Supporting Information

© Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2018

An Unusual Two-Step Hydrolysis of Nerve Agents by a Nanozyme

Kritika Khulbe, Punarbasu Roy, Anusree Radhakrishnan, and Govindasamy Mugesh*
Supplementary Information
for

An Unusual Two-step Hydrolysis of Nerve Agents by a Nanozyme

Kritika Khulbe, Punarbasu Roy, Anusree Radhakrishnan and Govindasamy Mugesh*
Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560012, India

*Corresponding author, Email: mugesh@iisc.ac.in
Chemicals

Cerium chloride heptahydrate (CeCl₃·7H₂O) was purchased from Avra synthesis pvt. Ltd. Zirconium oxychloride hexahydrate (ZrOCl₂·8H₂O), N-methylmorpholine (NMM), triethylamine, trimethylamine, diethyl ether, tetrahydrofuran, ethyl acetate, toluene, methanol and p-nitrophenol were purchased from S.D. Fine chemicals pvt. Ltd. Nerve agents which were checked for hydrolysis, i.e., paraoxon, parathion, methyl paraoxon, methyl parathion, dichlorvos, methyl chlorpyrifos, trimethyl phosphate along with ammonium hydroxide (NH₄OH, 30%) used for synthesis of nanomaterials were purchased from Sigma Aldrich chemical company. H₂O₂ (30 %) was purchased from Merck. Rest of the organophosphate triesters and diesters were synthesized in laboratory using chemicals methyl phosphorodichloridate and ethyl phosphorodichloridate purchased from TCI Co. Ltd.

**Caution:** The organophosphates described in this paper are highly hazardous in nature and necessary precautions should be taken at all stages when working with these compounds.

Analytical techniques

All ³¹P NMR spectra were recorded using 400 MHz Avance Bruker and ECX500 JEOL High Resolution Multinuclear NMR spectrometer. UV-Vis absorption spectra were acquired on SHIMADAZU UV-2600 spectrophotometer. X-ray photoelectron spectroscopy (XPS) was acquired on AXIS ULTRA, KRATOS ANALYTICAL, SHIMADAZU. Raman spectroscopy was performed on HORIBA JOBIN YVON LabRAM HR Raman spectrometer (532 nm laser) and Renishaw in-Via Raman Microscope (Renishaw Inc, UK) (excitation wavelength 785 nm, 50xL objective lens, 10 sec exposure and 5 accumulations (power < 10 mW)). Powder XRD was recorded on Philips PANalytical X-ray diffractometer by using a Cu-Kα (1.5406 Å) radiation. Transmission electron microscopy (TEM), HRTEM, X-ray Mapping and SAED pattern were recorded on JEOL transmission electron microscope operating at 200 kV after casting a drop of nanoparticle dispersion in acetone over Cu grid. EDX spectra were acquired on ESEM Quanta instrument. FT-IR was performed on a PerkinElemer FT-IR spectrometer. Softwares used for data analysis and plotting were CASA XPS, OriginPro 8, ChemDraw Professional 15.1, TopSpin 3.5pl6 and MestReNova.

1. Synthesis of VE CeO₂ (VEC) Nanoparticles

Vacancy engineered CeO₂ was prepared by a method reported in literature, with slight modification in the sequence of addition of the chemicals¹. First, 100 mg CeCl₃·7H₂O (0.268 mmol) was dissolved in deionized water (15 mL) and ~ 750 μL NH₄OH (30 %) was added to the solution under stirring. Then, 0.5 mL H₂O₂ (30 %) was added to the solution which turned
the colorless solution red. Immediately after addition of H₂O₂, another 750 μL of NH₄OH (30 %) was added to the solution and was stirred rapidly. The temperature was raised to 100 °C and kept stable until light yellow colored dispersion of CeO₂ was obtained (~2.0 hrs). The reaction mixture was cooled to RT and centrifuged to obtain the yellow precipitate (VEC), which was washed several times with deionized water till the pH was neutral. Finally, the precipitate was washed with acetone and dried in air.

2. Synthesis of Zr-doped CeO₂ Nanoparticles

Zr-doped CeO₂ was prepared by the standard co-precipitation nanomaterial chemical synthesis procedure. Typically, 100 mg CeCl₃.7H₂O (0.27 mmol) and ZrOCl₂.8H₂O was dissolved in deionised water (30 mL) and 1.5 mL NH₄OH (30 %) was added and stirred rapidly. The temperature was raised to 100 °C and kept stable for around 2.5 h until yellow coloured dispersion of Zr-doped CeO₂ was obtained. The reaction mixture was cooled and centrifuged (3,500 rpm for 15 min) to obtain the yellow precipitate, which was washed several times with deionized water till the pH was neutral. Finally, it was washed with acetone and dried in air. During the synthesis, 0.5 (0.45 mg, 1.4 μmol) mol %, 1.0 (0.9 mg, 2.79 μmol) mol %, 5.0 (4.3 mg, 0.133 mmol) mol %, and 10.0 (8.6 mg, 0.0267 mmol) mol % ratios with respect to CeCl₃.7H₂O resulting in four differently doped CeO₂ nanoparticles, i.e., 0.5%, 1%, 5% and 10% Zr-doped nanoceria, which were named as 0.5ZC, 1ZC, 5ZC and 10ZC, respectively.

3. ICP-MS analysis of percentage of elements present in Zr-doped nanoceria

Separate stock solutions of concentration 0.6 mg mL⁻¹ of different Zr-doped nanoceria catalysts were prepared in Eppendorf tubes using 1:1 H₂O:HNO₃. The final solutions for analysis was prepared after making serial dilutions from the stock solutions with water, to have the concentration of 1.0 μg mL⁻¹ Zr-doped nanoceria catalysts. The quantity of dissolved ions in all the solutions was quantified and summarized in Table S5.

