LETTERS TO THE EDITOR

SPECTROGRAPHIC DETERMINATION OF TRACE IMPURITIES IN STRONTIUM CARBONATE

IN electronics industry, strontium carbonate is required in a highly purified form for cathode spraying and thus it becomes necessary to analyse $SrCO_3$ for trace impurities. A spectrographic method is, therefore, developed for this purpose. A literature survey did not show any suitable method for the analysis of $SrCO_3$ as matrix for impurities in parts per million (ppm) range. The present method is simple, straight-forward and very convenient for routine and rapid analysis of Al, Ba, Ca, Cd, Cu, Fe, Mg, Na, Pb and Zn in parts per million range.

Standards are prepared synthetically by dry mixing the specpure grade oxides of above-mentioned impurities with specpure $SrCO_3$. All compounds used are supplied by Johnson Matthey and Co. The sample (standard) is thoroughly ground with equal quantity of specpure U.C.C. (Ultra Carbon Corporation) graphite containing 100 ppm of specpure Cr_2O_3 in order to use Cr as internal standard element. 50 mg of this mixture is loaded in the cavity of 6·15 mm diameter preformed 100–L U.C.C. graphite electrode and warmed under an infrared lamp for 10 minutes to drive away traces of moisture. The sample as anode is excited at 15 amps. d.c. for a period of 40 seconds. The spectrum is then photographed on Ilford R–40 emulsion and in the region 2750 Å–3375 Å in the first order of a 1,200 lines/mm grating blazed at 3,300 Å and employing Jaco 3·4 meter Ebert Spectrograph.

In order to improve the sensitivity of the method and to avoid selective volatilisation of the impurities, the experiments were conducted with different ratios (4 : 1) and (1 : 1) of sample to graphite and at 15 and 10 amperes d.c. excitation. It is found that the ratio of 1 : 1 at 15 amperes d.c. improved the sensitivity and volatilisation of the impurities. In addition Bi 2897·97 Å and Cr 2835·6 Å were tried as internal standard elements for volatile and non-volatile impurities. It is observed experimentally that the average standard deviation calculated with respect to these internal standard elements are of the same order. Consequently Cr 2835·6 Å is used as internal standard line. Other analytical details are given in Table I. The working curves are plotted for a set of standards for different impurities. The curves are linear and practically parallel to each other. Since the blank $SrCO_3$ contains residual amounts of Fe = 2 ppm and Mg = 2 ppm, the working curves are plotted by applying correction by method of trial additions.

TABLE I

Analytical data for determination of impurities in strontium carbonate

Sl. No.	Analytical line	(Å)	Concentration Range (ppm)	Precision ± %
1.	Al	3082·2	1–20	13
2.	Ba	3071·6	50–200	11
3.	Ca	3179·3	20–200	12
4.	Cd	3261·1	10–200	10
5.	Cu	3274·0	1–20	13
6.	Fe	2966·9	1–20	14
7.	Mg	2779·8	1–20	10
8.	*Na	3302·3	50–200	..
9.	Pb	2833·1	1–20	16
10.	*Zn	3303·0	50–200	..

* These elements are estimated semiquantitatively.

Cr 2835·6 is used as an internal standard line for above elements.

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POLYPHENOLS OF *SOLANUM TORVUM*

ALTHOUGH many investigators have reported the presence of alkaloids[1-3] and steroids[4,5] isolation and identification of polyphenols from *Solanum torvum* Swartz (Family : Solanaceae, Tamil name : Sundaikai) has not been reported. Hence it is thought interesting to study the polyphenolic system in the fruits of *S. torvum* that serves as good

substrate for the polyphenoloxidase present in the same plant.

Isolation of phenolic acids.—The fresh fruits (2 kg) were stirred for a few minutes with half their volume of hot water (80° C) to inactivate the polyphenoloxidase. The fruits were then blended with aqueous methanol (80% v/v ; 8 lit.). Chlorophyll and other fatty materials were removed by shaking the methanol extract with petroleum ether (b.p. 40–60° C). The phenols were precipitated with saturated neutral lead acetate solution. The precipitate thus obtained was centrifuged and the moist precipitate was stirred repeatedly with small quantities of Amberlite IR-120 (H+ form) till a clear solution was obtained. The solution was filtered free of the resin, concentrated under vacuum and extracted repeatedly with ether followed by ethylacetate. Thin layer chromatography on silica gel layers using the solvent system chloroform-ethyl acetate-formic acid (5 : 4 : 1 v/v) showed that the ether extract mainly contained three compounds with R_f values 0·07, 0·18 and 0·60 and ethylacetate extract, two only, those with R_f values 0·07 and 0·18.

Quantitative separation of the three major compounds was carried out by column chromatography using polyamide (Ultramid B 3, BASF) as adsorbent. The column was equilibrated with benzene. The mixture of phenols was adsorbed on a little polyamide and transferred to the top of the column. Elution was carried out with benzene-chloroform mixture .50 : 50 v/v) with increasing concentrations of methanol, Initially the compound with R_f 0·6 was eluted with 5% methanol in 50 : 50 benzene-chloroform mixture (Band I). With 15% methanol in 50 : 50 benzene-chloroform mixture, the compound with R_f 0·18 was eluted (Band II). In the later stage, the compound with R_f 0·07 (Band III) was eluted along with traces of Band II.

