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APPENDICES

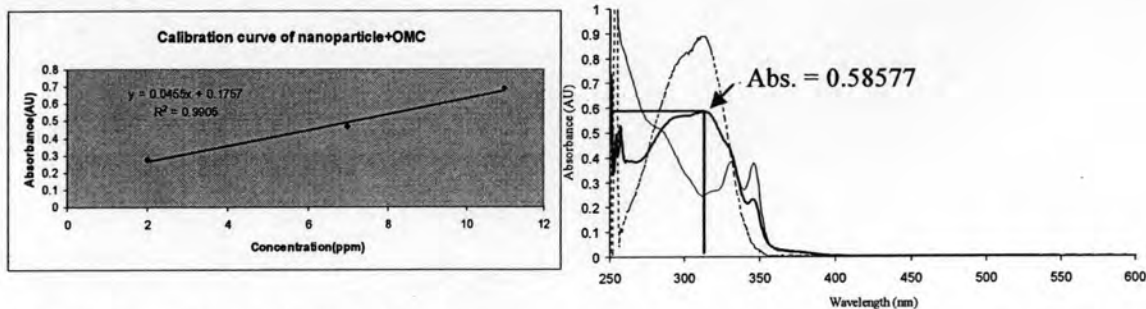
APPENDIX A

I. Content of cosmetic actives loaded into particle d**1) Solvent displacement method**

% Weight of the cosmetic actives in the particle d.

- EHMC

Calibration of EHMC + nanoparticle d



From the equation of calibration curve;

$$y = 0.0455X + 0.1757, R^2 = 0.9905$$

$$0.58577 = 0.0455X + 0.1757$$

$$X = 9.0125$$

Weight of the product 0.2 mg/10 mL gave content of EHMC in the particle d

9.0125 ppm

ppm \rightarrow mg/mL

$$\therefore 9.0125 \text{ ppm} = 9.0125 \text{ mg/L}$$

\therefore In volumetric 10×10^{-3} L gave content of EHMC in the particle d 0.090125 mg

From weight of the product 0.2 mg which gave content of EHMC in the particle d

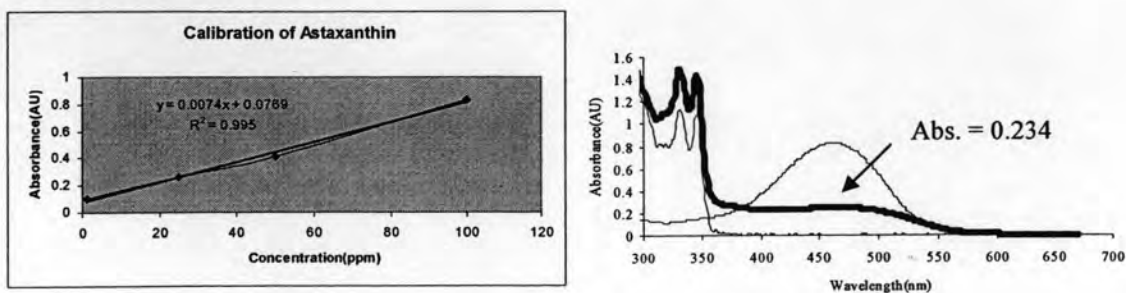
0.090125 mg, so percentage of EHMC in the particle d = $(0.090125 \times 100) / 0.2$

$$= 45.06 \%$$

Therefore, % Weight of the EHMC in the particle d is 45%.

