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Supplementary Materials for

Prevention of pesticide-induced neuronal dysfunction and mortality with nucleophilic poly-Oxime topical gel

Ketan Thorat, Subhashini Pandey, Sandeep Chandrashekharappa, Nikitha Vavilthota, Ankita A. Hiwale, Purna Shah, Sneha Sreekumar, Shubhangi Upadhyay, Tenzin Phuntsok, Manohar Mahato, Kiran K. Mudnakudu-Nagaraju, Omprakash Sunnapu, Praveen K. Vemula*

*Corresponding author. Email: praveenv@instem.res.in

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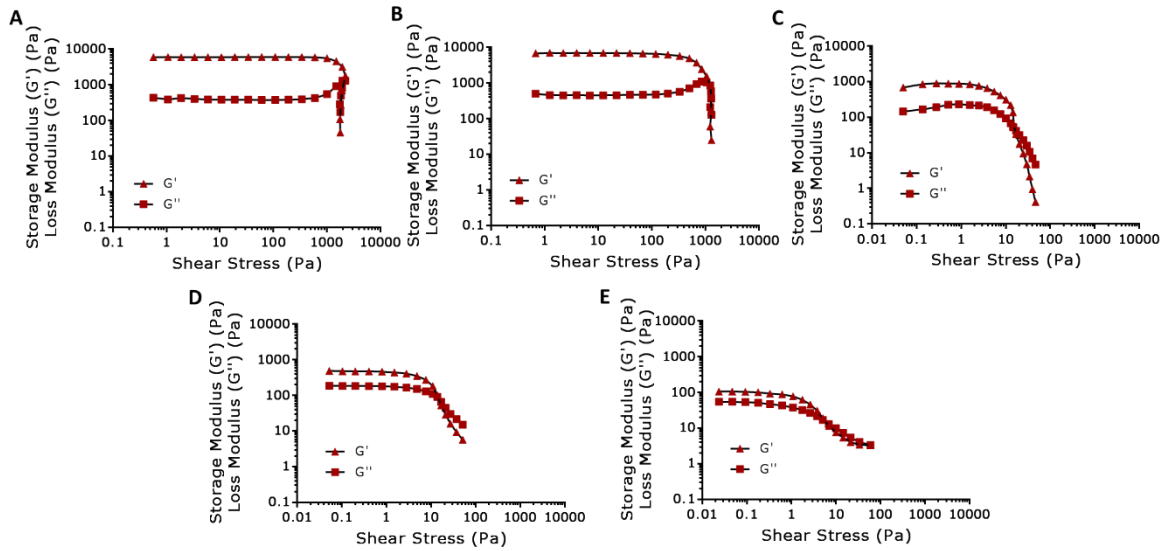


Fig. S1. Rheological analysis of *poly*-Oxime and sham gels. Amplitude sweep was performed to understand applicative behaviour of the dermal gels. (**A**) Carbopol gel (at 25˚C), (**B**) Sham gel (at 25˚C), (**C**) *poly*-Oxime gel at 25˚C, (**D**) *poly*-Oxime gel at 35˚C and (**E**) *poly*-Oxime gel at 45˚C.