Band I, crystallized from methanol, was found to melt at 210–212° C. Mixed m.p., and superimposability of I.R. spectra with an authentic sample (Koch-Light Laboratory) showed that Band I is caffeic acid.

Analysis

Found C, 60·22, H. 4·59 ;

Calculated for $C_9H_8O_4$: C, 60·02 ; H, 4·67%.

Band II could not be crystallized but was obtained as and amorphous powder by precipitation with butylacetate-chloroform mixture (1 : 10 v/v). This compound was characterized as isochlorogenic acid by comparison with authentic sample isolated from coffee beans following the method of Barnes *et al.*[6]. Though isochlorogenic acid behaves as a single compound during isolation or in chromatography in non-aqueous solvents, it was resolved into 4, 5–, 3, 4– and 3, 5–dicaffeoylquinic acids (designated as isochlorogenic acid A, B and C) by Corse *et al.*[7] by counter-current distribution. A 4th compound which appeared to be a mixture of the 3'-methylethers of 3, 5-dicafleoylquinic acid was also obtained by them. Earlier Lentner and Deatherage[8] also reported the resolution of isochlorogenic acid into four spots by paper chromatography. The resolution of isochlorogenic acid into four spots was also confirmed in the present study using thin layer chromatography over silica gel layers in the solvent system chloroform-ethylacetate-formic acid (5 : 4 : 1 v/v) (R_f 0·41, 0·35, 0·32 and 0·28). All these observations indicated the presence of dicaffeoylquinic acids which were supported by elementary analysis.

Analysis

Found C, 55·74 ; H. 4·98 ;

Caculated for $C_{25}H_{26}O_3.H_2O$: C, 56·17, H, 4·90%.

Band III was purified first by fractional precipitation with methanol-ethylacetate-petroleum ether mixture (1 : 3 : 10 v/v) and then by repeated crystallization with water, m.p. 208–210° C. Co-chromatography, mixed m.p. and superimposability of I.R. spectra with an authentic sample isolated from coffee beans confirmed its identity as chlorogenic acid.

The ethylacetate fraction containing only chlorogenic and isochlorogenic acids was partitioned between butylacetate and phosphate buffer[6] (pH 5·2, 2 M). The butylacetate fraction was found to contain isochlorogenic acid.

The aqueous solution was extracted with *n*-butanol and the extract was chromatographed on a polyamide column as described earlier. Elution with 15% methanol in benzene-chloroform (50 : 50 v/v) mixture yielded chlorogenic acid.

Neochlorogenic acid was eluted with 25% methanol in benzene-chloroform (50 : 50 v/v) mixture and it was identified by cochromatography with an authentic sample isolated from coffee beans m.p. 204–206° C.

The yield of acids : Caffeic acid, 100 mg ; Chlorogenic acid, 1·09 g ; Neochlorogenic acid, traces and Isochlorogenic acid, 150 mg.

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A NOVEL AND RAPID METHOD FOR *IN VITRO* TESTING OF ANTIAMOEBIC AGENTS AGAINST AEROBIC AND ANAEROBIC AMOEBAE GROWING AXENICALLY OR WITH BACTERIA

VARIOUS methods have been used in the past for screening antiamoebic compounds *in vitro* against anaerobic *E. histolytica, E. invadens*, etc., and aerobic free-living amoebae of pathogenic and non-pathogenic types[3-9]. Many conflicting results as to the efficacy of a given drug have been reported[1-2]. For the first time, a simple rapid and reliable cavity slide method of *in vitro* drug testing, against aerobic and anaerobic amoebae growing axenically or with bacteria, has been described in this communication.

Experimental.—Trophozoites of 24 to 48 hr old cultures growing in modified Boeck and Drbohlav medium[10] with mixed bacterial flora and rice starch were pooled and their number counted by haemocytometer. 1,000 and 2,000 trophozoites in 0·2 ml were added to hallow ground slides (Fig. 1). Each cavity was filled with 0·7 ml of liquid overlay of fresh B and D medium and a small quantity of sterile rice starch (Difco) was added. The cavities were covered with coverslips and put in moist chamber in petri dishes and incubated at 37° C for ½ to 1 hr for amoebae to become motile. 0·1 ml of the appropriate drug concentration was thin added taking care that no air bubble remained in the cavity. The edges of the cover slips were sealed with paraffin wax. In control 0·1 ml of distilled water was added.

