A STUDY ON ACTIVATION OF POLYETHYLENE GLYCOL AND ITS CHARACTERIZATION BY INFRARED SPECTROSCOPY AND THIN LAYER CHROMATOGRAPHY

R. Bashyal*
Agriculture and Forestry, University, Rampur, Chitwan, Nepal

*Corresponding author: rajendrabashyal@gmail.com
Received date: 25 March 2022, Accepted date: 5 April 2022

ABSTRACT

Polyethylene Glycol (PEG) is the most popular polymeric material used for alteration and control of biodistribution. PEG may increase the lifetime of “drug carrier” assembly which helps in administering lower concentration of the “drug carrier” composite. It has been used widely for the modification of carriers used in therapeutics because PEG offers a shielding character that avoids rapid renal clearance from the body. This study was carried out at Dolphin Institute of Biomedical and Natural Sciences, Dehradun and Indian Institute of Technology (IIT), Roorkee, India during May-July 2005. Activation of PEG of different molecular weight (400 Da, 4000 Da, 8000 Da and 20,000 Da) was done using dry benzene, Triethylamine, Ethylene dichloride and 4-nitrophenyl chloroformate. Reaction mixture was monitored on TLC using EDC: Methanol (7:3). Then reaction mixture was portioned between EDC & water. The lower fraction in separating funnel of EDC was collected & concentrated on rota evaporator to get activated PEG. From the above reaction and structure it was clear that 4-Nitrophenyl chloroformate contains two highly electronegative groups i.e. NO₂ and Cl. These two groups interact with each other resulting in neutralization of polarity. It then acts as non-polar molecule and shows high affinity for mobile phase (methanol & EDC). Therefore, 4-Nitrophenylchloroformate has the highest mobility and PEG has lowest mobility. From IR Spectroscopy it was found that the peak of Hydroxyl group- (OH) of PEG was at 3400-3450cm⁻¹. The peak of C-Cl bond was found at 746 cm⁻¹. But after the reaction between PEG and 4-Nitrophenyl Chloroformate the- OH peak was found not so deep as in PEG. The peak was somewhat short and broad.

Keywords: Polyethylene glycol, TLC, Rota evaporator, Nitrophenyl chloroformate, IR spectroscopy

INTRODUCTION

Polyethylene Glycol (PEG) is the neutral polymer and is now one of the most popular polymeric material used for alteration and control of biodistribution. The pharmacokinetics and toxicity of bio active molecule can be affected strongly by PEGylation, for example Cassettari et al.(2010), demonstrated that mPEG-g-chitosan conjugates exhibited reduced toxicity towards cells, as compared to unmodified chitosan counterparts (Cassetari, 2010). Moreover, PEG may increase the lifetime of “drug carrier” assembly and consequently lowering its toxicity (Duncan, 1984, Zhang, 2014). PEG holds a wide range of advantageous property that includes high solubility in aqueous media as well as organic solvents making it easy for end group modification (Ozdemir, 2007). It has been used widely for the modification of carriers used in therapeutics because PEG offers a shielding characters that avoids rapid renal clearance from the body (Kobaashi, 2014). This shielding property is also called “Stealth character” effect that PEG offers through its reduced interaction with blood components mainly “opsonin” that is well known to enhance phagocytosis and subsequently inhibiting its uptake by reticuloendothelial system (Pozzi, 2014; Shah, 2012). During 1930s, PEG was synthesized commercially by the base initiation of addition of ethylene oxide to ethylene glycol and diethylene glycol (Bailey, 1991) and it is now commercialised with different molecular weights and functionalities.

MATERIALS AND METHODS

This study was carried out at Dolphin Institute of Biomedical and Natural Sciences, Dehradun and Indian Institute of Technology (IIT), Roorkee, India during May-July 2005.

Reagents and chemicals

All reagents used were available commercially and were of highest purity grade. Polyethylene glycol (400, 4000, 8000&20000) was purchased from E.Merck (India) Limited Mumbai and used without further
purification. 4-Nitrophenyl chloroformate, Polyethleneimine (60,000 Da), ethylamine, ethylenedichloride,
acetic acid and acetone were also purchased from E. Merck (India) Limited Mumbai. Phenol was obtained
from SRL (Sisco Research Laboratories Pvt. Ltd) and NaCOOCH₃ was obtained from Qualigens. Water
was double- distilled and purified though a Milli –Q filter system. Transmission electron microscopy (TEM)
experiments were performed by using a JEOL 1200 EX -80 KV Microscope. Dialysis was carried out by
dialysis membrane with a cut off value of 12 kDa from SIGMA.