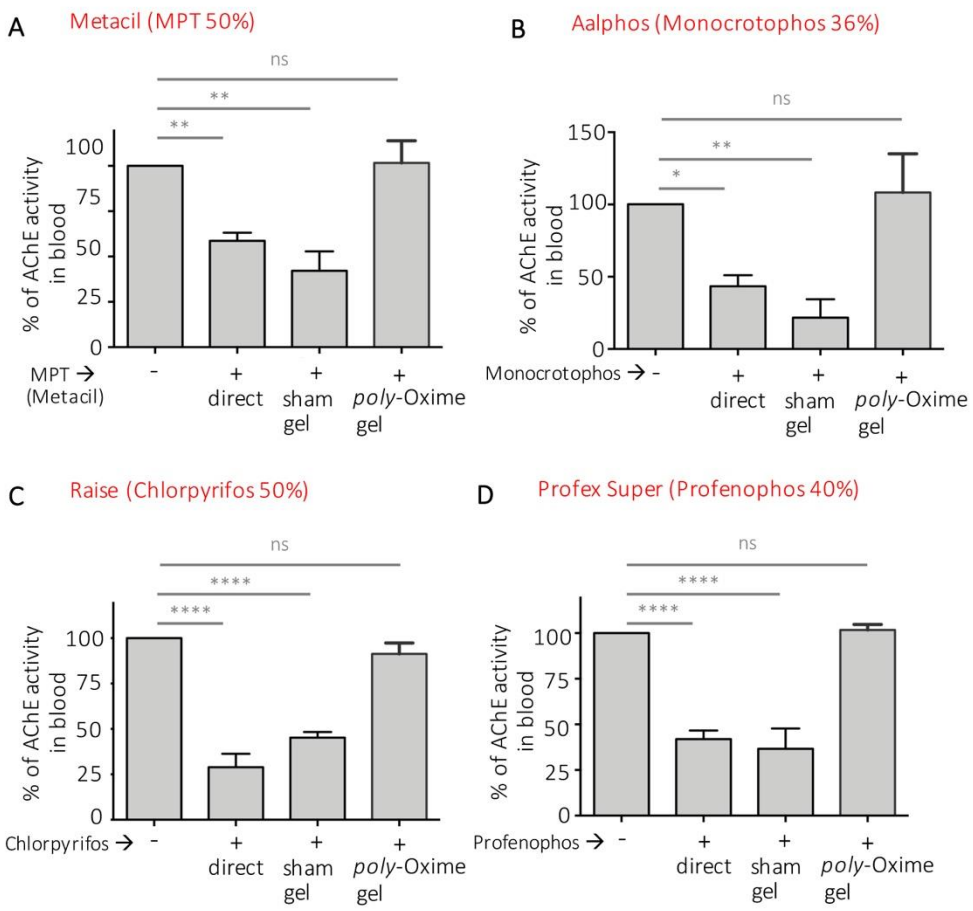


Fig. S2. Ex vivo Franz diffusion assay with commercial organophosphates. Franz diffusion assay was conducted with diluted rat blood in acceptor compartment separated from donor compartment by membrane (MWCO 3.5 kDa) without or with coating sham gel or *poly*-Oxime gel. 2 µmoles of each pesticide was added to donor compartment along with 500 µl phosphate buffer (pH 8.0). (**A**) Metacil (MPT), (**B**) Aalphos (Monocrotophos), (**C**) Raise (Chlorpyrifos) and (**D**) Profex Super (Profenophos) was used to study efficiency of *poly*-Oxime to prevent AChE inhibition. The thickness of gels were 2 mm for all experiments except for profenophos (3 mm). **Metacil** (Methyl parathion 50%, INSECTICIDES (INDIA) Ltd., Chopanki, Rajasthan, India. **Aalphos** (Monocrotophos 36%, Agastya Agro Ltd., Muraharipally, Telangana, India), **Raise** (Chlorpyrifos 50% + Cypermethrin 5% EC, Agastya Agro Ltd., Muraharipally, Telangana, India), **Profex Super** (Profenofos 40% + Cypermethrin 4% EC, Nagarjuna Agrichem Ltd., Punjagutta, Hyderabad, India). Values are mean ± SD; p values were determined by one-way ANOVA. * $p<0.05$; ** $p<0.01$; **** $p<0.0001$.

