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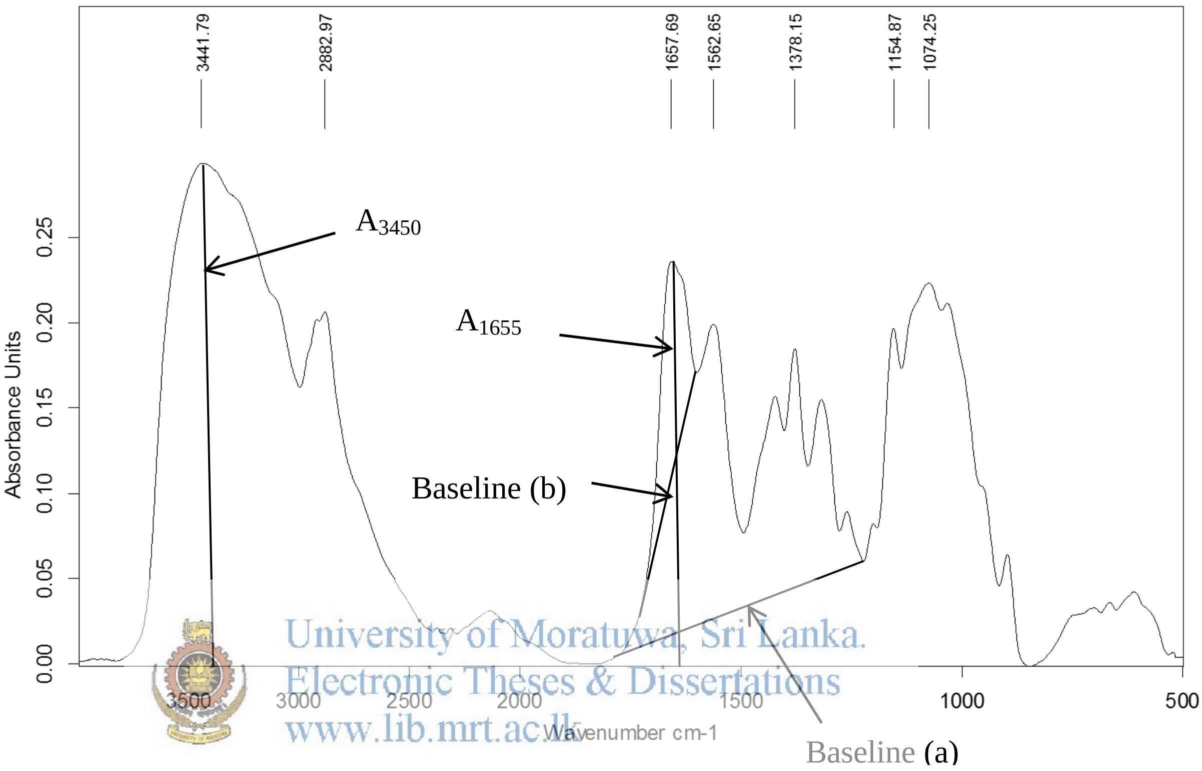
## [Appendix - I: Determination of Degree of deacetylation]






Baseline (a) equation

$$DD = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times \frac{100}{1.33}\right]$$

Baseline (b) equation

$$\mathrm{DD} = 100 - \left[\left(\frac{\mathrm{A}_{1655}}{\mathrm{A}_{3450}}\right) \times 115\right]$$

The acid base titration method was used to determine the DD from the amino group content in chitosan. Dry chitosan (0.3g) was dissolved in 30ml of HCL standard solution (0.1M). Methyl orange and aniline blue mixing indicators were added. A standard solution of NaOH (0.1M) was used for titration until the solution became blue green. The following formulas were used to calculate the DD of the product.

$$(-NH_2)\% = \frac{0.016(C_1V_1 - C_2V_2)}{W} \times 100$$

$$DD\% = \frac{203(-NH_2\%)}{16 + 42(-NH_2\%)} \times 100$$

Where $C_1$, $V_1$, $C_2$, and $V_2$ are the concentrations and volumes for the HCl standard solution and NaOH standard solution, respectively, and W is the weight of the sample.



| Sample | FTIR base line a method | FTIR base line b method | Titration method |
|---|---|---|---|
| Low DD | 72 | 88.6 | 94.2 |
| High DD | 85.6 | 93.6 | 98.5 |

## *[Appendix - II: FTIR Characterization of heavy metal adsorbed chitosan]*

Characteristic peaks which affect the adsorption of heavy metals are as follows,

- N-H stretching band of amine group – 3270- 3300 $cm^{-1}$
- C=O stretching of amide I – 1655 $cm^{-1}$
- C-N stretching of amide I – 1625 $cm^{-1}$
- N-H bending band due to the presence of $NH_2$ band – 1590 $cm^{-1}$

Due to the presence of number of peaks in the near wave number values, it is very difficult to find the actual intensity of the particular peak. For that, FTIR graphs were normalized relative to the O-H Stretching peak (Because OH doesn't involve with the adsorption process) and then the graph was deconvoluted to find the correct intensity.

