

QUALITY CONTROL OF INJECTABLE FAT EMULSION USING A COULTER COUNTER®

INTRODUCTION

Many injectable drugs, drug carriers, and imaging agents for clinical diagnostic equipment are in the form of emulsion. For example, nutritional supplement fat emulsion for parenteral nutrition, brucea javanica oil emulsion injection, vitamin E emulsion injection, coix lacryma-jobi seed oil injection, ultrasonic diagnostic emulsion, etc. Because these products directly enter the circulatory system through intravenous injection and are absorbed through micro-circulation, the stability and non-toxicity of these products have to be well controlled according to regulatory standards. The correct choice of emulsion particle analyzer is one of the key factors in quality control of these products.

FUNCTION OF FAT EMULSION

One type of important intravenous emulsions is fat emulsion. Most clinical fat emulsions use soybean oil as raw material. Other vegetable oils are also used, e.g., some fat emulsions that contain olive oils have been developed lately.

Fat emulsions with purified soybean oil-in-water as the main component have the advantages of solvency and non-toxicity. Such fat emulsion is an excellent nutritional supplement for parenteral nutrition. In addition, since it is highly dispersed and easily to be absorbed by human body, it can be

used as a carrier for targeted drug delivery or as a tracer for in vivo target allocation. More and more practices use fat emulsion as the carrier in intravenous injection to achieve better release, controlled release, or increase targeting for non-dissolvable drugs or drugs that cannot be directly injected intravenously.



Fat emulsions are commonly used in clinical as the carrier for dexamethasone, neuroleptic drugs, and propofol. Other intravenous injection drugs use fat emulsion as the carrier include diazepam, etomidate, PGE1, dexamethasone palmirate, fat dissolvable vitamins, and alprostadil. Many injectable emulsions are modifications of Intralipid, Liposyn II, Lipofundin fat emulsions. There are still more drugs, such as bupivacaine hydrochloride, tetrandrine, garlic oil, paclitaxel, itraconazole, amphotericin B, tirilazad, etc., being researched as candidates to be carried by fat emulsion through intravenous injection.

PROPERTY AND QUALITY REQUIREMENT OF FAT EMULSION

Emulsion is a thermodynamically instable and heterogeneous dispersion system. Its physical destabilization often results in separation, flocculation, phase transition, coalescence and rupture, and spoilage that may change emulsion particles and many properties of the system. Reducing globule size and maintaining uniform size within defined limits are the key approach to increase emulsion stability.



In addition to its stability, fat emulsion used in intravenous injection must be assured safety to recipients. Especially true is the globule size limits that have the greatest implications for infusion safety. The major safety issues involving injectable fat emulsions include impairments in plasma clearance in susceptible patients, and the infusion of an unstable emulsion containing large quantities of potentially embolic fat globules. Human blood capillaries have internal diameters of 6-8 μm . If too many fat globules larger than 5 μm are injected, the infused globules may lodge into the pulmonary capillaries and produce a fat embolism syndrome. In the earlier days of researching formulation for fat emulsion, globule size was not strictly controlled. During animal tests, because large fat globules blocked blood capillaries it resulted in

pulmonary edema to the test animals. Recent animal studies investigating the toxicity from the infusion of unstable injectable fat emulsions have shown evidence of oxidative stress and tissue damage to the liver when recommended globule size limits exceed 5 μm . After extensive experiments and clinical trials, it has been found that the optimum particle size for fat emulsion is in the range of mini-emulsion with diameters between 0.1 to 0.5 μm . These particles are then similar to human chyle particles in size (< 0.5 μm) and their osmotic pressure (310-350 mmol/L) and pH (8.0) are almost the same as human blood plasma.

An emulsion is normally analyzed for the following properties:

- 1) fat globule size
- 2) layer separation trend
- 3) coalescence rate
- 4) stability constant
- 5) viscosity

INDUSTRIAL STANDARD

There exist standards and designated measurement technology for this type of special medicine formulation (fat emulsion). In the Chinese Pharmacopoeia 2005, Volume 2, Chapter 6, it specifies that globules in fat emulsion injection need to "...use a counter to measure; most particles should have a size about 0.5 μm . Among particles larger than 0.5 μm , there should be no more than 3% of particles larger than 1 μm . There should be no particles larger than 5 μm ."

Despite such broad use, no pharmacopeia standards exist with respect to the optimal pharmaceutical characteristics of the formulation in the USA.