Esterification of PEG by 4-Nitrophenyl Chloroformate

Activation of PEG (400Da)

5g of PEG (12.5mM) was taken & 20ml of dry benzene was added two times & the dried solution of
PEG was separated .To the dried solution of PEG(taken in round button flask), 3x mol (37.5) of Triethylamine
(TEA) i.e. 5.209ml was added, along with 50ml Ethylene Dichloride(EDC). After that, 7.5g of 4-nitrophenyl
chloroformate was added slowly at room temperature & was kept for stirring for 5 hrs. Reaction mixture was
monitored on TLC using EDC: Methanol (7:3). Then reaction mixture was portioned between EDC & water.
The lower fraction in separating funnel of EDC was collected & concentrated on rota evaporator to get the
activated PEG.

Activation of PEG (4000Da)

5g of PEG (1.25mM) was taken & 20ml of dry benzene was added two times & the dried solution of
PEG was separated .To dried solution of PEG (taken in round button flask), 3x mol (3.75) of Triethylamine
(TEA) i.e. 5.209ml was added, along with 50ml Ethylene Dichloride (EDC). After that, 0.755g of 4-nitrophenyl
chloroformate was added slowly at room temperature & was kept for stirring for 5 hrs. Reaction mixture was
monitored on TLC using EDC: Methanol (7:3). Then reaction mixture was portioned between EDC & water.
The lower fraction in separating funnel of EDC was collected & concentrated on rota evaporator to get the
activated PEG.

Activation of PEG (8000DA)

5g of PEG (1.25mM) was taken & 20ml of dry benzene was added two times & the dried solution of
PEG was separated .To the dried solution of PEG (taken in round button flask), 3x mol (3.75) of Triethylamine
(TEA) i.e. 260µl was added, along with 50ml Ethylene Dichloride (EDC). After that, 0.379g of 4-nitrophenyl
chloroformate was added slowly at room temperature & was kept for stirring for 5 hrs. Reaction mixture was
monitored on TLC using EDC: Methanol (7:3). Then reaction mixture was portioned between EDC and water.
The lower fraction in separating funnel of EDC was collected & concentrated on rota evaporator to get the
activated PEG.

Activation of PEG (20,000DA)

5g of PEG (1.25mM) was taken & 20ml of dry benzene was added two times & the dried solution of
PEG was separated .To the dried solution of PEG (taken in round button flask), 3x mol (3.75) of Triethylamine
(TEA) i.e. 260µl was added, along with 50ml Ethylene Dichloride (EDC). After that, 0.379g of 4-nitrophenyl
chloroformate was added slowly at room temperature & was kept for stirring for 5 hrs. Reaction mixture was
monitored on TLC using EDC: Methanol (7:3). Then reaction mixture was portioned between EDC & water.
The lower fraction in separating funnel of EDC was collected & concentrated on rota evaporator to get the
activated PEG.
RESULTS AND DISCUSSION

Characterization of Activated PEG by TLC

The results of TLC are shown on the following diagrams:

![Figure 1. TLC of activated PEG in solvent system methanol: EDC (7:3) at different molecular weight](image)

A. 400 Da B. 4,000 Da C. 8,000 Da D. 20,000 Da

Note: The letters in the figures indicate: a. 4-nitrophenyl chloroformate, b. unactivated PEG, c. activated PEG

Characterization of activated PEG By IR spectroscopy

From IR Spectroscopy it was found that the peak of Hydroxyl group- (OH) of PEG was at 3400-3450cm\(^{-1}\). The peak of C-Cl bond was found as 746 cm\(^{-1}\). But after the reaction between PEG and 4-Nitrophenyl Chloroformate the- OH peak was found not so deep as in PEG. The peak was somewhat short and broad. This indicates that some portion of PEG was unreacted. Similarly, on activated PEG C-Cl bond was also present. There was another two peaks C=O at 1768cm\(^{-1}\) and C-O bond at1011cm\(^{-1}\), which clearly indicates that ester bond is present on the activated PEG. It means that 4-Nitrophenyl Chloroformate activated the PEG.