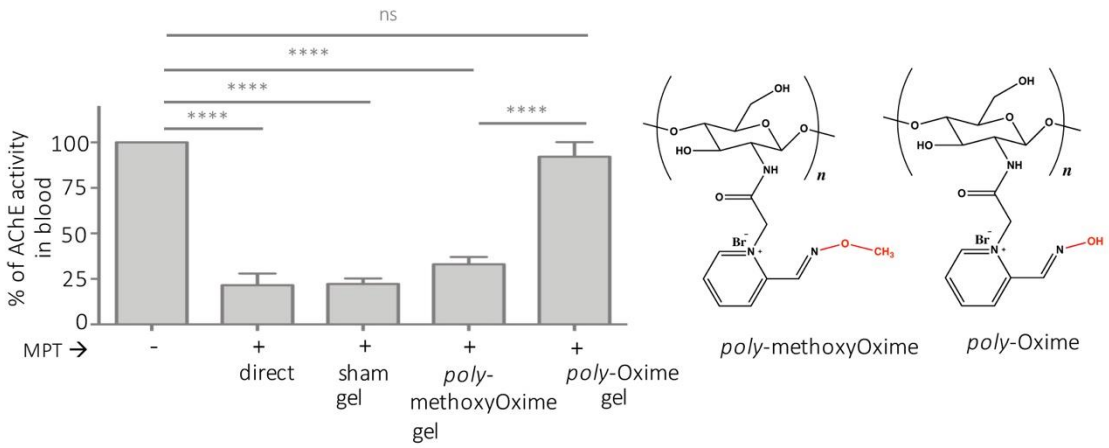


Fig. S3. Limiting MPT-induced AChE inhibition in blood using *poly*-Oxime and *poly*-methoxyOxime. To understand whether Oxime group (*N*-hydroxy) that was attached to chitosan is critical to deactivating MPT, we blocked oxime group by converting into a *N*-methoxy ether (*poly*-methoxyOxime). Akin to *poly*-Oxime gel, the *poly*-methoxyOxime gel was prepared and its ability to prevent MPT induced AChE inhibition in the blood was evaluated using Franz diffusion cell, *ex vivo*. An *ex vivo* assay was carried out using rat blood in Franz diffusion cell to evaluate the ability of *poly*-Oxime and *poly*-methoxyOxime gel to prevent MPT-induced AChE inhibition. Akin to the experiment shown in **Fig 2F**, four groups were taken (uncoated, sham gel, *poly*-methoxyOxime and *poly*-Oxime gel coated membranes between both chambers) along with no MPT group, and 1000x diluted rat blood was taken in acceptor chamber, and % of the AChE activity was quantified using modified colorimetric Ellman's assay. The addition of MPT (1µM) in the donor chamber led to a significant inhibition of AChE activity in blood. The presence of native membrane or sham gel coated membrane or *poly*-methoxyOxime gel coated membrane, did not reduce MPT mediated AChE inhibition. On the contrary, *poly*-Oxime gel deactivated MPT before entering into the acceptor chamber, thereby completely prevented MPT-induced inhibition of blood AChE activity. Interestingly, while *poly*-Oxime gel completely reduced the MPT-induced AChE inhibition, blocking of oxime nucleophile with methoxy ether (*poly*-methoxyOxime gel) led to the loss of its activity. The inability of *poly*-methoxyOxime gel to prevent AChE inhibition clearly suggesting the role of Oxime nucleophile to deactivate MPT. p values were determined by one-way ANOVA. **** $P<0.0001$.

