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論　文　名	「Development of Lipophilic Dye Liquids for Rapid and Highly-sensitive Optical Sensors Based on Solvent Polymeric Membrane」 （高速・高感度な高分子液膜型光センサーのための疎水性色素液体の開発）
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論文要旨

Optical sensors based on solvent polymeric membrane (SPM), which is mainly used plasticized poly(vinyl chloride) (PVC), have been utilized for various ion sensing applications by using functional molecules (dyes, ionophores) that were tailored to a target ion. These sensors have improved their sensing performance, and provided new response mechanisms by utilizing nanoparticle or nano-emulsion. Applications on microanalytical devices also have been suggested, such as single-step microanalytical devices using square glass capillary immobilizing PVC membrane optical sensors developed in our laboratory. Various applications such as ion-sensing, bioassay, and immunoassay were achieved by designing the spontaneous reaction in the devices triggered by capillary action-based sample introduction. Thus, SPM-based optical sensors are useful sensing media for designing analytical methods for various analytes. In general, to improve the sensitivity, increasing the thickness of the membrane and its dye concentration is necessary. However, increasing the membrane thickness leads to a slower response, and increasing the dye concentration causes the dye to precipitate due to its low solubility. Therefore, improving the sensitivity of conventional SPM-based optical sensors still remains the problems.

Ionic liquids (ILs) have received significant attention as the extraction media for chemical analyses that are based on solvent extraction. ILs composed of dye molecules have also received attention in physical chemistry for their unique optical properties. Among these, pH-sensing with ILs composed of a lipophilic phosphonium cation and a typical pH indicator dye anion was also proposed. Although such lipophilic ILs were observed to form interfaces between aqueous phases, bulk extraction processes such as ion extraction from the aqueous phase and back-distribution of the hydrophilic dye to the aqueous phase have not been discussed thoroughly. Thus, clarification of response phenomenon through the ion-extraction or ion-exchange between ILs phase and aqueous phase is important. By clarifying that, IL materials can be tailored for analytical use with better

sensing performance such as good selectivity for target ion.

In this thesis, the author focused on lipophilic ionic liquid structure, which can liquefy solid dye molecules possessing rigid chemical structures and have a potential to play a role as a plasticizer for SPMs. SPMs based on lipophilic dye liquid (LDL) contain an exceptionally high concentration of dye. Thus, highly sensitive measurements were expected. Moreover, a thin membrane could be prepared while maintaining enough sensitivity, thus, a fast response time was also expected. By systematic investigation of response to aqueous sample, ion-extraction mechanisms through the SPM surface was clarified. After that, example of functionalization of LDL, application to microanalytical devices and further use for fluorescence-based measurement with LDL characteristic were demonstrated.

In the first chapter, the introduction and motivation of my study were summarized by referring the previous related studies.

In the second chapter, the experimental results and discussion on the plasticized PVC membrane using LDL composed of trihexyltetradecylphosphonium (P_{66614}) cation and naphtholphthalein (NP) dianion ($[P_{66614}]_2[NP]$) were described. The prepared LDL contained approximately 40% dye relative to the formula $[P_{66614}]_2[NP]$, as confirmed by ^1H NMR measurements and elemental analysis. For clarification on response properties, the reversibility of response, correlation of thickness, response time were systematically investigated. Surprisingly, the membrane exhibited fully reversible responses to acidic and basic solutions, even though the dye was not lipophilized, that has been considered an indispensable chemical modification for dyes used in typical SPM-based optical sensors. A comparison with conventional plasticized PVC membranes using nitrophenyl octyl ether (NPOE) as a typical plasticizer was carried out in terms of the response time and sensitivity. The PVC membrane using $[P_{66614}]_2[NP]$ contained 5~60-fold higher concentration of dye, compared to those in previous reports, and exhibited approximately 4-fold faster 95% response time when both PVC membranes showed almost the same maximum absorbance value. Then, anion sensing was demonstrated and the PVC membrane successfully responded to anions through the coextraction of proton and anion with selectivity following the Hofmeister order.