When heavy metal adsorbed in to $NH_2$, shift and broadening of the peak can be observed. That can be analyzed by calculating the full width at half maximum of $NH_2$ absorption band as represent in Figure 1.



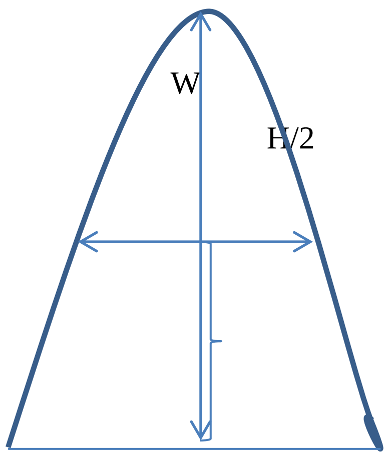



Figure 1. Representation of full width at half

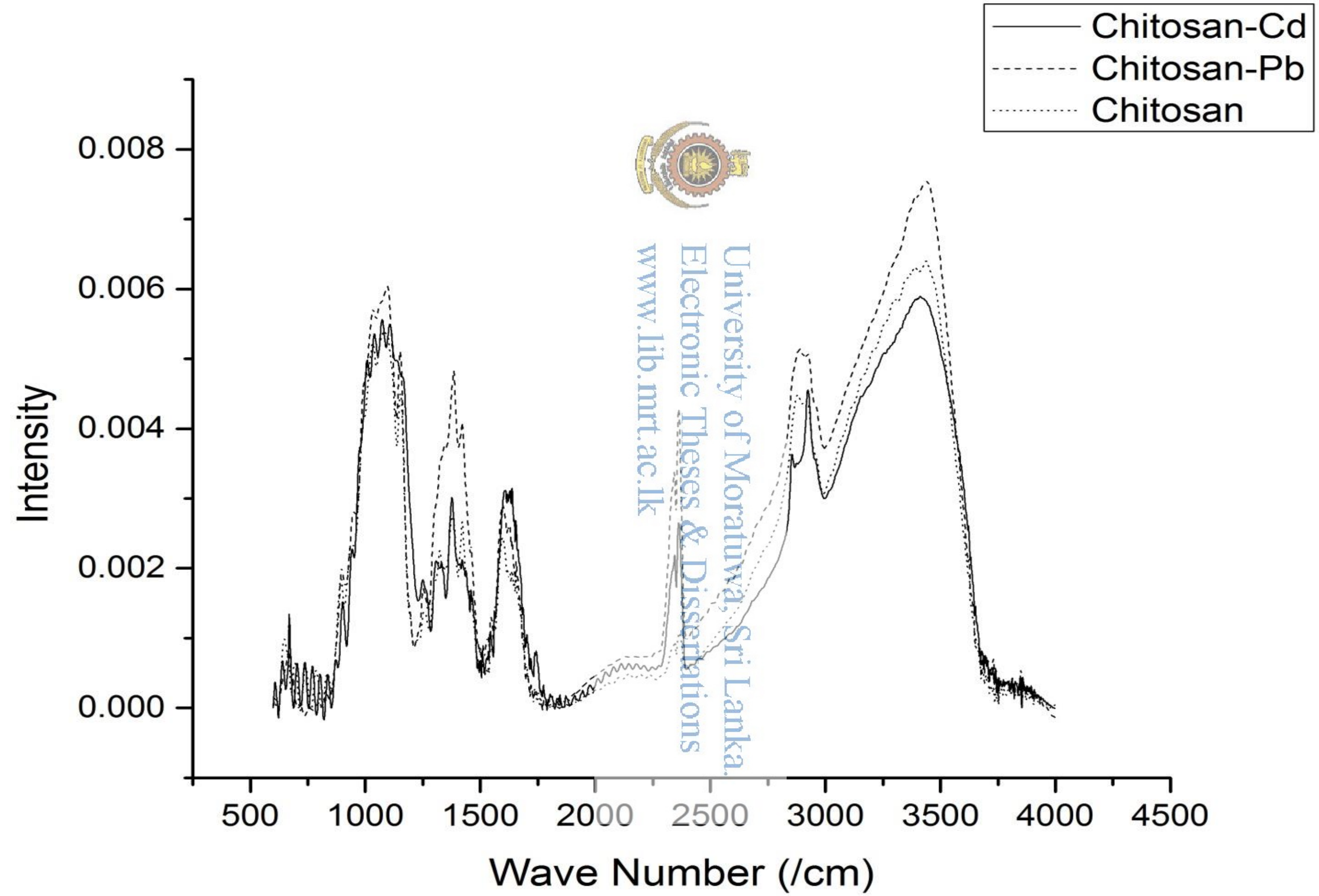





Figure 2. FTIR spectrum comparison of different chitosan samples

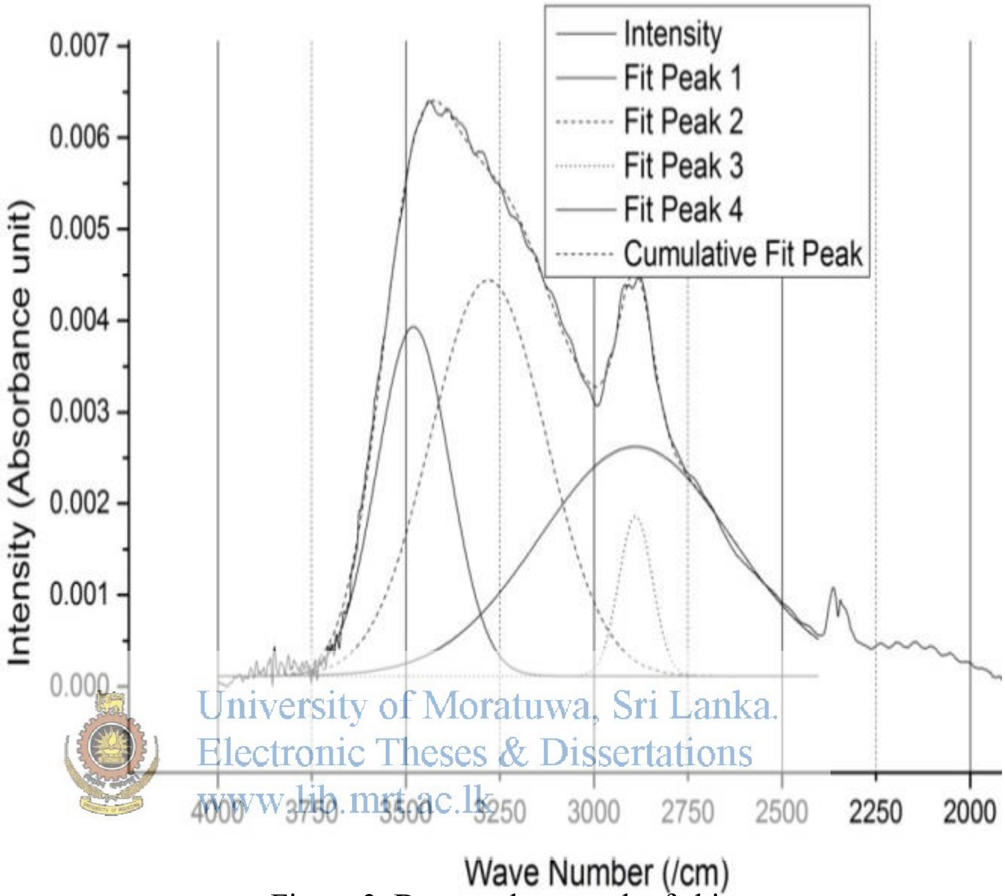




Figure 3. Deconvolute graph of chitosan

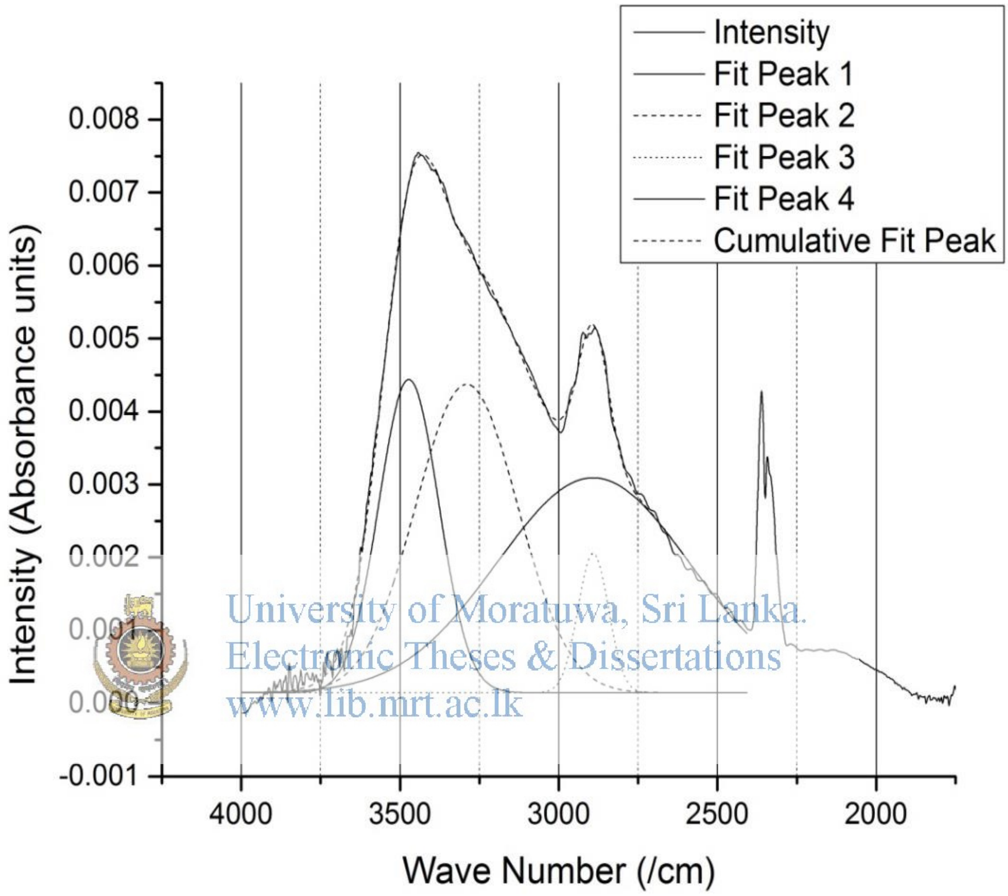




Figure 4. Deconvolute graph of Cd-chitosan

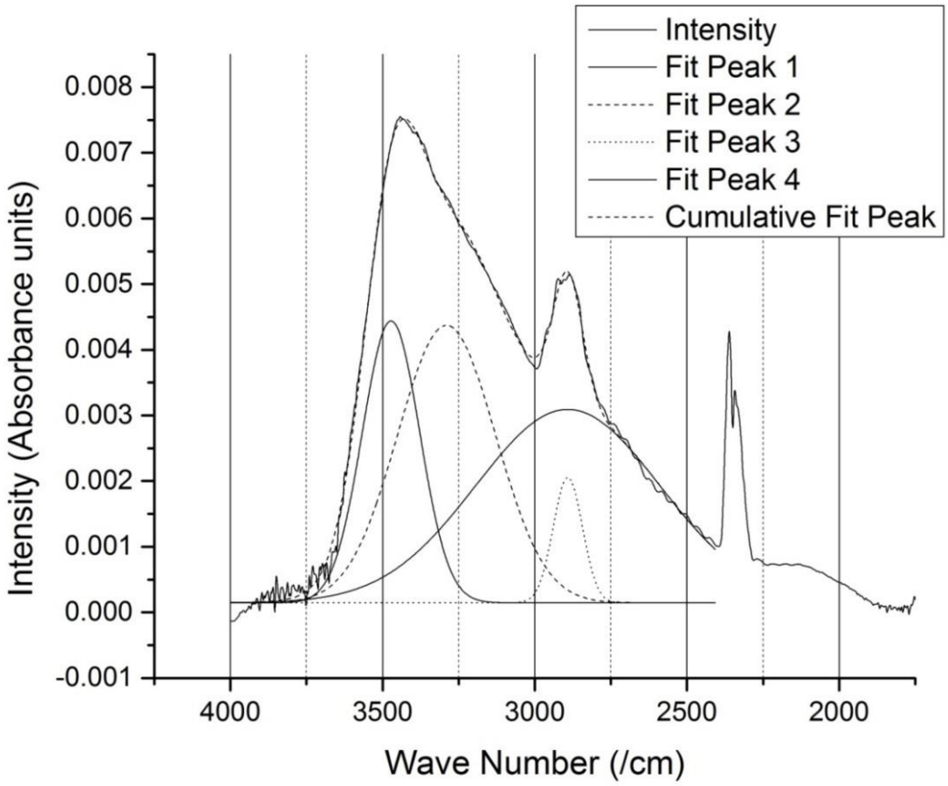



Figure 5. Deconvolute graph of Pb-chitosan

In all above three graphs, peak 2 is responsible for stretching of N-H bonds. So, if the Cd and Pb were connected to the $NH_2$ groups by forming complex structure, broadening of the peak can be observed. In the Following Table 1, full widths at half maximum (W) data are given.



