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A new determination method of anionic detergent in the seawater based on metachromasy and correlation between Azur A and methylene blue assays

Metakromaziye dayanan yeni bir metod ile deniz suyunda anionik deterjanın tayini ve Azur A ve metilen mavisi ile tayini arasında bağıntı

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Abstract

Anionic detergents in seawater have usually been determined by methylene blue method. In addition, Azur A assay was proposed in this work correlation was sought between the two methods. The assays were made at α-band and metachromatic band (β-band) of both dyes. The calibration curves were plotted at 652 nm and 600 nm for methylene blue and at 637 nm and 600 nm for Azur A.

Anionic detergent amounts in seawater found were 16.82 µg/L at 652 nm and 18.66 µg/L at 600 nm for methylene blue and 16.14 µg/L at 637 nm and 15.52 µg/L at 590 nm for Azur A. The results of the seawater plus 25µg/L LAS were: 39.30 µg/L, µg/L for methylene blue and 41.14 µg/L 39.46 µg/for Azur A respectively.

The results of detergent amount in seawater and LAS added seawater are similar for ∝ bands of both dyes and sligthly lower in metachromatic bands (β band).

The interfering compounds in seawater influence the assay when using methylene blue and Azur A. It was difficult to eliminate totally this problem. The measurement can be made separately on the α and β bands of Azur A and also methylene blue. It is difficult to say either method as exact.

Keywords: Anionic detergent. methylene blue Azur A. metachromatic assay. seawater

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Introduction

Organic contaminants originating from different countries are important pollutants in seawater. Detergents are major contaminants for ecosystem. They contain various anionic surfactants as linear alkylbenzene sulfonates (LAS). It is not a single compound and may comprise of 26 isomers and homologs as ($R^1C_6H_4SO_3$) Na^+. R^1 is an alkyl group ranging from 10 to 14 carbon atoms in length. It was prepared by sulfonation of benzene ring LAB. The final products are of complex mixture. During the sulfonation some LAB remain unsulfonated and carried into the final products. They are also pollutant for sea water. LAS was used 5.5 g per head per day in developed countries. It is in large amount and polluted the aquatic environment through waste water seawage to the rivers and sea water.

Various methods were proposed to determine anionic detergents in town-and seawater and rivers. The videly used method is methylene blue active subtances (MBAS) determination. The method depends on the formation of a ion pair formation by methylene blue (MB) with MBAS anions. The substance formed is in blue colour and soluble in chloroform and followed by an aquous back wash and measured by spectrophotometer at 652 nm. The detection limit is 0.025 mg/L anionic detergent (Standard Methods, 1995). This method is relatively simple but some subtances such as organic sulfonates, sulfates, carboxylates and phenols and inorganic substances as thiocyanates, cyanates, nitrates and chlorides also may transfer more or less methylene blue into the chloroform phase. The aqueous back wash step removes these positive interferences. Negative interference was due to the presence of cationic surfactants, other cationic materials and particulate matter. The latter was eliminated by filtration. The presence of sulfides in waste water may react with methylene blue to form a colourless reduction product. It was eliminated by hydrogen peroxide oxidation. The problems caused by dye-substrate interactions were investigated by authors. Positive and negative interferences with the organic and inorganic substances were demonstrated in the assay with methylene blue. (Sylven 1954; Balazs *et.al.*, 1968; Srivastava *et.al.*, 1977) The existence of interferring substances in river, town-and seawater influence the assay. The determination of anionic detergents with methylene blue depends on the pollution of sea water. The results vary depending on the pollution of sampling regions. MBAS results are the total anion active compounds plus interfered substances reacting this assay. It was difficult to eliminate totally the interferring substances in these assays. Also metachromatic dye-subtrates interactions are as intermolecular as hydrogen bonds, hydrogen bond plus oxygen -sulphur bridges, intermoleculer distance (Sylven 1954), ionic interaction (Singh, 1958, 1963), heteropolyacids (inorganic polymers).

