简易、快速检测有机磷农药的酶片和生色基质片

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摘要 研制出简易、快速检测有机磷农药的酶片和生色基质片。用胆碱酯酶抑制试验检测水中的有机磷农药，检测灵敏度在0.01－10 mg/L范围，适宜温度在25－35℃之间，分析周期约为15－20 min。甲胺磷和水胺硫磷等硫代磷酸酯类农药经溴水活化后检测灵敏度也达上述水平。实际水样检测表明，检样中农药含量在检测灵敏度范围内时，阳性结果相当明显，溶液吸光度比值 $A_{对照}/A_{检样}>2$。由于检测时无需配制试剂和使用仪器，本法特别适宜于现场检测。

关键词 有机磷农药，检测，酶片，生色基质片。

有机磷农药对胆碱酯酶特异性抑制的酶化学比色分析法，被广泛用于有机磷农药的定性和定量检测[1－3]，其检测最低极限可达10^{-9}水平。将酶和生色基质制成药片，使用方便，操作简单，特别适用于现场快速定性检测[4－6]。但用常见的生色基质制备的基质片，由于检测灵敏度和稳定性等原因，效果往往不够理想。Nanda Kumar N. V. 等用α-乙酸萘酯和固蓝B_2盐制备的生色基质片，稳定期只有15 d[4]，国内报道的生色基质片，其基质的水溶性可能欠佳，使用时需用手挤捏基质片促使基质溶解[6]，操作不便，并引起使用安全性问题。笔者用本实验室合成的基质制备的生色基质片，基质水溶性较好，稳定期长，与溴水配合使用，可用于包括甲胺磷和水胺硫磷等最常引起人畜中毒的有机磷农药的快速定性检测，为监测环境中残留农药的污染提供了一种简易、快速、可靠的检测手段。

1 材料与方法

1.1 实验材料

(1) 酶片 浸渍有胆碱酯酶及助剂的滤纸片，胆碱酯酶从动物组织中提取。

(2) 基质片 浸渍有生色基质及助剂的滤纸片，其中生色基质由本实验室合成。

(3) 农药标准溶液 将市售的农药标准溶液(国标GSB G23010-92等)用蒸馏水分步稀释至所需浓度，并保证最终稀液中有机溶剂的含量不超过1%。

(4) 稀释溴水 取1 ml饱和溴水用蒸馏水稀释至100 ml。

1.2 检测方法

取待检试样0.8 ml(约15滴)于小试管中(另1试管中加0.8 ml清洁水作为对照管)，加入酶片1片，振摇试管数次，3 min后加入基质片1片，保持作用10 min，其间振摇试管数次，然后观察试管中溶液的颜色。若对照管溶液呈蓝色而检样管溶液无色或蓝色明显变浅，定为阳性，表明检样中存在有机磷农药，此时由于检样管中胆碱酯酶的活性受到抑制而不能与生色基质作用产生颜色，而对照管中活性不受抑制的胆碱酯酶则与生色基质作用产生蓝色。若检样管与对照管颜色相同或接近则定为阴性，表明检样中不存在有机磷农药，或其含量低于最低检测极限。

为了提高硫代磷酸酯类有机磷农药的检测灵敏度，可先在检样管和对照管中各加入1－2滴稀释溴水活化5 min，然后再按上述程序进行检测。

2 结果和讨论

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2.1 溴水用量对酶促生色反应的影响

由于溴水是强氧化剂，化学性质活泼，加入溴水除了对硫代磷酸酯类有机磷农药起到活化作用外，对酶促生色反应本身也可能有影响。为此，在对照管中分别加入0—3滴稀释溴水，酶促反应15 min后用722光栅分光光度计于610 nm处测定其吸光度，结果列于表1。实验结果显示，溴水用量在1—3滴范围内对酶促生色反应的影响可以忽略。

表1 溴水用量对酶促生色反应的影响

溴水用量(滴)	0	1	2	3
吸光度	0.396	0.400	0.407	0.400

2.2 时间和温度对酶促生色反应的影响

按对照管操作，分别于25℃、30℃和35℃酶促反应经不同时间后，于610 nm测定溶液的吸光度，结果如图1所示。由图1可以看出，在相同温度下，溶液的吸光度随酶促反应时间的增加而增加；若反应时间相同，温度较高则溶液的吸光度较大。酶促反应于35℃进行时的反应速度明显高于30℃和25℃时的反应速度，这除了由于在35℃时酶的活性较高外，也可能与基质的溶解度随温度的升高而增加有关。实验结果也表明，在25—35℃范围内，反应10 min产生的颜色已能满足简易目视比色分析的需要，但较高的反应温度可缩短检测的时间。

