Supplementary material

Contents

1. Literature Review

1.1 Problem of normal drug administration

The current way of drug delivery is not optimal according to multiple scientific publications (1–3). A major problem that conventional pharmacotherapy encounters is the aspecific distribution of drugs where drugs enter healthy organs that should not be involved in the treatment and causing toxic effects (2). To maximize the response and reduce side effects, the medicine should ideally only be exposed to the tissues that contain the pharmacological target (4).

The concentration of drugs in the blood stream rises quickly after administration and short after already declines (1). That is why drugs might already be inactivated or cleared out of the body before they reach their targeted site of action (2). Furthermore lots of drugs have hydrophobic properties which makes it hard to be taken up by the body (5,6). Traditionally, the only goal of drug administration was to get the drug into the bloodstream, depending instead on tissue irrigation and drug affinities for access to the target. There are many obstacles that the medication alone finds difficult to overcome, including the enzymatic attack and low tissue permeability. As a result, the therapies typically entail the administration of relatively high dosages of medication in the hopes that some, albeit small, will reach the targeted tissues or cells (4).

These problems reduce the availability of drugs at the site of action and thus diminish the chance of an effective treatment while at the same time create possible unwanted side effects elsewhere in the body (1,2,7,8).

1.2 Smart DDSs

The last two decades a lot of attention has been directed towards the design of new methods for controlled drug delivery (1). The need for more effective and smart drug delivery systems (DDSs) led to the development of a number of potential techniques (9). A DDS can be considered a SMART DDS when it falls under one of the three types below (4).

– *Type 1. **S**ystems to **M**aximise Access, **R**etention and **T**herapy,*

These type of SMART DDSs are focused on improving targeting by enhancing permeability and retention of nanoparticles in pathological tissues. Examples are nanoparticles coated with human-like antibodies, magnetic nanoparticles and bioconjugates (4).

– *Type 2. **S**ystems that **M**onitor, **A**nalyze and **R**espond in **T**ime.*

These SMART DDSs work with a feedback drug release system. The DDS contains a biosensor that looks at the concentration of a biomarker. Release will be triggered if concentration of this biomarker is low, once the concentration increases again the drug release will stop (4).

– *Type 3. **S**ystems **M**ute until **A**ctivation by a **R**emote **T**rigger.*

These DDSs release drugs due to stimulation of a remote trigger. Stimuli can for instance be external such as infrared light, ultrasound or change in magnetic field or internal such as pH, temperature, ionic strength or concentration of other substances (4).

1.2.1 Retention and Release

A requirement of a SMART DDS according to researchers Alvarez-Lorenzo and Concheiro which they call the R2 challenge: *'drug Retention in blood circulation versus Release in target cells'*. When travelling towards targeted tissues or cells the DDS should be able to retain the drug without any

leakage. And when target is reached, the DDS should be able to quickly release 100% of the drug. Moreover, it should be capable to travel through the body without adhering to proteins or lipids that may cause the phagocytic system to recognise it. Furthermore it should not be taken up by non-target tissue but should engage vigorously with target tissue (4,5).

Various DDSs have been created for targeted and controlled release applications. The most often investigated nanoparticles include nanoshells, carbon nanotubes, dendrimers, superparamagnetic nanoparticles, liposomes, polymer conjugates and polymeric micelles. Liposomes and polymer-based DDSs are the more well-known types of polymer-based nanoparticles and have been used the most in clinical trials (10). This literature review will focus on polymer-based DDSs and polymeric micelles in particular.

1.3 Polymer-based drug delivery systems

Polymer-based DDSs are one of the most promising systems that show good results for tackling various problems with conventional medication delivery (5). Due to the large variety in polymers, polymer-based DDSs can be used for various drug delivery applications (11). Continuing progress made in nanotechnology field, polymer chemistry and chemical engineering make it possible for synthesis of new polymers and for current polymers to be modified which can be used in drug delivery applications (11,12).

Polymeric micelles (PMs), an example of a polymer-based DDS, have drawn a lot of scientific attention as a flexible nanomedicine platform with enhanced pharmacological and effective response in the area of drug delivery (5,7). PMs show promising results for drug delivery applications due to their separated core/shell structure and ability to respond to external or internal stimuli (2). First PMs and their structure will be explained and afterwards their application and promising abilities will be discussed.

1.4 Polymeric micelles

About thirty years ago, it was first thought that micelles produced by amphiphilic block copolymers in aqueous solution could serve as carriers for hydrophobic drugs. Since then, many studies have been conducted on the use of amphiphilic block copolymers in the synthesis of PMs as possible treatment administration. A number of new block copolymers have been developed for micelle-based DDSs. As a result of these developments, more PM based DDSs are undergoing either preclinical animal trials or clinical trials in preparation for regulatory approval or have already been approved (13).

1.4.1 Structure and characterization polymeric micelles

PMs are mostly formed of diblock, triblock or graft polymers or triblock amphiphilic polymers that have both hydrophilic and hydrophobic components. Amphiphilic polymers will form amphiphilic micelles formed by hydrophobic interactions. Other types of PMs are polyion complex micelles and these are based on ionic copolymers with oppositely charged parts (11). The focus of this literature review will be put on PMs that are based on amphiphilic copolymers. The hydrophobic part of an amphiphilic polymer can for instance be made up of polyesters like poly((ε-caprolactone), glycolic acid, lactic acid, poly(propylene oxide) or polystyrene. The hydrophilic segment can be poly(ethyleneglycole), poly(vinyl pyrrolidone), poly(methacrylic acid) or poly(trimethylene carbonate)

(5). The selection of the hydrophobic and hydrophilic segments of the copolymer can be adjusted for both site-specific delivery and controlled drug release (8).

