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REMOVAL AND RECOVERY OF LEAD (II) FROM SIMULATED SOLUTION USING ALGINATE IMMOBILIZED PAPAIN (*AIP)*

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Abstract

Lead (II) can bind with papain at its active site, destroying the enzymatic activity. Papain a sulfhydryl protease has been immobilized in calcium alginate bead for the purpose of removal of lead (II) from industrial waste water. Effect of lead (II) on the enzyme activity of immobilized papain, has been observed. A detailed kinetic study has been carried out and the data are fitted to different kinetic models. EDS (Energy Dispersive X-Ray Spectrometry) and desorption study suggest that metal can be successfully recovered from the enzyme metal complex and the adsorbent can be reused. To check the reusability of the immobilized enzyme, a detailed study has been carried out using Response Surface Methodology.

Key words: immobilization, lead removal, lead recovery, papain, Response Surface Methodology

1. Introduction

Heavy metals are introduced to the environment through a number of industrial processes and its amount is increasing with an increase in industrialization (Janos et al., 2007; Ngah and Hanafiah, 2008; Zhigang et al., 2009). Depending on the chemical form and exposure level, they can be very harmful to humans and have negative impact on the environment (David et al., 2012; Ismail and Beddri, 2009; Lee and Suh, 2000). Lead being a heavy metal is a nonbiodegradable, recirculating contaminant and gets accumulated in the environment (Al-Degs et al., 2001; Bailey et al., 1999). It is highly toxic to human and its poisoning may result in hypertension, nephritis, constipation, cramps, nausea, vomiting, behavioral changes, learning-disabilities, reading-problems, development defects and language difficulties (Ozcan et al., 2009). Its sources include effluents from industries like leather, paint, battery, ceramic, pottery and kitchen wares and automobile exhausts (Forstner and Wittman, 1981; Potgieter et al., 2006).

Lead occupies the second position of the priority list of hazardous materials prepared by the Agency of Toxic Substances and Disease Registry (ATSDR). It is necessary to remediate lead from heavily contaminated sites and to recover it from industrial effluents before it is released to the environment. Use of ion exchangers and removal by chelation with crown ethers or other macromolecules are often advocated to remove heavy metals from industrial effluent.

Recovery is possible from ion exchange columns but removal by this process is less due to the lack of selectivity in metal binding and weak binding characteristics. Crown ethers are both selective and strong binders, but they show poor recovery as they often exhibit a slow release-kinetics. Moreover, many crown ethers are very toxic, so using them may

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add to the problem of contamination (Malachowski et al., 2004). Bioremediation with microorganisms is another alternative (Lupea et al., 2012) but that has the problem of disposal of the biofilms or filters. In addition, few studies have been carried out for recovery of metals from the biofolms.

The binding ability of Poly Cysteine (Poly-Cys) immobilized on Controlled Pore Glass (CPG) for heavy metals like lead (II) and cadmium (II) has been demonstrated earlier ((Malachowski et al., 2004). Synthetic phytochelatin that contains a large number of –SH groups was fused with a Clostridium-derived cellulose binding domain (CBD). The purified fusion protein (CBD-EC20) immobilized on microcrystalline cellulose has been found to remove cadmium from simulated solution (Xu et al., 2002). A similar protein metallothionein (obtained from the algae *Neurospora crassa*) has been expressed and purified from a recombinant E. coli. The purified protein, designated as NCP, has demonstrated strong binding ability toward lead and cadmium on encapsulation in alginate and acrylamide beads (Pazirandeh, 1996).

The studies proved a potential of –SH groups for removal of heavy metals including lead. Several studies have also demonstrated stoichiometric binding of metal ions to papain, a property that can be attributed to the presence of –SH groups. Papain has been utilized by Shukor et al for development of an enzyme-based assay method for quantitative detection of contamination by heavy metals (Shukor et al., 2006). The group has demonstrated same property in another cysteine protease bromelain. Similar utility of bromelain as biosensor and bioindicator for heavy metal contamination has been demonstrated as well (Shukor et al., 2008). Similar inhibition of enzyme action has been used for detection of zinc using Serine proteases (Shukor et al., 2009). The above studies have shown a relation between the presence of –SH group, inhibition of enzyme activity and binding of heavy metals. The studies suggested use of –SH containing proteins/peptides for removal of heavy metals.

Bhattacharyya et al. have reported a significant decrease in mercury concentration from simulated waste by treatment with papain immobilized on calcium alginate where they have exploited property of papain to bind heavy metals due to presence of sulfhydryl groups for removal of mercury (II) (Bhattacharyya et al., 2010). However, the ability of immobilized papain to remove metals other than mercury (II) has not been tested. Moreover, thorough investigations have not been carried out to check the recovery of the bound metal and reusability of the spent beads.

No studies have yet been performed to optimize the recovery condition for lead (II) from alginate immobilized papain. In this study, an attempt has been made to check the ability of papain immobilized in calcium alginate for removal and recovery of lead (II) under the laboratory condition. Detailed study has been carried out to check its

ability for recovery of metals and to optimize the conditions for the same. Response Surface Methodology using Design Expert software has been applied to optimize the condition for recovery of lead (II).

2. Materials and methods

The procedure followed to carry out the experimental work in the present investigation along with the materials required for performing those experiments have been described below. All the experiments were carried out at least three times to ensure the reproducibility and accuracy of the data was checked by the standard deviation. Arithmetic means of the data have been presented. All chemicals, unless otherwise stated were of AR grade.

2.1. Immobilization of papain on calcium alginate beads under optimized condition for preparation of Alginate Immobilized Papain (AIP)

Papain (SRL Pvt. Ltd.) was immobilized in calcium alginate beads (sodium alginate and calcium chloride, both from Loba Chemie Pvt Ltd) by ionotropic gelation method under optimum conditions prescribed by Bhattacharyya et al. (2010) (Bhattacharyya et al., 2010). The conditions are: concentration of papain = 25.96 g/L, concentration of sodium alginate = 20 g/L, concentration of calcium chloride = 20 g/L and pH = 7. The temperature was maintained at $35°C$ and the hardening time of the beads was 30 minutes. The beads were then washed with distilled water and stored for further work. Papain immobilized under this optimized condition has been designated as Alginate Immobilized Papain (*AIP*) and used for further studies.