Table 1. Full width at half maximum values for chitosan samples

| | W (nm) |
|---|---|
| Pure Chitosan | 360.78 |
| Cd Adsorbed Chitosan | 377.97 |
| Pb Adsorbed Chitosan | 378.97 |

Finally, it can be concluded that the cadmium and lead were adsorbed by the $NH_2$ groups in chitosan.

## *[Appendix - III: Adsorption test results]*

**Cd**

| | Time (min) | Final concentration of solution (ppm) | Adsorbed amount into chitosan (ppm) | Adsorption capacity (mg/g) |
|---|---|---|---|---|
| **Low** | 5 | 41.8 | 8.2 | 4.1 |
| **DD** | 10 | 41 | 9 | 4.5 |
| pH- 5.5 | 15 | 38.333 | 11.667 | 5.8335 |
| 28±2$^0$C | 30 | 34.433 | 15.567 | 7.7835 |
| 0.1 g | 45 | 34.425 | 15.575 | 7.7875 |
| dose | 60 | 34.467 | 15.533 | 7.7665 |
| 50ppm | 90 | 33.966 | 16.034 | 8.017 |
| | 120 | 33.733 | 16.267 | 8.1335 |
| | 150 | 33.733 | 16.267 | 8.1335 |
| | 180 | 33.766 | 16.234 | 8.117 |
| | 240 | 30.425 | 19.575 | 9.7875 |
| | 300 | 30.426 | 19.574 | 9.787 |
| | | | | |
| **High** | 5 | 34.925 | 15.075 | 7.5375 |
| **DD** | 10 | 34.45 | 15.55 | 7.775 |
| pH- 5.5 | 15 | 31.266 | 18.734 | 9.367 |
| 28±2$^0$C | 30 | 29.867 | 20.133 | 10.0665 |
| 0.1 g | 45 | 29.4 | 20.6 | 10.3 |
| dose | 60 | 29.433 | 20.567 | 10.2835 |
| 50ppm | 90 | 29.233 | 20.767 | 10.3835 |
| | 120 | 28.867 | 21.133 | 10.5665 |
| | 150 | 27.433 | 22.567 | 11.2835 |
| | 180 | 27.467 | 22.533 | 11.2665 |
| | 240 | 26.874 | 23.126 | 11.563 |
| | 300 | 26.548 | 23.452 | 12.226 |
| | | | | |
| **High** | 5 | 39.15 | 10.85 | 5.425 |
| **pH-6.5** | 10 | 37.625 | 12.375 | 6.1875 |
| 28±2$^0$C | 15 | 32.533 | 17.467 | 8.7335 |
| 0.1 g | 30 | 28.123 | 21.877 | 10.9385 |
| dose | 45 | 26.98 | 23.02 | 11.51 |
| 50ppm | 60 | 26.15 | 23.85 | 11.925 |
| | 90 | 25.456 | 24.544 | 12.272 |
| | 120 | 24.98 | 25.02 | 12.51 |
| | 150 | 24.625 | 25.375 | 12.6875 |
| | 180 | 24.533 | 25.467 | 12.7335 |
| | 240 | 24.523 | 25.477 | 12.7385 |
| | 300 | 24.321 | 25.679 | 12.8395 |

