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# Effect of gamma-irradiation on in-vitro plasma compatibility of nanosuspension formulation

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**Introduction** : *Nanosuspension is an alternative dosage form for formulation of low solubility or insoluble drugs intended for intravenous administration. However, an appropriate sterilization process needs to be considered since the traditional filtration method may not be applicable. In this study, lyophilized nanosuspension formulation is developed for an anticancer compound. Gamma irradiation is performed for sterilization of the product. In vitro plasma compatibility of the formulations before and after gamma irradiation process is evaluated under a condition mimic to intravenous injection.*

**Objective** : *To study the effect of gamma irradiation on sterilization of in vitro plasma compatibility of nanosuspension formulation intended for intravenous infusion.*

**Design** : *In vitro experimental study.*

**Methods** : *Nanosuspension formulations, with and without gamma irradiation, were diluted with 0.9 % w/v NaCl to make a concentration of 1.5 mg/mL and then further diluted with plasma at ratio 1:1. After mixing with plasma, all samples (at 0, 5, 10, and 20 minutes) were observed under microscope for particle aggregation.*

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**Results** : *In most cases, the aggregation increases as a function of time at 37°C, and starts decreasing after 10 minutes. Gamma irradiation for sterilization has no effect on the aggregation of nanosuspension molecules after mixing with plasma.*

**Conclusions** : *Gamma irradiation used for sterilization of nanosuspension formulation intended for IV injection has no effect on the aggregation of drug molecule after mixing with plasma.*

**Keywords** : *Nanosuspension, Gamma irradiation, Plasma compatibility, Aggregation.*

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**พรอนงค์ อร่ามวิทย์. ผลของรังสีแกมมาที่มีต่อการเข้ากันได้ในพลาสมาของยาในรูปแบบนาโนซัสเพนชั่นที่ใช้ในการฉีดเข้าเส้นเลือดดำ. จุฬาลงกรณ์เวชสาร 2547 มี.ค; 48(3): 145 – 55**

**บทนำ** : *นาโนซัสเพนชั่นเป็นอีกทางเลือกหนึ่งของการตั้งตำรับยาที่ไม่ละลายน้ำหรือละลายน้ำได้น้อย เพื่อใช้ในการฉีดเข้าเส้นเลือดดำ อย่างไรก็ตาม กระบวนการทำให้ปราศจากเชื้อโดยวิธีการกรองอาจไม่เหมาะสมสำหรับยาฉีดในรูปแบบดังกล่าวส่งผลให้ต้องมีการค้นหาทางเลือกอื่นเพื่อใช้ในกระบวนการทำให้ปราศจากเชื้อ ซึ่งกระบวนการดังกล่าวอาจมีผลต่อการเกาะรวมตัวกันของโมเลกุลยาเมื่อมีการบริหารเข้าสู่เส้นเลือดดำ*

**วัตถุประสงค์** : *การศึกษานี้เป็นการศึกษาผลของการใช้รังสีแกมมาในกระบวนการทำให้ปราศจากเชื้อต่อสูตรตำรับยารักษาโรคมะเร็งที่อยู่ในรูปแบบนาโนซัสเพนชั่น โดยเป็นการดูผลของการเข้ากันได้ของโมเลกุลยา และพลาสมาในภาวะที่จำลองขึ้น*

**รูปแบบการศึกษา** : *เป็นการทดลองในภาวะที่จำลองขึ้น*

**วิธีการวิจัย** : *สูตรตำรับยาในรูปแบบของนาโนซัสเพนชั่นที่ได้ผ่าน และไม่ผ่านการทำให้ปราศจากเชื้อโดยรังสีแกมมาจะถูกนำมาทำให้มีความเข้มข้น 1.5 มิลลิกรัมต่อมิลลิลิตร โดยใช้น้ำเกลือที่มีความเข้มข้น 0.9 % หลังจากนั้นจะถูกนำมาผสมกับพลาสมาที่เตรียมไว้ด้วยอัตราส่วน 1:1 แล้วทำการศึกษาลักษณะของโมเลกุลยาในพลาสมาผ่านกล้องไมโครสโคปที่เวลา 0, 5, 10 และ 20 นาที ตามลำดับ*