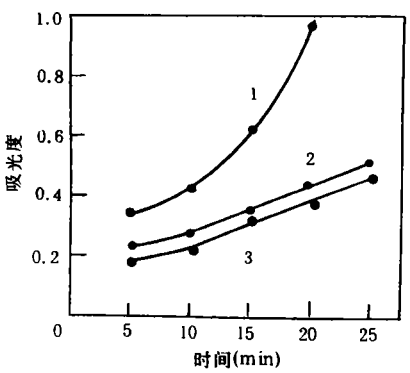

图1 吸光度与酶促反应温度、时间的关系

1. 35℃ 2. 30℃ 3. 25℃

2.3 检测灵敏度

用本检测法对目前常用的几种有机磷农药标准溶液进行试验，当对照管和检样管溶液于610 nm处的吸光度比值接近或大于2时，则认为该浓度的农药可以检出，试验结果见表2。

表2 几种有机磷农药的检测灵敏度

农药名称	最低检出极限 (mg/L)	
	不活化	经溴水活化
甲胺磷	>100	1
水胺硫磷	>100	0.01
甲基对硫磷	1	0.1
敌敌畏	0.01	0.01
敌百虫	1	1
乐果	10	10
氧乐果	5	5

从表2可见，目前几种常见的有机磷农药的最低检出极限在0.01－10 mg/L之间，并且农药的毒性和检测灵敏度之间有一定的对应关系，因此，从避免人畜急性中毒和亚急性中毒的角度出发，本检测法可为水中残留农药的危险程度提供可靠的判断依据。从表2还可发现，甲胺磷、水胺硫磷和甲基对硫磷等硫代磷酸酯类农药不经溴水活化时，检测灵敏度很低甚至完全不能检出。经溴水活化后，水胺硫磷和甲基对硫磷分子中的磷硫双键(P=S)转化为磷氧双键(P=O)，增强了对胆碱酯酶的抑制能力，从而提高了检测灵敏度。甲胺磷属于硫赶磷酸酯类，经溴水活化后检测灵敏度也大为提高，其溴水活化作用显然是以另一种方式进行。

2.4 干扰因素

溶液的pH值由酶片和基质片中的缓冲剂调节在7.4左右，检样的酸性或碱性过强会影响检测结果；强氧化剂或还原剂，浓度大于2%的乙醇、丙酮等亲水性有机溶剂会使检测结果出现假阳性；浓度低于20%的甲醇对检测影响不大，而浓度大于25%的甲醇会使酶促生色反应产生一个滞后期。

2.5 实际水样的检测

实际水样取自池塘、湖泊和菜田等处，稍经静置澄清或过滤后即进行检测，结果见表3。表3中列出了对照样和检样于610 nm处吸光度的比值 $A_{对照}/A_{检样}$，吸光度比值越大，表明酶的

抑制程度越高，阳性结果越明显。实际水样中有机磷农药的含量由广州分析测试中心用气相色谱法测定。由结果可见，实际水样中有机磷农药的浓度在检测灵敏度范围内时，$A_{对照}/A_{检样}$的比值均大于2，阳性结果是相当明显的。

表3 实际水样检测结果

编号	酶试纸法		气相色谱法	
	结果	$A_{对照}/A_{检样}$	农药名称	含量(mg/L)
1	+	2.6	甲胺磷	0.88
2	+	2.8	甲基对硫磷	0.082
3	+	3.1	敌敌畏	0.068
4	+	2.5	水胺硫磷	0.083
5	−	1.0		

2.6 酶片和基质片的稳定性

采取适当的稳定措施，干燥避光保存，酶片和基质片的稳定期均在2年以上，符合普通市售试纸稳定性要求。

2.7 实用性评价

由于酶片和生色基质片的稳定性好，材料价廉易得，使用方便，灵敏度基本符合普通残留农药监测的要求(0.1－10 mg/L)，能检测出甲胺磷等目前最常引起人畜中毒的有机磷农药，故本检测法不失为一种易于普及、快速可靠的残留农药检测方法。虽然在检测灵敏度和定量方面不如色谱法，但在简易、快速和可现场检测等方面却是色谱法和其它检测方法所不及的。采用适当的提取方法，本检测法还可推广用于蔬菜、水果、粮食等食物中有机磷残留农药的检测。

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(上接第11页)的条件，有机物在酸相反应器中的分解产物VFA是MPB利用的良好基质。因此，甲烷相反应器启动负荷高，提升快，产气指标正常。另外，废水中剩余部分SO_4^{2-}在此也得到彻底的去除。

甲烷相反应器出水水质，COD约600 mg/L，硫化物50－80 mg/L，如再增加一级生物脱硫对反应器出水进一步净化，不但硫化物可得到有效去除，有机物也可部分降解。出水水质可进一步得到改善。

4 结论

(1) 两相厌氧工艺处理含SO_4^{2-}有机废水整体工艺COD去除率可达到87.6%，SO_4^{2-}去除率为99.4%－100%。

(2) 当进水SO_4^{2-}为1000 mg/L、COD为5000 mg/L时，控制酸相反应器内pH值为5.8－6.2，SO_4^{2-}去除率可达到80%，容积负荷(SO_4^{2-})为5 kg/(m³·d)，COD去除率为26%－35%。

