul MS40 1%	10	0.0	0.0	0.0	0.0	0.0	0.0	47.5
Uvinul MS40 1% + PVP 1%	100	0.0	0.0	0.0	0.0	0.0	37.5	90.0
Uvinul MS40 1% + CMC 1%	1,000	0.0	0.0	0.0	0.0	0.0	38.1	90.0
PVP Alone 1%	10	76.9	91.3	92.5	92.5	93.6	96.3	96.9
CMC Alone 1%	0	51.9	33.1	37.5	50.0	83.8	90.6	92.5
Cyasorb 1% + CMC 0.5%	100	0.0	0.0	0.0	0.0	0.0	41.3	91.3
Cyasorb 1% + PVP 1%	100	0.0	0.0	0.0	0.0	0.0	40.6	90.6
Cyasorb 1% + CMC 1%	100	0.0	0.0	0.0	0.0	0.0	40.0	90.0
Blancophor 0.5%	10,000	0.0	0.0	0.0	0.3	0.0	0.0	0.3
Blancophor 1.0%	10,000	13.1	9.4	15.0	31.3	26.3	10.6	54.4

Table 12 Cont'd

SPECTRAL TRANSMITTANCE OF EXPERIMENTAL UV ABSORBERS
FOR B.T. AT THE FOLLOWING WAVELENGTHS (nm)

ABSORBERS	DILUTION FACTOR	% TRANSMITTANCE						
		250	280	290	300	320	360	380
Blancophor 2.5%	10,000	44.4	35.6	43.1	60.0	58.8	44.4	65.6
Blancophor 5% + CMC 0.5%	10,000	28.8	20.0	37.5	40.6	41.3	27.5	53.8
Univul MS40 0.5% + CMC 0.5%	100	1.3	0.0	0.0	0.6	1.9	68.1	100.0
Univul MS40 0.5% + PVP 1%	100	2.5	0.6	0.6	1.9	3.1	70.0	100.0
CIB 25% + 75% of CMC 0.5%	1,000	18.8	23.1	27.5	31.88	40.0	61.9	73.13
CIB 25% + PVP 1%	1,000	25.0	30.0	34.4	38.8	44.4	67.5	75.0
CIB 50% + 50% of CMC 0.5%	1,000	5.0	7.5	9.4	12.5	18.1	39.4	52.5
CIB 50% + PVP 1%	10,000	61.3	68.8	66.9	69.4	73.8	85.0	88.8
Cyasorb 10mg/1000ml	0	50.6	35.0	33.8	45.6	50.0	98.8	100.0
CIB 10% + Cyasorb 10mg/1000ml	1,000	62.5	66.9	70.6	73.1	79.4	88.1	95.6
MS40 2.5mg/100ml	0	4.4	1.3	1.3	3.1	3.8	73.8	100.0
CIB 10% + MS40 2.5mg/100ml	1,000	60.6	65.6	69.4	71.9	78.1	90.0	94.4
DS49 2.5mg/100ml	0	58.2	45.6	48.8	58.1	54.4	77.5	99.4
CIB 10% + DS49/100ml	1,000	56.3	62.5	65.0	68.2	74.4	88.2	93.8

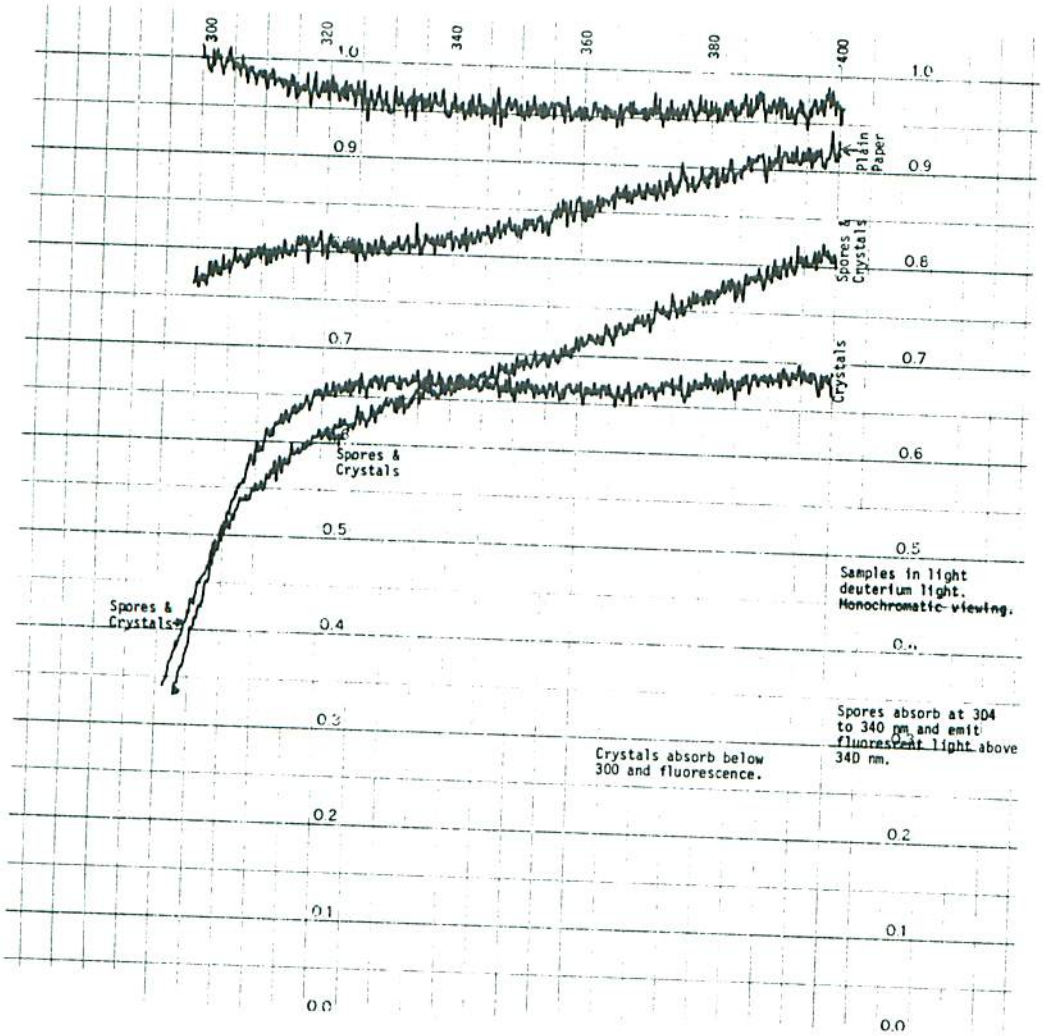

FIG. A1

Spectral reflectance of Bacillus thuringiensis spores and crystals -- deuterium light.