- Astaxanthin

Calibration of astaxanthin



From the equation of calibration curve;

$$y = 0.0074X + 0.0769$$

$$0.234 = 0.0074X + 0.0769$$

$$X = 21.229$$

Weight of the product 0.2 mg/10 mL gave content of astaxanthin in the particle **d** 21.229 ppm

ppm \rightarrow mg/mL

$$\therefore 21.229 \text{ ppm} = 21.229 \text{ mg/L}$$

\therefore In volumetric 10×10^{-3} L gave content of astaxanthin in the particle **d** 0.021229 mg

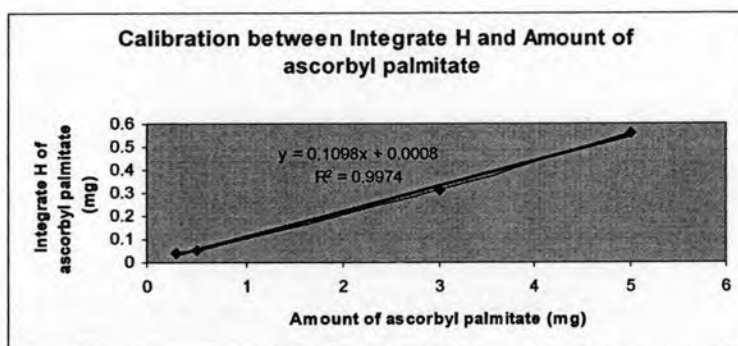
From weight of the product 0.55 mg which gave content of astaxanthin in the particle **d** 0.021229 mg, so percentage of astaxanthin in the particle **d** = $(0.021229 \times 100) / 0.55$
= 38.59 %

Therefore, % Weight of the astaxanthin in the particle **d** is 38.6 %.

- Ascorbyl palmitate

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle **d**, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 1.1

Calibration of Ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1098X + 0.0008, R^2 = 0.9974$$

$$1.1 = 0.1098X + 0.0008$$

$$X = 10.011$$

Weight of the product 10.7 mg gave content of ascorbyl palmitate in the particle **d** 10.011 mg

From weight of the product 10.7 mg which gave content of ascorbyl palmitate in the particle **d** 10.01 mg, so percentage of ascorbyl palmitate in the particle **d** = $(10.011 \times 100) / 10.7 = 93.56 \%$

Therefore, % Weight of the ascorbyl palmitate in the particle **d** is 93.56 %.

% Encapsulation efficiency (EE)

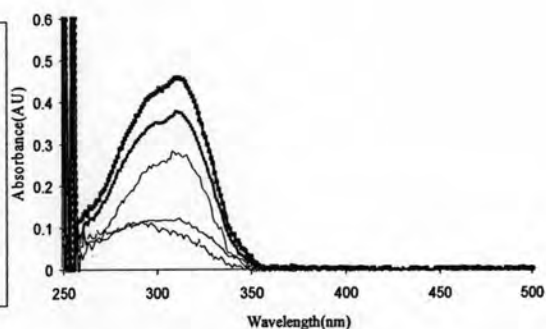
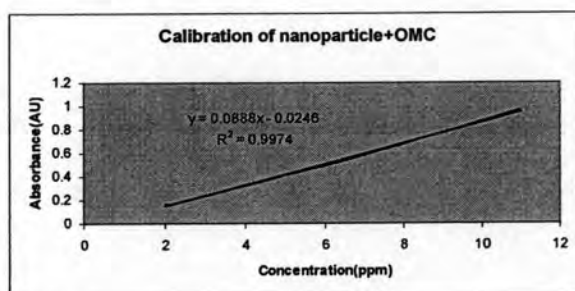
Calculation for content of the loaded active in particles

= the total amount of cosmetic actives – the remaining amount of free cosmetic actives outside of dialysis bag.

The results indicated that ascorbyl palmitate and astaxanthin wasn't found outside of dialysis bag. Therefore, % encapsulation efficiency (EE) of both ascorbyl palmitate and astaxanthin is 100 %. In contrast, EHMC was found outside of dialysis bag. From the calculation determined that % encapsulation efficiency of EHMC is 97.65 %.

2) Diffusion method

% Weight of the EHMC in the particle **d**.



