PART VI.

THE APPLICATION OF MICRO-METHODS TO THE ANALYSIS OF LAC PRODUCTS.

By C. R. Somayajulu.

INTRODUCTION.

In the study of the physiological significance of some constituents of rare stick-lacs, and particularly in an attempt to follow the development of lac on experimental host-plants grown in nutrient solutions of varying composition, it became necessary to use methods of micro-analysis. The quantities of material available were often so small as to be barely enough for the determination of a single constant in the ordinary way. The methods chosen were based on those of Gill and Simms.[1] According to these authors, as little as eleven milligrams of oil can be employed for the iodine value, and twenty milligrams for the saponification number without jeopardising the accuracy of the results, and they applied their methods successfully to the analysis of cotton seed, linseed, lard and olive oils. The present paper gives the results of using similar micro-analytical methods for the determination of the iodine for saponification and acid-values of the resin and wax occurring in stick lac; the values for other well-known substances being also determined for the purpose of comparison.

For micro-titrations, Gill and Simms describe a special burette made from an ordinary 10 c.c. pipette (30 c.ms. long) which terminates in a long, finely drawn-out tip, on to the lower end of which is fused a slender glass rod, down which the solution trickles when the burette is in use. This is connected to a wash bottle of solution provided with a ball or bead valve, by manipulating which the flow of liquid from the burette is regulated. With such a burette the authors state that drops can be withdrawn as small as 1/50 to 1/60 c.c.

For the determination of iodine value, a quantity of the substance, not less than eleven milligrams, is weighed out accurately into a small weighing bottle with a ground-glass stopper. It is dissolved in 1 c.c. of chloroform, 3 c.c. of Hanus iodine solution are added, and

[1] *Jour. Ind. Eng. Chem.*, 1921, 13, 547.

the bottle shaken at intervals for 15 minutes; 1.5 c.c. of a 10 per cent. solution of potassium iodide is then added, and the whole immediately titrated against 0·1 N sodium thiosulphate. The liquid is kept stirred during titration by a small coiled glass rod, a drop of starch being used as indicator at the end of the titration.

In the present investigation, the factors of quantity of reagent, and time of reaction were studied with various typical unsaturated compounds.

DESCRIPTION OF RESULTS.

Iodine values.—In Table I are given some figures for such substances as oleic acid, castor and linseed oils, and rosin. Except in the last case, where a peculiar variation is observed, and as to which a separate note is appended at the foot of Table III, the results are in fair agreement with those obtained by the several other methods. The best time of reaction was found in all cases to be about 30 minutes.

It was found necessary to use the same pipette and exactly the same volume of iodine solution for each set of experiments, otherwise with a burette such as the one used of 10 c.c. capacity, graduated into 0·02 c.c., but capable of delivering through the fine capillary tip, with a little manipulative practice, a drop of 0·01 c.c., a difference in the addition of one ordinary drop of the titre would introduce an error of 3–4 per cent., if the iodine value of the sample is small (e.g. about 10) as in the case of lac-wax. The strength of the thiosulphate may advantageously be reduced to 0·05 N, without detriment to the sharpness of the endpoint. Incidentally, the effect was observed of carrying out the determination in darkness, or at varying dilutions but no appreciable differences were noted.

In Table II are given a number of determinations of the iodine value of lac-wax obtained from *Shorea talura* (Mysore) seed-lac by extraction with petroleum ether. This is a good representative of insect waxes. The best results were obtained when using 10 milligrams of wax, 1 c.c. of carbon tetrachloride and 2 c.c. of Hanus' solution, allowing 20 minutes for the reaction.

The saponification value.—For the determination of the saponification value, Gill and Simms also recommend the use of one-tenth the customary quantities for analysis. The strength of alcoholic potash is kept semi-normal, but the acid strength is reduced to 0·1 N. The minimum quantity of an oil taken is 20 milligrams.

The present experiments show that with about 15 milligrams of an oil, fat or wax, and 2 c.c. of 0·5 N alcoholic potash, the values are very nearly correct. Conical flasks of 50 c.c. capacity were used for saponification, with small, specially designed, reflux condensers. In the case of an oil, fat or resin, 1·5 hours heating on the water bath would bring about complete saponification, whereas, in the case of waxes, about 3·5-4 hours heating is required. The values obtained are given in Table IV.

The Acid Value.—The acid value could be correctly determined by taking 15 milligrams of a fat, oil, wax or resin. Should, however, the acid value be very low, as in the case of oils, the samples are dissolved in alcohol (neutral 99 per cent.), 5 c.c. of 0·1 N alcoholic potash added, and the liquid immediately titrated against standard acid with phenol-phthalein as indicator. If, however, the acid value is not very low, as in the case of the solutions of lac under investigation, the alcoholic solution could be directly titrated against standard alcoholic potash. In the case of waxes, the samples are heated, when an emulsion is formed which can be titrated hot. In each of these determinations, two blanks, as a rule were made.

