4.10.4 Comparison of formulation C1 to C3

Formulation C	NVSC co-crystal	Carbopol 974 P	Sodium hydroxide 20%	Sorbitol	Polysorbate 80	methylparaben	propylparaben	Water	pH of formulation	Integrity of co-crystal (DSC)	Integrity of co-crystal (FTIR)
C1	50 mg	10.5 mg							4.12	✓	✓
C2	50 mg	10.5 mg	7 μL						5.80	✓	✓
C3	165 mg	33.6 mg	30 μL	3.7 mL	8 μL	28.8 mg	3.84 mg	q.s	8.08	✓	✓

Table 4.19 Comparison of pH and integrity of Formulation C1 , C2, C3

From table 4.19, it can be concluded that the integrity of NVSC co-crystal is not compromised in carbopol 974P. This is verified by the results of the DSC, FTIR and UV of the suspension. Furthermore, the pH of formulation C3 was within the target range. Hence, this suspension was scaled up and quality control tests were performed on it.

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Chapter 5

Quality control of suspension

Chapter 5 Quality control of suspension

Formulation C3 proved to be successful in the two criteria that were deemed necessary for co-crystal suspension formulation viz. co-crystal integrity and pH of the suspension. Hence, formulation C3 suspension in a concentration of 50 mg/5 mL was scaled up to 50 mL, characterization and quality control tests of this suspension was pursed. The results obtained were compared to the Viramune® suspension and the standard that was accepted according to literature.

5.1 Quality control of suspensions

5.1.1 Particle size of suspension

The particle size of the insoluble particle should be in a range of 10 to 1000 μm.[1] The particle size of the NVSC co-crystal prior to formulation was measured by SEM. The particle size of NVSC measured was 248.8 μm x 428.0 μm. As seen in figure 5.1, nevirapine had a particle size of 113.2 x 121.6 μm.

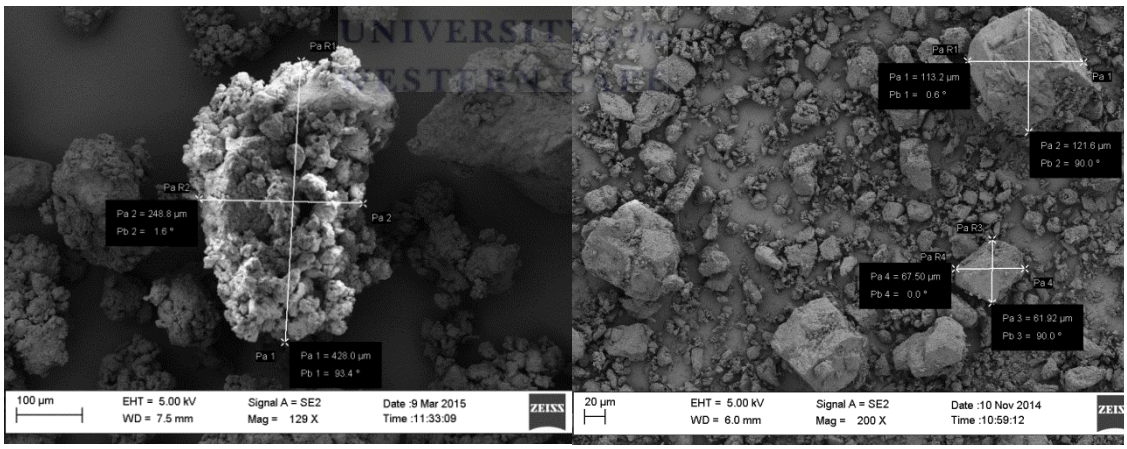


Figure 5.1 SEM of NVSC co-crystal and nevirapine (from left to right)

The particles size of the particles in the formulation was determined by a zeta sizer. The particle size determined for the Viramune® suspension was 935 nm while the NVSC co-crystal formulation revealed a much smaller size of 574.9 nm. The reason for a decrease in particle size after formulation could be due to the agitation the suspension undergoes through during formulation stages.

5.1.2 Polydispersity Index

The polydispersity index gives an indication on the particle size distribution that is measured through a zeta sizer. Suspensions are preferred to have a polydispersity index of 0.1 to 0.3. Viramune® suspension had a polydispersity index of 0.405 while the NVSC co-crystal suspension had a polydispersity index of 0.141. This suggested that the NVSC co-crystal suspension met the criteria of the polydispersity index however, the Viramune® suspension had a slightly higher polydispersity index which meant that the particle size distribution is slightly wider.

