การเปรียบเทียบโซลูพลัส® กับพอลิเมอร์อื่น ในการเตรียมพอลิเมอริกไมเซลล์ สำหรับนำส่งเออร์โลทินิบ
Comparison of Soluplus® with Other Polymers to Form Polymeric Micelles for Erlotinib Delivery

นิพนธ์ต้นฉบับ

Original Article

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วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ2564;16(2):149-155.

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Thai Pharmaceutical and Health Science Journal 2021;16(2):149-155.

บทคัดย่อ

วัตถุประสงค์: เพื่อเปรียบเทียบคุณสมบัติไมเซลล์ของยาเออร์โลทินิบที่เตรียมจากพอลิเมอร์โซลูพลัส และพอลิเมอร์อื่นอีก 4 ชนิด ก่อนการตั้งสูตรตำรับเพื่อนำส่งยาทางเข้าทางปอด **วิธีการศึกษา:** การพัฒนาระบบไมเซลล์ในระดับนาโนเมตร เพื่อนำส่งเออร์โลทินิบด้วยพอลิเมอร์ 5 ชนิด ได้แก่ polyvinyl caprolactame-polyvinyl acetate- polyethylene glycol graft copolymer (Soluplus®), macrogal 15 hydroxysterate (Kolliphor® HS 15), poloxamer 188 (Lutrol® micro 68), D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) และ polyethylene glycol 5000–distearoylphosphatidylethanolamine (mPEG 5000- DSPE) เตรียมโดยวิธีทินฟิล์มไฮเดรชั่น ทุกสูตรตำรับที่เตรียมขึ้นจากสัดส่วนโดยมวลของยาต่อพอลิเมอร์ เท่ากับ 1:20 จะถูกประเมินคุณสมบัติต่าง ๆ คือ ขนาดและประจุของไมเซลล์ ตลอดจนประสิทธิภาพในการกักเก็บเออร์โลทินิบ เพื่อประเมินว่าพอลิเมอร์ชนิดใดมีความเหมาะสมในการนำส่งตัวยาดังกล่าวมากที่สุด **ผลการศึกษา:** โซลูพลัสมีประสิทธิภาพในการกักเก็บเออร์โลทินิบมากที่สุด เมื่อเทียบกับพอลิเมอร์อีก 4 ชนิด โดยคุณสมบัติของระบบนำส่งยามีความเหมาะสมต่อการนำส่งทางปอด คือ ขนาดของไมเซลล์ที่เล็กกว่า 200 นาโนเมตร และการกระจายของขนาดอนุภาคน้อยกว่า 0.1 อาจแปลผลได้ว่าประสิทธิภาพในการกักเก็บยาแปรผันตรงกับพื้นที่/ปริมาตรของส่วนที่ไม่ชอบน้ำของพอลิเมอร์ โดยบริเวณดังกล่าวจะเพิ่มโอกาสในการที่ตัวยาจะเข้าแทรก และ/หรือ ละลายเป็นเนื้อเดียวกันกับพอลิเมอร์ นอกจากนี้ความสามารถในการแทรกเข้าไปในระบบไมเซลล์ของเออร์โลทินิบถูกจำกัด อันเนื่องมาจากความเกะกะของโครงสร้างที่เป็นสายไฮโดรคาร์บอนและพันธะสามที่ติดกับวงเบนซีน **สรุป:** โซลูพลัสอาจนำไปพัฒนาเป็นระบบนำส่งเออร์โลทินิบเพื่อนำส่งยาทางปอด และอาจใช้เป็นพอลิเมอร์หลักในการนำส่งยาที่ละลายน้ำยากตัวอื่น ๆ ได้

คำสำคัญ: โซลูพลัส, เออร์โลทินิบ, พอลิเมอริก ไมเซลล์

Editorial note
Manuscript received in original form: November 16, 2020;
Revised: December 15, 2020;
Accepted in final form: December 21, 2020;
Published online: June 29, 2021.