- 0 min

From the equation of calibration curve;

$$y = 0.0888X - 0.0246, R^2 = 0.9974$$

$$0.089 = 0.0888X - 0.0246$$

$$X = 1.28$$

Weight of the product 1 mg/10 mL gave content of EHMC in the particle **d**

1.28 ppm

ppm \rightarrow mg/ mL

$\therefore 1.28 \text{ ppm} = 1.28 \text{ mg/ L}$

∴ In volumetric 10×10^{-3} L gave content of EHMC in the particle **d** 0.0128 mg
 From weight of the product 1 mg which gave content of EHMC in the particle **d** 0.0128 mg, so percentage of EHMC in the particle **d** = $(0.0128 \times 100) / 1$
 = 1.28 %

Therefore, % Weight of the EHMC in the particle **d** is 1.28 %.

- 4 hour

From the equation of calibration curve;

$$y = 0.0888X + 0.0246, R^2 = 0.9974$$

$$0.119 = 0.0888X + 0.0246$$

$$X = 1.62$$

Weight of the product 1 mg/10 mL gave content of EHMC in the particle **d** 1.62 ppm

ppm → mg/mL

∴ 1.62 ppm = 1.62 mg/L

∴ In volumetric 10×10^{-3} L gave content of EHMC in the particle **d** 0.0162 mg
 From weight of the product 1 mg which gave content of EHMC in the particle **d** 0.0162 mg, so percentage of EHMC in the particle **d** = $(0.0162 \times 100) / 1$
 = 1.62 %

Therefore, % Weight of the EHMC in the particle **d** is 1.62 %.

- 5 day

From the equation of calibration curve;

$$y = 0.0888X + 0.0246, R^2 = 0.9974$$

$$0.274 = 0.0888X + 0.0246$$

$$X = 2.81$$

Weight of the product 1 mg/10 mL gave content of EHMC in the particle **d** 2.81 ppm.

ppm → mg/mL

∴ 2.81 ppm = 2.81 mg/L

∴ In volumetric 10×10^{-3} L gave content of EHMC in the particle **d** 0.0281 mg
 From weight of the product 1 mg which gave content of EHMC in the particle **d** 0.0281 mg, so percentage of EHMC in the particle **d** = $(0.0281 \times 100) / 1$
 = 2.81 %

Therefore, % Weight of the EHMC in the particle **d** is 2.81 %.

- 10 day

From the equation of calibration curve;

$$\begin{aligned} y &= 0.0888X + 0.0246, R^2 = 0.9974 \\ 0.378 &= 0.0888X + 0.0246 \\ X &= 4.544 \end{aligned}$$

Weight of the product 1 mg/10 mL gave content of EHMC in the particle **d** 4.544 ppm.

ppm \rightarrow mg/mL

$$\therefore 4.544 \text{ ppm} = 4.544 \text{ mg/L}$$

\therefore In volumetric 10×10^{-3} L gave content of EHMC in the particle **d** 0.04544 mg

From weight of the product 1 mg which gave content of EHMC in the particle **d** 0.04544 mg, so percentage of EHMC in the particle **d** = $(0.04544 \times 100) / 1$
= 4.544 %

Therefore, % Weight of the EHMC in the particle **d** is 4.544 %.

- 20 day

From the equation of calibration curve;

$$\begin{aligned} y &= 0.0888X + 0.0246, R^2 = 0.9974 \\ 0.458 &= 0.0888X + 0.0246 \\ X &= 5.446 \end{aligned}$$

Weight of the product 1 mg/10 mL gave content of EHMC in the particle **d** 5.446ppm.

ppm \rightarrow mg/mL

$$\therefore 5.446 \text{ ppm} = 5.446 \text{ mg/L}$$

\therefore In volumetric 10×10^{-3} L gave content of EHMC in the particle **d** 0.05446 mg

From weight of the product 1 mg which gave content of EHMC in the particle **d** 0.05446 mg, so percentage of EHMC in the particle **d** = $(0.05446 \times 100) / 1$
= 5.45 %

Therefore, % Weight of the EHMC in the particle **d** is 5.45 %.

