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Pinacolyl methyl phosphonate (PMP) detection by molecularly imprinted polymers (MIP): A labile covalent bonding approach

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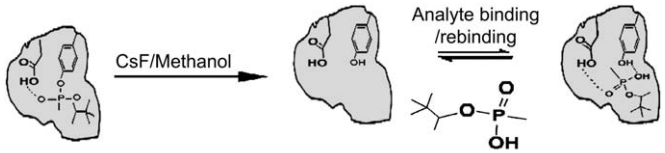
Abstract

Molecularly imprinted polymers (MIP) have become increasingly important in the area of separation and detection. They are emerging tools for the design of structured porous materials having a precise arrangement of functional groups within pores of controlled size and shape. In the present study, covalent molecular imprinting was achieved by using styrene and di-acrylate monomers together with covalently tethered nerve agent (NA) analogs. Due to the nonpersistent nature of this class of nerve agents, the covalent attachment ensures the integrity of the analyte as opposed to a non-covalent approach where the decomposition products of pinacolyl methyl phosphonate (PMP) can lead to a variety of binding cavities based upon the nature of the decomposed products. The binding affinity of the imprinted polymers to the NA analogs, as studied by colorimetric methods, was found to be efficient and highly selective.



Keywords: Pinacolyl methyl phosphonate; Molecular imprinting; Covalent bonding

1. Introduction

A critical issue to homeland security is the development of a broad range of detectors for highly toxic nerve agents. There is a significant interest in developing materials that effectively adsorb and/or degrade organophosphorus compounds and for differentiating nerve agents from pesticides [1]. Various methods of adsorption and detection of organophosphorus compounds in the environment have also been investigated [2]. The nerve gas agents of particular concern are the organophosphonate compounds such as Sarin and Soman [3]. In the present study, pinacolyl methyl phosphonate (PMP), a degradation product and an active analog of Soman, was chosen as an analyte to demonstrate the viability of the molecularly imprinted polymers (MIP) technique.

MIP have become increasingly important in the area of separation and detection. They have found applications in the design of structured porous materials having a precise arrangement of functional groups within pores of controlled size and shape [4]. Such controlled selectivity in principle can offer a scope of opportunities for molecular recognition applications [5]. Because of the capability of forming stable, robust

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materials with molecular selectivity for a wide variety of compounds, molecular imprinting has become an attractive method for the formation of sensor components and for other applications such as analysis and separation of trace levels of compounds, and enzyme mimics [6]. MIPs are highly cross-linked polymers synthesized in the presence of print molecules. After the removal of the print molecule, recognition sites suitable for the selective rebinding of the print molecule are obtained. MIPs also offer several other advantages such as stability at high temperatures and in organic solvents [7].

In this work, molecular imprinting was achieved by the use of styrenic monomers, which were first tethered covalently to the nerve agent (NA) analog. After polymerization, the tethered analog is then selectively hydrolyzed by using CsF, thus creating an imprint cavity inside the polymer matrix. Several research groups in the past have shown other methods of hydrolyzing a variety of phosphorus ester bonds, and its efficacy in detoxifying pesticides and chemical warfare agents using both enzymatic and non-enzymatic approaches [8]. The present covalent imprinting approach is different from most of the conventional MIP methods which mainly involve a non-covalent approach [9]. Due to the nonpersistent nature of this class of nerve agents, the covalent attachment ensures the integrity of the analyte as opposed to the non-covalent approach, where the decomposition products of PMP [10] can lead to a variety of binding cavities based upon the nature of the decomposed products. Although a plethora of literature is available on non-covalent imprinting, only a handful of research has been targeted towards covalent imprinting. The covalent imprinting itself is advantageous in that the interaction between the template and functional monomer is much less affected by solvent polarity and temperature during the imprinting process [11]. Furthermore, the functional groups are only situated inside the cavities and not statistically distributed all over the polymer matrix as is usually the case with non-covalent imprinting in which a large excess of polymerizable binding sites have to be used. Once the polymer was formed, the template was chemically cleaved leaving behind an imprinted cavity. The rebinding of the analyte was then tested in a non-covalent manner to test the ability and selectivity of the imprinted polymer.