Abstract

Objective: To compare micellar properties of erlotinib by using Soluplus® and 4 other polymers to achieve desired characteristics for pulmonary delivery. **Method:** Erlotinib-incorporated polymeric micelles were prepared from five common polymers; namely, polyvinyl caprolactame- polyvinyl acetate- polyethylene glycol graft copolymer (Soluplus®), macrogal 15 hydroxysterate (Kolliphor® HS 15), poloxamer 188 (Lutrol® micro 68), D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) and polyethylene glycol 5000–distearoylphosphatidyl ethanolamine (mPEG 5000- DSPE) using thin-film hydration method, with drug:polymer mass ratio of 1:20. All formulations were then characterised for their hydrodynamic diameter, zeta potential and encapsulation efficiency to investigate the appropriate polymer for erlotinib delivery. **Results:** The highest encapsulation efficiency was from Soluplus® with desirable hydrodynamic diameter smaller than 200 nm and a PDI value of less than 0.1. It might be assumed that more polymer chains or more hydrophobic groups provide more entrapment sites leading to increased solubilisation and thus the incorporation of erlotinib. Steric hindrances of two branches aliphatic chain and three benzene rings attached to alkyne of erlotinib play an important role in encapsulation efficiency, resulting in the difficulty to be packed into other alternative polymers. **Conclusion:** Based on the results of characterisation of erlotinib-loaded polymeric micelles, Soluplus® might be considered as a promising polymer for producing nanocarriers for nebulised delivery, and may be applicable for other hydrophobic drugs.

Keywords: soluplus, erlotinib, polymeric micelles

Journal website: http://ejournals.swu.ac.th/index.php/pharm/index

Introduction

Erlotinib is a new promising drug for non-small cell lung carcinoma (NSCLC). With its low aqueous solubility, low bioavailability, certain toxicities, relatively poor stability, and sub-therapeutic drug level, erlotinib faces certain challenges before it can offer the optimal effectiveness and safety. A new drug delivery and targeting using new technology including nanotechnology is needed to improve efficacy and safety profilfe of the drug. Soluplus® and other polymers were subject to comparisons in forming desirable polymeric micelles for pulmonary delivery.

Lung cancer is one of the most cancer-related death worldwide, and is the second most common cancer in both males and females. Lung cancer is divided into two types where 85% of the cases are diagnosed as non-small cell lung carcinoma (NSCLC), while 15% of total cases are classified as small-cell lung carcinoma (SCLC).[1,2] Pulmonary drug

delivery has been widely investigated to enhance therapeutic efficacy of drug at its site of action.[3]

Erlotinib, N-(3-ethynylphenyl)-6,7-bis (2-methoxyethoxy)-4-quinazolinamine, has been extensively studied in patients with NSCLC.[4] This molecule is a reversible tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR). Therefore it is useful in the treatment of proliferative disorders and is currently marketed for the treatment of locally advanced or metastatic NSCLC in film-coated tablet form under various trade names; for example, Erlocip® (Cipla), Tarceva® (Roche) and Erloshil® (Raichem).[5]

However, erlotinib has low aqueous solubility (3 - 6 μg/mL at pH 6.8) and has problems of low bioavailability, with a molecular weight of 393.44 g/mol and a log P value of 2.75. Also, the toxicity of erlotinib, the stability and sub-therapeutic drug level have been still remaining. The solvents in which this drug can be dissolved are limited, thus erlotinib encapsulation in drug-carriers is likely to be problematic. Therefore, a suitable drug formulation with appropriate physicochemical properties should be taken into consideration to maximise erlotinib therapeutic effectiveness. The potential benefits of nanotechnology have been increasingly attractive since it provides many improvements in drug delivery and drug targeting, for example, reducing drug toxicities and improving efficacy of the treatments.[6]

From previous reports, this problem had been addressed in the field of formulation sciences. Pharmaceutical technology has been employed to achieve the most effective treatment of erlotinib as follows.

- Liquid self-emulsifying drug delivery system consisting of oil, surfactant and co-surfactant was firstly prepared, then solid self-emulsifying drug delivery system was developed by a spray drying method, using solid carriers.[7]
- Erlotinib-loaded albumin core with phospholipid bilayer shell using bovine serum albumin and lipid mixture containing dipalmitoyl-phosphatidylcholine (DPPC), cholesterol and 1,2-distearoyl-sn-glycero-3-phospho- ethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000).
- Polycaprolactone-polyethylene glycol-polycaprolactone (PCEC) nanoparticle-loaded erlotinib.[8]
- Reverse micelle-loaded lipid nanocarriers containing erlotinib by preparing reverse micelle and then adding the known amount of reverse micelles to a mixture of surfactant, oil and water.[9]
- PLGA nanoparticles of erlotinib and quinacrine prepared by multiple emulsion (w/o/w) with solvent evaporation method.
- Erlotinib and doxorubicin-loaded liposomes containing a mixture of distearoylphosphatidylcholine (DSPC), cholesterol and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (POPG) by lipid film hydration method.[10]

