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APPENDICES

APPENDIX A

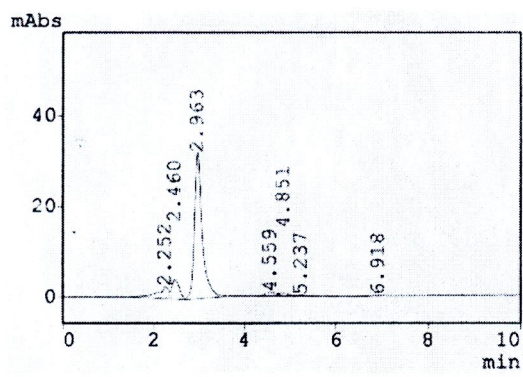
Validation of Analytical Method

1. Analysis of xyloglucan by high performance liquid chromatographic (HPLC) method

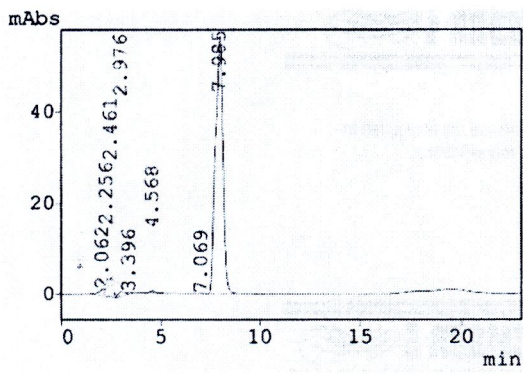
The validation of analytical method is the process by which it is established that the performance characteristics of the method meet the requirements for the intended analytical applications. The performance characteristics are expressed in term of analytical parameters. For HPLC assay validation, these include specificity, linearity, accuracy and precision.

1.1.1 Specificity

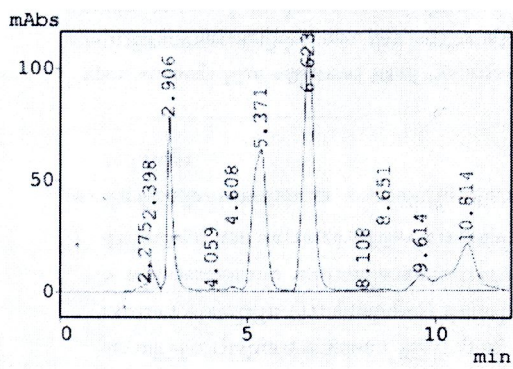
The specificity of an analytical method is the ability to measure the analyte accurately and with specificity in the presence of other components in the sample. Figures 1A were shown typical chromatogram of asiaticoside standard solution, internal standard solution, titrated extract of *Centella asiatica* (TECA) solution, blank sample solution (blank film formulation), respectively. The chromatograms demonstrated that the HPLC condition used in the study had a suitable specificity.



(a)



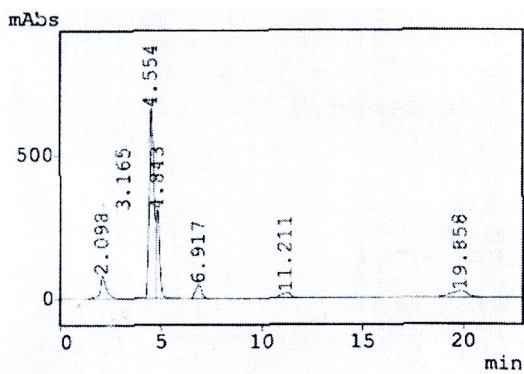
(b)



(c)

Fig 16 HPLC chromatograms of mobile phase

- (a) asiaticoside standard solution
- (b) triamcinolone acetonide solution
- (c) *Centella* extract solution
- (d) Blank film formulation



(d)

Fig 16 HPLC chromatograms of mobile phase (continued)

- (a) asiaticoside standard solution
- (b) triamcinolone acetonide solution
- (c) TECA solution
- (d) Blank film formulation

1.1.2. Linearity

Table 1A was displayed the calibration curve data of standard asiaticoside. The plot of standard asiaticoside concentrations versus the peak height ratio (Figure 2A) shown the linear correlation in the concentration range studied of 10-100 µg/ml. The coefficient of determination (R^2) of this line was 0.9996. These results indicated that HPLC method was acceptable for quantitative analysis of asiaticoside in the range studied.

Table 1A Data for calibration curve of asiaticoside by HPLC method

Concentration (µg/ml)	Peak height ratio			Mean	SD	%CV
	Set1	Set2	Set3			
10	0.4903	0.4985	0.4997	0.4962	0.0051	1.03
20	1.0119	1.0361	1.0279	1.0253	0.0123	1.20
40	2.0353	2.0470	2.0313	2.0378	0.0081	0.40
60	3.0187	3.0398	3.0425	3.0336	0.0130	0.43
80	4.0820	4.0793	4.0709	4.0774	0.0058	0.14
100	5.2119	5.1913	5.2225	5.2085	0.0158	0.30
R²	0.9994	0.9997	0.9995	0.9996	-	-

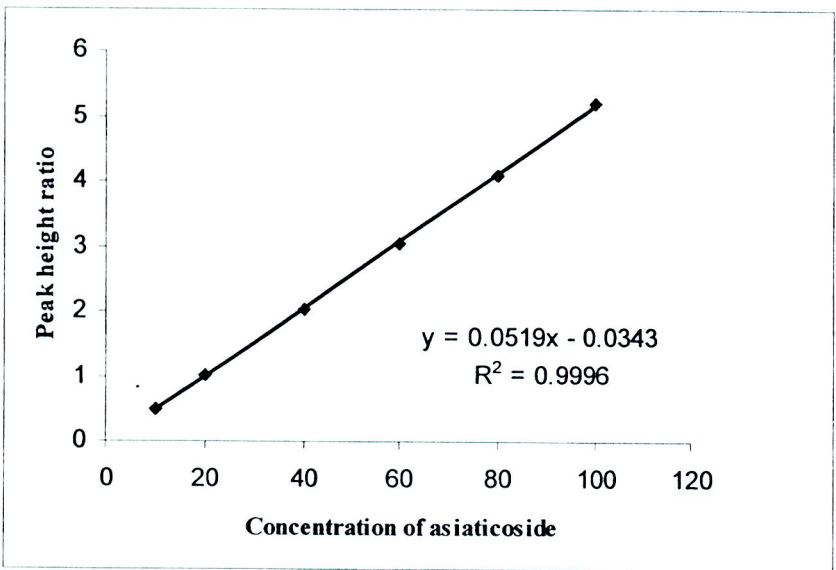


Fig 2A Calibration curve of a by HPLC method

1.1.3 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy is calculated as percent recovery by the assay of known added amount of analyses. The percentages of analytical recovery of asiaticoside solution and film formulation were shown in Table 7 and 8. The percentages analytical recovery of asiaticoside was in the range of 98.13-101.96% and 98.20-101.18%, respectively which indicated that this method could be used for analysis in all concentrations studied with a high accuracy.

Table 2A The percentages of analytical recovery of asiaticoside solution by HPLC method

Concentration (µg/ml)	%Analytical recovery					Mean ± SD
	1	2	3	4	5	
30	100.78	101.96	99.65	100.27	101.55	100.84±0.93
50	100.11	100.38	101.50	101.75	100.50	100.85±0.73
90	99.48	98.13	100.74	101.66	99.70	99.94±1.33

Table 3A The percentages of analytical recovery of asiaticoside film formulation by HPLC method

Concentration (µg/ml)	%Analytical recovery					Mean ± SD
	1	2	3	4	5	
30	99.31	99.29	99.48	100.16	99.01	99.45±0.43
50	99.37	100.57	98.38	99.93	98.20	99.29±1.01
90	100.17	100.99	100.85	101.18	100.81	100.80±0.38

1.1.4 Precision

The precision of asiaticoside analyzed by HPLC method were determined both within run precision and between run precision as illustrated in Tables 9 and 10. All coefficients of variation values were small, as 0.38-1.02% and 0.98-1.23%, respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the HPLC method was precise for quantitative analysis of asiaticoside in the range studied.

Table 4A Data of within run precision by HPLC method

Concentration (µg/ml)	Peak height ratio					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
30	29.79	29.79	29.84	30.05	29.70	29.83	0.13	0.43
50	49.69	50.29	49.19	49.97	49.10	49.65	0.50	1.02
90	90.15	90.89	90.76	91.06	90.73	90.72	0.34	0.38

Table 5A Data of between run precision by HPLC method

Concentration (µg/ml)	Peak height ratio					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
30	29.47	28.92	29.83	29.67	29.22	29.42	0.36	1.23
50	49.45	48.46	49.64	49.05	49.52	49.22	0.48	0.98
90	88.93	89.81	90.71	90.22	91.25	90.18	0.88	0.98

2. Analysis of protein by UV Spectrophotometric Method

2.1. Validation of UV Spectrophotometric Method

The validation of analytical method is the process for evaluation that the method is suitable and reliable for the intended analytical applications. The analytical parameters used for the UV spectrophotometric assay validation were linearity, accuracy and precision.

2.1.1 Linearity

The data for calibration curve of BSA are shown in Table 1B. Linear regression analysis of the absorbance versus the concentration curve was performed and the coefficient of determination (R^2) was calculated. The coefficients of determination were 0.9998 (Figure 1B).

Table 6A Data for calibration curve of BSA

Concentration (µg/ml)	Absorbance			Mean	SD	%CV
	Set1	Set2	Set3			
50	0.1705	0.1707	0.1674	0.1695	0.0019	1.09
100	0.3064	0.3062	0.2999	0.3042	0.0037	1.22
150	0.4316	0.4354	0.4368	0.4346	0.0027	0.62
200	0.5571	0.5564	0.5671	0.5602	0.0060	1.07
250	0.6973	0.7112	0.6921	0.7002	0.0099	1.41
300	0.8153	0.8363	0.8127	0.8214	0.0129	1.58
R^2	0.9996	0.9992	0.9995	0.9998	-	-

2.1.2 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy is calculated as percent recovery by the assay of known added amount of analyses. Table 7A displayed the percentage of analytical recovery of BSA. All percentages analytical recovery of BSA was in the range of 91.39-105.17% which indicated the high accuracy of this method. Thus, it could be used for analysis of BSA in all studied concentrations.

