ESTABLISHING PHYSICOCHEMICAL **BIOEQUIVALENCE** IN OPHTHALMIC MICROEMULSIONS

Establishing bioequivalence is a necessary step for US FDA approval of new generic drug products. Here, Paul Kippax, PhD, Director, Product Management: Morphology, Malvern Panalytical, describes the methods by which bioequivalence can be established for ophthalmic microemulsions by *in vitro* testing, saving the need for costly clinical trials.

The use of microemulsions has increased significantly over recent decades as the knowledge base associated with their successful formulation has rapidly grown. Microemulsions are now used as drug delivery vehicles, as exemplified by topical products for eye complaints. Key benefits of these ophthalmic microemulsions include their excellent thermodynamic stability and fine droplet size, which can aid optical clarity, drug delivery, retention and absorption. However, formulating such products remains challenging, particularly with respect to ensuring product stability over a long shelf life.

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Ophthalmic microemulsions are classified as complex generic products on the basis of their formulation structure and route of delivery. However, the challenge of proving bioequivalence with a reference drug product is significant for such products, as pharmacokinetic data obtained via clinical trials may not provide a realistic measure of bioavailability at the point of local action. As a result, the US FDA has set out the requirements for assessing bioequivalence *in vitro* via the use of appropriate analytical techniques, with the goal of providing sponsors with a faster route to market for new generics by avoiding costly clinical endpoint studies.

In this article, we discuss the value of particle size, rheology measurements and zeta potential in the characterisation of microemulsions and the *in vitro* demonstration of Q3 bioequivalence – physicochemical equivalence between a test and a reference product – for ophthalmic microemulsions. Relevant analytical techniques are introduced and their application is discussed with reference to case study data for cyclosporine, the active pharmaceutical ingredient in Restasis®, an ophthalmic emulsion for the treatment of dry eye disease.

INTRODUCTION TO MICROEMULSIONS AND OPHTHALMICS

Aqueous eye drops are the most common ophthalmic formulations. However, with conventional dosage forms like ophthalmic solutions and suspensions, demonstrating the bioavailability of such formulations can be tricky, especially given their low residence time in combination with the

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eye's natural defences (e.g. lachrymal fluid secretion, lachrymal fluid-eye barriers, and blood-ocular barriers).

Microemulsions normally consist of an aqueous phase, an oil phase, a surfactant and a co-surfactant (usually an alcohol). When the concentrations of these components are favourable they spontaneously emulsify to form a monodisperse, thermodynamically stable, transparent microemulsion. The low surface tension and small droplet size (5-200 nm) of microemulsions may result in increased drug absorption and permeation and, hence, an improved possibility of drug delivery to the posterior segment of the eye (vitreous humour, retina, choroid and optic nerve).[1] Microemulsions are appealing to ophthalmics formulators not only due to these benefits, but also because of their ability to solubilise and deliver otherwise immiscible liquids by, for example, loading a hydrophobic drug into the oil phase. They also allow for a phase transition to a high viscosity liquid-crystal state, which can increase residence time and thus bioavailability.

The potential of microemulsions to increase the effectiveness of ophthalmic drugs means that they are the subject of many current R&D efforts, by innovators and complex generics manufacturers alike. In the case of generics, identifying optimal analytical strategies for demonstrating bioequivalence is an important goal, with FDA guidance highlighting the benefits of applying orthogonal analytical methods.[2]

BIOEQUIVALENCE AND BIOAVAILABILITY

To gain FDA approval, a test generic drug must be shown to be bioequivalent to a reference innovator drug.[3] Bioequivalence includes bioavailability – the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action (21 CFR 320.1(a)). How efficiently a drug is released in the system differs between dosage forms. For example, if a drug is ingested orally, it may be only partially absorbed and metabolised, leaving less of the drug to act upon the target site. Drugs that are administered intravenously, however, are generally found to be much more bioavailable. In the case of conventional ophthalmics, such as solutions and suspensions administered topically to the eye, low residence time means bioavailability can be as little as just 5%.[4]

Abbreviation	Terminology	Definition
Q1	Qualitatively the same	The generic and innovator products contain the same active and inactive ingredients (i.e. they have the same components)
Q2	Quantitavely the same	The generic and innovator products contain the same amounts of active and inactive ingredients (i.e. they have the same amounts of the same components)
Q3	Physicochemical attributes of a specific dosage form	The generic and innovator products have the same physicochemical properties (i.e. they have the same amounts of the same components arranged in the same way)

Table 1: Bioequivalence categories.