In the third chapter, the experimental results and discussion on the systematic investigation of LDLs composed of P_{66614} cation and dianion of typical commercial pH-indicator from the view point of increasing the dye content were described. From the results of chapter 2, synthesized $[P_{66614}]_2[NP]$ contained only 40% NP molecules compared with ideal amount. Therefore, to improve sensitivity, many other pH indicator-based LDLs still have the potential to contain more amount of dye. Here, several triphenylmethane dyes were chosen and LDLs were synthesized. As the result, LDL composed of bromothymol blue (BTB) realized the highest dye content of approximately 100% just after synthesis. Absorbance response of the PVC membrane using BTB-based LDL was unfortunately decreased ca. 37% by repetitive introduction of acid and base solutions, however, interestingly, it reached to exhibit stable and reproducible response. Concentration of dye was 20-70-fold higher than that of conventional ones. In addition, the PVC membrane was also applied for anion sensing based on coextraction of proton and anion in aqueous solutions, and found that the ion selectivity followed Hofmeister order as well as the result in chapter 2.

In the fourth chapter, the experimental results and discussion on the development of LDL composed of P_{66614} cation and fluorescein anion possessing long alkyl chain (n-FL) ($[P_{66614}][n\text{-FL}]$: n is alkyl number) to ensure the lipophilicity of dye and application to rapid and highly-sensitive heparin sensing were described. Systematic investigation of the alkyl chain length proved the significance of lipophilicity for obtaining the reversible absorbance measurements, where dodecyl substituent was lipophilic enough to retain dye in the PVC membrane phase. A PVC membrane prepared with $[P_{66614}][12\text{-FL}]$ contained an unusually high dye concentration (915 mmol/kg). The

sensitivity of the presented PVC membrane was 26-fold higher than that of a conventional optode membrane with the same membrane thickness and the same lipophilic dye of typical dye content (1 wt%). The response time was observed to be >120-fold faster by using a significantly thinner PVC membrane (approx. 140 nm). Further, the presented PVC membrane exhibited an extremely fast response (20-150 seconds) to the heparin in diluted serum within the required concentration region. Thus, the LDL could significantly improve the sensor performance in conventional SPM-based optical sensors, especially for an analyte showing slow diffusion such as macromolecular heparin.

In the fifth chapter, the experimental results and discussion on the development of calcium selective LDL ([KD-M13][OP_2P]) were described. In this study, the strategy for development of LDL that responds to calcium ion by ion-exchange mechanism was demonstrated. [KD-M13][OP_2P] was composed of merocyanine dye possessing bulky substituents and long alkyl chain (KD-M13) and bulky anionic charged ionophore (bis(4-n-octylphenyl) phosphate : OP_2P), which appeared as a liquid at room temperature. The [KD-M13][OP_2P]-based PVC membrane contained very high concentrations of dye and ionophore (872 mmol/kg), 17~170-fold higher than that in a conventional one. Further, fully reversible response to calcium ion concentration was obtained. Ion selectivity was investigated by using typical cations and found that the selectivity in the order of $Ca^{2+} > Mg^{2+} >> K^+ \approx Na^+$, indicating that the Ca^{2+}-selective response was successfully achieved. Sensitivity, evaluated as the absorbance for a 100 nm-thick membrane, was significantly enhanced 13-fold as compared to that for a conventional PVC membrane.

In the sixth chapter, the experimental results and discussion on fabrication of microanalytical devices based on combining convex and concave shaped poly(dimethylsiloxane) (PDMS) microchannel immobilizing LDL-based PVC membrane were described. The main purpose of this chapter was the development of regioselective and intact immobilization method of very thin (~200 nm) and soft LDL-based PVC membrane optical sensors on a microanalytical device. The proposed method using poly(vinyl alcohol) film as a sacrificial layer allowed successful immobilization of intact LDL-based PVC membrane only on the convex-shaped PDMS surface without any deformation or increase of inhomogeneity. In addition, different kinds of PVC membranes were successfully immobilized simultaneously toward multiplexed detection. As the demonstration, simultaneous Ca^{2+} and Cl^- measurement was succeeded with microchannel array devices immobilizing [P_{66614}][12-FL]-based and [KD-M13][OP_2P]-based PVC membranes.