(3) 当脱硫化物反应器容积负荷(S^{2-})约为0.4 kg/(m³·d)时，控制溶解氧为1.5 mg/L，硫化物去除率大于90%，其中95%转化为S^0。

(4) 甲烷相反应器COD容积负荷可达到15.8 kg/(m³·d)，COD去除率为83.3%，COD产气率为0.464 m³/kg。

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Hygienic Evaluation of Indoor Air Pollution Caused by Domestic Natural Gas. Wang Juning et al. (Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, Beijing 100050): *Chin. J. Environ. Sci.*, **16**(3), 1995, pp. 44—48

Natural gas (NG) combustions was compared with coal gas and liquid petrol gas (LPG) in the causation of indoor air pollution. The routine pollutants including B(a)P have been monitored and the 1-hydoxy pyrene in urine of the representative subjects measured. Radon concentration and its change in the NG used, starting from the source plant through transfer station to the end users, has been investigated and measured during the four seasons of the year. To analyse organic components in 3 gases, a big volume sample collector has been designed. The semi-volatile organic components contained in the combustion products of the 3 gases have been identified by GC/MC. The results show that the levels of particles and CO in the heating season were much higher than the standards, but they were the lowest in NG; NO_2 and CO_2 were a little higher in NG; B(a)P in particles and the 1-hydoxy pyrene were the lowest in NG. The organic compositions of coal gas and LPG were more complicated than that of NG. Radon in the NG from Beijing contributed less than 1‰ of effective dose to indoor air quality of the population. Compared with the traditional fuels, the gas fuel are the cleanest ones, and the NG is cleaner than other two.

Key words: natural gas, indoor air pollution, organic components, radon, 1-hydoxy pyrene.

Rapid Determination of Trace Arsenic in Water and Wastewater by Using an Arseno-antimono-molybdenum Blue Spectrophotometric Method. Qiu Xingchu et al. (Ganzhou Prefectural Institute of Environ. Sci. Research, Ganzhou, Jiangxi 341000): *Chin. J. Environ. Sci.*, **16**(3), 1995, pp. 49—51

It was found that the colour producing products have a Soret band at 865 nm, an apparent molar absorptivity of 2.1×10^4, an over 24 hour stability at room temperature, a linearity range of 0—80 μg As in a volume of 10 ml and a correlation coefficient of 0.9990. The use of the Qiu's Arsenic Analyzer allows arsenic in water to be reduced as AsH_3 giving off from water. None of Al, Mn, Zn, Cd, Fe, Co, Ni, Cu, Sn, Sb, Bi and S^{2-} in a reasonable amount can interfere with the determination. This method has been applied to determining arsenic at a microgramme level in surface water and wastewater with satisfactory results.

Key words: arsenic, Qiu's Arsenic Analyzer, arseno-antimono-molybdenum blue, spectrophotometry, surface water, wastewater.

Enzyme Ticket and Chromophoric Substrate Ticket for the Convenient and Rapid Detection of Organophosphorus Pesticides. Huang Yan et al. (Guangzhou Medical College, Guangzhou 510182): *Chin. J. Environ. Sci.*, **16**(3), 1995, pp. 52—54

The cholinesterase inhibition test made it possible to detect organophosphorus pesticides (OPs) compounds in water at 25—35℃ in 15—20 min, and the sensitivity of the method was in the range of 0.01—10 mg/L. Most thiophosphorus pesticides, such as methamidophos and optunal, can also be detected in those levels after they are oxidized with bromine water. This enzyme inhibition assay has been applied to the detection of pesticide in natural waters, and it was found that the positive results were quite obvious as soon as the concentration of OPs were not lower than the detection limits. The method is particularly suitable for field dtection because there is no need for expensive instruments and preparation of reagents.

Key words: organophosphorus pesticides, detection, enzyme ticket, substrate ticket.

β-Correction Spectrophotometric Determination of Silver in Wastewater with Malachite Green. Gao Hongwen (Huaibei Environ. Monitoring Station, Anhui 235000): *Chin. J. Environ. Sci.*, **16**(3), 1995, pp. 55—57

Silver has been found to react with both malachite green (MG) and potassium iodide to form a green chelate at pH 5. β-correction spectrophotometry was studied for the determination of trace amounts of silver. This method can eliminate completely the excess MG in its Ag(I) colored solution to give out the real absorbance of produced Ag-MG-I^- chelate. The reaction of Ag-MG-I^- is selective in the presence of EDTA to mask other metal ions. The β-correction method has higher sensitivity, precision and accuracy than a conventional single wavelength spectrophotometry. Beer's law was obeyed over the concentration range of 0—2.0 mg/L of silver and the detection limit for Ag is 0.05 mg/L which is suitable for the analysis of wastewater. The recovery was found to be between 97.3%—107%,