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Iodometric Estimation of Sucrose in Purified Duck Embryo Vaccine for Rabies

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A simple and rapid iodometric titration method was developed and validated for the determination of sucrose in purified duck embryo vaccine for rabies. The lyophilized formulation of rabies vaccine containing NMT 25 mg sucrose per vial was detected by iodimetry titration. The formulation containing sucrose, on acid hydrolysis gives glucose, which can be estimated by iodometric titration. The percentage RSD was found to be NMT 10 %. The method was linear over the concentration range from 60 to 140 % of target concentration (25 mg per vial) with coefficient of correlation 0.9997. Accuracy and precision results were in acceptable limit.

Key Words: Iodometry, Vaccine, Sucrose.

INTRODUCTION

Vaccine is a preparation of one or more microbial antigens used to induce protective immunity. These are suspensions of killed, living, or attenuated (having weakened virulence) cultures of microorganisms used as antigens to produce immunity against infection due to the particular microorganisms. Rabies vaccine is an active immunizing agent used to prevent infection caused by the rabies virus[1].

Sucrose is used as an excipient in rabies vaccine. Rate zonal centrifugation in sucrose gradients revealed that the rabies virus has a sedimentation coefficient about 600 s^2. The lyophilized vaccine should contain not more than 25 mg sucrose per vial. Sucrose is a disaccharide carbohydrate, chemically, β-D-fructofuranosyl-α-D-glucopyranoside[3], is used as pharmaceutical aid[3,4]. It has specific optical rotation in between + 65.9° and 67.0° and is determined in a 10 % w/v solution by measuring specific optical rotation[3]. Generally, the concentration of sucrose in the sample is determined refractorimetrically and the corresponding values of density and viscosity are measured[3,5,6].

Literature review reveals that there is no method for titrimatric estimation of sucrose, so it is worthwhile to develop and validate method for estimation of sucrose in purified duck embryo vaccine for rabies.

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EXPERIMENTAL

Principle: When sucrose is treated with dilute hydrochloric acid, it undergoes hydrolysis. Mild oxidizing reagents such as bromine water or an alkaline solution of iodine (sodium carbonate + KI + I_2 solution) oxidize only the aldonic sugar to respective aldonic acids (Fig. 1). When sucrose is hydrolyzed by dilute hydrochloric acid and hydrolyzed product is oxidized by alkaline iodine[5,6]. Iodine used in oxidation can be estimated by back titration of iodine with sodium thiosulphate. Each mL of 0.025 N iodine is equivalent to 2.2525 mg of dextrose. Sucrose is equivalent to 1.9 times of estimated dextrose (Fig. 2)[7].

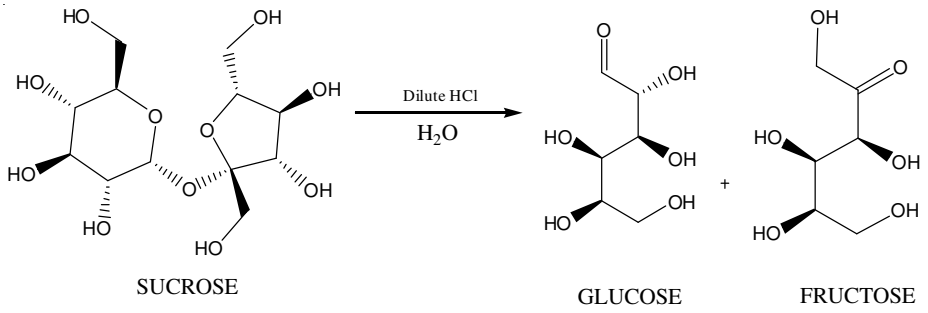

Fig. 1. Hydrolysis of the sucrose

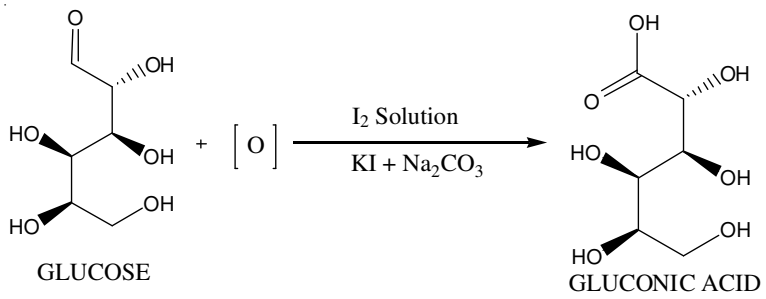

Fig. 2. Oxidation of the glucose

Lyophilized rabies vaccine was purchased from market. Analytical grade iodine, potassium iodide, hydrochloric acid, sodium thiosulphate and sodium carbonate were obtained from S.D. Fine Chemicals Ltd.