In the seventh chapter, the experimental results and discussion on development of highly fluorescent LDL composed of P_{66614} cation and pyrene modifying sulfonate anion ([P_{66614}][HP-SO_3]) and fluorescence amplification based on Förster resonance energy transfer (FRET) in the LDL-based PVC membrane were described. The purpose in this chapter was how the fluorescence sensitivity was improved by LDL, which was generally higher than that of absorption-based measurement. As one approach achieving this objective, FRET-based fluorescence amplification system reported previously was applied for [P_{66614}][HP-SO_3]-based PVC membrane optical sensor. Here, [P_{66614}][HP-SO_3] was used as plasticizer and donor, and [P_{66614}][12-FL] was used as acceptor. Systematic investigation on the donor/acceptor ratio clarified the effects on amplification factor and sensitivity. At an acceptor doping level of 0.5 mol% (vs donor), approximately 22-fold higher sensitivity was obtained compared to that of conventional PVC membrane optical sensor. During anion measurement based on the coextraction, selectivity following Hofmeister order was observed, which was controlled by the addition of ionophore. Thus, proposed FRET system based on a fluorescent LDL has the potential to significantly improve the sensitivities of optical sensor using SPM with high selectivities for various target analytes.

In the eighth chapter, the results and findings obtained in this study were summarized.

審査結果の要旨

本論文は、従来固体粉末であることが常識であった色素分子を溶媒に溶解させることなく液化し、従来型センサーを凌駕する性能を持ったオプティカルイオンセンサーを開発した研究であり、以下の成果を得ている。

疎水性ホスホニウムカチオンとナフトールフタレインジアニオンから成る疎水性色素液体を開発し、色素濃度が従来比約 5〜60 倍となる高濃度薄膜の作製および、それに伴う高感度・高速検出を実現した。また。その膜-水溶液界面で生じる応答機構が、プロトンとアニオンの協同抽出であることを明らかにした。

種々の市販トリフェニルメタン系色素の液化を検討し、色素の疎水性・静電相互作用が高分子液膜中色素の保持に重要であること示した。ブロモチモールブルーを用いた色素液体においてほぼ理想的なイオン交換率になることを明らかにし、ここでは色素濃度約 20〜70 倍の高濃度化および、アニオンの高感度・高速検出を実現した。

疎水化色素を利用した色素液体開発とそのヘパリン分析への応用を検討し、色素分子への炭素数 12 のアルキル鎖導入により、色素流出のほぼない可逆応答を示すことを明らかにした。また、膜厚 140 nm の極薄膜の利用により、色素高濃度化に基づく高い吸光感度かつ従来比 120 倍もの高速応答を実現し、従来応答時間の長かったヘパリン等の高分子イオンに対しても高速・高感度な分析を実現できることを明らかにした。

Ca^{2+}選択的色素液体の開発では、嵩高い置換基を持つメロシアニン色素カチオンと、嵩高いリン酸系イオノフォアアニオンの組み合わせにより、Ca^{2+}選択的色素液体の開発に成功し、色素液体構造の設計でアニオン分析のみならず選択的カチオン分析にも適用できることを示した。

マイクロ分析デバイスへの色素液体薄膜固定化法開発では、ポリビニルアルコール基板を犠牲層とする位置選択的な高分子液膜固定化法を開発した。また、異なる色素液体を用いた高分子液膜の同時固定により、1 デバイスでのマルチイオンセンシングの例を示した。

色素液体の高濃度色素環境を利用した、フェルスター共鳴エネルギー移動型の蛍光増強に基づく高感度蛍光検出法を提案した。ここでは従来比約 20 倍の高感度化を実現し、高濃度な色素環境の利用で、従来と同等の低濃度のセンサー分子でも、より高感度・高選択的な分析が実現できることを明らかにした。

以上の諸成果は、光検出型化学センサーの課題となっている高感度化・分析時間短縮・選択性向上を実現するために重要な知見を与えるとともに、新たな分析デバイス開発についても有益な情報を提供したものであり、本分野の学術的・産業的な発展に貢献するところ大である。また、申請者が自立して研究活動を行うのに必要な能力と学識を有することを証したものである。学位論文審査委員会は、本論文の審査および最終試験の結果から，博士（工学）の学位を授与することを適当と認める。