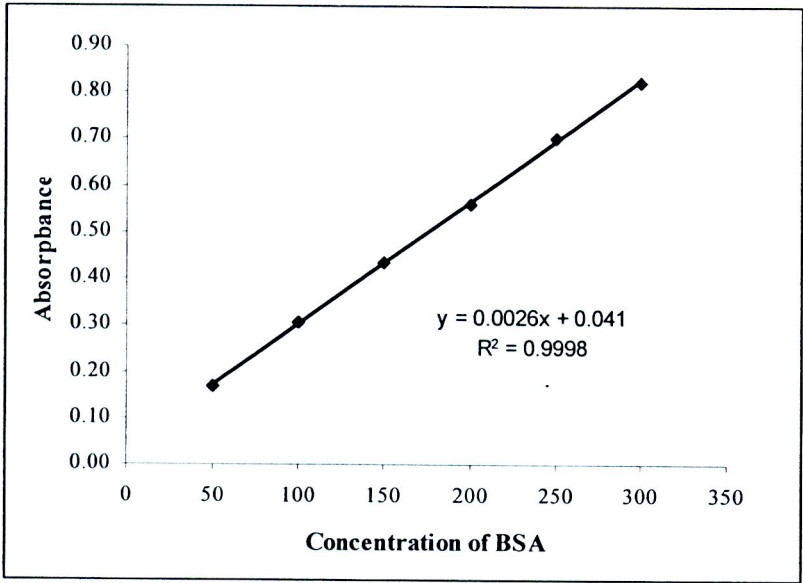


Fig 3A Calibration curve of BSA by UV spectrophotometric method

Table 7A The percentages of analytical recovery of BSA by UV spectrophotometric method

Concentration (µg/ml)	%Analytical recovery					Mean ± SD
	1	2	3	4	5	
80	94.62	94.38	91.39	98.17	98.80	95.47±3.04
160	104.21	105.17	102.52	100.63	104.98	103.50±1.92
240	102.00	99.29	103.09	101.36	101.30	101.41±1.38

2.1.3 Precision

The precision of BSA analyzed by UV spectrophotometric method were determined both within run and between run precisions as depicted in Table 3B and 4B, respectively. The coefficients of variation were in the range of 1.40-1.86% for within run precision and 1.93-3.81% for between run precision. Both within run and between run precisions of BSA provided the coefficient of variation less than 15%. Thus, the UV spectrophotometric method was accurate for the quantitative analysis of BSA in the range of studied.

Table 8A Data of within run precision by UV spectrophotometric method

Concentration (µg/ml)	Absorbance					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
80	79.28	79.12	82.00	81.84	82.00	80.85	1.51	1.86
160	165.76	171.76	169.76	166.88	168.36	168.50	2.36	1.40
240	237.32	236.28	237.76	236.28	244.40	238.40	3.41	1.43

Table 9A Data of between run precision by UV spectrophotometric method

Concentration (µg/ml)	Absorbance					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
80	80.85	88.33	81.84	80.75	82.27	82.81	3.15	3.81
160	168.50	168.23	161.71	170.14	167.43	167.20	3.22	1.93
240	238.41	238.63	247.58	254.22	243.70	244.51	6.64	2.71

APPENDIX B

Experimental data of xyloglucan tamarind seed extract and optimal spray dried condition

Table 1B The percentage yield of xyloglucan spray dried powder from four different methods

	%Xyloglucan			Mean	SD
	set 1	set 2	set 3		
method1	43.56	41.81	43.17	42.85	0.92
method2	44.12	43.87	43.17	43.72	0.49
method3	42.13	44.17	42.41	42.90	1.11
method4	43.57	45.26	45.03	44.62	0.92

Table 2B The percent of total protein of xyloglucan spray dried powder from four different methods and tamarind seed powder

	%Protein			Mean	SD
	set 1	set 2	set 3		
Tamarind seed powder	15.72	15.62	16.27	15.87	0.35
method1	14.21	14.39	14.44	14.35	0.12
method2	14.15	14.10	14.25	14.17	0.08
method3	14.27	14.75	14.89	14.64	0.33
method4	14.92	15.49	15.17	15.19	0.29

Table 3B The percent of fat of xyloglucan spray dried powder from four different methods and tamarind seed powder

	%Fat			Mean	SD
	set 1	set 2	set 3		
Tamarind seed powder	7.99	7.69	7.50	7.73	0.25
method1	0.99	1.29	0.99	1.09	0.17
method2	0.98	1.29	1.19	1.15	0.16
method3	0.70	1.29	0.69	0.89	0.34
method4	1.09	1.09	1.38	1.19	0.17

Table 4B ANOVA for the percentage xyloglucan of four different methods

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.253	3	2.084	2.848	0.120
Within Groups	6.297	8	0.787		
Total	12.550	11			

Table 5B ANOVA for the %total protein of four different methods and tamarind seed powder

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.771	4	1.443	21.839	0.000
Within Groups	0.661	10	0.066		
Total	6.432	14			

Table 6B ANOVA for the %fat of four different methods and tamarind seed powder

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106.156	4	26.539	506.212	0.000
Within Groups	0.524	10	0.052		
Total	106.680	14			

Table 7B Tukey HSD test of the percentage xyloglucan of four different methods

Method	Method	Sig.
I	II	0.640
	III	1.000
	IV	0.144
II	I	0.640
	III	0.684
	IV	0.620
III	I	1.000
	I	0.684
	IV	0.166
V	I	0.144
	II	0.620
	III	0.161

* The mean difference is significant at the 0.05 level.



Table 8B Tukey HSD test of the %total protein of four different methods and tamarind seed powder

Method	Method	Sig.
I	II	0.906
	III	0.651
	IV	0.016*
	Tamarind seed powder	0.000*
II	I	0.906
	III	0.241
	IV	0.004*
	Tamarind seed powder	0.000*
III	I	0.651
	II	0.241
	IV	0.133
	Tamarind seed powder	0.001*
IV	I	0.016*
	II	0.004*
	III	0.133
	V	0.055*
Tamarind seed powder	I	0.000*
	II	0.000*
	III	0.001*
	IV	0.055

* The mean difference is significant at the 0.05 level.

Table 9B Tukey HSD test of the %fat of four different methods and tamarind seed powder

Method	Method	Sig.
I	II	0.997
	III	0.826
	IV	0.984
	Tamarind seed powder	0.000*
II	I	0.997
	III	0.646
	IV	1.000
	Tamarind seed powder	0.000*
III	I	0.826
	II	0.646
	IV	0.546
	Tamarind seed powder	0.000*
IV	I	0.984
	II	1.000
	III	0.546
	V	0.000*
Tamarind seed powder	I	0.000*
	II	0.000*
	III	0.000*
	IV	0.000*

* The mean difference is significant at the 0.05 level

Table 10B A face centered design matrix of two parameters, the viscosity, outlet temperature, %yield and %moisture content (n=3)

Code	Temperature (°C)	Aspirator	Outlet Temperature (°C)	Viscosity (mPas)	%Moisture content			Mean	SD
					1	2	3		
C1	120 (-)	80 (-)	74±1	329.9	7.77	7.77	7.95	7.83	0.10
C2	200 (+)	80 (-)	117±2	329.3	5.98	5.99	6.19	6.05	0.12
C3	120 (-)	100 (+)	77±2	325.7	6.97	7.20	6.99	7.05	0.13
C4	200 (+)	100 (+)	99±2	323.3	4.80	4.99	5.17	4.99	0.19
C5	120 (-)	90 (0)	73±2	322.1	7.36	7.16	7.17	7.23	0.11
C6	200 (+)	90 (0)	120±2	304.1	5.98	5.79	5.80	5.86	0.11
C7	160 (0)	80 (-)	96±2	335.9	7.17	7.00	7.00	7.06	0.10
C8	160 (0)	100 (+)	82±3	316.7	6.40	6.39	6.20	6.33	0.11
C9	160 (0)	90 (0)	100±3	304.1	7.16	6.97	6.97	7.03	0.11
C10	160 (0)	90 (0)	100±3	323.9	6.56	6.56	6.39	6.50	0.10
C11	160 (0)	90 (0)	101±1	309.5	6.59	6.59	6.79	6.66	0.12
C12	160 (0)	90 (0)	101±1	298.1	6.37	6.19	6.39	6.32	0.11
C13	160 (0)	90 (0)	101±2	296.9	6.60	6.57	6.60	6.59	0.02

Table 11B The optimum region by overlay plot of two parameters, the viscosity, outlet temperature and %moisture content (n=3)

Code	Temperature (°C)	Aspirator	Outlet Temperature (°C)	Viscosity (mPas)	%Moisture content			Mean	SD
					1	2	3		
O1	178	100	114±2	382.1	6.18	6.39	6.19	6.25	0.12
O2	178	100	113±2	411.5	6.37	6.39	6.20	6.32	0.10
O3	178	100	112±2	5.59	5.77	5.59	5.65	0.10	5.59