4. Half-life measurement for methyl paraoxon and methyl parathion hydrolysis by 1ZC

UV-Vis study of methyl paraoxon and methyl parathion degradation was carried out in a 1mL quartz cuvette. In a typical experiment, 0.025 nmol Zr-doped nanoceria catalyst (equivalent to 5 mol% of the catalyst) was taken from a pre-dispersed 7 mg mL⁻¹ stock solution and 0.05 nmol of methyl paraoxon (50.12 μM) or methyl parathion (49.43 μM) was added in 0.9 M NMM at 45 °C. Formation of p-nitrophenolate was monitored at 405 nm at fixed time intervals and the percentage conversion was plotted as a function of time. Experiments were also performed by taking 1 mol% concentration of 1ZC (0.005 nmol) for monitoring the rate
of hydrolysis of methyl paraoxon and methyl parathion under standard assay conditions. (Figure 2b, c and S11).

5. Preliminary experiments to monitor hydrolysis of methyl paraoxon by $^{31}$P NMR Spectroscopy

Reactions were carried out in 4.0 mL glass vials and 2.5 mg mL$^{-1}$ catalyst (0.035 mol %) were pre-dispersed in 0.9 M NMM solution for 30 minutes prior to reaction. Then, methyl paraoxon (4.7 mg, 27.0 mM) was added in the reaction vial and stirred at 45 °C. Control experiments were carried out in the absence of nanocatalyst and in the presence of other materials (bulk CeO$_2$ or ZrO$_2$ nanomaterial). Results were compared with hydrolysis performed by different nanoceria catalysts (VEC, 0.5ZC, 1ZC, 5ZC and 10ZC). Extent of hydrolysis was monitored after 24 h by recording $^{31}$P NMR spectra at constant no. of scans (102) and delay time (1.0 s). (Figure 2d, S12 and S15b)

6. Calibration plot for concentration vs. peak integral value of dimethyl phosphate (10) and monomethyl phosphate (11)

Solutions of dimethyl phosphate (DMP, 10) and monomethyl phosphate (MMP, 11) were prepared in 0.9 M NMM in 0.5 mL water in known concentrations and $^{31}$P NMR spectra was recorded for solutions of each concentration at constant number of scans (250) and delay time (5.0 s) taking TOPO in DMSO-$d_6$ as an external standard. From the integration values of the NMR peaks and the corresponding known concentrations of DMP and MMP, two calibrations plots were obtained for DMP and MMP. (Figure S13)

7. Effect of concentration of base (NMM) on hydrolysis of methyl paraoxon by $^{31}$P NMR Spectroscopy

In seven 4.0 mL glass vials, reactions were kept by using 2.5 mg mL$^{-1}$ catalyst (0.035 mol %) pre-dispersed in the following seven concentrations of NMM solution: 0.0 M, 0.15 M, 0.30 M, 0.45 M, 0.60 M, 0.75 M and 0.9 M, for 30 minutes prior to reaction. Then, methyl paraoxon (4.7 mg, 27.0 mM) was added to each reaction vial and stirred at 45 °C. Extent of hydrolysis after 24 h reaction was probed by $^{31}$P NMR spectroscopy at constant no. of scans (102) and delay time (1.0 s). (Figure 3b, S14, S15a)

8. Time-dependent study of hydrolysis of methyl paraoxon by $^{31}$P NMR spectroscopy

Time course profile for the hydrolysis of nerve agent methyl paraoxon (4) by different nanocatalysts, i.e., VEC, 1ZC, 5ZC and 10ZC was checked. In a 4.0 mL glass vial, 0.035 mol% of nanocatalyst was taken and stirred in 0.9 M NMM solution for 30 min prior to reaction in order to obtain a homogeneous pre-dispersed solution. Then, methyl paraoxon
(4.7 mg, 27.0 mM) was added to the reaction vial and stirred at 45 °C. The extent of hydrolysis at different time intervals was monitored by $^{31}$P NMR spectroscopy by maintaining constant number of scans (250) and delay time (5.0 s). The percentage conversion of starting material to different species in the reaction mixture was obtained using standard calibration plots and plotted as a function of time. External standard containing TOPO in DMSO-$d_6$ was used as external lock while recording the NMR spectra. At each time point, separate vial was kept and once the reaction was over, the solution was transferred to an eppendorf tube and frozen at liquid $N_2$ temperature to stop the reaction and preserved at -20 °C until NMR was recorded (Figure 3c-e, S15c, S17 and S16).

9. Kinetic studies for hydrolysis of methyl paraoxon

In 4.0 mL glass vials, 2.5 mg mL$^{-1}$ 1ZC nanocatalyst (0.035 mol %) were pre-dispersed in 0.9 M NMM solution for 30 minutes prior to reaction. Then, different concentrations of methyl paraoxon was added to each of the different reaction vials and stirred at 45 °C. Extent of hydrolysis of methyl paraoxon, in each concentration was analysed by $^{31}$P NMR spectroscopy of the reaction mixtures, recorded after time intervals of 5 mins, 30 mins, 75 mins, 120 mins, and 360 mins, at constant number of scans (250) and delay time (5.0 s) using TOPO in DMSO-$d_6$ as external standard. Same studies were carried out with 10ZC and VEC also under similar conditions. (Figure S18, Table S6)

10. Reaction of different organophosphates by 1ZC

In 4.0 mL glass vials, 2.5 mg mL$^{-1}$ 1ZC nanocatalyst (0.035 mol %) were pre-dispersed in 0.9 M NMM solution for 30 minutes prior to reaction. Then, organophosphate triester (27.0 mM) was added to the reaction vial and reaction was carried out by stirring at 45 °C. Various organophosphates whose hydrolysis was studied in this experiment were: methyl parathion (Figure S20), dichlorvos (Figure S21), methyl chlorpyrifos (Figure S22). After continuing the reaction for 24 h, $^{31}$P NMR spectra were recorded at constant number of scans (250) and delay time (5.0 s) using TOPO in DMSO-$d_6$ as external standard. Rate of hydrolysis of organophosphates methyl parathion (6), dichlorvos (8) and methyl chlorpyrifos (9) was analysed by $^{31}$P NMR spectroscopy. (Figure S20-S23)