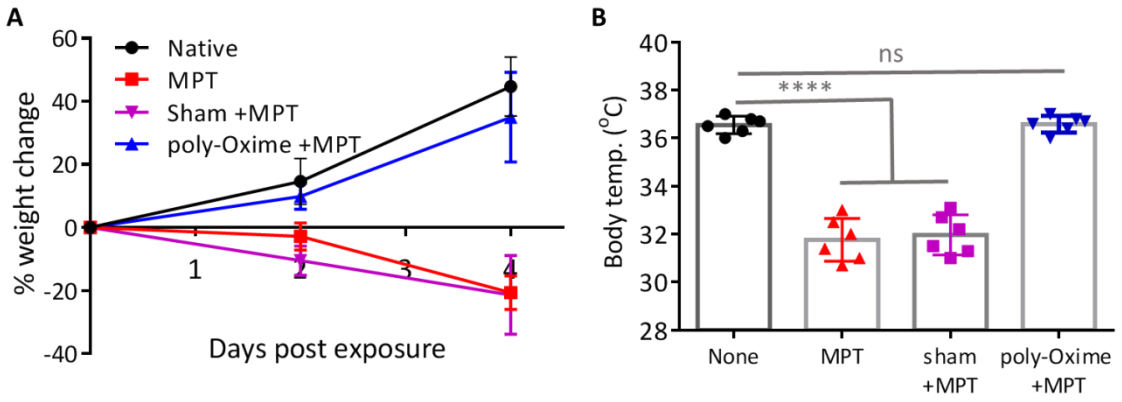


Fig. S4. Change in body weight and body temperature following acute exposure. (**A**) Upon exposure to 150 mg/kg of MPT dermally, either directly or in the presence of sham gel, animals showed reduction of body weight by almost 20% in four days which represents severe toxicity. When protected with *poly*-Oxime cream, this weight loss was completely prevented, and the animals showed increase in the body weight similar to naïve animals. (**B**) When exposed to 150 mg/kg of MPT, animals showed marked reduction in body temperature on day 3. Animals protected with *poly*-Oxime gel showed no decrease in body temperature as compared to unexposed animals. Values are mean ± SD (n = 6 rats per group); p values were determined by one-way ANOVA. **** P<0.0001.

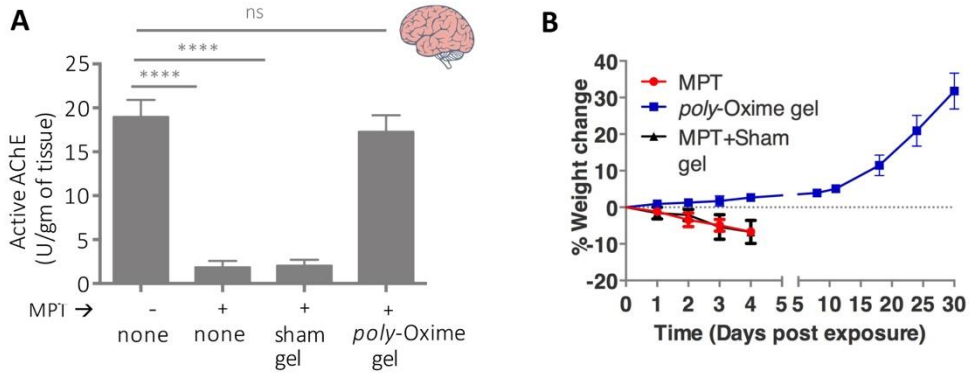


Fig. S5. Brain AChE and body weight decrease in repeated exposure of MPT with daily application of *poly*-Oxime gel. The *poly*-Oxime gel completely reduced the loss of brain AChE activity and body weight loss when animals were subjected to repeated exposure of 100mg/kg/day with daily gel application. This scenario partly mimics the real life where the agriculture worker would be expected to apply gel every day before spraying pesticides in the farm. Values are means±SD (n = 6 rats per group); p values were determined by one-way ANOVA. **** P<0.0001.