APPENDIX C

Experimental data of size and size distribution of
spray drying condition

Figure 1C Size and size distribution of C1

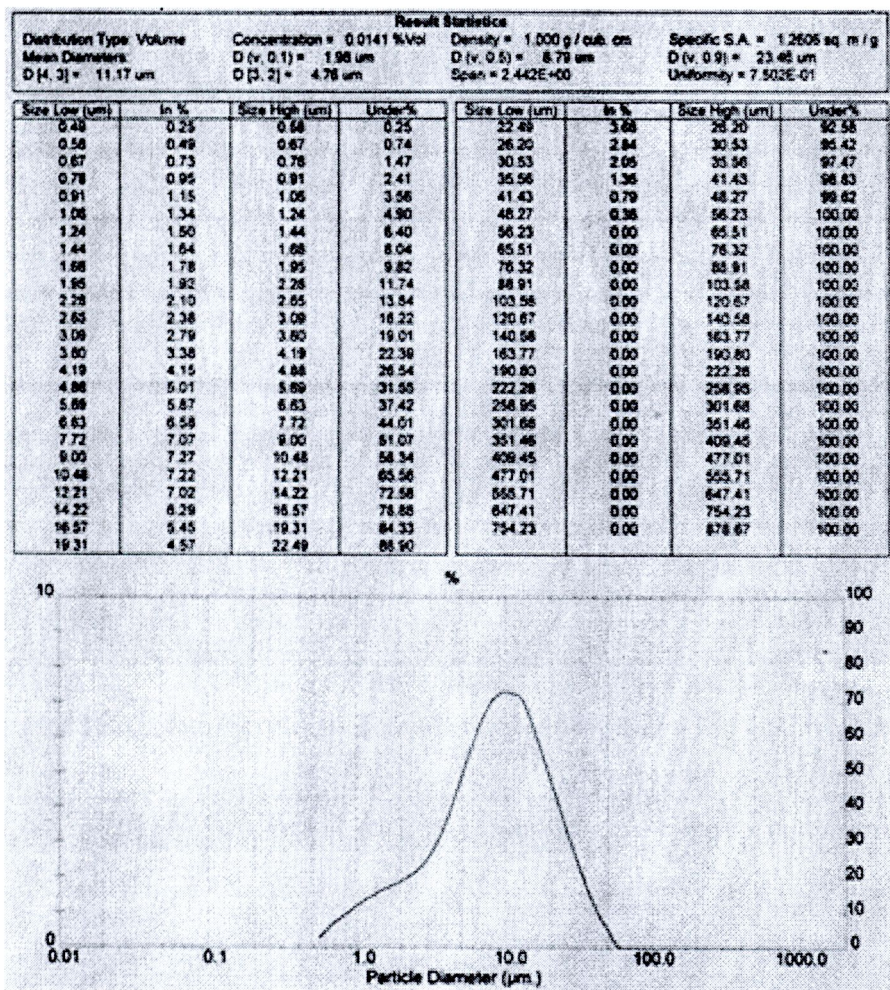


Figure 2C Size and size distribution of C2

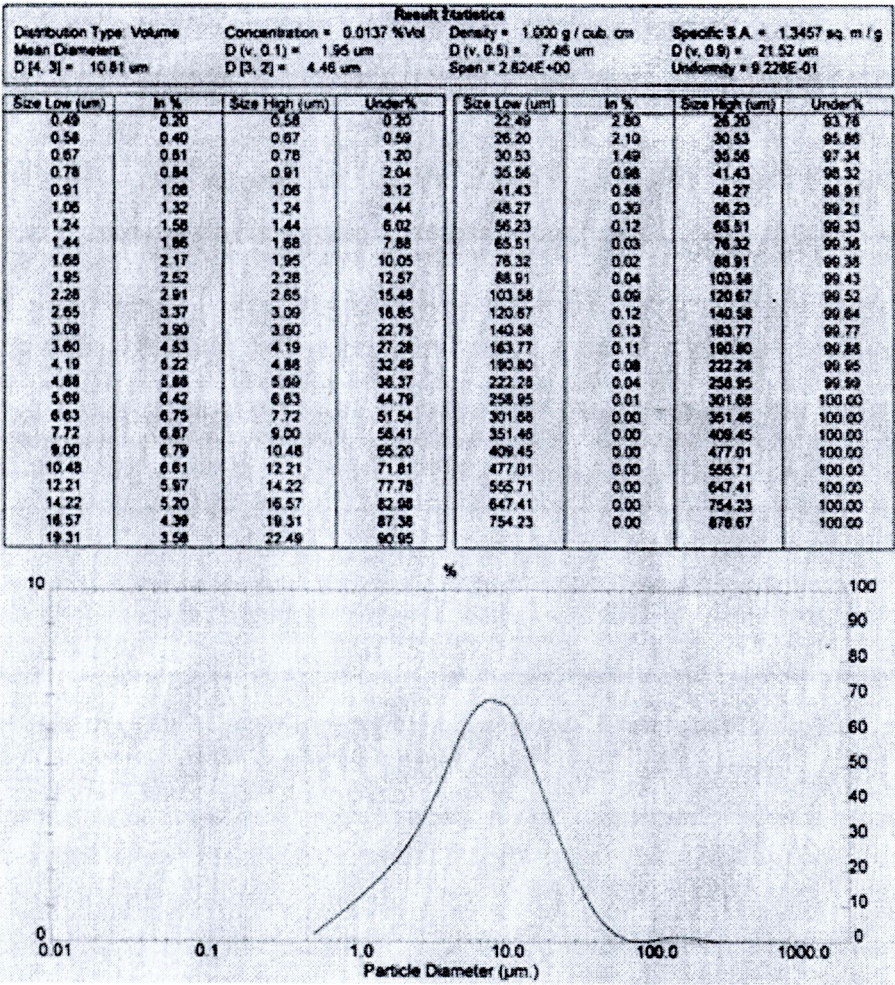


Figure 3C Size and size distribution of C3

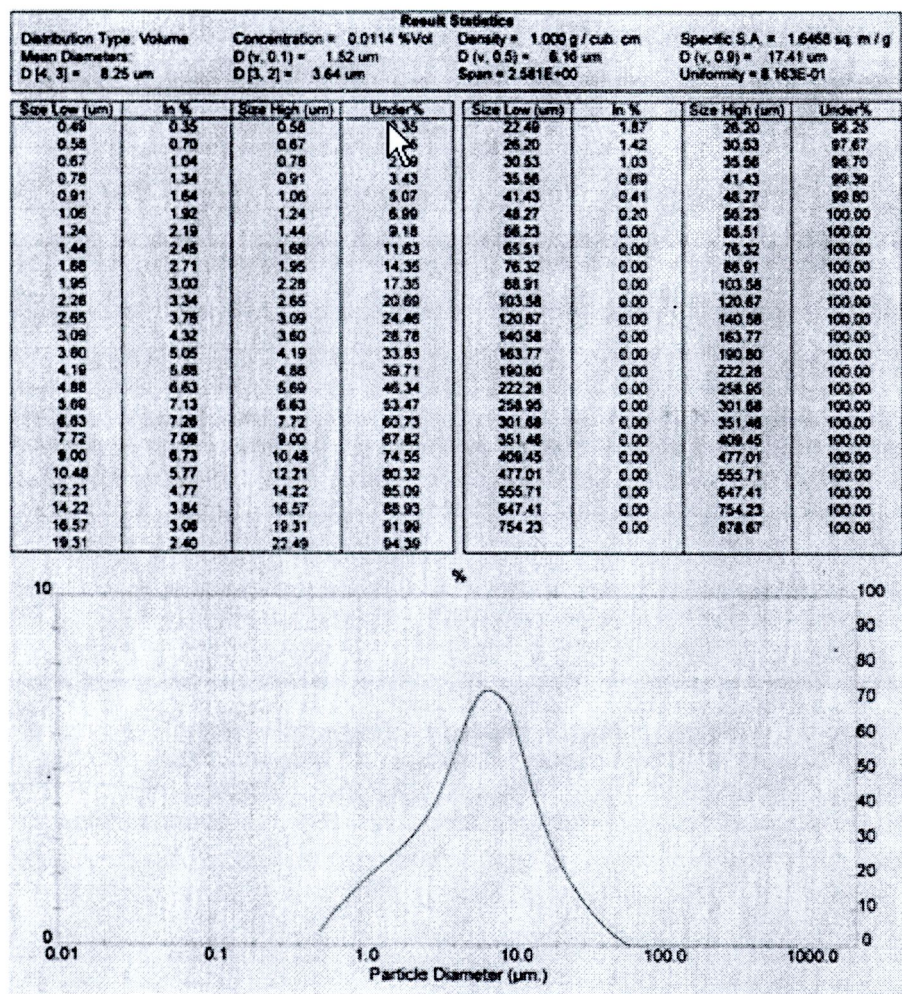


Figure 4C Size and size distribution of C4

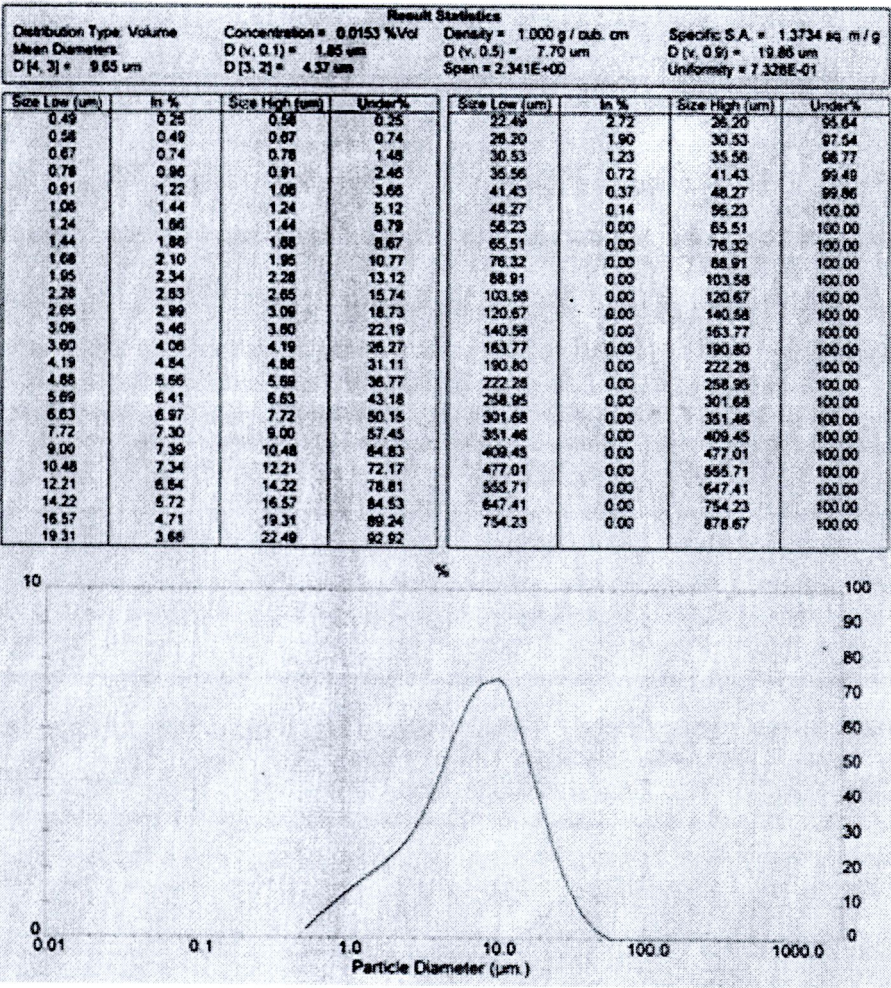


Figure 5C Size and size distribution of C5

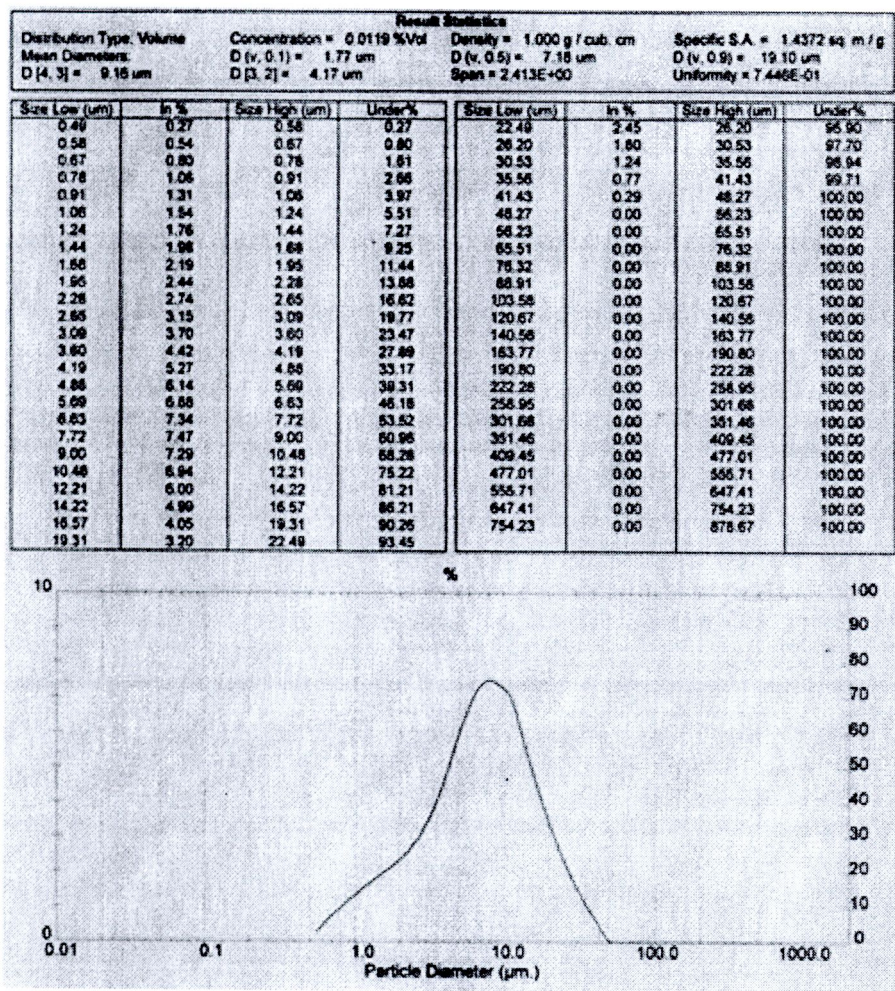


Figure 6C Size and size distribution of C6

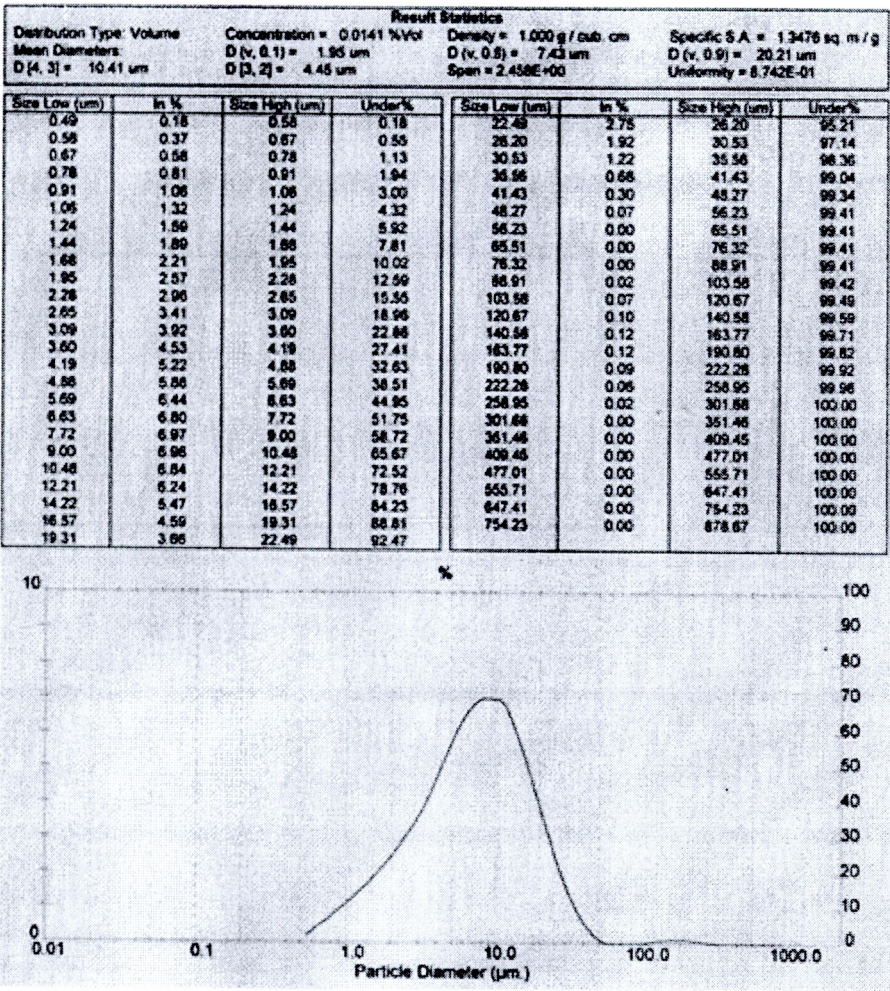


Figure 7C Size and size distribution of C7

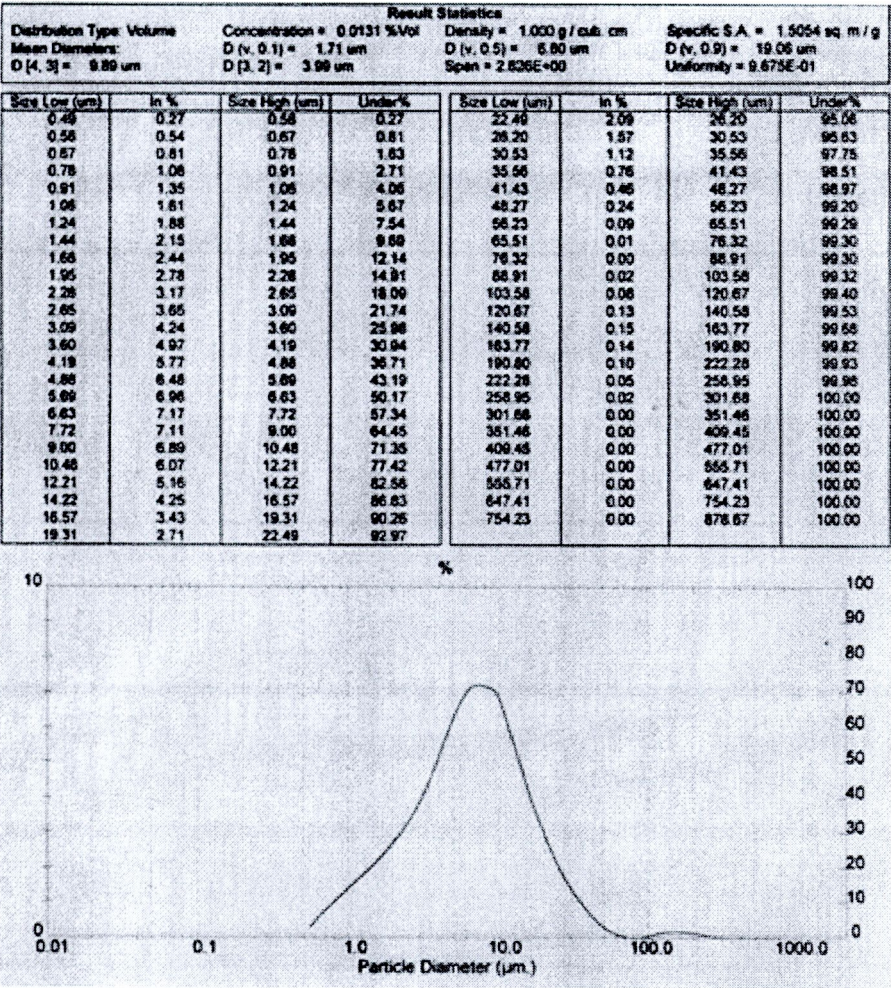