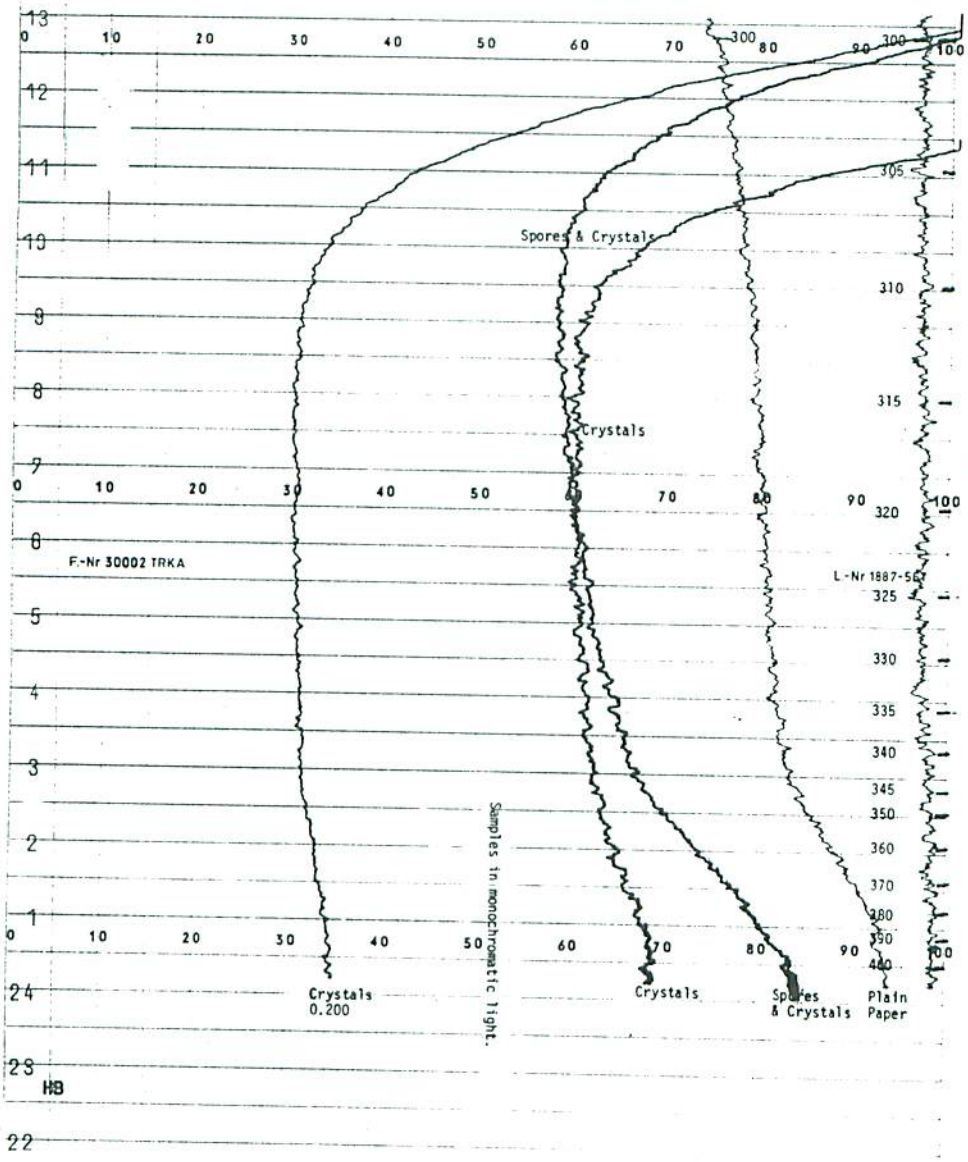

FIG. A2

Spectral reflectance of Bacillus thuringiensis spores and crystals - monochromatic light.

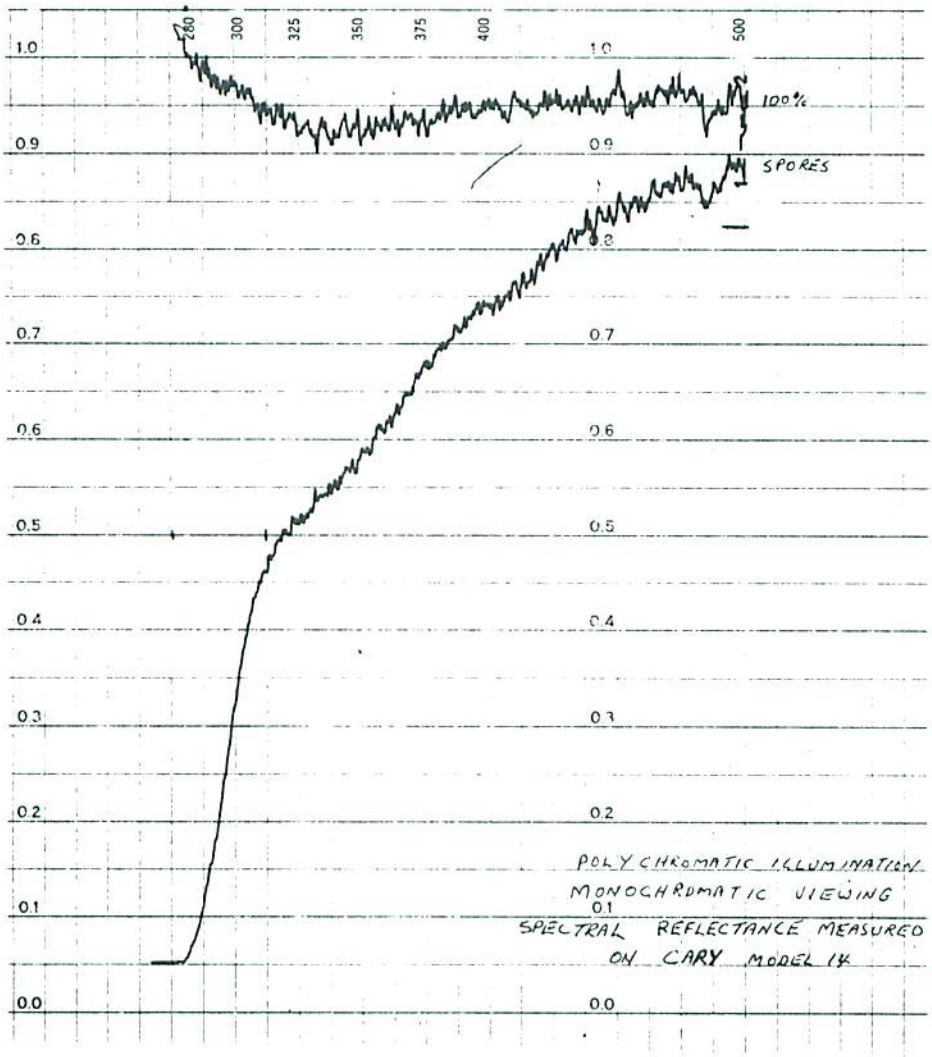

FIG. A3

Spectral reflectance of B. thuringiensis spores - polychromatic light with monochromatic viewing.