When PEG was reacted with 4-Nitrophenyl Chloroformate, hydrochloric acid (HCl) was released and the ester bond was formed between PEG and 4-Nitrophenyl Chloroformate. The released HCl increased the acidity of reaction mixture and Triethylamine (TEA) neutralized that acidity. From the above reaction and structure it was clear that 4-Nitrophenyl Chloroformate contains two highly electronegative groups i.e NO\(_2\) and Cl. These two groups interact with each other to cancel the polarity. It then acts as non-polar molecule and has high affinity for mobile phase(methanol & EDC), which is organic in nature. Therefore, 4-Nitrophenyl Chloroformate has the highest mobility and PEG has lowest mobility. The activated PEG also contains electronegative groups NO\(_2\) on both sides but the polarity was not cancelled as in 4-Nitrophenyl Chloroformate because the chain length of activated PEG is greater than 4-Nitrophenyl Chloroformate. Therefore, the mobility of activated PEG was somewhat intermediated between inactivated PEG and 4-Nitrophenyl Chloroformate. This indicates that the PEG was activated by 4-Nitrophenyl Chloroformate. Higher molecular weight PEI has much greater DNA binding capacity (Kataoka et al., 1996; Wolfert et al., 1996). Complicating this result is the fact that the molecular weight of PEI affects the transfection efficiency and the cytotoxicity. Although PEI with a molecular weight greater than 25 kDa provides high transfection efficiency, it is usually highly toxic, non-biodegradable, and cannot be excreted (Bailey, 1991). On the other hand, PEI carriers with molecular weights below 18KDa are not toxic, but have much lower transfection efficiency. Thus, even though the nanoparticles with high molecular weight of PEI shells bind DNA more effectively, the nanoparticles prepared from a low molecular weight of PEI (25 kDa) will probably have more potential for use in gene delivery. When the branched PEI25K was complexed with pDNA using N/P ratios of 4.4:1, 5.5:1, and 11:1, bimodal particle size distributions were obtained in all cases. The diameters of the complexed particles ranged from 70-to100 nm, while those of the larger particles were between 400 and 650 nm. The findings are in agreement of (Duncan, 1984). Thus, use of the core-shell nanoparticles as the carrier
has obvious an advantage over the branched PEI with regards to the nano size and mono dispersity. To evaluate the non-ionic polymer, poly (ethylene glycol) (PEG), as a component in cationic copolymers for non-viral gene delivery systems, PEG was coupled to polyethylenimine (PEI) (Pozzi, 2014). The effects of different degrees of shapes of PEGylation of PEI effects on cytotoxicity, water solubility and transfection efficiency. This work reports the synthesis and characterization of a series of cationic copolymers on the basis of the conjugates of PEI with PEG. The modified molecules were significantly less toxic than the original polymer. Moreover, the chemical modification led to enhancement of their solubility (Shah, 2012). The comparison of PEGylated PEIs with different degrees of derivation showed that all the polymers tested reached comparable levels of transgene expression that of native PEI. As assessed by to agarose gel electrophoresis, even highly substituted PEI derivatives were still able to form polyionic complexes with DNA. However, aside from an increase in solubility and retention of the ability to condense DNA, methoxy-PEG-modified PEIs resulted in a significant decrease in the transfection activity of the DNA complexes (Ozdemir, 2007). In fact, the efficiency of the copolymer was compromised even at a low degree of modification suggesting that the PEG action resulting from its shape is important for efficient gene transfer. The mode of PEG grafting and the degree of modification influenced the transfection efficiency of PEI.

CONCLUSION

The activation of PEG of different molecular weight was done and their characteristics were studied using IR spectroscopy and Thin layer chromatography (TLC). The peak was somewhat short and broad. From IR Spectroscopy it was found that the peak of Hydroxyl group- (OH) of PEG was at 3400-3450cm⁻¹. But after the reaction between PEG and 4-Nitrophenyl Chloroformate the- OH peak was found not so deep as in PEG. This indicates that some portion of PEG was unreacted. 4-Nitrophenyl Chloroformate acts as non-polar molecule and has high affinity for mobile phase (methanol & EDC), which is organic in nature. Therefore, 4-Nitrophenyl Chloroformate has the highest mobility and PEG has lowest mobility. The activated PEG also contains electronegative groups NO₂ on both sides but the polarity was not cancelled as in 4-Nitrophenyl Chloroformate. Therefore, the mobility of activated PEG was somewhat intermediated between inactivated PEG and 4-Nitrophenyl Chloroformate.

ACKNOWLEDGEMENTS

The author would like to thank all the laboratory staffs of Dolphin Institute of Biomedical Science, Dehradun and Indian Institute of Technology, India for their support in laboratory works.

REFERENCES