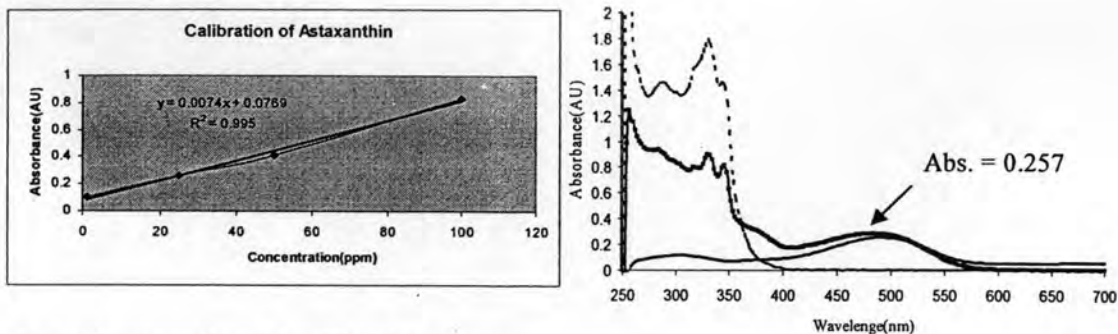
II. Content of cosmetic actives loaded into particle e.

1) Solvent displacement method

% Weight of the cosmetic actives in the particle e.

- Astaxanthin

Calibration of astaxanthin



From the equation of calibration curve;

$$y = 0.0074X + 0.0769$$

$$0.257 = 0.0074X + 0.0769$$

$$X = 24.34$$

Weight of the product 1.1 mg/10 mL gave content of astaxanthin in the particle e 24.34 ppm.

ppm \rightarrow mg/ mL

$$\therefore 24.34 \text{ ppm} = 24.34 \text{ mg/ L}$$

\therefore In volumetric 10×10^{-3} L gave content of astaxanthin in the particle e 0.2434 mg.

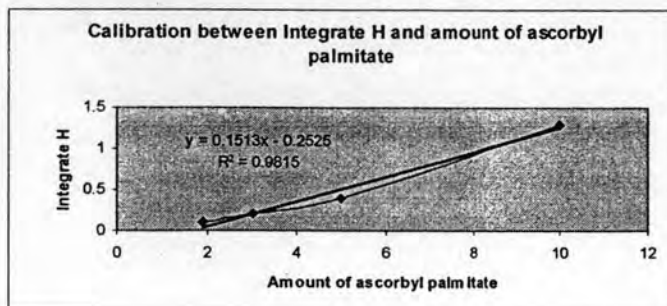
From weight of the product 1.1 mg which gave content of astaxanthin in the particle e 0.2434 mg, so percentage of astaxanthin in the particle e = $(0.2434 \times 100) / 1.1 = 22.13\%$

Therefore, % Weight of the astaxanthin in the particle e is 22.13 %.

- Ascorbyl palmitate

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle e, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.8

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$\begin{aligned} y &= 0.1513X - 0.2525, R^2 = 0.9815 \\ 0.8 &= 0.1513X - 0.2525 \\ X &= 6.9564 \end{aligned}$$

Weight of the product 10.1 mg gave content of ascorbyl palmitate in the particle e 6.9564 mg.

From weight of the product 10.1 mg which gave content of ascorbyl palmitate in the particle e 6.9564 mg, so percentage of ascorbyl palmitate in the particle e = $(6.9564 \times 100) / 10.1 = 68.88\%$.

Therefore, % Weight of the ascorbyl palmitate in the particle e is 69 %.

% Encapsulation efficiency (EE)

Calculation for content of the loaded active in particles

= the total amount of cosmetic actives – the remaining amount of free cosmetic actives outside of dialysis bag.

The results indicated that ascorbyl palmitate and astaxanthin wasn't found outside of dialysis bag. Therefore, % encapsulation efficiency (EE) of both ascorbyl palmitate and astaxanthin is 100 %.