As can be seen from Scheme 1, three polymers were synthesized namely Poly-A, Poly-S and Poly-C to comparatively study molecular imprinting inside the polymer matrix. Poly-A was synthesized selectively to study the selectivity against PMP. Poly-S was purposely functionalized with a chemically similar group, diethyl phosphite (DEP), although it bears a different spatial arrangement. The cavity thus formed after cleavage from Poly-S was found to be less selective towards PMP, while the imprinted Poly-A shows the highest selectivity. We have also synthesized Poly-C, which acts as a control and was found to be least selective. The results obtained herein were quite predictable, nevertheless, the synthetic strategy and imprinted polymer holds a promise for future viability and use of these materials as selective adsorbers for PMP. The results, therefore, demonstrate the effectiveness of the covalent imprinting in the light of maintaining the nonpersistent nature of the nerve agent analog PMP.

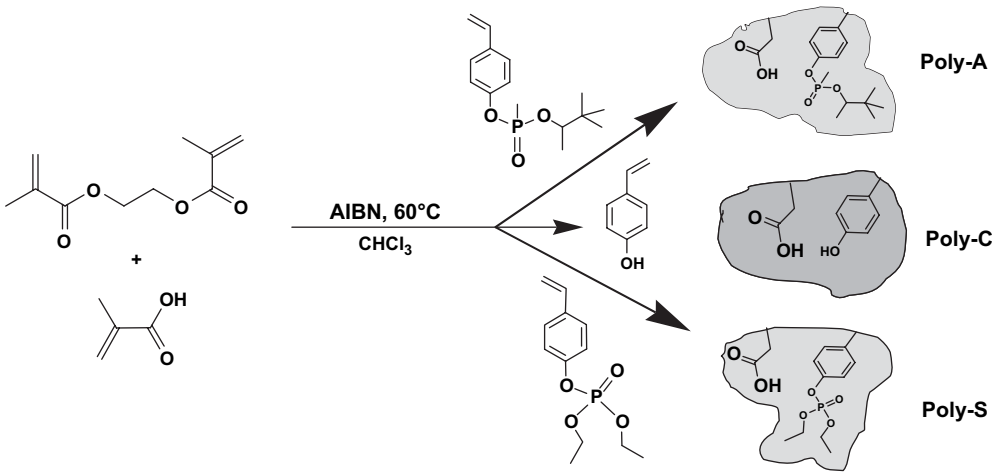
2. Experimental section

2.1. Instrumentation

NMR spectra were obtained using General Electric QE 300 spectrometer ($^{1}H/^{13}C$ 300 MHz). UV–vis spectra were recorded using an Agilent 8453 spectrophotometer. All FTIR measurements were done using a Digilab FTS 7000 step scan spectrometer. Phosphorous elemental analysis was performed using a phosphorous trace analysis method.

2.2. Reagents

All chemicals were purchased from Aldrich Chemical Company. Methacrylic acid (MAA) with modified vinylphenol was used as the functional monomer. Azobis(isobutyronitrile) (AIBN) after recrystallizing from ethanol was used as the radical initiator. Ethylene glycol dimethacrylate (EDMA) which was purified to remove the inhibitor and serves as the



Scheme 1. Synthesis of covalently bound polymers with the template.

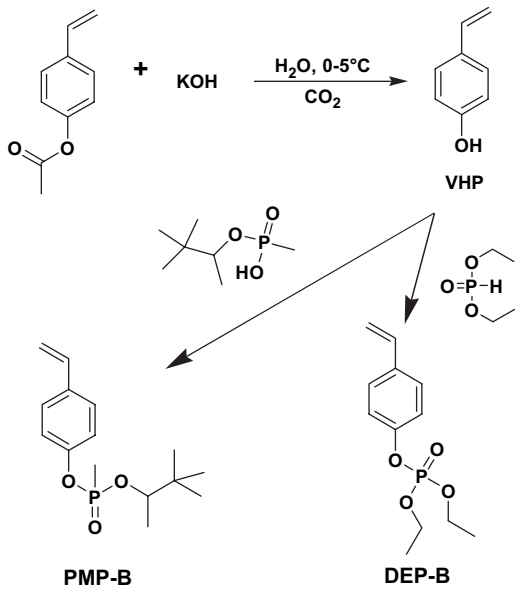
cross-linking monomer; $CHCl_3$ was used as the porogenic solvent. Distilled methanol and deionized water were used as solvents for the stock and test solutions.