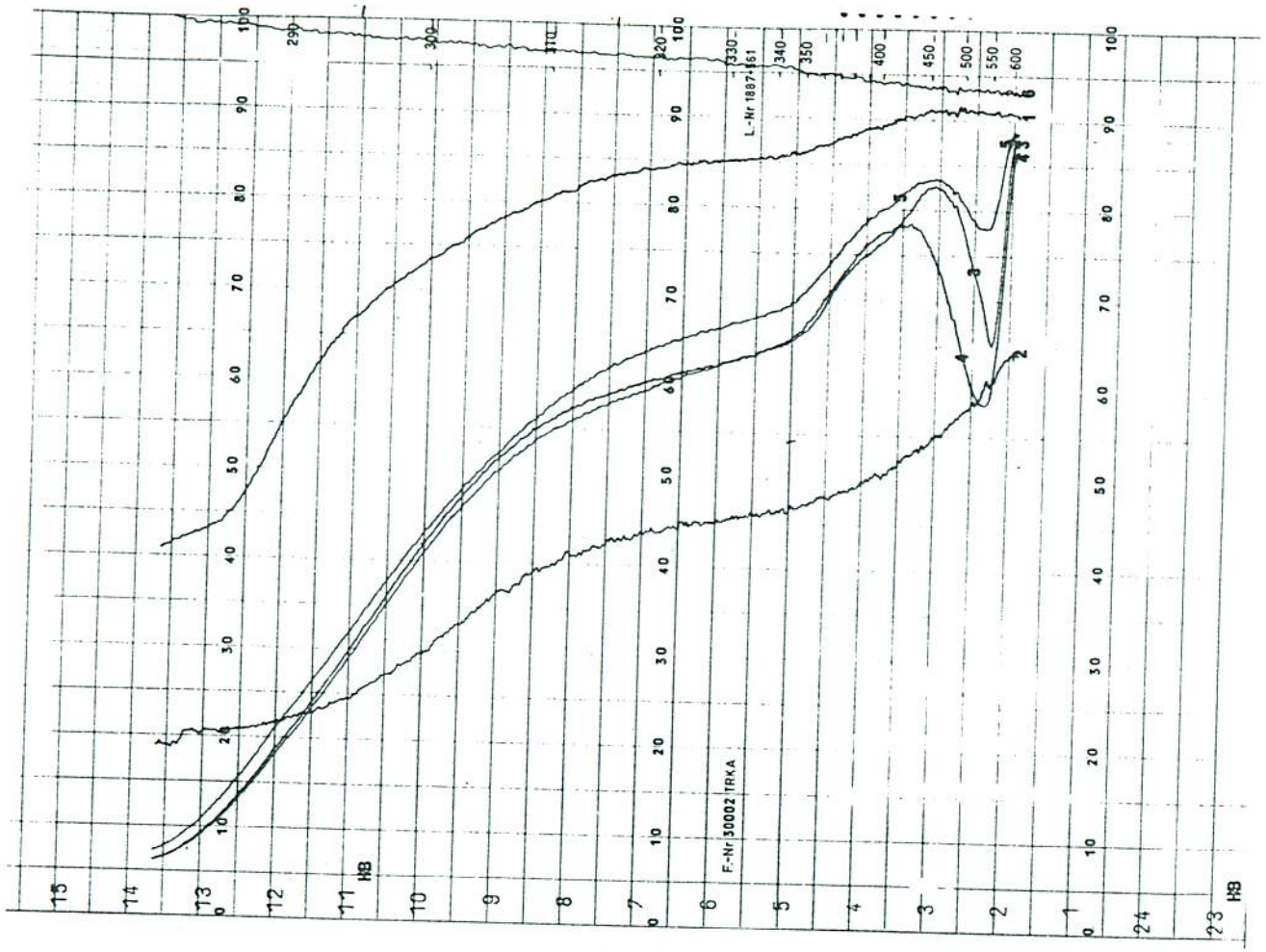

FIG. B1

Monochromatic Illumination - Curve 1, Plain filter; Curve 2, NPV; Curve 3, Erio Acid Red XB .01% and spores; Curve 4, Maxilon Brilliant Pink B .001% and spores; Curve 5, Maxilon Brilliant Pink B .0001% and spores; Curve 6, 100%.

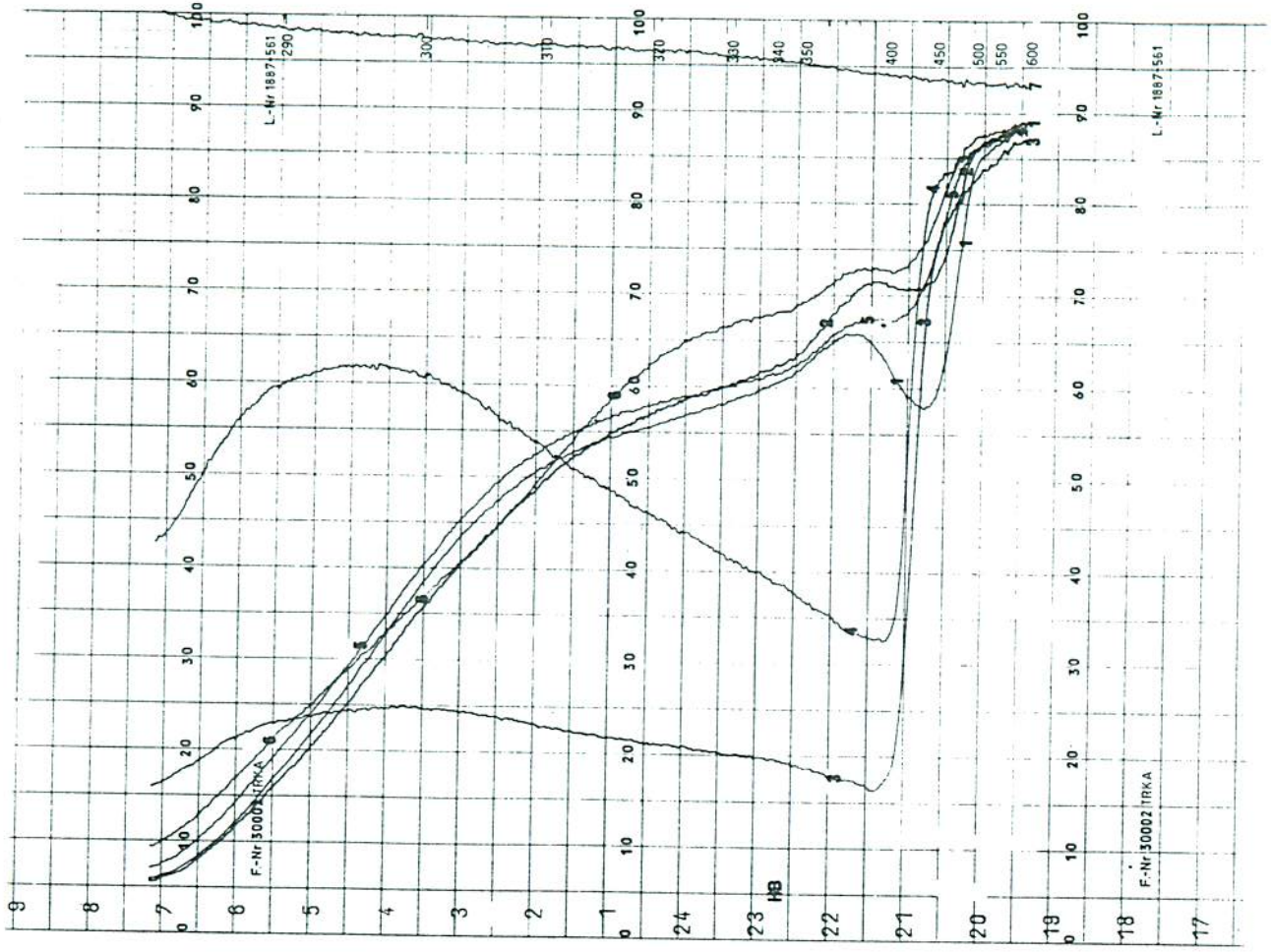

FIG. B2

Monochromatic Illumination. All these spores plus the following:

Curve 1 - Maxilon Brilliant Flavine .01%; Curve 2 - Maxilon Brilliant Flavine .001%;

Curve 3 - Uvitex EFN P 0.1%; Curve 4 - Uvitex EBF 0.1%; Curve 5 - Erio Yellow FF 0.1%;

Curve 6 - BSF 0.1%; Curve 7 - 100%.