Polymeric micelles are nano-sized colloidal carrier system. They are self-assembled of amphiphilic copolymers to provide spherical inner core, which encapsulates poorly water soluble drugs, while the outer shell of the hydrophilic part protects the drug from the aqueous environment. The two most crucial characteristics of the polymeric micelles for pulmonary drug delivery are the mean particle size and surface charge. The nano-sized polymeric micelles (10 - 200 nm) are advantageous for enhancing the solubility of hydrophobic drugs as well as avoiding alveolar macrophage.[11] Furthermore, this size range of nanocarriers can be easily incorporated into the aerosol droplets upon aerosolisation and achieve alveolar deposition[11,12] For surface charge, a high value is suggested for long-term physical stability. However, positive charge is preferable as it can interact with the negatively-charged mucus layer in the airways through electrostatic force.[13]

In terms of preparation techniques, there are a number of methods, e.g., direct dissolution, oil in water emulsification, thin-film hydration, dialysis and freeze-drying. Among all, thin-film method has been the most popular method in polymeric micelle preparation as it yields high drug-loading capacity and it is feasible for scaling- up.[13]

When selecting the suitable polymers, a few considerations including biocompatibility and biodegradability should be made in order to avoid acute and/or chronic toxicity.[13] In previous research, polyvinyl caprolactame-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®), macrogal 15 hydroxysterate (Kolliphor® HS 15) and poloxamer 188 (Lutrol® micro 68), D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) and polyethylene glycol 5000–distearoylphosphatidylethanolamine (mPEG 5000-DSPE) have been used as major and/or mixed components in the drug-carrier for pulmonary drug delivery, i.e., rifampicin-loaded Soluplus micelles[14], pacitaxel-incorporated TPGS mixed micelles[15] and beclomethasone dipropionate-encapsulated PEG 5000-DSPE carriers.[16] These

five polymers were consequently considered appropriate in this study.

Since hydrophobic drug can be loaded in the amphiphilic micelle with high drug loading, in the diameter ranging from 10 to 100 nm, with a narrow size distribution.[17,18] Polymeric micelles have therefore been developed as novel colloidal carriers for increasing solubility and bioavailability of hydrophobic drugs for pulmonary drug delivery. The aim of this study was therefore to investigate suitable polymers for the preformulation of polymeric micelles to achieve suitable encapsulation efficiency for erlotinib. These micelles should have desirable properties for pulmonary delivery including hydrodynamic diameter (less than 200 nm), good stability (high value of surface charge), high encapsulation efficiency, reduced systemic side effects, and therapeutic drug level at the target site. Specifically, we compared micellar properties of erlotinib (hydrodynamic diameter, polydispersity index, Zeta potential and encapsulation efficiency) using Soluplus® and 4 other different polymers to achieve desired characteristic for pulmonary delivery.

Methods

Materials

Erlotinib 99% (LC Laboratories, USA) was used as a hydrophobic drug in the formulations. The following polymers were used in the formulation. Polyvinyl caprolactame-polyvinyl acetate- polyethylene glycol graft copolymer (Soluplus®), macrogal 15 hydroxysterate (Kolliphor® HS 15) and poloxamer 188 (Lutrol® micro 68) were purchased from D-BASF (Germany) and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) was obtained from Sigma-Aldrich Co. (St. Louis, USA). Polyethylene glycol 5000–distearoylphosphatidylethanolamine (mPEG 5000-DSPE) was provided by Ludwigshafen (Germany) and used as a lipid mixed polymer in the formulations. Absolute ethanol (Sigma Aldrich Co., St. Louis, USA) was used as a solvent for HPLC analysis. For quantification of erlotinib, acetronitrile (99.9%, gradient grade for HPLC), HPLC grade water and trifluoroacetic acid (TFA, > 99.0%) were used as a mixture of mobile phase.