Specific Enzymatic Activity (*SEA*) of *AIP* was determined spectrophotometrically using casein as the substrate following standard protocol modified for application on the solid immobilized sample (Arson, 1970; Bhattacharyya et al., 2010). To eliminate the external mass transfer resistance for bulk diffusion, the solution was stirred using magnetic stirrer (Remi Equipments Pvt. Ltd., Model No. 1MLH) during the process. Concentration of the small peptides formed due to enzymatic action of papain immobilized on alginate beads was measured spectrophotometrically (UV 2300, TECHCOM) at 280 nm. *SEA* of immobilized papain sample has been expressed as [(g peptide formed) / (g papain × hr)]. Micro-Kjeldahl method was used to determine the loading of papain in alginate bead (Miller and Houghton, 1945).

2.2. Effect of lead (II) on the SEA of AIP under equilibrium condition

The effect of lead (II) on *AIP* was studied by incubating *AIP* with lead acetate (MERCK) for 30 minutes. The following parameters were varied during the period of incubation viz. initial

concentration of lead (II) (0.1-10 mg/L); weight of *AIP* (0.3-0.7 g); pH (4-9) and temperature (15-70°C). The beads were then taken out and washed thrice with distilled water to remove residual lead (II) on it. *SEA* was determined using the *AIP* treated with lead (II).

2.3. Scanning electron microscopy and Energy dispersive X-ray spectrometry

Scanning Electron Microscopy (SEM) was performed for calcium alginate, *AIP* and *AIP* treated with lead (II) separately to obtain the surface topography of these samples. The samples mounted on brass stubs using double-sided adhesive tape after coating them with platinum for 60 seconds. SEM was performed with a scanning electron microscope (JSM 6700F, JEOL, Japan) maintaining a working distance of 25 mm and 20 KV acceleration voltage. The image was taken at the required magnification at room temperature with the secondary electron image (SEI) as a detector. Energy dispersive X-ray spectrometry (EDS) study was done for *AIP* bound with lead (II) using the same instrument.

2.4. Batch study for removal of lead (II)

To examine the efficacy of *AIP* for lead (II) removal, *AIP* was incubated with the simulated solution of lead (II) in a batch contactor. Four parameters viz., initial concentration of lead (II), weight of *AIP*, pH and temperature were varied individually in the range of 0.13-3.95 mg/L, 3-8 g, 4-9 and 20-50°C respectively. Finally the beads were separated from the solution and residual metal concentrations in the solutions were measured using Atomic Absorption Spectrophotometry (AAS) (JSM 6700F JEOL, Japan).

2.5. Equilibrium study for removal of lead (II)

For equilibrium study initial concentration of lead (II) was varied in the range of 0.13 to 3.95 mg/L. 1.0 g *AIP* was contacted with 30 mL of lead acetate (MERCK) solution for 20 minutes at 30°C temperature and pH 7. The beads were separated from the solution and residual metal concentrations in the solutions were measured using Atomic Absorption Spectrophotometer (AAS) (JSM 6700F JEOL, Japan).

2.6. Study of recovery of lead (II) through desorption and reusability of AIP

To study metal recovery, *AIP* was incubated with lead (II) solution of 10 mg/L for 20 minutes. The beads were then taken out, washed with water for removal of excess metal ions. After that the beads were taken for the study of recovery of lead (II).

Design Expert Software (Version 8.0.4.1) was used to optimize the condition for recovery of lead (II) from *AIP*. An extensive study has been performed to optimize experimental process-parameters using Response Surface Methodology (RSM). Weight of spent bead, pH of solution and time of contact were considered as operating (independent) parameters for recovery of lead (II) and their +1 and -1 levels were fixed separately at 3 and 8 g, 3 and 7, 20 and 60 min respectively. Percentage desorption of lead (II) and percentage recovery of *SEA* both were selected as response. The most popular RSM is the Central Composite Design (CCD). Normally CCD consists of a 2^n factorial runs with 2n axial runs and n_c centre runs where n is the number of input parameters or numeric factors. To assess the effects of these four input independent parameters on the activity of immobilized papain sample, CCD was employed to design experiments statistically which consisted of 8 factorial points, six axial points and six replicates at the centre points. This resulted in a total of 20 experiments which would have to be performed. Factorial points consist of all possible combination of +1 and -1 levels of the factors. The axial points were located at (±a, 0, 0,); (0, ±a, 0,) and (0, 0, ±a,), where a was the distance of the axial point from the centre. The centre points (0, 0, 0) were repeated six times to determine the experimental error and reproducibility of the data. The condition having weight of spent bead 5.5 g, pH of solution 5 and time of contact 40 min was considered as the centre point.

Different amount of spent *AIP* was incubated in solutions with different pH for different time periods as prescribed by the Design Expert Software. Metal recovered was examined by measuring the metal concentrations in the solution using AAS (Atomic Absorption Spectroscopy) .The spent beads after recovery of lead (II) were checked for *SEA*.

On completion of 20 experiments, ANOVA was used to fit the data in a suitable model, depending on which the best condition for lead (II) recovery was determined by the software. Metal recovery has been reconfirmed by Energy Dispersive X-Ray Spectroscopy (EDS).

3. Theoretical analysis

In the present study, simulated solution of lead acetate has been contacted with papain, immobilized in alginate bead, in a batch contactor. The kinetic study shows an increase in percentage removal of lead (II) $[((C_0 - C_t)/C_0) \times 100]$ with time up to a certain period and after that there is no further decrease in residual lead (II) concentration indicating the attainment of equilibrium/saturation value.

3.1. Kinetic model

The heterogeneous separation processes are generally characterized by three steps, viz., bulk diffusion or film diffusion, intraparticle diffusion and chemical reaction, occurring sequentially in the process. To assess the sorption mechanism vis-à-vis the rate determining step, i.e., the slowest one among

these three, different kinetic models have been used to fit the experimental data obtained from batch study for the removal of lead (II) using *AIP*. These models have been categorized as (a) reaction based model and (b) diffusion based model, considering the rate of chemical reaction as the slowest one for the first case and rate of diffusion as the slowest one for the second case respectively (Svilovic et al., 2010).