| | | | | |
|---|---|---|---|---|
| | | | | |
| **Low pH-3.5** $28\pm2^0C$ 0.1 g dose 50ppm | 5 | 45.725 | 4.275 | 2.1375 |
| | 10 | 44.625 | 5.375 | 2.6875 |
| | 15 | 44.499 | 5.501 | 2.7505 |
| | 30 | 44.267 | 5.733 | 2.8665 |
| | 45 | 43.733 | 6.267 | 3.1335 |
| | 60 | 43.267 | 6.733 | 3.3665 |
| | 90 | 42.2 | 7.8 | 3.9 |
| | 120 | 41.266 | 8.734 | 4.367 |
| | 150 | 40.267 | 9.733 | 4.8665 |
| | 180 | 40.266 | 9.734 | 4.867 |
| | 240 | 40.261 | 9.739 | 4.8695 |
| | 300 | 40.256 | 9.744 | 4.872 |
| | | | | |
| **106 microns** pH- 6.5 $28\pm2^0C$ 0.1 g dose of chitosan 50ppm | 5 | 38 | 12 | 6 |
| | 10 | 35.64 | 14.36 | 7.18 |
| | 15 | 35.36 | 14.64 | 7.32 |
| | 30 | 32.64 | 17.36 | 8.68 |
| | 45 | 31.42 | 18.58 | 9.29 |
| | 60 | 29.26 | 20.74 | 10.37 |
| | 90 | 28.4 | 21.6 | 10.8 |
| | 120 | 26.06 | 23.94 | 11.97 |
| | 150 | 24.86 | 25.14 | 12.57 |
| | 180 | 22.36 | 27.64 | 13.82 |
| | 240 | 22.3 | 27.7 | 13.85 |
| | 300 | 22.06 | 27.94 | 13.97 |
| | | | | |
| **75 microns** pH- 6.5 $28\pm2^0C$ 0.1 g dose of chitosan 50ppm | 5 | 35.8 | 14.2 | 7.1 |
| | 10 | 35.32 | 14.68 | 7.34 |
| | 15 | 34.12 | 15.88 | 7.94 |
| | 30 | 32.02 | 17.98 | 8.99 |
| | 45 | 31.58 | 18.42 | 9.21 |
| | 60 | 28.16 | 21.84 | 10.92 |
| | 90 | 27.36 | 22.64 | 11.32 |
| | 120 | 25.02 | 24.98 | 12.49 |
| | 150 | 23.06 | 26.94 | 13.47 |
| | 180 | 21.06 | 28.94 | 14.47 |
| | 240 | 21.16 | 28.84 | 14.42 |
| | 300 | 20.8 | 29.2 | 14.6 |
| | | | | |

| | | | | |
|---|---|---|---|---|
| **25 ppm** | 5 | 19.19 | 5.81 | 2.905 |
| pH- 6.5 | 10 | 19.4 | 5.6 | 2.8 |
| 28±2$^0$C | 15 | 17.18 | 7.82 | 3.91 |
| 0.1 g dose of | 30 | 11.41 | 13.59 | 6.795 |
| chitosan | 45 | 10.35 | 14.65 | 7.325 |
| | 60 | 10.81 | 14.19 | 7.095 |
| | 90 | 10.32 | 14.68 | 7.34 |
| | 120 | 9.3 | 15.7 | 7.85 |
| | 150 | 8.5 | 16.5 | 8.25 |
| | 180 | 7.85 | 17.15 | 8.575 |
| | 240 | 7.64 | 17.36 | 8.68 |
| | 300 | 6.5 | 18.5 | 9.25 |
| | | | | |
| **5 ppm** | 5 | 0.806 | 4.194 | 2.097 |
| pH- 6.5 | 10 | 0.798 | 4.202 | 2.101 |
| 28±2$^0$C | 15 | 0.66 | 4.34 | 2.17 |
| 0.1 g dose of | 30 | 0.798 | 4.202 | 2.101 |
| chitosan | 45 | 0.706 | 4.294 | 2.147 |
| | 60 | 0.652 | 4.348 | 2.174 |
| | 90 | 0.672 | 4.328 | 2.164 |
| | 120 | 0.602 | 4.398 | 2.199 |
| | 150 | 0.638 | 4.362 | 2.181 |
| | 180 | 0.592 | 4.408 | 2.204 |
| | 240 | 0.576 | 4.424 | 2.212 |
| | 300 | 0.562 | 4.438 | 2.219 |
| | | | | |
| **0.05 g** | 5 | 42.65 | 7.35 | 7.35 |
| pH- 6.5 | 10 | 40.875 | 9.125 | 9.125 |
| 28±2$^0$C | 15 | 41.725 | 8.275 | 8.275 |
| 50ppm | 30 | 41.4 | 8.6 | 8.6 |
| | 45 | 37.95 | 12.05 | 12.05 |
| | 60 | 39.375 | 10.625 | 10.625 |
| | 90 | 38.7 | 11.3 | 11.3 |
| | 120 | 37.625 | 12.375 | 12.375 |
| | 150 | 36.25 | 13.75 | 13.75 |
| | 180 | 35.85 | 14.15 | 14.15 |
| | 240 | 33.94 | 16.06 | 16.06 |
| | 300 | 33.175 | 16.825 | 16.825 |
| | | | | |

| | | | | |
|---|---|---|---|---|
| **0.025g**<br>pH- 6.5<br>$28\pm2^0C$<br>50ppm | 5 | 43.9 | 6.1 | 12.2 |
| | 10 | 43.725 | 6.275 | 12.55 |
| | 15 | 42.725 | 7.275 | 14.55 |
| | 30 | 42.6 | 7.4 | 14.8 |
| | 45 | 42.625 | 7.375 | 14.75 |
| | 60 | 42.075 | 7.925 | 15.85 |
| | 90 | 40.98 | 9.02 | 18.04 |
| | 120 | 40.175 | 9.825 | 19.65 |
| | 150 | 40.7 | 9.3 | 18.6 |
| | 180 | 39.35 | 10.65 | 21.3 |
| | 240 | 39.15 | 10.85 | 21.7 |
| | 300 | 38.95 | 11.05 | 22.1 |
| | | | | |

