

NOVEL SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BENZOYL PEROXIDE FROM THE WHEAT FLOUR SAMPLE

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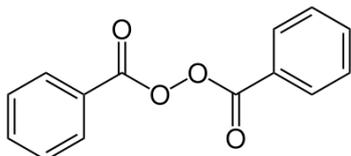
ABSTRACT

A novel spectrophotometric method for the determination of Benzoyl peroxide from the wheat flour was developed. The detection principle is based on the reaction between Benzoyl peroxide & potassium iodide in alcoholic medium. Here in this reaction potassium iodide oxidized by Benzoyl peroxide oxidation, it generates coloured iodine. There was maximum absorption peak in 580 nm wavelength. Potassium iodide system determine Benzoyl peroxide (Result). Wheat flour dissolved in ethanol by extraction of ethanol & centrifugal of Benzoyl peroxide. Under the selected conditions, the linear range for quantification of Benzoyl peroxide was observed between 10 mg/L to 50 mg/L. The limit of detection (LOD) was 30 mg/L. The developed method obtained superior precision using 10 repeatability. The proposed methodology was successfully applied to determine Benzoyl peroxide in wheat flour samples.

Keywords: Spectrophotometer, Benzoyl peroxide, Ethanol and Potassium Iodide.

1. INTRODUCTION

Benzoyl peroxide is an organic compound in the peroxide family. It consists of two benzoyl groups bridged by a peroxide link. Its structural formula is $(C_6H_5CO)_2O_2$. It is one of the most important organic peroxides in terms of applications and the scale of its production. Benzoyl peroxide is used as an acne treatment, for bleaching flour, hair and teeth, for cross-linking polyester resins, and for many other purposes. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.



Uses

Other common uses for Benzoyl peroxide include

- ✓ Bleaching hair
- ✓ Tooth whitening systems
- ✓ The preparation of bleached flour
- ✓ As a convenient oxidant in organic chemistry
- ✓ An initiator and catalyst for polyester thermoset resins, as an alternative to the much more hazardous methyl ethyl ketone peroxide.
- ✓ A hardener in order to start the polymerization process in resins. For instance, PMMA resins can be polymerized with Benzoyl peroxide.
- ✓ Removing ink and dye stains on vinyl dolls.

In the U.S., the typical concentration for Benzoyl peroxide is 2.5% to 10% for both prescription and over-the-counter drug preparations that are used in treatment for acne. Higher concentrations are used for hair bleach and teeth whitening. Benzoyl peroxide, like most peroxides, is a powerful bleaching

agent. Contact with fabrics or hair can cause permanent color dampening almost immediately. Even secondary contact can cause bleaching; for example, contact with a towel that has been used to wash off Benzoyl peroxide-containing hygiene products.¹¹

Side effect

Concentrated Benzoyl peroxide is potentially explosive like other organic peroxides, and can cause fires without external ignition. The hazard is acute for the pure material, so the compound is generally used as a solution or a paste. For example, cosmetics contain only a small percent of Benzoyl peroxide and pose no explosion risk.

The carcinogenic potential of Benzoyl peroxide has been investigated. A 1981 study published in the journal *Science* found that although Benzoyl peroxide is not a carcinogen, it does promote cell growth when applied to an initiated tumor. The study concluded, "caution should be recommended in the use of this and other free radical-generating compounds".

In a 1977 study using a human maximization test, 76% of subjects acquired a contact sensitization to Benzoyl peroxide. Formulations of 5% and 10% were used.

The U.S. National Institute for Occupational Safety and Health has developed criteria for a recommended standard for occupational exposure to Benzoyl peroxide, Benzoyl peroxide has been used for over 50 years as a bleaching agent in flour, whey processing and milk for Italian cheese making. It was used for bleaching flour and cheese at concentrations of up to 40 mg/kg, while bleaching of Cheddar cheese whey has been done successfully using 20 mg/kg Benzoyl peroxide and holding for an hour at 60-63 °C. As Benzoyl peroxide is almost totally converted (> 91%) to benzoic acid during cheese making and any remaining traces would further be reduced by processing of whey. Therefore the intake assessment should be made on the additional benzoic acid incorporated in the diet from the use of Benzoyl peroxide to bleach whey. JECFA has evaluated the use of Benzoyl peroxide as a bleaching agent in flour and concluded that treatment at concentrations up to 40 mg/kg was acceptable (WHO, 1964) & as per FSSAI guideline we can use maximum 40 p.p.m. Benzoyl peroxide in wheat flour (or in Maida). Moreover, at the 59th meeting JECFA concluded that Benzoyl peroxide was of "no safety concern" when used as a flavouring agent (based on current levels of intake) (WHO, 2002).