In aqueous solutions amphiphilic block copolymers at a certain concentration of polymers start to self assemble into PMs. Amphiphilic block copolymers function independently as surfactants in aqueous solution, however when the concentration of copolymers rises, they begin to assemble as a result of the bulk solution's saturation (14). The hydrophilic part of the polymer will direct outwards which forms the shell while the hydrophobic part will direct inwards and forms the core (11). The concentration at which this happens is called the critical micelle concentration (CMC) and is different for every polymer. PMs are stable at concentrations above CMC but disassemble at concentrations below CMC (14).

The separated core/shell structure makes PMs able to capture unstable and insoluble drugs. The primary factor influencing how well a PM can saturate less water-soluble drugs is its hydrophobic core. Longer hydrophobic chains mean larger hydrophobic cores and the ability for more hydrophobic drugs to be encapsulated in the core (15,16).

1.4.1.1 Size, shape and surface charge

The chemical composition, length of the hydrophilic and hydrophobic segments as well as the molecular weight of the block copolymer all have an impact on the size and morphology of the eventual PMs (14,17). Most PMs have a diameter ranging within 5 and 100 nm (15,18). Above 200 nm they can still be considered PMs however this might lower the in vivo performance which will be explained under the section ‘1.5.1 Retention’ (19).

Usually PMs are spherically shaped but other possible shapes are rod-like, worm-like, disk-like, cylindrical or other morphologies depending on the copolymers chosen. Besides the copolymer itself, the characteristics of the surrounding environment (solvent, pH, temperature etc.) have an impact on the structure of the PM (5,14).

Surface characteristics such as surface charge (zeta potential) have an influence on the behaviour of PMs in vivo. When a micelle solution is exposed to the effects of an electric field, micelle surface charge impacts the dispersion of particles (14). A stable system that inhibits nanoparticle aggregation is thought to consist of nanoparticles with zeta potentials greater than +30 mV or less than -30 mV (20).

The most used methods for measuring the hydrodynamic size, surface charge and polydispersity of PMs while also defining their shape are dynamic light scattering, atomic force microscopy, and transmission electron microscopy (14).

1.4.1.2 Encapsulation Efficiency and Loading Capacity

To determine the effectiveness of PMs as DDSs the terms Encapsulation Efficiency (EE) and Loading Capacity (LC) especially have been introduced. The EE is the percentage of actual encapsulated drug divided by the total initial drug added. Loading capacity is the amount of mass percentage that the encapsulated drug takes up in the nanoparticle suspension (14).

1.4.1.3 Methods of preparation

The technique used to prepare PMs is dependent on the properties of the block copolymer, with factors such as the method of addition, ratio of aqueous to organic materials, and copolymer

concentration playing a crucial role. Hence, it is essential to optimize these factors to achieve a standard recipe that can produce PMs with suitable physicochemical and functional properties (11,21). The process of encapsulating drugs in PMs can be performed during synthesis or in a subsequent stage, depending on the technique and properties of the drugs. Direct dissolving is the simplest technique for preparation of PMs; others include dialysis, (oil-in-water) solvent evaporation, freeze drying, the nanoprecipitation method and more (5,11,21).

Direct Dissolution Method

- Copolymer has high aqueous solubility

For making PMs out of copolymers with high aqueous solubility the Direct Dissolution Method is used. This technique involves mixing copolymers and drugs in aqueous solvents and uses methods such as sonication, stirring and applying heat to encapsulate the drugs.(11,21)

Simple mixing

This method is usually used for PMs that are made of oppositely charged block copolymers which as mentioned before are called polyion complex (PIC) micelles. Self-assembly happens due to electrostatic interactions in aqueous environment (11)

Solvent Evaporation Method

- Copolymer is soluble in water

Solvent evaporation method also known as film hydration method. Drugs and copolymers are both dissolved in a common solvent. Methanol, ethanol, acetone, acetonitrile and other volatile organic solvents are commonly employed for dissolving the copolymer and drug. Then solvents are evaporated, afterwards a thin layer of a drug-copolymer is left. Drug-loaded PMs spontaneously develop in response to the addition of water or buffers (11,21).

Oil-in-water solvent evaporation method

This technique is based on the creation of an oil-in-water emulsion. In the organic phase containing the polymer, the drug dissolves. The organic phase is then emulsified with an aqueous phase. A suspension of drug-loaded PMs in aqueous solution is left by evaporating the organic solvent (22).

Dialysis Method

- Copolymer has low aqueous solubility

When the hydrophobic part of the amphiphilic polymer is long and very hydrophobic the two aforementioned methods are not useable. Then the dialysis method is used. In this method, the copolymer and drug are dissolved together in a solvent such as N,N-dimethylformamide, dimethylsulfoxide, acetone, acetonitrile, tetrahydrofuran, dioxane or similar. Then to promote the formation of the micelles, an aqueous solvent containing the copolymer and drug is introduced into the solution. The solution with micelles is added to a dialysis bag and placed in water. This is usually left for a long time to remove the organic solvent. It is a highly effective method although very time-consuming.(11,21)

Freeze-drying method

The freeze-drying process makes use of organic solvents that can be frozen and dried. The drug and the copolymer are dissolved in a solution of water and organic solvent. This mixture is then freeze-dried to remove the organic solvent. The PMs start to form after adding water to the resulting powder. However, this preparation technique has the disadvantage that the organic solvents utilised in the procedure may still be present in the finished product (14).

Nanoprecipitation method

The nanoprecipitation method involves combining two miscible solvents, one of which acts as a good solvent (typically an organic solvent like ethanol, isopropanol or acetone) and the other as a non-solvent for the polymer, such as water (23). The organic solvent is then removed via dialysis or evaporation, resulting in the formation of PMs in the solution (24,25).

1.5 Retention and Release

As mentioned prior, according to researchers Alvarez-Lorenzo and Concheiro PMs should be able to Retain in the blood circulation and Release in target cells. When travelling towards targeted tissues the PM should be able to retain the drug without any leakage and at target the PM should be quickly release the drug. Here different techniques will be explained that can be used to create PMs that are able to abide to the Retain and Release challenge.