Figure 8C Size and size distribution of C8

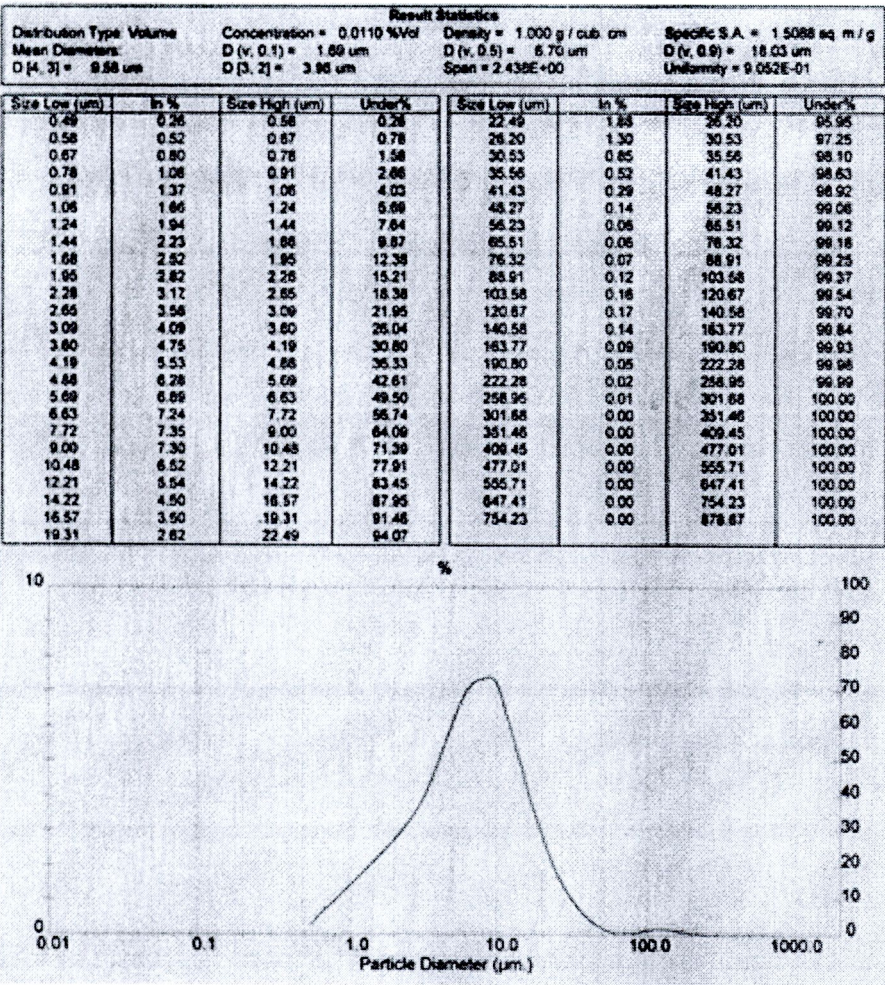


Figure 9C Size and size distribution of C9

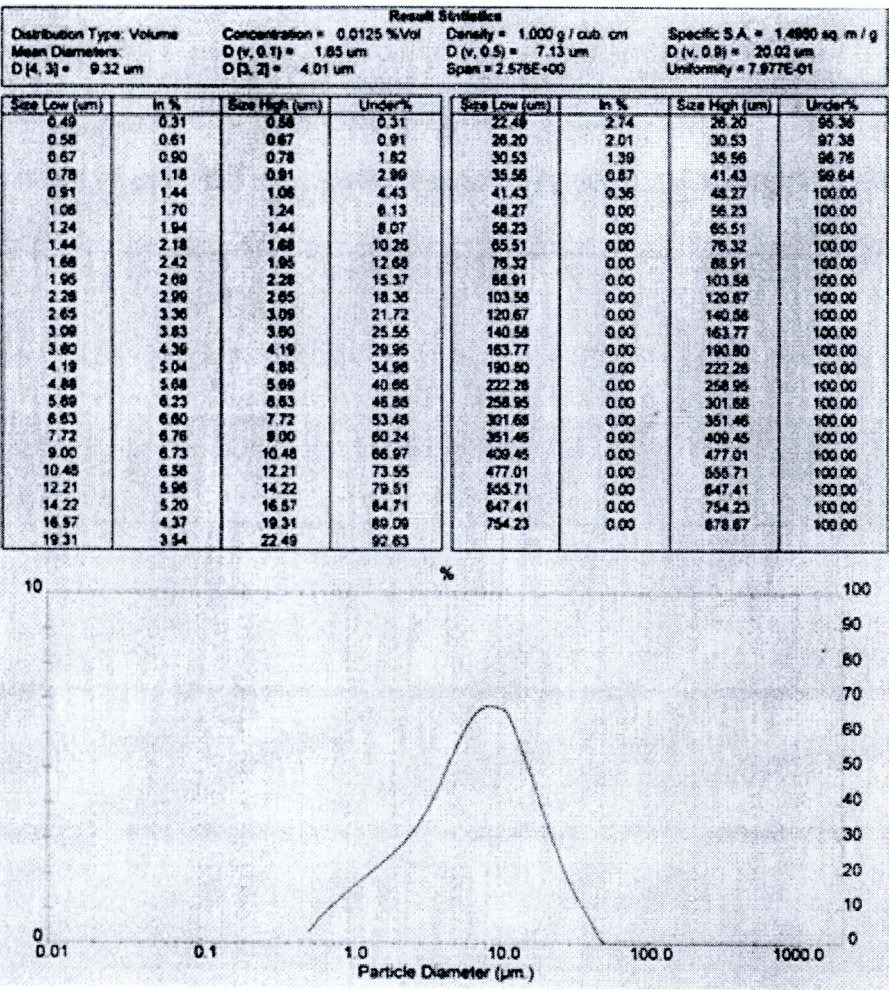


Figure 10C Size and size distribution of optimization spray dried condition

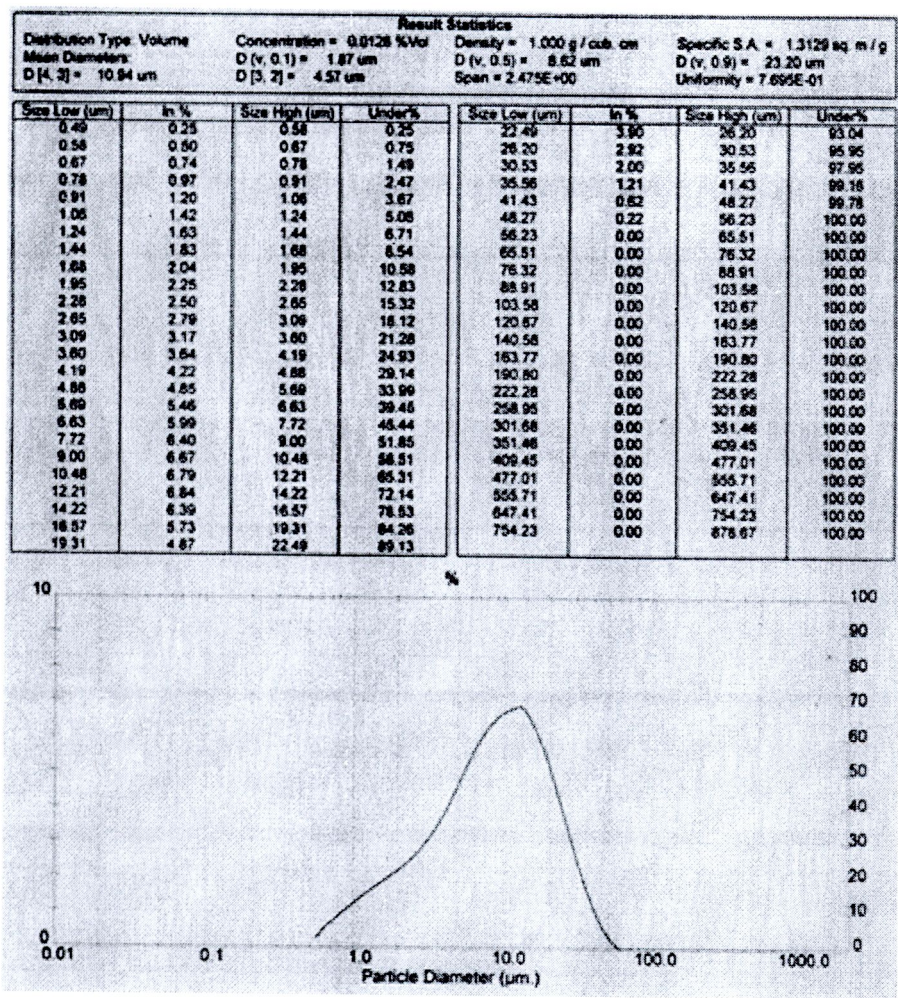
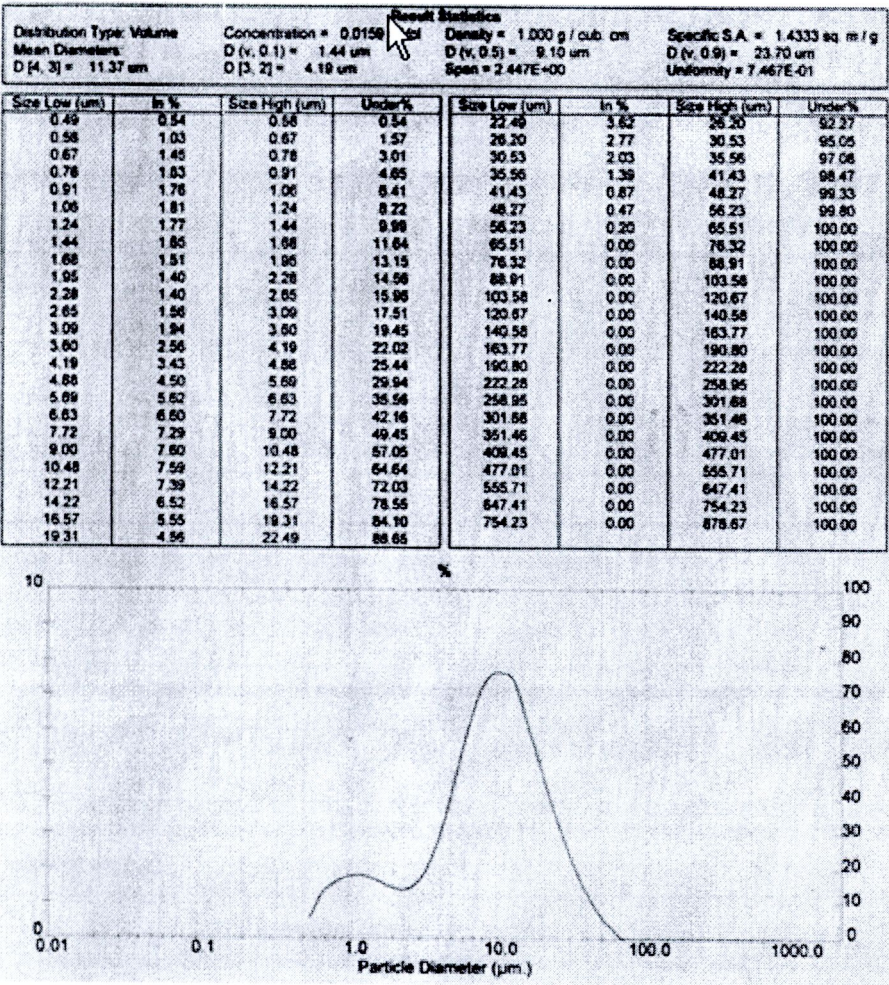


Figure 11C Size and size distribution of xyloglucan spray dried powder



APPENDIX D

Experimental data of physicochemical properties of xyloglucan powder from tamarind seeds

Table 1D The percentage of xyloglucan in xyloglucan spray dried powder

Run	%Xyloglucan			mean	SD
	set1	set2	set3		
1	42.21	42.99	43.35	42.85	0.58
2	41.96	42.00	41.71	41.89	0.16
3	41.98	42.18	42.32	42.16	0.17

Table 2D The pH value of xyloglucan spray dried powder

	set 1	set 2	set 3	AVG	SD
pH	7.61	7.93	7.94	7.83	0.1877

Table 3D The viscosity value of 1%, 1.5% and 2%w/v of xyloglucan spray dried powder

conc.	Viscosity (mPas)			Mean	SD
	set 1	set 2	set 3		
1%	40.135	39.742	38.513	39.463	0.8461
1.5%	94.264	92.630	94.150	93.681	0.9123
2%	166.729	170.146	167.292	168.056	1.8320

Table 4D The percentage of remained xyloglucan after added ethanol

%Ethanol	%Remained xyloglucan			Mean	SD
	set 1	set 2	set 3		
1	97.82	97.45	100.26	98.51	1.53
2	95.85	94.49	95.07	95.14	0.68
3	91.51	91.56	94.06	92.38	1.46
4	85.14	85.04	88.31	86.16	1.86
5	83.14	80.72	83.74	82.53	1.60

