APPLICATION OF IMMUNOASSAY FOR SCREENING OF POLYCHLORINATED BIPHENYLS (PCBS) IN TRANSFORMER OILS

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Introduction

Polychlorinated biphenyls (PCBs), which are one of persistent organic pollutants (POPs), are having been issued world-widely. Due to its chemical stability and insulating characteristic, PCBs had been used in many parts of industries, especially in electric power industry[1,2,3]. But, as environmental adverse effect of PCBs has been known, production and usage of PCBs was banned in various advanced countries.

For its well-management, the analysis of PCBs concentration is extremely crucial[4]. However, it is difficult to analyze PCBs concentration rapidly and quickly in Korea because of large amount of transformers and high cost and long analytical time of Waste Official Test Method (WOTM). So it is getting necessary to develop a new method for screening of PCBs concentration.

There are several methods for PCBs screening in some countries, but they cannot be adopted directly in Korea due to largely low regulatory level and difference of transformer oil's physiochemical properties. Among them, Immunoassay is regarded to be one of useful method for PCBs screening in Korea. Immunoassay uses compound specific antibody which has been produced by immunological reaction in animal's body. After performing appropriate pre-treatment of oil, anti-PCBs antibody captures PCBs and PCBs concentration can be calculated from the amount of free-antibodies.

Therefore, in this study, application of immunoassay to screening of PCBs in 500 transformer oils was presented by modifying pre-treatment procedure for antigen-antibody reaction and comparing results of WOTM and immunoassay.

Materials and Methods

In WOTM of Korea, cleanup procedure includes alkali treatment and multi-layer acid silica-gel column. Since aim of this study is rapid, simple and cheap analysis of PCBs, the amount of absorbent should be decreased, but stronger acid (sulfuric acid, fuming) than WOTM was used in acid coated silica-gel column. And, silver nitrate ($AgNO_3$) coated silica-gel was added to remove interference for calculating amount of free-antibodies.

After cleanup column elusion with n-Hex, the extract was reacted with anti-PCBs antibody solution, of which antibodies were labeled by gold colloid. In this stage, anti-PCBs antibodies can capture extracted PCBs. To separate free antibodies from antibody-PCBs complex, the solution was penetrated through the membrane. Since antibodies were labeled by gold colloid, the color of membrane is changed according to the amount of free-antibodies captured in membrane. After membrane filtration, the membrane was dried and the color of membrane was measured by colorimeter. The detailed analytical procedure was described in Fig. 1.