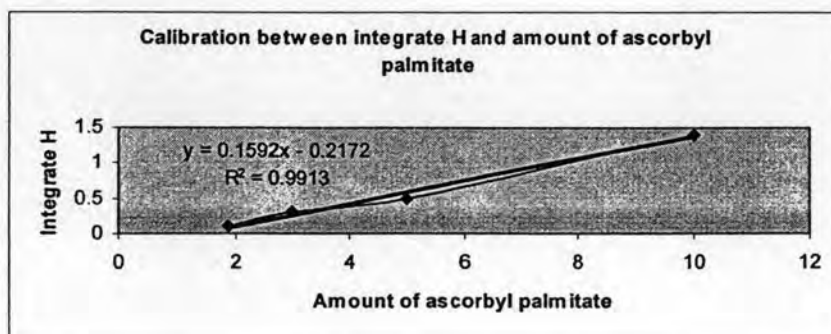
2) Diffusion method

% Weight of the ascorbyl palmitate in the particle e.

- 0 min

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle e, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.5

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1592X - 0.2172, R^2 = 0.9913$$

$$0.5 = 0.1592X - 0.2172$$

$$X = 4.51$$

Weight of the product 6.1 mg gave content of ascorbyl palmitate in the particle e 4.51 mg.

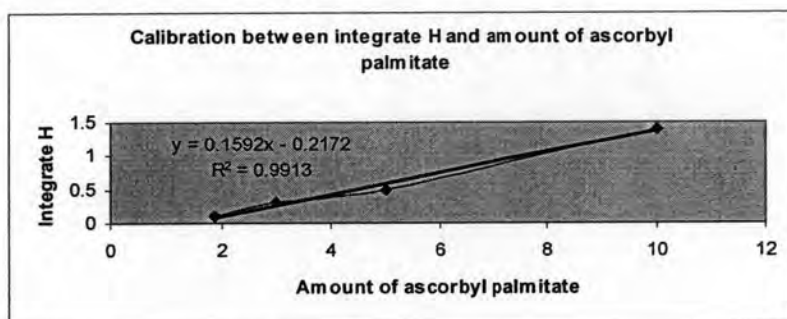
From weight of the product 6.1 mg which gave content of ascorbyl palmitate in the particle e 4.51 mg, so percentage of ascorbyl palmitate in the particle e = $(4.51 \times 100) / 6.1 = 73.93\%$.

Therefore, % Weight of the ascorbyl palmitate in the particle e is 74 %.

- 5 day

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle e, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.6.

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1592X - 0.2172, R^2 = 0.9913$$

$$0.6 = 0.1592X - 0.2172$$

$$X = 5.13$$

Weight of the product 6.1 mg gave content of ascorbyl palmitate in the particle e 5.13 mg.

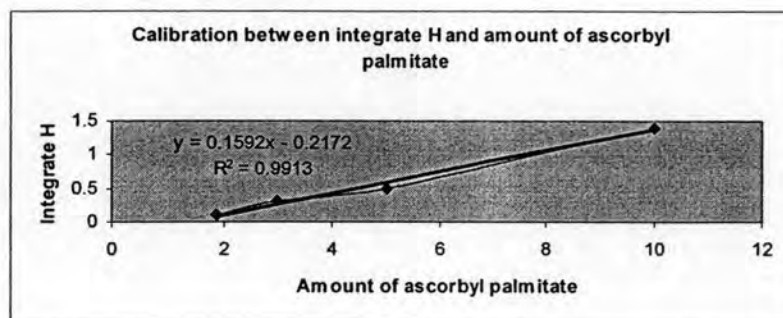
From weight of the product 6.1 mg which gave content of ascorbyl palmitate in the particle e 5.13 mg, so percentage of ascorbyl palmitate in the particle e = $(5.13 \times 100) / 6.1 = 84.15\%$.

Therefore, % Weight of the ascorbyl palmitate in the particle e is 84.15 %.

- 10 day

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle **e**, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.7.

