A simple and efficient method for synthesis of carboxymethylated polyethyleneglycol

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A simple and efficient method for the synthesis of carboxymethylated polyethylene glycol (CM-PEG) by the oxidation of the corresponding monomethoxy polyethylene glycol (mPEG) with catalytic amounts of TEMPO and hypobromide as a regenerating oxidant and water as solvent was developed.

Keywords: preparation, carboxymethylated PEG, TEMPO

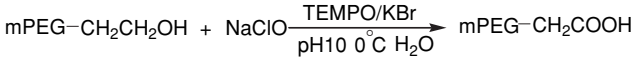
Poly(ethylene glycol) (PEG) is a linear or branched neutral polyether. It possesses an ideal array of properties: very low toxicity,[1] excellent solubility in aqueous solutions[2] and extremely low immunogenicity and antigenicity[3]. PEG also exhibits excellent pharmacokinetic and biodistribution behaviour.[4] All these properties make it ideal to be used in pharmaceutical applications.

PEGylation of protein drugs has become one of the most widely used and well-established drug enhancement strategies in the biopharmaceutical industry. The common route for preparing PEG–protein conjugates is to transform hydroxyl group of monomethoxy polyethylene glycol (mPEG) to functional groups which are suitable for reaction with the -NH_2 of lysine and the *N*-terminal amino acid group of the protein. The carboxymethylated poly(ethylene glycol) (CM-PEG) has often been used for reaction with the –NH_2 of lysine and the *N*-terminal amino acid group of a protein in the presence of *N*-hydroxysuccinimide and *N*,*N*-dicyclohexysuccinimmide. This is because the CM-PEG as a modifier of the protein has some advantages: (1) The CM-PEG does not contain a degradable linkage to the PEG backbone; differently from mPEG-chloroformate and mPEG succinate, the PEG–protein conjugate is stable. (2) The form of CM-PEG can be used to purify mPEGs, Commercially available mPEG, especially those with a large molecular weight, contain a considerable amount of diol PEG that would yield unwanted cross-linked conjugates with the protein. A effective route to remove the diol impurity is to convert the PEG into PEG carboxylic acids; PEG carboxylic diacid can be easily removed from PEG carboxylic monoacid by ion-exchange chromatography.[5] Up to date, a number of methods for the synthesis of CM-PEG have been reported, for example, CM-PEG is prepared by the oxidation of the corresponding mPEG in the presence of a stable free radical nitroxide and molecular oxygen.[6] CM-PEG is synthesised in a two-step process, first reacting mPEG with potassium *t*-butoxide and then reacting the resultant mixture with ethyl bromoacetate.[7] Although these methods have their merits individually, they suffer from disadvantages, such as low yield, the use of organic solvents and the requirement of severe conditions. Therefore, an efficient, convenient and clean method for synthesis of CM-PEG is of practical importance.

We now report an efficient, convenient and clean method for synthesis of CM-PEG by the oxidation of corresponding mPEG with catalytic amounts of 2,2,6,6-teramethyl–1-piperidineoxyl (TEMPO) and hypobromide, formed by reaction of hypochlorite and bromide, as the regenerating oxidant and water as solvent (Scheme 1). This method only requires simple instruments, a cheap oxidant and an easy work-up procedure and gives an excellent yield. The use of non-toxic and non-polluting water as solvent is noteworthy.

Although the detailed mechanism of the reaction (Scheme 1) has not yet been clarified, a possible mechanism for the formation of CM-PEG may be proposed as shown in Scheme 2, according to the previous literature.[8, 9]

In this study, mPEG with molecular weight 750 Da/mol was used as a model. The optimum reaction conditions were sought by using 1 mmol of mPEG (750) and the experimental results are summarised in Table 1. The optimum conditions



Scheme 1

Table 1 Effect on CM-PEG yield of different reaction conditions for the oxidation of mPEG (750)