1.5.1 Retention

When PMs are administered in the bloodstream, the micellar concentration might fall below CMC. As a result, the equilibrium will change in favour of the unimer state and thus result in (partial) disintegration of the micelles. Furthermore, copolymers show affinity to blood constituents such as albumin, other proteins and cells which can cause early micelle disruption and drug loss. To make sure that the loaded drug will reach the target site at reasonable concentrations to perform its function, PMs must be able to retain the drug for a specific amount of time as well as have a long circulation time in the bloodstream (26). Stable PMs will reduce early leakage of encapsulated drugs and improve targeted drug delivery. The main goal is to make PMs that are sufficiently stable when administered in vivo (27). To achieve this, many techniques can be used.

In the first generation of PMs retention of the drug was primarily due to hydrophobic interactions between drug and the core-forming segment of the copolymer (15,16). The hydrophobic interactions between the core and the encapsulated hydrophobic drug help to stabilize the micelle (2,18,21). Since then more techniques have been developed to stabilize the drug during transport, for instance to bind the drug via reversible bonds to the copolymer (26). An example of this is the work by Bae et al., who synthesized polymers that were conjugated with anti-tumour drug doxorubicin (DOX) and contained hydrazones that responded to changes in pH. The hydrazone linkage was designed to break apart within lysosomes, releasing DOX in an acidic environment (28).

Stabilizing PMs can also be achieved via crosslinking, where bonds are created between neighbouring copolymers either in the core or shell. Crosslinking is an easy and effective way to prevent early disassembly, ensuring longer circulation durations and effective target site accumulation. In this method, the assembly of the unimers is sustained not only by hydrophobic contacts but also by the production of crosslinks in the PMs (12).

Core-crosslinking is typically accomplished after micelle production using PMs made of side-chain or end-group-functionalized block copolymers. It is important that the reactive groups utilised for core-crosslinking do not disrupt the micellization process. This means that they must be sufficiently hydrophobic or few in number to not interfere with the development of micelles (12). The same applies to crosslinking in shell-forming segments of copolymers (29).

The structure and cohesiveness of the hydrophobic core affect how easily micelles dissolve. By increasing the hydrophobicity of a certain copolymer, the hydrophobic core of the micelle will then be more cohesive and dense and therefore more stable. Increasing hydrophobicity of a copolymer

can for instance be done by altering the molecular weight ratio of hydrophobic to hydrophilic segments of the copolymer (18).

Furthermore, increasing hydrophobic chain length also leads to increasing stability of the micelle. Hussein and Youssry studied poly(ethylene glycol) (PEG) copolymers with different hydrophobic segments. Results of their study were that at a constant hydrophilic block length but with a higher hydrophobic/hydrophilic ratio, CMC of the copolymer decreased and thus more stable micelles were created (30).

Besides length of the hydrophobic segment, the hydrophobicity of the core also influences the stability of micelles (18). Ranger et al. measured CMC of PEG-block-poly(alkylmethacrylates) with different degrees of hydrophobicity and found that the most hydrophobic copolymer showed the lowest CMC and thus the most stable micelles (31). The same was found by Vangeyte et al. where PEG was bound to strongly hydrophobic polystyrene (PS) even addition of surfactants did not lead to destabilization of micelles (32).

Certain drugs can also be encapsulated in the shell structure of PMs. Drugs with intermediate polarity are able to situate between the PM core and the shell surface or even on the surface of the PM itself (27). The hydrophilic shell serves to camouflage the drug within the body and thereby hinder its interaction with cells (5).

Another difficulty that PMs seem to have in vivo is the adherence to bio-components such as plasma proteins. PMs might be easily eliminated from the body by biocomponent adsorption and/or complement activation, which activates the reticuloendothelial system (RES) to remove the PM as well as the drug within its core. In healthy individuals the RES consists of phagocytic cells that eliminate any foreign bodies in the blood or tissues (13).

A benefit that most PMs have is their very tiny particle size and diameter, which mostly ranges between 5 and 100 nm as earlier stated. For smaller particles it is easier to achieve stability and they have a greater range of movement in the bloodstream. Biocomponents such as proteins adsorb less easily to smaller particles (15).

According to findings on solid polymeric nanoparticles, a hydrophilic and neutral surface prolongs circulation time after intravenous injection and decreases the development of the protein corona. As a result of non-specific protein binding and enhanced aggregation in vivo, micelles with positive zeta potential, on the other hand, are characterised by relatively low stability in biological fluids (5).

Besides producing smaller PMs there seems to be another way to avoid this problem. Certain hydrophilic blocks have been added into the structure of block copolymers to give polymeric micelles anti-fouling capabilities. These anti-fouling properties reduce binding of biocomponents and therefore protects the PM (13).

1.5.2 Release

For a PM to be able to release all of the encapsulated drugs many different techniques have been employed. The construction of stimuli-responsive PMs is a very efficient method for ensuring targeted drug administration and sufficient release. Stimuli-responsive PMs can react to a variety of extracellular and intracellular biological stimuli (such as pH, altered redox potential and enzyme concentration) as well as external stimuli (such as magnetic field, light, temperature and ultrasound) (33).

Temperature sensitive PMs

Temperature-sensitive polymers are one of the most well-known materials that have been used in DDSs. They can alter their structure from a shrunken to a swelled form (or vice versa) in response to a change in temperature. A change in temperature leads to a change in solubility of the copolymer which causes the release of the drug that is encapsulated. A phase transition that results in swelling or shrinking happens when the temperature changes (34). Temperatures in most organisms beyond baseline values (37 °C) are present in many inflammatory or diseased areas, as well as tumors. Thermo-responsive may therefore be stimulated by rising temperatures, enabling stimuli-responsive DDSs to be activated by both internal and exterior temperature changes. Fast responsiveness to heat changes is just one benefit of a thermo-responsive PM (35).