The determination of anionic detergents with MB method in sea water and rivers were investigated by various authors (Barr *et al*, 1948; Topçuoğlu *et al.*, 1992; Yaramaz and Tuncer, 1988; Aboul-Kasım, 1990).

The assay method of active anionic subtances based on metachromasy was investigated firstly by Akıncı (1992) and later a paper was published by Güven *et. al.* (1994).

Metachromasy which is used in this assay was found first by Ehrlich (1887) in the course of histological staining of particular tissue elements in animals. This phenomenon was defined by Lison (1935) as the change of the absorption band of the dyes from long to shorter wavelengths. The substance that can induce metachromasia in a dye is called a chromotrope and also metachromatic dyes as methylene blue, Azur A, Nile blue, night blue etc. The usual chromotropic substances of biological origin are high. molecular weight polyanions as mucopolysaccharides like heparin (Mac Intosh, 1991; Güven and Ertan 1984) and others as nucleic acids, carboxymethyl cellulose, algal polysaccharides as agar, alginic acid and carrageenans (Güven and Güvener, 1985) and some inorganic substances and synthetics as silicates, polyphosphates, detergents and some ions (Sylven, 1954; Balazs *et al.*, 1968; Singh, 1959, 1963; Srivastava *et al.*, 1977).

Methachromatic method was also used for identification of anionic detergent on thin-layer chromatography (Akıncı and Güven ,1992).

The other techniques used for the determination of anionic detergents are GC/MS (Hon-Nami and Hanya, 1978, 1980; Raymundo and Preston, 1992) HPLC (Marcomimi and Giger, 1987; Terzic *et.al.*, 1991; Terzic and Ahel, 1993).

This paper reports the result on correlation of the assay methods between Azur A. and methylene blue at α-and β band.

Materials

Seawater samples were taken from Kumkapı (the Sea of Marmara). They were collected in dark glass bottles and analysed immediately.

LAS was obtained from a detergent manufacturing plant, Lever, (Gebze, Turkey). Stock LAS solution: 0.100 g LAS calculated on active subtances 95.6 % in 100 ml distilled water.

Standard LAS solution: Dilute 1 ml stock solution adjusted to 100 ml in distilled water, prepared daily.

Phenolphtalein solution: Phenolphtalein 1/100 in alcohol.

Sodium hydroxide: 1.N NaOH

Sulfuric acid: 1. N H_2SO_4 and 6 N H_2SO_4

Methylene blue (MB) solution: 30 mg Methylene blue dissolved in 50 ml water.

Azur A solution: 30 mg Azur A dissolved in 100 ml water.

Wash solution (Back wash): 41 ml 6N H_2SO_3 500 ml mixed with 500 ml distilled water and added 50 g $Na_2PO_4H_2O$ and the volume was adjusted to 1000 ml in a volumetric flask.

Glass wool to filter

Spectrophotometer (Shimadzu UV-1601)

Method

1. Calibration curve

The calibration curve was plotted with LAS in conc. of 20-100 µg/L calculated from active subtances.

20, 50, 100 µg/L LAS was added to 800 ml distilled water in a separatory funnel. 5 drops of phenolpthalein solution added and alkalinized with 0.1 N. NaOH and then neutralized with 0.1 N. H_2SO_4, 4 ml dye solution (methylene blue or Azur A) was added. They were extracted 3 times with 30 ml chloroform. The extracts were combined and washed with acid solution. It was filtered through glass wool. The volume of filterate was adjusted to 100 ml with chloroform. Its absorption graph was drawn using a spectrophotometer and the standard curves were plotted at 652 nm for methylene blue and 637 nm for Azur A. and also for methacromatic β-band at 600 nm for BM and at 590 nm for Azur A.

The equation of each standard curves was taken from spectrophotometer.

2. The determination of the anionic detergent (LAS) in seawater.

800 ml seawater was taken and the anionic detergent content was determined as described above. The amount of LAS µg/ml was taken from spectrophotometer and calculated as µg/L according to the curve

plotted for α-band and metachromatic band (β-band) of each dye.