5.1.3 Measurement of pH

The ideal pH for a suspension for oral consumption should be between 5–8. Viramune® suspension had a pH of 6.31, whereas the NVSC co-crystal suspension had a pH of 8.01. Thus, both the Viramune® suspension and the NVSC co-crystal suspension were within the desired pH range. The differences in pH range could be attributed to the variation in the formulation process of the two suspensions, as the NVSC co-crystal suspension required more volume of sodium hydroxide than the Viramune® suspension. The NVSC co-crystal required a greater volume of sodium hydroxide because the NVSC co-crystal was acidic in nature, which significantly reduced the pH of the suspension.

5.1.4 Viscosity

For a suspension to have ideal flow properties, it should have high viscosity at low shear rates (during storage) and low viscosity at high shear rates. Viramune® suspension was measured in a small sample adapter at 25 ºC with a SC4-16 spindle and had torque values between 41–99 %. The Viramune® suspension has a maximum viscosity of 2513 cP at a shear rate of 5.8 sec^{-1}. This was in accordance to what was required, as it obtained a high viscosity at a low shear rate. As the speed of the spindle

increases, so does the shear rate, this resultant force causes a decrease in the viscosity of the suspension.

Viscosity (cP)	Speed (RPM)	Torque (%)	Shear stress (D/cm^2)	Shear rate (1/sec)
2513.46	20.00	41.90	145.78	5.80
1939.59	30.00	48.50	168.74	8.70
1616.66	40.00	53.90	187.53	11.60
1406.10	50.00	58.60	203.88	14.50
1253.73	60.00	62.70	218.15	17.40
1144.90	70.00	66.80	232.41	20.30
1057.27	80.00	70.50	245.29	23.20
981.12	90.00	73.60	256.07	26.10
915.40	100.00	76.30	265.47	29.00
868.18	110.00	79.60	276.95	31.90
823.82	120.00	82.40	286.69	34.80
787.22	130.00	85.30	296.78	37.70
750.70	140.00	87.60	304.78	40.60
723.85	150.00	90.50	314.87	43.50
697.35	160.00	93.00	323.57	46.40
676.80	170.00	95.90	333.66	49.30
651.86	180.00	97.80	340.27	52.20
629.55	190.00	99.70	346.88	55.10

Table 5.1 Viscosity of Viramune suspension

As seen in figure 5.1, Viramune exhibits a Bingham's plastic type of flow, where the fluids that have a linear shear stress/shear strain require a finite yield stress before they begin to flow. This is apparent as the graph does not begin from the origin, indicating that the suspension requires force for it to flow.

