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APPENDICES

Appendix A Calculation for the Degree of Purification of Purified Biopolymer by Fourier Transform Infrared Spectroscopy (FT-IR)

This method was used to analyze the functional groups of the purified biopolymers (Figure A1) in order to find out amide groups, which are the major functional groups in determining degree of deacetylation.

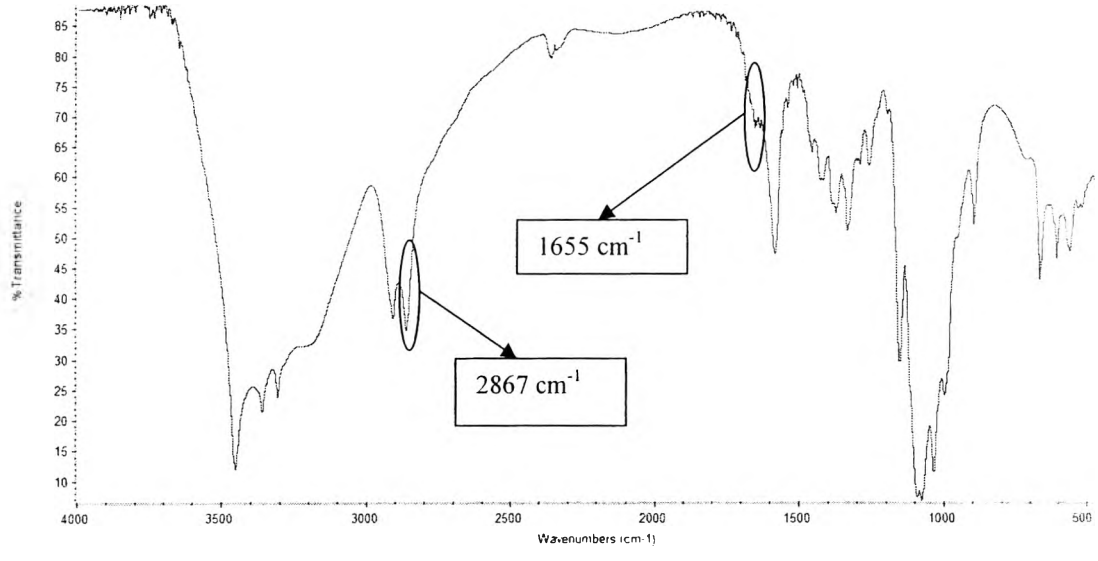

Figure A1 The IR spectra of purified biopolymer used to find degree of purification.

The degree of purified biopolymer can be determined by using the relation of the absorbance ratio of the amide band I at 1655 cm^{-1} to the CH stretching band at 2867 cm^{-1}(Miya *et al.*, 1980). The absorbance ratio can be calculated by this equation

$$\text{The absorbance ratio} = \frac{(A)_{\text{amide } 1655\,cm^{-1}}}{(A)_{\text{CH stretching } 2867\,cm^{-1}}}$$

Whereas:

$$(A)_{\text{amide } 1655\ \text{cm}^{-1}} = \log_{10}\left(\frac{DF}{DE}\right)$$

$$(A)_{\text{CH stretching } 2867\ \text{cm}^{-1}} = \log_{10}\left(\frac{AC}{AB}\right)$$

The values of DF, DE, AC and AB are showed in Figure A2.