2.3. Synthesis of 3,3-dimethylbutan-2-yl-4-vinylphenyl methyl phosphonate (PMP-B)

PMP-B was synthesized by a modified Castors method [12] as shown in Scheme 2. *p*-Vinylphenol was prepared in 92% yield by using the method described by Corson et al. [13]. *p*-Vinylphenol (0.48 g, 4 mmol), pinacolyl methyl phosphonate (PMP) (0.72 g, 4 mmol), triethylamine (1.12 g, 8 mmol), and Castro's BOP (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) reagent (1.76 g, 4 mmol) were dissolved successively in CH_2Cl_2 (15 mL). After 20 h an additional 0.5 equiv. of BOP and triethylamine were added, and the reaction was left overnight. Saturated brine (50 mL) was added and the product extracted into ethyl acetate. The organic phase was washed with 1 N HCl, $NaHCO_3$ (saturated), and brine. The product was purified by column chromatography (silica, eluent: $CHCl_3$/MeOH, 9:1), giving 0.66 g (60%) of PMP-B as a yellow oil. The diastereomers of PMP-B were taken as is without further separation. ^{1}H NMR (δ) in $CDCl_3$: 7.30 (d, 2H), 7.14 (d, 2H), 6.60–6.62 (m, 1H), 5.64 (dd, 1H), 5.17 (dd, 1H), 4.25–4.12 (m, 1H), 1.58 (dd, 3H), 1.0–1.3 (m, 3H), 0.88 (d, 9H). ^{13}C NMR (δ) in $CDCl_3$: (2d, C–P) 12.92–12.98, 17.8, 26.21, (2d, $C(CH_3)$) 32.56–32.59, (2d, O–CH_3) 79.17–79.37, (vinyl) 113.3, (2d, C2,6-phenol) 120.06, 120.11, (2d, C3,5-phenol) 129.31, 129.43, (C4-phenol) 134.61, (vinyl) 136.18, (2d, C1-phenol) 149.32, 149.36.

2.4. Synthesis of diethyl 4-vinylphenylphosphate (DEP-B)

A 100 mL round bottom flask was charged with diethyl phosphite (DEP) (2.39 g, 17.3 mmol) and CCl_4 (7.91 g, 51.4 mmol). The mixture was allowed to cool to 0 °C under constant stirring and nitrogen atmosphere and *p*-vinylphenol (2 g, 16.6 mmol) was then added and the mixture was allowed to stir for another 30 min. Triethylamine (1.75 g, 17.3 mmol) was then added drop-wise via a syringe and the resulting solution was allowed to stir overnight. The resulting milky solution so formed was found to have formed $NHEt_3$ crystals on the bottom of the flask. The precipitate was filtered off and washed with a very small amount of water. The collected supernatant was then extracted using ethyl acetate. After drying with Na_2SO_4 and evaporating the solvent, the residue was separated by column chromatography using hexane:ethyl acetate (3:1) to give the pure product in 56% yield (Scheme 2). ^{1}H NMR (δ) in $CDCl_3$: 7.34 (d, 2H), 7.14 (d, 2H), 6.65–6.59 (dd, 1H), 5.64 (dd, 1H), 5.19 (dd, 1H), 4.23–4.14 (m, 4H), 1.32 (dt, 6H). ^{13}C NMR (δ) in $CDCl_3$: (2d, CH_2CH_3) 16.11, 16.19, (2d, O–CH_2CH_3) 62.28, 62.33, (vinyl) 113.62, (2d, C2,6-phenol) 120.13, 120.24, (2d, C3,5-phenol) 127.31, 127.43, (C4-phenol) 134.33, (vinyl) 136.18, (2d, C1-phenol) 149.95, 150.03.



Scheme 2. Synthesis of PMP-B and DEP-B.