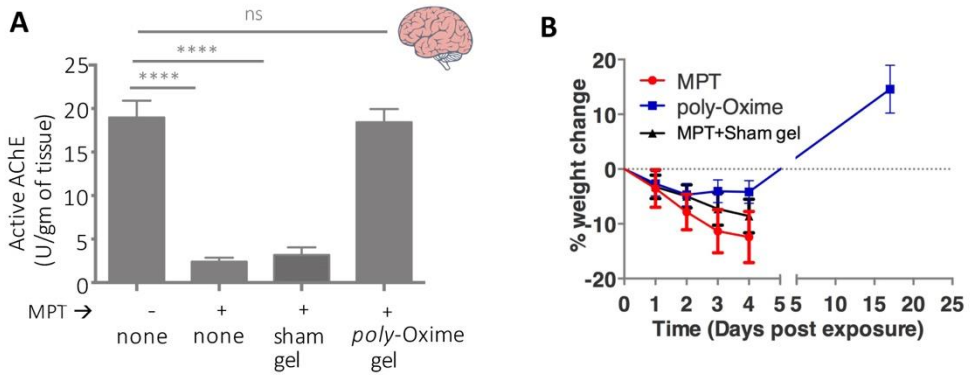


Fig. S6. Brain AChE and body weight decrease in repeated exposure of MPT with single application of gel. (A) Brain AChE, and (B) body weight decrease in repeated exposure of MPT with single application of sham gel or poly-Oxime gel. To study the robust nature of *poly*-Oxime gel *in vivo*, we applied 220 mg of *poly*-oxime or sham gel only once to a group of animals and exposed them with 100 mg/kg/day for 4 instances. The direct exposure group received the same dose of MPT directly on the skin. The *poly*-Oxime gel reduced the loss of AChE activity and weight as compared to sham or direct exposure group, reinstating the robust nature of *poly*-Oxime. Values are mean ± SD (n = 6 rats per group); p values were determined by one-way ANOVA. **** P<0.0001.

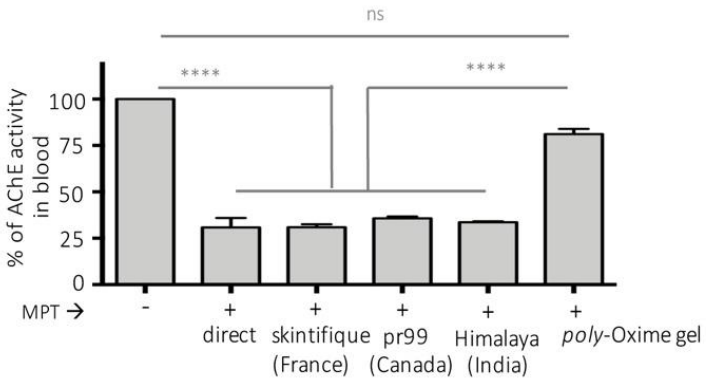


Fig. S7. Ex vivo Franz diffusion assay with various barrier creams to measure AChE inhibition. To study performance of our non-barrier chemically active *poly*-Oxime gel compared to few barrier creams available in the market, we used Franz diffusion assay, *ex vivo*. Briefly, 220 mg of each of skintifique (France), pr99 (Canada), Himalaya (India) or *poly*-Oxime cream was applied on donor compartment side of membrane. Upon addition of 1µmole of MPT in donor compartment, inhibition of AChE activity in 1000x diluted rat blood in acceptor compartment was studied at 3 h. The results clearly show that *poly*-Oxime gel is significantly better at limiting AChE inhibition as opposed to commercial physical barrier creams. p values were determined by one-way ANOVA. **** P<0.0001.

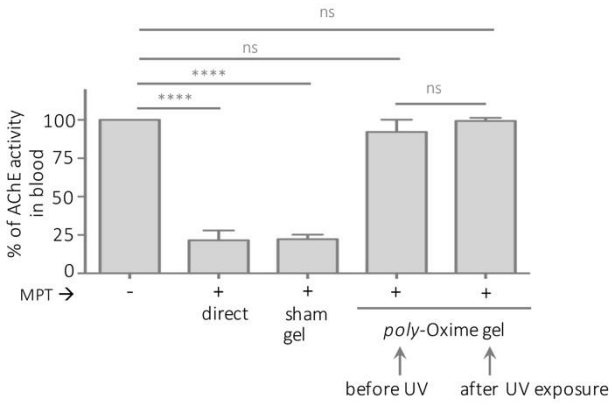
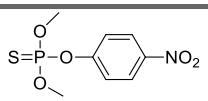
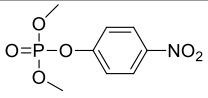
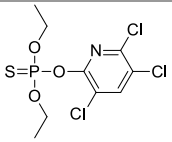


Fig. S8. Ex vivo Franz diffusion assay with *poly*-Oxime gel before and after exposure to UV light. We exposed *poly*-Oxime gel to UV light in an enclosed UV chamber for 3 hours and performed *ex vivo* Franz diffusion assay using diluted rat blood to study if the efficiency of the gel was affected. As evident in the results, exposure to UV did not affect ability of *poly*-Oxime gel to limit pesticide exposure *ex vivo*. p values were determined by one-way ANOVA. **** P<0.0001.