Every enterprise manufacturing and marketing this type of product must use

a counter to analyze each lot and provide reports to show that the product meets the pharmacopoeia standard. If it is a new formulation or a new product, samples have to be submitted to a regulatory agent such as a provincial institute for drug control for the approval before being produced in large scale and marketed. For example, in China, Guangdong Institute for Drug Control uses a Coulter Counter Multisizer 3 from Beckman Coulter to analyze fat emulsion injection samples, submitted from pharmaceutical companies, according to the standard set by the Chinese Pharmacopoeia.



WHY USING A COULTER COUNTER

According to the Chinese Pharmacopoeia 2005, the instrument used for quality assurance of fat emulsion has to be a counter, not a particle size analyzer because it has to assure that there is no particle larger than 5 μm . It has to be an absolute particle number measurement not relative size distribution measurement.

Fat emulsion injection is a nutritional supplement used intravenously. It is very important that there are no particles larger than 5 μm . Coulter Counter can be used to sensitively and accurately detect existence of any 5 μm or larger particle. Other methods, such as laser diffraction and photon correlation spectroscopy, cannot be used as the

standard means for quality control since measurements using these technologies are affected by number, color, shape, other optical properties of particles and none of these technologies can achieve the resolution of Coulter Counter. More significantly are these technologies not directly detecting particles but deriving particle size under theoretical assumption from measured signal.

For example, even though laser diffraction has a broad dynamic measurement range from tens of nanometer to a few millimeters, the entire process is based on optical theory and mathematical modeling. One has to make several assumptions, e.g., particles have to be spherical and no multiple scattering occur, and uses matrix conversion or functional fitting to obtain relative particle size distribution. When there are particles larger than 5 μm but the total amount is very small, laser diffraction measurement often will miss them and give a wrong message of large particle absence. This is dangerous to intravenous products. Laser diffraction does not measure particle individually so it cannot tell yes or no for the presence of small amount of large particles. When it reports the presence of large particles, it won't tell how many; when it reports no large particles, often there are.

The same is true for photon correlation spectroscopy. It 'derives' particle size and size distribution using scattering theory from the measured ensemble signal. It is an intrinsically low resolution technology limited for submicron particles. It cannot measure absolute particle number. Therefore it cannot be used in detecting a few large particles in the sample even though it may be used in formulation and stability study of fat emulsion particle size distribution.

The Coulter Principle (electrical sensing zone method) utilizes the proportionality between the volume of a particle and the height of the electric pulse generated from passage of the particle through a small aperture to directly count it and measure its size [1]. The measurement is not affected by color, shape, and optical characteristics of particles. It can detect particles even in extremely low concentration, which is especially advantageous to measure emulsion injection with very few particles.

APPLICATIONS OF COULTER COUNTER

Beckman Coulter, Inc., as the inventor of Coulter Principle, has been serving particle characterization field for more than half a century. During the past fifty plus years, there have been more than twenty models of Coulter Counter developed and marketed. The latest Coulter Counter Multisizer 3 uses digital pulse processing technology, which enables the instrument to more accurately record and analyze every electrical pulse. It also enables the user monitoring sample changes, such as flocculation, decomposition, and cell size change, through pulse analysis.

As the global leader of medicinal nutrition supplements research and manufacture, Sino-Swed Pharmaceutical has been using Coulter Counter as the major means in product research and quality control since its establishment in China. The presence of particles larger than 5 μm in the products is beyond the issue of emulsion stability; it is more importantly a concern to the safety of patients. Sino-Swed Pharmaceutical fully realizes that Coulter Counter is the best means to directly detect absolute number of particles, not through model conversion of measured signals. Therefore it has always been using Coulter Counter as

the irreplaceable choice in quality control, from the earlier models, such as TA II, Multisizer IIe, to the latest model Multisizer 3.



In China, many major fat emulsion manufacturers, such as Sino-Swed Pharmaceutical, Guangzhou Qiaoguang Pharmaceutical, and Mitsubishi Pharma (Guangzhou), and many other pharmaceutical companies, such as Chongqing Yaoyou Pharmaceutical, Zhejiang Kanglaite Pharmaceutical, and Shanghai Xinxing Medicine, use Coulter Counter as their major means for quality control and R&D of fat emulsion products.

REFERENCE

- [1] International Standard ISO 13319: Determination of particle size distributions — Electrical sensing zone method

