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Reflection paper on the pharmaceutical development of intravenous medicinal products containing active substances solubilised in micellar systems (non-polymeric surfactants)

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Scope

This paper describes a basic package of information which could be relevant to confirm the sound pharmaceutical development and full characterisation of products of this type.

In particular, this text applies in the following contexts cumulatively:

- medicinal products for intravenous injection or infusion which contain active substances, which have a low aqueous solubility and which are therefore solubilised in an aqueous micellar system[1], where the main objective of the development is to solubilise the drug, and not to create a system where special size- or surface-dependent properties of the disperse phase are critical factors in the kinetics and disposition of the drug.
- it is particularly relevant to 'traditional' or 'established' 'small molecule, non-polymeric' surfactants which are sensitive to dilution effects during slow intravenous administration, which are quickly metabolised and which therefore do not have a long halflife in plasma, e.g. polysorbate 80.

Additional considerations may apply to certain polymeric surfactant systems developed to create a 'delivery system' in addition to a solubilising system and which have special properties affecting kinetics and distribution *in vivo*, e.g. very low critical micelle concentration (cmc) or prolonged half-life[2] (e.g. innovative block co-polymer surfactants) or those oncology products designed to utilise the EPR effect[3] (Enhanced Permeability and Retention). Such systems *may* indeed be characterised in part by the following tests, but it is likely that additional studies could be needed to fully characterise these products in a relevant way.

In micellar solutions, there exists, in rapid dynamic equilibrium, different species of the drug substance (as aqueous solute or drug substance solubilised in the micelle) and surfactant (as monomer or in micelle form). In this way, the drug substance is maintained in solubilsed form and precipitation is avoided. These drug products are normally presented as stable sterile concentrates (e.g. powder and solvent for concentrate solution for infusion, sterile concentrate and solvent for solution for infusion, concentrate for solution for infusion).

The micellar solution is normally prepared for intravenous infusion by dilution with a large volume of aqueous 0.9% sodium chloride or 5% dextrose injection.

On slow intravenous infusion of the products described by the bullet points above, the micellar solution is deaggregated such that the drug substance is presented to the blood compartment in a 'free' rather than 'solubilised' form. Nevertheless, it should be acknowledged that these are generalisations and that the specific qualities and attributes of the both the drug substance and micellar and formulation excipients need to be specifically considered on a case by case basis.

Given the complexity of micelle systems, a comprehensive pharmaceutical development is necessary, needing an understanding of what happens to the product after administration. It is acknowledged that this development may involve some tests which are not currently well-reported, and applicants are encouraged to develop and validate such techniques for themselves, particularly those which give information on the likely state of these systems *in vivo.* Therefore, applicants are advised to discuss

1 Hoiland H and Blokhus M : Solubilisation in aqueous surfactant systems, in Birdi KS Handbook of Surface and Colloid Chemistry, CRCPress, 1997

2 Matsumura Y : Polymeric micellar delivery systems in oncology *Jpn J Clin Oncol.* 38(12), 793-802, Dec 2008

3 Duncan, R. (2006) Polymer conjugates as anticancer nanomedicines. Nature Reviews Cancer, 6, 688-701

the pharmaceutical development with the regulatory authorities and/or to seek Scientific Advice from the CHMP.

1. Properties of the medicinal product ingredients

The lipophilicity and solubility of the drug substance should be fully characterised, and the known *in vivo* disposition should be described. Each excipient should be described and its function fully justified. Relevant critical quality parameters should be described, including characterisation, e.g. surfactant polydispersity and purity.

The proposed pharmaceutical form should also be justified and the point at which the micellar solution is prepared for administration should be fully described.

2. The critical micelle concentration (cmc) of the surfactant

To begin with, it is useful to know the critical micelle concentration (c.m.c. or just cmc) of the surfactant used[4]. Calculations based on the cmc are useful to indicate that a micellar component is *likely* to be present to solubilise the drug substance.

For single molecular mass surfactants, the cmc depends upon the structure and purity, and in particular the characteristics which determine surface activity must be controlled – e.g. the alkyl chain length of the lipophilic region and the characteristics of the hydrophilic region e.g. polyoxyethylene chain length although this latter does not have such a great effect as the former on the cmc of most non-ionic surfactants.