11. Synthesis of 12 (ethyl methyl $p$-nitrophenyl phosphate)

Ethyl methyl $p$-nitrophenyl phosphate was synthesized following a procedure reported earlier with minor modifications.$^{[1]}$ A solution of ethyl phosphodichloridite (400.0 mg, 3.0 mmol) in THF (8.0 mL) was added to the $p$-nitrophenol (1 eq.) followed by addition triethylamine (1 eq.) at 0 °C. The reaction mixture was stirred for 8 hours at room temperature followed by the addition of a solution of methanol (1 eq.) and triethylamine (1 eq.) in THF (8.0 mL). After
stirring for another 8 hours, the triethylamine hydrochloride was filtered from the reaction mixture. The filtrate was dissolved in ethyl acetate and separated from the unreacted substrate by washing with ethyl acetate, then water and finally with brine solution. Product collected in ethyl acetate layer was condensed and dried to obtain crystallized product. Yield was in the range from 60-85%. (Figure 4b)

12. Synthesis of 13 (methyl p-nitrophenyl phosphate)

Methyl p-nitrophenyl phosphate was synthesized following a procedure reported earlier with minor modifications. Methyl phosphorodichloridate (125 μL, 1.25 mmol) was reacted with p-nitrophenol (2 eq.) in anhydrous ethyl ether (~ 25 mL) at 0 °C and triethylamine (2.1 eq.) was added dropwise slowly in the solution to produce bi (p-nitrophenyl) methyl phosphate which was then hydrolysed by stirring in 1 eq. NaOH in acetone for 12 h to produce methyl p-nitrophenyl phosphate. Solid white precipitate formed at the end of the reaction was filtered and washed with diethyl ether. The solid was then allowed to dry at room temperature overnight. Yield ~ 63%. (Figure 4c)

13. Hydrolysis of substrates 12 and 13 by 1ZC

In two 4.0 mL glass vials, 2.5 mg mL⁻¹ 1ZC was pre-dispersed in 0.9 M NMM solution for 30 minutes prior to reaction. Then, substrate 12 and 13 (20.0 mM) were added in either of the reaction vials and stirred at 45 °C. Products formed and progress of reaction was analysed by ³¹P NMR spectroscopy at constant number of scans (250) and delay time (5.0 s) in the presence of external standard TOPO in DMSO-d₆. (Figure 4b and 4c)

14. Hydrolysis of dimethyl phosphate (DMP, 10), i.e., organophosphate diester by Zr-doped nanoceria under various conditions

(a) In 4.0 mL glass vials, 2.5 mg mL⁻¹ 1ZC nanocatalyst (0.035 mol %) were pre-dispersed in 0.9 M NMM solution for 30 minutes prior to reaction. Then, 27.0 mM dimethyl phosphate (DMP, 10) was added in the reaction vial and reaction was carried out by stirring at 45 °C. Extent of hydrolysis of dimethyl phosphate was monitored after continuing the reactions for 24 h by recording ³¹P NMR spectra of the reaction mixture. Similar experiments were performed under identical conditions with other catalysts, i.e., 5ZC, 10ZC and VEC and hydrolysis was monitored after 24 h by ³¹P NMR. In none of the cases, hydrolysis of DMP was observed. (Figure 4d, S24a, b and e)

(b) In order to simulate the reaction condition of methyl paraoxon hydrolysis, hydrolysis of dimethyl phosphate was carried out in the presence of p-nitrophenol, which is a by-product from the hydrolysis of methyl paraoxon. Dimethyl phosphate and p-nitrophenol were reacted
with a pre-dispersed solution of 2.5 mg mL\(^{-1}\) 1ZC nanocatalyst (0.035 mol %) in 0.9 M NMM 45 \(^\circ\)C. 2.5 mM dimethyl phosphate (DMP, 10) and 2.5 mM \(p\)-nitrophenol was used for this experiment. Extent of hydrolysis of DMP was monitored after 24 h and 48 h by recording \(^{31}\)P NMR spectra of the reaction mixture. No hydrolysis of DMP was observed indicating the \(p\)-nitrophenol has no role in the hydrolysis of dimethyl phosphate to monomethyl phosphate. (Figure 4d and Figure S24c)

(c) Hydrolysis of dimethyl phosphate imidazolium ion pair (25.0 mM) by 1ZC was monitored under same assay conditions. In 4.0 mL glass vial, 0.035 mol % 1ZC was pre-dispersed in 0.9 M NMM solution. Then, 27.0 mM substrate was added in the reaction vial and reaction was carried out by stirring at 45 \(^\circ\)C. Hydrolysis of DMP was monitored by recording \(^{31}\)P NMR spectra of the reaction mixture after 24 h. No hydrolysis of DMP was observed. (Figure 4d)

(d) Hydrolysis of dimethyl phosphate by 1ZC was monitored under similar assay conditions with higher concentrations of NMM also. In a 4.0 mL glass vial, 0.035 mol % 1ZC was pre-dispersed in 1.8 M NMM solution. Then, 27.0 mM of dimethyl phosphate was added in the vial and reaction was carried out by stirring at 45 \(^\circ\)C. Extent of hydrolysis was monitored by recording \(^{31}\)P NMR spectra of the reaction mixture after 24 h. (Figure S24d)