To control, 25µg/L LAS was added in to seawater and the total amount of detergent was determined by both methods.

Results and Discussion

1. The standard curves plotted at 652 nm and 600 nm for methylene blue and 637 nm and 590 nm for Azur A are shown in Fig.1-2. Their absorption spectra are shown in Fig.3-4.

The amounts of anionic detergent in seawater and also LAS added seawater are shown in Table 1.

Dyes	Anionic detergent in sea water	Anionic detergent, 25 µ/LAS added in seawater
Methylene blue		
α-band 652 nm	16.86	39.30
β-band 600 nm	18.66	43.11
Azur A		
α-band 637nm	16.14	41.14
β-band 590 nm	15.52	39.46

Table 1. The amount of LAS in seawater and 25 µg/LAS added seawater (µg/L) at α-and β- band of dyes.

As can be seen in Table I the LAS amounts in seawater at α-band and β-band for Azur A were 16.14 and 15.52 µg/L and for methylene blue 16.86, 18.66 µg/L respectively. The amounts in LAS added seawater were found as 43.11 and 39.43 µg/L for methylene blue and 41.14 and 39.46 for Azur A.

A correlation was found between the two determination methods which were based on the determination at α-and β band for both dyes. Both methods can be used separately or together. The values at β-band were higher than that at α-band of Azur A and methylene blue. As can be explaine in the introduction, various substances and factors influence the assay.