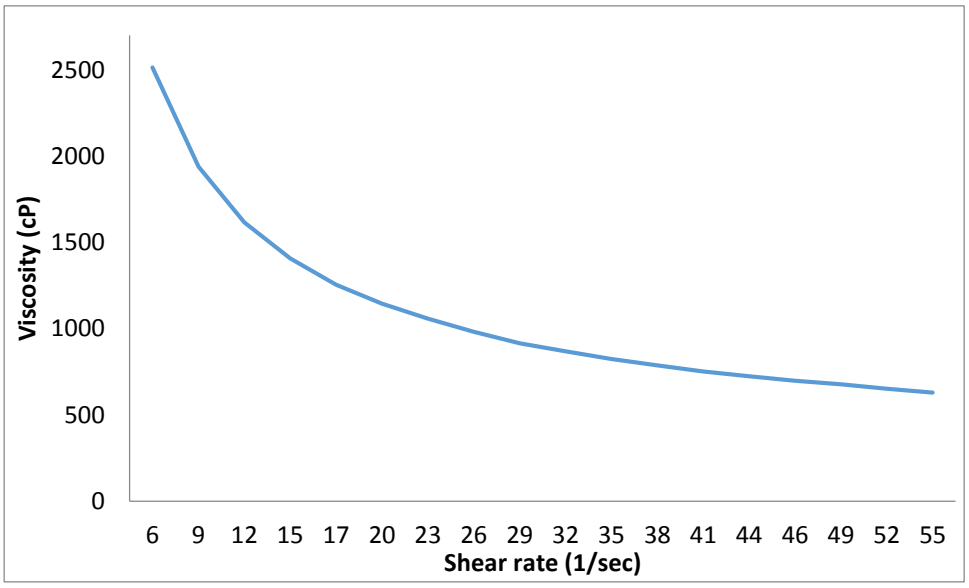


Figure 5.1 Viscosity versus shear rate of Viramune® suspension

The viscosity results of the NVSC co-crystal suspension is outlined in table 5.2. The viscosity of the NVSC co-crystal suspension was also measured in a small sample adapter at 25 ºC with a SC4-18 spindle and obtained torque values between 32 – 82 %. The NVSC co-crystal suspension has a maximum viscosity of 65 cP at a shear rate of 20 sec^{-1}. Although the viscosity of this suspension is significantly lower than Viramune®, it still meets the specification which states that a high viscosity should be obtained at a low shear rate.

Figure 5.2 displays the viscosity of the NVSC co-crystal suspension which exhibits a similar flow to the Viramune® suspension. It exhibits a Bingham's plastic type flow, which requires a finite yield stress before the suspension begins to flow.

Viscosity (cP)	Speed (RPM)	Torque (%)	Shear stress (D/cm^2)	Shear rate (1/sec)
64.59	15.00	32.30	12.79	19.80
47.99	25.00	40.00	15.84	33.00
38.65	35.00	45.10	17.86	46.20
34.13	45.00	51.20	20.27	59.40
31.25	55.00	57.30	22.69	72.60
29.07	65.00	63.00	24.94	85.80
27.23	75.00	68.10	26.96	99.00
25.37	85.00	71.90	28.47	112.20
24.09	95.00	76.30	30.21	125.40
23.45	105.00	82.10	32.50	138.60

Table 5.2 Viscosity of NVSC co-crystal suspension

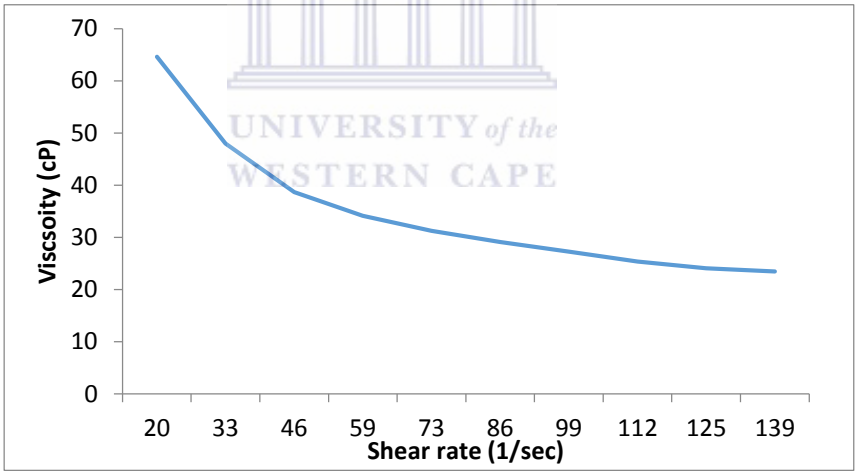


Figure 5.2 Viscosity versus shear rate of NVSC co-crystal suspension

5.1.5 Zeta potential

Zeta potential of the suspension gives an indication of physical stability of the suspension. A value more than +30 mV or less than -30mV is preferred for suspensions. Viramune® obtained a value of -14.2 mV while the NVSC co-crystal suspension obtained a value of -2.37 mV. Both the suspensions did not meet this requirement,

however, the Viramune® suspension was closer to the desired zeta potential range indicating that it was physically more stable. The Viramune® suspension had greater viscosity thus, it was physically more stable as it allowed the particles to be dispersed whereas the NVSC co-crystal had a lower viscosity thus the zeta potential was also significantly lower. Due to the low viscosity of the NVSC co-crystal suspension the particles were not dispersed continually throughout the suspension, hence it had a low zeta potential.

