Small World

by John Garner

A novel approach to microparticles and nanoparticles

Chapter 1

Nicole Klein shifted nervously as she rode the elevator up towards her arranged meeting. The elevator dinged softly as it arrived to the appropriate floor. A man stood waiting just outside it. She nodded slightly as they shook hands. Mike Rometer gave her a slight smile and said "Miss Klein, thanks for joining me for a tutorial. I see you came dressed the way I told you to." She replied "Absolutely, closed-toe shoes with full-length pants. No loose clothing and nothing flammable." Mike replied "Great, you're ready for the lab."

Mike said "I'm excited to show this to you. Have you ever worked with anything in the micro to nano scale before?" Nicole shook her head "No. I've heard of them, of course, but never really worked with them before." Mike handed over a lab coat which they dawned along with safety glasses. "I still don't understand how you can work with and even fabricate something smaller than what you can see. It's almost like magic." Mike chuckled "Ahh… you will soon see there is no magic at all. Unless you consider interfacial tension and controlled nucleation magical. Fabricating microparticles and nanoparticles is actually quite simple. I'll show you more inside."

Mike opened the lab doors revealing rows of black-epoxy topped benches laying under overhead fluorescent lights. Along one wall, ventilated fume hoods hummed away busily sending potentially hazardous chemicals to disperse in the outside world down to safe concentrations. Mike said "So, which would you like to learn about first. Microparticles or nanoparticles." Nicole replied "What's the difference?" Mike said "Honestly, it is simply an issue of scale. As you know we start with meter and then $1/100^{th}$ is a centimeter and $1/1000^{th}$ is a millimeter. A micrometer is $1/1000^{th}$ of a millimeter and a nanometer is even smaller, being $1/1000^{th}$ of a micrometer. To give you an idea a typical human cell is 30 µm in diameter. So, by comparison, to a nanosized object a human cell would be as large as a house but for a micro-sized object it would be about the same size to slightly smaller. This is a critical distinction. Nanoparticles can easily penetrate into cells. Cells can also uptake microparticles as well by endocytosis, but to a much lesser extent." Nicole shrugged and said "Well, I guess we'll start big and work our way down."

Mike said "Great. We'll start with a fairly simple emulsion microparticle technique." He reached into his freezer and pulled out a jar marked "Poly(lactide-co-glycolide) (75:25 LA:GA Mn 75,000-85,000) PolyVivo AP125" He withdrew some pieces of the polymer and weighed them out, dissolving one gram of the polymer in 10 ml of dichloromethane. Nicole asked "Why dichloromethane?" Mike replied "Several reasons, mostly because of its unique properties. First off, it's a good solvent for a wide range of polyesters of this type including not only poly(lactide-co-glycolide, or PLGA, as well as poly(DL lactide), or PDLLa, but also the highly crystalline Poly(L-lactide), or PLLA. Second off, it is highly hydrophobic so when mixed with water it will tend to separate away from the water. Finally, with a boiling point of only 39 C, it is highly volatile so it will easily evaporate away." Mike motioned towards a bottle of polyvinyl alcohol mowiol 4-88 and said "Please make a 0.5% w/v solution of the PVA mowiol 4-88 in water for me." He said. Nicole asked "How much do you need?" Mike replied "About 1 L." Nicole blinked "Really? That much?" Mike nodded "Oh yes, for a proper emulsion there always needs to be a large excess of the aqueous phase." Nicole prepared the solution using some heating to get the PVA to dissolve well.

She returned and saw Mike put the PVA under an overhead stirrer. Mike said "There's many different ways to do an emulsion, so, for today, I'll show you a very simple and conventional method. Typically the exact emulsion method used is optimized to generate microparticles for drug delivery applications." Nicole chuckled "I thought

drug-delivery was illegal." Mike rolled his eyes "Not that kind of drug-delivery. When we talk about drug delivery here, I mean a carrier system that allows for a pharmaceutical agent to be injected, eaten, or transferred into the patient in such a way that it has some therapeutic effect. Different medicines require different kinds of delivery. For instance, Risperdal Consta is a PLGA microparticle that contains risperidone. It allows for a single monthly injection to maintain clinical effect.