Preparation of erlotinib -loaded polymeric micelles

Erlotinib-loaded polymeric micelles were prepared using thin-film hydration method as shown in Figure 1. 2 mL of a transparent stock solution of erlotinib (0.5 mg/mL) in absolute ethanol was added to 20 mg of different polymers (TPGS, Lutrol® 68, Soluplus®, DSPE-PEG, Kolliphor® HS 15) dissolved in 20 mL absolute ethanol. The mixture of a stock solution of erlotinib and the alcoholic solution of polymer was clear and the solvent was subsequently removed under low pressure at 50 - 200 bars, at 70 °C by rotary vacuum evaporation (Knf Lab, USA) for 20 min to obtain a thin film of drug/polymer. The film was then hydrated by adding 10 mL deionised water and rotated with the rotary evaporator at 250 rpm to form a polymeric micelles. The formulation was bath sonicated for 3 min to reduce particle size and non-incorporated drug was removed by filtration through a 0.45 µm syringe filter membrane (Merck Millipore Ltd., UK) to obtain the clear polymeric micelles. All formulations from different 3 batches were characterised for particle size distribution, polydispersity index (size distribution, PDI), zeta potential, and encapsulation efficiency. The data are expressed as mean values ± standard deviation.

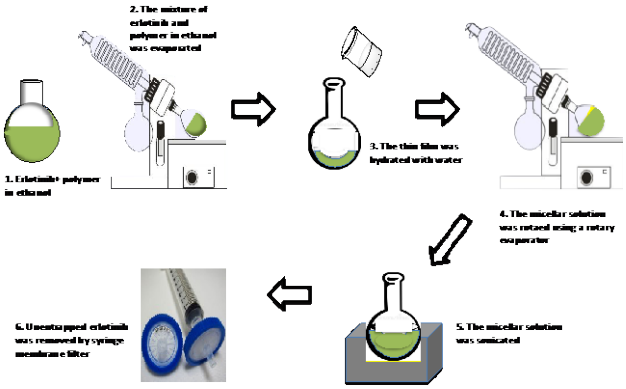

Figure 1 Preparation of erlotinib-loaded polymeric micelles

Characterisation of polymeric micelles

1. Particle size distribution

The hydrodynamic diameter and polydispersity index of the polymeric micelles were assessed using the Malvern Nano ZS Zetasizer (Malvern Instruments Ltd, UK). Briefly, 1.0 mL erlotinib-loaded micelles were directly placed into a disposable quartz cuvette to measure the hydrodynamic diameter in triplicate of particle undergoing Brownian motion within the dispersion at 25 °C. A PDI value of less than 0.1 is determined a monodispersed sample, a value smaller than 0.3 indicates a relatively monodispersed system, while a PDI value of greater than 0.7 is considered as a polydispersed system.[19,20]

2. Zeta potential

1.0 mL erlotinib loaded polymeric micelles were determined using the same instrument as particle size distribution by laser doppler velocimetry, which involves applying an electric field of known value to the sample and measuring the velocity of the particles, correlating against this value, by applying the Smoluchowski equation. The measurement was determined in triplicate at 25 °C.

3. Encapsulation efficiency

The quantification of erlotinib in the micelles was determined by using a high performance liquid chromatography (HPLC) system equipped with auto sampler and UV/Vis detvector (Agilent 1100 Series, USA) at wavelength 246 nm to determine the quantity of erlotinib. Synergi 4 µm Polar-RP 80 °A HPLC column (250 x 4.6 mm x 4 µm), which has an ether-linked phenyl phase, was used as the stationary phase for separation. It is noted that HPLC method was developed by adapting existing methods.[21,22]

The 0.1 mL polymeric micelles was diluted with methanol 10 times prior to determination. The encapsulation efficiency (%EE) of erlotinib in polymeric micelles was calculated from the following equation:

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{mass of drug in micelles}}{\text{mass of drug weight for encapsulation}} \times 100\%$$

Statistical analysis

All experiments within this study were carried out in triplicate and data were presented as mean with standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test by GraphPad Prism 6 software. A *P*-value of less than 0.05 was considered as statistical significant.

Results and Discussions

Particle size distribution, zeta-potential and encapsulation efficiency of erlotinib nanocarriers with five different polymers

Micelles less than 200 nm were aimed so as to passively target the leaky vasculature associated with cancer cells and to avoid being easily phagocytosed by alveolar macrophage.[23,24] Hence, TPGS, Kolliphor® HS 15 and Soluplus® may be suitable polymers for delivery of erlotinib. No significant difference (*P*-value > 0.05) in hydrodynamic diameter and zeta potential among these polymers was evident. However, encapsulation efficiency of erlotinib in Soluplus® micelles (95% EE) was significantly higher (*P*-value < 0.01) when compared to the encapsulation efficiency of erlotinib in other polymers. Hence, Soluplus® was likely to be the best polymer to serve as a nanocarrier for delivery of erlotinib. The data are shown in Table 1.

Table 1 Characterisation of erlotinib- loaded polymeric micelles using five different polymers (n = 3, mean ± S.D.).