Methodology

Linear calibration graph: Working standard solution of sucrose was prepared with purified water and a series of dilutions 5, 10, 15, 20, 25, 30 and 35 mg/mL were prepared. Each concentration was treated with 5 mL of 5 M hydrochloric acid and heated on water bath for 1 h. Dilutions were made to 100 mL with water and filtered. Each filtrate (50 mL) were treated with 25 mL buffer solution (14.3 % of

sodium carbonate and 4 % of potassium iodine solution) and 50 mL of 0.025 N iodine solution for 0.5 h at 20 ºC. Excess iodine was titrated with sodium thiosulphate (0.025 M) with adding 30 mL hydrochloric acid. The linear calibration curve was plotted for the sucrose concentration against iodine volume used in glucose oxidation (Fig. 3). The linear regression equation was found to be y = 2.2465x + 0.1341 with r^2 = 0.9997.

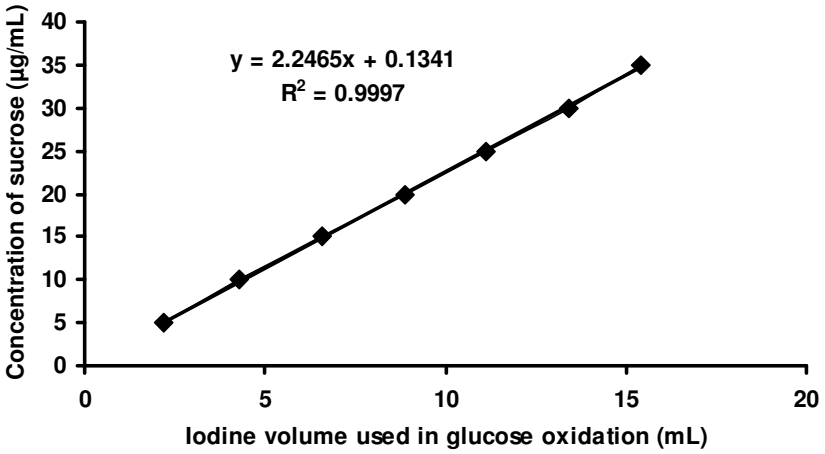

Fig. 3. Calibration graph for the sucrose estimation

Validation: Developed method was validated for the accuracy by recovery method, linearity, precision and ruggedness.

Accuracy: Various concentration of sample in the range of 50 to 150 % (50, 100 and 150 %) of the sample concentration in triplicate were prepared and analyzed. All analytes were spiked with a standard concentration of the sample. Spiked analyted were analyzed for recovery of the sample and RSD of recovery was found to be 0.57.

Linearity: Linearity was performed in the range of 20 to 160 % of standard concentration (5, 10, 15, 20, 25, 30 and 35 mg/mL in triplicates). The calibration was plotted for the sucrose concentration against iodine volume used for oxidation. The linear regression equation was found to be y = 2.2465x + 0.1341 with r^2 = 0.9997.

Precision: Repeatability was performed for intra-day and inter-day analysis. A series of dilutions were analyzed in triplicates in the same day at the interval of 2 h and at different days. The RSD values were found to be 1.49 and 1.76, respectively.

Ruggedness: Samples of the same batch were prepared and analyzed separately six times by two different analysts on different days. The RSD was found to be 2.2.

Estimation of sucrose in vaccine: Sample quantity equivalent to one dose was diluted with 20 mL of water treated with 5 mL of 5 M HCl on boiling water bath for 1 h. The solution was cooled, made to 100 mL with water and filtered. The filtrate (50 mL) was added to 25 mL of buffer solution (14.3 % of sodium carbonate

and 4 % of potassium iodide solution) and 50 mL of 0.025 N iodine solution mixture and allowed to stand for 0.5 h at 20 ºC. The excess of iodine was titrated with 0.025 M sodium thiosulphate by adding 30 mL HCl. The above process was repeated with 1 mL of duck embryo stabilizer as blank. The difference between the titration was the amount of iodine required to oxidize glucose. The amount of sucrose was calculated as per principle.

RESULTS AND DISCUSSION

Developed method was validated for precision, linearity, accuracy (by recovery studies) and ruggedness were within acceptance criteria (Table-1). The linearity was 15-35 mg/mL with correlation coefficient, $r^2 = 0.9997$. Sucrose was estimated in vaccine the range of 7.99-8.67 mg/vial with 3.582 % relative standard deviation (Table-2).

TABLE-1
VALIDATION PARAMETERS

Validation parameter	RSD value	Acceptance criteria
Accuracy	0.57 %	Not more the 5 %
Linearity	y = 2.2465x + 0.1341 $r^2 = 0.9997$	Not less than 0.995
Precision		
Repeatability		
Intra-day	1.49 %	Not more than 10 %
Inter-day	1.76 %	Not more than 10 %
Ruggedness	2.20 %	Not more than 10 %

TABLE-2
ESTIMATION OF SUCROSE IN VACCINE

Replicate	Iodine volume used in glucose oxidation in mL	Sucrose present (mg)
1	3.5	7.99
2	3.6	8.22
4	3.5	7.99
5	3.8	8.67
6	3.5	7.99
	Mean	8.176
	SD	0.292
	RSD (%)	3.582

Conclusion

The developed method was found to be accurate, precise and reproducible. It may be useful for routine analysis of sucrose in vaccines, syrup dosage form and sugar estimation in sugar industry.

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