**ผลการวิจัย** : *เมื่อเวลาผ่านไป โมเลกุลของยาจะมีการเกาะตัวรวมกันทำให้มีขนาดใหญ่ขึ้นในทุกกรณี แต่จากการสังเกตพบว่าเมื่อมีการผสมกับพลาสมาเป็นเวลานาน 10 นาทีที่อุณหภูมิร่างกาย โมเลกุลของยาที่เกาะกันอยู่จะมีขนาดลดลง นอกเหนือจากนี้การทำให้ยาปราศจากเชื้อโดยใช้รังสีแกมมาไม่มีผลทำให้การเกาะตัวกันของโมเลกุลยามากขึ้นหรือน้อยลงเมื่อเข้าสู่พลาสมา*

**สรุป** : *การใช้รังสีแกมมาในกระบวนการทำให้ปราศจากเชื้อของยาในสูตรตำรับนาโนซัสเพนชั่นไม่มีผลต่อการเกาะตัวรวมกันของโมเลกุลยาเมื่อมีการผสมรวมกับพลาสมา*

**คำสำคัญ** : *นาโนซัสเพนชั่น, รังสีแกมมา, การเข้ากันได้กับพลาสมา, การเกาะรวมตัวกันของโมเลกุลยา*

As a part of the study of toxicological effects, the studies of plasma compatibility have become a regulatory requirement for all intravenously (IV) administered drug products even before Phase I clinical studies.[1,2] This is due to the fact that there have been several problems caused by precipitation of drug molecules in blood vessels upon IV injection, especially water-insoluble drugs formulated with non-aqueous vehicle.[3,4] The precipitated drug particles may lead to severe adverse events, such as phlebitis, thrombosis or even death.[5,6]

There are several methods already developed to assess the potential precipitation of drugs from true solution formulations upon mixing with blood. [4,7-9] The key variables controlling the precipitation process include the drug solubility in the plasma, the concentration of the drug in the formulation during infusion, infusion rate and the rate of blood flow in the injected vein. Nanosuspension formulation is an alternative for poorly soluble drugs. This dosage form has been used for intravenous administration for several preparations. [10,11] However, there is no report on models to evaluate the plasma compatibility of a nanosuspension formulation intended for IV injection. When a nanosuspension formulation is being infused by IV, the drug precipitation is no longer an issue because the drug is already in the solid form. Normally, the minimum diameter of individual particles in nanosuspension is about 5 micrometers which is small enough to go through capillary vessels.[12] The main issue is the aggregation of drug particles since the aggregation of drug particles may form significant size and cause blocking effect which leads to cell damage.

Gamma irradiation is a potential sterilization technique for suspensions intended for IV injection.[13] However, this sterilization process may lead to severe problems after infusion of the drug into circulation. Several reports indicate chemical degradation, cross linking and, the most common effect, the aggregation of drug molecules happen after gamma irradiation.[14-16] On the condition that gamma irradiation is contraindicated, other sterilization methods which are more complicated and less cost-effective may be applied.

Compound A ($C_{15}H_{14}N_2O$), an aromatic and heterocyclic compound, is intended for anticancer use. The compound is selected as a drug model in this study due to its extremely low solubility (<10 ng/mL) in water or other pharmaceutically acceptable solvents, and its non-ionize, highly crystalline solid state. Compound A was formulated as Nanocrystal Colloidal Dispersion (NCD) intended for IV injection by wet milling process in the presence of certain stabilizers and then lyophilized. The final product was sterilized by gamma irradiation. Upon IV infusion, the lyophilized cake was reconstituted with sterile water for injection then further diluted with 0.9 % sodium chloride (NaCl) solution. In this paper, we evaluated the effect of gamma irradiation and the incubation time as contributing factors for the particle aggregation upon mixing with plasma.