In the case of drug screening against axenically grown *E. histolytica*, trophozoites of 48 to 72 hr old cultures growing in modified Diamond's medium[11,12] were collected by centrifugation following the method of Das and Prasad[13]. 0·2 ml inoculum containing about 2,000 amoebae was put into cavity slide and filled with fresh medium (0·8 ml) containing the required concentration of the drug dilutions. The cavity was covered with cover slips, and the edges of cover slip were sealed with paraffin wax. The slides were put in moist chamber at 37° C (Fig. 1). Observations were taken after 6, 18, 24, 48 and 72 hr under inverted microscope to find out whether the amoebae were dead or alive. In doubtful cases subcultures were made in fresh culture medium. Petri dishes served as moist chamber were sealed from outside by adhesive tape to avoid contamination. In the case of the control, no drug was added. Duplicate sets were run for each drug dilution.

Cavity Slides Inside Moist Chamber

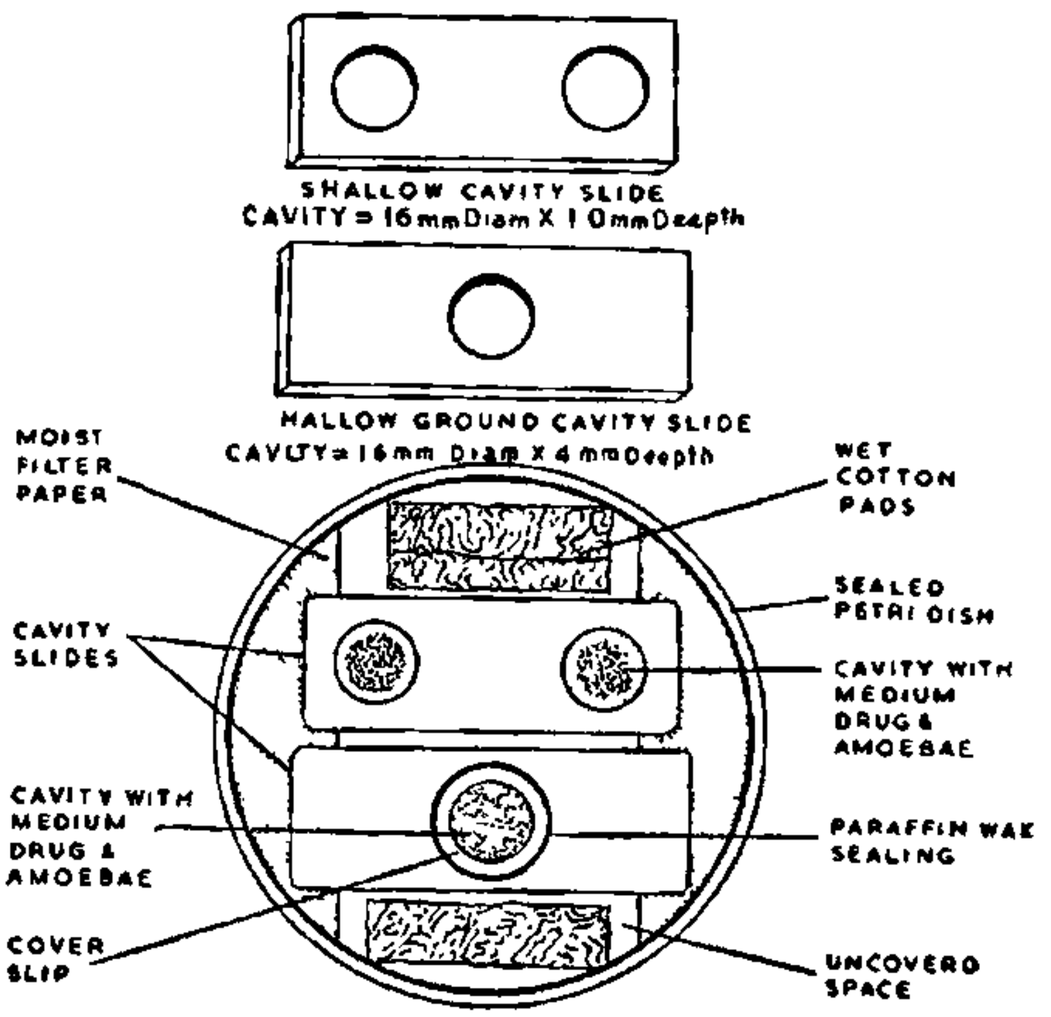

FIG. 1. Diagrammatic presentation of cavity slide method for antiamoebic drug screening.

In the case of the aerobic free-living amoebae, trophozoites from 24 to 48 hr old cultures growing on non-nutrient agar and bacteria (14) were washed with sterile distilled water by centrifugation and suspended in water containing *Escherichia coli* bacteria. The number of amoebae was determined by haemocytometer. The suspension (0·1 ml) containing about 1000 trophozoites was added to a cavity slide. (16 mm in diameter and 1·0 cm deep). The slides were put in moist chamber in Petri dishes and incubated at 25° C or 37° C for 30 minutes for amoebae to become motile. Drug (0·1 ml) was then added to a slide and slides were incubated for 6, 18, 24 and 48 hr. In the control 0·1 ml distilled water was added. Microscopic examination was done to find out whether the amoebae were dead or alive. In doubtful cases subcultures were made on non-nutrient agar plates