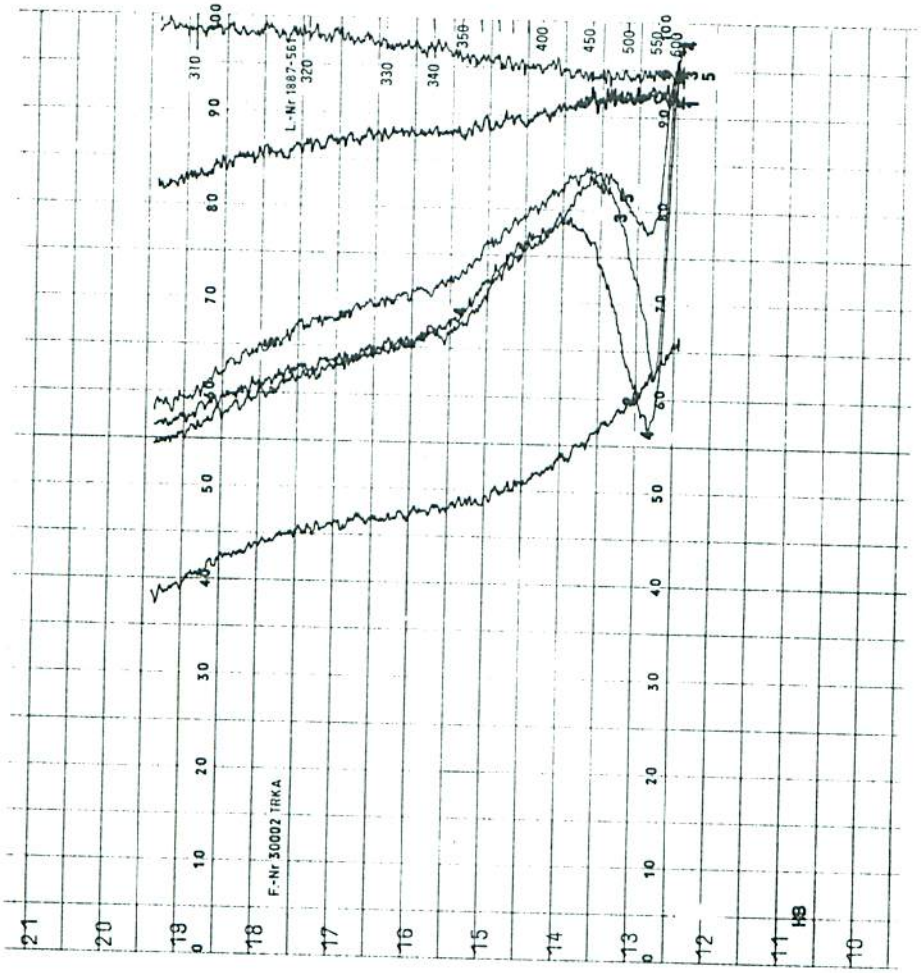

FIG. B3

Polychromatic Illumination.
Sample spores plus:

Curve 1 - Plain filter.
" 2 - NPV
" 3 - Erio Acid Red .01%
" 4 - Maxilon Brilliant Pink .001%
" 5 - Maxilon Brilliant Pink .0001%
" 6 - 100%

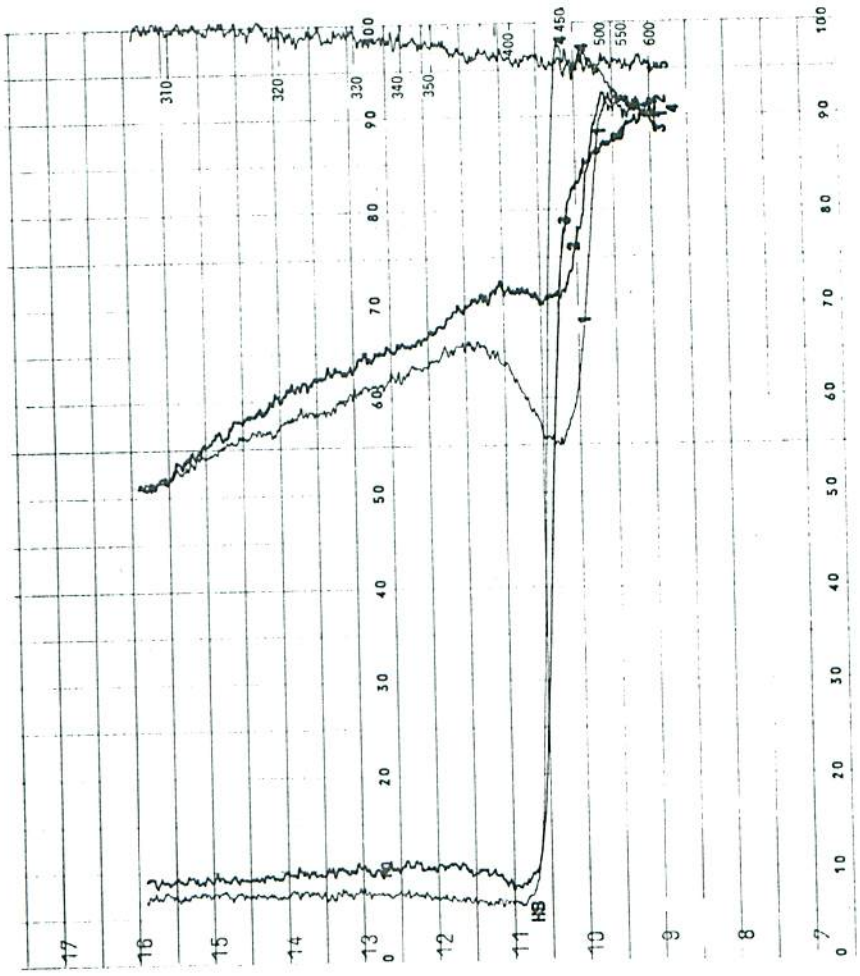

FIG. B4

Polychromatic Illumination.
Sample plus smores.