There are several definitions of the cmc, but for the purposes of this paper it may be taken to represent the maximum limit of solubility of the (monomeric) surfactant in the bulk solvent, at the point where self-assembly into micellar structures begins. Given the variability in composition of industrially manufactured surfactants, many of them may be classified as *polydisperse*, and the cmc may be expected to vary from batch to batch. However, in contrast to heterodisperse systems, their solubilising properties may be expected to be close to that of a homogeneous compound having the composition of the mean. Therefore, while pure surfactants are normally required for physical chemical studies, useful information may still be obtained from solutions of polydisperse materials of this type.[5]

A wide range of techniques are described for the measurement of cmc, for example:

surface tension[6]

- light scattering[7]
- dye solubilisation[8]
- conductivity (but since the majority of surfactants for clinical use are probably nonionic in nature, conductivity methods may not be so useful as the others.)

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4 Hiemenz, P: Principles of Colloid and Surface Chemistry, CRC Press, 1997

5 Paul Becher, in 'Nonionic Surfactants' , ed. M.J Schick, Surfactant Science Series Vol. 1, Ch 15, Marcel Dekker Inc., New York, (1967)

6 Cosgrove T, 'Colloid Science: Theory, Methods and Applications'. Wiley Blackwell, 2005

7 Mazer N A, Laser Light Scattering in Micellar systems in 'Dynamic Light Scattering: Applications' by R Pecora, Springer 1985

8 Das A K, Hajra A K , *J Biol Chem*, 267 (14) 9731 (1992) http://www.jbc.org/cgi/reprint/267/14/9731.pdf

The cmc of a surfactant is different depending on the composition of the solution in which it is dissolved, therefore the media chosen for measurement should be as close as possible to the composition of any diluted solutions immediately prior to infusion. It cannot be assumed that the cmc in water or saline is the same as the cmc in plasma, so the cmc should be recorded for information only, to indicate the presence of the micellar component prior to infusion of the product.

3. Solubilising Capacity

Given the presence of any micellar system, it is useful to know its 'capacity' to solubilise the active substance. The Maximum Additive Concentration (MAC) or other similar attribute provides this assurance with regard to the active substance as the additive in question. Micellar media relevant to infusion solutions should be challenged by incorporating increasing concentrations of drug and noting the point at which phase separation occurs, at 15°C and 25 °C. This gives an indication of how great is the margin of safety before the crystallisation of the drug becomes a possible danger for the patient, taking into account a 'cold' and 'warm' hospital environment.

The MAC is linked to the concept of 'cargo capacity' which is a feature of novel drug delivery systems, especially those based on micellar or liposomal systems. New surfactants are being developed for pharmaceutical applications having a greatly increased cargo capacity for water-insoluble drugs, and a prolonged half-life *in vivo*.

The recommendation of an intravenous in-line filter to remove precipitated drug arising from a badly-developed product with no margin of safety represents bad practice. It should be demonstrated that the pharmaceutical development leads to a robust product with the drug in solution and a margin of safety such that an in-line filter would not be necessary under normal conditions of use. However, accepting this, it would not preclude the use of a filter for other reasons, e.g. extra margin of microbiological safety.

4. Physical stability of diluted infusion solutions

In addition to the normal in-use stability studies, and taking into account the principles mentioned above, it is useful to confirm that the principle of the MAC is confirmed and drug crystallisation does not occur over a time interval relevant to the preparation and administration process. Diluted product at the extremes of the range of concentrations likely to be encountered in clinical practice should be used, representative of a cold and warm hospital environment.

Applicants should pay particular attention to the wording in the current guideline maximum shelflife of sterile products after first opening (CPMP/QWP/159/96 corr):

"… from a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would not normally be longer than 24 hours at 2 – 8°C, unless reconstitution/dilution has taken place in controlled and validated aseptic conditions…"

The effect of temperature on these complex systems is difficult to predict but it is likely that refrigeration will lead to precipitation of the poorly-soluble drug. Therefore, in order to further assist users, the ability of the diluted product to resist phase separation if refrigerated after dilution but prior to infusion should be investigated to see what is the margin of safety in this regard, given the realities of clinical and pharmaceutical practice. It may be necessary for the product literature to specifically advise against refrigeration.