Magnetic field-responsive PMs

Magnetic nanoparticles (MNPs) can be triggered by an alternating magnetic field (AMF) for targeted release. MNPs are able to form heat under influence of a high-frequency AMF due to internal rotational motion (Brownian) and external motion (Neel) (34). The increased local temperature caused by MNPs might be used to either directly induce apoptosis in tumors or make them more vulnerable when combined with chemotherapy (33). MNPs can have a size of around 10nm which makes it able for PMs to encapsulate them. For instance MNPs encapsulated by thermos-responsive PMs can undergo conformational changes due to higher temperatures as mentioned above. Drugs can thus be released in a remotely controlled manner (34).

Ultrasound-responsive PMs

There has been considerable interest in utilizing ultrasound (US) as a means of triggering the release of drugs from nanoparticles at the intended site. When exposed to ultrasound, PMs have demonstrated enhanced drug release due to the physical/chemical rupture. Besides triggering release, US can create cavitation bubbles and increase temperature in vivo, which increases permeability of cell membranes etc (34).

Light-responsive PMs

Near-Infrared (NIR) light is particularly advantageous for drug delivery because it can penetrate deep into tissues (up to approximately 10 cm within the human body) without causing significant tissue damage. PMs that respond to light are often produced by integrating chromophores into their structure, including azobenzene, pyrene, cinnamoyl, spirobenzopyran or nitrobenzyl groups. When illuminated, the nanostructure of the PM changes and they dissolve, releasing the encapsulated drugs (33).

Redox responsive PMs

Glutathione (GSH) is an antioxidant that is produced in lots of organisms in inflamed tissues to prevent damages caused by reactive oxygen species, free radicals etc. Due to the difference in GSH concentration GSH is a possible biomarker to discriminate between the extracellular and intracellular environments, as well as between the tumour/inflamed and normal tissues. GSH levels in the cytoplasm may be 100 to 1,000 times higher than in extracellular environment. Disulfide bonds, which GSH can cleave, are frequently employed to increase redox sensitivity. Disulfide bonds have been included into a variety of redoxresponsive PMs thus far, either between the hydrophobic and hydrophilic segments or in the hydrophobic blocks. These PMs can thus be reductively degradable or quickly deconstructed to release encapsulated drugs in a redox environment (33).

Enzyme responsive PMs

Enzymes play a crucial role in numerous biological and metabolic processes in vivo due to their catalytic properties. Dysregulated expression and activity of enzymes have been observed in various pathological sites and are associated with several diseases. Enzyme responsive PMs are prepared by

incorporating specific groups in the main chain or side groups of the polymer that can be recognised and degraded by overexpressed enzymes (33).

pH sensitive PMs

Sensitivity to pH changes can be effectively utilized for designing PMs that can disassemble and release their load when encountering a change in pH in the environment (3). It is well acknowledged that the pH levels vary in different parts of the human body (34). For instance in the different parts of the gastrointestinal tract, where the pH is highly acidic in the stomach (~1-2) and in the other parts more neutral (6-7) (34,36). At a more cellular level there are also variations in pH values, for instance the pH of cytosol is neutral (7.4) compared to a more acidic environment in intracellular organelles such as the Golgi apparatus (6.4), endosomes (5-6) and lysosomes (4-5) (3,34). Furthermore certain abnormal tissues like tumour tissue exhibit higher pH levels (4-5) than blood and normal tissues (7.4) due to rapid proliferation causing a deficit in oxygen and therefore an overproduction of lactic acid. The same applies to healing wounds, where after 60h into an inflammatory process a drop in pH (6.5) can be seen (4,34).

It has been mentioned in previous paragraphs that PMs are very suitable for delivering chemotherapy medication. Due to their small size (<100 nm) they are able to penetrate tumour tissue via extravasation. Tumour tissue in general has a higher amount of blood vessels than 'normal' tissue, this is called the Enhanced Permeability and Retention (EPR) effect. A general explanation for this is that in order for tumours to grow very rapidly the production of blood vessels needs to be stimulated. The same applies to inflamed tissue. PMs will therefore accumulate more in tumour tissue than in healthy tissue if they circulate in the blood stream for a sufficient amount of time. Additionally, these tissues have poor lymphatic outflow, which also helps to retain the PM at the target location (26). This combined with the previously mentioned lowered pH levels makes pH responsive PMs the perfect candidate for delivering chemotherapeutics.

There are two main strategies for making pH-sensitive PMs. The first is to use a block copolymer where the hydrophilic part contains an ionizable chemical group such as an amino or carboxylic group (34). At physiological pH of 7.4 these groups are deprotonated and the polymer is hydrophilic which makes the core/shell structure hold its structure in an aqueous environment. In contrast in an acidic environment the ionic groups of these polymers are protonated which makes them al positively charged. This positive charge on the backbones of all these polymers in the shell makes them repel each other and the core/shell structure opens up (1,13).

The second strategy uses chemical groups called acetals or hydrazones to form linkages between the hydrophobic and hydrophilic blocks of the micelles. When these PMs encounter an acidic pH, the linkages degrade, causing the PMs to disassemble and release the drug (27).

1.6 Current state and future prospects

There have been various preclinical studies conducted on PMs, which have shown that they can serve as a promising nanomedicine platform for drug delivery and cancer therapy. Compared to traditional anticancer drugs, PMs-based DDSs can enhance the effective dose of the drug and decrease systemic side effects (27,33).

So far, nine PMs have been researched in clinical trials. All of these PMs passively target cancer tissue through the EPR effect,. For the treatment of two different cancers, Genexol-PM (poly(ethylene glycol)-b-poly(D,L-lactide)) has received approval (33,37). PMs still need to be improved in order to have the intended therapeutic benefits. Because of the complexity of cancers and the human body,

passive tumour targeting using the EPR effect of existing PMs may not be as successful in humans (27,33).