3.1.1. Reaction based models

Lagergren pseudo first-order model, proposes a first-order kinetic rate equation based on surface reaction (Al-Asheh et al., 2003; Svilovic et al., 2010). The differential form of the Lagergren pseudo first order kinetic model equation is given by Eq. (1).

$$\frac{dq_t}{dt} = k_L (q_e - q_t) \tag{1}$$

where, k_L is the overall constant rate in Lagergren model, (h^{-1}). By integration using the boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_e$ at $t = t$, it gives Eq. (2).

$$\log(q_e - q_t) = \log q_e - \frac{k_L t}{2.303} \tag{2}$$

The plot of log $(q_e - q_t)$ vs. t gives a straight line with the slope $\frac{k_L}{2.303}$ and the intercept of log q_e. If the present system is chemical reaction rate controlled and if it follows first order rate kinetics, the experimental data will fit to the above equation satisfactorily and $q_{e,experimental}$ will match with $q_{e,theoretical}$. Similarly, Ho and McKay Equation or Pseudo Second Order model (PSOM) assumes that the sorption process is of pseudo-second-order and that the rate-limiting step is of chemisorption nature (Al-Asheh et al., 2003). The mechanism may involve valence forces by sharing or through the exchange of electrons between sorbent and sorbate (Al-Asheh et al., 2003). The differential form of the model equation is given by Eq. (3).

$$\frac{dq_t}{dt} = k_P (q_e - q_t)^2 \tag{3}$$

The integration with the same boundary condition as mentioned above will give the form expressed by Eq. (4).

$$\frac{t}{q_t} = \frac{1}{k_P q_e^2} + \frac{t}{q_e} \tag{4}$$

If the plot of t/q_t vs. t gives a straight line with the slope $1/q_e$ and the intercept of $1/k_p q_e^2$ and the values of $q_{e,experimental}$ matches with $q_{e,theoretical}$, it can be concluded that the present system is characterized by chemical reaction rate controlled and the reaction rate follows second order dynamics.

3.1.2. Diffusion based model

Generally, a sorption process is said to be diffusion rate controlled when the rate of diffusion of components through film or particle is the slowest one and the rate of uptake of solute from its aqueous solution is solely dependent on the rate of diffusion through film or intraparticular space. Morris-Weber model has been used to explore the mechanism of sorption in terms of whether film diffusion controlled or intraparticle diffusion controlled. The equation for Morris-Weber model is given by Eq. (5).

$$q_t = I + k_M t^{1/2} \tag{5}$$

where k_M is the rate constant of intraparticle transport in Moris Weber model, (mg g^{-1} h$^{-1/2}$) (Svilovic et al., 2010). This intercept has been used to judge the predominating/governing diffusional resistance between film resistance and intraparticle diffusional resistance.

If the Morris-Weber plot of q_t versus $t^{0.5}$ gives a straight line through the origin, which means that $I = 0$, intraparticle diffuson is considered to be the rate limiting step, while, at $I > 0$ both film and intraparticle diffusion are said to be rate limiting steps (Al-Asheh et al., 2003; Svilovic et al., 2010). Film diffusion resistance can be eliminated by increasing the turbulence in the medium which can be done by stirring the medium thoroughly in a batch contactor.

4. Results and discussion

4.1. Immobilization of papain on calcium alginate beads under optimized condition for preparation of Alginate Immobilized Papain (AIP)

AIP has been prepared successfully under the conditions prescribed by Bhattacharyya et al. (2010). Presence of papain on alginate beads has been checked by *SEA* of *AIP*. It has been found to be 0.033 g peptide produced per g of *AIP* per hour. Determination of total nitrogen content using micro-Kjeldahl method shows that enzyme loading in alginate is 4.5% w/w with respect to weight of *AIP*, or in other words, 0.045 g papain is immobilized in 1 g of *AIP*.

4.2. Effect of lead (II) on the SEA of AIP under equilibrium condition

To see the effect of metal solution in the *SEA* of *AIP*, the beads were incubated with lead (II) solution for 30 minutes by varying four parameters individually viz. different initial concentration of lead (II), with different weight of *AIP* at different pH and temperature (Table 1).

The *SEA* of *AIP* was found to be decreased after incubation with lead (II). The result indicates an inhibition of enzyme activity in presence of lead (II) which in turn, indicates an interaction between lead (II) and papain. The inhibition results from either by

direct binding of lead (II) with the active site or by alteration of conformation of the active site due to binding of lead (II) at some other points of papain. Maximum inhibition has been obtained at 10 mg/L of lead (II), 0.3 g of *AIP*, pH 9 and at 30˚C. Enzyme was relatively active at lower concentration of lead (II) and higher weight of *AIP* which indicate that at this concentration and weight, *AIP* was not saturated with lead (II). However, the change in *SEA* due to interaction with lead (II) at pH 4 is lowest indicating a very weak interaction of the metal at low pH.

The highest change in *SEA* has been obtained when *AIP* was incubated with lead (II) at pH 7.0 and 9.0 indicating a strong binding of lead (II) with papain. When temperatures were varied keeping the other parameters constant, inhibition of the enzyme by lead (II) was found to be minimum at 15˚C. This may be due to the lower diffusion rate of lead (II) through *AIP* which prevents the ions enter the beads. Maximum Inhibition was found at the temperature of 30°C which may be contributed by the higher mass transfer rate of lead (II) at this temperature.

Mass transfer rate is even higher at 70°C but at the same time, there is a chance for higher rate of dissociation of lead (II) from *AIP* at this temperature. As a result of these two combined effects, binding of lead (II) with *AIP* is less at this temperature and papain is less inhibited.

4.3. Scanning electron microscopy and Energy dispersive X-ray spectrometry:

Figs. 1-4 show the SEM photograph of calcium alginate, *AIP*, *AIP* with bound lead (II) and *AIP* after recovery of lead (II). From the Figures it is evident that immobilization of papain in calcium alginate bead smoothens the surface of the bead (Figs. 1 and 2). The surface becomes even smoother when lead (II) is bound with *AIP* as seen in Fig. 3. After recovery of lead (II) from *AIP,* smoothening effect of the surface was lost (Fig. 4).

Fig. 5 shows the EDS picture of *AIP* after binding of lead (II). EDS study reconfirms the binding of lead (II) with *AIP*. The mass percentage of lead (II) in different samples has been shown in Table 2. Data presented in Table 2 give the evidence of removal of lead (II) from simulated solution and recovery of lead (II) from *AIP*-lead (II) complex.

4.4. Kinetic study for removal of lead (II)

To assess the lead (II) removal efficacy of *AIP*, simulated solution of lead acetate has been contacted with *AIP* in a batch contactor by varying four parameters viz., initial concentration of lead (II) (0.13 - 3.95 mg/L), weight of *AIP* (3 – 8 g), pH (4 - 9) and temperature (20^0C – 50^0C) individually in a systematic manner.