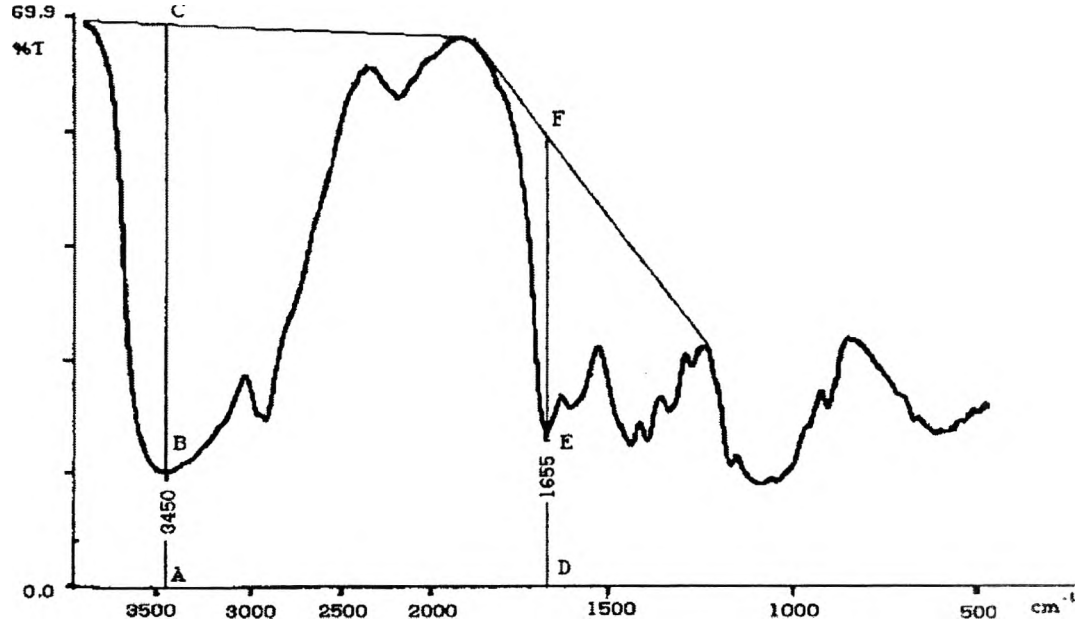

Figure A2 The IR spectrum that show baselines for determine the peak absorbance.

For example :

$$(A)_{\text{amide } 1655\ \text{cm}^{-1}} = \log_{10}\left(\frac{76.12}{74.45}\right)$$

$$= 0.009$$

$$(A)_{\text{CH stretching } 2867\ \text{cm}^{-1}} = \log_{10}\left(\frac{77.54}{57.54}\right)$$

$$= 0.128$$

Thus :

$$\text{The absorbance ratio} = \frac{0.009}{0.128}$$

$$= 0.075$$

The absorbance ratio of the amide band I at 1655 cm^{-1} to the CH stretching band at 2867 cm^{-1} can be determined the degree of purification by using Figure A3. The result is 96.99 % degree of purification.