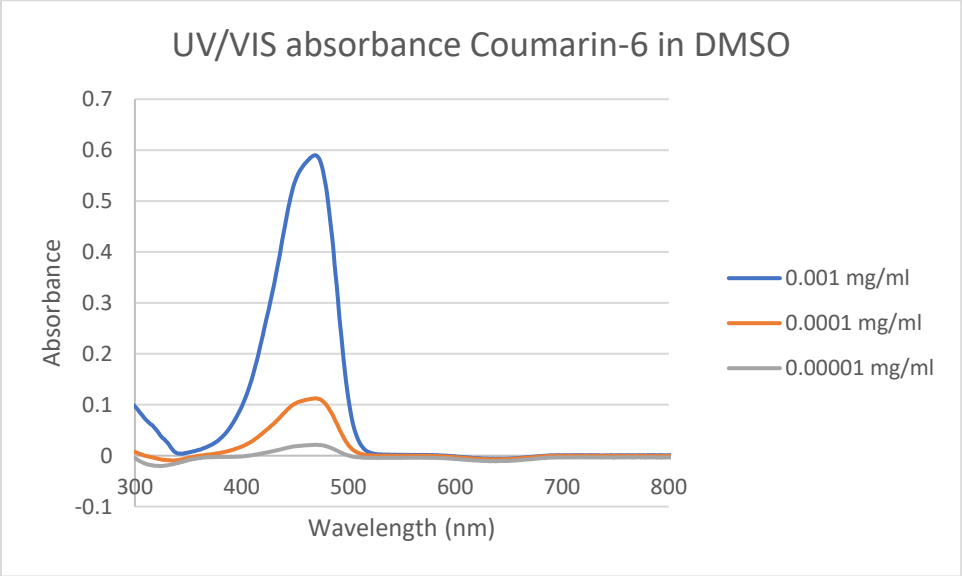
For PMs to be successful, modifications seem to be required. In comparison to conventional drug administration and non-specific targeted PMs, stimuli-sensitive PMs exhibit a number of benefits. However, it is crucial to remember that there are still many serious problems and difficulties that need to be resolved since they are not perfect yet (5,38). Firstly, the biostability of PMs in vivo is limited. PMs come into touch with a variety of biocomponents after being administered in vivo, including cells, tissues and organs. The biodistribution and cellular reactions of PMs will be influenced by their surface charge and size. (13,33,38). Secondly, it is important to increase the stimuli sensitivity of PMs in target areas in order to prevent off-target effects (38). In order to control the amount of release and rate to the target areas, it is crucial to identify the degree of acidic pH that PMs will respond to. For instance, some normal tissues might also display low pH values (33,38). Additionally, more attention needs to be directed towards the variety of tumour kinds and stages, so PMs can be designed more specifically (38).

In order to achieve targeted therapeutic delivery for cancer treatment, it is crucial to gain a deeper understanding of the physiological microenvironments of tumours and to continue developing PMs that can be tailored to very specific stimuli (38).

2. Supplementary results

2.1 UV/VIS Absorbance

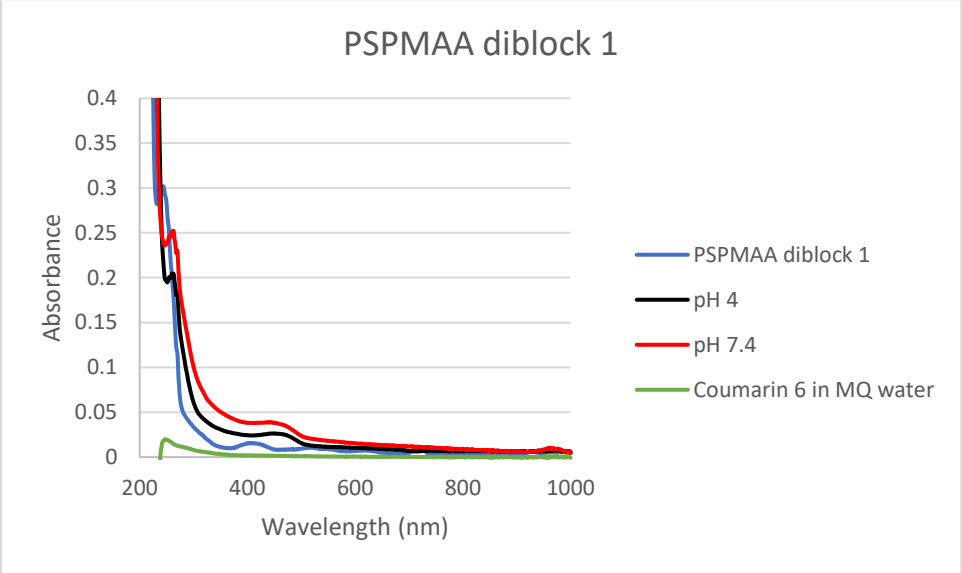
2.1.1 Calibration curve



Appendix 1: The UV/VIS absorbance of samples with different concentrations of Coumarin-6 in dimethylsulfoxide (DMSO).