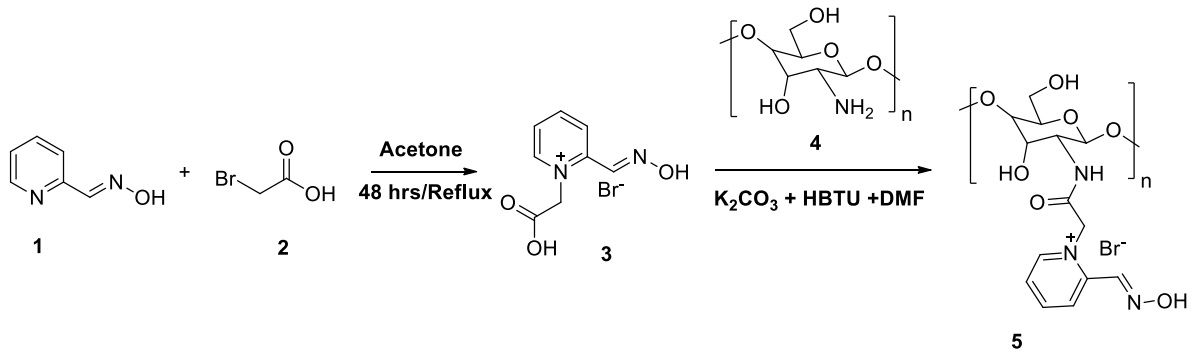
Table S1. Pseudo–first-order rate constants for hydrolysis of organophosphates by *poly*-Oxime polymer. Reactions were carried out in 50mM HEPES Buffer (pH 8.2). Temperature 25˚C, Polymer concentration: 2mg/ml, substrate concentration: 2.5×10^{-5} M.

Entry	Substrate	Catalyst	$k_\psi \times 10^{-5}$, (s^{-1})	Relative rate
1	 Methyl Parathion	HEPES (pH 8.2)	0.16	1
2		*poly*-Oxime	44.5	278.15
3	 Methyl Paraoxon	HEPES (pH 8.2)	0.22	1
4		*poly*-Oxime	22.8	100.4
5	 Chlorpyrifos	HEPES (pH 8.2)	0.29	1
6		*poly*-Oxime	55.4	191

Supplementary methods

A detailed synthesis methods and spectral characterization of *poly*-Oxime and *poly*-methoxyOxime polymers.

Scheme for the synthesis of *poly*-Oxime:



Synthesis of 1-(carboxymethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide (3):

To a stirred solution of 2-picolinaldehyde oxime **1** (5g, 40mmol) in 175 ml of acetone, 2-bromoacetic acid 2 (5.68g, 40mmol) was added at room temperature. The reaction mixture was refluxed for 48 hours. The product formation of reaction was monitored by TLC. Upon cooling the reaction mixture to room temperature, light brown colour solid was formed. The solid was filtered under vacuum and washed with acetone (4x50ml) and dried under vacuum which gave 2g of 1-(carboxymethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide **3** as a light brown solid (19% yield).

Characterization:

^{1}H-NMR (DMSO-d6 600 MHz) δ = 13.22 (1H, s), 9.05-9.04 (1H, m), 8.67 (1H, s), 8.66-8.65 (1H, m), 8.46-8.45 (1H, m), 8.15-8.15 (1H, m), 5.75 (2H, s).

^{13}C-NMR (DMSO-d6 150 MHz) δ= 167.68, 148.12, 147.74, 146.77, 142.77, 127.68, 126.66, 59.64.