5.1.6 Dissolution

Dissolution tests are an indication of the cumulative amount of API that passes into solution which is studied as a function of time. The test describes the overall rate of all the processes involved in the release of the API into a bioavailable form. Dissolution studies evaluates the potential effect of formulation and process variables on the bioavailability of an API and ensures that preparations comply with product specification. It gives an indication of the performance of the preparation under *in vitro* conditions.[2]

To analyse the concentration of nevirapine in the Viramune® suspension and the co-crystal suspension, a calibration curve of nevirapine was constructed in a phosphate buffer of pH 6.8. A sixteen point calibration curve, of nevirapine ranged from a concentration of 0.3 mg/mL to 0.00234375 mg/mL in triplicate was plotted. A straight line with a regression value of R^2=1 was achieved (Fig 5.3). Nevirapine compound was eluted at approximately 3 minutes at 280 nm. HPLC chromatogram and the peak area observed for the dissolution results can be seen in Appendix B.

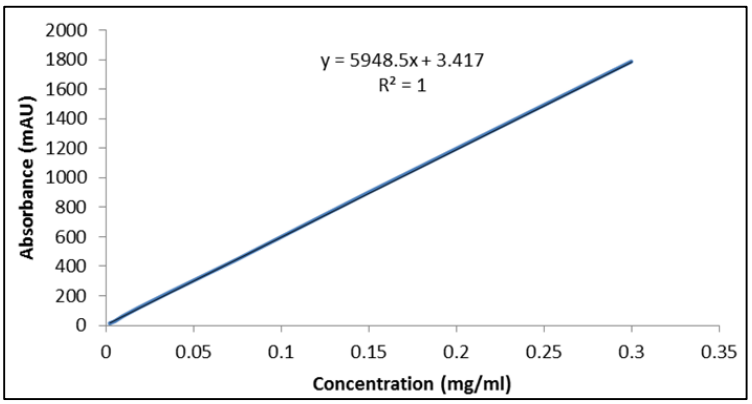


Figure 5.3 Standard curve of nevirapine in phosphate buffer with a pH of 6.8

The dissolution media that was used was phosphate buffer with a pH of 6.8. This is the pH at which nevirapine is absorbed in the body. To simulate *in vivo* conditions the temperature of the dissolution media was 37 °C. Six dissolution vessels were used for the study. 5 mL of the suspension was inserted in the vessel by means of a syringe. 5 mL samples were extracted at 10, 20, 30, 45 and 60 minute intervals and replaced with 5 mL of phosphate buffer. 1 mL of extracted samples was then placed in HPLC vials. Concentration of samples were determined using the peak area of samples obtained at 3 minutes in the equation obtained in the standard curve. Cumulative amount of API was calculated as a percentage for Viramune® suspension and NVSC co-crystal suspension as seen in table 5.3 and 5.4.

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	40	35	40	45	34	50
20	70	62	69	77	60	69
30	86	78	93	93	79	89
45	98	91	98	98	97	95
60	100	100	100	100	100	100

Table 5.3 Percentage release of nevirapine in Viramune® in phosphate buffer at pH 6.8

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	76	81	84	87	78	87
20	85	88	92	92	87	93
30	91	92	96	95	93	96
45	97	97	98	100	97	98
60	100	100	100	100	100	100

Table 5.4 Percentage release of nevirapine in NVSC suspension in phosphate buffer at pH 6.8

According to the USP 32 criteria, 81-100 % of drug release must occur within 30 minutes of the dissolution study.[2] Both the branded version, Viramune® and the NVSC co-crystal suspensions met this criteria, with the Viramune® achieving a 86 % drug release while the NVSC co-crystal suspension achieved a drug release of 94 % within 30 minutes of dissolution (table 5.5 and figure 5.4).

Time	**Viramune ® suspension (average % dissolved)**	**NVSC suspension (average % dissolved)**	**Standard deviation**
10	41	82	20
20	68	89	11
30	86	94	4
45	96	98	1
60	100	100	0

Table 5.5 Comparison of average percentage drug release of Viramune ® suspension and NVSC co-crystal suspension