Table 1. Inhibition of activity of *AIP* after incubation with lead (II) under varying condition

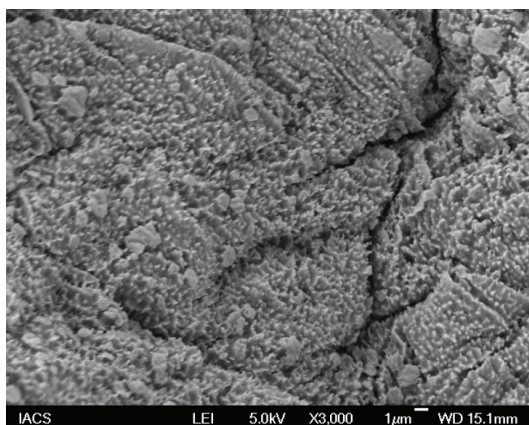| *Properties*		*Percentage Inhibition*
Initial Concentration (mg/L)	0.1	45.45
	1	70
	10	93.52
Weight of *AIP* (g)	0.3g	100
	0.5g	93.52
	0.7g	62.07
pH	pH 4	72.59
	pH 7	93.52
	pH 9	100
Temperature (˚C)	15	47.61
	30	93.52
	70	69.96

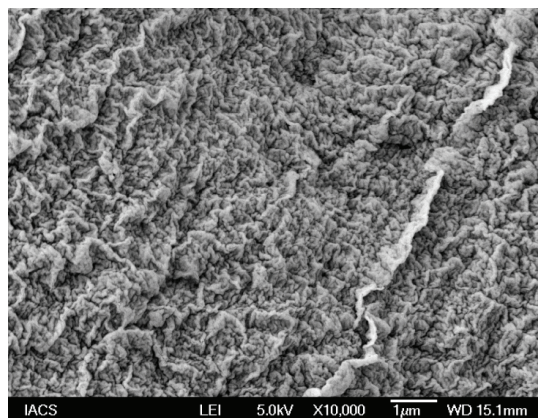**Fig. 1.** SEM micrograph of calcium alginate bead

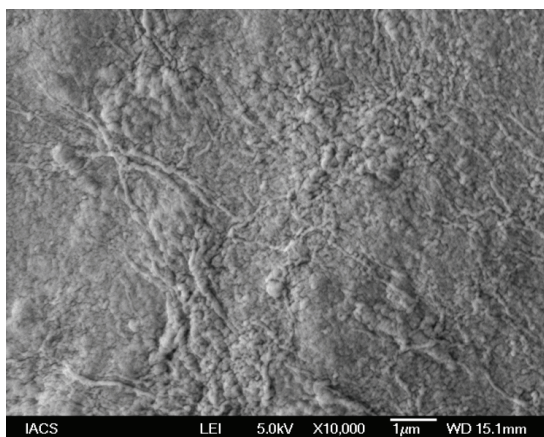**Fig. 2.** SEM micrograph of AIP

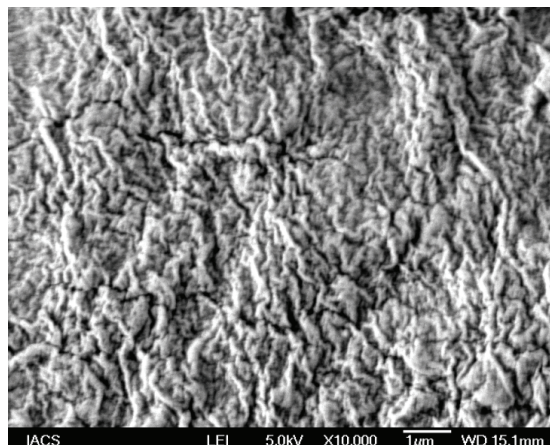**Fig. 3.** SEM micrograph of *AIP* with lead (II)

Fig. 4. SEM micrograph of *AIP* after recovery

From the batch study it is evident that in every circumstance there is gradual increase in percentage removal of lead (II) with time irrespective of parameters studied. In most of the cases, this trend has been followed up to 8 minutes.

After that the percentage removal curves become invariant with time indicating the attainment of saturation/equilibrium values.

Table 2. Mass percentage of lead (II) according EDS study of different samples

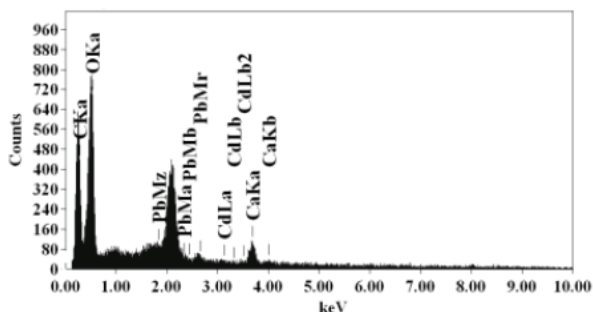| Sample	Lead (Mass percentage)
Calcium alginate bead	0.0
AIP	1.05
AIP with bound lead (II)	3.04
AIP after recovery of lead (II)	1.48

Fig. 5. EDS spectrum of alginate immobilized papain with bound lead (II)

Fig. 6 represents the percentage removal- time histories of lead (II) for various initial concentration of lead (II) in solution ranging from 0.13 mg/L to 3.95 mg/L keeping all other parameters viz. weight of *AIP*, pH and temperature constant at 5 g, 7 and 30^{0}C respectively.

From Fig. 6 it is evident that for higher initial concentrations of 1.8 and 3.95 mg/L, the percentage removal of lead (II) are higher than those at lower concentration like 0.13 mg/L and 1.08 mg/L. This is contrary to the expected outcome. With lower concentrations of lead (II), *AIP* is likely to have more space for accommodating lead (II) and is expected to

remove it from the solution at least to the same extent as with the higher concentrations. This observation can be scrutinized in the following manner. At higher concentrations of lead (II), *AIP* experiences higher mass transfer driving force expressed as the concentration difference between bulk solution and at the surface.

