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# Supplementary Materials: Development of a Resveratrol Nanosuspension Using the Antisolvent Precipitation Method, Based on a Quality by Design (QbD) Approach

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**Table S1.** Initial risk assessment of the resveratrol nanosuspension.

| CQAs | Parameters | Risk Level | Justification |
|---|---|---|---|
| Particle size (z-average, d90) | Resveratrol concentration | High | If the concentration of resveratrol is excessively high, particles grow very quickly during the manufacturing process. Therefore, the risk level was high. |
| | Stabilizer type | High | The ability to inhibit particle growth depends on the type of stabilizer. Therefore, the risk level was high. |
| | Stabilizer concentration | High | An appropriate concentration of stabilizer has an effective ability to inhibit particle growth. Therefore, the risk level was high. |
| | Solvent type | High | The solvent and anti-solvent should be sufficiently miscible, and the solvent should have a solubilization effect. Therefore, the risk level was high. |
| | Ratio of solvent/anti-solvent | High | The solubility of resveratrol for the mixture solvents depends on the ratio of the solvent/anti-solvent. The solubility affects particle growth. Therefore, the risk level was high. |
| | Mixing speed | High | Depending on the mixing speed, the mixing speed of the anti-solvent and solvent vary, and can affect particle growth rate. Therefore, the risk level was high. |
| | Mixing time | High | Mixing time can affect particle growth. Therefore, the risk level was high. |
| | Injection rate (solvent) | High | Depending on the rate of injection of solvent, the mixing speed of the anti-solvent and solvent vary, which can affect the particle growth rate. Therefore, the risk level was high. |
| | Temperature | High | The solubility of resveratrol changes when the temperature of the solvent changes, which can affect particle growth. Therefore, the risk level was high. |
| Zeta Potential | Resveratrol concentration | Low | The effect of changes in resveratrol concentration on the zeta potential is insignificant. Therefore, the risk level was low. |
| | Stabilizer type | High | Depending on the type of stabilizer, the surface charge of the particles differs. Therefore, the risk level was high. |
| | Stabilizer concentration | High | Depending on the concentration of stabilizer, the surface charge of the particles differs. Therefore, the risk level was high. |

| | | | |
|---|---|---|---|
| | Solvent type | Low | The influence of the type of solvent on the surface charge of the particles was insignificant. Therefore, the risk level was low. |
| | Ratio of solvent/anti-solvent | Medium | Depending on the solvent/anti-solvent ratio, the concentration of the stabilizer in the mixture solvent varies, which affects the surface charge of the particles. Therefore, the risk level was medium. |
| | Mixing speed | Low | The effect of mixing speed on the zeta potential is insignificant. Therefore, the risk level was low. |
| | Mixing time | Low | The effect of mixing time on the zeta potential is insignificant. Therefore, the risk level was low. |
| | Injection rate (solvent) | Low | The effect of injection rate on the zeta potential is insignificant. Therefore, the risk level was low. |
| | Temperature | Low | The effect of temperature on the zeta potential is insignificant. Therefore, the risk level was low. |
| Drug content | Resveratrol concentration | Low | Resveratrol is chemically stable when light is blocked. Nanosuspensions were prepared in a space where light was blocked, and the possibility of drug loss during the manufacturing process is insignificant. Therefore, the risk level was low. |
| | Stabilizer type | Low | |
| | Stabilizer concentration | Low | |
| | Solvent type | Low | |
| | Ratio of solvent/anti-solvent | Low | |
| | Mixing speed | Low | |
| | Mixing time | Low | |
| | Injection rate (solvent) | Low | |
| | Temperature | Low | |

