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Thermal detection of histamine with a graphene oxide based molecularly imprinted polymer platform prepared by reversible addition–fragmentation chain transfer polymerization

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ABSTRACT

Graphene oxide (GO), with its small dimension and high surface-to-volume ratio, can enhance the binding capacity and sensitivity of molecularly imprinted polymers (MIPs). Therefore, a straightforward and fast method was developed to graft MIPs onto GO by reversible addition–fragmentation chain transfer (RAFT) polymerization. First, the initiator was linked to the GO in a simple two-step process which was verified via UV–vis spectroscopy. Subsequently, a MIP layer for histamine was grown onto the functionalized surface by RAFT crosslinking polymerization, enabling control over the imprint structure. The formation of a hybrid GO–MIP structure, particles surrounded with a polymer network of ~2.4 nm thick, was verified by atomic force microscopy (AFM). Classical batch rebinding experiments demonstrated the specificity of the MIP towards its original template histamine. Next, the heat-transfer method (HTM) was applied, a novel sensing technique requiring only two thermocouples and an adjustable heat source. This method has been employed for the detection of small organic molecules with bulk MIPs, but never with a GO-hybrid structure. For proof-of-principle purposes, silicon substrates were functionalized with the GO–MIPs and sensing was performed on histamine in buffer solutions. The designed sensor platform could detect histamine in the nanomolar regime, similar to conventional techniques. In summary, we have developed a fast and straightforward method to prepare MIP–GO hybrids which were able to measure histamine in buffer solutions by thermal detection. Since GO exhibits excellent thermal properties, this opens the window to sensing of small organic molecules in relevant biological samples.

1. Introduction

Molecularly Imprinted Polymers (MIPs) are synthetic receptors which can recognize target molecules based on shape, size and functionality [1,2]. Their binding mechanism is similar to the "key-lock" principle of enzymes, thereby allowing high affinity and specificity for its template [3]. As compared to natural antibodies, they offer several advantages such as chemical inertness [4], almost unlimited shelf life [5], and a cheap and straightforward production process [6]. Due to these properties, the use of MIPs have become increasingly attractive for bio-analytical and biosensing purposes [7].

Traditionally, MIPs are prepared from bulk polymerization by which micron sized particles are obtained [8]. While they exhibit excellent specificity [9], there are also several disadvantages including their dimensions, high diffusion barrier, incorporation into sensing devices is non-straightforward, and there is no control over the structure of the binding sites [10]. To overcome these disadvantages, it might be interesting to consider grafting MIPs onto graphene. Graphene has a small dimension with a high surface-to-volume ratio [11], it possesses unique mechanical and electrical properties [12], and can be selectively deposited onto surfaces [13]. The benefits for molecular imprinting are that nanoscale sized graphene enables miniaturization of the system and thereby lowers the diffusion barrier. Additionally, the large surface area

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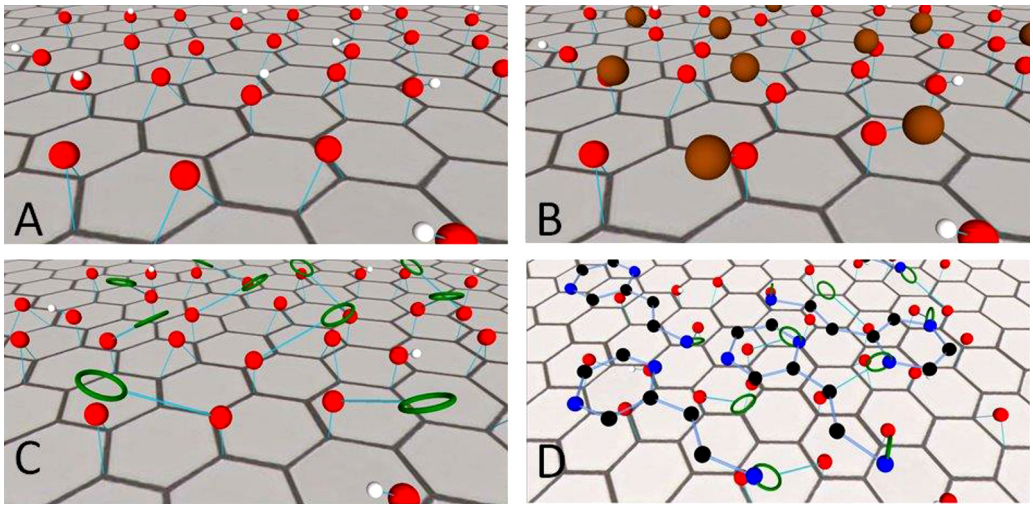