CONCLUSIONS.

i. The iodine, saponification and acid values of oils, fats, waxes and resins can be correctly determined by micro-methods.

ii. In the case of the iodine value, 10 milligrams of material serves for each determination; in the case of saponification and acid values about 15 milligrams are required.

iii. For the determination of iodine values, the best period to allow was found to be 30 minutes, 2 c.c. of Hanus' reagent being used.

iv. For determination of saponification values of oils and fats, 1-1·5 hours' heating on the water-bath with 2 c.c. of 0·5 N alcoholic potash is recommended. For waxes, 3·5 to 4 hours is required.

TABLE I.

Iodine values for different substances.

Method	Oleic acid	Linseed oil	Castor oil	Rosin	Time (in minutes)
I. Micro-(Hanus)	72·8	...	...	...	10
	72·6	...	...	...	10
	80·0	...	...	...	15
	80·1	...	...	...	15
	82·2	...	82·2	...	20
	83·3	...	82·2	...	20
	84·8	164·5	82·4	175·4	30
	84·9	164·0	82·0	175·9	30
	84·9	168·7	81·9	179·80	45
	84·8	168·5	81·7	...	45
II. Macro-(Hanus)	83·2	168·3	...	182·5	60
	82·8	167·6	86·2	182·3	30
	83·0	165·8	86·7	...	45
III. Wij's	77·0	170·6	84·9	169·1	30
	77·1	170·5	84·9	169·8	30
	79·4	172·4	85·7	174·6	60
	79·6	171·8	...	175·4	60
IV. Winkler	75·3	...	...	...	10
	74·8	...	...	...	10
	84·4	...	...	...	20
	83·9	...	...	...	20
	86·0	171·4	85·9	171·6	30
	85·8	171·6	86·3	172·6	30
V. Hubl	79·5	...	...	...	18
	80·0	...	...	...	18
	81·2	172·3	86·0	174·9	24
	81·4	172·1	86·2	175·2	24

TABLE II.

IODINE VALUES.

Shorea talura lac-wax.

Method and quantity		Time in minutes	Solvent. Carbon tetrachloride c.c.	Iodine solution c.c.	Iodine value
Micro Hanus ...	0·0100	10	1	3	9·5
	0·0114	10	1	2	9·6
	0·0138	15	1	3	10·2
	0·0098	15	1	2	10·2
	0·0111	20	1	3	10·4
	0·0104	20	1	2	10·4
	0·0108	30	1	3	10·5
	0·0100	30	1	2	10·5
Macro Hanus ...	0·4086	10	10	30	9·5
	0·4162	15	10	10	9·9
	0·4080	15	10	15	10·2
	0·4108	15	10	20	10·4
	0·4064	15	10	25	10·5
	0·4186	15	10	30	10·4
	0·4224	20	10	30	10·5
	0·4150	30	10	30	10·7
Winkler ...	0·3246	30	30	30	10·5
	0·4180	60	30	30	10·6
Hubl ...	0·4250	18 hrs.	30	30	10·4
	0·5340	24 ,,	30	30	10·5

TABLE III.

IODINE VALUES OF ROSIN.

Quantity in gms.	Time in minutes	c.c. Iodine solution	Iodine value
0·0302	30	2	143
0·0222	30	2	174
0·0220	30	2	175
0·0196	30	2	186
0·0116	30	2	228
0·0116	45	2	241
0·0116	45	2	245
0·0114	30	2	246
0·0111	30	2	231
0·0109	30	2	240
0·0106	15	2	237
0·0102	15	1	165
0·0100	30	1	176
0·0100	45	1	180
0·0100	60	1	185
0·0079	30	2	280

Note.—It will be seen that if the volume of iodine solution is kept constant at 2 c.c. the iodine value markedly increases, not only with time but with the decreasing quantities of rosin taken. As the quantity of rosin diminishes the iodine value tends to increase almost in the inverse ratio. But if the volume of the iodine solution is reduced to 1 c.c. the values for about 10 milligrams of rosin tend to approach more nearly the values obtained by other methods. For comparable results, therefore, a definite ratio must be maintained between the weight of rosin and the volume of iodine solution taken.

TABLE IV.

Saponification values for different substances by the micro-method.

Time in hours	Seed-lac	Lac-resin	Castor oil	Linseed oil	Rosin
1	211	201	181	190	178
1	211	202	183	191	180
1	212	200	184	192	180
1·5	210	202	184	192	180
1·5	211	201	185	192	180
1·5	211	200	184	192	180
2	211	202	184	190	199
2	211	202	183	191	180
2	212	201	184	191	180

TABLE V.

Saponification values for Shorea talura lac-wax.

Time in hours	1·0	1·5	2·0	2·5	3·0	3·5	4·0
	Saponification values.						
	45·8	52·2	58·6	66·2	75·8	79·8	80·4
	46·4	51·4	58·4	66·6	75·6	79·6	80·8
	45·6	52·4	59·0	66·8	75·4	80·4	81·0
	45·6	52·3	58·6	66·4	76·0	80·2	80·2
	45·9	52·6	58·8	67·0	75·2	80·6	79·9
	45·4	52·0	58·5	67·2	75·9	79·9	80·5