According to the these findings it is difficult to say which of the either

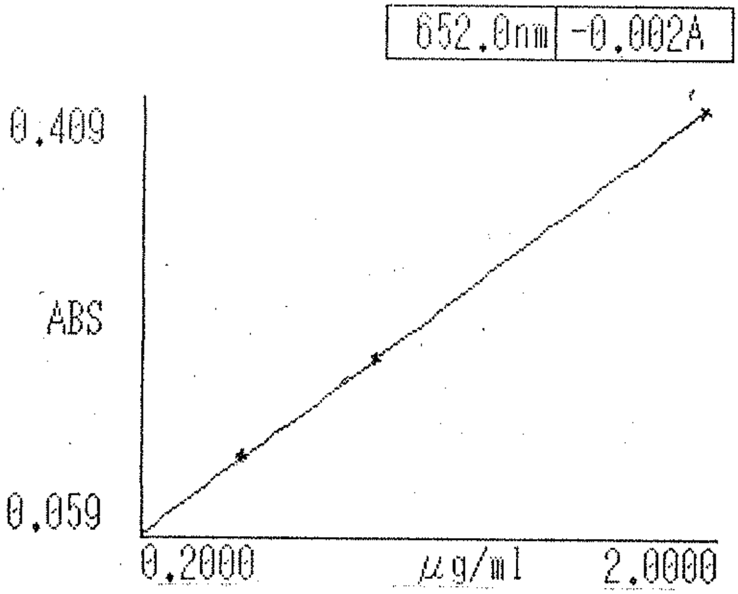

Fig.1. Standard curve of methylene blue assay of LAS at 652 nm,

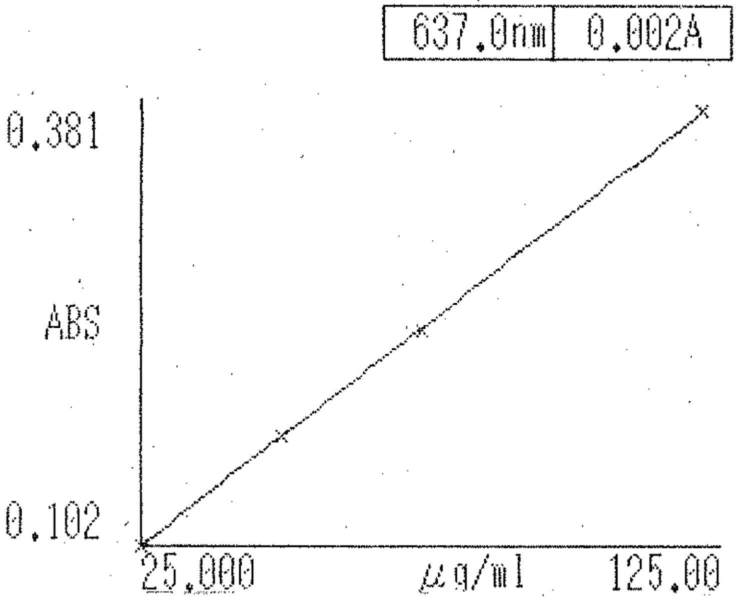

Fig.2. Standard curve of Azur assay of LAS at 637 nm, b) 590 nm

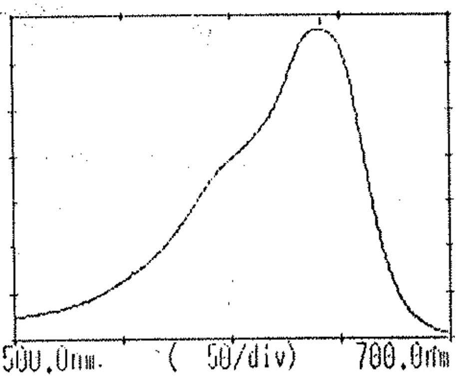

Fig.3. Absorption spectrum of LAS + methylene blue.

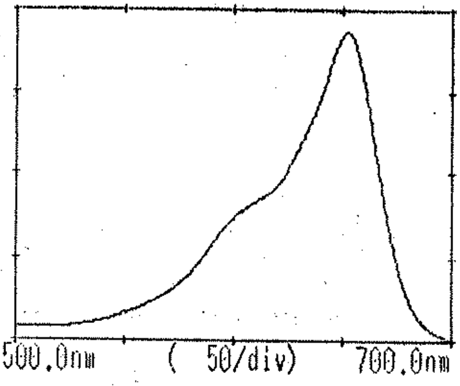

Fig.4. Absorption spectrum of LAS + Azur A.

Özet

Deniz suyunda anionik deterjanlar genelde metilen mavisi metodu ile tayin edilir. Evvelce anionik deterjanların ilk defa yapılan metakromatik metod ile tayinine (Akıncı, 1991) ek olarak metilen mavisi ve Azür A. tayinlerdeki bağıntı incelenmiştir. Bu tayinler her iki boyanın λ-max.(α-band) ile metakromatik (β-bandl)da ölçümler yapılmıştır. α ve β bantlara ait metilen mavisi için λmax. 652 nm ve 600 nm ve Azur A için 637 nm ve 590 nm bulunmuştur. Her iki boyanın λmax.da yapılan tayinde birbirine yakın sonuçlar alınmıştır. Metakromatik bandda yapılan tayinde küçük bir fark görülmüştür. Metilen mavisi tayin metodu da birçok madde deneye girişim yapar ve dolayısı ile sonuç metilen mavisine aktif maddeler diye tanımlanır. Bu girişim tayin esnasında bütünü ile önlenemez. Buna benzer olarak Azur A ile de yapılan tayin aynı problem mevcuttur. Zira metakromaziye de birçok maddeler etki eder. Bu sebeplerden tayinin metilen mavisinde α-band yanında β-bant'ta ve keza buna ek olarak Azur A ile de her iki band'ta tayin yapılması ve sonuçların karşılaştırılması bu çalışmada önerilmiştir.

References

Aboul-Kassım, T.A. (1990). Detergent as indices of organic pollution in Alexandria costal waters, *Rapp. Comm. Int., mer Médit.* 32:124.

Akıncı, S. (1992). Anyonik deterjanların yeni bir metod ile deniz suyunda tayini, Ms. Thesis İ.Ü. Deniz Bilimleri ve İşletmeciliği Enstitüsü. İstanbul.

Akıncı, S. and Güven, K.C. (1992). The identification of anionic detergents by thin-layer chromatography. *Acta Pharm. Turcica*, 34:121-126.

Balazs, E.A., Davies, J.V., Phillips, G.O. and Scheufele, D.S. (1968). Polyanions and their complexes, part IV. *J. Chem. Soc.* 12:1424-1429.