Mike turned on and carefully adjusted the overhead stirrer. He said "For a hydrophobic drug like risperidone, it can be dissolved directly into the polymer organic solvent. Hydrophilic drugs require what is called a double emulsion, where the water soluble drug is emulsified into the polymer solution first and then that emulsion is emulsified with water." As the overhead stirrer spun, the soapy, PVA solution took on a bubbly appearance and a steady vortex formed. Mike pipetted the PLGA dichloromethane solution in and Nicole watched as the PVA solution turned white. "It looks like milk." She said. Mike nodded "Yes, milk is a good example of an emulsion. As you know, oil and water don't mix. Here, too, dichloromethane and water don't mix. Without the PVA surfactant and the rapid stirring, we would just have a layer of DCM sink to the bottom of the water layer, but, with enough stirring and some surfactant, the DCM forms tiny micro-sized bubbles inside the water layer. Over time, the DCM partitions out into the water, not much but a little, and then evaporates off the top leaving the polymer behind inside the microsized bubble." Nicole's eyes lit up "And, that's what forms the microparticle!"

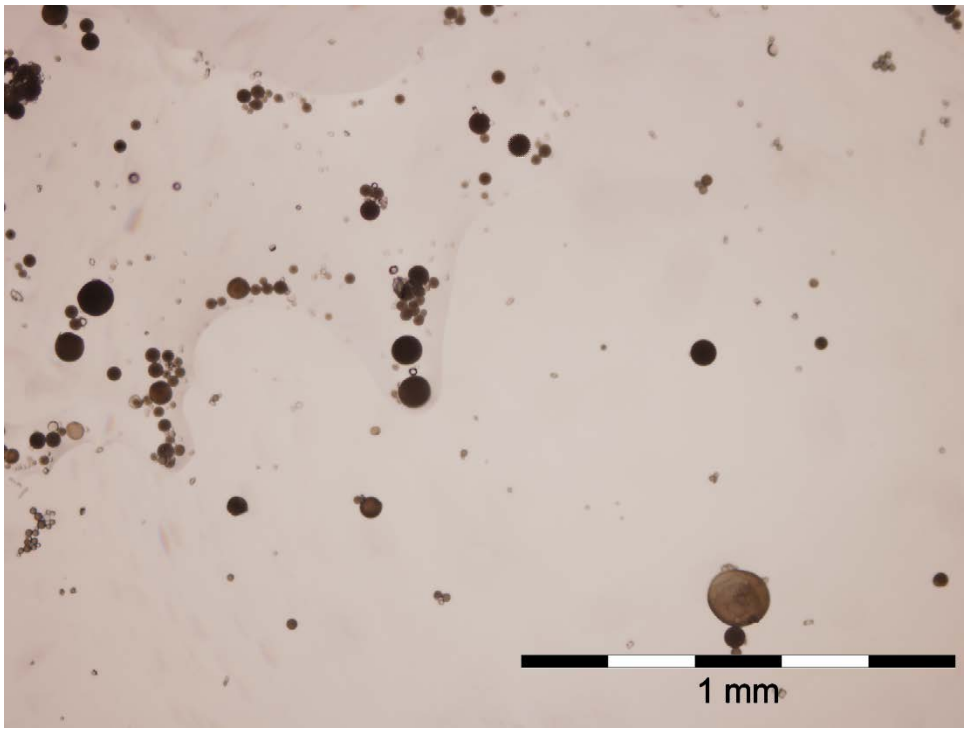
Mike nodded "Exactly. This, of course, is just one of a plethora of techniques to form microparticles. There's homogenization, static mixing, template based, and microfluidic techniques, to name a few." Nicole asked "Is it always PLGA, or some other polyester like it?" Mike shook his head. "No, microparticles can be made by many different polymers including polysaccharides like chitosan which form the particles based on charge interactions."

Nicole seemed impressed "Wow, micro can do so much." Mike chuckled "Yes, for our particular batch, it has been stirring for some time and so is ready for next step. For a real batch there could be many processes between formation and collection such as forced air drying, heated annealing, or other treatments. Here, however, we will filter and collect." Mike pulled out a piece of nylon mesh "This is simplest way to filter. This particular one, readily available, has a mesh size of 200 µm." Nicole asked "Why that size?" Mike replied "Because below that is the best size for fitting down fine-gauge needles without plugging them."

