MethodsX 5 (2018) 609–612

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

2-Aminoethanaminium 2-(ethoxycarbonyl)-4,6-dinitrophenolate as a greener route in reducing sugar quantification

Abdel-Naser A. Zohri[a], Mohamed Abdelazim[b,*], Sara Ibrahim[b]

[a] Faculty of Science, Botany and Microbiology Department, Assiut University, Assiut, 71516, Egypt
[b] Chemical and Biotechnological Laboratories, Assiut University, Sugar Industry Technology Research Institute, Assiut, 71516, Egypt

A B S T R A C T

3,5-dinitrosalicylic acid (DNS) reducing sugar assay is the most convenient method for quantification of total reducing sugar in biomass hydrolysate, fermentation samples, sugar industry and biotechnology laboratories. The dimeric proton transfer salt 2-aminoethanaminium 2-(ethoxycarbonyl)-4,6-dinitrophenolate (AED) is an intensely colored derivative of DNS and in turn its reduced form intense color showed a superior properties in reducing sugar quantification eliminating phenol and rochelle salt additives using the same practical methodology of DNS giving an overall methodology advantageous than using DNS assay as a greener route.

The proton transfer salt has already been X-ray imaged and deposited in Cambridge Crystallographic Data Centre CCDC 1441586 and a comparison was done between DNS and this salt using a salt and sodium hydroxide concentrations as the same as DNS assay as well as the latter phenol-rochelle salt free environment giving correlation coefficient 0.999 and absorptivity nearly two thirds the obtained in case of use DNS assay with added phenol for enhancing absorptivity and rochelle salt for produced color stabilization for the same detection range 0.1-0.5 mg/ml according to Miller procedures. The sensitivity and the reduced form color stability of this proton transfer salt could be interpreted on both of its molecular structure has a double oxidizing groups as well as it has an intense color as compared with DNS and a good reduced form solubility respectively.

- DNS in reducing sugar quantification, Miller procedures involving phenol and rochelle salt addition.
- AED in reducing sugar quantification as the same detection range as DNS with the elimination of phenol and rochelle salt from the assay.
- A greener convenient route in reducing sugar quantification.

A R T I C L E I N F O

Method name: AED in reducing sugar quantification
Keywords: Organic proton transfer salts, AED, DNS, Application in reducing sugar determination, Greener route
Article history: Received 29 January 2018; Accepted 18 May 2018; Available online 5 June 2018

* Corresponding author.
E-mail address: maboulela@icloud.com (M. Abdelazim).

https://doi.org/10.1016/j.mex.2018.05.017

Specification Table

Subject area	Chemistry
More specific subject area	*Spectrophotometric determination of reducing sugars.*
Method name	*AED in reducing sugar quantification.*
Name and reference of original method	*Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. G. L. Miller, Anal. Chem, 1959, 31(3), 426-428*

Method details

DNS, Original Miller Method.
Reagents and Equipments:

- 3,5-dinitrosalicylic acid.
- Sodium hydroxide.
- Phenol.
- Rochelle salt, sodium potassium tartrate.
- Sodium bisulfite.
- Water bath.
- Spectrophotometer.

Procedures:
3 ml of glucose solution 0.1-0.5 mg/ml is mixed with 3 ml of DNS reagent (1 gm of DNS dissolved in 100 ml 1% sodium hydroxide contains 0.2 gm phenol and 0.05 gm sodium bisulfite), the reaction mixture was heated at 90 °C for 15 min and 1 ml of 40% sodium potassium tartrate is added then cooled and the absorbance is measured at 540 nm.

AED method.
Reagents and Equibments:

- AED is prepared by the reaction of ethyl 3,5-dinitrosalicylate [1] using ethyl salicylate instead of methyl salicylate with ethylenediamine in hot ethanol for 5 min then collecting the yellow precipitate and recrystalized from water.
- Sodium hydroxide.
- Sodium bisulfite.
- Water bath.
- Spectrophotometer.

Procedures:
3 ml of glucose solution 0.1-0.5 mg/ml is mixed with 3 ml of AED reagent (1 gm AED dissolved in 100 ml 1% sodium hydroxide contains 0.05 gm sodium bisulfite), the reaction mixure was heated at 90 °C for 15 min then cooled and the absorbance is measured at 545 nm.

In this laboratory [2] the reducing sugar is extensively and routinely analyzed in sugarcane molasses, biomass pretreatment hydrolysate, and woody residual enzymatic hydrolysate and due to the lower Pk_a of DNS that might be in relation to skin irritation in case of contact and its relatively high price we investigated this reagent AED under study in comparison with DNS in reducing sugar quantification prepared simply starting with a cheap available chemicals, ethyl salicylate, sulfuric acid, nitric acid and ethylenediamine and its intense non reduced and reduced color was successfully utilized to substitute DNS as well as the elimination of phenol a skin burning compound and rochelle salt from the redox reaction mixture as shown in the following table.

Comparison of DNS and its ethyl ester ethylendiamine proton transfer salt, AED, in assay parameters.