## Material and Method

### Materials

All materials were used as received from the suppliers. Lyophilized compound A NCD with (10 kGy) and without gamma irradiation were obtained from a contracting lab. Sterile water for injection, USP (lot # 332028) was from American Pharmaceutical Partners, Inc., Schaumburg, IL. Normal saline (0.9 % NaCl)

solution for injection, USP (lot # 95-209-JT) was manufactured by Abbott Laboratories, North Chicago, IL.

**Preparation of human plasma**

Human plasma was obtained from human whole blood, collected from a 29-year-old healthy male volunteer, in heparin-containing tubes. After gently mixing with heparin, the whole blood sample is centrifuged at 1500G for 5 min and the supernatant is removed. The plasma is kept at 37°C, shaken occasionally, and is used within 6 hours.

**Dosing suspension preparation**

Lyophilized compound A NCD with and without gamma irradiation were reconstituted with sterile water for injection to make a concentrated stock of nanosuspension (50 mg/mL). This stock of nanosuspension was further diluted with 0.9 % w/v NaCl solution to concentrations of 1.5 mg/mL.

**Plasma compatibility test**

The dosing suspension prepared above was first mixed with the plasma at an appropriate ratio and then the mixtures were incubated at 37°C. The mixtures were examined under a microscope (Zeiss Universal Pol, Plan Fluor lens 20 X 0.4 NA Objective) for particle aggregation at 0, 5, 10, 20 minutes. Photomicrographs of three views per sample were taken to ensure representative assessment of the particle aggregation.

**Results**

The nanosuspension is expected to be injected through the antecubital vein, the site often used for drawing blood or drug infusion. [17] The recommended dose of the compound is 145 mg/m$^2$, and the intended rate of infusion is 300 mg/hr or 200 mL/hr for 1-2 hours. Based on the targeted dose and dosing time, the concentration of 1.5 mg/mL of dosing suspension is selected for evaluation. Since the formulation is intended for slow infusion, mixing drug with plasma is estimated at 1:1 ratio. Because the nanosuspension particles will not be diluted immediately upon injection, the assessment of the particle aggregation in the suspension/plasma mixture is carried out over time to simulate the *in vivo* situation.

In general, different levels of aggregation were observed in all conditions even at time zero. Figure 1-4 showed the photomicrographs of compound A nanosuspension with no gamma irradiation in normal saline solution as a function of time, from time zero to 20 minutes. The average size of the drug molecule at time zero was about 5 μm which was then increased to about 20 μm after 10 minutes of incubation. However, the size of aggregated particle became much smaller, similar to the size at the initiation stage, after 20 minutes of incubation. Figure 5-8 indicated the photomicrographs from the same conditions of nanosuspension with gamma irradiation. The average size of the drug particle at time zero and after incubation for 10 and 20 minutes were also similar to the case of formulation without any gamma irradiation. The average size of the drug particle at time zero and after incubation for 10 and 20 minutes were 5 μm, 10 μm and 5 μm, respectively; they were similar to the formulation that had no gamma irradiation. There is no significant difference between the sizes of the particle of the two formulations at time zero, 5, 10 and 20 minutes ($p>0.05$).