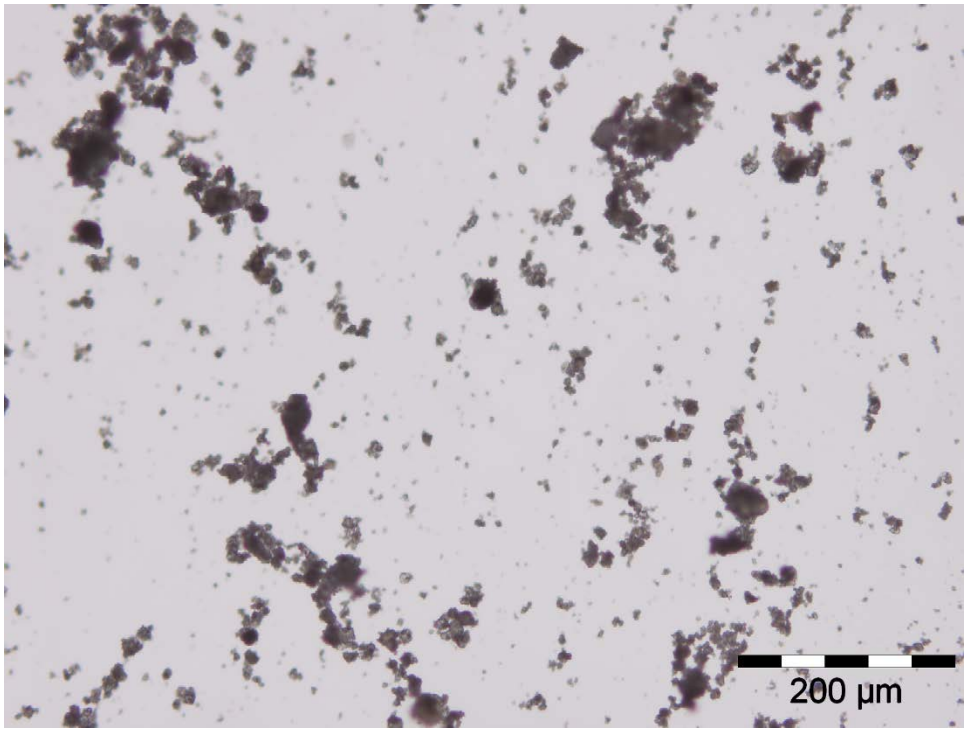
Mike held the mesh while Nicole poured the white slurry through it. She watched as a few larger chunks and pieces collected on top. Mike said "Now, we can either collect the particles by centrifuging them down, or by simply using a smaller filter to collect the particles on top." Mike pulled out another mesh. "This one has a pore size of 1 µm." Once more they filtered the slurry. Nicole watched as most the white particles collected on top while the solution which poured through still had a slightly whitish appearance, like water milk. Mike motioned towards the liquid portion and said "And there are some nanoparticles." Nicole frowned "But, I thought we were making microparticles." Mike nodded "We did. But emulsion methods create an incredibly broad range of particles. That's why filtering is important to screen out what's too big and too small, respectively."

Nicole looked at their harvest. A puddle of still slightly-wet white powder on top of the cloth. She said "So, these are the microparticles then." Mike nodded and said "Yes, in crude form. Typically, there would be some additional washing steps to remove the residual PVA and other impurities but those are the microparticles." She squinted at them "How many are there?" Mike chuckled "Thousands, millions, billions, trillions, perhaps. We stop counting with microparticles after the first few hundred and just deal with them in terms of weight." Nicole smiled "Seems reasonable. Can we see some under a microscope?"

"Sure." Mike replied. The pulled a smidge of particles off the top of the pile with a metal spatula and mixed it with a small amount of water to help disperse the particles out. Mike said "Here's a look at 4X magnification."



Nicole asked “Ok, I see it makes a pretty disperse set of small spheres, but, is this really any different than just having a really fine powder of the polymer.” Mike reached in the fridge and pulled out a different jar. Mike said “Here I have some fine-ground, low molecular weight PLLA, which I requested from PolySciTech. This one wasn’t formulated into microparticles, just ground up and passed through a small 53 µm mesh.” Nicole marveled “Wow, that’s pretty small. Can you do that with any polymer?” Mike shook his head “Not really, only crystalline types with low molecular weight will grind easily. Amorphous or high molecular weight polymers are tough and gummy so they can’t really be broken down as well. Anyhow, take a look.”



Mike said “As you can see, it is not microspheres or microparticles but rather just jagged chunks that happen to be a very small size. Microparticles at their formation undergo several complex interactions which define their properties. For well-made microparticle formulations, a great deal of effort goes into defining the conditions for optimal drug distribution throughout the particles for optimized release properties. For ground powders, however, well… they’re just ground powders with no particular distribution or surface characteristics.”