"Particle size and polymorphism, along with viscosity and rheology, are important examples of the physicochemical attributes which enable us to understand how a drug will be released and behave in the system."

In bioequivalent products, there is no significant difference in the rate and extent to which the active ingredient or moiety becomes available at the site of drug action, when administered at the same molar dose under similar conditions in an appropriately designed study (21 CFR 320.1(e)). Showing the bioequivalence of a reference and test product satisfies one of the FDA's key requirements for generic drug approval.

To establish the bioequivalence of two drug products, they must be compared qualitatively (Q1), quantitatively (Q2) and also physicochemically (Q3) as shown in Table 1.

Particle size and polymorphism, along with viscosity and rheology, are important examples of the physicochemical attributes which enable us to understand how a drug will be released and behave in the system. This type of information can be especially useful when a drug is administered via a complex formulation, such as a microemulsion, applied topically to the eye. It is these types of characteristics which must be analysed in order to establish Q3 bioequivalence.

For ophthalmic cyclosporine emulsions, some of the Q3 bioequivalence attributes required for generic approval are as follows:[5]

- globule/particle size distribution
- viscosity profile as a function of applied shear
- zeta potential.

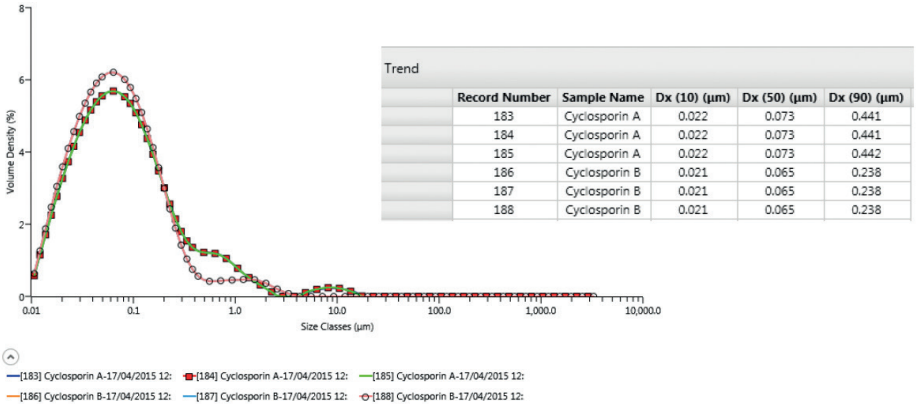
Effective methods for analysing such characteristics to demonstrate Q3 bioequivalence for ophthalmic microemulsions will be considered in the following sections.

Particle Size Characterisation

Determining the globule or particle size distribution of ophthalmic microemulsions and suspensions provides information on drug release, formulation clearance and product stability. Dynamic light scattering (DLS) and laser diffraction are particle size measurement techniques suggested by the FDA as useful for establishing bioequivalence of these product types *in vitro*.[5] The most appropriate technique for the particle size characterisation of microemulsions depends on the physical attributes of each sample, with DLS being more suitable for characterising particles in the submicron range and laser diffraction better suited to those in the micron range. It is typically preferable to avoid diluting microemulsion systems for analysis; for some techniques, such as laser diffraction, it is necessary however to disperse particles in a medium.

An example of the comparability data that can be obtained for test and reference ophthalmic formulations is provided in Figure 1 (next page), which shows measurement of globule size distribution

for cyclosporine using laser diffraction. These data confirm that the primary particle size for microemulsion globules within the reference and test formulations are similar. In addition, both formulations show the presence of large particles which may represent the onset of globule flocculation. The differences in the percentage of large globules is shown in the reported values for the Dv50 (median) and Dv90 (particle size below which 90% of the volume of material exists). This may have an impact on bioavailability, as it suggests that there may be stability differences between the formulations. However, to confirm this, the particle size data must be considered alongside the other Q3 physicochemical parameters advised by the FDA.



Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
183	Cyclosporin A	0.022	0.073	0.441
184	Cyclosporin A	0.022	0.073	0.441
185	Cyclosporin A	0.022	0.073	0.442
186	Cyclosporin B	0.021	0.065	0.238
187	Cyclosporin B	0.021	0.065	0.238
188	Cyclosporin B	0.021	0.065	0.238

Figure 1: Globule size distribution for two cyclosporine products, labelled A and B.

Rheological Characterisation

In rotational rheometry, stress is applied to a sample that is sandwiched between two plates. By rotating, oscillating or applying a step function to the measuring system, and by controlling the force (stress-controlled rheometry) or the speed (strain-controlled rheometry) applied, various rheological characteristics of the sample can be determined. Under such conditions, the sample will experience some manner of shear deformation. Rheology testing therefore involves measuring some standard variables:

- shear stress (force per area)
- shear strain (displacement divided by height)
- shear rate (change in strain with time).

From this shear profile, the sample's typical material properties can be calculated. A common test mode is rotation, used to measure shear viscosity, calculated as:

$$\frac{\textit{Shear stress}}{\textit{Shear rate}}$$

Viscosity measurements can provide a wealth of information about the stability of the suspension/emulsion. The higher the viscosity, the stronger the suspended particle interactions, and therefore the more stable the formulation.

Another common test mode is oscillation, which is used to measure viscoelastic modulus, calculated as:

$$\frac{\textit{Shear stress}}{\textit{Shear strain}}$$

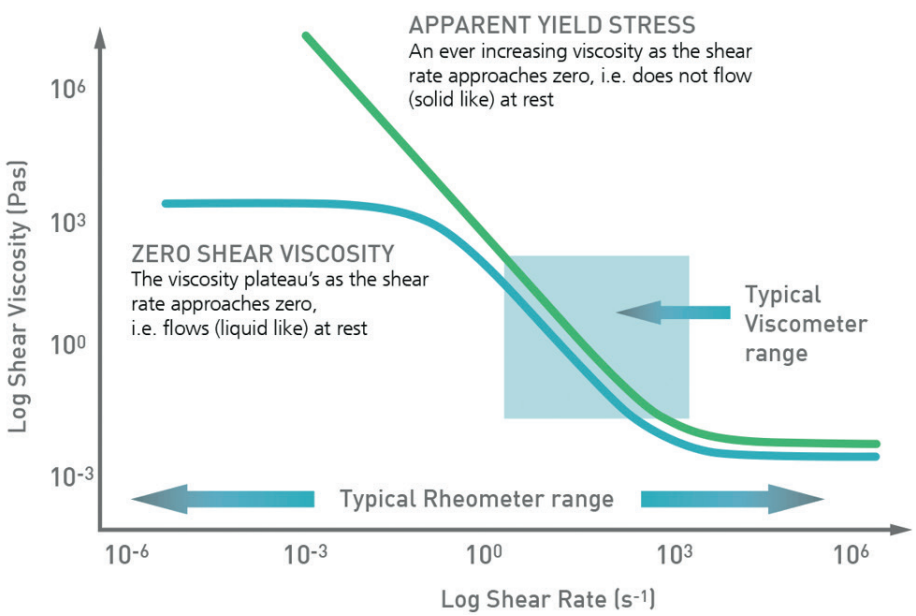


Figure 2: Rheological analysis showing how a material behaves at rest (whether it is solid- or liquid-like).

Other characteristics can also be determined through rheological analysis, such as:

- **yield stress**: the stress that must be applied for the material to break down and flow.
- **thixotropy**: the dependence of the viscosity on the timespan of the applied shear or how long it takes for the microstructure to rebuild after breakdown.
- **viscoelasticity**: how solid- or liquid-like the material is, and how this property changes with time, temperature, stress or strain.

Low shear rates show how a material behaves at rest. For example, a sample with an ever increasing viscosity as the shear rate approaches zero is solid-like, i.e. does not flow at rest, whereas if a sample's viscosity plateaus as the shear rate approaches zero it is liquid-like, i.e. flows at rest (Figure 2).

As well as helping explain the microstructural changes that occur in microemulsion systems as a result of dilution, rheological characterisation can also provide insights into the sample's responses to processes such as storage and delivery.