Curve 1 - Maxilon Brilliant Flavine .01%
" 2 - Maxilon Brilliant Flavine .001%
" 3 - Uvitex ERN P 0.1%
" 4 - Uvitex EBF 0.1%
" 5 - 100%

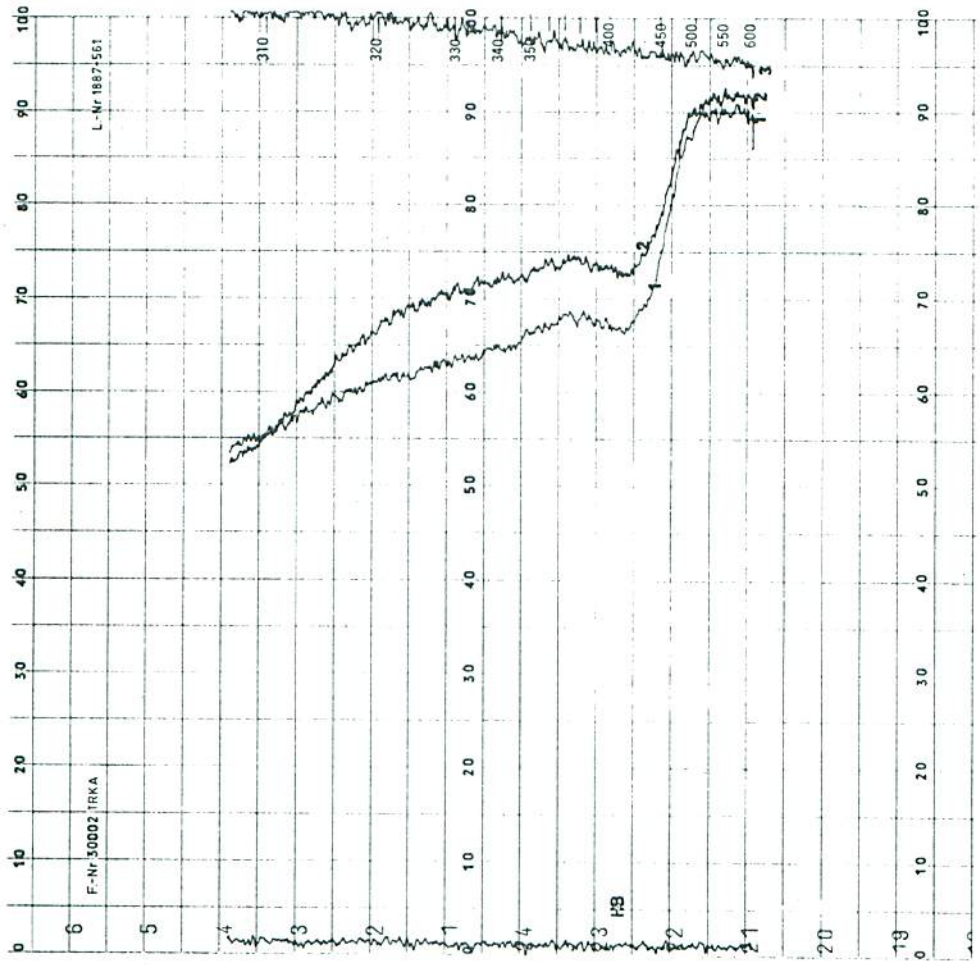

FIG. B5

Polychromatic Illumination.
Samples plus spores.
Curve 1 - Erio Yellow FF 0.1%
" 2 - BSF 0.1%
" 3 - 100%

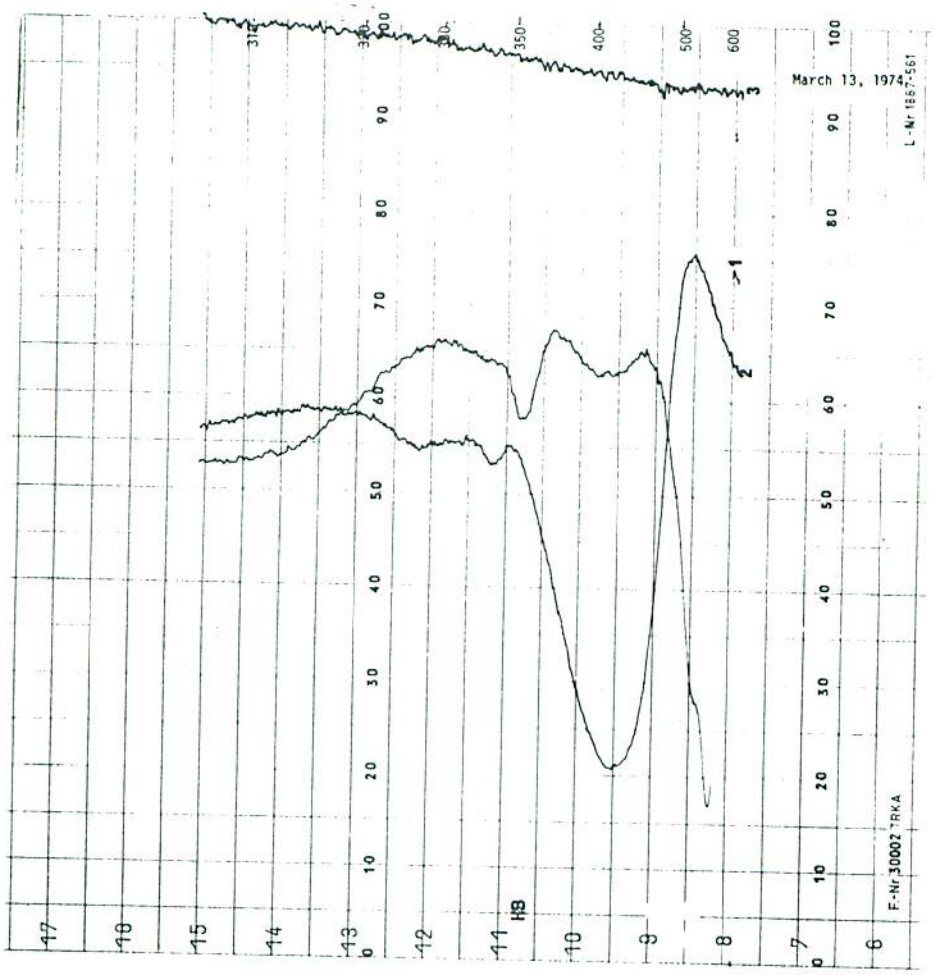

FIG. B6

Polychromatic Illumination.

Samples with no spores.