Entry	TEMPO/mmol×10^{-3}	NaClO/mmol	KBr/mmol	Temperature/°C	pH	Time/h	Yield(%)
1	0	1	0	0	9	5	0.8
2	0	2	0.1	9	10	5	26.67
3	0	3	0.2	17	11	5	4.8
4	0	4	0.3	25	12	5	3.65
5	1	1	0.1	17	12	5	20.17
6	1	2	0	25	11	5	21.68
7	1	3	0.3	0	10	5	75.47
8	1	4	0.2	9	9	5	68.86
9	2	1	0.2	25	10	5	16.9
10	2	2	0.3	17	9	5	18.6
11	2	3	0	9	12	5	55.43
12	2	4	0.1	0	11	5	92.6
13	5	1	0.3	9	11	5	20.22
14	5	2	0.2	0	12	5	53.8
15	5	3	0.1	25	9	5	71.79
16	5	4	0	17	10	5	87.5
17	5	3	0.1	0	10	5	>99

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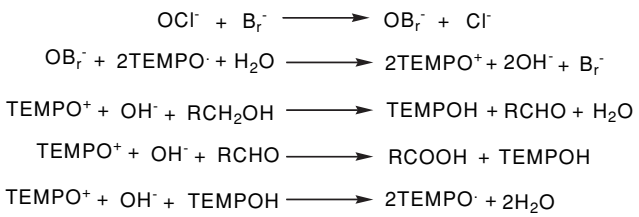

Scheme 2

for the best yield was that mPEG : TEMPO : NaClO : KBr was 1 : 0.005 : 3 : 0.1(molar ratio) at pH10 and 0 °C.

CM-PEG (5000) was also synthesised under the above optimum conditions. CM-PEG (750) and CM-PEG (5000) were characterised by ^{1}H NMR, IR and MS spectra. Both compounds gave well-resolved ^{1}H NMR and IR spectra, which were consistent with their structure. The results of MS differing by 14Da between CM-PEG and mPEG matched the calculated values. CM-PEG, titrated with NaOH, gave >99% free carboxyl group.

In conclusion, we recommend this efficient, convenient and clean method for the synthesis of CM-PEG. The advantages of this method are the single product, simple manipulation, the non-organic solvent and mild conditions.

Experimental

IR spectra were obtained on an FT/IR-400/600 (JASCO) instrument using KBr pellets. ^{1}H NMR spectra were recorded in $CDCl_3$ on a 400MHz, Bruker DMX-300 NMR spectrometer, with TMS as internal standard. Mass spectra and MALDI-TOF-MS were measured on LCQ-DecaXP (ThermoFinnigan) and BIFLEXIII spectrometers.

General procedures: mPEG (750) (7.5 g, 10 mmol primary alcohol), TEMPO (7.8×10^{-3} g, 0.05 mmol) and KBr (1 mmol, 0.119 g) were dissolved in water (70 ml). An 8% sodium hypochlorite solution (3 mmol sodium hypochlorite/mmol primary alcohol) was adjusted to pH10 by adding aq 4M HCl. After both solutions were cooled to the required temperature in an ice-bath, they were mixed at once. The temperature was maintained during the reaction and pH was maintained at pH10 by adding 0.5M NaOH and using a pH-stat. After reaction was continued for 5h, excess hypochlorite was quenched by adding ethanol (5 ml) and the pH was adjusted to 3 by adding 4 M HCl. The oxidised mPEG was extracted from the mixture three times with CH_2Cl_2, dried, concentrated, precipitated with diethyl ether, and crystallised from ethanol.

Characterisation of products: CM-PEG(750): white waxy solid; IR (KBr) ν_{max}: 3447, 2907, 1738, 1471, 1352, 1251, 1105(cm^{-1}). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.37 (s, 3H, terminal mPEG methoxy), 3.64–3.66(s, mPEG backbone methoxylene), 4.16 (s 2H, $–CH_2COO–$). *m/z* calcd. 750.81 [CH_3O-$(CH_2CH_2O)_{15}CH_2COOH$], found 751.71. CM-PEG(5000): white powder; IR (KBr) $\nu_{max:}$ 3433, 2886, 1735, 1467, 1343, 1280, 1111(cm^{-1}). ^{1}H NMR, 3.41(s, 3H, terminal mPEG methoxy), 3.66-3.69(s, mPEG backbone methoxylene), 4.18(s, 2H, $–CH_2COO–$). *m/z* (MALDI-TOF) calcd. 5046.65 (M^+ + Na), [CH_3O-$(CH_2CH_2O)_{112}CH_2COOH$], found 5041 ($M^+$ + Na)

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