(e) Hydrolysis of dimethyl phosphate by Ce\(^{3+}\), Ce\(^{4+}\) and Zr\(^{4+}\) ion mixture in catalytic concentrations (using 50 \(\mu\)M Ce(NH\(_4\))\(_2\)(NO\(_3\))\(_6\), 16 \(\mu\)M CeCl\(_3\)7H\(_2\)O and 0.5 \(\mu\)M ZrOCl\(_2\).8H\(_2\)O) was also monitored. In a 4.0 mL glass vial, calculated amounts of these salts were added in 0.9 M NMM solution. Then, 27.0 mM dimethyl phosphate was added in the vial and reaction was carried out by stirring at 45 \(^\circ\)C. Extent of hydrolysis was monitored by \(^{31}\)P NMR spectra of the reaction mixture after 24 h. No hydrolysis of DMP was observed under these conditions. (Figure S24f)

15. Comparison of rate of hydrolysis of methyl paraoxon in the presence and absence of externally added dimethyl phosphate

Effect of dimethyl phosphate in the hydrolysis of methyl paraoxon was investigated by carrying out reaction in the presence of externally added dimethyl phosphate and comparing the rate of hydrolysis with that of hydrolysis observed in the standard conditions. In six 4.0 mL glass vials, 0.035 mol % 1ZC was pre-dispersed in 0.9 M NMM. Methyl paraoxon of concentration 15.0 mM, 30.0 mM and 40.0 mM was added, each in a pair of vials. In three vials, each containing a different concentration of methyl paraoxon, 25.0 mM dimethyl phosphate was added. In remaining three vials dimethyl phosphate was not added. Rate of hydrolysis of methyl paraoxon was monitored by recording \(^{31}\)P NMR spectra at different time points (5 mins, 30 mins, 100 mins, and 480 mins) for each of the cases. During NMR
measurements, constant number of scans (250) and delay time (5.0 s) were maintained in the presence of external standard TOPO in DMSO-d<sub>6</sub>. (Figure S25)

16. FT-Raman spectroscopic analysis of nanozyme-diester intermediate

Hydrolysis of 78.0 mM methyl paraoxon and 76.0 mM methyl parathion was carried out under normal assay conditions as described before. 2.0 mg/mL stock solution of catalyst (1ZC, 10ZC or VEC) was used for the experiments. Fixed volume of reaction mixture (50 µL) was collected and the solution was centrifuged to collect the precipitate of catalyst (pellet). This pellet was washed with ultrapure water, resuspended in 10 µL water and then dropcasted on a glass slide to record FT-Raman spectra of the pellet collected at fixed time points during the course of the reaction. (Figure 5a-d and S26)

17. FT-IR spectroscopic analysis of the reaction mixture

Binding of dimethyl phosphate with catalyst was monitored by FT-IR spectroscopy. In two 4.0 mL glass vial, 2.0 mg mL<sup>-1</sup> 1ZC was pre-dispersed in 0.9 M NMM solution. Hydrolysis of 78.0 mM methyl paraoxon and 76.0 mM methyl parathion was carried out at 45 ºC. 50 µL of the reaction mixture was collected from the reaction vial at different time points to record the IR spectra. Same method was followed to monitor the formation of dimethyl phosphate bound catalyst over time, for hydrolysis of methyl paraoxon 10ZC and VEC. (Figures 5e, S27 and S28)

18. Hydrolysis of the nanocatalyst (1ZC) pellet recovered from reaction mixture during hydrolysis of methyl paraoxon and methyl parathion

Nanocatalysts were recovered by centrifugation from the reaction mixture, at different time points, during hydrolysis of methyl paraoxon and methyl parathion by 1ZC. The nanocatalyst pellet was then hydrolysed, i.e., completely dissolved by treating with 10.0 M NaOH. The product of the hydrolysis was monitored by 31P NMR spectroscopy with constant number of scans (200) and fixed delay time (3.0). (Figure 5g and S29)

19. Inductively coupled plasma mass spectrometry (ICP-MS) studies to detect stability of catalyst

Stability of 1ZC during hydrolysis of organophosphates was monitored by ICP-MS analysis of reaction mixture. Hydrolysis of 25.0 mM methyl paraoxon, dichlorvos, paraoxon, and dimethyl phosphate were carried out separately by 0.035 mol % 1ZC using the reaction conditions described above. After continuing the reaction for 24 h, supernatant solution was separated from the pellet by centrifugation at 10,000 rpm for 30 minutes. One-fourth part of supernatant was taken in falcon tube and used for preparing serial dilutions with an acidic
solution (containing HNO₃ and H₂O₂) of double distilled deionized water. The pellet comprising of undissolved nanomaterial was treated with 1.5 mL of 1:1 HNO₃: H₂O₂ overnight. The serial dilutions from the stock solution (0.6 mg/mL) of 1ZC (in pellet) were prepared with the acidic solution (containing HNO₃ and H₂O₂) of double distilled deionized water. Ce and Zr content in the supernatant and pellet (after the reaction with the different substrates) was quantified and summarized in Table S7. Blank condition corresponds to the catalyst solution where no substrate was used.

20. Column preparation using 1ZC

1% Zr-doped nanoceria (~100 mg) based nanocatalyst (1ZC) dispersed in petroleum ether was loaded onto a glass column and maintained at 45 °C. 1% solution of methyl paraoxon in water was passed through the column @ 0.1 mL min⁻¹ using 0.9 M NMM as eluent and filtrate was collected in eppendorf placed at the bottom opening. Filtrate was subjected to ³¹P NMR spectroscopy analysis. (Figure S31)

![Figure S1](image-url)  
**Figure S1.** Hydrolysis of the neurotransmitter, acetylcholine (ACh) by acetylcholinesterase (AChE) and its irreversible inhibition (aging) at serine203 by monophosphate complex. [³]
Figure S2. (a) XRD pattern of bulk CeO$_2$, 5ZC and 10ZC nanoparticles. In contrast to bulk CeO$_2$, the peak broadening observed for all the Zr-doped CeO$_2$ confirms the nano-form of the materials. Owing to a similar charge to size ratio, Ce$^{4+}$ ions can be substituted by Zr$^{4+}$ in CeO$_2$ without affecting the overall structure. Variation of (b) crystallite size, (c) lattice parameter and (d) average diameter of nanocatalyst particle with increase in Zr % doping. Correlation between the crystallite size obtained from XRD and average diameter obtained from HRTEM is observed.

