

STUDY OF CATALYTIC DEPHOSPHORYLATION USING CERIUM DIOXIDE NANO-CRYSTAL

by

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Abstract:

Cerium oxides emerge as a competitive candidate for wastewater treatment due to its exceptional ability to cleave P-O ester bond. In this paper, we want to validate its universal catalysis ability on wider range of organic phosphates; furthermore, we want to probe what factor(s) inhibits and/or promotes the reaction, and what pathway this dephosphorylation reactions take. Understanding the reaction mechanism helps commercialize cerium oxide nano-particles.

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Introduction:

Organic phosphate compound (OPs) is ubiquitous in biological system as well as industrial application.

Phosphate monoester hydrolysis reaction is used to regulate protein function, energy production, and many metabolic pathways [1]. Living organism produces alkaline phosphatase (AP) to help form and break P-O ester bond with ease. Without enzyme, however, Ops take months or even years to decompose [2]. Abundance of phosphorus could lead to rampant growth of plant and algae known as eutrophication. When this happens, algae grows and blocks sunlight for other plants. And as plant decompose by bacteria, remaining oxygen is further consumed, asphyxiating fish and other wildlife, thus forming a positive feedback loop pushing the ecosystem future away from stability. Eutrophication is very hard to remedy by itself [3]. It is a global problem as well: a survey by Japanese International Lake Environment Committee reveals that 54% of Asian lakes, 53% of those in Europe, 48% of those in North America, 41% of those in South America and 28% of those in Africa are affected by eutrophication [4].

Most commonly used water treatment methods include precipitation, oxidation, biological treatment, and reactive barrier [5]. These methods are not portable, expensive, and complicate to implement. It would be helpful to use catalyst to facilitate the dephosphorylation process.

On the other hand, despite of the ubiquity of phosphorus in nature, the utilizable concentration could fail to meet demand in near future. Some estimated that we will be facing phosphorus shortage within twenty years [6]. Phosphorus is not renewable source and cannot be synthesized in the lab. Even today, 80% phosphorus fertilizers come from phosphate rocks [7]. Cordell, D and White, S predicted that the production of phosphorus rocks will peak around 2040, after which the reserve will soon be depleted.

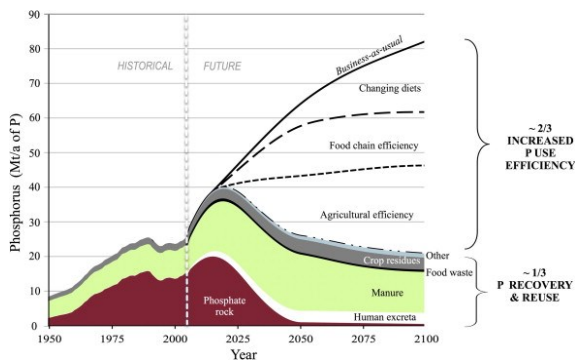


Figure 5: Long term historical and future sources of phosphorus fertilizers for global food demand through phosphorus use efficiency and recovery. Source: Redrawn from Cordell et al. (2009b)

Combining these two problems, our targeted problem seems clear. We want to extract phosphorus from wastewater, capture them, and recycle for industrial use. Although some solutions exist, traditional methods in achieving this involves non-renewable feedstocks such as Mg, NaOH, $\text{NH}_3 \cdot \text{H}_2\text{O}$ [8] [9].

An improvement is heterogenous catalytic dephosphorylation. Hydrolytic cleavage aided by catalyst breaks the phosphate ester bond, releasing free phosphate. Later, it can be separated and captured as chemical streams. If there is a compound that can mimic phosphatases' efficacy yet does not require such stringent condition.

Rare earth elements have traditionally been used for heterogeneous catalyst Cerium oxide (or ceria) stands out with its unique oxygen co-ordination numbers. At fully oxidized state, cerium has an oxygen co-ordination number of eight. When a defect is incurred, usually in the form of oxygen vacancy, Ce^{4+} can be easily reduced to Ce^{3+} due to its narrow 4f band electron orbital; thus, create local electron excess on surrounding Ce^{4+} . That is the sites that bind substrate and promote the hydrolysis reaction [10].