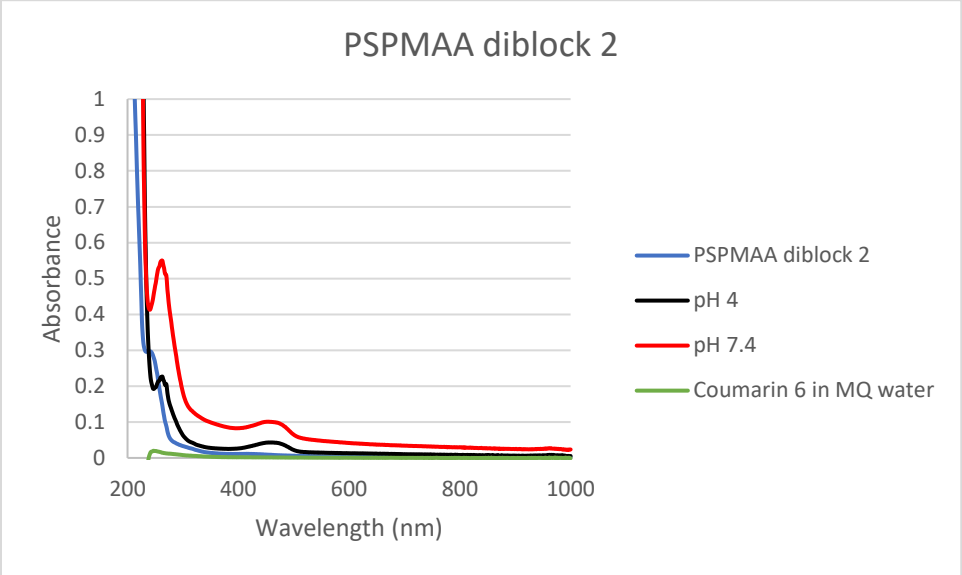
2.1.1 Polymers

PSPMAA diblock 1



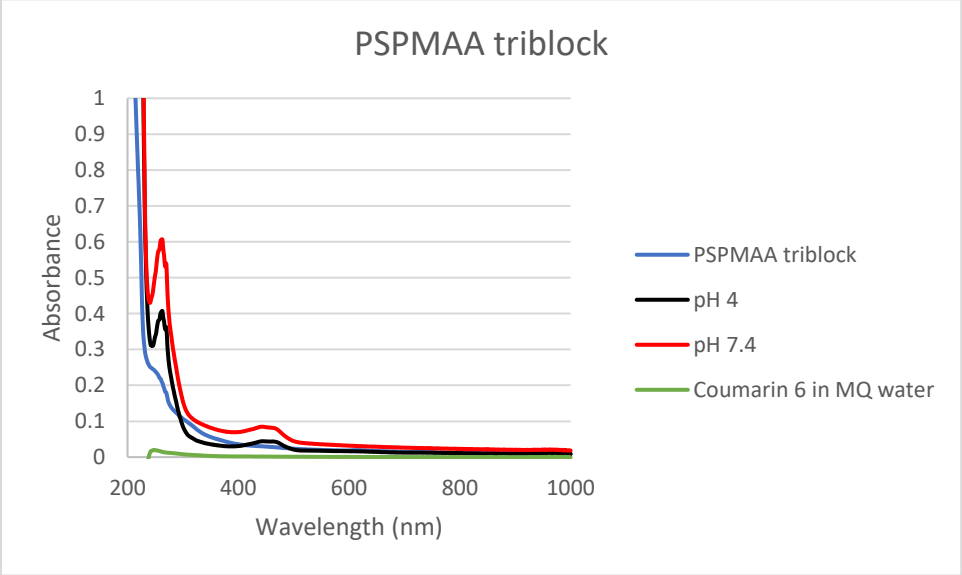
Appendix 2: The UV/VIS absorbance PSPMAA diblock 1 polymer in basic water shown in blue. The UV/VIS absorbance of the micelles in black (pH 4) and red (pH 7). The UV/VIS absorbance of Coumarin-6 in MQ water in green.

PSPMAA diblock 2



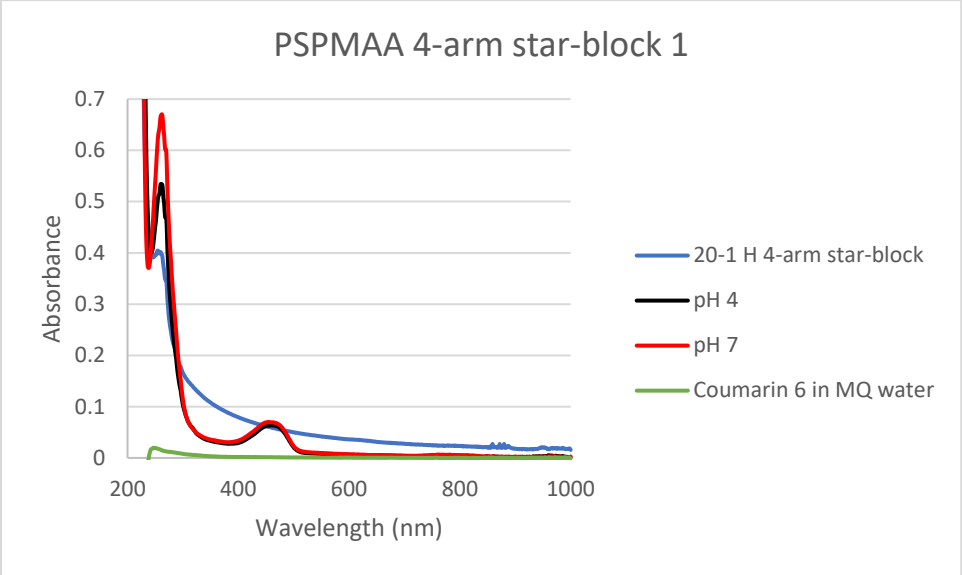
Appendix 3: The UV/VIS absorbance PSPMAA diblock 2 polymer in basic water shown in blue. The UV/VIS absorbance of the micelles in black (pH 4) and red (pH 7). The UV/VIS absorbance of Coumarin-6 in MQ water in green.