<table>
<thead>
<tr>
<th>Material</th>
<th>2θ (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk CeO$_2$</td>
<td>59.24</td>
</tr>
<tr>
<td>VEC</td>
<td>59.83</td>
</tr>
<tr>
<td>1ZC</td>
<td>58.75</td>
</tr>
<tr>
<td>5ZC</td>
<td>58.60</td>
</tr>
<tr>
<td>10ZC</td>
<td>58.57</td>
</tr>
</tbody>
</table>

Table S1. Shifts in the peak position of (222) plane in XRD pattern of different materials. With increase in Zr %, 2θ value decreased which indicates incorporation of Zr atoms in the CeO$_2$ cubic fluorite lattice.
Figure S3. TEM images and indexed SAED pattern (inset). (a) VEC. (b) 1ZC. (c) 5ZC (d) 10ZC. The images were obtained at 50 nm resolution and particle like morphology of all the nanomaterials can be observed for all nanocatalysts.

Figure S4. Size distribution of particle diameter. (a) VEC. (b) 1ZC. (c) 5ZC. (d) 10ZC. Average diameter of the nanoparticles, measured from HRTEM images, were found to match well with the crystallite size obtained from XRD patterns.
Figure S5. High-resolution TEM (HRTEM) images of nanocatalysts. (a) VEC. (b) 1ZC. (c) 5ZC. (d) 10ZC. (111) planes were observed in all HRTEM images. The images show that pure ceria (111) planes are retained in all the ZCs, indicating that there is no major morphological variation after Zr-doping.

Figure S6. EDS spectra of nanocatalysts. (a) VEC. (b) 1ZC. (c) 5ZC. (d) 10ZC confirming elemental purity of these nanoparticles. Peaks corresponding to Ce and Zr were observed for only 1ZC, 5ZC and 10ZC. No peak for Zr was found in the EDS spectra of VEC.
Figure S7. SABF and X-ray elemental mapping images different nanocatalyst. (a) – (c) VEC. (d) – (g) 1ZC. (h) – (k) 5ZC. (l) – (o) 10ZC. X-ray mapping images indicate homogenous distribution of the elements.
Figure S8. Raman spectra of VEC, 5ZC, 10ZC. To understand the structural differences between doped nanoceria and bulk CeO$_2$, the possibility of any changes in Ce-O bonds in the structure was studied by FT-Raman spectroscopy. Along with the characteristic peak of Ce-O bond at 460 cm$^{-1}$ another peak at 600 cm$^{-1}$ (inset) for vacancies on CeO$_2$ surface was observed for all the nanocatalysts except bulk CeO$_2$.

<table>
<thead>
<tr>
<th>Material</th>
<th>$F_{2g}$ Raman mode shift (cm$^{-1}$)</th>
<th>Oxygen vacancy Raman shift (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk CeO$_2$</td>
<td>464.1131</td>
<td>-</td>
</tr>
<tr>
<td>VEC</td>
<td>458.9</td>
<td>611.8</td>
</tr>
<tr>
<td>1ZC</td>
<td>459.8</td>
<td>612.8</td>
</tr>
<tr>
<td>5ZC</td>
<td>460.8</td>
<td>613.3</td>
</tr>
<tr>
<td>10ZC</td>
<td>460.9</td>
<td>614.2</td>
</tr>
</tbody>
</table>

Table S2. Peaks obtained from Raman spectra of different nanocatalysts. Shifting of the peak corresponding to Ce-O bond to higher wavenumber for Zr-doped CeO$_2$ compared to VEC confirms of structural stability obtained of the nanomaterial upon doping with Zr.
Figure S9. XPS spectra of nanocatalysts. Wide spectra for (a) VEC, (c) 1ZC, (i) 5ZC and (m) 10ZC. Ce 3d deconvoluted spectra for (b) VEC, (f) 1ZC, (j) 5ZC and (n) 10ZC. O 1s deconvoluted spectra for (c) VEC; (g), 1ZC; (k) 5ZC and (o) 10ZC. Zr 3d deconvoluted spectra for (d), VEC; (h) 1ZC; (l) 5ZC and (p) 10ZC respectively. The atomic ratio (Zr/Ce) as well as vacancies on the surface of different nanoceria was calculated by X-ray photoelectron spectroscopy (XPS) analysis.
<table>
<thead>
<tr>
<th></th>
<th>VEC</th>
<th>1ZC</th>
<th>5ZC</th>
<th>10ZC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce$^{3+}$ 3d$5/2$</td>
<td>884.6</td>
<td>880.0</td>
<td>885.4</td>
<td>885.9</td>
</tr>
<tr>
<td>Ce$^{4+}$ 3d$5/2$</td>
<td>881.9, 882.4, 888.6, 898.1</td>
<td>881.8, 885.4, 895.4, 898.1</td>
<td>882.2, 890.1, 897.7</td>
<td>881.8, 890.6, 897.8</td>
</tr>
<tr>
<td>Ce$^{3+}$ 3d$3/2$</td>
<td>902.7</td>
<td>901.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ce$^{4+}$ 3d$3/2$</td>
<td>900.8, 902.7, 907.2, 916.5</td>
<td>907.4, 914.2</td>
<td>900.5, 904.5, 917.0</td>
<td>901.7, 905.1, 916.3, 920.0</td>
</tr>
<tr>
<td>Zr$^{(4-x)+}$ 3d$5/2$</td>
<td>-</td>
<td>180.6</td>
<td>181.6</td>
<td>181.9</td>
</tr>
<tr>
<td>Zr$^{4+}$ 3d$5/2$</td>
<td>-</td>
<td>182.5</td>
<td>182.7</td>
<td>184.2</td>
</tr>
<tr>
<td>Zr$^{(4-x)+}$ 3d$3/2$</td>
<td>-</td>
<td>-</td>
<td>184.0</td>
<td>185.4</td>
</tr>
<tr>
<td>Zr$^{4+}$ 3d$3/2$</td>
<td>-</td>
<td>184.4</td>
<td>184.6</td>
<td>188.3</td>
</tr>
<tr>
<td>O 1s$_a$</td>
<td>529.3</td>
<td>526.7</td>
<td>529.0</td>
<td>528.9</td>
</tr>
<tr>
<td>O 1s$_b$</td>
<td>531.2</td>
<td>529.6</td>
<td>531.5</td>
<td>531.2</td>
</tr>
<tr>
<td>O 1s$_c$</td>
<td>533.3</td>
<td>532.1</td>
<td>534.6</td>
<td>533.6</td>
</tr>
</tbody>
</table>