**Pb**

| | Time (min) | Final concentration of solution (ppm) | Adsorbed amount into chitosan(ppm) | Adsorption capacity(mg/g) |
|---|---|---|---|---|
| **Low DD**<br>pH- 3.5<br>28±2$^0$C<br>0.1 g dose of chitosan<br>50 ppm | 5 | 27.665 | 22.335 | 11.1675 |
| | 10 | 26.795 | 23.205 | 11.6025 |
| | 15 | 27.325 | 22.675 | 11.3375 |
| | 30 | 26.955 | 23.045 | 11.5225 |
| | 45 | 24.605 | 25.395 | 12.6975 |
| | 60 | 22.65 | 27.35 | 13.675 |
| | 90 | 21.38 | 28.62 | 14.31 |
| | 120 | 17.265 | 32.735 | 16.3675 |
| | 150 | 20.525 | 29.475 | 14.7375 |
| | 180 | 16.48 | 33.52 | 16.76 |
| | 240 | 17.55 | 32.45 | 16.225 |
| | 300 | 17.45 | 32.55 | 16.275 |
| | | | | |
| **High DD**<br>pH- 3.5<br>28±2$^0$C<br>0.1 g dose of chitosan<br>50 ppm | 5 | 25.135 | 24.865 | 12.4325 |
| | 10 | 25.14 | 24.86 | 12.43 |
| | 15 | 25.37 | 24.63 | 12.315 |
| | 30 | 23.055 | 26.945 | 13.4725 |
| | 45 | 23.075 | 26.925 | 13.4625 |
| | 60 | 20.715 | 29.285 | 14.6425 |
| | 90 | 17.65 | 32.35 | 16.175 |
| | 120 | 14.526 | 35.474 | 17.737 |
| | 150 | 17.95 | 32.05 | 16.025 |
| | 180 | 12.42 | 37.58 | 18.79 |
| | 240 | 12.96 | 37.04 | 18.52 |
| | 300 | 12.76 | 37.24 | 18.52 |
| | | | | |
| **High pH- 4.5**<br>28±2$^0$C<br>0.1 g dose of chitosan<br>50 ppm | 5 | 25.135 | 24.865 | 12.4325 |
| | 10 | 25.14 | 24.86 | 12.43 |
| | 15 | 25.37 | 24.63 | 12.315 |
| | 30 | 23.055 | 26.945 | 13.4725 |
| | 45 | 23.075 | 26.925 | 13.4625 |
| | 60 | 20.715 | 29.285 | 14.6425 |
| | 90 | 17.65 | 32.35 | 16.175 |
| | 120 | 14.526 | 35.474 | 17.737 |
| | 150 | 17.95 | 32.05 | 16.025 |
| | 180 | 12.42 | 37.58 | 18.79 |
| | 240 | 12.96 | 37.04 | 18.52 |
| | 300 | 12.76 | 37.24 | 18.52 |
| | | | | |

| | | | | |
|---|---|---|---|---|
| **Low pH- 2.0** 28±2$^0$C 0.1 g dose of chitosan 50 ppm | 5 | 34.805 | 15.195 | 7.5975 |
| | 10 | 32.57 | 17.43 | 8.715 |
| | 15 | 31.595 | 18.405 | 9.2025 |
| | 30 | 33.935 | 16.065 | 8.0325 |
| | 45 | 30.455 | 19.545 | 9.7725 |
| | 60 | 28.615 | 21.385 | 10.6925 |
| | 90 | 26.205 | 23.795 | 11.8975 |
| | 120 | 22.905 | 27.095 | 13.5475 |
| | 150 | 24.895 | 25.105 | 12.5525 |
| | 180 | 22.815 | 27.185 | 13.5925 |
| | 240 | 21.8 | 28.2 | 14.1 |
| | 300 | 21.68 | 28.32 | 14.16 |
| | | | | |
| **106 microns** pH- 4.5 28±2$^0$C 0.1 g dose of chitosan 50 ppm | 5 | 24.5 | 25.5 | 12.75 |
| | 10 | 23.676 | 26.324 | 13.162 |
| | 15 | 23.652 | 26.348 | 13.174 |
| | 30 | 22.724 | 27.276 | 13.638 |
| | 45 | 22.752 | 27.248 | 13.624 |
| | 60 | 21.608 | 28.392 | 14.196 |
| | 90 | 19.0925 | 30.9075 | 15.45375 |
| | 120 | 18.9142 | 31.0858 | 15.5429 |
| | 150 | 17.6168 | 32.3832 | 16.1916 |
| | 180 | 16.593 | 33.407 | 16.7035 |
| | 240 | 16.9824 | 33.0176 | 16.5088 |
| | 300 | 15.732 | 34.268 | 17.134 |
| | | | | |
| **75 microns** pH- 4.5 28±2$^0$C 0.1 g dose of chitosan 50 ppm | 5 | 24.98 | 25.02 | 12.51 |
| | 10 | 24.4 | 25.6 | 12.8 |
| | 15 | 23.2167 | 26.7833 | 13.39165 |
| | 30 | 22.4466 | 27.5534 | 13.7767 |
| | 45 | 21.349 | 28.651 | 14.3255 |
| | 60 | 19.621 | 30.379 | 15.1895 |
| | 90 | 18.315 | 31.685 | 15.8425 |
| | 120 | 17.7111 | 32.2889 | 16.14445 |
| | 150 | 17.5329 | 32.4671 | 16.23355 |
| | 180 | 16.869 | 33.131 | 16.5655 |
| | 240 | 15.732 | 34.268 | 17.134 |
| | 300 | 15.032 | 34.968 | 17.484 |
| | | | | |