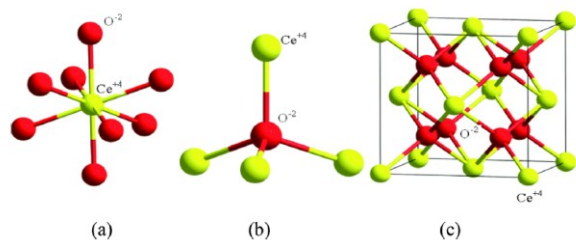


Figure 6: Structure of ceria. (a) Each cerium ion coordinates with eight oxygen ions; (b) each oxygen ion binds with four cerium ions; (c) fluorite structure of perfect CeO_2 lattice.

For decades, CeO₂ has been used in treating toxic emissions from vehicle engines, removal of organics in wastewater, combustion catalysts, along with many other redox reactions [11]. Mike Manto did an intensive research on the catalytic effect of ceria on the dephosphorylation of p-NPP, a very spontaneous reaction that can take place without elevated temperature or catalyst. He concluded that the reaction rate is positively enhanced by increasing basicity; oxygen vacancies are the active sites for dephosphorylation; and the strength of activity on different facets is: (111) > (110) > (100) [12]. With that said, a systematic study of ceria dephosphorylation on a broad range of organic phosphate in aqueous solution is still lacking and the mechanistic pathway of it is still under debate. Understanding how substrates interact with ceria nanoparticles can help us edge closer to applying ceria to purify phosphate-polluted waste in nature.

Approach to the Problem:

It is of interest to first look at alkaline phosphatase (AP), whose mechanism has been thoroughly studied. The active sites for AP usually contain two Zn²⁺ ions binding to inorganic phosphate both covalently and noncovalently to form a transition state in analogue of pentavalent vanadyl. This is a powerful mechanism that follows an associative reactive pathway, meaning the potency of AP is not affected by its substrate.

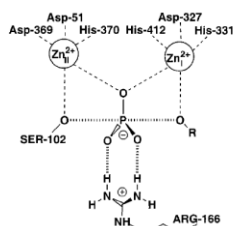


Figure 7: Pentavalent vanadate ester model in alkaline phosphatase. [13]

In this paper, I will try to investigate CeO₂ catalytic reaction by using traditional reaction mechanistic tools such as Arrhenius equation and Brønsted catalysis correlation.

Based on Mike Manto's work, we know that ordered facet (111) and surface defects such as Ce³⁺ and/or oxygen vacancies are active sites for dephosphorylation reaction. Now, we hypothesized four possible rate limiting factors for this reaction:

- 1) Adsorption of phosphate esters onto catalyst surface
- 2) P-O bond scission
- 3) Chemical transformation to form dephosphorylation product (nucleophilic attack)
- 4) Desorption of product

To accentuate the limiting effect of factor (2) and (3), we kept substrate at low concentration with respect to catalyst.

Experiment Design:

To start off, we carefully selected six organic phosphate compounds with various leaving group pK_a . Since we want to generalize the reaction potency, both alkane phosphate and aryl phosphate are chosen (three each). In order for the reaction to proceed and product detectable in aqueous solution, we made sure both the reactants and their respective leaving alcohol groups to be soluble in water at room temperature.

50mL 1 mM of each phosphate will be mixed with 3.5 mg cerium (IV) oxide nano powder from Alfa Aesar (LOT: D06X036). After the reaction starts, a stir bar is kept stirring at 600 rpm, and sample is drawn from the reactor every 3 mins up to 30 mins. We set the reaction time to 30 min so that the substrate concentration can be treated as constant. Silicone oil bath is used instead of water bath to provide better temperature stability, especially at higher temperature.

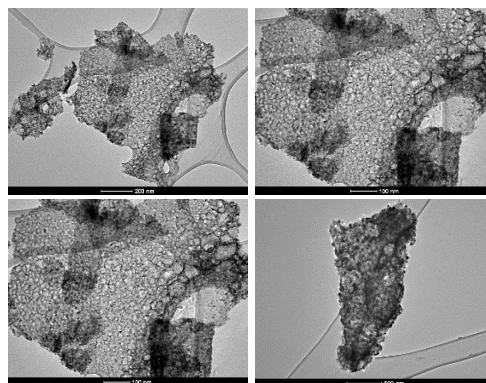


Figure 8: TEM image of cerium oxide nano particles used.