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1592X - 0.2172, R^2 = 0.9913$$

$$0.7 = 0.1592X - 0.2172$$

$$X = 5.761$$

Weight of the product 6.1 mg gave content of ascorbyl palmitate in the particle **e** 5.761 mg.

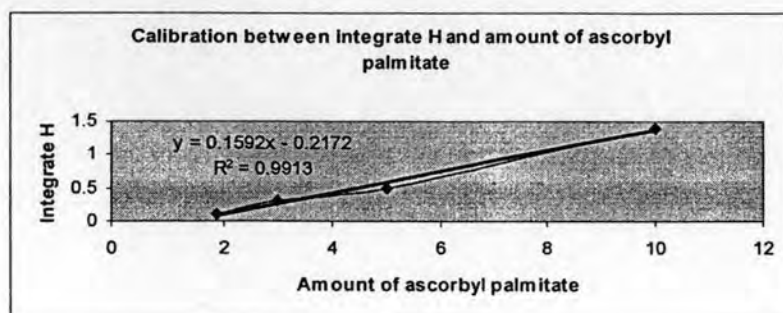
From weight of the product 6.1 mg which gave content of ascorbyl palmitate in the particle **e** 5.761 mg, so percentage of ascorbyl palmitate in the particle **e** = $(5.761 \times 100) / 6.1 = 94.44\%$

Therefore, % Weight of the ascorbyl palmitate in the particle **e** is 94.44 %.

- 20 day

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle **e**, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.7.

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1592X - 0.2172, R^2 = 0.9913$$

$$0.7 = 0.1592X - 0.2172$$

$$X = 5.761$$

Weight of the product 6.1 mg gave content of ascorbyl palmitate in the particle e 5.761 mg.

From weight of the product 6.1 mg which gave content of ascorbyl palmitate in the particle e 5.761 mg, so percentage of ascorbyl palmitate in the particle e =

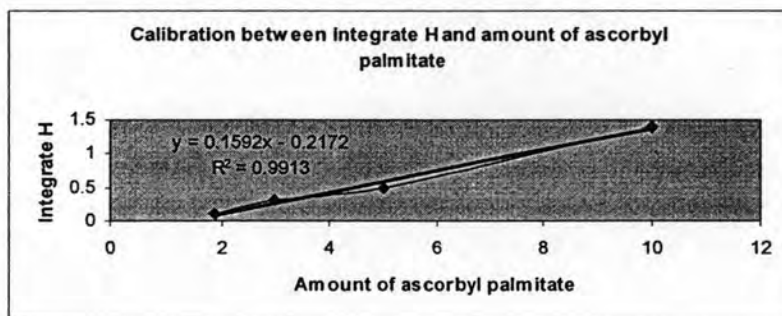
$$(5.761 \times 100) / 6.1 = 94.44 \%$$

Therefore, % Weight of the ascorbyl palmitate in the particle e is 94.44 %.

- 30 day

From $^1\text{H-NMR}$ of ascorbyl palmitate-loaded-particle e, integration of aromatic protons = 1, and integration of methyl protons of 4-methoxycinnamoyl moieties on N-phthaloylchitosan = 0.7.

Calibration of ascorbyl palmitate



From the equation of calibration curve;