**Table S3.** Peaks of deconvoluted XPS spectra of Ce$^{4+}$ 3d$5/2$, Ce$^{4+}$ 3d$3/2$, Ce$^{3+}$ 3d$5/2$, Ce$^{3+}$ 3d$3/2$, Zr$^{(4-x)+}$ 3d$5/2$, Zr$^{4+}$ 3d$3/2$, Zr$^{4+}$ 3d$5/2$, Zr$^{4+}$ 3d$3/2$, and O 1s$\_a$ (surface adsorbed), O 1s$\_b$ (lattice oxygen) and O 1s$\_c$ (chemisorbed). With increase in Zr % in CeO$_2$ lattice, peaks for Ce$^{3+}$ became less distinct but more broadened which is because of increase in the Ce$^{3+}$ content. Presence of Zr$^{(4-x)+}$, where x<0.5, shows that partially reduced Zr is present on CeO$_2$ surface which is be possible in the vicinity of electronically dense vacancies on CeO$_2$ lattice.
Table S4. XPS analysis of Ce 3d deconvoluted spectra: Comparison of peak areas of Ce$^{3+}$ and Ce$^{4+}$ (3d$_{5/2}$ and 3d$_{3/2}$) by deconvoluted Ce 3d X-ray photoelectron spectra. The %age vacancies (from Ce$^{3+}$ content) were found to increase with increase in Zr % content. a = area of peaks corresponding to Ce$^{3+}$ and Ce$^{4+}$; b = normalized % concentration Ce$^{3+}$ and Ce$^{4+}$ ions. c = Ratio of atomic %age of Zr and Ce obtained by selecting the corresponding Zr 3d and Ce 3d regions in wide XPS spectra.

<table>
<thead>
<tr>
<th>Nanocatalyst</th>
<th>Total Area of Ce$^{3+}$ deconvoluted peaks$^a$</th>
<th>Total Area of Ce$^{4+}$ deconvoluted peaks$^a$</th>
<th>Ratio Ce$^{3+}$/Ce$^{4+}$ (% concentration)$^b$</th>
<th>Ratio Zr/Ce (%)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEC</td>
<td>4974.8</td>
<td>32475.2</td>
<td>13.32</td>
<td>0</td>
</tr>
<tr>
<td>1ZC</td>
<td>2595.5</td>
<td>10298.0</td>
<td>20.11</td>
<td>1.15</td>
</tr>
<tr>
<td>5ZC</td>
<td>6182.1</td>
<td>14606.9</td>
<td>29.89</td>
<td>5.55</td>
</tr>
<tr>
<td>10ZC</td>
<td>6480.4</td>
<td>17886.8</td>
<td>31.54</td>
<td>10.89</td>
</tr>
</tbody>
</table>

Table S5. ICP-MS analysis of Ce and Zr elements composition in Zr-doped CeO$_2$ nanoparticles: Nanomaterial stock solutions of concentration 0.6 mg mL$^{-1}$ of different Zr-doped nanoceria catalysts were prepared in 1:1 H$_2$O:HNO$_3$. After serial dilutions, the final concentration of Ce and Zr ions in 1.0 μg mL$^{-1}$ Zr-doped nanoceria catalysts was quantified through ICP-MS analysis. The ratio of Zr/Ce content from XPS analysis was found to correlate with that of ICP-MS elemental analysis of these elements.
Figure S10. Characterization of 0.5ZC nanoparticles. (a) TEM image. (b) HRTEM image. (c) SABF image. (d) – (f) X-ray elemental mapping images. (h) XRD pattern. (h) Raman spectra (500 to 750 cm\(^{-1}\) in inset).

Figure S11. Hydrolysis of organophosphate triesters by Zr-doped nanoceria catalysts. Conditions: 1ZC, 4, 0.9 M NMM in water, 45 °C. (a) Representative UV-Vis spectra for the hydrolysis of 4 by 5 mol% of 1ZC, showing an increase in the intensity of peak at 405 nm for \(p\)-nitrophenol. (b) Variation in the rate of conversion of 4 by different mol % of 1ZC.
Figure S12. $^{31}$P NMR spectra of the reaction mixture for the hydrolysis of methyl paraoxon by different materials. (a) - (c) In the absence of nanocatalysts or bulk material after 0h, 12h, and 24h respectively. (d) In the presence of 2.5 mg mL$^{-1}$ bulk CeO$_2$ pre-dispersed in 0.9 M NMM after 24h. (e) In the presence of ZrO$_2$ nanomaterial (2.5 mg mL$^{-1}$) pre-dispersed in 0.9 M NMM after 24h. **Conditions:** methyl paraoxon (4.7 mg, 27.0 mM), 0.9 M NMM in water, 45 °C.