Lastly, because each phosphate salt has different leaving group pK_a , they have different pH when dissolved in water. 0.12M HCl and 0.1M NaOH solution is used to adjust pH to neutral in the beginning of the reaction.

Table 1: Phosphate esters chosen in this experiment. To make it general, we chose three phosphate alkyl and phosphate aryl, respectively.

Phosphate ester	Leaving group	pKa of leaving group
Butyl acid phosphate	Butyl alcohol	16.1
O-Phosphoryl-ethanolamine	Ethanol amine	15.61
β-Glycero-phosphate	Glycerol	14.1
Phenyl phosphate	Phenol	10.0
1-Naphthyl phosphate	1-Naphthol	9.3
p-Nitrophenyl phosphate	p-Nitrophenol	7.2

Colorimetric Assay:

We elect using ultraviolet-visible spectroscopy (UV-vis) to measure the concentration of end product—phosphate. According to Beer-Lambert law:

$$A = \epsilon cl$$

Light absorbed by the solution is linearly correlated with the concentration of species in it. Different species absorb at different wavelength. For our purpose, all tests are performed under 880 nm wavelength (figure 5).

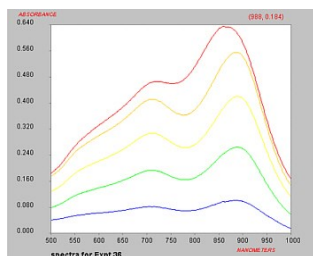


Figure 9: Absorbance of phosphate at various concentration. The peak shows maximum absorbance, which generally gives best correlation.

Molybdenum blue assay is used to determine the ortho-phosphate concentration in solution: 2 mL sample solution mixed with 1mL 1.0M H₂SO₄, 1mL 3.85mM ammonium molybdate tetrahydrate solution, and 1mL L-

ascorbic acid + antimony potassium tartrate mixing reagent. This assay binds with phosphate ions displaying blue color detectible under UV-vis spectroscopy at 880 nm wavelength.

Results and Discussion:

Two numbers are particularly pertinent to our investigation:

Activation energy (E_A) can be calculated by measuring reaction rate under different temperature. It is given by Arrhenius equation:

$$k = Ae^{-\frac{E_a}{RT}}$$

This can be rearranged to give a linear format:

$$\ln(k) = -\frac{E_a}{RT} + \ln(A)$$

Plotting $\ln(k)$ against $1/RT$ would give us E_a for the substrate.

Bronsted catalysis equation gives insights to reaction mechanistic pathway. Bronsted equation for general catalysis is given by:

$$\ln(k_{obs}) = \beta \cdot pK_a$$

Bronsted coefficient (β) indicates how the observed rate of reaction is affected by the leaving group. β ranges between 0 and -1. When β equals 0, the reaction is considered to have a transition state closer to the reactant. The rate of reaction is irrelevant to the property of the leaving group; in other words, the activation energy of the overall reaction is determined by that of the chemical transformation step. This is an indication of an associative pathway. On the other side of spectrum is total dissociative pathway, where the activation energy of the reaction is restricted by cleavage of P-O ester bond while chemical transformation ensues easily afterwards.

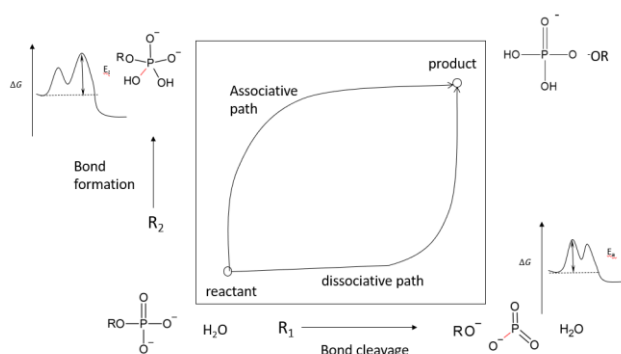


Figure 10: More O'Ferall-Jencks plot showing two types of pathways. First peak represents the activation energy for P-O bond cleavage; second peak represents the energy barrier for chemical transformation. The higher peak will decide which step is the rate limiting step.