Figure 1D Rheogram of 1%w/v xyloglucan spray dried powder

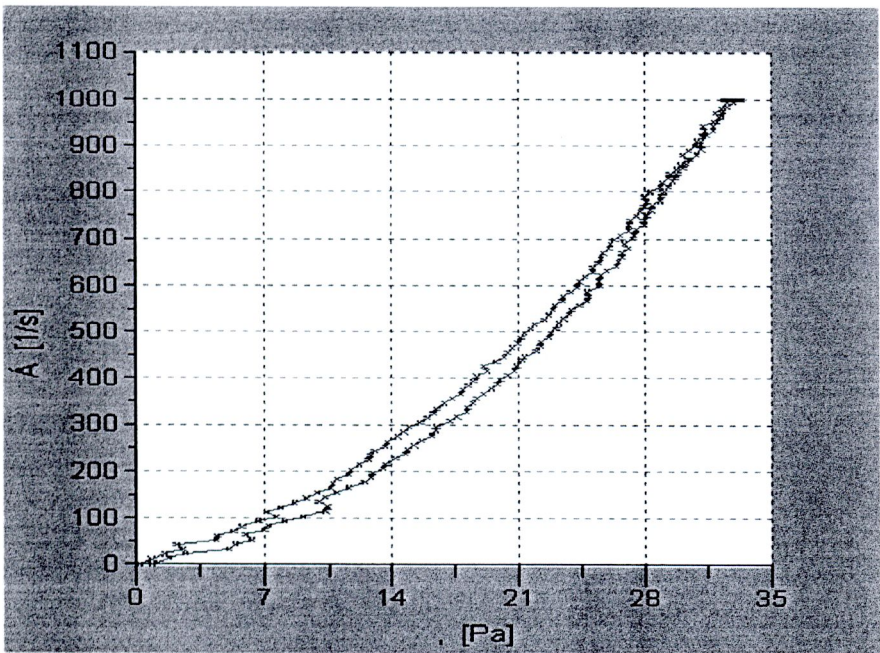


Figure 2D Rheogram of 1.5%w/v xyloglucan spray dried powder

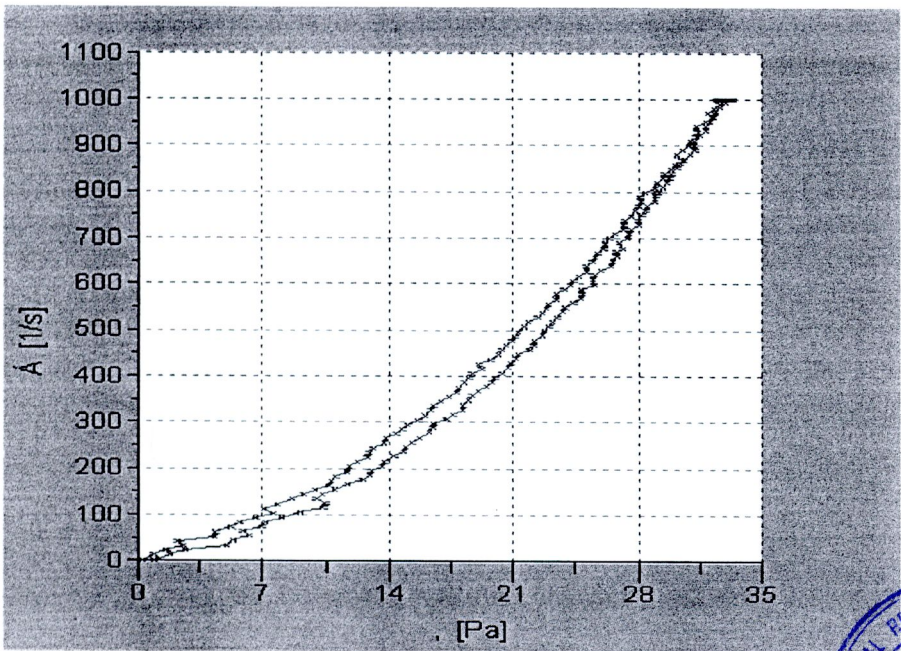
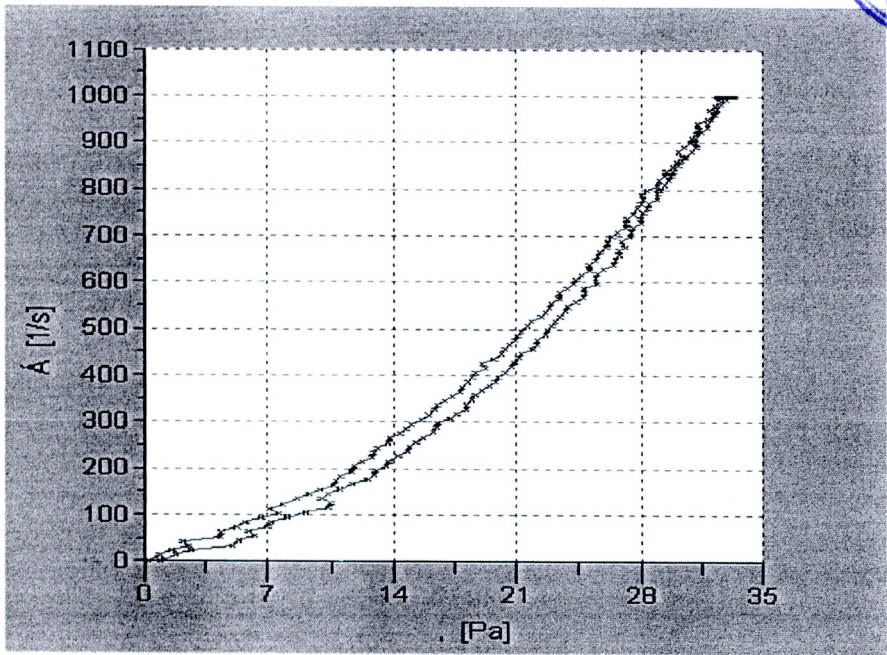


Figure 3D Rheogram of 2%w/v xyloglucan spray dried powder



APPENDIX E

Experimental data of mechanical properties of film formulations and film containing *Centella Asiatica* extract

Table 1E The film weights of film formulations and film containing *Centella Asiatica* extract

Formulation	Film weight (mg)					mean	SD
	1	2	3	4	5		
F1	11.92	11.60	11.88	11.03	11.27	11.54	0.39
F2	10.85	11.28	11.47	11.58	10.97	11.23	0.31
F3	15.50	14.89	15.13	14.76	14.68	14.99	0.33
F4	4.68	5.04	4.97	4.89	4.45	4.81	0.24
F5	9.39	9.55	9.60	9.88	10.20	9.72	0.32
F6	8.79	8.74	8.49	8.91	8.71	8.73	0.15
F7	7.60	8.15	7.60	7.51	7.75	7.72	0.25
F8	13.02	12.94	12.85	13.30	13.26	13.07	0.20
F9	7.14	6.65	7.02	6.65	7.05	6.90	0.23
F10	10.15	10.50	10.18	10.33	11.02	10.44	0.35
F11	9.50	9.76	10.13	9.34	9.16	9.58	0.38
F12	8.41	9.50	9.76	8.88	8.79	9.07	0.55
F13	9.21	9.78	9.67	9.26	8.89	9.36	0.36
F14	8.05	8.13	7.72	7.75	7.70	7.87	0.20
F15	8.35	8.33	7.93	8.64	7.80	8.21	0.34
Film with extract 1	11.63	12.55	13.21	11.83	13.15	12.474	0.73
Film with extract 2	11.67	11.63	12.02	11.98	11.56	11.772	0.21
Film with extract 3	12.72	13.42	12.08	12.12	13.50	12.768	0.68

Table 2E The max force, force of adhesive, work of adhesive and thick value of F1

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0357	2.856	0.0286	0.05
2	0.0230	1.840	0.0136	0.05
3	0.0227	1.816	0.0149	0.05
4	0.0577	4.616	0.0346	0.05
5	0.0713	5.704	0.0319	0.05
Mean	0.0421	3.366	0.0247	0.05
SD	0.02	1.73	0.01	0

Table 3E The max force, force of adhesive, work of adhesive and thick value of F2

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0383	2.553	0.0243	0.06
2	0.0553	3.687	0.0326	0.06
3	0.0370	2.467	0.0156	0.06
4	0.0403	2.687	0.0216	0.06
5	0.0253	1.687	0.0159	0.06
Mean	0.0392	2.616	0.022	0.06
SD	0.01	0.71	0.01	0

Table 4E The max force, force of adhesive, work of adhesive and thick value of F3

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.2777	37.02	0.027	0.03
2	0.2543	33.91	0.0502	0.03
3	0.2593	34.58	0.019	0.03
4	0.1534	20.45	0.0767	0.03
5	0.1643	21.91	0.0436	0.03
Mean	0.2218	29.58	0.0433	0.03
SD	0.06	7.77	0.02	0

Table 5E The max force, force of adhesive, work of adhesive and thick value of F4

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0747	9.96	0.0495	0.03
2	0.0470	6.27	0.0220	0.03
3	0.0490	6.53	0.0240	0.03
4	0.0267	3.56	0.0193	0.03
5	0.0287	3.83	0.0158	0.03
Mean	0.0452	6.029	0.0261	0.03
SD	0.02	2.58	0.01	0.00

Table 6E The max force, force of adhesive, work of adhesive and thick value of F5

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0357	4.76	0.0140	0.03
2	0.0563	7.51	0.0208	0.03
3	0.0220	2.93	0.0133	0.03
4	0.0400	5.33	0.0276	0.03
5	0.1143	15.24	0.0300	0.03
Mean	0.0537	7.156	0.0211	0.03
SD	0.04	4.81	0.01	0.00

Table 7E The max force, force of adhesive, work of adhesive and thick value of F6

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.5403	36.02	0.0906	0.06
2	0.2083	13.89	0.0598	0.06
3	0.0700	4.67	0.0316	0.06
4	0.1787	11.91	0.0405	0.06
5	0.5893	39.29	0.0746	0.06
Mean	0.3173	21.16	0.0594	0.06
SD	0.23	15.49	0.02	0.00

Table 8E The max force, force of adhesive, work of adhesive and thick value of F7

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0373	4.973	0.0257	0.03
2	0.0233	3.107	0.0145	0.03
3	0.0243	3.240	0.0141	0.03
4	0.0310	4.133	0.0202	0.03
5	0.0230	3.067	0.0150	0.03
Mean	0.0278	3.704	0.0179	0.03
SD	0.01	0.83	0.01	0.00

Table 9E The max force, force of adhesive, work of adhesive and thick value of F8

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0223	1.274	0.0142	0.07
2	0.0137	0.781	0.0078	0.07
3	0.0290	1.657	0.0147	0.07
4	0.0217	1.238	0.0097	0.07
5	0.1157	6.610	0.0480	0.07
Mean	0.0405	2.312	0.0189	0.07
SD	0.04	2.42	0.02	0.00