MS (M+1) = 181.2

Synthesis of *poly*-Oxime (5):

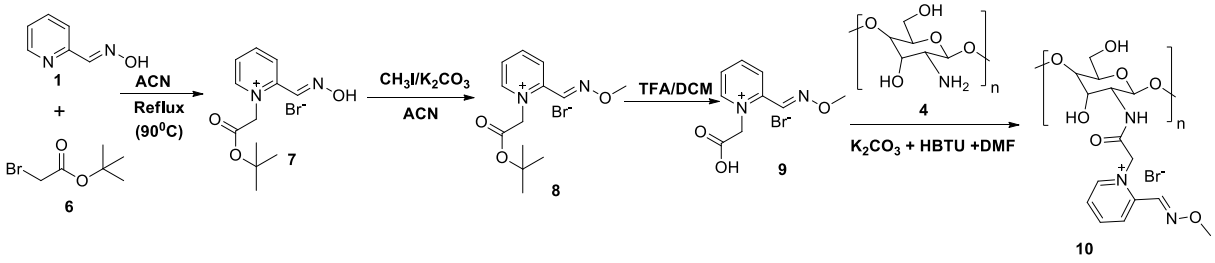
To a stirred solution of 1-(carboxymethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide **3** (2g, 7.6 mmol) in 40 ml of DMF, Potassium Carbonate (4.2 g, 30.7 mmol), HBTU (3.4g,

9.23 mmols) was added. Stirring was continued at room temperature for 15 mins. To this activated carboxylic acid, Chitosan (1.66 g 9.23 mmols, which was dissolved in 65 ml of 1% acetic acid) was added dropwise using a dropping funnel over a period of 15 mins, and stirred it at room temperature for 48 hours. Initially the reaction mixture was completely dissolved and slowly it was precipitated. The reaction mixture was transferred to a dialysis bag (MWCO 3.5 kDa) to remove unreacted reactant. After dialyzing in 1M NaCl for 24 hours, dialysis was continued in deionized water for 48 hrs. After completion of dialysis the reaction mixture was Lyophilized to get 2.4 g of ***poly*-Oxime** (**5**).

^{1}H-NMR (TFA 600 MHz): 8.2-7.8 (2.5H, br, Aromatic Protons), 5.57 (0.9H, br, -CH2-), 5.34 (1H, br, Chitosan protons), 4.0-3.2 (6H, Chitosan Protons).

Based on the NMR data, the calculated conjugation of the nucleophile to chitosan polymer is ~40%.

Scheme for the synthesis of *poly*-methoxyOxime:



Synthesis of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide (7):

To a stirred solution of 2-picolinaldehyde oxime **1** (5g, 40 mmol) in 150 ml of acetonitrile, *tert*-butyl 2-bromoacetate **6** (7.9 g, 40 mmol) was added at room temperature. The reaction mixture was refluxed at 90 °C for 48 hours. The product formation of reaction was monitored by TLC. Upon cooling the reaction mixture to room temperature, solid was precipitated. The solid were filtered under vacuum and washed with acetonitrile (4x50 ml) and dried under vacuum to get 5.5 g of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide **7** (43% Yield).

Characterization:

^{1}H-NMR (DMSO-d6 600 MHz) δ= 13.16 (1H, s), 9.05-9.04 (1H, m), 8.69 (1H, s), 8.46-8.44 (1H, m), 8.33-8.32 (1H, m), 8.19-8.17 (1H, m), 5.77 (2H, s), 1.43 (9H, s).

^{13}C-NMR (DMSO-d6 150 MHz) δ= 165.21, 147.96, 147.03, 142.69, 127.77, 127.19, 84.37, 79.69, 60.03, 27.95.