**Table S2.** Particle size of resveratrol nanosuspensions prepared using a polymer.

| Polymer | Concentration (%, w/v) | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|---|---|---|---|---|
| PVP K12 | 2.0 | N.D[a] | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |
| PVP K17 | 2.0 | N.D | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |
| PVP K25 | 2.0 | N.D | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |
| PVP K30 | 2.0 | N.D | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |
| PVP K90 | 2.0 | N.D | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |
| PVP VA64 | 2.0 | N.D | N.D | N.D |
| | 1.0 | N.D | N.D | N.D |
| | 0.5 | N.D | N.D | N.D |
| | 0.1 | N.D | N.D | N.D |

| | | | | |
|---|---|---|---|---|
| HPMC 3cp | 2.0 | 515[b] | 520 | 6402 |
| | 1.0 | 867[b] | 1004 | 2034 |
| | 0.5 | 1357[b] | 1760 | 3198 |
| | 0.1 | N.D | N.D | N.D |
| HPMC 6cp | 2.0 | 331[b] | 357 | 1290 |
| | 1.0 | 618[b] | 573 | 7441 |
| | 0.5 | 978[b] | 1008 | 1646 |
| | 0.1 | N.D | N.D | N.D |
| HPMC 15cp | 2.0 | N.D | N.D | N.D |
| | 1.0 | 663[b] | 820 | 1756 |
| | 0.5 | 1122* | 1337 | 2318 |
| | 0.1 | N.D | N.D | N.D |

[a]N.D indicates that data cannot be measured and there is no data. [b]Precipitation occurs due to particle agglomeration.

**Table S3.** Particle size of resveratrol nanosuspensions prepared using a polymer/polymer combination.

| Polymer / Polymer | Concentration (%, w/v) | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|---|---|---|---|---|
| PVP VA64 / PVP K12 | 2.0 / 1.0 | N.D[a] | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | 1151[b] | 1058 | 6760 |
| | 0.5 | N.D | N.D | N.D |
| PVP VA64 / PVP K17 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | 940[b] | 877 | 7053 |
| PVP VA64 / PVP K25 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | 1516[b] | 1326 | 2245 |
| | 1.0 / 0.5 | 500[*] | 572 | 5782 |
| PVP VA64 / PVP K30 | 2.0 / 1.0 | 18[b] | 15 | 239 |
| | 2.0 / 0.5 | 436[b] | 196 | 7521 |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | N.D | N.D | N.D |
| PVP VA64 / PVP K90 | 2.0 / 1.0 | 258[b] | 50 | 3681 |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | 948[b] | 1173 | 2339 |
| | 1.0 / 0.5 | 1014[*] | 1500 | 8217 |
| HPMC 6cp / PVP K12 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | 380[b] | 351 | 2301 |
| HPMC 6cp / PVP K17 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | 507[b] | 451 | 7239 |

| | | | | |
|---|---|---|---|---|
| HPMC 6cp / PVP K25 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | N.D | N.D | N.D |
| HPMC 6cp / PVP K30 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | N.D | N.D | N.D |
| HPMC 6cp / PVP K90 | 2.0 / 1.0 | N.D | N.D | N.D |
| | 2.0 / 0.5 | N.D | N.D | N.D |
| | 1.0 / 1.0 | N.D | N.D | N.D |
| | 1.0 / 0.5 | N.D | N.D | N.D |

[a]N.D indicates that data cannot be measured and there is no data. [b]Precipitation occurs due to particle agglomeration.

**Table S4.** Particle size of resveratrol nanosuspensions prepared using various polymer/polymer/surfactant combinations.