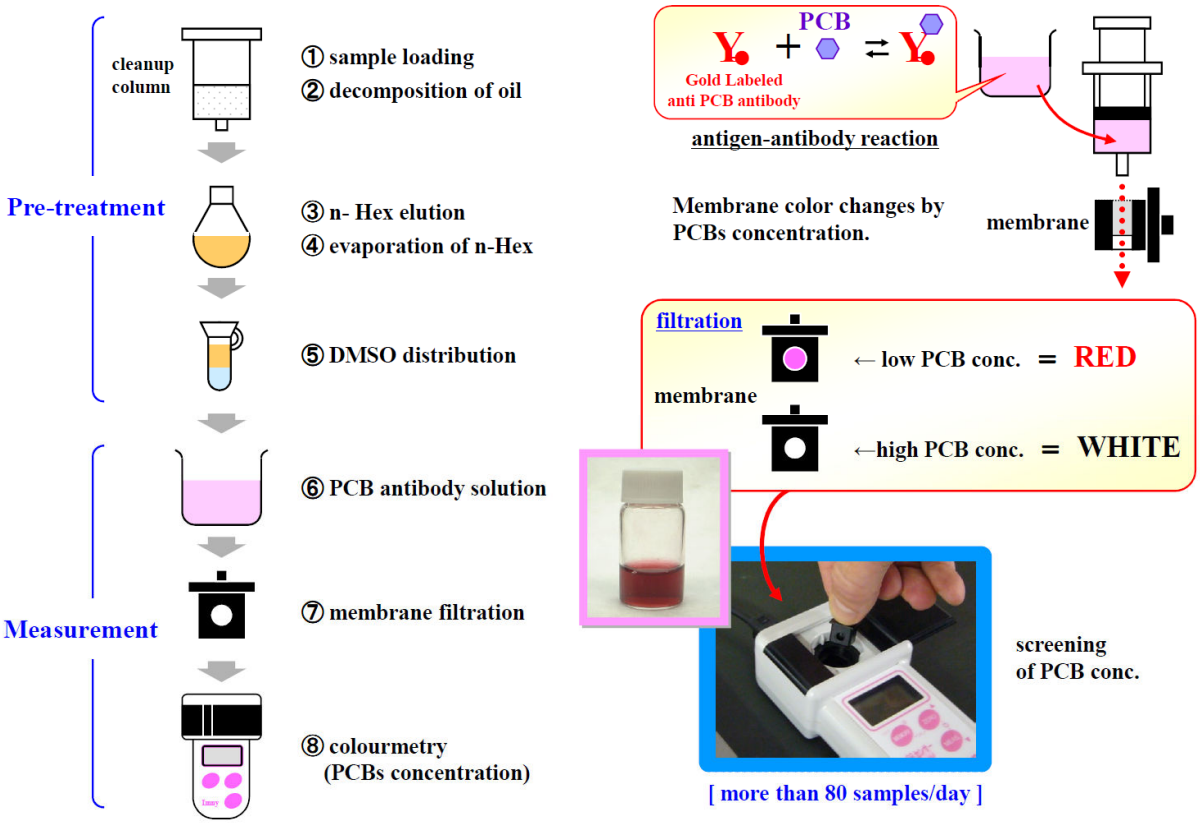

Fig. 1. Analytical procedure of immunoassay for PCBs screening

Results and Discussion

Antibodies are produced from B-cell of mouse's spleen by injecting an antigen. Since PCBs are hydrophobic and have relatively small molecular weights (less than 500 except for deca-chlorinated biphenyl), they can hardly perform a role as an antigen or an immunogen in mouse's body. Thus, antigen with an epitope of which molecular formula is similar with PCBs was injected into mouse. An epitope is a part of antigen which can bind to antibody. Production of antibodies was dependent on the dose of antigen and frequency or period of injection. The antibody used to measure PCBs in this study is monoclonal.

The total time required for analysis was largely decreased comparing to previous WOTM. First of all, elution time of cleanup column was decreased to only about 10 min since smaller amount of absorbent was used. Also, in this study, portable device (colorimeter) was used for quantification of PCBs and it took a few seconds for measurement; which was drastically decreased comparing to gas chromatography / electron capture detector (GC/ECD) which takes more than 20 min. Moreover, the analytical cost was lower than WOTM. WOTM takes about 70,000~80,000 KRW for one oil sample, but immunoassay applied in this study is expected to take less than 50,000 KRW. If a larger amount of oil samples were analyzed, the reduction effect of cost and time required would be much bigger.

The quantification method used in this study is colourimetry. So, the analytical result can be easily affected by interferences. The most probable interference was shown to be alkyl-benzenes as a result of gas chromatography / mass spectroscopy (GC/MS). Alkyl-Benzenes were extracted when mixing antibody solution with PCBs extract. They had a white color, thus it caused inaccurate measurement of free antibodies which were labeled with gold colloid. The more amount of free antibody is underestimated, the more concentration of PCBs is overestimated (false positive). In Korea, synthetic oils have been used in some kinds of transformer or capacitor. It is regarded to

be mixed with mineral oils through re-usage procedure of transformers, which was the most probable cause of PCBs contamination problem in Korea. As a result of GC/MS analysis after pre-treatment for synthetic oil, similar peaks were appeared in the chromatogram. Since transformers or capacitors haven't been managed well in Korea, it is difficult to separate which transformer contains synthetic oils. Therefore, silver nitrate ($AgNO_3$) coated silica-gel was added in pre-treatment procedure which has been used to remove aliphatic hydrocarbons.

For quantification using anti-PCB antibody, there are several parameters to be considered carefully and deeply. It includes dilution factor of solutions, preparing membrane and its kind, velocity and volume of antibody solution for membrane filtering, etc. We have assumed a number of cases and the optimized condition could be established. Fig. 2 shows screening result of 500 transformer oil samples.