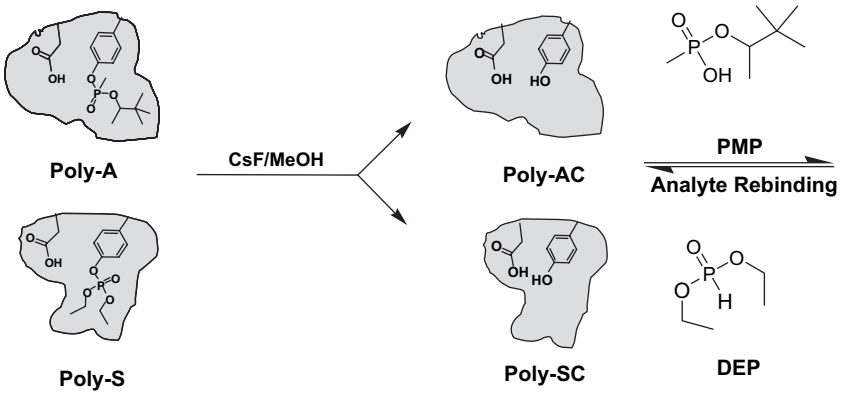
2.5. Synthesis of Poly-A/Poly-S/Poly-C

In a typical preparation of Poly-A, template PMP-B (28.2 mg, 0.1 mmol) was dissolved in $CHCl_3$ (3.76 mL) and methacrylic acid (MAA) (0.17 mL, 2 mmol), and ethylene glycol dimethacrylate (EDMA) (3.39 mL, 18 mmol) and the initiator AIBN (33 mg, 0.2 mmol) in 1 mL of $CHCl_3$ were added followed by the transfer of the mixture to a thick-walled glass tube. This ratio was found to be optimum after several trials. Oxygen was removed by two freeze–thaw cycles and the tube was sealed under vacuum. The sealed tube was then heated at 60 °C for 12 h (F_m = volume porogen/(volume porogen + volume monomer) = 0.56).

For the synthesis of Poly-S and Poly-C, all the conditions and concentration of the reagents were kept similar to that of Poly-A, except the template molecules. For Poly-S and Poly-C, DEP-B and VHP were used as templates, respectively. DEP-B and VHP were taken in the same molar quantities as PMP-B (0.1 mmol).

2.6. Workup and template splitting

The template splitting (Scheme 3) was performed by crushing the polymers and then passing them through a 100 mesh steel disc. The splitting of the templates was attempted in the following order. (1) The polymers were placed in flasks and mixed with 100 mL of $CHCl_3$/MeOH, 1:1 (v/v), for 5 h followed by filtration into Soxhlet extraction thimbles. The filtrate was evaporated and saved for analysis. (2) Soxhlet extraction was performed in MeOH overnight. The extract was evaporated and saved for analysis. (3) The polymers were vacuum dried; and 5 mL of a solution of CsF (50 mg/mL) in MeOH was added to 0.5 g of each polymer. The resultant mixture was shaken at room temperature and subsequently heated at 60 °C on a hot plate for 24 h [14]. The supernatant was removed (saved for analysis), the polymers were repeatedly washed with MeOH and oven dried for 24 h. The P-elemental analysis of the



Scheme 3. Synthesis of imprinted polymers and their binding studies.

polymers confirmed that more than 95% of the template was cleaved based on a total incorporation of template before the splitting, which corresponds to 0.079% of phosphorous in the polymer matrix. The supernatant was also analyzed by using NMR to further confirm the presence of hydrolyzed product.

3. Results and discussion

Three cross-linked network polymers Poly-A, Poly-S and Poly-C were synthesized using free radical polymerization. The polymer Poly-A, which contains covalently attached pinacolyl methyl phosphonate group, was hydrolyzed using CsF as a catalyst. CsF was found to be more efficient in cleavage and handling as compared to aqueous bases such as 15% NaOH solution or KOH solution. Moreover it becomes more difficult to analyze the supernatant for NMR analysis due to the workup procedures, which involves neutralization and extraction of the hydrolyzed product PMP. Under such conditions one has to always worry about maintaining the pristine condition of PMP as PMP can rapidly degrade under high pH conditions [15].