Curve 1 - Erio Acid Red .1%
" 2 - Erio Yellow .1%
" 3 - 100%

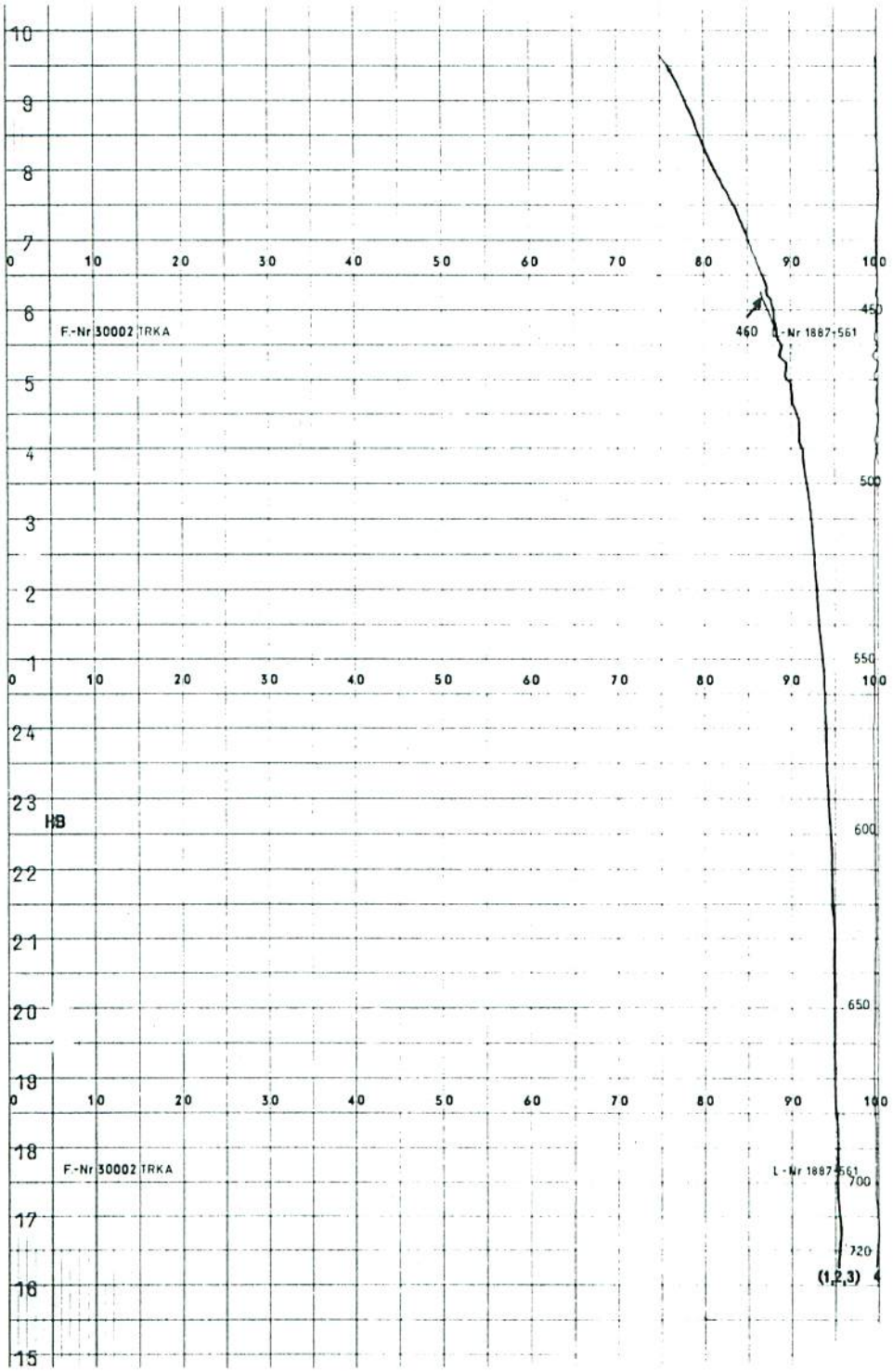

FIG. B7

Polychromatic Illumination. Spores only.

Curve 1 - No filter; Curve 2 - 400 nm cutoff filter;
Curve 3 - 460 nm cutoff filter; Curve 4 100%

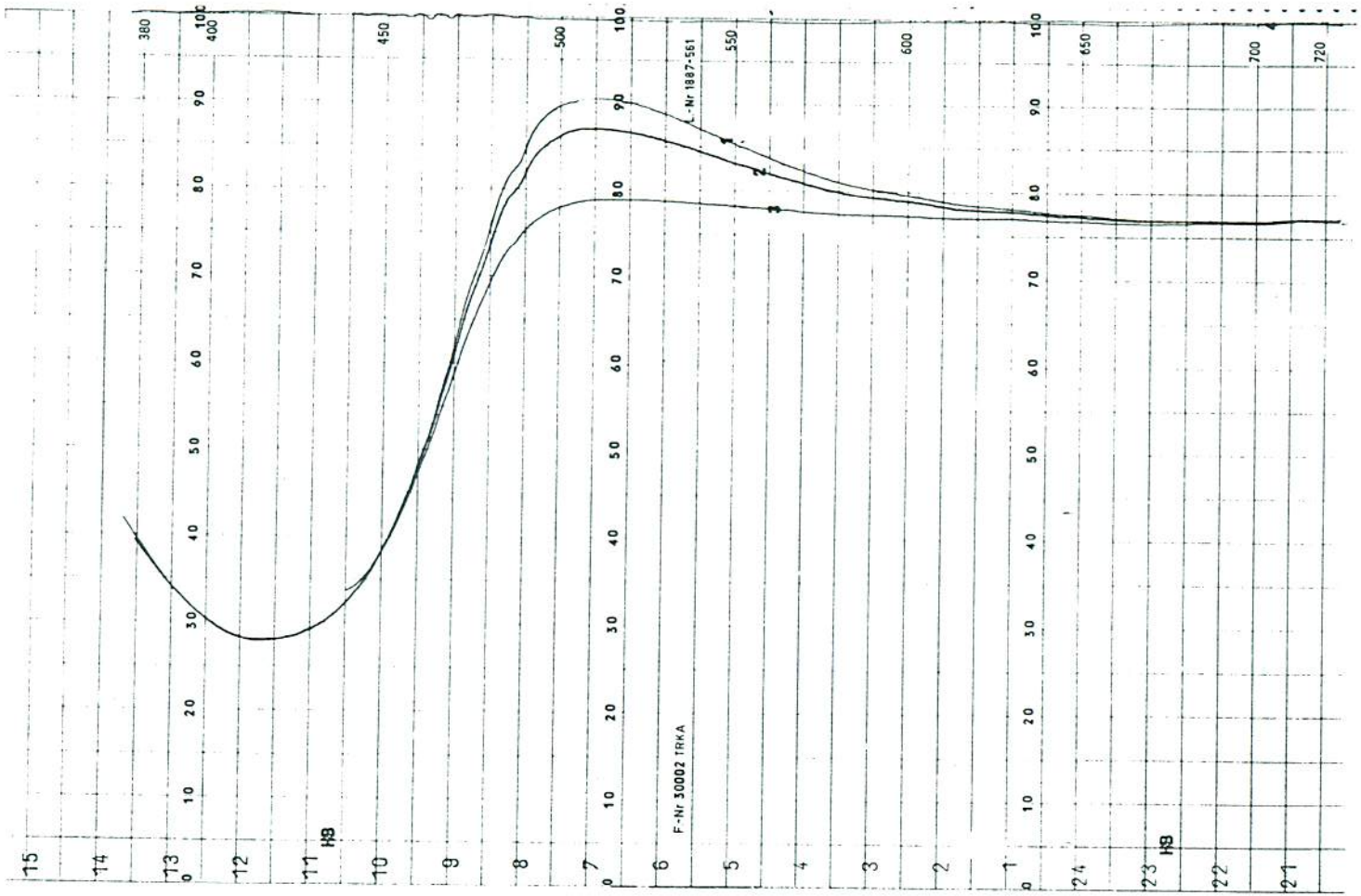

FIG. B8

Polychromatic Illumination. BSF only, 0.1%.

Curve 1 -- Xenon arc, no filter.
" 2 - 400 nm cutoff filter plus arc.
" 3 - 460 nm cutoff filter plus arc.
" 4 - 100%.

