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海水中超痕量活性磷的检测方法研究

Study on the Determination Techniques
for Ultra-trace Orthophosphate in Seawater

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摘 要

活性磷是海洋浮游植物生长所必需的物质基础,在磷的海洋生物地球化学过程中扮演着重要角色。寡营养盐海域海洋表层活性的磷浓度极低,通常在 nmol/L 水平。现有的活性磷测定方法已不能满足日益发展的海洋环境科学研究的需要,发展灵敏可靠的分析方法已成为研究的技术基础和迫切需求。本论文针对现有方法的不足,研发灵敏度高、可靠性好、操作相对简单、可用于现场测定的超痕量活性磷分析方法,主要内容和结果如下:

- (1) 建立了海水中超痕量活性磷的沉淀分离富集-分光光度测定方法。磷钼蓝 (phosphomolybdenum blue, PMB) 可与一定浓度的十六烷基三甲基溴化铵 (cetyltrimethylammonium bromide, CTAB) 反应生成难溶于水的 PMB-CTAB 缔合物沉淀,该沉淀易溶于 0.50 mol/L 硫酸乙醇溶液,溶液在 700 nm 有强吸收。采用单因素法对溶剂、试剂用量、显色与沉淀时间、反应温度等实验参数进行了优化选择。在优化的实验条件下,以盐度为 35 的人工海水为工作曲线溶液的基底,方法的线性范围为 0.010-0.259 $\mu\text{mol/L}$,方法检测限为 0.003 $\mu\text{mol/L}$,实际海水基底加标的平均回收率为 95.2%。对实际海水中超痕量活性磷进行测定,相对标准偏差 (relative standard deviation, RSD) 为 4.4%-7.1%,与 $\text{Mg}(\text{OH})_2$ 共沉淀 (magnesium hydroxide-induced coprecipitation, MAGIC) 法比较,结果无非常显著差异。1-4 个样的分析时间约需 45 min。
- (2) 建立了海水中超痕量活性磷的 PMB-CTAB 离子对-固相萃取-分光光度测定方法。PMB 与适当浓度的 CTAB 生成均匀分散于水相的疏水性 PMB-CTAB 离子对缔合物,被 Sep-Pak C18 小柱固相萃取;吸附在 C18 上的 PMB-CTAB 被 0.56 mol/L 硫酸乙醇溶液迅速洗脱,洗脱液在 700 nm 和 791 nm 处有最大吸收。采用单因素法对试剂用量、反应时间、反应温度、洗脱剂及萃取流速等实验参数进行了优化选择。在优化的实验条件下,以盐度为 35 的人工海水为

工作曲线溶液的基底，方法的线性范围为 0.006-0.129 $\mu\text{mol/L}$ ，方法检测限为 0.002 $\mu\text{mol/L}$ ，实际海水基底加标的平均回收率为 98.4%。考察了试样中盐度的影响。对实际海水中痕量活性磷酸盐进行测定，RSD 为 2.7%-5.6%，与 MAGIC 法比较，结果无显著差异。1-6 个样的分析时间约需 60 min。

(3) 在方法 (2) 的基础上，建立了海水中超痕量活性磷的流动注射-固相萃取-分光光度测定方法。利用停流技术使试样溶液中的活性磷与磷钼蓝试剂及 CTAB 充分反应，生成 PMB-CTAB 离子对化合物。采用单因素法和正交试验设计对反应温度、试样过柱流速、停留时间和洗脱流速等实验参数进行了优化选择。实验结果表明，反应温度与停流时间是影响 PMB-CTAB 形成的重要因素。在优化的实验条件下，以盐度为 35 的人工海水作为工作曲线溶液的基底，方法的线性范围为 0.003-0.048 $\mu\text{mol/L}$ ，检测限为 0.002 $\mu\text{mol/L}$ ，实际海水基底加标回收率平均值为 96.4%。考察了试样中硅的影响，5000 倍的硅对活性磷的测定无干扰。对含磷 0.032 $\mu\text{mol/L}$ 的人工海水加标样每天测定一次，连续 8 天测定的 RSD 为 4.52%。本法对实际海水的测定结果与 MAGIC 法无显著差异。每个样品的分析时间为 30 min。本法成功测定了取自南海的 21 个表层海水中的活性磷含量，测定值介于 0.011-0.078 $\mu\text{mol/L}$ 之间，结果令人满意。本法尤其适合船上现场在线分析。