Erlotinib- loaded polymeric micelles	Hydrodynamic diameter (nm)	Polydispersity Index (PDI)	Zeta potential (mV)	Encapsulation efficiency (%EE)
TPGS	24.50 ± 9.29	0.21 ± 0.05	-1.75 ± 2.55	14.83 ± 4.41
mPEG 5000-DSPE	952.15 ± 470.72	0.73 ± 0.23	-4.61 ± 6.81	19.35 ± 1.80
Kolliphor® HS 15	95.20 ± 76.91	0.30 ± 0.07	-1.08 ± 1.01	8.54 ± 2.83
Soluplus®	64.97 ± 0.27	0.06 ± 0.02	0.74 ± 1.20	95.93 ± 5.30
Lutrol® micro 68	610.43 ± 237.27	0.47 ± 0.20	-6.77 ± 5.94	1.07 ± 0.16

Hydrophobic drugs can be loaded into polymeric micelles by two predominant factors, i.e., chemical bonding and physical entrapment.[25] Physical interaction between drug and carrier is commonly considered to dominate for micelle formation, particularly in the case of lipophilic compounds.[23] According to previous reports, encapsulation efficiency of drug by physical entrapment depends on various factors; for example, the drug solubility in water, the preparation method (solvent evaporation, emulsification, etc.), the steric hindrance properties of drug and polymeric micelles and the physical interactions between the drug molecule and the inner core of micelles. As demonstrated in Figure 2, it can be evidently seen that hydrogen bonding, π-π parallel stacking interaction, Van

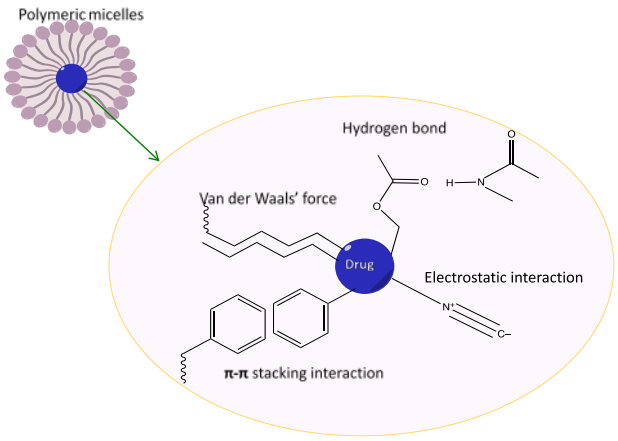

Figure 2 Physical interactions between drug and inner core of polymeric micelles (adapted from ref. 26).

der Waals' bonding interaction and electrostatic force critically impact the compatibility between the drug and micelle core. All of these can significantly affect drug-loading capacity of polymeric micelles and hence micellisation may not be suitable for all drugs.[25-28]

In this work, the parameters such as the preparation method (thin-film hydration) and the mass ratio of erlotinib and polymers (1:20) were exactly the same. The physicochemical properties of erlotinib and various polymers seem therefore impact encapsulation efficiency. For erlotinib, the main factor which interferes with drug encapsulation may be steric hindrance from the two branch aliphatic chains as well as and three benzene rings attached to alkyne (triple bond) of erlotinib (Figure 3). Moreover, oxygen atom from methoxyethoxy chains and nitrogen atom from quinazolinamine ring can possibly form weak electrostatic interactions and/or dipole-dipole interactions with the hydrophilic segment (on the surface) of micelles. Hence, it might be the barrier for erlotinib to penetrate to the inner core of micelle. To determine molecular interactions between erlotinib and polymers, X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC) and Fourier-Transform Infrared (FT-IR) are commonly used. Since XRD and DSC are limited to detecting specific drug-polymer bonds, i.e. hydrogen bond (H-bond), FT-IR is useful to identify molecular interations resulted from the infrared absorption.[29] Combined techniques are necessary for the clearer understanding of drug-polymer interactions.

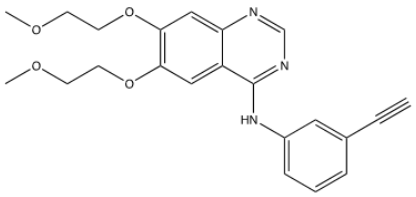

Figure 3 Structure of erlotinib.