Figure S13. (a) $^{31}$P NMR calibration plots for monomethyl phosphate (MMP, 11), and (b) $^{31}$P NMR calibration plots for dimethyl phosphate (DMP, 10) in 0.9 M NMM (0.5 mL) at constant number of scans (250) and delay time (5.0 s) using TOPO in DMSO-d$^6$ as an external standard.
Figure S14. Effect of the concentration of NMM on hydrolysis of methyl paraoxon. Shown above are the $^{31}$P NMR spectra recorded after 24 hours of reaction, in different concentrations of NMM: (a) 0.00 M, (b) 0.15 M, (c) 0.30 M, (d) 0.45 M, (e) 0.60 M, (f) 0.75 M and (g) 0.90 M. Conditions: 2.5 mg mL$^{-1}$ 1ZC (0.035 mol%), NMM (0.00 – 0.90 M), methyl paraoxon (4.7 mg, 27.0 mM), 45 °C.

Role of concentration of NMM on the extent of hydrolysis as well as the specificity towards product was checked by conducting the hydrolysis reaction of methyl paraoxon at different concentrations of NMM. With increase in the concentration of NMM, intensity of the peak for 11 (Monomethyl phosphate, peak at 4.5 ppm) was found to increase intensity of the peak for 10 (Dimethylphosphate, peak at 2.5 ppm) decreased. Quantification of the extent of hydrolysis in all these cases is presented in Figure S13.
Figure S15. (a) Quantification of extent of hydrolysis of methyl paraoxon (4) by 1ZC and the products formed at the end of reaction conducted in different concentrations of NMM. (b) Relative ratio of [MMP] to [DMP] in the reaction mixture after 24 h hydrolysis of 4 by different nanocatalysts. (c) Comparison of the initial rates of MMP formation from hydrolysis of methyl paraoxon in the presence of different nanocatalysts.

Figure S16. Time course $^{31}$P NMR spectra for hydrolysis of methyl paraoxon (4) by (a) VEC, (b) 5ZC, (c) 10ZC. **Conditions:** Nanocatalyst (0.035 mol%), methyl paraoxon (27.0 mM), 0.9 M NMM in water, 45 °C. As the reaction progressed, intensity of peak for methyl paraoxon (4) at -4.3 ppm reduced and peaks for dimethyl phosphate (DMP, 10) at 2.5 ppm and monomethyl phosphate (MMP, 11) at 4.5 ppm appeared.
Figure S17. Change in the amount (%) of different species in the reaction mixture during hydrolysis of methyl paraoxon (4) (27.0 mM) catalysed by (a) 5ZC and (b) 10ZC, 0.035 mol% each, in 0.9 M NMM in water at 45 °C. In both 5ZC and 10ZC catalysed reactions, the amount (%) of MMP (11) was observed to increase with time. Amount of DMP (10) remained mostly constant throughout the reaction at a basal value (~10-15%).

Figure S18. Sigma (left) and corresponding Lineweaver-Burk plots (right) with varying concentration of methyl paraoxon for different nanocatalysts. The plots for the rate of MMP formation vs. concentration of methyl paraoxon obtained for all the three nanocatalysts (VEC, 1ZC and 10ZC) showed sigmoidal nature and their corresponding Lineweaver-Burk plot was found to be linear which indicated that the nanocatalyst mediated hydrolysis reaction follows Michaelis-Menten like kinetics. Reactions were carries out by 0.035 mol% nanocatalyst in 0.9 M NMM at 45 °C.
Table S6. Kinetic parameters $K_M$ (substrate binding affinity) and $V_{\text{max}}$ (maximum velocity) for different nanocatalysts were obtained from the sigma and Lineweaver-Burk plot with varying concentration of methyl paraoxon. With increase in percentage of Zr in the catalysts, the substrate binding affinity increased but maximum velocity for reaction decreased.

<table>
<thead>
<tr>
<th>Nanocatalyst</th>
<th>$K_M$ (mM)</th>
<th>$V_{\text{max}}$ (mM min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEC</td>
<td>60.29 ± 6.37</td>
<td>0.87 ± 0.17</td>
</tr>
<tr>
<td>1ZC</td>
<td>10.51 ± 2.21</td>
<td>1.28 ± 0.14</td>
</tr>
<tr>
<td>10ZC</td>
<td>12.08 ± 1.49</td>
<td>0.57 ± 0.08</td>
</tr>
</tbody>
</table>

Figure S19. Structure of some more organophosphates used in this work.

Figure S20. Hydrolysis of methyl parathion (6) by 1ZC. Conditions: 1ZC (0.035 mol%), methyl parathion (27.0 mM), 0.9 M NMM in water, 45 °C. $^{31}$P NMR spectra of the reaction mixture after (a) 0 h and (b) 24 h. Hydrolysis of methyl parathion resulted in formation of dimethyl thiophosphate (DMTP, 16) and monomethyl thiophosphate (MMTP, 17). The spectra obtained for uncatalysed hydrolysis of methyl parathion (6) without catalyst after 24 h, was subtracted from both the spectra (a, b).
Figure S21. Hydrolysis of dichlorvos (8) by 1ZC. Conditions: 1ZC (0.035 mol%), dichlorvos (27.0 mM), 0.9 M NMM in water, 45 °C. $^{31}$P NMR spectra of the reaction mixture after (a) 0 h and (b) 24 h. Hydrolysis of dichlorvos resulted in the formation of monomethyl phosphate (MMP, 11). The spectra obtained for uncatalyzed hydrolysis of dichlorvos (8) without catalyst after 24 h, was subtracted from both the spectra (a, b).

Figure S22. Hydrolysis of methyl chlorpyrifos (9) by 1ZC. Conditions: 1ZC (0.035 mol%), methyl chlorpyrifos (27.0 mM), 0.9 M NMM in water, 45 °C. $^{31}$P NMR spectra of the reaction mixture after (a) 0 h and (b) 24 h. Hydrolysis of methyl chlorpyrifos (9) resulted in formation of dimethyl thiophosphate (DMTP, 16) and monomethyl thiophosphate (MMTP, 17).