Nicole nodded “Well that makes sense.” Mike asked “Ready to go smaller?” Nicole smiled “Yes. Let’s see some nano.”

Mike said “Ok, let’s start with a dialysis technique.” Nicole twisted her mouth in confusion “But, I thought dialysis was for purification?” Mike nodded “Typically, yes, but it can also be used for nanoparticle formation.

Mike pulled out another jar from the freezer and said “For this one, we will use poly(DL-lactide) from PolySciTech.” Nicole asked “What is the difference between the PLLA and PDLLa?” Mike replied “Although they are technically the same chemistry, they have a difference in the chiral arrangement of the side methyl unit. Poly(L-lactide) chains are isotactic, with the methyl units on the same side, and so they lay close to each other. This gives Poly(L-lactide) superior crystallinity, mechanical strength, and a longer degradation time than the poly(DL-lactide), which I have here. But, it has a drawback.” Nicole asked “Which is?” Mike brought the bottle over to one of the benches and set it down. He reached into a flammables cabinet and removed a bottle of dimethylformamide.

Mike said “The very crystallinity itself is the drawback of Poly(L-lactide). For only powerful solvents, such as dichloromethane, can reach deep into the core of these crystalline domains and pull the chains apart from one another. Poly(DL-lactide), however, does not stack as closely and has no such difficulty. A wide variety of organic solvents, including dimethylformamide, may pull the chains apart, same as for the PLGA we used earlier. For now, we will use the PDLLa here along with dimethylformamide (DMF) to make nanoparticles by dialysis.” Mike pulled out from the jar a small, off-white series of small chunks and carefully weighed them on a scale. He weighed out 20 milligrams of the polymer pieces into the vial and mixed them with 10 mL of dimethylformamide. “Can other solvents be used for making nanoparticles by dialysis as well?” Nicole asked. Mike replied “Yes, this process can be done with a variety of water-miscible organic solvents including dimethylformamide, dimethylsulfoxide, dimethylacetamide, or acetone. Naturally, the choice of solvent has an effect on the nanoparticles size and characteristics.”

Mike and Nicole prepared the next step. He drew forth from the refrigerator a roll of regenerated cellulose dialysis tubing and Nicole sliced it with a pair of scissors removing a section of appropriate length. “What is the molecular weight cut off for this?” Asked Nicole. Mike replied “For this use, the tubing has a molecular weight cut off of 12,000Da.” Nicole dawned nitrile gloves and doused the membrane in deionized water to clean away any preservatives or additives from it.

Mike clamped one end and Nicole held the other end open. Mike pipetted the PLA/DMF solution repeatedly into the tube. Nicole held it up as Mike put on another clamp sealing the tube closed at both ends. They placed the tube in a large beaker full of water and Nicole watched it as it spun lightly, floating in the water. Over time, she saw the contents of the tubing transition from clear to opaque white. Mike said “It will take many exchanges of water over the course of twenty-four hours to finish, but the slow replacement of dimethylformamide with water induces the PLA to precipitate out of solution into an uncountable number of nanoparticles.”

Mike continued “Microfluidic techniques can also be applied to nanoparticles by using PEG-PLGA, where the polymer itself acts as a surfactant.” Nicole replied, “Well, what polymer is good for that?” Mike said “PolyVivo AK010, it’s a pretty robust mPEG-PLGA 5000-10,000Da diblock. It’s been used before for making nanoparticles

by microfluidic techniques." Also PLGA nanoparticles can also be prepared in an emulsion using surfactants like Sodium Cholate.

Nicole marveled and said "So, what kind of things are nanoparticles used for?" Mike said "Well, a wide array of applications, but mostly targeted delivery. For this application they typically have their surface chemistry modified to make them target towards specific cells. Nanoparticles have been used for delivery of docetaxel to prostate cancer, delivering siRNA across the blood-brain-barrier, delivery of SN-38, pancreatic cancer treatment, photodynamic therapy applications, mesiothelioma treatment, breast cancer treatment, glioblastoma treatment, brain cancer treatment, antibiotic resistant bacterial infectin treatment, liver cancer treatment, bone cancer treatment, oral insulin delivery, ovarian cancer treatment, and inhaled schizophrenia treatment to name a few.

"Wow!" said Nicole. She stared at the bag and said "But, what if I just want to get the microparticles and nanoparticles premade?" Mike nodded and said "You can order that. Of course, it would be a custom order and cost and lead time will depend on how much you want and what drugs or other parameters you would like loaded in. You should contact PolySciTech to learn more."