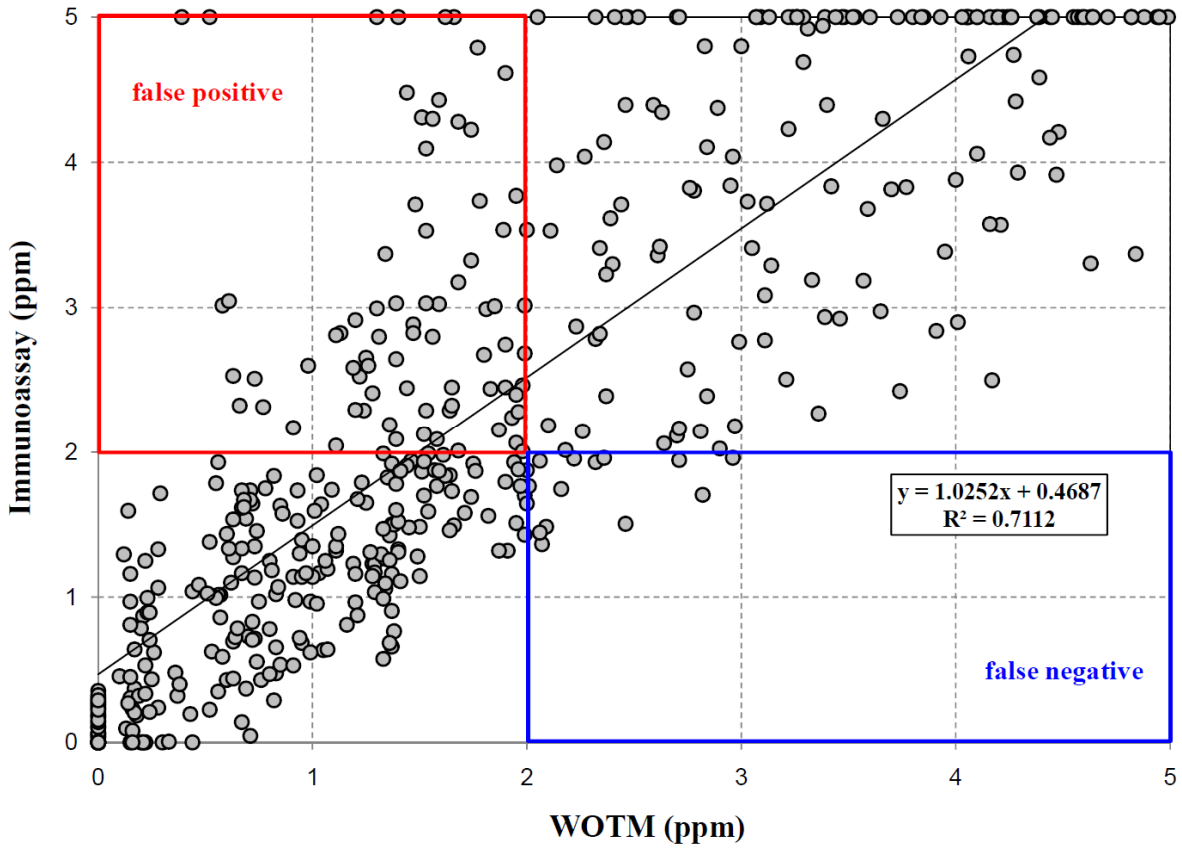

Fig. 2. Screening result of 500 transformer oil samples by immunoassay

At this point of time, the result shows relatively moderate correlation between two methods and it can be seen the variation of analytical result exists to some extent. Especially, the concentration of some samples was highly overestimated. However in case of N.D. (not detected) samples, they were all less than about 0.5ppm (immunoassay) and most of highly overestimated samples were over 2ppm (WOTM). Since the regulatory level of PCBs in Korea is 2ppm, it was regarded to be meaningful result (R^2 = 0.7112) to some extent for screening of PCBs in transformer oils.

This screening method could be easily influenced by skillfulness of analyst. For the maintenance of precision and accuracy of analytical result, meticulous care is needed for handling antibodies, preparing membrane, pre-treatment, etc. And it is regarded further modification and consideration for analytical procedure is required for application.

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