(4) 建立了海水中超痕量活性磷的流动注射-固相萃取-鲁米诺化学发光测定方法。磷钼黄 (molybdophosphoric heteropoly acid, MoP) 与 CTAB 生成的离子对缔合物 MoP-CTAB 可被 C18 小柱固相萃取富集，因而与海水基底分离；吸附在 C18 上的 MoP-CTAB 用 0.3 mol/L 硫酸乙醇溶液迅速洗脱，与鲁米诺溶液在碱性条件下发生化学发光反应，采用单因素法对鲁米诺溶液的浓度、洗脱剂酸度和洗脱流速等实验参数进行优化。以盐度为 35 的人工海水为工作曲线溶液的基底，方法的线性范围为 0.005-0.194 $\mu\text{mol/L}$ ，检测限为 0.002 $\mu\text{mol/L}$ ，南海海水基底加标回收率平均值为 92.5%。考察了试样中盐度和硅的影响。至少 4000 倍的硅对磷的测定无干扰。对含磷 0.097 $\mu\text{mol/L}$ 的人工海

水加标样 8 小时内连续测定 7 次的 RSD 为 4.52%。本法对实际海水的测定结果与 MAGIC 法无显著差异。每个样品的分析时间为 10.5 min。

本研究将 CTAB 与磷钼蓝（或磷钼黄）的离子对反应成功应用于超痕量活性磷的富集及其与高盐基底的分离中，很大程度上提高了磷钼蓝（或磷钼黄）的分离富集效果，基于此建立的四种测定海水中超痕量活性磷的方法，相对于被广泛采用的 MAGIC 法，具有灵敏度高、省时、省力和省试样的优点。为海水中超痕量活性磷的分析提供了多种选择，可满足寡营养海区海水样品的测定要求。

关键词：超痕量活性磷；海水；磷钼蓝；磷钼黄；十六烷基三甲基溴化铵；沉淀分离富集；固相萃取；流动注射分析；鲁米诺化学发光

ABSTRACT

Orthophosphate is an essential nutrient element for phytoplankton in ocean environment, and plays an important role in the marine biogeochemical cycle of phosphorus. In oligotrophic open-ocean waters orthophosphate concentrations are down to nanomolar levels. At present, most of the reported determination techniques for orthophosphate have not succeeded in the field of marine environmental science because of their drawbacks as regards sensitivity, reproducibility, chemical interference and ease of use. It is an urgent need to establish sensitive and reliable detection technique for orthophosphate in the oligotrophic open-ocean waters. To meet these requirements, the following studies have been done, and some successful results have been received.

- (1) A novel method had been set up to determine ultra-trace amount of orthophosphate in seawater with precipitate preconcentration and colorimetric detection. An ion-pair compound of phosphomolybdenum blue (PMB) with cetyltrimethylammonium bromide (CTAB) precipitated from aqueous solution could be easily dissolved in 0.50 mol/L sulfuric acid ethanol solution, and thus determined with a spectrophotometer at 700 nm. Experimental parameters, including the solvent, the reagent concentrations, the time for color developing and precipitation and temperature, were optimized with a univariate experimental design. Under the optimized conditions, using an artificial seawater with salinity of 35 as a matrix, the linearity, the detection limit and the recovery of the proposed method were found to be 0.010 to 0.259 $\mu\text{mol/L}$, 0.003 $\mu\text{mol/L}$ and 95.0%, respectively. The relative standard deviation (RSD) ranged from 4.4 to 7.1% for the real seawater samples. Seawater samples were analyzed with both the proposed method and magnesium hydroxide-induced coprecipitation

(MAGIC) method, and the results showed no significant difference with t test at the confidence interval 99%. 45 minutes were needed to analyze up to 4 samples.

(2) A novel method had been established for the analysis of ultra-trace orthophosphate in seawater with PMB-CTAB ion-pair solid phase extraction and colorimetric detection. An ion-pair compound of PMB with CTAB could be first extracted on solid phase C18, and then the PMB-CTAB adsorbed could be rapidly eluted by 0.56 mol/L sulfuric acid ethanol solution, and determined with a spectrophotometer at 700 nm. Experimental parameters, including the reagent concentrations, the reaction time, the temperature, the eluent and the extracting flow rate, were optimized using a univariate experimental design. Under the optimized conditions, using an artificial seawater with salinity of 35 as a matrix, the linearity, the detection limit and the recovery of the proposed method were found to be 0.006 to 0.129 $\mu\text{mol/L}$, 0.002 $\mu\text{mol/L}$ and 98.5%, respectively. The effect of salinity had been studied. The RSD ranged from 2.7 to 5.6% for the real seawater samples. Seawater samples were analyzed with both the proposed method and the MAGIC method, and the results showed no significant difference with t test at the confidence interval 95%. It took 60 minutes to analyze up to 6 samples.

(3) Based on method (2), a novel on-line solid phase extraction method coupled with flow injection (FI) analysis and colorimetric detection had been developed for the determination of ultra-trace orthophosphate in seawater. Stopped flow technique was employed to assure the complete formation of the PMB-CTAB. Experimental parameters, including the reaction temperature, the sample loading flow rate, the stopped flow time and the eluting flow rate, were optimized with the experiments based on univariate experimental design and orthogonal array design. The results showed that reaction temperature and stopped time were the

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