Figure S23. Initial rates for the hydrolysis of various nerve agents by 1ZC. Decrease in the concentration of the triesters was followed by $^{31}$P NMR spectroscopy. Conditions: 1ZC (0.035 mol %), Organophosphate substrate (27.0 mM), 0.9 M NMM in water, 45 °C.

24
Figure S24. Hydrolysis of dimethyl phosphate (DMP, 10) under various conditions: (a) 0.035 mol% 5ZC catalysed hydrolysis of DMP monitored after 24 h. (b) 0.035 mol% 10ZC catalysed hydrolysis of DMP monitored after 24 h. (c) 0.035 mol % 1ZC mediated hydrolysis of DMP in the presence externally added p-nitrophenol (in 1:1 mol ratio with DMP) checked after 48 h. (d) 0.035 mol% 1ZC mediated hydrolysis of DMP in 1.8 M NMM after 24 h. (e) 0.035 mol% 1ZC mediated hydrolysis of DMP in 1.8 M NMM after 48 h. (f) Hydrolysis of DMP mediated by Ce$^{3+}$, Ce$^{4+}$ and Zr$^{4+}$ ions in catalytic concentrations (50.0 µM, 16.0 µM and 0.5 µM respectively).

Figure S25. Comparison of the rate of 1ZC (0.035 mol %) catalysed hydrolysis of methyl paraoxon in the presence and absence of externally added dimethyl phosphate.
Figure S26. Time course Raman spectroscopic analysis of nanocatalysts during hydrolysis of methyl paraoxon and methyl parathion. (a) 1ZC during the hydrolysis of methyl paraoxon. (b) 1ZC during the hydrolysis of methyl parathion. (c) 10ZC during the hydrolysis of methyl paraoxon. (d) VEC during the hydrolysis of methyl paraoxon. **Conditions:** Substrate (78.0 mM), Catalyst (2.0 mg mL⁻¹), 0.9 M NMM in water at 45 °C. Small fraction (50 µL) of the total reaction mixture from the reaction mixture was collected at different time points as the reaction progressed and the solution was centrifuged to collect the precipitate of catalyst (pellet) which was washed with ultrapure water and resuspended in 10 µL water which was drop-casted on a glass slide to record FT-Raman spectra of the nanocatalyst.
Figure S27. Time course FT-IR analysis of nanocatalysts during hydrolysis of methyl paraoxon and methyl parathion. (a) 1ZC during the hydrolysis of methyl paraoxon. (b) 1ZC during the hydrolysis of methyl parathion. (c) 10ZC during the hydrolysis of methyl paraoxon. (d) VEC during the hydrolysis of methyl paraoxon. **Conditions:** Substrate (78.0 mM), Catalyst (2.0 mg mL⁻¹), 0.9 M NMM in water at 45 °C. Small fraction (50 µL) of the total reaction mixture from the reaction mixture was collected at different time points to record the FT-IR spectra.
Figure S28. Probing the binding of phosphodiester intermediate with nanocatalyst surface from time dependent FT-IR spectroscopy of nanocatalysts. (a) 1ZC during hydrolysis of methyl parathion. (b) VEC during hydrolysis of methyl paraoxon. (c) 10 ZC during hydrolysis of methyl paraoxon. The peak at 1100 cm\(^{-1}\) was monitored to understand the strength of the binding of phosphodiester intermediate with catalyst surface.

Figure S29. Hydrolysis of nanocatalyst (1ZC) and the surface bound intermediate species using 10M NaOH. Shown above are \(^{31}\)P NMR spectra of (a) hydrolysed solution of the 1ZC pellet used for hydrolysis of methyl parathion and (b) products formed after hydrolysis of methyl paraoxon by 0.035 mol% 1ZC in the presence of 10 M NaOH at 45 °C. \(^{31}\)P NMR spectra recorded using constant number of scans (200) and delay time (3.0) using TOPO in DMSO-d\(^{6}\) as standard.
Figure S30. Characterisation of the nanomaterial after hydrolysis: (a) XRD and (b) HRTEM image of 1ZC after completion of hydrolysis of methyl paraoxon, respectively.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Supernatant</th>
<th>Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ce content (ppb)</td>
<td>Zr content (ppb)</td>
</tr>
<tr>
<td>1.</td>
<td>Blank</td>
<td>118.4</td>
<td>1.694</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>49.22</td>
<td>0.394</td>
</tr>
<tr>
<td>3.</td>
<td>4</td>
<td>87.96</td>
<td>0.847</td>
</tr>
<tr>
<td>4.</td>
<td>10</td>
<td>32.49</td>
<td>0.417</td>
</tr>
<tr>
<td>5.</td>
<td>8</td>
<td>69.7</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Table S7. ICP-MS studies to detect stability of catalyst after hydrolysis of various organophosphate triesters. Conditions: Hydrolysis of 25.0 mM methyl paraoxon, dichlorvos, paraoxon, and dimethyl phosphate were carried out separately by 0.035 mol % 1ZC and after 24 h reaction, supernatant solution was separated from the pellet by centrifugation. Serial dilutions from the stock solution (0.6 mg/mL) of 1ZC (in pellet) and supernatant solution were analyzed for Ce and Zr content by ICP-MS. It was observed that although supernatant was taken in approximately 1000 times greater concentration compared to pellet, the elemental %age of Ce and Zr in supernatant was negligible compared to pellet.
Figure S31. 1ZC for the removal of pesticide/insecticide residues from drinking water: To confirm this, a column was designed using 1ZC nanomaterial as a bed to treat water containing 1% methyl paraoxon (56.0 mM, $10^5$ times greater concentration than usually found in polluted water). (a) miniature water purification setup. The glass column was loaded with Zr-doped nanoceria (1ZC). (b) left: schematic of the purification setup; right: $^{31}$P NMR spectra of the products obtained after passing a solution of methyl paraoxon through the column. Within one run, most of the methyl paraoxon is hydrolysed to the products. TOPO in DMSO-d$_6$ was used as internal standard.

References