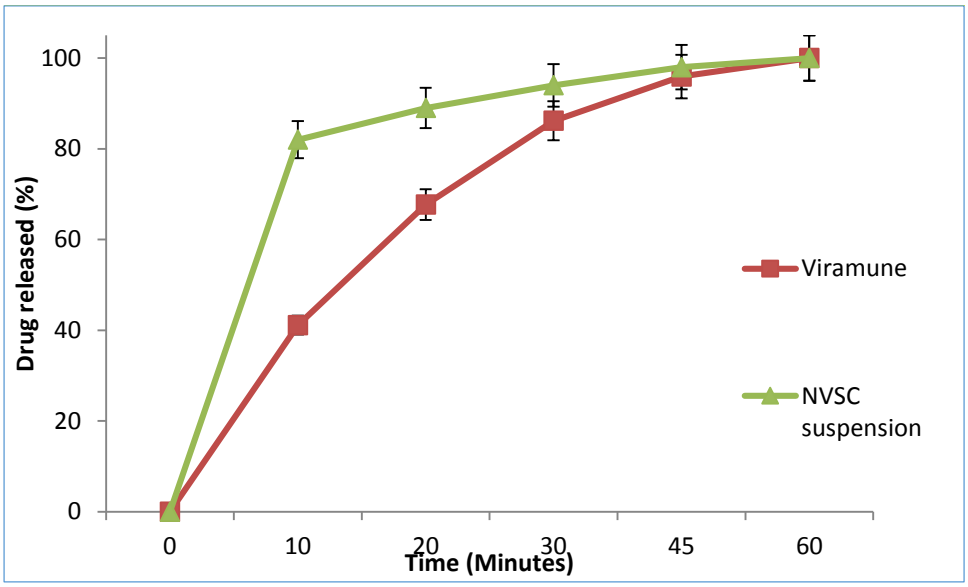
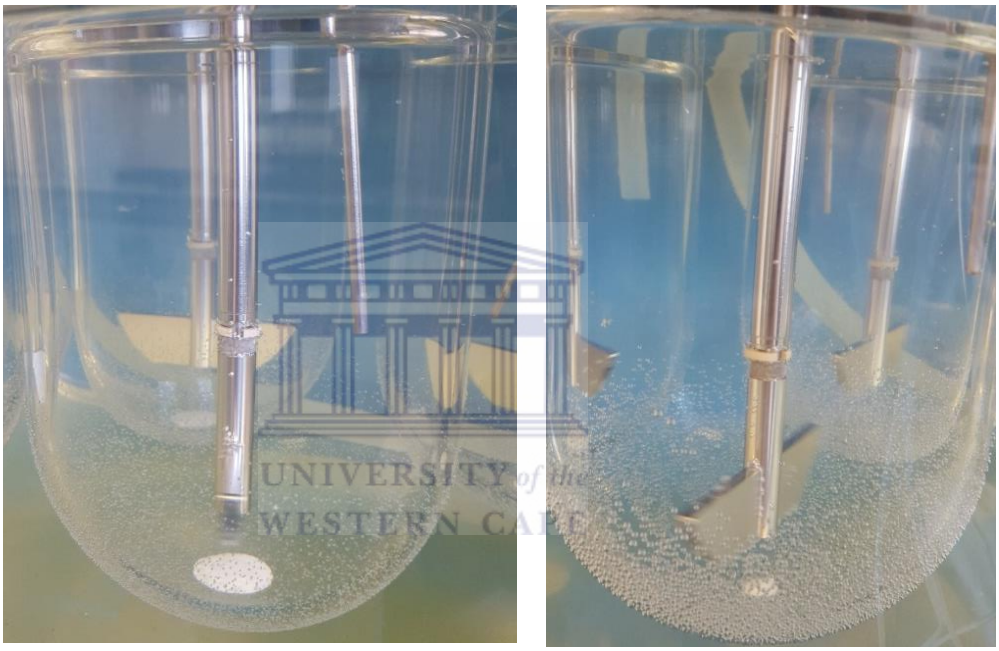


Figure 5.4 Dissolution profiles of Viramune® suspension and NVSC co-crystal suspension

The NVSC co-crystal suspension dissolved more quickly, this is substantiated by the ten minute point, where only 41 % of nevirapine is released in the Viramune® suspension whereas 82 % of nevirapine is released in the NVSC co-crystal suspension within ten minutes of dissolution. This is twice the amount of nevirapine that is released from the Viramune® suspension, indicating that the NVSC co-crystal releases more nevirapine at the ten minute time interval.