Concentration of Benzoyl peroxide commercially used is much lower than 100 mg/kg. Only 15% of the world's cheese

production is coloured and hence is subject to use Benzoyl peroxide. Besides, not all of the coloured whey undergoes bleaching process before drying.

2. METEIRALS AND METHODS

Here, from the study of different determination methods, have check samples of wheat flour which was borrowed from the market & have checked Benzoyl peroxide's level by different spectrophotometric methods. In the different methods researchers have used different chromogenic agents like 3-ethylbenzothiazoline-6-sulfonic (ABTS), TMB (tetra methyl benzidine), Adapalene etc. Here we have used common solvent-KI for the determine Benzoyl peroxide, these method will be used wide because KI is common solvent & it is available easily. It is common iodometry reaction. Here spectrophotometer is used for the determine new method.

Proposed methodology

The proposed methodology was successfully applied to determine Benzoyl peroxide in wheat flour samples. This method has been used for quantification of Benzoyl peroxide in flour samples. The sample preparation procedures are described in the Materials and methods section. In order to eliminate the matrix interference, ethanol was utilized as extraction solvent for the real sample. Because of inorganic salts, starch and fat are poorly dissolved in ethanol that is used as the extraction solvent and detection media. The samples were spiked with Benzoyl peroxide standard at different concentrations. The quantification of Benzoyl peroxide content depends on the formation of cation radical. However, there are non oxidative agents or other bleaching agents such as ammonium per sulfate, calcium phosphate, nitrogen dioxide, chlorine dioxide, nitrogen dichloride, and calcium peroxide that can be affected by the determination of Benzoyl peroxide by the approach method. Because the solubility of inorganic salts were poor in ethanol extract solutions other peroxides cannot be reacted with KI without adding other enzyme peroxidases. The detectable reaction of the proposed method for examination of Benzoyl peroxide in flour samples is based on redox reaction between KI and Benzoyl peroxide in an alcoholic medium.

3. REAGENTS AND CHEMICALS

The chemical reagents used throughout this study were analytical grade and utilized without any further purification.

The deionized water was purified by Milli-Q, Millipore apparatus. KI was from LR grade

used. 97% pure(dried weight) Benzoyl peroxide & 94% pure ethanol used. 1000 mg /L Benzoyl peroxide stock solution was generated by dissolving Benzoyl peroxide 0.1 g in 100 mL of 94% ethanol. The working solution of Benzoyl peroxide was prepared fresh by diluting appropriate volume of stock Benzoyl peroxide in 94% ethanol. Here 3M KI stock solution used in following procedure.

Apparatus

Manti Lab MT-128 Digital Double Display Spectrophotometer was used.

Sample preparation for benzoyl peroxide

Non additive wheat flour (flour blank) and flour samples were purchased from kalapur local markets in Ahmedabad, Gujarat, India . All samples were stored at 4^oC until prepared for quantitative assay. Flour sample (0.5 g) and spiked samples were transferred to a centrifuge tube and 5 mL of ethanol was added. Then, sonication of the sample for 5 minutes was completed followed by shaking of the solution for 5 minutes with a vortex mixer. The supernatant was collected after being centrifuged at 2000 rpm for 15 minutes.

Analytical method

The analytical procedures for the quantification of Benzoyl peroxide were started by pipette 10 mL of extracted solution to 100 ml volumetric

flask. Then, 10 ml of 3 M KI solution was added. Finally, the solution was made up to 10 ml with 94% ethanol and the solution was reacted for 1 minute. The solution developed from blue to dark blue or violete color immediately without any catalyts, which provided maximum absorbance at 580 nm. The content of Benzoyl peroxide in the real sample was calculated using the linear regression equation of standard curve. Afterward, the 94% ethanol was utilized to adjust volume to 10 mL.

RESULTS AND DISCUSSION

The detectable reaction of the proposed method for examination of Benzoyl peroxide in flour samples is based on redox reaction between liberated KI and starch in an alcoholic medium. After Benzoyl peroxide was reduced by a chromogenic agent such as KI. It became benzoate anion. Therefore, the possibility of this color reaction was shown. The complex of violet colour formed because of starch & iodine. Here maximum absorption peak we have observed at 580 nm & maximum absorbance was 0.24 at 30 ppm.

The calibration graph was created by plotting absorbance (y-axis) with concentration of Benzoyl peroxide in mg/L (x-axis). Here 10ppm to 30 ppm sample sets were prepared & got the linear regression with $r^2=0.8$. Repeatable results have also got.