For example, when looking at the shear viscosity versus the shear rate of cyclosporine, the yield point, i.e. the point at which the material breaks down and flows (Figure 3), has an impact on ocular retention time and drug release. The more difficult it is to "break" the microemulsion, the higher the ocular retention. Moreover, if the "cohesive energy" (the energy required to break the suspension) is calculated, the stability of the microemulsion can be quantified. The data obtained for the reference and test formulations in this case are similar, suggesting that the formulations have a similar structure.

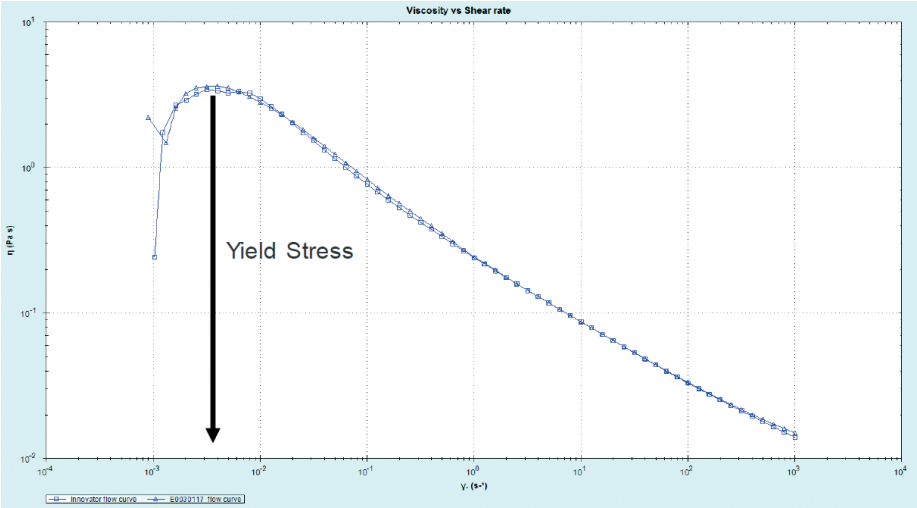


Figure 3: Yield point of cyclosporine – test and reference samples showing near-identical results.

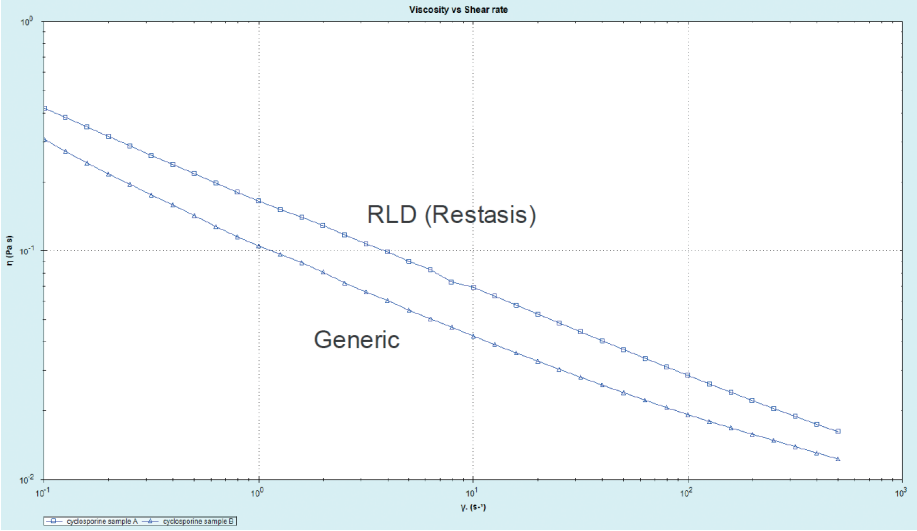


Figure 4: Cyclosporine reference and test products: viscosity versus shear rate.