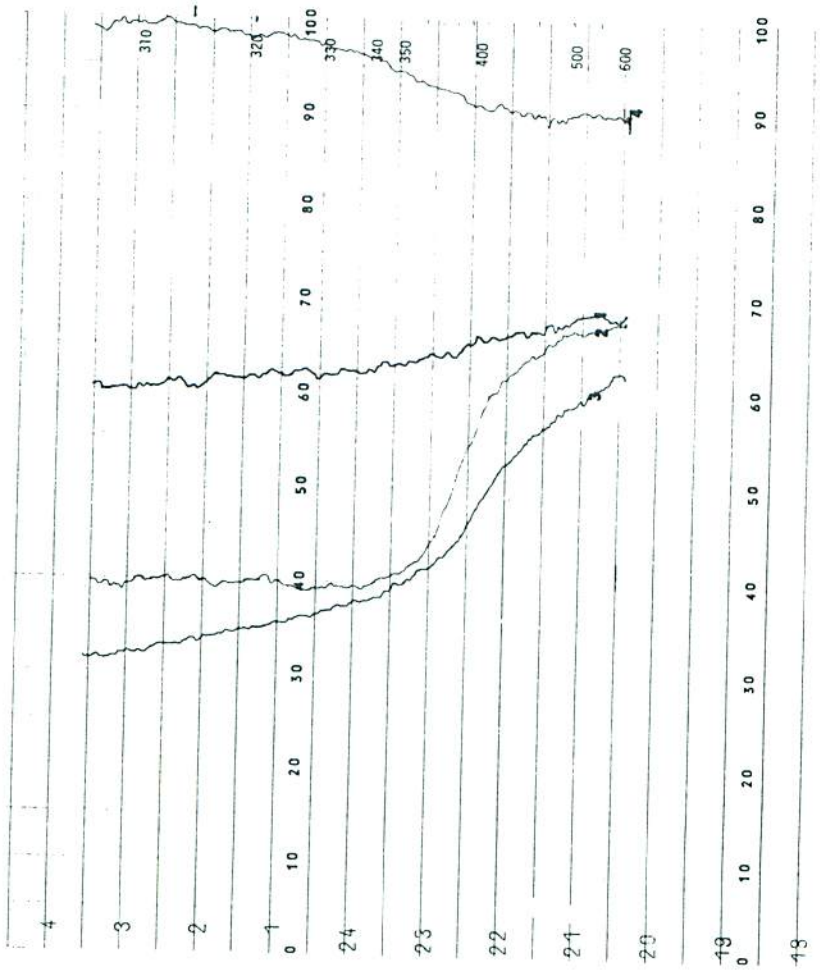

FIG. B9

Polychromatic Illumination. Samples on paper.

Curve 1 - Soy sauce.
" 2 - Molasses 10%
" 3 - Tea
" 4 - 100%

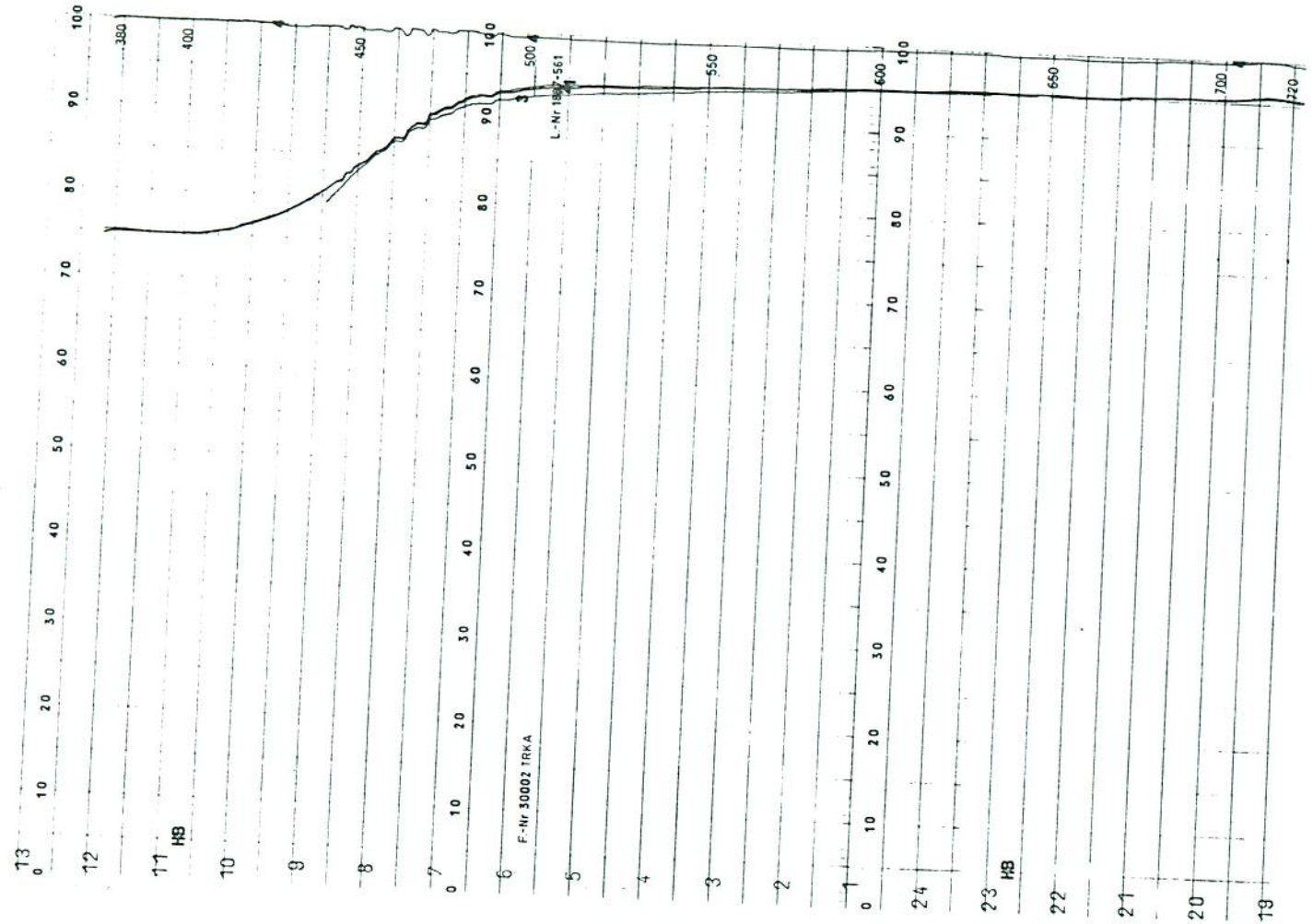

FIG. B10

Polychromatic Illumination. BSF and spores.