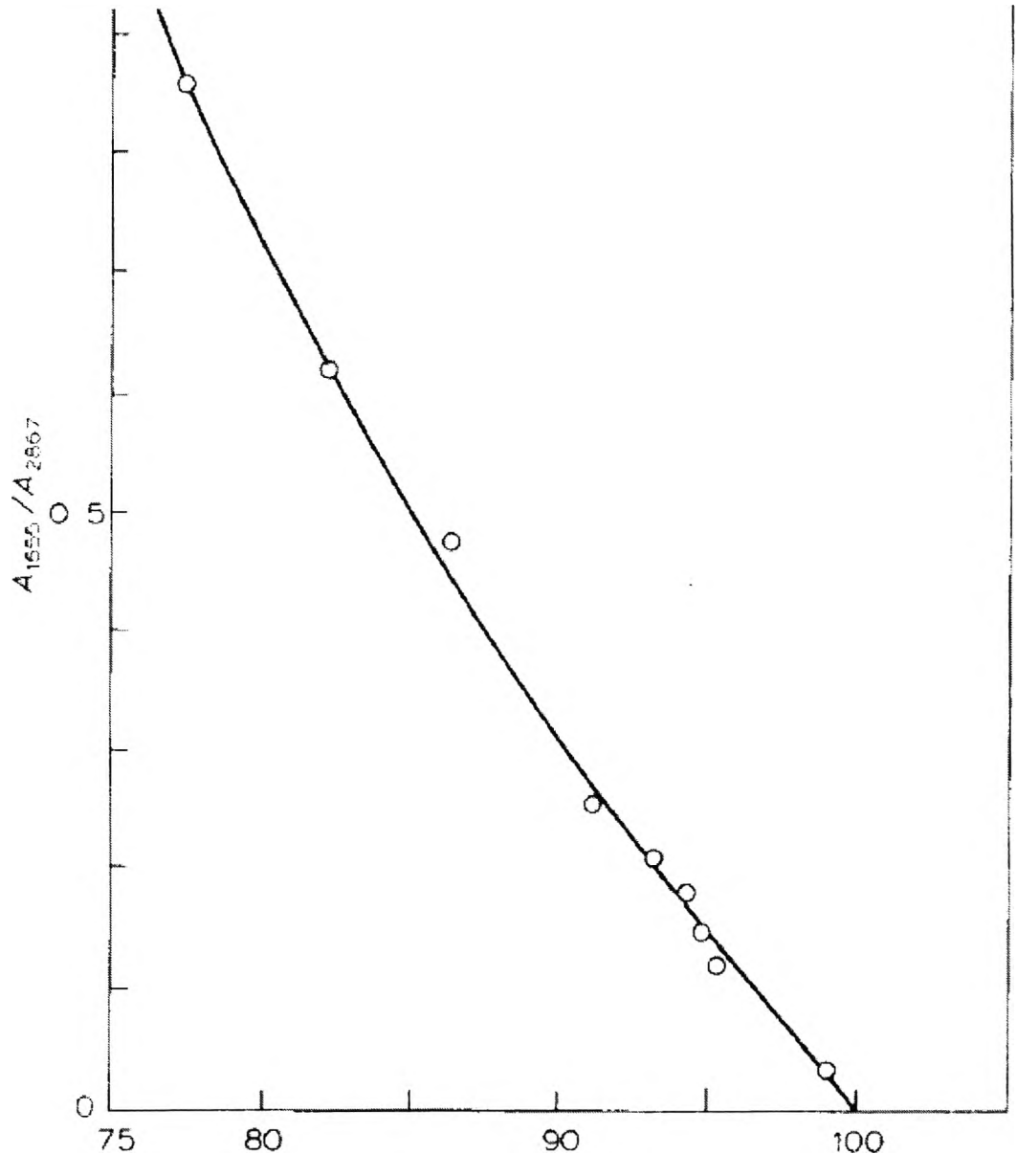

Figure A3 The relation of the ratio of the absorbance of the band at 1655cm to that of the band at 2867cm 1 (A_{1655}/A_{2867}) against the degree of deacetylation.

Raw data for determining degree of deacetylation of purified biopolymer by FTIR method was showed in Table A1.

Table A1 Raw data from FTIR method.

Batch	Relative intensity		$A_{2867\ cm^{-1}}$	Relative intensity		$A_{1655\ cm^{-1}}$	Absorbance ratio	%DD
	AB	AC		DE	DF			
1	57.72	77.54	0.128	74.45	76.12	0.009	0.075	96.99
2	59.85	79.23	0.122	73.23	74.83	0.009	0.077	96.39
3	58.76	81.34	0.141	74.44	76.15	0.009	0.070	97.03
	Average							96.80

The degree of purification was 96.80% with 0.358% of standard deviation.

Appendix B Calculation Degree of Purification of Purified Biopolymer by Titration Method

This method used titration curve of 0.1 M NaoH and 0.05 g purified biopolymer dissolved in 0.1 M HCl. Titration curve are shown in Figure B1.

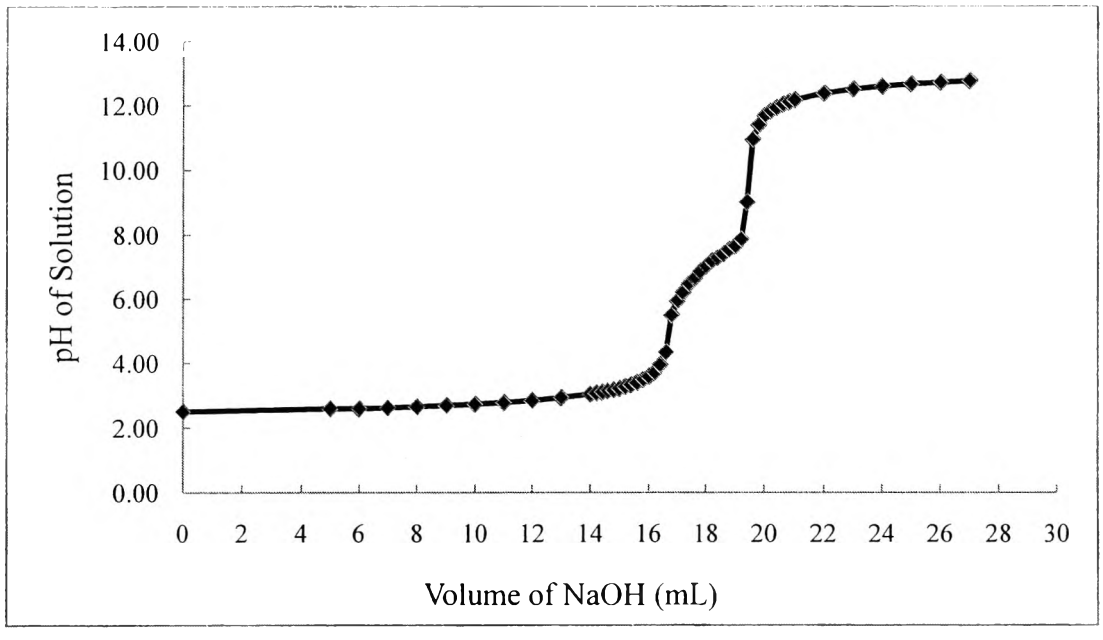

Figure B1 Titration curve for determining degree of deacetylatio of purified biopolymer.

A titration curve shows that there are two equivalent points those are related to excess of HCl and protonated amino groups. The first equivalent point is due to neutralization of the excess HCl with NaOH solution and the second equivalent point is due to displacement of HCl bound to the primary amino groups of biopolymer. The correct position of two equivalent points can be determined by linear extrapolation of the adjacent portions of the titration curve. The degree of deacetylation calculated from the two equivalent points (Avadi *et at.*, 2004).