Due to higher mass transfer driving force, rate of diffusion of lead (II) will be higher at higher initial concentrations and hence not the rate determining step for binding of lead (II) with *AIP*. Thus, rate of uptake of lead (II) by *AIP* will be governed by the rate of chemical reaction between lead (II) and papain molecule for the process with higher initial concentration.
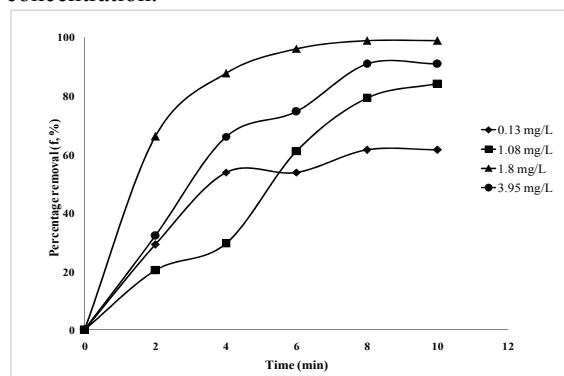

Fig. 6. Time histories of percentage removal of lead (II) with varying initial concentration of lead (II). In each case pH, weight of *AIP*, temperature are 7, 0.5 g and 30^{0}C respectively

On the other hand, rate of diffusion is the rate determining step with lower initial concentrations of lead (II). This assessment has been reconfirmed when the experimental data have been fitted to different kinetic models and the values of correlation coefficient (R^2) have been obtained as shown in Table 3.

From Table 3, it is seen that the experimental data obtained with higher initial concentration of lead (II) i.e., with 1.8 mg/L and 3.95 mg/L, have been fitted well with Lagergren pseudo first order model

(R^2= 0.996 and 0.98 respectively). This is a clear indication that the process is controlled by chemical reaction. From Table 3, it is also observed that the experimental data obtained with low initial concentration of 0.13 mg/L and 1.08 mg/L, do not match with any of the models based on chemical reaction rate, instead they match well with Morris Weber model. When q_t has been plotted with $t^{0.5}$ for these cases, the straight line obtained passes through origin giving the intercept value as zero (Figure is not shown). This observation leads to the conclusion that the rate of abatement of lead (II) with lower initial concentration is not controlled by film diffusion but by intra-particle diffusion. This is quite expected as at the higher rate of stirring, film diffusion cannot be a rate limiting step. The ratio of initial weight of lead (II) in solution to the weight of immobilized papain is 1.35×10^{-7}(mg/mg).

It has been observed that at lower ratio of weight of lead (II) to *AIP* the results fit the Morris Weber best, whereas at the higher ratio the results fit the Lagergren model. The influence of ratio of weight of lead (II) to papain is more pronounced when weight of *AIP* is varied keeping the initial concentration of lead (II) constant. The results indicate that not only the initial concentration of lead (II) but also the ratio of weight of lead (II) to *AIP* plays an important role in controlling removal of lead (II) by *AIP*. From Fig. 6, it is observed that maximum removal (98%) took place with the initial concentration of 1.8 mg/L.

Fig. 7 represents the variation of percentage removal of lead (II) with time when weight of *AIP* has been varied in the range of 3 g to 8 g keeping other parameters viz., initial concentration of lead (II), pH and temperature constant at 1.8 mg/L, 7 and 30°C respectively. Best removal was achieved when 5.0 g of *AIP* had been allowed to interact with lead (II) solution with a concentration of 1.8 mg/L. Comparatively lower removal has been obtained with 3.0 g *AIP* indicating that 3.0 g of *AIP* got saturated and the amount is not sufficient for removal of 1.8 mg/L lead (II). In all cases, saturation level has been reached by 8 minutes. About 90% removal was obtained when 8.0 g *AIP* had been allowed to interact with the same concentration of lead (II). The lower ratio of initial weight of lead (II) to the weight of immobilized papain [1.35×10^{-7}(mg/mg)] fits the Morris Weber model better than the Lagergren model (Table 4).

On the other hand, at the higher ratio of lead (II) to *AIP*, the reaction fits the Lagergren model better indicating a first-order chemical reaction between lead (II) and *AIP*. With the higher ratio of lead (II) to *AIP*, the rate of adsorption of lead (II) is controlled by the rate of chemical reaction leading to a better removal. The effect of pH on the percentage removal has been shown in Fig. 8. Removal of lead (II) by *AIP* is 72% in 10 minutes. The process is fast at the initial stage, 52% being removed in the first 4 minutes. It indicates a rapid adsorption rate of lead (II) by *AIP* at pH 9.0.

Table 3. Kinetic parameters with varying initial concentration of lead (II)

Model	Parameters	Parameter varied: Initial concentration of lead (II)			
		0.13 mg/L	1.08 mg/L	1.8 mg/L	3.95 mg/L
		WR = 1.56×10^{-8}	WR = 1.29×10^{-7}	WR = 2.16×10^{-7}	WR = 4.74×10^{-7}
Lagergren	q_{eExp}	48×10^{-5}	546×10^{-5}	1068×10^{-5}	215×10^{-4}
	q_{eTh}	45×10^{-5}	789×10^{-5}	1129×0^{-5}	224×10^{-4}
	k_L	0.38	0.33	0.59	0.3
	R^2	0.89	0.85	0.99	0.98
PSOM	q_{eTh}	5279×10^{-7}	8062×10^{-6}	1124×10^{-4}	256×10^{-4}
	k_P	1626.01	19.53	165.29	17.52
	R^2	0.95	0.41	0.99	0.85
Morris Weber	k_M	0.0	1×10^{-3}	3×10^{-3}	7×10^{-3}
	D_e	0.0	0.19	0.29	0.34
	R^2	0.97	0.88	0.89	0.97

Table 4. Kinetic parameters with varying weight of *AIP*

Model	Parameters	Parameters varied: Weight of AIP		
		3 g	5 g	8 g
		WR=3.6×10^{-7}	WR=2.16×10^{-7}	WR=1.35×10^{-7}
Lagergren	q_{eExp}	163×10^{-4}	1068×10^{-5}	53×10^{-4}
	q_{eTh}	218×10^{-4}	1129×10^{-5}	49×10^{-4}
	k_L	0.34	0.59	0.21
	R^2	0.94	0.99	0.9
PSOM	q_{eTh}	247×10^{-4}	112×10^{-4}	61×10^{-4}
	k_P	6.81	165.29	95.97
	R^2	0.44	0.99	0.88
Morris Weber	k_M	5×10^{-3}	3×10^{-3}	1×10^{-3}
	D_e	0.32	0.29	0.19
	R^2	0.92	0.90	0.94

Table 5. Kinetic parameters with varying pH

Model	Parameters	Parameters varied: pH		
		4	7	9
Lagergren	q_{eExp}	141×10^{-4}	106×10^{-4}	37×10^{-4}
	q_{eTh}	228×10^{-4}	1129×10^{-4}	59×10^{-4}
	k_L	0.55	0.59	0.26
	R^2	0.88	0.99	0.98
PSOM	q_{eTh}	264×10^{-3}	112×10^{-4}	69×10^{-4}
	k_P	0.02	165.29	131.09
	R^2	0.00	0.99	0.96
Morris Weber	k_M	4×10^{-3}	3×10^{-3}	2×10^{-3}
	D_e	0.30	0.29	0.55
	R^2	0.83	0.90	0.97

After 4 minutes, the rate of binding decreased which may be attributed to some conformational changes of papain at pH 9. At the initial stage, when this conformational change due to increase in pH is incomplete, binding of lead (II) takes place, resulting in 52% removal in the first 4 minutes.