Table 10E The max force, force of adhesive, work of adhesive and thick value of F9

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.1393	0.929	0.0295	0.07
2	0.1833	1.222	0.0431	0.07
3	0.2353	1.569	0.0582	0.07
4	0.4250	2.833	0.0864	0.07
5	0.1917	1.278	0.0387	0.07
Mean	0.2349	1.5662	0.0512	0.07
SD	0.11	0.74	0.02	0

Table 11E The max force, force of adhesive, work of adhesive and thick value of F10

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.1223	8.16	0.0214	0.06
2	0.0597	3.98	0.0298	0.06
3	0.2870	19.13	0.0468	0.06
4	0.0993	6.62	0.0270	0.06
5	0.4427	29.51	0.0667	0.06
Mean	0.2022	13.48	0.0383	0.06
SD	0.16	10.66	0.02	0

Table 12E The max force, force of adhesive, work of adhesive and thick value of F11

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0373	2.984	0.0243	0.05
2	0.0343	2.744	0.0179	0.05
3	0.0297	2.376	0.0162	0.05
4	0.0157	1.253	0.0092	0.05
5	0.0213	1.704	0.0143	0.05
Mean	0.0277	2.212	0.0164	0.05
SD	0.01	0.72	0.01	0

Table 13E The max force, force of adhesive, work of adhesive and thick value of F12

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0460	4.600	0.0250	0.04
2	0.0413	4.133	0.0233	0.04
3	0.0410	4.100	0.0181	0.04
4	0.0333	3.333	0.0145	0.04
5	0.0213	2.130	0.0126	0.04
Mean	0.0366	3.659	0.0187	0.04
SD	0.01	0.97	0.01	0

Table 14E The max force, force of adhesive, work of adhesive and thick value of F13

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0210	1.680	0.0133	0.05
2	0.0280	2.240	0.0153	0.05
3	0.0617	4.936	0.0285	0.05
4	0.0413	3.304	0.0219	0.05
5	0.0373	2.984	0.0224	0.05
Mean	0.0379	3.029	0.0203	0.05
SD	0.02	1.24	0.01	0

Table 15E The max force, force of adhesive, work of adhesive and thick value of F14

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0233	1.864	0.0193	0.05
2	0.0220	1.760	0.0195	0.05
3	0.0237	1.896	0.0171	0.05
4	0.0180	1.440	0.0165	0.05
5	0.0213	1.704	0.0172	0.05
Mean	0.0217	1.7328	0.0179	0.05
SD	0.002	0.18	0.001	0

Table 16E The max force, force of adhesive, work of adhesive and thick value of F15

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0317	3.170	0.0159	0.04
2	0.0410	4.100	0.0188	0.04
3	0.0557	5.567	0.0210	0.04
4	0.0263	2.630	0.0142	0.04
5	0.0150	1.500	0.0039	0.04
Mean	0.0339	3.393	0.0147	0.04
SD	0.02	1.54	0.01	0

Table 17E The max force, force of adhesive, work of adhesive and thick value of
Film with extract 1

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.04530	4.530	0.0181	0.04
2	0.03900	3.900	0.0219	0.04
3	0.06270	6.270	0.0279	0.04
4	0.05430	5.430	0.0324	0.04
5	0.02270	2.267	0.0109	0.04
Mean	0.0448	4.479	0.0223	0.04
SD	0.02	1.53	0.01	0

Table 17E The max force, force of adhesive, work of adhesive and thick value of
Film with extract 2

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.06270	5.0160	0.0320	0.05
2	0.03870	3.0960	0.0223	0.05
3	0.07800	6.2400	0.0387	0.05
4	0.02370	1.8930	0.0129	0.05
5	0.05930	4.7440	0.0240	0.05
Mean	0.0525	4.198	0.026	0.05
SD	0.02	1.71	0.01	0

Table 17E The max force, force of adhesive, work of adhesive and thick value of
Film with extract 3

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.03230	2.5840	0.0218	0.05
2	0.01330	1.0640	0.0107	0.05
3	0.03570	2.8560	0.0236	0.05
4	0.03070	2.4530	0.0149	0.05
5	0.04000	3.2000	0.0257	0.05
Mean	0.0304	2.431	0.0193	0.05
SD	0.01	0.82	0.01	0

APPENDIX F

Experimental data of adhesive properties of film
formulations and film containing *Centella Asiatica* extract

Table 1F Mechanical properties data of F1

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	15.03	178.20	10.31	7.80
2	18.00	198.80	13.65	8.05
3	12.32	161.00	7.48	7.19
4	12.15	176.20	8.24	6.52
5	19.73	170.00	13.12	10.87
Mean	15.447	176.84	10.559	8.086
SD	3.38	13.98	2.78	1.6664

Table 2F Mechanical properties data of F2

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	3.378	368	10.54	2.403
2	4.978	356	13.19	3.271
3	5.306	336	13.21	3.315
4	5.382	448	19.45	3.062
5	4.639	368	12.99	2.730
Mean	4.736	375.2	13.876	2.956
SD	0.81	42.75	3.32	0.39

Table 3F Mechanical properties data of F3

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	2.167	416.0	5.83	3.267
2	3.683	524.0	8.03	3.097
3	5.400	542.4	10.06	4.208
4	3.633	500.0	11.13	6.011
5	5.211	474.0	13.00	7.911
Mean	4.019	491.3	9.611	4.899
SD	1.32	49.31	2.78	2.04

Table 4F Mechanical properties data of F4

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	14.80	138.20	5.68	17.53
2	15.33	149.10	6.20	13.68
3	14.17	120.20	5.04	24.70
4	13.95	116.00	4.43	17.20
5	14.23	117.20	4.47	16.47
Mean	14.497	128.1	5.163	17.917
SD	0.56	14.78	0.77	4.09

Table 5F Mechanical properties data of F5

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	5.242	352	7.83	5.32
2	3.928	348	5.93	7.60
3	4.789	382	7.13	4.99
4	4.692	368	7.32	5.04
5	4.983	354	8.01	6.44
Mean	4.727	360.8	7.245	5.88
SD	0.49	14.04	0.82	1.13

Table 6F Mechanical properties data of F6

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	13.01	420.0	30.49	4.731
2	12.39	450.0	30.99	4.220
3	12.58	300.0	19.59	4.765
4	10.93	285.9	15.89	4.059
5	11.21	332.0	21.39	4.530
Mean	12.026	357.6	23.67	4.461
SD	0.91	73.39	6.76	0.31

Table 7F Mechanical properties data of F7

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	3.483	528	16.75	3.342
2	3.694	548	20.37	3.362
3	3.847	466	17.60	3.334
4	3.372	576	18.29	3.144
5	4.800	618	28.73	4.025
Mean	3.839	547.1	20.35	3.441
SD	0.57	56.45	4.87	0.33

Table 8F Mechanical properties data of F8

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	9.56	184.2	8.59	4.421
2	4.66	150.7	5.17	3.952
3	8.16	157.0	5.92	3.883
4	5.98	145.1	4.12	3.791
5	6.11	187.2	5.89	3.097
Mean	6.893	164.83	5.941	3.829
SD	1.95	19.54	1.65	0.48

Table 9F Mechanical properties data of F9

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	11.7	96.8	6.62	17.59
2	10.62	95.6	6.46	17.20
3	12.23	82.4	6.33	23.66
4	7.73	66.8	3.27	34.05
5	11.19	98.0	6.96	19.12
Mean	10.695	87.92	5.926	22.33
SD	1.76	13.38	1.50	7.04

Table 10F Mechanical properties data of F10

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	2.600	510.4	11.76	2.554
2	3.040	580.0	16.00	2.405
3	2.988	508.0	13.13	2.635
4	2.550	404.0	8.29	2.139
5	2.683	468.0	10.57	2.688
Mean	2.772	494.1	11.951	2.484
SD	0.23	64.49	2.88	0.22

Table 11F Mechanical properties data of F11

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	8.57	646.4	35.73	5.860
2	3.48	540.0	13.89	2.516
3	7.13	532.0	30.93	5.931
4	7.43	600.0	33.77	6.790
5	10.32	488.0	29.29	4.725
Mean	7.386	561.3	28.72	5.164
SD	2.52	62.10	8.66	1.65

Table 12F Mechanical properties data of F12

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	4.542	488	16.34	4.439
2	3.313	526	15.47	4.183
3	4.342	522	15.72	3.791
4	3.225	522	13.59	2.570
5	2.642	566	14.30	4.007
Mean	3.613	525	15.083	3.798
SD	0.80	27.82	1.12	0.73

Table 13F Mechanical properties data of F13

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	4.46	618	22.90	4.99
2	6.99	576	21.57	4.79
3	4.89	594	22.78	4.82
4	8.33	484	25.64	6.44
5	9.60	572	33.02	6.08
Mean	6.853	568.6	25.18	5.424
SD	2.20	50.62	4.63	0.78

Table 14F Mechanical properties data of F14

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	5.31	498	17.93	3.393
2	6.32	426	16.43	4.390
3	6.08	568	24.32	4.236
4	4.96	580	22.39	3.701
5	6.28	496	22.21	4.220
Mean	5.789	513.5	20.66	3.988
SD	0.62	62.47	3.32	0.42

Table 15F Mechanical properties data of F15

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	6.13	510	22.38	5.21
2	3.52	520	13.14	4.34
3	8.21	512	20.54	5.57
4	10.43	500	41.75	12.84
5	6.94	474	19.79	6.22
Mean	7.044	503.3	23.52	6.835
SD	2.56	17.85	10.77	3.43

Table 16F Mechanical properties data of Film with extract n 1

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	11.89	249.9	12.75	5.575
2	13.11	255.9	10.95	4.486
3	10.39	210.0	6.87	4.086
4	10.89	198.0	7.21	4.285
5	15.95	252.0	12.70	4.176
Mean	12.446	233.2	10.095	4.522
SD	2.22	27.04	2.89	0.61

Table 17F Mechanical properties data of Film with extract 2

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	17.81	202.0	11.90	7.89
2	16.75	232.0	13.47	6.67
3	14.00	222.0	10.82	5.76
4	16.05	183.2	9.44	6.68
5	18.72	236.0	15.08	7.14
Mean	16.666	215	12.143	6.829
SD	1.80	22.13	2.21	0.78