MS (M+1)=237.2

Synthesis of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((methoxyimino)methyl)pyridin-1-ium bromide (8):

To a suspension of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide **7** (2g, 6.3 mmols) in acetonitrile, Potassium Carbonate (0.88g, 6.3 mmols) and methyl iodide (1.07g, 7.59 mmols) were added, and stirred at 100 ^{0}C for 12 hrs in a pressure tube. The product formation was confirmed by mass spectroscopy. Upon cooling the reaction mixture to room temperature, solid was formed. Subsequently, filtered the reaction mixture to separate solid potassium carbonate, washed the residue with acetonitrile (4x50 ml) and evaporated the filtrate with reduced pressure to get 1.8 g of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((methoxyimino)methyl)pyridin-1-ium bromide, **8** (86% Yield).

Characterization:

^{1}H-NMR (DMSO-d6 600 MHz) δ= 8.97-9.95 (1H, m), 8.69 (1H, s), 8.65-8.62 (1H, m), 8.35-8.34 (1H, m), 8.15-8.12 (1H, m), 5.65 (2H, s), 4.01 (3H, s), 1.34 (9H, s).

^{13}C-NMR (DMSO-d6 150 MHz) δ= 165.12, 148.53, 147.44, 146.57, 143.33, 128.36, 128.04, 79.66, 60.52, 49.02, 28.00.

MS (M+1)=251.2.

Synthesis of (E)-1-(carboxymethyl)-2-((methoxyimino)methyl)pyridin-1-ium bromide (9):

To a stirred solution of (E)-1-(2-(tert-butoxy)-2-oxoethyl)-2-((methoxyimino) methyl)pyridin-1-ium bromide **8** (1.6g, 4.84mmols) in 10 ml of Dichloromethane, 10 ml of TFA was added at 0 ^{0}C. The reaction mixture was stirred at room temperature for overnight. Completion of the reaction was monitored by mass spectroscopy. After completion of the

reaction, the solvent was evaporated under reduced pressure to get a gummy residue. Upon addition of *n*-hexane solid was precipitated. The solid was filtered and dried with vacuum to get 1.2 g of (E)-1-(carboxymethyl)-2-((methoxyimino)methyl)pyridin-1-ium bromide, **9** (90% yield).

Characterization:

^{1}H-NMR (DMSO-d6 600 MHz) δ= 9.06-9.05 (1H, m), 8.79 (1H, s), 8.69-8.67 (1H, m), 8.45-8.43 (1H, m), 8.21-8.19 (1H, m), 5.73 (2H, s), 4.08 (3H, s).

^{13}C-NMR (DMSO-d6 150 MHz) δ= 167.74, 149.87, 146.18, 144.89, 142.90, 128.29, 127.42, 60.02, 49.10

MS (M+1)=195.2

Synthesis of *poly*-methoxyOxime (10):

To a stirred solution of 1-(carboxymethyl)-2-((hydroxyimino)methyl)pyridin-1-ium bromide **3** (1g, 3.64 mmol) in 20 ml of DMF, Potassium Carbonate (2 g, 14.5 mmol) and HBTU (1.65g, 4.37 mmols) were added. The reaction mixture was stirred at room temperature for 15 mins. To this reaction mixture, Chitosan (0.788 g 4.37 mmols which was dissolved in 32 ml of 1% acetic acid) was added dropwise using a dropping funnel over a period of 10 mins, and stirred at room temperature for 48 hours. After completion of the reaction, the reaction mixture was transfered to a dialysis bag (MWCO 3.5 kDa) to remove the unreacted reactant. After dialyzing in 1M NaCl for 24 hours, dialysis was continued in deionized water for 48 hrs. After completion of dialysis the reaction mixture was lyophilized to get 1.2 g of ***poly*-methoxyOxime** (**10**)**.**

Characterization:

^{1}H-NMR (TFA 600 MHz): 8.2-7.9 (5H, br, Aromatic Protons), 5.61 (1.88 H, br, -CH2-), 5.38 (1H, br, Chitosan protons), 4.06 (2.8H, s, OCH3), 4.0-3.3 (9H, Chitosan Protons).

Based on the NMR, the calculated conjugation of the methoxy-oxime to chitosan polymer is between ~50% to 55%.