As the process progresses, binding capacity of papain with lead (II) decreases gradually with a gradual change in conformation leading to 72% removal of lead (II) at the end 10 minutes. Maximum removal (98%) was obtained at pH 7.0, after 10 minutes.
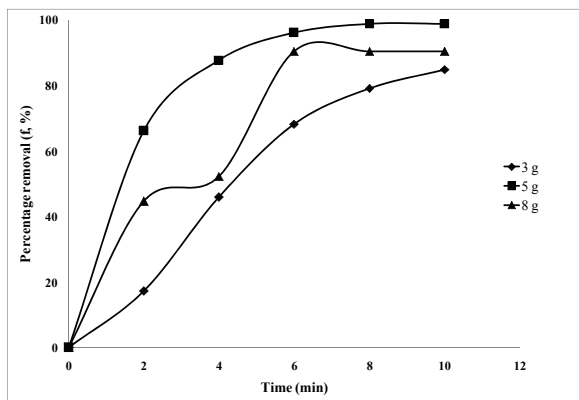

Fig. 7. Time histories of percentage removal of lead (II) with varying weight of *AIP*. In each case pH, initial concentration of lead (II) and temperature are 7, 1.8 mg/L and 30°C respectively

Removal was less (81%) at pH 4.0. According to the molecular modeling of papain, binding of a metal ion is accompanied by a proton release (Sluyterman and Wijdenes, 1976). Therefore, at low pH the process is kinetically driven to the dissociation of papain-metal complex resulting in lower removal of lead (II). The kinetic data fits the Lagergren model better than the Morris Weber model indicating a first-order chemical reaction between lead (II) and *AIP* at different pH (Table 5). Fig. 9 represents the dependence of the percentage removal of lead (II) on temperature when initial concentration of lead (II), weight of *AIP* and pH have been kept constant at 1.8 mg/L, 5g and 7 respectively. From Fig. 9 it is seen that removal of lead (II) is highest at 30°C followed by 50°C using same condition.

The extents of removal are 98% and 81% respectively at these two temperatures. Only 60% removal has been obtained at 20°C. At low temperature, diffusion of metal to the inner core of *AIP* is lower resulting in lower extent of removal. At higher temperatures like 30°C and 50°C, higher removal of lead (II) has been obtained due to higher diffusion rate. At the same time, higher temperature is able to provide the activation energy for dissociation of lead (II) from *AIP*. Therefore, the chance of dissociation of lead (II) from papain is higher at 50°C.
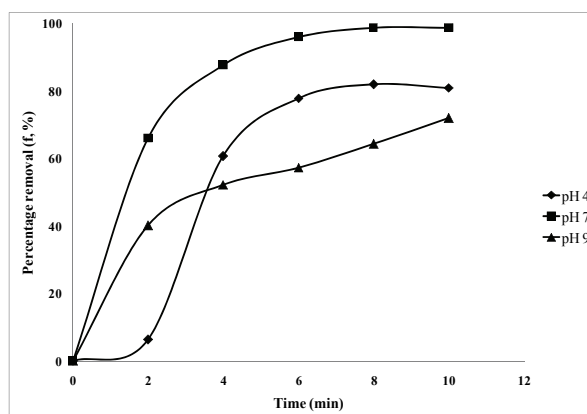

Fig. 8. Time histories of percentage removal of lead (II) with varying pH. In each case initial concentration of lead (II), weight of *AIP* and temperature are 1.8 mg/L, 0.5 g and 30°C respectively
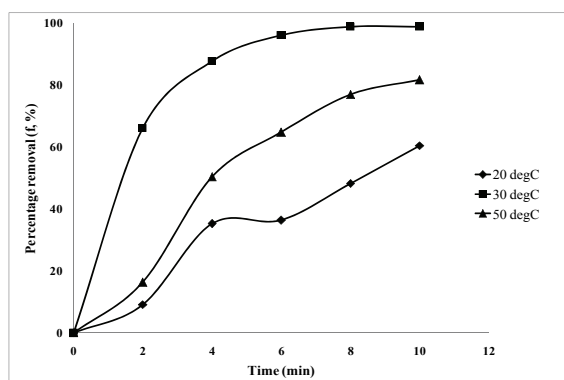

Fig. 9. Time histories of percentage removal of lead (II) with varying temperature. In each case initial concentration of lead (II), pH and weight of *AIP* are 1.8 mg/L, 7 and 0.5 g respectively

The dissociation of lead (II) may be the reason for which removal of lead (II) is slightly less at 50°C than at 30°C. In this case, kinetic data fits the Lagergren model indicating a first-order chemical reaction (Table 6).

4.5. Equilibrium study for removal of lead (II)

Equilibrium data has been fitted to different adsorption isotherm models viz., Langmuir model, Freundlich model etc. Table 7 represents the values of equilibrium parameters. It can be said that the equilibrium data fit most satisfactorily with Langmuir adsorption isotherm model ($R^2 = 0.988$).

4.6. Metal recovery and reusability of AIP:

Optimum condition for recovery was determined using Design Expert software. The design of experiment is shown in Table 8. As suggested by the software, the data for desorption were transformed into natural log to fit a model. No transformation was required for data of percentage recovery of *SEA* to fit a model. The data for both percentage recovery of lead (II) and percentage recovery of *SEA* have been best fitted to quadratic model. The final regression functions for desorption of lead (II) and percentage recovery of *SEA* in terms of actual factors are given below (Eqs. 6-7). ANOVA analysis showed that three input parameters viz. weight of adsorbent, pH, time and the quadratic

effect of pH and time have significant effect on percentage desorption of lead (II).