| | | | | |
|---|---|---|---|---|
| **25 ppm** pH- 4.5 $28\pm2^0C$ 0.1 g dose of chitosan | 5 | 6.04 | 18.96 | 9.48 |
| | 10 | 5.326 | 19.674 | 9.837 |
| | 15 | 5.136 | 19.864 | 9.932 |
| | 30 | 4.864 | 20.136 | 10.068 |
| | 45 | 4.624 | 20.376 | 10.188 |
| | 60 | 4.806 | 20.194 | 10.097 |
| | 90 | 4.214 | 20.786 | 10.393 |
| | 120 | 3.56 | 21.44 | 10.72 |
| | 150 | 3.97 | 21.03 | 10.515 |
| | 180 | 3.19 | 21.81 | 10.905 |
| | 240 | 2.282 | 22.718 | 11.359 |
| | 300 | 2.012 | 22.988 | 11.494 |
| | | | | |
| **5 ppm** pH- 4.5 $28\pm2^0C$ 0.1 g dose of chitosan | 5 | 0.775 | 4.225 | 2.1125 |
| | 10 | 0.551 | 4.449 | 2.2245 |
| | 15 | 0.513 | 4.487 | 2.2435 |
| | 30 | 0.54 | 4.46 | 2.23 |
| | 45 | 0.346 | 4.654 | 2.327 |
| | 60 | 0.318 | 4.682 | 2.341 |
| | 90 | 0.271 | 4.729 | 2.3645 |
| | 120 | 0.119 | 4.881 | 2.4405 |
| | 150 | 0.062 | 4.938 | 2.469 |
| | 180 | 0.008 | 4.992 | 2.496 |
| | 240 | 0.008 | 4.992 | 2.496 |
| | 300 | 0.007 | 4.993 | 2.4965 |

## *[Appendix – IV: Publications]*

1. IEEE Conference Publications

Unagolla, J. M., Adikary, S. U., “Adsorption of cadmium and lead heavy metals by chitosan biopolymer: A study on equilibrium isotherms and kinetics”, *Moratuwa Engineering Research Conference (MERCon), 2015*, pp 234-239, 2015, DOI: 10.1109/MERCon.2015.7112351

2. Unagolla J. M., Adikary S. U.; “Adsorption Characteristics of Cadmium and Lead heavy metal into Locally Synthesized Chitosan Biopolymer” *Tropical Agricultural Research Journal*, Vol 26(2), pp395-401,2014; ISSN: 1016.1422.

Available online at, http://www.pgia.ac.lk/files/Annual_congress/ journel/v26

3. Unagolla J.M., Adikary S. U.;“Adsorption of Lead heavy metal ions by Chitosan Biopolymer: Kinetics and Equilibrium” *108th Annual transactions of Institute of Engineers Sri Lanka*, Vol 1 – Part B, pp 155-162, 2014.



4. Unagolla J. M., Adikary S. U.; “Study of Adsorption Characteristics of Cadmium into Chitosan Biopolymer to be used for Waste Water Treatments” *107th Annual transactions of Institute of Engineers Sri Lanka,* Vol 1- Part B, pp 313-319, 2013.

Available online at, http://www.scribd.com/doc/202126465/IESL-Technical-Papers-Oct-2013#scribd