PSPMAA triblock



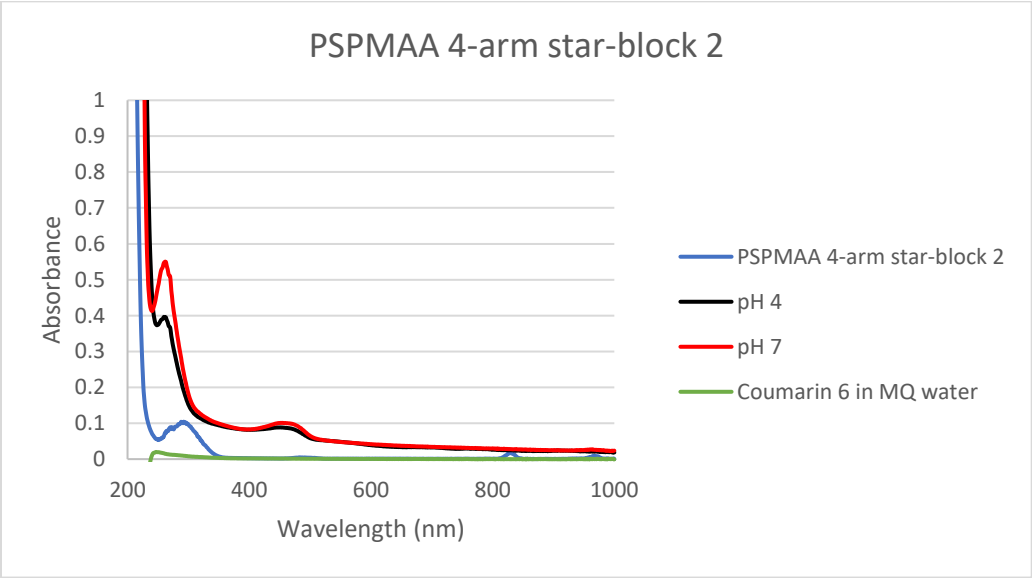
Appendix 4: The UV/VIS absorbance PSPMAA triblock polymer in basic water shown in blue. The UV/VIS absorbance of the micelles in black (pH 4) and red (pH 7). The UV/VIS absorbance of Coumarin-6 in MQ water in green.

PSPMAA 4-arm star-block 1



Appendix 5: The UV/VIS absorbance PSPMAA 4-arm star-block 1 polymer in basic water shown in blue. The UV/VIS absorbance of the micelles in black (pH 4) and red (pH 7). The UV/VIS absorbance of Coumarin-6 in MQ water in green.

PSPMAA 4-arm star-block 2



Appendix 6: The UV/VIS absorbance PSPMAA 4-arm star-block 2 polymer in basic water shown in blue. The UV/VIS absorbance of the micelles in black (pH 4) and red (pH 7). The UV/VIS absorbance of Coumarin-6 in MQ water in green.

2.2 Statistical Analysis

2.2.1 Micelles

Encapsulation Efficiency

Within group

Encapsulation Efficiency

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	358.2327	71.64655	44.17956
Column 2	5	373.1987	74.63974	56.33722

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	22.39803	1	22.39803	0.445657	0.523191	5.317655
Within Groups	402.0671	8	50.25839			
Total	424.4651	9				

Appendix 7: Results of the one-way ANOVA test for the encapsulation efficiency within each group of micelles.

Between groups

Encapsulation Efficiency

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	146.0627	73.03137	12.83678847
Row 2	2	126.6831	63.34156	5.507349473
Row 3	2	149.256	74.628	58.4829777
Row 4	2	152.6252	76.31261	37.1524586
Row 5	2	156.8044	78.4022	38.50268179

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	271.9829	4	67.99572	2.229627315	0.20125	5.192168
Within Groups	152.4823	5	30.49645			
Total	424.4651	9				

Appendix 8: Results of the one-way ANOVA test for the encapsulation efficiency between all groups of micelles.

Size

Within group

Size						
Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	1131	226.2	8745.2		
Column 2	5	905.1	181.02	8424.802		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5103.081	1	5103.081	0.594418	0.462876	5.317655
Within Groups	68680.01	8	8585.001			
Total	73783.09	9				

Appendix 9: Results of the one-way ANOVA test for the size measurements within each group of micelles.

Between groups

Size						
Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Row 1	2	401	200.5	144.5		
Row 2	2	311	155.5	60.5		
Row 3	2	638	319	18		
Row 4	2	193.1	96.55	1734.605		
Row 5	2	493	246.5	13944.5		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	57880.98	4	14470.25	4.549789478	0.063857	5.192168
Within Groups	15902.11	5	3180.421			
Total	73783.09	9				

Appendix 10: Results of the one-way ANOVA test for the size measurements between all the groups of micelles.

Zeta potential

Within group

Zeta potential

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	-182.3	-36.46	6.493
Column 2	5	-152.9	-30.58	72.332

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	86.436	1	86.436	2.193111	0.176903	5.317655
Within Groups	315.3	8	39.4125			
Total	401.736	9				

Appendix 11: Results of the one-way ANOVA test for the zeta potential within each group of micelles.

Between groups

Zeta potential

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	2	-68.7	-34.35	2.205
Row 2	2	-73.8	-36.9	0.98
Row 3	2	-73.7	-36.85	0.845
Row 4	2	-57.9	-28.95	248.645
Row 5	2	-61.1	-30.55	43.245

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	105.816	4	26.454	0.446978913	0.77232	5.192168
Within Groups	295.92	5	59.184			
Total	401.736	9				

Appendix 12: Results of the one-way ANOVA test for the zeta potential between all groups of micelles.

2.2.2 Release

UV-absorbance probe in micelles measurements

PSPMAA diblock 1

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	100.1176	116.8182
Variance	31.428	186.2391
Observations	7	7
Hypothesized Mean Difference	0	
df	8	
t Stat	-2.99491	
P(T<=t) one-tail	0.008602	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.017205	
t Critical two-tail	2.306004	

Appendix 13: Two-sample t-test assuming unequal variances for the UV absorbance measurements for the release of the PSPMAA diblock 1 micelles in pH 4 and pH 7 solution.