| **Polymer / Polymer / Surfactant** | | **Concentration (%, w/v)** | **Particle size (z-average, nm)** | **Particle size (d50, nm)** | **Particle size (d90, nm)** |
|---|---|---|---|---|---|
| PVP VA64 / | PVP K12 / SLS | 1.0 / 0.5 / 0.1 | 46.5 | 66.8 | 179.2 |
| PVP VA64 / | PVP K17 / SLS | 1.0 / 0.5 / 0.1 | 59.2 | 80.5 | 178.6 |
| PVP VA64 / | PVP K25 / SLS | 1.0 / 0.5 / 0.1 | 66.2 | 61.3 | 552.9 |
| PVP VA64 / | PVP K30 / SLS | 1.0 / 0.5 / 0.1 | 57.5 | 88.0 | 212.6 |
| PVP VA64 / | PVP K90 / SLS | 1.0 / 0.5 / 0.1 | 72.6 | 108.3 | 255.3 |
| HPMC 6cp / | PVP K12 / SLS | 1.0 / 0.5 / 0.1 | 210.4 | 217.1 | 516.2 |
| HPMC 6cp / | PVP K17 / SLS | 1.0 / 0.5 / 0.1 | 203.4 | 205.3 | 362.6 |
| HPMC 6cp / | PVP K25 / SLS | 1.0 / 0.5 / 0.1 | 236.2 | 230.5 | 525.7 |
| HPMC 6cp / | PVP K30 / SLS | 1.0 / 0.5 / 0.1 | 236.8 | 232.0 | 548.1 |
| HPMC 6cp / | PVP K90 / SLS | 1.0 / 0.5 / 0.1 | 208.2 | 209.7 | 406.6 |

**Table S5.** Particle size of resveratrol nanosuspensions prepared using various resveratrol concentrations in Transcutol HP and various ratios of solvent/antisolvent using PVP VA64/PVP K12/SLS (1.0%/0.5%/0.1%, *w*/*v*).

| Resveratrol concentration in Transcutol HP | Ratio of solvent/anti-solvent | Resveratrol in nanosuspension | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|---|---|---|---|---|---|
| 100 mg/mL | 1/9 | 10 mg/mL | 140.3 | 101.4 | 1180.9 |
| 100 mg/mL | 1/19 | 5 mg/mL | 44.7 | 60.0 | 165.6 |
| 200 mg/mL | 1/19 | 10 mg/mL | 1293.5 | 1792.1 | 34516.2 |
| 200 mg/mL | 1/39 | 5 mg/mL | 225.7 | 117.2 | 2780.4 |

**Table S6.** Updated risk assessment of resveratrol nanosuspension after preformulation and screening study.

| CQAs | Parameters | Risk level | Justification |
|---|---|---|---|
| Particle size (z-average, d90) | Resveratrol concentration | Low | In a preliminary experiment, the resveratrol concentration was fixed at 100 mg/mL to satisfy the define QTPP. Therefore, the risk level was reduced to low. |
| | Stabilizer type | Low | Based on preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low. |
| | Solvent type | Low | Based on preliminary experiments, Transcutol® HP was selected as a solvent. Therefore, the risk level was reduced to low. |
| | Ratio of solvent/anti-solvent | Low | Based on preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19 to satisfy the define QTPP. Therefore, the risk level was reduced to low. |
| | Mixing speed | Medium | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at a mixing speed of 750 rpm. However, the mixing speed can still affect particle size. Therefore, the risk level was reduced to medium. |
| | Mixing time | Low | In preliminary experiments, the effect of mixing time on the particle size distribution was insignificant. Therefore, the risk level was reduced to low. |
| | Injection rate (solvent) | Medium | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at an injection rate of 1.0 mL/min. However, the injection rate can still affect the particle size. Therefore, the risk level was reduced to medium. |
| | Temperature | Medium | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at 25°C. However, the temperature can still affect the particle size. Therefore, the risk level was reduced to medium. |

| | | | |
|---|---|---|---|
| Zeta Potential | Stabilizer type | Low | In preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low. |
| | Stabilizer concentration | High | Depending on the concentration of stabilizer, the surface charge of the particles is different. Therefore, the risk level was high. |
| | Ratio of solvent/anti-solvent | Low | In preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19 to satisfy the define QTPP. Therefore, the risk level was reduced to low. |