5. Characteristics of the micelle component & free drug fraction prior to administration

It is useful to know the important characteristics of the micelle component in solutions immediately prior to injection/infusion, according to the dilution/administration instructions in the SPC, in particular:

- mean size and size distribution of the dispersed micellar component
- estimated concentration of micellar entities, reflecting the extent (amount) of the micellar component.
- [free] *vs* [solubilised] fractions of the active substance

Dynamic Laser light scattering is often the most useful method to give information on size and extent, or the resulting fluorescence arising from a fluorescent probe by dye solubilisation may be used to give an indication of the extent of any micellar component which may be present, normally a function of how much surfactant is there and its cmc. Again, since it cannot be assumed that the micellar properties prior to infusion are the same as the micellar state *in vivo*, this information should be used only to indicate the 'pharmaceutical' state of the product prior to infusion. It should not be used to predict how the product will exist *in vivo.*

Concerning the relative amounts of free and solubilised drug that are being administered to the patient, equilibrium dialysis[9] can be used. However, since in most cases the drug will have a very low aqueous solubility, the free drug concentration is expected to be very small since the partitioning equilibrium is in favour of the micelle interior. Furthermore the possibility of surfactant monomer crossing the membrane and re-assembling should be kept in mind and this can introduce further methodological difficulties.

In certain cases, depending on the claims made by the applicant, it could be relevant to have some knowledge of the surface characteristics e.g. surface charge as reflected in the zeta-potential, although this would probably not be a standard requirement for most non-polymeric micellar systems, or those with a very short half-life *in vivo*. Concerning the test methods mentioned above, any other relevant and justified validated methods could be considered.

6. Modelling studies indicating the persistence and extent of the micelle component *in vivo*[10]

Normally, micellar parenteral drug products are administered by slow intravenous infusion, and are therefore subject to dilution effects which may promote the deaggregation of the micellar component.

The method and rate of administration may affect the disposition of the drug substance and drug product excipients. (Note that this may not necessarily be the case with a rapid or bolus intravenous injection, where rapid deaggregation of the micellar component may not occur to the same extent).

9 Smith G A and others, Use of the semi-equilibrium dialysis method in studying the thermodynamics of solubilization of organic compounds in surfactant micelles. *J Solution Chemistry*, 15, 6, (1986).

10 In Modules 4 & 5, such models/hypotheses should be supported by *in vivo* pharmacokinetic studies , possibly in animals but preferably in humans, showing entrapped/free drug levels and the fate of the micellar component. It is essential that thiese pharmacokinetic studies are undertaken within the correct time frame and results analysed as biodistribution (% dose) not just as classical kinetic parameters. Tissue distribution of drug will influence efficacy/safety and guide preclinical toxicological and clinical studies/protocols.

The clinical dossier should justify the time and conditions of the infusion process, taking into account such issues as potential haemolysis, CNS effects, etc.

As an extended aspect of the pharmaceutical development of these complex systems, it is essential to gather as much information as possible concerning what is likely to happen to the drug and the micelle component *in vivo*. In general, the 'persistence' and extent of the micelle component are probably of more interest than the size or electrostatic properties in the small molecule non-polymeric surfactants. It is possible that micelles will disappear during a slow infusion, simply due to dilution and metabolism of the surfactant, but applicants need to consider a number of competing equilibria to arrive at a better understanding of what is happening.

In this regard, the overall picture emerging from the recent literature (mostly on polymeric micelles) is that the micellar component is much more transient *in vivo* than may be imagined, and evidence points to rapid loss of micelle integrity and dispersal of the 'free' drug systemically. It is likely that this observation will be even more marked with non-polymeric micelles where no attempt has been made to increase the biological persistence of the system and there is evidence that in the case of polysorbate 80, the dilution effects coupled with rapid metabolim by plasma carboxyesterases may lead to the surfactant having little or no significant effect on the disposition of the drug.