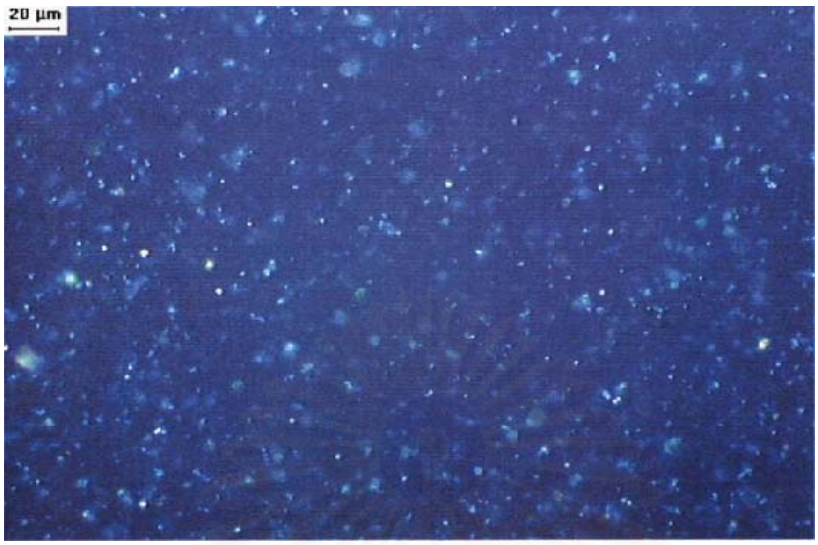


**Figure 1.** Compound A nanosuspension reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at time zero.


**Figure 2.** Compound A nanosuspension reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 5 minutes.

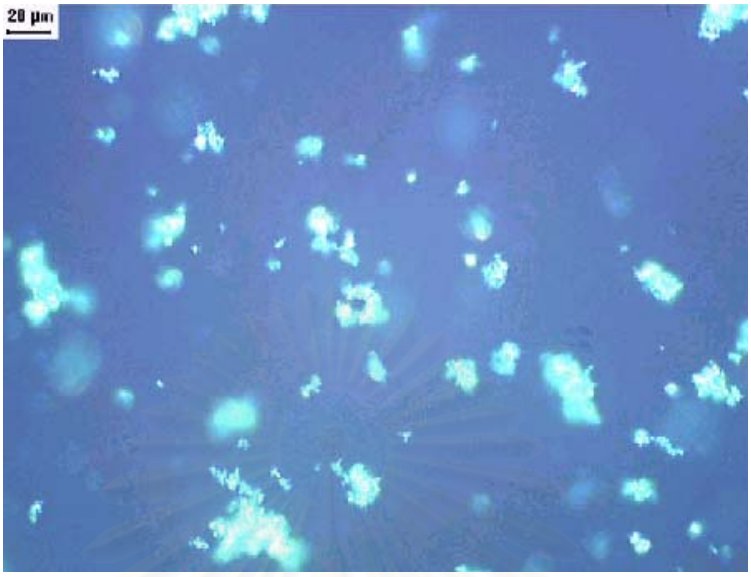


**Figure 3.** Compound A nano suspension reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 10 minutes.

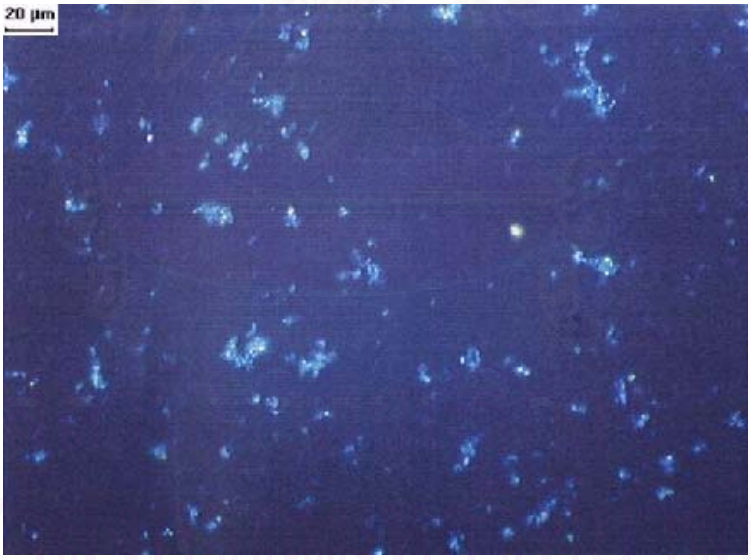