**Table S7.** Summary of results of regression analysis for the fitted model of the full factorial design.

| Response | $R^2$ | Pred. $R^2$ | PRESS | %CV | p-value | Remark |
|---|---|---|---|---|---|---|
| $Y_1$ | 0.9901 | 0.4876 | 58.36 | 1.50 | 0.0295 | Significant |
| $Y_2$ | 0.9912 | 0.9271 | 7.70 | 0.41 | 0.0028 | Significant |
| $Y_3$ | 0.7011 | 0.1557 | 47.78 | 1.18 | 0.0882 | Not significant |
| $Y_4$ | 0.4176 | −0.5642 | 73.12 | 1.44 | 0.4009 | Not significant |
| $Y_5$ | 0.3285 | −0.0400 | 1166.64 | 5.90 | 0.1067 | Not significant |
| $Y_6$ | 0.9959 | 0.9602 | 7.60 | 0.26 | 0.0122 | Significant |
| $Y_7$ | 0.3503 | −0.1103 | 517.28 | 2.55 | 0.0932 | Not significant |
| $Y_8$ | 0.7269 | 0.4196 | 168.17 | 1.36 | 0.0204 | Significant |
| $Y_9$ | 0.9496 | 0.7110 | 10.73 | 1.86 | 0.0074 | Significant |
| Regression equation of the fitted model | | | | | | |
| $Y_1 = 49.97 + 0.97X_1 - 2.12X_2 - 0.93X_3 - 0.92X_1X_2 - 1.12X_1X_3 - 2.39X_2X_3$ | | | | | | |
| $Y_2 = 134.54 - 0.10X_1 - 2.28X_2 - 0.16X_3 - 1.85X_1X_2 - 2.10X_2X_3$ | | | | | | |
| $Y_3 = 155.63 - 0.03X_1 - 0.72X_2 - 2.11X_1X_2$ | | | | | | |
| $Y_4 = 162.25 + 0.17X_1 - 0.15X_2 - 1.55X_1X_2$ | | | | | | |
| $Y_5 = 175.88 + 6.79X_1$ | | | | | | |
| $Y_6 = 241.87 + 1.50X_1 + 0.71X_2 - 1.96X_3 - 0.85X_1X_2 + 4.05X_1X_3 + 0.30X_2X_3$ | | | | | | |
| $Y_7 = 258.09 - 4.52X_3$ | | | | | | |
| $Y_8 = 266.96 + 4.74X_2 + 1.97X_3$ | | | | | | |
| $Y_9 = -36.74 - 0.90X_1 - 0.10X_2 - 0.22X_3 + 1.88X_2X_3$ | | | | | | |

$R^2$, coefficient of determination; PRESS, predicted residual error sum of squares; CV, coefficient of variation.

**Table S8.** Predicted values (95% prediction interval) for responses ($Y_1$–$Y_9$).

| Response | 95% PI (low) | 95% PI (high) | Predicted mean | Actual mean |
|---|---|---|---|---|
| $Y_1$ | 35.4 | 58.1 | 46.7 | 46.3 |
| $Y_2$ | 134.3 | 149.3 | 141.8 | 139.2 |
| $Y_3$ | 149.0 | 169.6 | 159.3 | 154.6 |
| $Y_4$ | 167.5 | 183.7 | 175.6 | 169.7 |
| $Y_5$ | 147.6 | 169.4 | 158.5 | 157.7 |
| $Y_6$ | 241.1 | 260.0 | 250.5 | 255.0 |
| $Y_7$ | 234.2 | 281.6 | 257.9 | 252.9 |
| $Y_8$ | 240.3 | 286.8 | 263.5 | 250.9 |
| $Y_9$ | −43.37 | -35.45 | −39.41 | −38.02 |

PI, prediction interval.