Nevertheless, as a point of departure it is useful to consider the several mechanisms in the human body acting on intravenous lipophilic drugs solubilised in micelles formed from a non-polymeric surfactant :

1. Initially, the micelle component disappears due to simple dilution below the cmc of the surfactant[11], and the drug is released. Theoretically, micelles may re-form as more product is infused, and this equilibrium is in competition with 2. below.

2. surfactant monomer may be exposed to metabolic clearance by plasma enzymes[12]. This leads to a re-adjustment of the micelle -> monomer equilibrium, accelerating the deaggregation of the micelles and release of 'free' drug

3. the free drug may bind to plasma proteins, or may otherwise be 'transferred' out of the micellar system. If the affinity for plasma protein or other *in vivo* lipophilic domain is greater than the affinity for the micelle interior then the drug will be depleted from the micellar component, even though the latter may persist *in vivo*.[13]

4. the free drug may be passively dissolved by the greatly increased volume of water outside the restricted confine of vascular compartment without exceeding the solubility limit.

5. free drug may partition itself into the many lipophilic domains in the body without exceeding the solubility limit.

In the *in vitro* modelling of the administration process in human plasma, the first three of the above mechanisms are retained, but 4 & 5 are not. Equilibration into total body water is probably slow, and disregarding this aspect makes modelled i.v. infusion into a restricted closed volume a more

[11] Although it cannot be assumed that the cmc in plasma is the same as the aqueous cmc

12 Tellingen O, Beijnen J H, Verweij J, and others, Rapid Esterase-sensitive Breakdown of Polysorbate 80 and Its Impact on the Plasma Pharmacokinetics of Docetaxel and Metabolites in Mice , *Clinical Cancer Research*, Vol. **5**, 2918-2924, October 1999 http://clincancerres.aacrjournals.org/cgi/content/full/5/10/2918

[13] There is evidence that the biodistribution patterns of the radiolabels of tritium-labelled paclitaxel solubilised in 14C-labelled polymeric micelles are different, and that the patterns diverge rapidly after administration, paclitaxel showing a wide diffuse distribution. This indicates that paclitaxel is rapidly released from the micelles after administration.

discriminating test. Equilibrium 5 is more rapid, and is probably what happens to most lipophilic water-insoluble drugs released from micelles in any case.

The main experimental difficulty in trying to model the infusion process with a medicinal product is that the drug would precipitate in dilute solutions below the cmc of the surfactant. For new drug products, formulations *minus* the drug could be prepared and studied, yielding 'indirect' information since the drug loading could indeed disturb micellar equilibria. Nevertheless, it would be useful to see these studies for new products (Generics present additional difficulties, see later.). As a way round this problem in order to facilitate more relevant information from the final (drug-loaded) product itself, a 'sink' for the drug could be considered, e.g. a lipophilic or immiscible solvent layer in which the drug is soluble but the surfactant is not, in order to partition the drug and prevent it reaching its aqueous solubility limit during the study (substituting for mechanism 5 above). This could in theory enable the complete medicinal product to be studied on the condition that such a sink does not significantly disturb micellar equilibria.

Ideally, the infusion process may be replicated by infusion into a closed system of 3 litres saline or preferably plasma at 37oC. However, especially for plasma studies, the process may be scaled down, i.e. maintaining the same concentrations of the components but using smaller volumes.

Plasma-based studies would be more biologically relevant but the investigational methods are obviously more restricted, e.g. light-scattering techniques could be applied to drug-free systems in saline, but probably not in plasma. On the other hand, micelle properties may be investigated in plasma by using fluorescent-labelled surfactants, or a dye uptake method, e.g. a fluorescent probe like TNS [6-*p*-Toluidino-2-naphthalene Sulfonate] which fluoresces in the hydrocarbon interior of a micelle (or indeed any other probe), corrected for any dye that may be taken up by endogenous amphiphiles in the plasma. It may be possible to use the product itself, since there may be some residual cargo capacity remaining for the uptake of fluorescent dye.