The NVSC co-crystal suspension had a consistently higher percentage release than the Viramune® suspension during the dissolution study. At the ten minute time interval, the standard deviation between the Viramune ® suspension and the NVSC co-crystal suspension indicated that the dissolution rate of the NVSC co-crystal suspension is significantly higher. However, at the 45 minute time interval, the standard deviation was low indicating that the dissolution rates of the NVSC co-crystal and the Viramune® suspension is similar. The Viramune® suspension is released gradually over time whereas 82 % of the NVSC co-crystal suspension is released within ten minutes.

Upon placing the suspensions in the vessels, the suspensions are seen as small circular mass, this is illustrated in figure 5.4. Viramune® suspension remains as a circular masses of powder throughout the dissolution study, whereas comparatively the NVSC co-crystal suspension reduces its mass over time. Furthermore, this mass of powder is greater in the Viramune® suspension than the NVSC co-crystal suspension. This can be recognized due to the Viramune® containing an extra excipient -sucrose- which is not in the formulation of the NVSC co-crystal suspension.

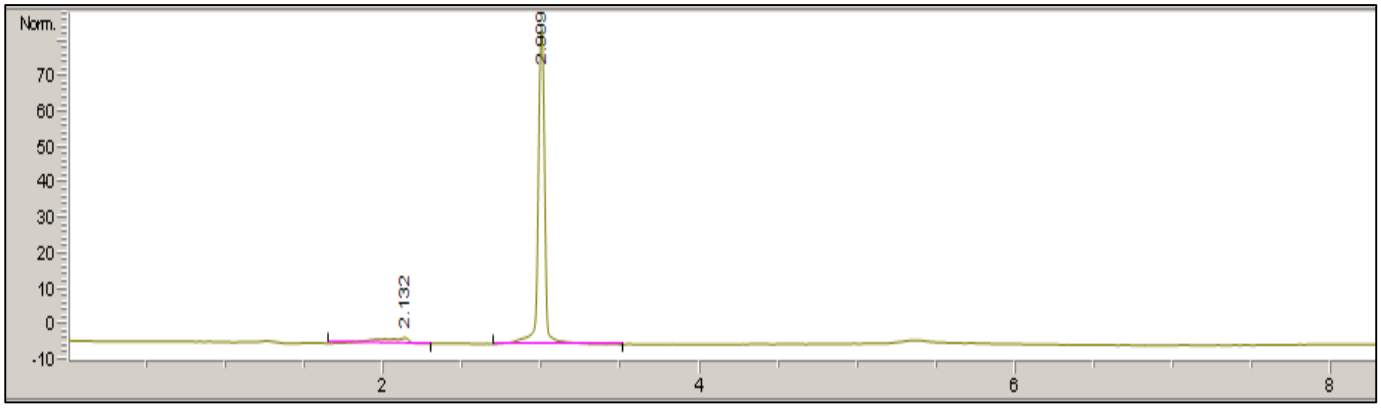


Viramune® suspension NVSC co-crystal suspension

Figure 5.4 Dissolution of Viramune suspension and NVSC co-crystal suspension at thirty minutes

Table 5.6 summarizes the quality control tests of Viramune® suspension and NVSC co-crystal suspension. Both the suspensions met the criteria of particle size, polydispersity index, pH and dissolution. Viramune® had a better viscosity while the NVSC co-crystal suspension did not meet this criteria. The zeta potential for both the suspensions did not meet the standard required.

Appendix B



HPLC chromatgram of nevirapine at 280 nm in phosphate buffer

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	717.2	680.1	660	761.4	578	1162.8
20	1247.8	1207	1124.3	1297.1	1005.9	1596.3
30	1519.2	1521.4	1520.8	1562	1340.3	2052.4
45	1739.3	1779.1	1609.7	1643.6	1628.7	2191.9
60	1772.4	1950.7	1638.4	1674.2	1685.3	2318.5

Peak area of Viramune® suspension at 280 nm

Time (min)	Vessel Number					
	1	**2**	**3**	**4**	**5**	**6**
10	1798.3	2013.3	1228.2	1947.3	1848.2	2238.5
20	2013.5	2200	1334.9	2072.7	2057.4	2396.9
30	2175.2	2299.1	1395.2	2140.0	2207.4	2453.2
45	2315.7	2401.8	1423.6	2240.3	2297.2	2516.5
60	2380.9	2484	1455.8	2242.7	2376.9	2570.7

Peak area of NVSC co-crystal suspension at 280 nm