Plotting the phosphorus concentration profile against reaction time, the slope is the initial rate of reaction (k) in the unit of mM/min. Using these data, we can proceed to plot $\ln(k)$ vs $1/RT$, the slope of which gives us the inverse of activation energy (fig. 6).

Expectedly, the activation energy is lower with increasing pK_{a1g} , signifying that, at least to some extent, this reaction is dissociative.

Table 2: Rate of reaction (k) for each phosphate at different temperature.

Temp [K]	323	333	343	353	363
Phosphate					
Butyl acid phosphate	0.010	0.026	0.041	0.13	0.34
Glycerol phosphate	0.019	0.028	0.062	0.091	0.37
Phenyl phosphate	0.077	0.12	0.25	0.39	1.0
O-Phosphoryl-ethanolamine	0.011	0.058	0.089	0.19	0.36
Naphthyl	0.11	0.14	0.27	0.39	1.1
pNPP	0.74	0.65	1.1	1.8	6.3

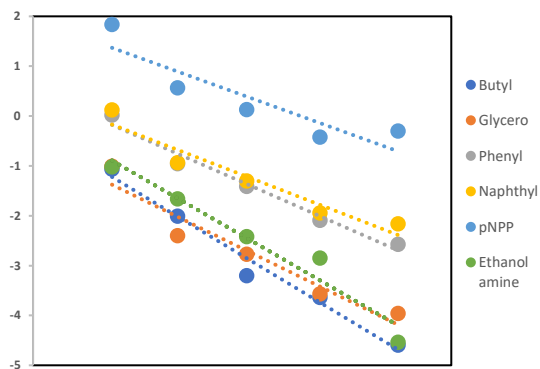


Figure 11: Linear correlation of $\ln(k)$ -y axis vs $1/RT$ -x axis. Slope is $-E_a$ and intercept is $\ln(A)$.

Table 3: Activation energy calculated from k across temperature range.

	Activation Energy [kJ/mol]
Butyl acid phosphate	84.4
Glycerol phosphate	68.5
Phenyl phosphate	61.4
O-Phosphoryl-ethanolamine	80.6
Naphthyl	54
pNPP	50.6

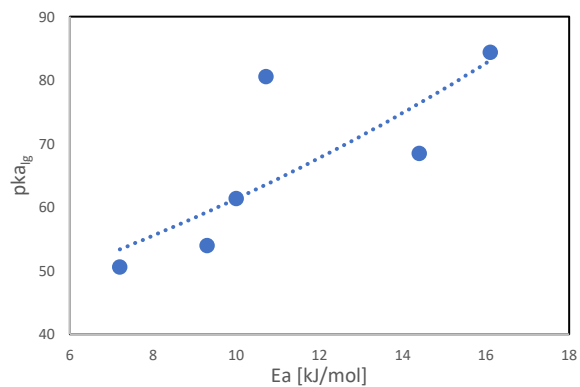


Figure 12: Correlation between pK_a and activation energy. They appear to have exponential relationship, which is agreed by LFER.

But how much it depends on the dissociative pathway? To answer that question, we need to resort to Bronsted catalysis equation (fig. 8). It is shown that the average β_{lg} for this series of reactions is ~ -0.33 , mildly dissociative with other factors limiting the reaction as well.

It should be noted that this β is the average of the five phosphates calculated, not for each individual phosphate compound. From the plot, we can see a small but consistent deviation trend: pNPP ($pK_{alg} = 7.6$) is always above the trend line while naphthyl and phenyl phosphate are consistently below the trendline at certain temperature. It is surprising that these three aryl phosphates deviate in different directions. This systematic deviation can be considered a strong indication that the transition state is slightly different for each phosphate affected by the electron density of leaving group attached to P atom. This hypothesis has been confirmed in a similar study by Fernanda Duarte et al., where they used computational method to calculate the activation energy of spontaneous hydrolysis reaction of different aryl phosphates. Based on their model, they concluded that good leaving groups would favor dissociative solvent-assisted transition state whereas the transition state for phosphates with poor leaving group is more concerted and ambiguous [14].