A recent *in vitro / in vivo* study of what happens to the micellar state after injection in animals has shown an apparent disintegration of micellar structure within 15 minutes, using Förster Resonance Energy Transfer between a lipophilic donor/acceptor pair solubilised in a polymeric surfactant. In order for transfer of this resonance energy to be optimised, the donor and receptor couple must be confined in close proximity (typically ~10nm) as is the case in the restricted interior of a micelle. Loss of resonance energy transfer indicates a greater separation of the couple, as happens when the micelle structure is lost. This rapid loss of micelle integrity and release of core-loaded molecules was initiated by the presence of α- & β- globulins and interestingly occurred at concentrations of surfactant above the aqueous cmc[14]. FRET methods could indeed have some value in helping to understand what happens to the micellar system *in vivo*. Ideally, both lipophilic FRET probes, or fluorescent dye probes could be used with the product and a drug sink to indicate the presence or absence of a micellar system.

Furthermore, there is evidence that the biodistribution patterns of the radiolabels from ^{3}H-labelled paclitaxel solubilised in ^{14}C-labelled polymeric micelles are different, and that the patterns diverge rapidly after intravenous administration, paclitaxel showing a wide diffuse distribution different from the micelles. This indicates that they are not travelling together, the most likely explanation being that paclitaxel is rapidly released from the micelles after administration[15]. It is likely this also happens with non-polymeric surfactant micelles.

14 Chen H and others, *Langmuir*, 24. 5213-5217 (2008)

15 Burt H M, and others, Colloids and Surfaces B : Biointerfaces 16, 161-171 (1999)

Nuclear (nmr) methods may also be able to give information on the state of any exogenous micelles in the complex medium of plasma.

Finally, it is again acknowledged that the methods described above are non-standard techniques which are not in common use, and they need to be carefully validated. They need to be justified by applicants on the basis of a sound understanding of colloidal and physical chemistry processes applied to a complex biological environment.

7. Generic Products and Post-Authorisation changes

In this section we mean generics of those reference products containing active substances which are also solubilised in micellar systems; both products should meet the bullet points listed in the Scope of this paper. Concerning the Pharmaceutical Development of these generics, Sections 1 to 5 of this paper could be considered applicable in all cases, and Section 6 depending on the composition of the generic compared to the reference product.

Furthermore, very similar principles also apply to post-authorisation changes, e.g. variations. The basic questions are:

- what is the likely effect of formulation/manufacturing changes on bioavailability ? and
- how can reassurance be provided in the form of *in vitro* studies ?

To avoid repetition, the following text refers only to generics.

7.1. Bioequivalence Surrogate Markers

According to EU guidance on bioavailability and bioequivalence intravenous micellar injections can be regarded as 'complex' parenterals in which case a bioequivalence study may need to be performed regardless of the intravenous route. The basis for this classification as complex is linked to the (theoretical) introduction of an additional micellar compartment or 'lipohilic phase' into the patient, i.e. the lipophilic interior of the micelle, compared to a 'simple' aqueous solution. The real extent and persistence of the micellar component *in vivo* may be questioned, since during the initial stages of a slow infusion the micelles will probably disappear due to dilution and thereafter by metabolism of the surfactant. Consequently the real effect of the surfactant on the bioavailability of the active substance may also be questioned.

The principles outlined below, although emphasised for generics, are equally applicable to any post-authorisation modification that might affect bioavailability.

If an applicant wishes to apply for an exemption from the need to perform a bioequivalence study - i.e. a 'biowaiver' - a complete biopharmaceutical argument should be proposed together with the results of relevant physicochemical tests as bioequivalence surrogate markers. In this regard, satisfactory development pharmaceutics, as described in sections 1 to 4 and some of the *in vitro* studies under sections 5 & 6 could be useful if performed in a comparative manner against the reference product.

Data requirements to support a biowaver are briefly described in the guideline on the investigation of bioequivalence. Satisfactory development pharmaceutics data could support the submission of a reduced extent of clinical or non-clinical data, or its absence.

The level of testing depends on how similar the generic is to the reference product in terms of qualitative and quantitative composition. However, since it is unlikely that the detailed composition of the reference product will be known, applicants will need to decide and justify the amount of

information which is necessary to support any claim for a biowaiver. Therefore, applicants are strongly advised to seek scientific advice from the CHMP and / or National Competent Authorities.

7.1.1. Differences in the formulations

It would be unreasonable to require extensive testing of a generic micellar injectable which is *identical* to the reference product. However formulation differences tend to be the norm rather than the exception.