Curve 1 - Xenon arc, no filter.
" 2 - 400 nm cutoff, filter plus arc.
" 3 - 460 nm cutoff, filter plus arc.
" 4 - 100%

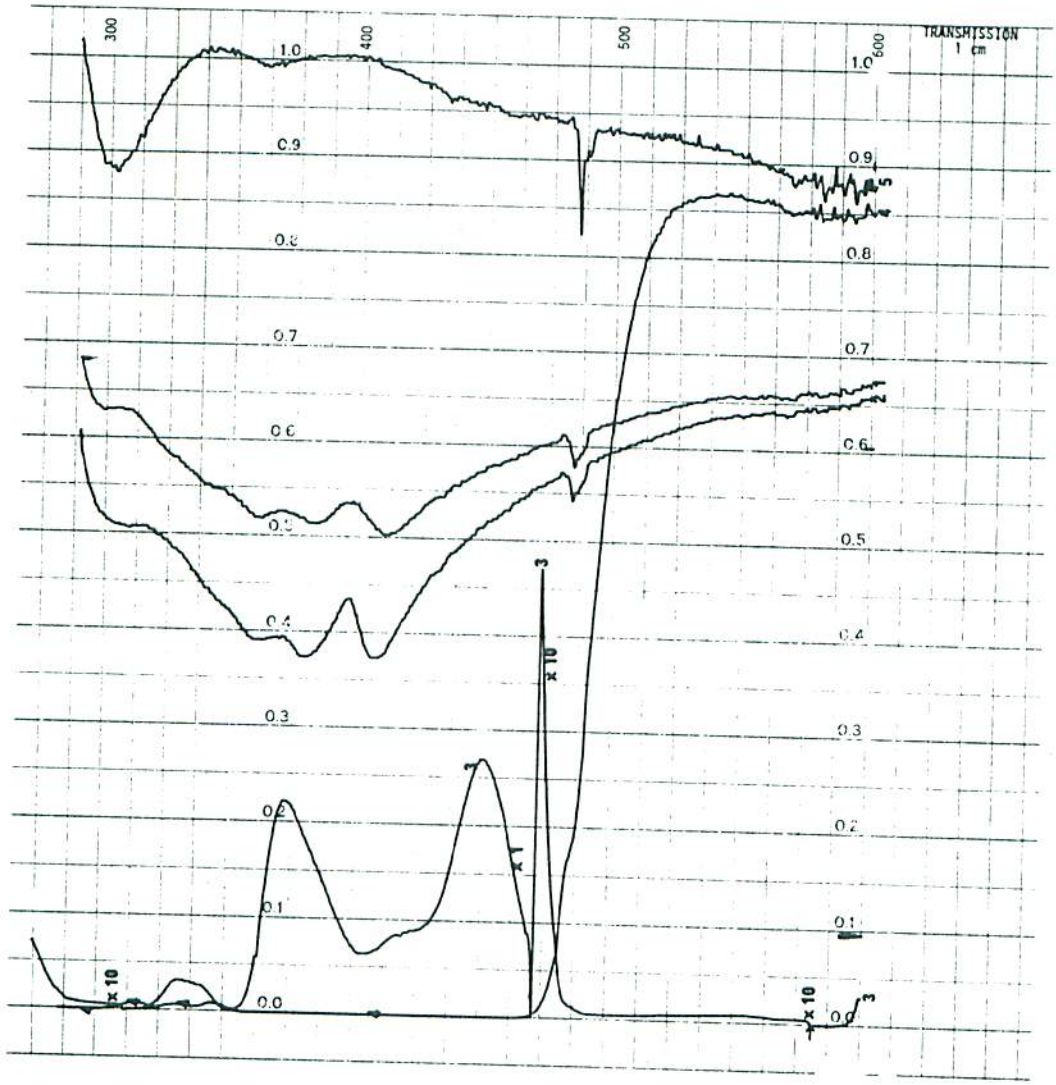

FIG. B11

Transmittance 1 cm cell.

Curve 1 - Uvitex EBF .01%
" 2 - Uvitex ERN .01%
" 3 - Erio Acid Red .1%
" 4 - BSF .01%
" 5 - H_2O

Curve 1 - Polychromatic Illumination, BSF alone on paper.
" " 2 - Monochromatic Illumination, BSF alone.
" " 3 - 100%

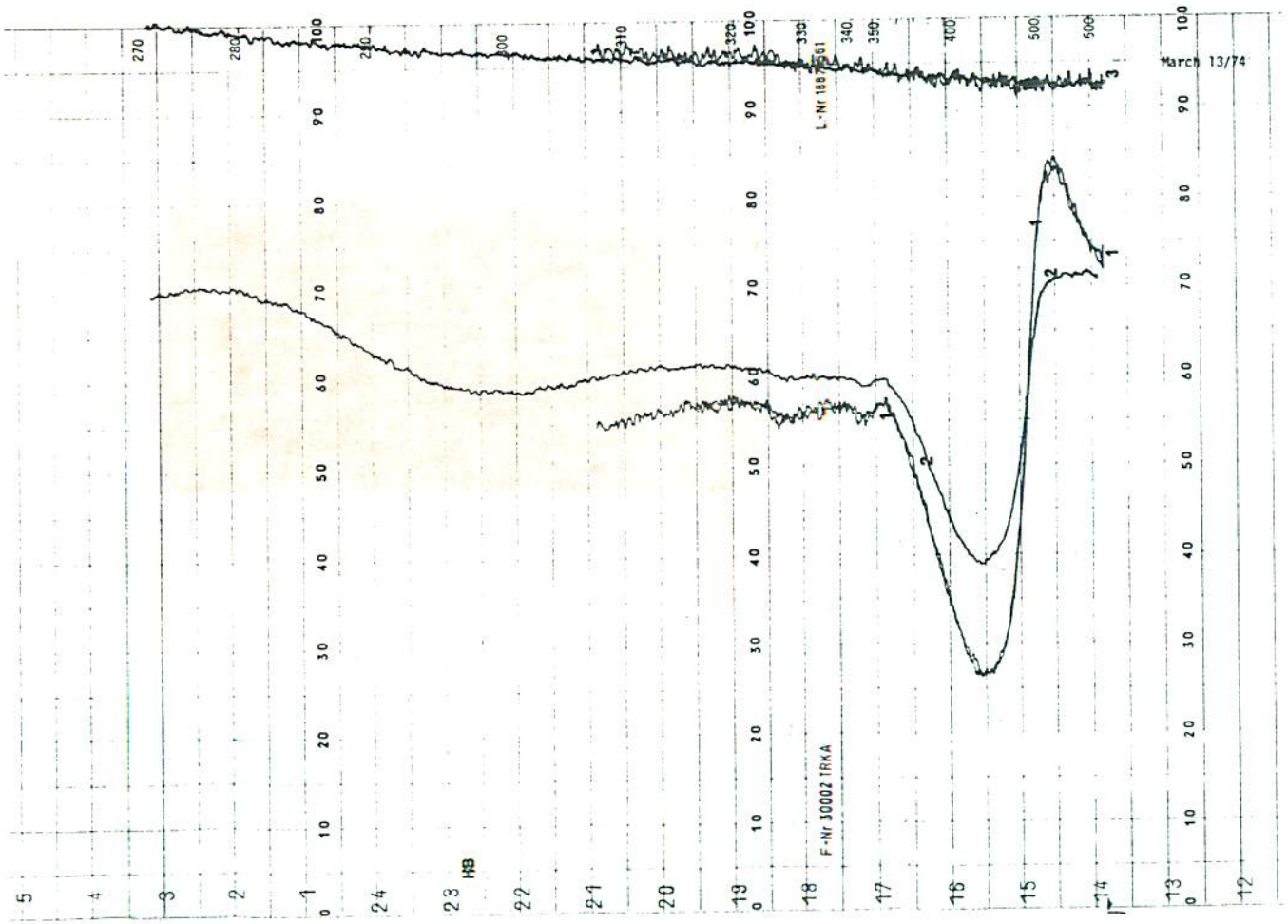

FIG. B12