**Figure 4.** Compound A nanosuspension reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 20 minutes.

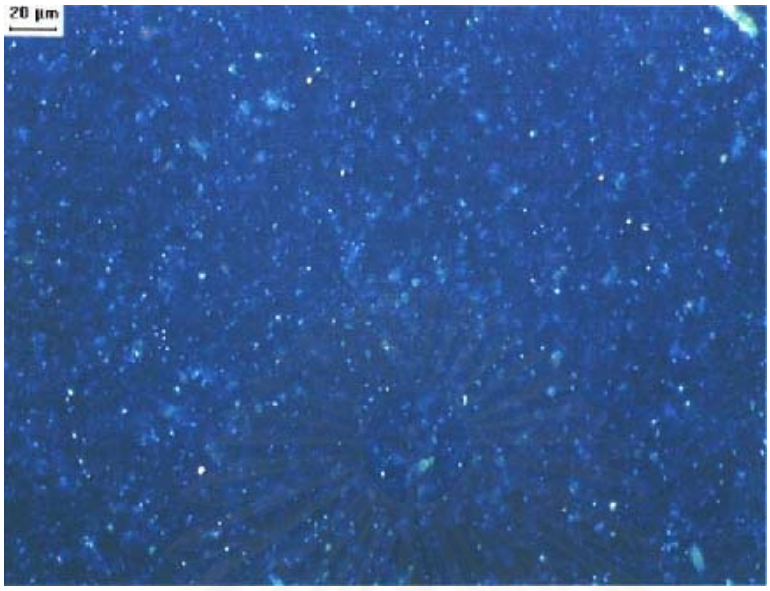


**Figure 5.** Compound A nanosuspension formulation with gamma irradiation reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug:plasma = 1:1 incubated at 37°C at time zero.

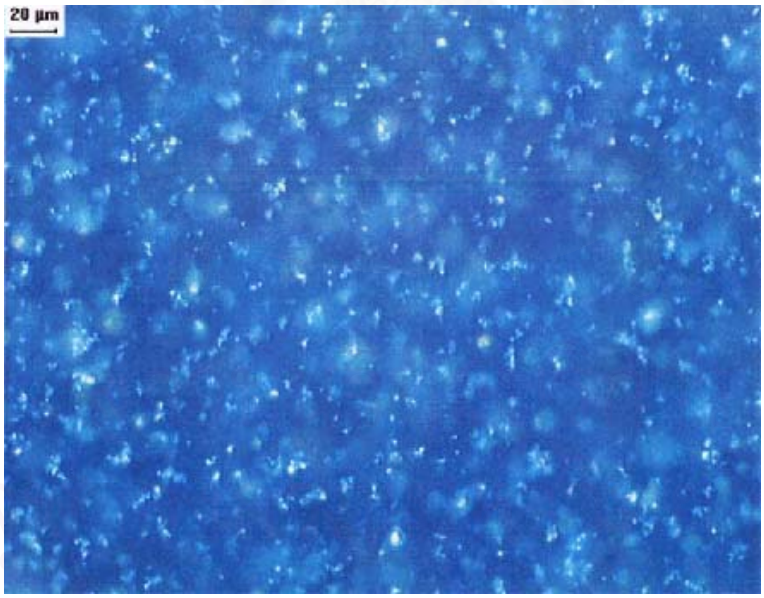


**Figure 6.** Compound A nanosuspension with gamma irradiation reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 5 minutes.