**Table S9.** Updated risk assessment of resveratrol nanosuspension after optimization study.

| CQAs | Parameters | Risk level | Justification |
|---|---|---|---|
| Particle size (z-average, d90) | Resveratrol concentration | Low | Based on a preliminary experiment, the resveratrol concentration was fixed at 100 mg/mL. Therefore, the risk level was reduced to low. |
| | Stabilizer type | Low | In preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low. |
| | Stabilizer concentration | Low | The particle size distribution in the optimized nanosuspension satisfies the set target range. Therefore, the risk level was reduced to low. |
| | Solvent type | Low | Based on preliminary experiments, Transcutol® HP was selected as a solvent. Therefore, the risk level was reduced to low. |
| | Ratio of solvent/anti-solvent | Low | Based on preliminary experiments, the ratio of solvent/anti-solvent was fixed at 1/19. Therefore, the risk level was reduced to low. |
| | Mixing speed | Low | The particle size distribution of the nanosuspension prepared at 500 rpm–1000 rpm was within the set target range. Therefore, the risk level was reduced to low. |
| | Mixing time | Low | Based on preliminary experiments, the effect of mixing time on the particle size distribution was insignificant. Therefore, the risk level was reduced to low. |
| | Injection rate (solvent) | Low | The particle size distribution of the nanosuspension prepared at an injection rate of 1.0 mL/min was within the set target range. Therefore, the risk level was reduced to low. |

| | | | |
|---|---|---|---|
| | Temperature | Low | The particle size distribution of the nanosuspension prepared at 20°C ~ 30°C was within the set target range. Therefore, the risk level was reduced to low. |
| Zeta Potential | Resveratrol concentration | Low | The effect of changes in the resveratrol concentration on the zeta potential is insignificant. Therefore, the risk level was low. |
| | Stabilizer type | Low | Based on preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low. |
| | Stabilizer concentration | Low | In the optimized nanosuspension, the zeta potential value was -32.9 mV to -39.6 mV and satisfied the target range. Therefore, the risk level was reduced to low. |
| | Solvent type | Low | The influence of the type of solvent on the surface charge of the particles was insignificant. Therefore, the risk was low. |
| | Ratio of solvent/anti-solvent | Low | Based on preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19. Therefore, the risk was reduced to low. |
| | Mixing speed | Low | The effect of mixing speed on the zeta potential was insignificant. Therefore, the risk was low. |
| | Mixing time | Low | The effect of mixing time on the zeta potential was insignificant. Therefore, the risk was low. |
| | Injection rate (solvent) | Low | The effect of injection rate on the zeta potential was insignificant. Therefore, the risk was low. |
| | Temperature | Low | The effect of temperature on the zeta potential was insignificant. Therefore, the risk was low. |
| Drug content | Resveratrol concentration | Low | Resveratrol is chemically stable when light is blocked. Nanosuspensions were prepared in a space where light is blocked, and the possibility of drug loss during the manufacturing process is insignificant. Therefore, the risk level was reduced to low. |
| | Stabilizer type | Low | |
| | Stabilizer concentration | Low | |
| | Solvent type | Low | |
| | Ratio of solvent/anti-solvent | Low | |
| | Mixing speed | Low | |
| | Mixing time | Low | |
| | Injection rate (solvent) | Low | |
| | Temperature | Low | |

**Table S10.** Summary of long-term stability test results for the optimized resveratrol nanosuspension.

| Days | Response | | | |
|---|---|---|---|---|
| | Particle size (z-average, nm) | Particle size (d90, nm) | Zeta potential (mV) | Drug content (%) |
| 0 | 46.3 | 157.7 | −38.02 | 100.02 |
| 1 | 139.2 | 255.0 | N.D[a] | N.D[a] |
| 3 | 154.6 | 252.9 | N.D[a] | N.D |
| 7 | 169.7 | 250.9 | N.D[a] | N.D |
| 30 | 178.7 | 302.9 | −37.61 | 99.62 |
| 60 | 189.8 | 311.3 | N.D[a] | 99.21 |
| 90 | 197.9 | 312.1 | N.D[a] | 98.87 |
| 120 | 204.4 | 311.1 | N.D[a] | 98.55 |
| 150 | 209.4 | 320.7 | N.D[a] | 98.14 |
| 180 | 212.6 | 321.0 | −37.29 | 97.81 |

[a]N.D indicates that data cannot be measured and there is no data.