Table 18F Mechanical properties data of Film with extract 3

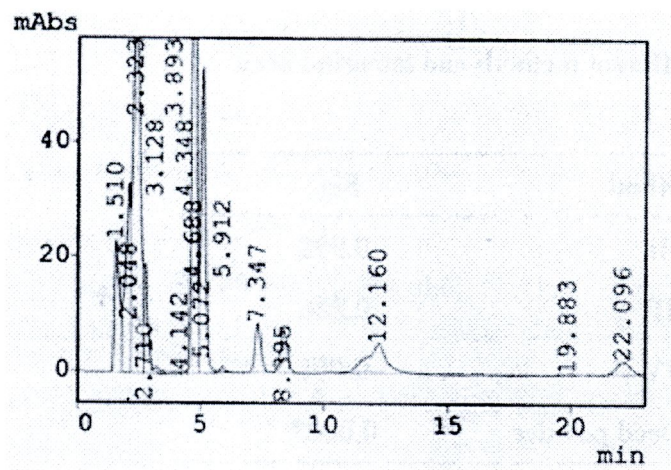
Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	15.43	252.0	12.97	5.40
2	15.90	240.0	13.03	5.29
3	17.23	254.1	15.14	6.62
4	15.70	261.9	17.70	6.93
5	16.85	242.0	13.55	6.08
Mean	16.223	250	14.475	6.064
SD	0.78	9.03	2.00	0.73

APPENDIX G

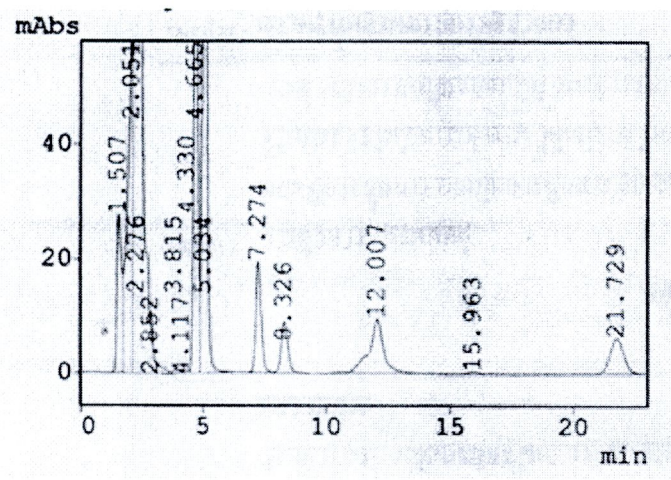
Experimental data of release and permeation study

Table 1G The release data of asiaticoside from film formulations

Time (hr)	%Cumulative release of asiaticoside			Mean	SD
	set 1	set 2	set 3		
0	0	0	0	0	0
0.25	5.97	4.34	6.77	5.69	1.24
0.5	7.10	8.04	9.24	8.13	1.07
0.75	8.80	8.65	12.41	9.95	2.13
1	10.81	12.34	16.74	13.30	3.08
1.5	14.20	14.97	20.23	16.47	3.28
2	19.10	23.89	25.74	22.91	3.43
3	29.14	31.42	33.27	31.28	2.07
4	35.74	36.49	38.22	36.82	1.27
5	34.12	35.96	42.80	37.63	4.57
6	39.96	40.19	44.77	41.64	2.72
8	42.83	45.05	49.17	45.68	3.22
10	48.33	50.91	56.60	51.95	4.23
12	52.53	59.05	61.20	57.60	4.51
15	55.97	61.12	60.87	59.32	2.90
18	60.33	65.18	64.50	63.34	2.63
21	63.13	65.69	67.29	65.37	2.10
24	70.61	62.67	69.40	67.56	4.28
28	70.57	62.42	69.62	67.54	4.45
32	72.61	61.94	73.57	69.37	6.45
36	77.37	71.61	74.86	74.61	2.89
40	79.05	72.23	80.84	77.37	4.54
44	79.72	74.46	82.85	79.01	4.24
48	80.98	76.65	84.62	80.75	3.99



(a)



(b)

Figure 1G HPLC chromatograms of asiatic acid from permeation study at different time.

(a) Peak of asiatic acid at 2 hours (retention time at 12.160 minutes)

(b) Peak of asiatic acid at 24 hours (retention time at 12.007 minutes)

APPENDIX H **Experimental data of stability study**

Table 1H The percentages labeled amount of asiaticoside in film formulations containing *Centella asiatica* extract in stability test

Periods	% Asiaticoside			Mean	SD
	set 1	set 2	set 3		
Initial	89.4506	99.392	92.7002	93.85	5.07
1 st month	84.0113	92.1003	84.6858	86.93	4.49
2 nd month	80.5896	86.0523	76.629	81.09	4.73
3 rd month	76.2121	83.6113	75.5291	78.45	4.48

Table 2H Adhesive properties data of film formulations in initial period

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0448	4.479	0.0223	0.04
2	0.0525	4.198	0.0260	0.05
3	0.0304	2.431	0.0193	0.05
Mean	0.0426	3.703	0.0225	0.047
SD	0.01	1.11	0.003	0.01

Table 33H Adhesive properties data of film formulations in 1st month period

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0274	2.191	0.0208	0.05
2	0.0415	3.322	0.0229	0.05
3	0.0409	3.269	0.0216	0.05
Mean	0.0366	2.927	0.0218	0.05
SD	0.01	0.64	0.001	0

Table 4H Adhesive properties data of film formulations in 2nd month period

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0529	5.287	0.0174	0.04
2	0.0357	2.853	0.0217	0.05
3	0.0365	2.917	0.0212	0.05
Mean	0.0353	2.825	0.0215	0.05
SD	0.001	0.11	0.0003	0

Table 5H Adhesive properties data of film formulation in 3rd month period

Run	Max Force (N)	Force of adhesion (N/cm ²)	Work of Adhesion (mJ)	Thick (mm)
1	0.0285	2.854	0.0176	0.04
2	0.0300	2.402	0.0216	0.05
3	0.0289	2.314	0.0211	0.05
Mean	0.0291	2.523	0.0201	0.0467
SD	0.001	0.29	0.002	0.006

Table 6H ANOVA for the adhesive forces data of film formulation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	3	0.000	0.793	0.531
Within Groups	0.000	8	0.000		
Total	0.000	11			

Table 7H Tukey HSD test of the adhesive forces data of film formulation

Periods	Method	Sig.
Initial	1 st month	0.028*
	2 nd month	0.018*
	3 rd month	0.006*
1 st month	Initial	0.028*
	2 nd month	0.989
	3 rd month	0.632
2 nd month	Initial	0.018*
	1 st month	0.989
	3 rd month	0.800
3 rd month	Initial	0.006*
	1 st month	0.632
	2 nd month	0.800

* The mean difference is significant at the 0.05 level

Table 8H Mechanical properties data of film formulation in initial period

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	12.446	233.2	10.095	4.522
2	16.666	215	12.143	6.829
3	16.223	250	14.475	6.064
Mean	15.11	232.73	12.24	5.81
SD	2.32	17.50	2.19	1.18

Table 9H Mechanical properties data of film formulation in 1st month period

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	7.362	334.4	10.678	4.027
2	5.934	386.4	12.947	3.155
3	6.829	372	13.834	3.426
Mean	6.71	364.27	12.49	3.54
SD	0.72	26.85	1.63	0.45

Table 10H Mechanical properties data of film formulation in 2nd month period

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	6.797	539.7	24.97	4.361
2	2.922	500.9	10.676	2.226
3	7.896	481.8	25.16	4.682
Mean	5.87	507.47	20.27	3.76
SD	2.61	29.50	8.31	1.33

Table 11H Mechanical properties data of film formulation in 3rd month period

Run	Tensile Strength (MPa)	%Elongation	Work of failure (mJ)	E-Mod (MPa)
1	3.836	490.7	12.621	4.101
2	3.621	451.3	11.631	3.2
3	3.61	475.9	11.828	3.073
Mean	3.69	472.63	12.03	3.46
SD	0.13	19.90	0.52	0.56

Table 12H ANOVA for the tensile strength of film formulation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	225.789	3	75.263	23.625	0.000
Within Groups	25.485	8	3.186		
Total	251.274	11			

Table 13H Tukey HSD test of the tensile strength of film formulation

Periods	Method	Sig.
Initial	1 st month	0.002*
	2 nd month	0.001*
	3 rd month	0.000*
1 st month	Initial	0.002*
	2 nd month	0.937
	3 rd month	0.240
2 nd month	Initial	0.001*
	1 st month	0.937
	3 rd month	0.481
3 rd month	Initial	0.000*
	1 st month	0.240
	2 nd month	0.481

* The mean difference is significant at the 0.05 level

Table 14H ANOVA for the %elongation of film formulation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	137845.776	3	45948.592	80.126	0.000
Within Groups	4587.607	8	573.451		
Total	142433.38	11			

Table 15H Tukey HSD test of the %elongation of film formulation

Peroids	Method	Sig.
Initial	1 st month	0.001*
	2 nd month	0.000*
	3 rd month	0.000*
1 st month	Initial	0.001*
	2 nd month	0.000*
	3 rd month	0.002*
2 nd month	Initial	0.000*
	1 st month	0.000*
	3 rd month	0.347
3 rd month	Initial	0.000*
	1 st month	0.002*
	2 nd month	0.347

* The mean difference is significant at the 0.05 level

Table 16H ANOVA for the work of failure of film formulation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	144.982	3	48.327	2.519	0.132
Within Groups	153.500	8	19.188		
Total	298.483	11			

Table 17H Tukey HSD test of the work of failure of film formulation

Peroids	Method	Sig.
Initial	1 st month	1.000
	2 nd month	0.191
	3 rd month	1.000
1 st month	Initial	1.000
	2 nd month	0.209
	3 rd month	0.999
2 nd month	Initial	0.191
	1 st month	0.209
	3 rd month	0.176
3 rd month	Initial	1.000
	1 st month	0.999
	2 nd month	0.176

* The mean difference is significant at the 0.05 level

Table 18H ANOVA for the Young’s modulus of film formulation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.248	3	3.749	4.079	0.050
Within Groups	7.353	8	0.919		
Total	18.601	11			

Table 19H Tukey HSD test of the Young’s modulus of film formulation

Peroids	Method	Sig.
Initial	1 st month	0.077
	2 nd month	0.114
	3 rd month	0.067
1 st month	Initial	0.077
	2 nd month	0.992
	3 rd month	1.000
2 nd month	Initial	0.114
	1 st month	0.992
	3 rd month	0.980
3 rd month	Initial	0.067
	1 st month	1.000
	2 nd month	0.980

* The mean difference is significant at the 0.05 level

VITA

Miss. Jirunya Assanee was born on December 8, 1981 in Bangkok, Thailand. She received her Bachelor degree of Science in Pharmacy from the Faculty of Pharmaceutical Sciences, Srinakarinwirot University in 2003. She worked for the Rajvitee hospital, Thailand.