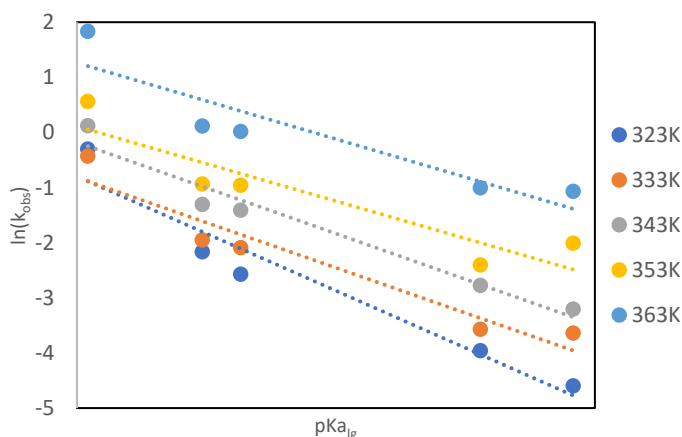


Figure 13: $\ln(k_{obs})$ vs pK_{alg} at different temperature. According to Bronsted equation, the slopes are β_{lg} : -0.23 (363K), -0.29 (353K), -0.35 (343K), -0.34 (333K), -0.44 (323K). Because phosphoryl ethanolamine is an anomaly, it is excluded in this plot.

Unanswered Questions and Future Work:

Despite the positive results, many questions are yet to be studied.

One of the most discussed issue is the pH effect onto the mechanism. It is more than possible that the same substrate would behave differently under different pH. One way it can be achieved is via different ionization number. At higher pH, some phosphate could lose two protons and become dianionic. In this case, it is difficult to determine the overall $pK_{a_{lg}}$ since the second deprotonation usually has higher pK_a value and it is elusive to know the exact ionized composition in solution. Fernanda Kirby et al recorded a Bronsted coefficient (β_{lg}) of -1.23 for phosphate monoester dianions, an indicator of extreme dissociative pathway, while β_{lg} of -0.27 for phosphate monoester monoanions [15]. This is drastic discrepancy cannot be negligible. This is possibly the biggest hurdle in finding the correlation between $pK_{a_{lg}}$ and reaction mechanism.

More interestingly, during the experiment, it is accidentally discovered that pH has different effect to different phosphate. For example, for all the aryl phosphate, basic environment is favored by the dephosphorylation reaction; however, the reverse trend is observed with butyl acid and glycerol phosphates. When adding NaOH into these phosphates, the reaction rate is slowed compare to without. On the other hand, HCl accelerates the dephosphorylation rate in the case of butyl acid and glycerol phosphate yet it suppresses the reaction of pNPP, phenyl and naphthyl phosphate. This trend has not been investigated by any peer reviewed source and might shed light into the mechanistic research.

Shape and active sites. In Manto's investigation, many different shapes of nano particles are synthesized and tested. He found that nanosphere has the best catalytic capability [12]. It would be interesting to know if nanosphere has lower Bronsted coefficient (β_{lg}) and lower activation energy for the same phosphates.

Overview and Conclusions:

The benefit to capture and recycle phosphorus is numerous, one of the best ways to achieve that is using cerium (IV) oxide nanoparticle as artificial phosphatase. Understanding its catalytic mechanism and limitation is first and foremost step towards its industrial application. With the support of Arrhenius equation, Bronsted catalysis equation, and TEM, we showed, within acceptable error, that dephosphorylation reaction is favored

by good leaving group (lower $pK_{a_{lg}}$) with a correlation coefficient of ~ -0.4 at neutral pH condition. Since lower β_{lg} suggests the catalyst is less affected by worse leaving group, lower β_{lg} is desired in industrial application setting¹. The next step, we could try synthesizing different shaped nanoparticles to find the ideal dephosphorylation catalyst.

¹ To put in perspective, natural phosphatase has a correlation of ~ -0.013 [18]

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