The conjugate effect of pH and weight of adsorbent has been shown in Fig. 10. It is evident from the figure that percentage desorption is maximum at lower weight of adsorbent which suggests that as the volume of solution is fixed, with increase of weight of spent *AIP* the solution get saturated and it cannot recover metal any more. On the other hand percentage desorption increases due to decrease in pH up to a certain point after that it decreases. Fig. 11 shows the combined effect of time and pH. When the contact time is less pH has little effect on percentage desorption. It may be due to the fact that in this short time of contact, lead (II) cannot come out from *AIP*. At lower pH percentage desorption increases gradually with time, whereas at pH 7.0, there is a little change in percentage desorption with time. Fig. 12 represents the pooled effect of weight of spent adsorbent (g) and time (min). At lower weight of spent beads, percentage desorption of lead (II) changes with time. On the other hand, with a higher weight of spent beads, percentage desorption remains almost constant with time. The effects of weight of spent adsorbent, pH and time of contact on *SEA* of spent beads after recovery of metal are shown in Figs. 13, 14 and 15. *SEA* of the spent beads decreases with decreasing pH during treatment of the beads for recovery of lead (II) (Fig. 13).

Table 6. Kinetic parameters with varying temperature

Model	Parameters	Parameters varied: Temperature		
		20	*30*	*50*
Lagergren	q_{eExp}	918×10^{-5}	1068×10^{-5}	1656×10^{-5}
	q_{eTh}	0.01	0.11	0.02
	k_L	0.19	0.59	0.36
	R^2	0.94	0.99	0.94
PSOM	q_{eTh}	148×10^{-4}	112×10^{-4}	249×10^{-4}
	k_P	0.13	165.29	7.07
	R^2	0.26	0.99	0.44
Morris Weber	k_M	2×10^{-3}	3×10^{-3}	5×10^{-3}
	D_e	0.23	0.29	0.32
	R^2	0.89	0.90	0.92

Table 7. Equilibrium parameters for lead (II) removal using *AIP*

Adsorption isotherm model	Expression	Temperature 30°C	
		Constants	R^2
Langmuir model	$q_e = \dfrac{K'_L q^0 C_e}{1 + K'_L C_e}$	$q^0 = 0.5$ $K'_L = 0.113$	0.988
Freundlich model	$q_e = K'_F C_e^{1/n'_F}$	$K'_F = 0.278$ $n'_F = 1.838$	0.919

$\ln(PercentageDesorption) = -4.04409 - 0.27855 * Adsorbent + 2.55144 * pH + 0.16455 * Time$
$- 0.34359 * pH^2 - 1.74660E - 003 * Time^2$

(6)

$$\% \mathrm{Re\,cov}\,eryofSEA = -28.97175 + 1.25925 * Adsorbent + 10.71543 * pH + 0.43728 * Time -$$
$$0.031540 * Adsorbent * pH + 0.023641 * Adsorbent * Time + 0.014396 * pH * Time$$
$$-0.17768 * Adsorbent^2 - 0.92294 * pH^2 - 7.28953E - 003 * Time^2 \tag{7}$$

Table 8. Experimental design and results

| *Weight of adsorbent (g) **	*Ph*	*Time (min)*	*Percentage desorption*	*Percentage recovery of SEA*
8.00	7.00	20.00	0.05	9.29
5.50	8.36	40.00	0.02	11.65
3.00	7.00	20.00	0.24	11.8
5.50	5.00	40.00	12.88	16.08
3.00	7.00	60.00	0.24	12.98
8.00	3.00	60.00	3.46	5.37
9.70	5.00	40.00	3.73	15.04
8.00	7.00	60.00	0.2	14.36
5.50	5.00	73.64	8.85	10.91
3.00	3.00	60.00	14.14	2.52
5.50	5.00	40.00	12.88	16.08
5.50	5.00	40.00	12.88	16.08
5.50	5.00	40.00	12.88	16.08
5.50	1.64	40.00	10	1.36
3.00	3.00	20.00	12.5	4.49
1.30	5.00	40.00	32.05	12.57
5.50	5.00	6.36	0.94	6.49
5.50	5.00	40.00	12.88	16.08
5.50	5.00	40.00	12.88	16.08
8.00	3.00	20.00	0.76	1.77
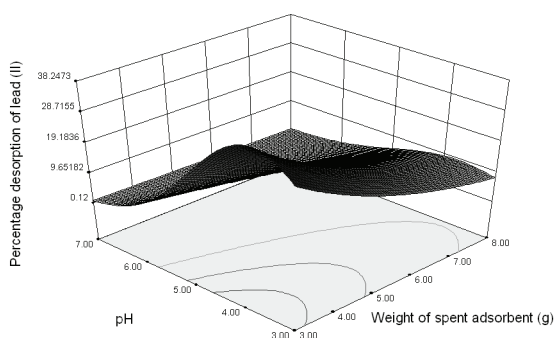
Fig. 10. Combined effect of pH and spent adsorbent on percentage desorption of lead (II)
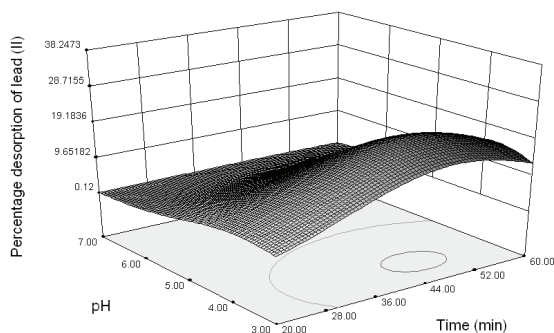

Fig. 11. Combined effect of pH and contact time on percentage desorption of lead (II)

It may be possible that lower pH has altered the conformation of the enzyme for which *SEA* has been decreased. Change in weight of adsorbent during the recovery process, has no effect on *SEA* of the spent beads. Fig. 14 illustrates the combined effect of time of incubation and weight of spent

adsorbent. The observation was in accordance with the earlier one (Fig. 13) the *SEA* remained unaltered with a change in weight of *AIP*. *SEA* increases moderately with time at the beginning, and decreases afterwards. Leaching of metal ions from the spent beads leads to a higher *SEA* at the beginning.
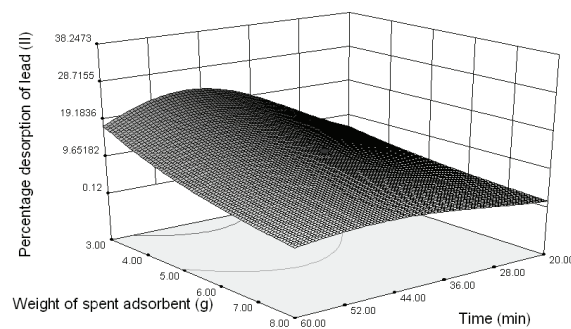