In the case of generic products applicants are advised to copy exactly the composition of the reference product as far as is known to them. Applicants should provide clear tables showing the concentrations of all ingredients, not only in their product as presented for the market, but also in the infusion solution(s) immediately prior to administration to the patient.

7.1.2. Identical Formulations

Same excipients, same amounts.

This is the simplest case, and minimum testing is required, utilising diluted solutions according to the SPC, immediately prior to administration. If the generic product is presented in a different pharmaceutical form compared to the reference, but the qualitative and quantitative composition of the diluted infusion is the same, such a product can be included in this section.

Comparative studies according to Section 5 could provide useful information in support of a biowaiver.

7.1.3. 'Similar' Formulations

The same surfactant is a condition.

Formulations may be qualitatively identical, with small quantitative changes only.

Or, there may be qualitative differences in excipients which are judged to be 'noncritical' with regard to their influence on micelle stability and bioavailability of the drug. In this regard the following should be noted:

- small differences in the relative amounts of pH adjusting agents (e.g. citric acid) are not likely to have a significant impact on micellar stability or disposition of the drug *in vivo*, due to the great dilution in the plasma on administration. Ionic micelles are sensitive to the ions in their aqueous environment (ionic strength) but in general the non-ionic surfactants most likely to be used in biological applications e.g. polysorbates, cremophors, are much less sensitive to differences in ionic strength
- small differences in the content of added co-solubulising substances such as PEG (same Mol.Wt. in both products) or alcohol (ethanol) are not likely to have a significant impact on the micellar stability or disposition of the drug *in vivo*, due to the great dilution in the plasma on administration. The hydration corona plays a part in the stabilisation of non-ionic micelles and this can be reduced by alcohol and other polar solvents. However, alcohol only has this inhibitory effect when the concentration rises above ~ 10% v/v. Concentrations in an infusion solution immediately prior to administration (and *in vivo* post-administration) are very much less than this.

As with identical formulations, comparative testing according to Section 5 could be useful information in support of a biowaiver, but there is an increased risk that further comparative *in vitro* testing would be required according to Section 6, or even additional nonclinical/clinical studies if the noncritical nature of the qualitative/quantitative differences in the formulations proves difficult to decide conclusively.

7.1.4. Different Formulations

Chemically different surfactant / micelle-forming system.

Or, the same surfactant but used at a significantly different concentration compared to the reference product.

Or, there may be qualitative/quantitative differences in other excipients which may have an impact on micelle stability and/or bioavailability of the drug.

The published transience and observed rapid loss of integrity of certain micelle systems *in vivo* supports the general view that relevant physicochemical testing to support a biowaiver for intravenous micellar systems should not be ruled *a priori* out of the question. However, if the generic product has a different surfactant to the reference product, then it is unlikely that an *in vitro* comparison alone, according to Section 6, could be used to support a biowaiver for the generic. With different surfactant systems there is a risk that *at least* a bioequivalence study would be needed, and also the possibility of additional nonclinical or clinical studies confirming safety, if necessary. But these are clinical decisions, taking into account the following issues, and others:

- The metabolism of the surfactants may be different, and this may have an effect on the extent and persistence of the micellar compartment *in vivo*.
- There may be differential drug binding to surfactant monomer and consequent effects on drug clearance.
- The different safety profiles of the surfactants *per se* needs to be considered, together with the possibility of different interactions with co-administered drugs.
- Surfactants may affect transporters and enzyme systems thus modifying drug disposition.

These investigations are not strictly part of Pharmaceutical Development in Module 3, although a summary could be made back to Module 3 in order to support the findings of any physico-chemical studies.

Since these physicochemical studies in Section 6 are non-standard, applicants are strongly advised to discuss their development with the competent authorities and/or to seek scientific advice from the CHMP or National Competent Authorities, in order to reach an agreement not only on the level of testing which is required, but also what is feasible.

If no convincing evidence can be obtained from *in vitro* studies, applicants have to provide relevant *in vivo* efficacy and safety studies. With respect to efficacy, this may be addressed by appropriate pharmacokinetic studies showing equivalent disposition.