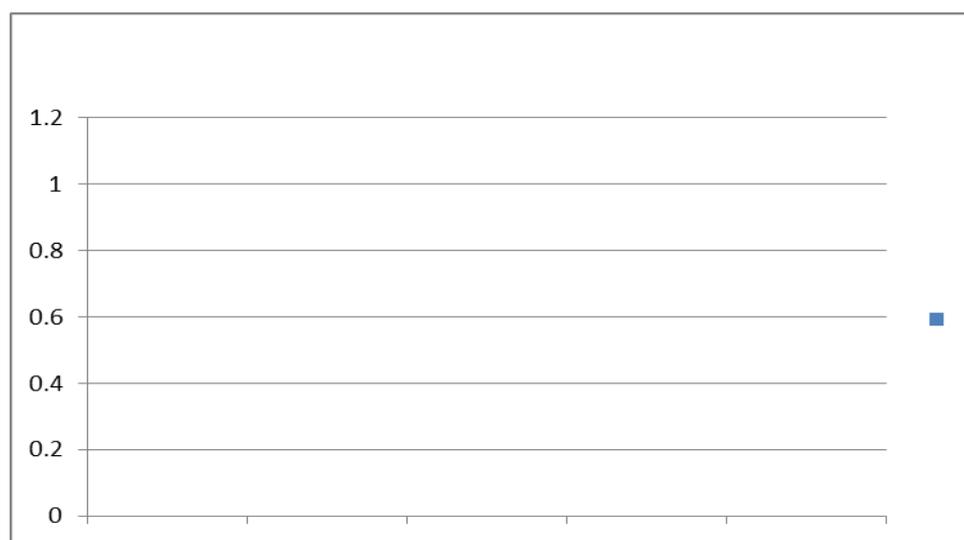
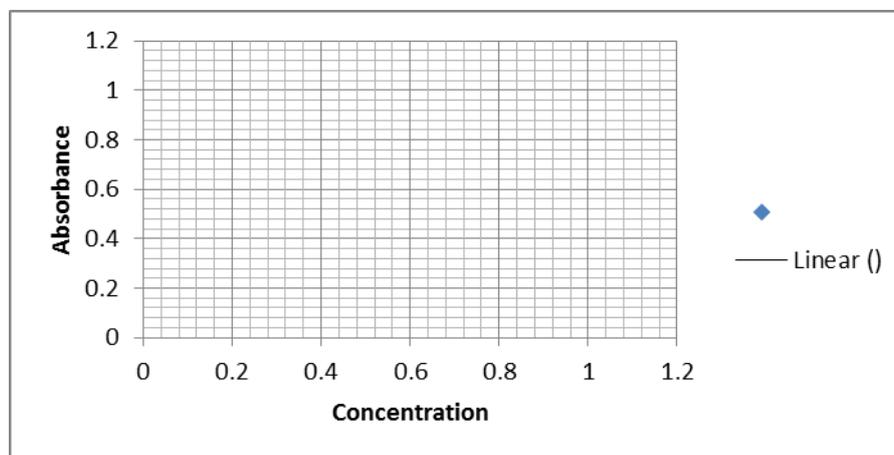


Fig. Absorbance → Concentration

Table 1: Experimental results

Number	concentration	Absorbance
1	10 ppm	0.7
2	20 ppm	1.2
3	30 ppm	2.4
4	40 ppm	2.3
5	50 ppm	1.8



$$r^2 = 0.77 = 0.8$$

Table 2: Repeatable results

Concentration	Absorbance
10 PPM	0.64
20 PPM	1.32
30 PPM	2.41
40 PPM	2.37
50 PPM	1.75

4. CONCLUSION

Many determination methods used in different modes. Different methods have different agents used in it & it have its own characteristic property. Freshly milled wheat flour has a pale yellow color due to its carotenoids content. Benzoyl peroxide is a bleaching agent typically used to give such flour a better appearance. Benzoyl peroxide has been used for over 50 years as a bleaching agent in flour. Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated the use of Benzoyl peroxide as a bleaching agent in flour and concluded that treatment at concentrations up to 40 mg/kg was acceptable (WHO, 1964). The colorimetric reaction for the determination of Benzoyl peroxide using KI as the chromogenic reagent was successfully developed. This procedure provided a simple, rapid, and sensitive method for the determination of Benzoyl peroxide in wheat flour samples. The results were satisfactory when compared with the other methods. Therefore, the proposed method is an alternative procedure for application to determine Benzoyl peroxide in flour samples.

5. ACKNOWLEDGEMENT

Government authorities like FSSAI, FDA etc. have decided the level of Benzoyl peroxide in wheat flour that's why it is mandatory to know its concentration in wheat flour, this method is easy, cheap & accurate so it will be used widely in industrialist & Researchers. There are some chromogenic agents which react with Benzoyl peroxide so By using this method Researchers can experiment. Benzoyl peroxide widely used in pharmaceutical industries so reference from the above research work researchers can develop new methods.