Barr, T., Oliver, J. and Stubbing, W.V. (1948). The determination of surface active agents in solution. *J.soc. Chem.Ind.* (Lond) 65:45.

Ehrlich, P. (1887). Baiträge zur Kenntniss der Anilin färbungen and ihrer Vervendung in der mikroskopischen Technik. *Archiv F. Mikrosk Anatomie*, 13:263-277.

Güven. K.C. and Ertan. G. (1984). The correlation equations for the assays of heparin in metachromatic and thrombin time methods. *Thrombosis Research*, 33:197-201.

Güven. K.C. and Güvener. B. (1985). A metachromatic method for the identification of alginic acid. agar and carrageenan, *Fette.Seifen. Anstrichmittel.* 87:172-176.

Güven. K.C.. Gürakin, N., Akıncı. s., Bektaş. A. and Öz. V. (1994). Metachromatic method for the assay of LAS in town -and sea water, *Acta pharm. Turc.* 34: 136-140.

Hon-Nami. H. and Hanya. T. (1978). Gas-liquid chromatographic-mass spectrophotometric determination of alkylbenzene sulphonates in river water. *J.Chromatog.* 161:205-212.

Hon-Nami, H. and Hanya. T. (1980). Linear alkylbenzene sulfonates in river, estuary and bay water. *Water Research*. 14:1251-1256.

Lison L. (1935) Etude sur métachromasie colorants métachromatiques et substances chromotropes. *Archive de Biologie*. 46: 599-668.

MacIntosh. F.C. (1941). A metod for estimating the potency of heparin preparations. *Biochem.* J. 35: 776-775.

Marcomimi. A. and Giger. W. (1987). Simultaneous determination of linear alkylbenzene sulphanates. alkylphenol polyethoxylates. and nonyl phenol by high-performance liquid chromatography. *Anal.Chem.* 59:1709-1715.

Raymundo. C.C. and Preston, M.R. (1992). The distribution of linear alkylbenzenes in coastal and Estuarine sediments of the Western North Sea. *Mar.Poll.Bull.* 24:138-146.

Singh. C. (1958). Use of basic dyes for the paper chromatographic detection of various polyanionic compounds, *J. Sci.Industr. Res.* 19 C. 12:306-301.

Singh. C. (1963). Heteregeneity and metachromasy of some commercial anionic dyes. *Stain Technology*, 38: 103-110.

Singh. C. (1963). Heteregeneity and metachromasy of some commercial anionic dyes. *Stain Technology*, 38: 103-110:

Srivastava. V.K.. Misra, B.B. and Tripathi. A.M. (1977). Effect of acid and inorganic salts on metachromasia induced by heteropolyanions. *Transition Met. Chem.* 2: 106-108.

Standard Methods 19 th edition (1995) (A.D. Eaton. L.S. Clesceri, A.E. Greenberg Eds): American Public Healt Ass. Washington D.E. 1015 p.

Sylvén. B. (1954). Metachromatic dye-substrate interactions. *Quart. J. micr. Sci.* 95: 327-358.

Terzic. S.Hrsak. D.. Ahel. M. (1992). Enrichment and isolation of linearalkylbenzene sulphonate (LAS) degrading bacteria from estuarine and coastal water. *Mar.Poll.Bull.* 24:199-204.

Terzic, S. and Ahel. M. (1993). Determination of linear alkylbenzene sulphonates in the Krka River estuary. *Bull.Environ.Contam.Toxicol.* 50:241-246.

Topcuoğlu. S.. Ünlü. M.Y. and Bulut. A.M. (1992). Pollution of anionic detergents in Küçükçekmece Lagooh and its toxicity. In.VIII^th^ National Symposium on Chemistry and Chemical Engineering Book (Marmara Univ.Publ.) 518:183-187.

Yaramaz. O.. and Tuncer. S. (1988). Analyse spectrophotometrique des surfactants sur la cote Egéenne. Rapp. Comm. int. Mer Médit. 31.

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