For preparation method, this is chosen mainly based on the solubility of the polymers and drugs. For example, a solvent-diffusion technique has been used for the preparation of Kolliphor® HS micelles[30], while Soluplus® micelles prepared by classical thin-film hydration method have been previously reported.[31] Consequently, preparation method may be one of the predominant factors significantly affecting the encapsulation efficiency of erlotinib. Another factor leading to these significant differences may be due to inappropriate process of non-incorporated erlotinib removal. In this work, filtration method was used to remove unentrapped erlotinib, using a 0.45 μm cellulose acetate membrane filter. It can be possible that the majority of erlotinib-incorporated TPGS/mPEG5000-DSPE/Kolliphor HS15 and Lutrol micro 68 micelles have hydrodynamic diameter larger than 0.45 μm. These large drug-carriers cannot pass through a small pore size of the hydrophilic membrane filter, resulting in low encapsulation efficiency of erlotinib. From these observations, Soluplus® is likely to be the best polymer candidates under the same experimental conditions throughout the study.

In order to differentiate the polymeric formation and molecular bonding between erlotinib and Soluplus®, additional techniques are required to investigate further. Critical Micellar Concentration (CMC) is the minimum concentration of polymers required for the formation of micelles. This parameter is necessary to define the characteristics of micelles based on a sudden change in physical properties upon self-assembly micelle formation. Transmission Electron Microscope (TEM) is also a useful method to study the morphology of the polymeric micelles. TEM image is expected to demonstrate individual spherical micelles with narrow size distribution for micelle formation, which is in a good agreement with the results of hydrodynamic diameter and size distribution. The results of size distribution of erlotinib loaded-polymeric micelles obtained from Nano zetasizer are also suggested. The size distribution curve is supposed to show a narrow peak for erlotinib-loaded micelles, indicating a relatively uniformly dispersed population.

Subsequent to this initial study, five polymers (Figure 4) with different polymer chains, i.e., D-α-tocopherol polyethylene glycol 1000 succinate (TPGS), mPEG 5000-DSPE, polyvinyl caprolactame-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®), Macrogal 15 hydroxysterate (Kolliphor® HS 15) and poloxamer 188 (Lutrol® micro 68), were employed with erlotinib to study the factors affecting the incorporation of the drug. From Table 1, it is clearly seen that the highest EE, i.e., 95% EE, was from Soluplus®, while EE was approximately 20% for TPGS and DSPE-PEG. It might be assumed that more polymer chains or more hydrophobic groups provide more entrapment sites leading to increased solubilisation and thus the incorporation of erlotinib. This may be because more lipid had more possibilities for hydrophobic drug dispersed inside the micellar

core.[32] Therefore, the strategy for erlotinib delivery by adding lipids or more hydrophobic regions for inner core should be studied and explored further.

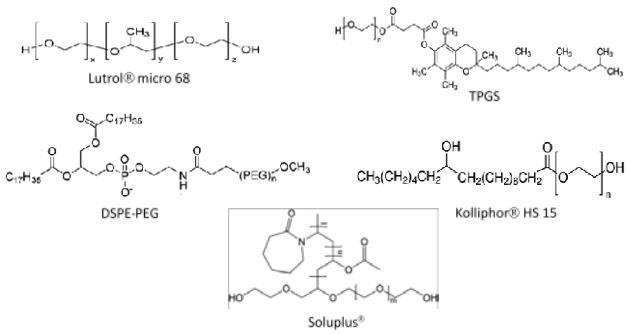

Figure 4 Structure of alternative polymers used for erlotinib-loaded polymeric micelles

Conclusion

From this preliminary study, % EE of erlotinib was found to increase when using more lipid or hydrophobic content, for example, TPGS, DSPE-PEG and Soluplus®. Hydrodynamic diameter and zeta potential has not shown any significant difference among these alternative polymers, encapsulation efficiency of erlotinib in Soluplus® micelles (95% EE) was significantly higher (*P*-value < 0.01).

In this study, it may allow a conclusion that Soluplus® was the best polymer for delivery of erlotinib with the good results of hydrodynamic diameter (65 nm) and polydispersity index (PDI = 0.06), presenting a stable micellar formulation and suitable for aerosolisation to the airways. Moreover, the optimal formulation of erlotinib related lipid component will be explored in the future work. The in vitro cytotoxic effect of Soluplus® and other materials is also required for the assessment of cell viability to reflect the inhibition of cell growth for human lung carcinoma cells.[33]

Acknowledgements

The author would like to sincerely thank Professor Kevin M.G. Taylor for his proofreading, and Dr. Satyanarayana Somavarapu for providing laboratory space.

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