3.1. FTIR studies

The FTIR analysis of the polymers was performed before and after the hydrolysis of phosphorous ester. Fig. 1 shows the FTIR spectra of all the polymers. The P=O peak [16a] appears at 1150 cm^{-1}, but the peak intensity is not very sharp. This is reasonable because hydrogen bonds not only lower the frequency but also cause peak broadening in the case of Poly-A and Poly-S. In addition the polymers Poly-A and Poly-S show a splitting pattern in the range of 1100–1200 cm^{-1}, while the imprinted polymers (Poly-AC and Poly-SC) and the control show a broad peak in the same region, indicating the absence or negligible phosphorous ester content. This cleavage can be further confirmed by the appearance of 4-substituted phenolic peak at 1260 cm^{-1} occurring only in the case of Poly-AC, Poly-SC and the control. The peak around 1700 cm^{-1} corresponds to the carboxylic acid (COOH) dimer [16b] which, is almost absent in all the polymers indicating a statistical distribution of the carboxylic acid functionality under the present choice of reaction conditions. In addition there is a peak at 1728 cm^{-1} corresponding to the hydrogen bonding of the COOH groups to either phenolic groups or the phosphorous ester groups if present.

3.2. Colorimetric and imprinting studies

One milliliter of varying concentrations (0.1–20 mM) of PMP in anhydrous isopropanol was added to different microcentrifuge tubes containing 20 mg of imprinted polymers (Poly-AC/Poly-SC) and the control polymer (Poly-C). The mixture was shaken vigorously on a vortex mixer for 45 min. The equilibrium adsorption time (E.A.T.) was determined by analyzing the supernatant phosphorus content as a function of vortex time. After centrifugation for 1 min at 4400 rpm, an aliquot of clear supernatant was transferred into a glass test tube to determine the phosphorous content. The E.A.T. was

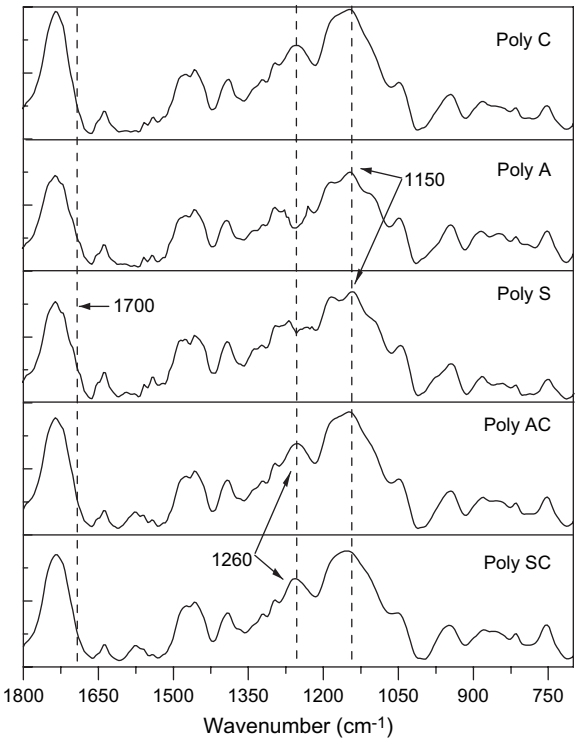


Fig. 1. FTIR spectra of the imprinted and non-imprinted polymers.

found to be 15 min based on an average of three trials for each data point.

The phosphorous analysis was performed using a colorimetric detection method [17]. A 100 μL 10 wt% aqueous $Mg(NO_3)_2$ solution was added into test tubes containing 5 μL supernatant and KH_2PO_4 standard solutions (0.5 mM, 1.0 mM, 2.5 mM, 5.0 mM, 10.0 mM and 20.0 mM), respectively, in six different test tubes. The tubes were heated on a gas burner thoroughly to give a white residue. The residue was then dissolved by adding 0.5 mL of 0.1 M HCl and shaken on a vortex mixer. All the test tubes were then heated in boiling water bath for 30 min. Next, the colorimetric reagent was prepared by mixing 10 wt% ascorbic acid in water and six parts of 0.5 wt% ammonium heptamolybdate tetrahydrate in 1.0 N H_2SO_4. After the addition of 1.7 mL of the colorimetric reagent, each test tube was incubated at 45 °C for 30 min. The test tubes were then cooled to room temperature and the absorbance was measured at 825 nm. The phosphorous content was calculated based on the absorbance at 825 nm using a calibration curve determined with standard KH_2PO_4 solutions. Each data point was calculated based on the average of three trials.