Degree of purification observed from equation :

$$DD = 16.1(Y - X)\frac{f}{W}$$

X is slope of point X in Figure B1 correspond to consumed NaOH volume of the first equivalent point

Y is slope of point Y in Figure B1 correspond to consumed NaOH volume of the first equivalent point

f is concentration of NaOH (molar)

w is the initial purified biopolymer weight (grams)

For example : Weight of biopolymer was 0.0502 g

Concentration of NaOH was 0.1000 N

Volume of NaoH for the first equivalent points was 16.505 mL

Volume of NaoH for the second equivalent points was 19.500 mL

Therefore;

$$DD = 16.1(19.5 - 16.505)\frac{0.1}{0.0502}$$

$$DD = 96.05\ \%$$

Raw data for determining degree of deacetylation of purified biopolymer by titration method was showed in Table B1.

Table B1 Raw data from titration method

Batch	Volume of NaOH at first equivalent point (mL)	Volume of NaOH at second equivalent point (mL)	Initial purified biopolymer (g)	Concentration of NaOH (N)	%DD
1	16.505	19.5	0.0502	0.1000	96.05
2	15.31488	18.3	0.0500	0.1000	96.12
3	15.91303	18.9	0.0501	0.1000	95.99
Average					96.05

The degree of purification was 96.05% with 0.066% of standard deviation.

Appendix C Calculation the Amount of Biopolymer in PolyHIPE

This method used to determine the amount of biopolymer in polyHIPE using calculation of nitrogen (N) in structure of biopolymer and the results were showed in Table C1.

Table C1 CHN test results of each condition of polyHIPE contained biopolymer solution

Sample		Sample Weight (g)	%			Weight (mg)		
wt% of biopolymer	Trial		C	H	N	H	C	N
30	1	0.0566	70.95	8.174	1.253	4.627	40.16	0.709
	2	0.0586	71.01	8.247	0.934	4.833	41.61	0.548
50	1	0.0568	69.06	8.038	1.918	4.566	39.22	1.089
	2	0.0569	68.25	8.181	1.858	4.655	38.83	1.057
	3	0.0405	72.08	8.293	1.344	3.359	29.19	0.544
70	1	0.0533	66.64	8.047	2.457	4.289	35.52	1.309
	2	0.0608	65.47	7.911	2.546	4.810	39.81	1.548
	3	0.0579	65.73	7.965	2.442	4.612	38.06	1.414
100	1	0.0540	65.54	7.793	3.148	4.208	35.39	1.700
	2	0.0542	65.19	8.008	3.114	4.340	35.33	1.687
	3	0.0582	64.75	7.996	2.981	4.654	37.69	1.734
120	1	0.0546	62.29	7.753	3.487	4.233	34.01	1.904
	2	0.0592	61.81	7.757	3.573	4.592	36.59	2.115
	3	0.0525	62.97	7.727	3.506	4.057	33.06	1.841
150	1	0.0586	59.99	7.651	3.959	4.484	35.16	2.320
	2	0.0560	60.59	7.709	3.926	4.317	33.93	2.198
	3	0.0638	59.6	7.650	3.983	4.881	38.03	2.540

For example : calculation at 30 wt% of biopolymer (0.2601 g)

From theory,

In Figure C1, there was nitrogen 14 g in biopolymer 161 g (1mol) therefore there was nitrogen 0.0226 g in biopolymer 0.2601 g and polyHIPE that obtained from DVB monomer 1 mL was 0.8669 g. Total weight of polyHIPE – biopolymer was 1.127 g.

PolyHIPE – biopolymer 1.127 g had nitrogen 0.0226 g.

PolyHIPE – biopolymer 1 g had nitrogen 0.0201 g or 20.10 mg.

From CHN results,

Sample 0.0576 g had nitrogen 0.63 mg.

Sample 1 g had nitrogen 10.91 mg.

% Loading of biopolymer, $\frac{10.91\ mg}{20.10\ mg} \times 100 = 54.36\ \%$.

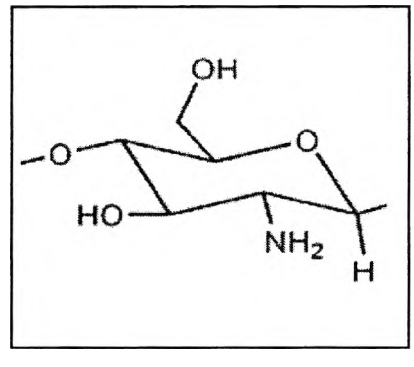

Figure C1 Structure of biopolymer.

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