$$y = 0.1592X - 0.2172, R^2 = 0.9913$$

$$0.7 = 0.1592X - 0.2172$$

$$X = 5.761$$

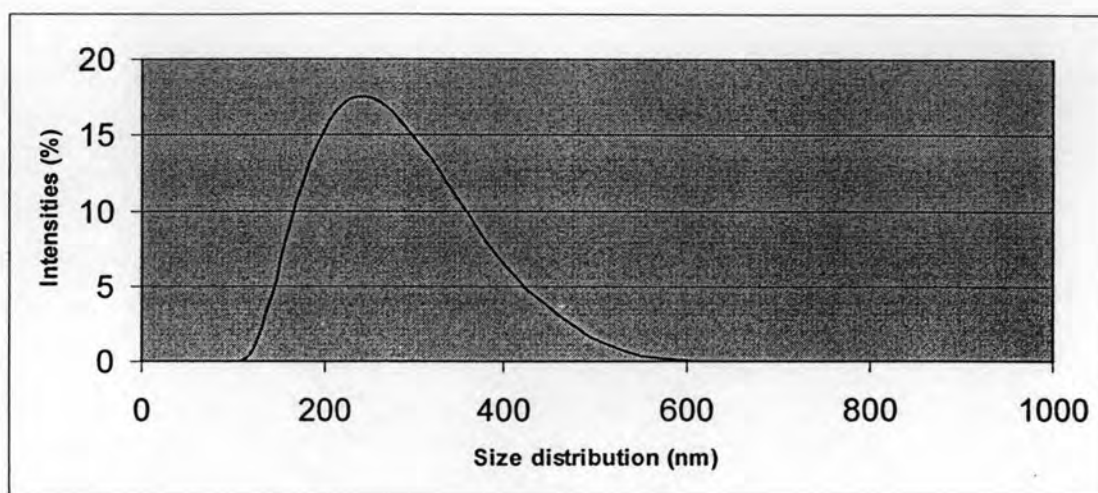
Weight of the product 6.1 mg gave content of ascorbyl palmitate in the particle e 5.761 mg.

From weight of the product 6.1 mg which gave content of ascorbyl palmitate in the particle e 5.761mg, so percentage of ascorbyl palmitate in the particle e =

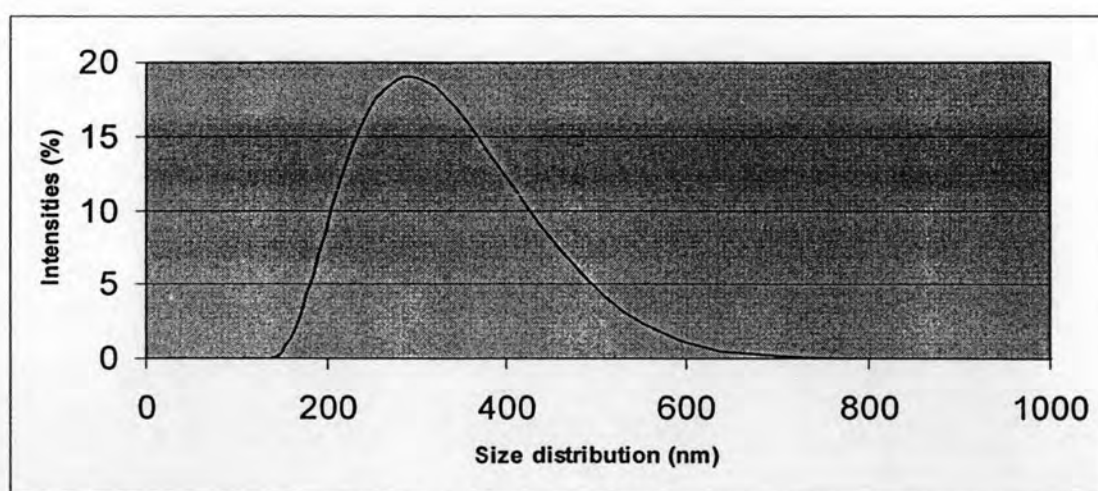
$$(5.761 \times 100) / 6.1 = 94.44 \%$$

Therefore, % Weight of the ascorbyl palmitate in the particle e is 94.44 %.

APPENDIX B

Particle size distribution*mPEG-phthaloylchitosan nanoparticles*

Average particle size distribution = 255 nm

mPEG-4-methoxycinnamoyl-phthaloylchitosan nanoparticles

Average particle size distribution = 295 nm

VITA

Ms. Nattaporn Anumansirikul was born on October 1, 1981 in Bangkok. She received a Bachelor's Degree of Science in Chemistry from Mahidol University in 2004. After that, she started her graduate study a Master's degree in the Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University.

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