The selectivity studies were performed by adding 1 mL of 7.5 mM stock solutions of PMP/DEP in anhydrous isopropanol into separate microcentrifuge tube containing 20 mg of imprinted polymers (Poly-AC/Poly-SC) and non-imprinted polymer (Poly-C). After centrifugation, an aliquot of clear supernatant (100 μM) was transferred into six separate glass test tubes. Once again the phosphorous analysis was performed and the phosphorous content was calculated based on the absorbance at 825 nm, using KH_2PO_4 as a calibration standard.

The binding ratio (B.R.) [B.R. = (adsorbed analyte)/(analyte remaining in solution)] and imprinting efficiency (I.E.) [I.E. = (B.R. for PMP imprinted Poly-AC)/(B.R. for non-imprinted polymer Poly-C)] calculations were performed on Poly-AC/Poly-SC and Poly-C. The binding abilities of the imprinted polymers towards PMP are shown in Fig. 2. The nature of B.R. and I.E. obtained from Poly-AC and Poly-C, (Table 1) clearly suggests that the covalent approach creates very selective binding sites with respect to the interacting functional groups and are not scattered throughout the polymer matrix.

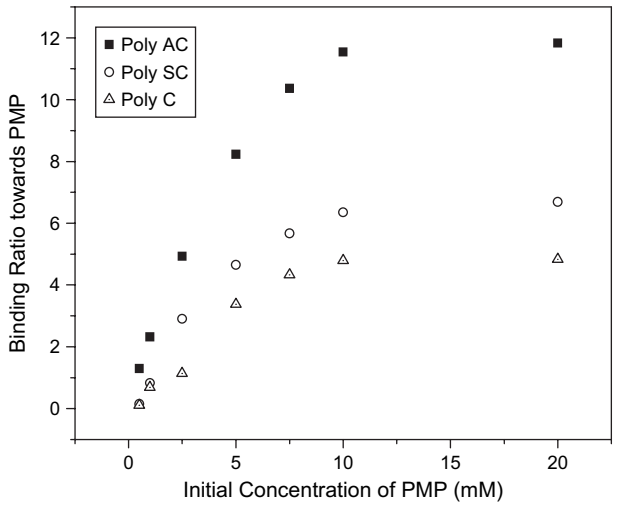


Fig. 2. Binding ratios of imprinted polymers (Poly-AC and Poly-SC) and control polymer (Poly-C).

Table 1
Binding ratios for imprinted polymers and their imprinting efficiency

Imprinted polymers/control	Binding ratio[a]	Imprinting efficiency
Poly-AC	10.37	2.39
Poly-SC	6.87	1.58
Poly-C	4.33	–

[a] At 7.5 mM PMP concentration.

Table 2
Rebinding selectivity of imprinted polymers

Imprinted polymers/control	B.R. against PMP (RP)[b]	B.R. against DEP (RD)[b]	Binding selectivity[a]
Poly-AC	8.23	2.81	2.92 (RP/RD)
Poly-SC	4.65	11.5	2.47 (RD/RP)
Poly-C	3.37	3.10	0.91 (RD/RP)

[a] At 5 mM concentration of analyte (PMP/DEP).
[b] The RP and RD are defined here as B.R. against PMP and DEP, respectively.

The binding selectivity (Table 2) of the imprinted polymers to their respective analyte was calculated by dividing the binding ratios of PMP and DEP. It was observed that the PMP imprinted polymer Poly-AC shows the highest binding ratio towards PMP while Poly-SC and Poly-C show intermediate and lowest binding ratios, respectively. In the case when DEP was exposed to the polymers, it was found that Poly-SC shows the highest affinity towards DEP as expected, however, Poly-C was found to show higher affinity than Poly-AC. These results also confirm that the strength and integrity of the binding pocket so formed in the case of Poly-AC are very selective towards PMP and the non-imprinted polymer Poly-C shows no selectivity. These observations are clear evidence that the binding site suitable for the rebinding of the template molecule was created through the covalent imprinting method.

In conclusion the present work has demonstrated a novel molecular imprinting approach bearing functional groups for intermolecular interaction with a covalently tagged PMP as a template molecule. The imprinted polymers were found to show high and selective binding affinity towards nerve agent analogs. Future work will involve applications of this approach to thin films and the use of surface plasmon resonance approaches for detection.

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