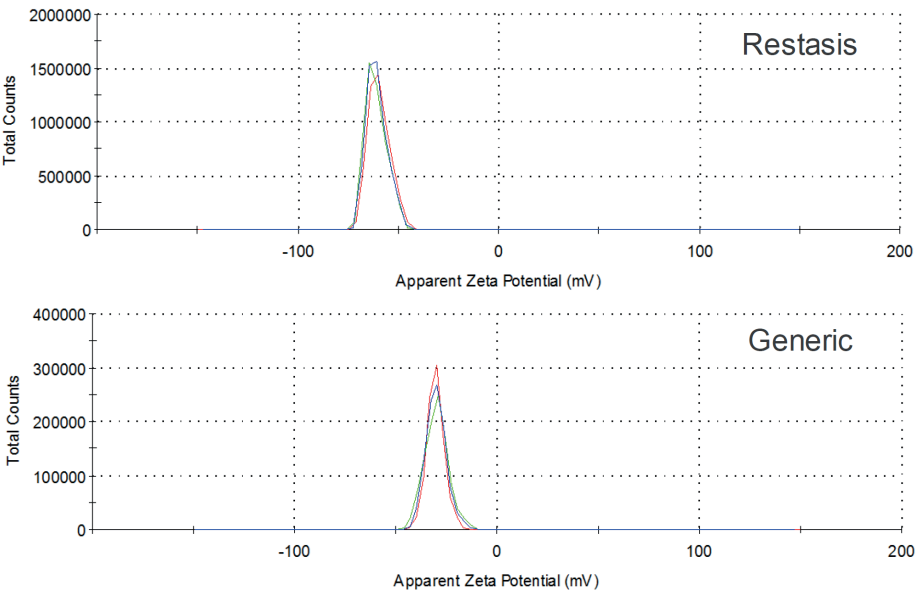


Figure 5: A comparison of zeta potential in cyclosporine reference and test products – the reference product has a more negative zeta potential, suggesting that it may be a more stable product.

It is also important to consider the flow behaviour of the formulations, as this can impact delivery of the formulation and also its dispersion following delivery. Viscosity versus shear rates for test and reference cyclosporine products can be seen in Figure 4, which shows that the reference listed drug (RLD) is more viscous than the generic. This may impact its ocular retention time and drug release characteristics.

Zeta Potential

Zeta potential is a parameter which relates to the charge a particle acquires in a particular medium. It can be related to formulation stability and dispersion, as well as to the adhesion of particles to cell membranes.

The results of the determination of the zeta potential of cyclosporine (in the RLD and test products) can be seen in Figure 5. This shows that the reference product has a more negative zeta potential compared to the test product, suggesting that the reference product may be more stable over time. This difference may also have an impact on the way in which the two formulations are absorbed following delivery.

CONCLUSION

FDA guidance recommends that an orthogonal approach is applied to pharmaceutical bioequivalence testing. For example, dynamic light scattering or laser diffraction techniques provide the particle size distribution of the sample which, in turn, gives an idea of drug release properties, formulation clearance and product stability. However, this needs to be considered alongside rheological characterisation, which can provide information relating to suspension/emulsion stability, as well as an understanding of the behaviour of the formulation during storage, delivery and drug release. And to further complement this analysis, measuring a product's zeta potential gives an additional prediction of its stability.

The establishment of particle size distribution, viscosity profile and zeta potential are some of the Q3 bioequivalence attributes required as part of the approval process for generic ophthalmic cyclosporine products. By applying complementary analytical methods such as these in combination – that is, by taking an orthogonal approach – a well-rounded picture can be created of the physicochemical comparability

of ophthalmic microemulsions. This can yield significant benefits to sponsors by enabling bioequivalence to be assessed *in vitro*, thus avoiding complex and time-consuming clinical endpoint studies.

ABOUT THE COMPANY

Malvern Panalytical technologies are used by scientists and engineers in a wide range of industries and organisations to solve the challenges associated with maximising productivity, developing better quality products and decreasing time to market. The company's focus is on creating innovative, customer-focused solutions and services to enhance efficiency and deliver tangible economic impact through chemical, physical and structural analysis of materials.

Malvern Panalytical was formed by the merger of Malvern Instruments Limited and PANalytical B.V. in January 2017, has headquarters in both Almelo (Netherlands) and Malvern (UK), and employs over 2,000 people worldwide. The combined entity is a strong player and innovator in the materials characterisation market and leverages the strengths of the individual companies in their end markets, having applications laboratories around the world, a global sales and service presence and a strong distributor network.

Malvern Panalytical is part of Spectris plc, the productivity-enhancing instruments and controls company.

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