Fig. 12. Combined effect of weight of spent adsorbent and contact time on percentage desorption of lead (II)
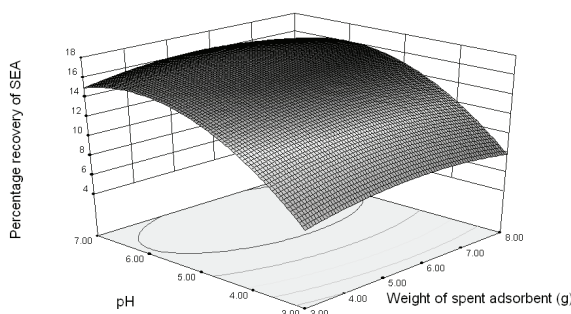

Fig. 13. Combined effect of pH and weight of spent adsorbent during recovery of lead (II) on *SEA* of spent adsorbent after desorption

Long incubation at low pH may alter the conformation of papain and results in a decreased *SEA*. The effect of pH and time of incubation is same as the previous observations (Fig. 15). A comparison of results obtained from EDS study shows that mass percentage of lead (II) has been reduced in lead (II)-impregnated *AIP* after treatment with an acidic solution (pH 4.0) (Table 2).
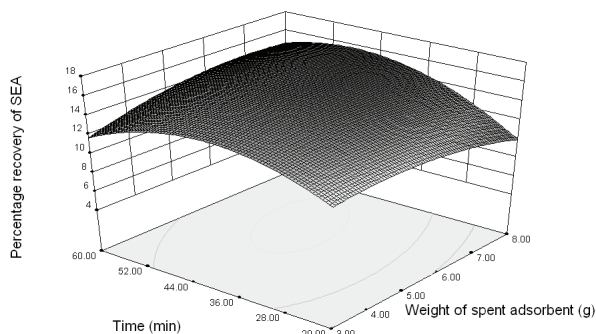

Fig. 14. Combined effect of time of contact and weight of spent adsorbent during desorption of lead (II) on *SEA* of spent adsorbent after desorption
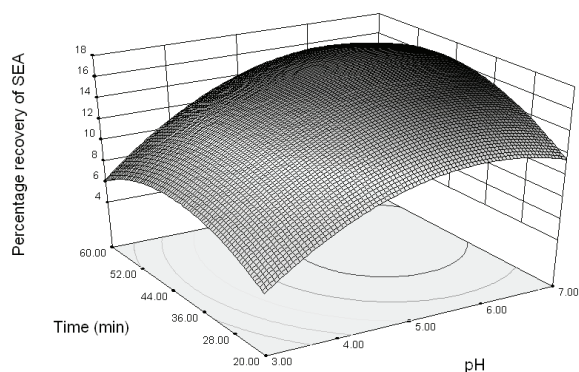

Fig. 15. Combined effect of time of contact and pH during desorption of lead (II) on *SEA* of spent adsorbent after desorption

Molecular modeling of papain shows that binding of metal ions is associated with a release of proton (Sluyterman and Wijdenes, 1976).

$$EH = E^- + H^+ \tag{8}$$

$$E^- + M^{+2} = EM^+ \tag{9}$$

The observation of the EDS study is in accordance with the above equations (Bhattacharyya et al., 2010). In the low pH, papain remains in the protonated form which shifts the equilibrium to the left hand side of the equations resulting in release of lead (II). The observation is similar to a previous study where the authors reported recovery of mercury from *AIP* at low pH (Bhattacharyya et al., 2010).

The optimized condition obtained from RSM for recovery of lead (II): Weight of spent adsorbent = 3 g, pH = 4.59, Time of incubation = 46.43 min. In this condition predicted percentage desorption is 32.0514 and percentage recovery of *SEA* is 13.4602.

Highest percentage of recovery was obtained with the minimum weight of spent *AIP*. In the present study 30 mL of lead (II) solution was used in all the experiments for the study of recovery of metals. As a result, volume of solution was comparatively less for higher weights of *AIP* containing higher amount of adsorbed lead (II). This is turn, resulted in lower rate of diffusion of the leached ions from *AIP* which might be responsible for lower extent of recovery. The amount of lead (II) recovered from *AIP* could be increased by increasing the volume of solution to *AIP* ratio.

Recovery of lead (II) from *AIP* is important to avoid disposal problem and to make *AIP* reusable. Disposal of the *AIP* beads saturated with lead (II) would introduce the toxic metal in the environment and add cost to the process. Metal ions thus recovered may be utilized for other industrial purposes. After recovery of lead (II), beads were reused and satisfactory results have been obtained.

5. Conclusions

SEA of *AIP* is decreased due to incubation with lead (II) under equilibrium condition indicating binding of lead (II) with *AIP* which is confirmed by the EDS study. The rate of abatement of lead (II) with lower initial concentration is not controlled by film diffusion but by intra-particle diffusion. Not only the initial concentration of lead (II) but also the ratio of weight of lead (II) to *AIP* plays an important role in controlling removal of lead (II) by *AIP*. Optimum percentage recovery of lead and percentage recovery of *SEA* was obtained at the following condition: weight of spent adsorbent = 3 g, pH = 4.59, Time of incubation = 46.43 min.

Nomenclature

C_0 = initial concentration of lead (II), mg/L
C_t = concentration of lead (II) after time t, mg/L
EH = undissociated papain
E^- = deprotonated papain
EM^+ = metal bound with papain
H^+ = hydrogen ion
k_M = rate constant of intraparticle transport in Moris Weber model, (mg g^{-1} h$^{-1/2}$).
K'_F = adsorption equilibrium constant used in Freundlich adsorption isotherm model, (mg adsorbate/g *AIP*) (mg adsorbate/L)^n
K'_F = adsorption equilibrium constant used in Langmuir isotherm, (L/mg adsorbate)
k_L = overall rate constant in Lagergren model, (h^{-1}).
k_P = pseudo-second-order rate constant of sorption, (g mg^{-1} h^{-1}).
M^{2+} = divalent metal ion
n'_F = adsorption equilibrium constant used in Freundlich adsorption isotherm model.
q_t = Solid phase concentration of adsorbate at time t, (mg/g).

q_e = solid phase concentration of adsorbate at equilibrium, (mg/g).
q^0 = solid phase concentration of adsorbate for complete monolayer formation (mg/g).
SEA = Specific Enzymatic Activity, ((g peptide formed)/(g papain ×hr)]
WR = Weight ratio of lead (II) to *AIP* (mg/mg).

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