Fig. 1. Schematic illustration of the functionalization of the GO surface starting from (A) pure GO particles to (B) esterification of the surface with a bromide group, GO–Br (C) substitution of the bromide by the RAFT agent, GO–RAFT (D) after growing of the MIP layer, nanocavities are formed where the template histamine can be bound. The white balls represent hydrogen atoms, the red oxygen atoms, brown is bromine, black is carbon, blue is nitrogen and the green circles correspond to the RAFT agent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ensures easy removal of the template and offers a potentially high rebinding capacity [14]. By employing a controlled polymerization technique, the imprinting structure can be defined and the MIPs can be directly deposited onto the GO without any additional procedure such as stamping. Several methods to functionalize graphene have been developed, however it is challenging to control the density and thickness of materials grafted onto the surface [15,16]. A MIP-graphene hybrid has been prepared by Mao et al. [17]. They synthesized a MIP for dopamine by dispersing graphene sheets with template and functional monomers in an organic solvent followed by free radical polymerization. While with electrochemical sensing detection limits of 100 nM in buffer were obtained, this technique is not able to obtain a homogeneous imprinted structure or define the dimensions of the imprints. Therefore, the use of graphene oxide (GO) compared to graphene is a viable alternative since the existence of residual oxygen-containing functional groups allow facile chemical modification of the surface [18]. MIP–GO hybrids have been synthesized by various controlled polymerization techniques including atom-transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer (RAFT) polymerization. Chang et al. [19] have studied a MIP for 2,4-dichlorophenol prepared by ATRP and demonstrated its selectivity by performing sensing measurements with a HPLC device coupled to a UV detector. The drawbacks of this method however are the long preparation time and the difficulty of removing the copper catalyst afterwards, which could potentially hinder when measuring biological samples. The RAFT polymerization technique, first reported by Rizzardo et al. in 1998 [20], has the benefits that it is more versatile since it can be used for a wide variety of monomers. Furthermore, it operates under mild reaction conditions. The first MIP on functionalized GO by RAFT polymerization was described by Li et al. [21]. They have transferred a protocol designed for functionalized RAFT silica gels [22] to GO and were able to show its potential use as building blocks in nano electromechanical devices. In recent years, several improvements have been done to facilitate easier synthesis methods for grafting polymers onto GO [23–25] but the drawback remains the tremendous preparation time. In this manuscript we will present a straightforward and fast synthesis method to obtain MIP–GO hybrid structures by RAFT polymerization. We start from commercially available GO in contrast to preparing GO by the Hummers method which involves vigorous treating of graphite with an anhydrous mixture of sulfuric acid, sodium nitrate, and potassium permanganate [26]. The functionalization of the GO and the subsequent crosslinking polymerization of the MIP are schematically shown in Fig. 1.

First, the RAFT agent is attached to the surface by a simple two-step procedure. The number of functional groups is determined by a sacrificial initiator experiment and is similar to what can be found in literature [27,28]. Next, a MIP for histamine is polymerized onto the functionalized GO by a slight adaptation of the bulk MIP protocol by Horemans et al. [29]. The presence of the network is demonstrated with atomic force microscopy (AFM) measurements and showed that a polymer network with a thickness of 2.4 nm was formed around the GO particles. Subsequently, sensing experiments with histamine in buffer solutions were performed. In first instance, the binding capacity was determined by traditional UV–vis batch rebinding experiments. These results showed a significant difference between the MIP and its reference, the non-imprinted polymer (NIP). Moreover, the results of the saturation banding capacity are in the same range as was obtained by Li et al. [21] with a GO–MIP for 2,4 dichlorophenol. The novel sensing part is the employment of the heat-transfer method (HTM) which requires only two thermocouples, an adjustable heat-source and a proportional-integral-derivative (PID) controller, thereby ensuring fast, straightforward and low-cost detection. This concept was previously applied in the molecular imprinting field for DNA mutation analysis [30], the screening of cancer cells [31] and the detection of neurotransmitters by bulk MIPs [32]. This method has not been extended to graphene based systems while graphene, with a better thermal conductivity than an excellent thermal conductor such as copper [12], can enhance the detection limit of HTM. To investigate this, silicon electrodes were functionalized with the GO–MIPs by simply dispersing the particles followed by spin coating of the solution. After stabilization of the sensor in phosphate buffered saline (PBS) solutions, increasing concentrations of histamine in PBS were added. Due to the binding of the template to the MIP, the thermal resistance increased and histamine could be detected in the nanomolar regime. This is only a proof-of-principle measurement, GO exhibits excellent thermal properties and with fine tuning of the sample preparation the detection limit could be further enhanced. In summary, we have developed an easy and straightforward preparation process for the synthesis of GO–MIP hybrid materials. When combining this concept with the HTM sensing approach, fast and low-cost detection of histamine in buffer