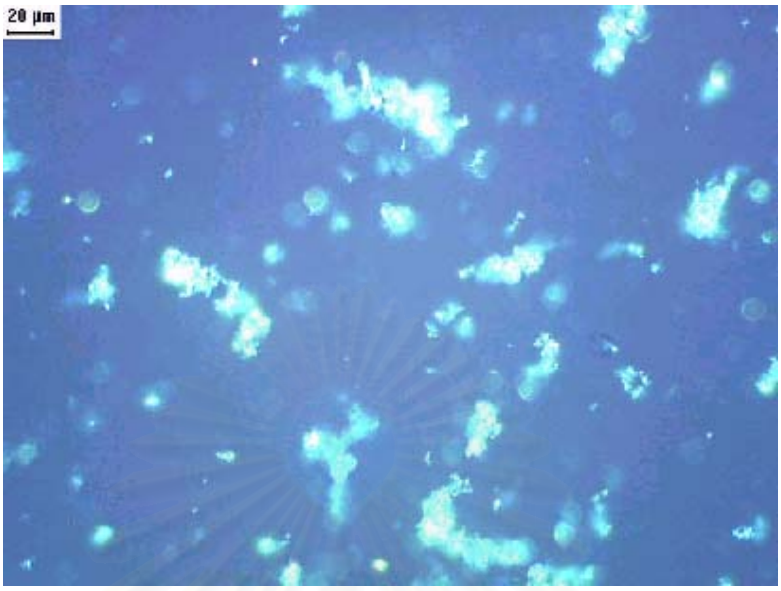


**Figure 7.** Compound A nanosuspension with gamma irradiation reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 10 minutes.

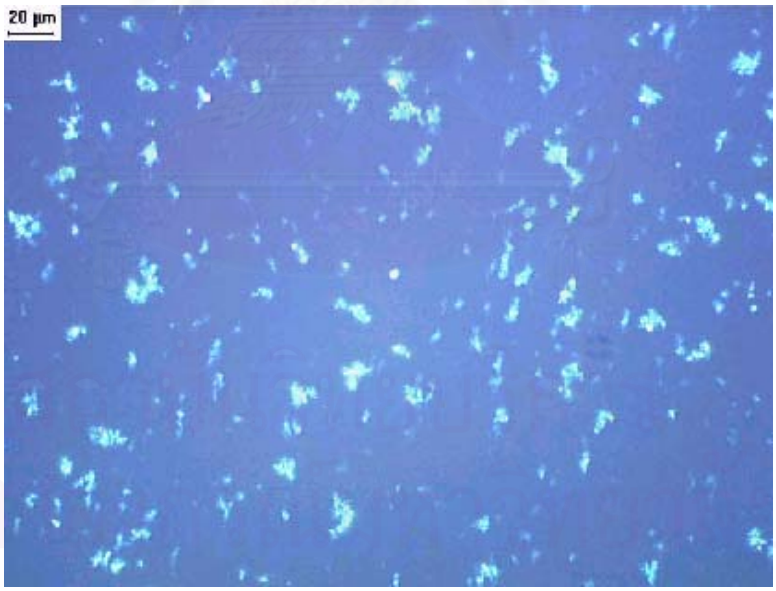


**Figure 8.** Compound A nanosuspension with gamma irradiation reconstituted with 0.9 % NaCl to 1.5 mg/mL and further diluted with plasma at the ratio of drug: plasma = 1:1 incubated at 37°C at 20 minutes.

## Discussion

The results from both formulations, with and without gamma irradiation, indicated that aggregation increased as a function of time, but start decreasing after 10 minutes. This is probably due to desorption of the stabilizers of the formulations from the particle surface, leading to initial increase in aggregation, followed by adsorption of new stabilizers in the plasma, such as human albumin, leading to a decrease in aggregation. Furthermore, the aggregation pattern did not show significantly difference between formulations with and without gamma irradiation. This suggests that gamma irradiation cause no aggregation in nanosuspension particles and can be used for sterilization process of compound A nanosuspension formulation and may be applied for other nanosuspension formulations intended for IV injection as well. During the microscopic evaluation, it was observed that all the aggregates were “soft” aggregates, which can be easily broken down by mechanical force such as tapping on the slides. These aggregates should not have difficulty passing through blood vessel and become monomeric much easier than “solid” aggregates.

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