REFERENCES

1. Kraingkrai P and Sam-ang Supharoek WS. A rapid and sensitive spectrophotometric method. Journal of food and drug analysis. 2015;23:652-65.
2. Abe-Onishi YYC. Determination of Benzoyl peroxide and benzoic acid by the H.P.L.C. and identification by H.P.L.C.M.S. Journal of chromatography A. 2004;1040:209-214.

3. Bevington JC. D. C. Anomalous behaviour of Benzoyl peroxide as an initiator of polymerization. *Polymer*. 1975;16:938-949.
4. Chen QC. MS. Determination of peroxide in wheat flour by ion chromatography with precolumn derivatization. *Journal of Liquid chromatography & Related Technology*. 1998;21:705-716.
5. Chen W SW. Simple and fast fluorescence detection of BENZOYL PEROXIDE in wheat flour by N-methoxy rhodamine-6G spirolactam based on consecutive chemical reactions. *Analytical Chemica Acta*. 2011;708:84-88.
6. Childs RE. BW The steady state kinetics of peroxidase with 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) as chromogen. *Biochemistry*. 1975;145:93-103.
7. Okieimen EF. Non-ideal kinetics in vinyl polymerization. *Polymer*. 1981;1:1737-1739.
8. Feigl F. DC. Contributions to organic spot test analysis. *Analytical Chem*. 1961;132:419-428.
9. Fennema OR. Food chemistry. Newyork: Marcel deccer. 1985.
10. Hu J and Dong YL. Naked eye detection of Benzoyl peroxide from wheat flour using 3,3',5,5'-tetramethylbenzidine as a chromogenic agent. *RSC Advance*. 2013;3:26307-26312.
11. Pharr DY and Tomsyck JA. Flow injection analysis of Benzoyl peroxide using TMPDA and surfactants. *Analytical Letter*. 2009;42:821-832.
12. Dugan PR. Rapid spectrophotometric determination of microgram amounts of lauroyl and Benzoyl peroxide. *Analytical Chemistry*. 1961;12:696-708.
13. Ren Q and Huang J. Determination of Benzoyl peroxide by peroxide, as benzoic acid, in wheat flour by capillary electrophoresis compared with HPLC. *Journal of Science Food Agriculture*. 2004;2:221-224.
14. Saiz, Manrique G and Fritz. Determination of Benzoyl peroxide and benzoic acid levels by HPLC during wheat flour bleaching process. *Journal of Agriculture and food chemistry*. 2001;49:98-102.
15. Sudhakar D, Srinivasan KSV, Joseph KT and Santappa M. Grafting of methyl methacrylate onto cellulose nitrate initiated by Benzoyl peroxide. *Polymer*. 1981;22:491-503.
16. Wang C and Hu X. Determination of Benzoyl peroxide levels in wheat flour and pharmaceutical preparations by differential pulse voltammetry in no aqueous media. *Analytical letter*. 2005;38:2175-2187.
17. Zhao J, Peng Y, Chao K, Qin J, Dhakal S and Xu T. Rapid detection of Benzoyl peroxide in wheat flour by using Raman scattering spectroscopy. *Sensing for Agriculture and Food Quality and Safety*. 2015;VII:94880S.
18. Yao XR, Yu J and Guo ZX. Preparation of isotactic polypropylene/polystyrene blends by diffusion and subsequent polymerization of styrene in isotactic polypropylene pellets. *Polymer*. 2011;52: 667-675.
19. Kozan JVB, Silva RP, Serrano SHP, Lima AWO and Angnes L. Amperometric detection of Benzoyl peroxide in pharmaceutical preparations using carbon paste electrodes with peroxidases naturally immobilized on coconut fibers. *Biosen Bioelectron*. 2010;25:1143-1148.
20. Wei L, Zhujun Z and Liu Y. Chemiluminescence microfluidic chip fabricated in PMMA for determination of Benzoyl peroxide in flour. *Food Chem*. 2006;95:693-698.
21. Wada M, Inoue K, Ihara A, Kishikawa N, Nakashima K and Kuroda N. Determination of organic peroxides by liquid chromatography with on-line post-column ultraviolet irradiation and peroxyoxalate chemiluminescence detection. *J Chromatogr A*. 2003;987:189-195.
22. Gaddipati N, Volpe F and Anthony G. Quantitative determination of Benzoyl peroxide by high-performance liquid chromatography and comparison to the iodometric method. *J Pharm Sci*. 1983;72:1398-1400.
23. Su SC, Chu H, Chen CM, Lee SC and Chou SS. Determination of Benzoyl peroxide and azodicarbonamide in flour. *J Food Drug Anal*. 1996;4:229-231.