PSPMAA diblock 2

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	96.75639	127.1304
Variance	420.7245	278.4797
Observations	7	7
Hypothesized Mean Difference	0	
df	12	
t Stat	-3.03913	
P(T<=t) one-tail	0.005145	
t Critical one-tail	1.782288	
P(T<=t) two-tail	0.01029	
t Critical two-tail	2.178813	

Appendix 14: Two-sample t-test assuming unequal variances for the UV absorbance measurements for the release of the PSPMAA diblock 2 micelles in pH 4 and pH 7 solution

PSPMAA triblock

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	87.19747	93.71032
Variance	76.30126	21.70369
Observations	7	7
Hypothesized Mean Difference	0	
df	9	
t Stat	-1.74059	
P(T<=t) one-tail	0.057872	
t Critical one-tail	1.833113	
P(T<=t) two-tail	0.115744	
t Critical two-tail	2.262157	

Appendix 15: Two-sample t-test assuming unequal variances for the UV absorbance measurements for the release of the PSPMAA triblock micelles in pH 4 and pH 7 solution

PSPMAA 4-arm star-block 1

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	93.17414	97.75337
Variance	41.94386	3.620658
Observations	7	7
Hypothesized Mean Difference	0	
df	7	
t Stat	-1.79485	
P(T<=t) one-tail	0.057877	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.115753	
t Critical two-tail	2.364624	

Appendix 16: Two-sample t-test assuming unequal variances for the UV absorbance measurements for the release of the PSPMAA 4-arm star-block 1 micelles in pH 4 and pH 7 solution

PSPMAA 4-arm star-block 2

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	98.29973	97.75337
Variance	49.02789	3.620658
Observations	7	7
Hypothesized Mean Difference	0	
df	7	
t Stat	0.199221	
P(T<=t) one-tail	0.423877	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.847753	
t Critical two-tail	2.364624	

Appendix 17: Two-sample t-test assuming unequal variances for the UV absorbance measurements for the release of the PSPMAA 4-arm star-block 2 micelles in pH 4 and pH 7 solution

Size

PSPMAA diblock 1

Size

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	192.15	96.075	8.61125
Column 2	2	400	200	512

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10800.41	1	10800.41	41.49125	0.023264	18.51282051
Within Groups	520.6113	2	260.3056			
Total	11321.02	3				

Appendix 18: Results of the one-way ANOVA test for the size measurements during the release experiment of the PSPMAA diblock 1 micelles in pH 4 and pH 7 solution.

PSPMAA diblock 2

Size

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	125.075	62.5375	40.36511
Column 2	2	279	139.5	760.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5923.226	1	5923.226	14.79207	0.061439	18.51282051
Within Groups	800.8651	2	400.4326			
Total	6724.092	3				

Appendix 19: Results of the one-way ANOVA test for the size measurements during the release experiment of the PSPMAA diblock 2 micelles in pH 4 and pH 7 solution.

PSPMAA triblock

Size

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	123.2495	61.62475	6637.882
Column 2	2	605.5	302.75	21.125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	58141.39	1	58141.39	17.46248	0.052773	18.51282051
Within Groups	6659.007	2	3329.503			
Total	64800.39	3				

Appendix 20: Results of the one-way ANOVA test for the size measurements during the release experiment of the PSPMAA triblock micelles in pH 4 and pH 7 solution.

PSPMAA 4-arm star-block 1

Size

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	197.945	98.9725	3965.397
Column 2	2	477.5	238.75	0.125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19537.75	1	19537.75	9.853811	0.088256	18.51282051
Within Groups	3965.522	2	1982.761			
Total	23503.27	3				

Appendix 21: Results of the one-way ANOVA test for the size measurements during the release experiment of the PSPMAA 4-arm star-block 1 micelles in pH 4 and pH 7 solution.

PSPMAA 4-arm star-block 2

Size

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	58.795	29.3975	728.6653
Column 2	2	477.5	238.75	120.125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43828.47	1	43828.47	103.2728	0.009545	18.51282051
Within Groups	848.7903	2	424.3952			
Total	44677.26	3				

Appendix 22: Results of the one-way ANOVA test for the size measurements during the release experiment of the PSPMAA 4-arm star-block 2 micelles in pH 4 and pH 7 solution.

Zeta potential

PSPMAA diblock 1

Zeta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	-49.265	-24.6325	1.029613
Column 2	2	-80.65	-40.325	0.06125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	246.2546	1	246.2546	451.486	0.002208	18.51282
Within Groups	1.090863	2	0.545431			
Total	247.3454	3				

Appendix 23: Results of the one-way ANOVA test for the zeta potential measurements during the release experiment of the PSPMAA diblock 1 micelles in pH 4 and pH 7 solution.

PSPMAA diblock 2

Zeta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	-25.91	-12.955	1.1552
Column 2	2	-56.5	-28.25	4.205

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	233.937	1	233.937	87.28668	0.011263	18.51282
Within Groups	5.3602	2	2.6801			
Total	239.2972	3				

Appendix 24: Results of the one-way ANOVA test for the zeta potential measurements during the release experiment of the PSPMAA diblock 2 micelles in pH 4 and pH 7 solution.

PSPMAA triblock

Zeta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	-20.98	-10.49	146.0341
Column 2	2	-77.85	-38.925	1.71125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	808.5492	1	808.5492	10.94518	0.080488	18.51282
Within Groups	147.7453	2	73.87265			
Total	956.2945	3				

Appendix 25: Results of the one-way ANOVA test for the zeta potential measurements during the release experiment of the PSPMAA triblock micelles in pH 4 and pH 7 solution.

PSPMAA 4-arm star-block 1

Zeta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	-38.255	-19.1275	0.137812
Column 2	2	-85.9	-42.95	0.98

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	567.5115	1	567.5115	1015.397	0.000983	18.51282
Within Groups	1.117813	2	0.558906			
Total	568.6293	3				

Appendix 26: Results of the one-way ANOVA test for the zeta potential measurements during the release experiment of the PSPMAA 4-arm star-block 1 micelles in pH 4 and pH 7 solution.

PSPMAA 4-arm star-block 2

Zeta

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	2	-10.455	-5.2275	16.67531
Column 2	2	-70.65	-35.325	0.21125

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	905.8595	1	905.8595	107.2876	0.009192	18.51282
Within Groups	16.88656	2	8.443281			
Total	922.7461	3				

Appendix 27: Results of the one-way ANOVA test for the zeta potential measurements during the release experiment of the PSPMAA 4-arm star-block 2 micelles in pH 4 and pH 7 solution.

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