THE PREPARATION OF TRITIUM LABELLED BIOCOMPATIBLE POLYMERS

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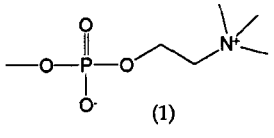
SUMMARY

The preparation of tritiated phosphoryl choline containing linear polymers by the reduction of an acetylenically unsaturated polymer with tritium gas is described. Homogeneous catalysis was used and the product was analysed by ^{1}H and ^{3}H-nmr spectroscopy. The use of lanthanide shift reagents showed that 98% of the isotope was located in the 7 and 8 positions of the hydrophobic chains.

Key Words: Biocompatibility, Phosphoryl Choline, Polymers, Lanthanide Shift Reagent, Tritium-nmr.

In the search for biocompatible materials, Chapman proposed that the incorporation of phosphoryl choline (PC) (1) onto a material would provide a haemocompatible surface by mimicking the major component of the outer membrane of the red blood cell [1,2]. It has since been shown that such surfaces suffer from minimal protein adsorption and thereby prevent many of the problems associated with conventional biomaterials such as thrombolysis, encrustation and bacterial infection [3-5].



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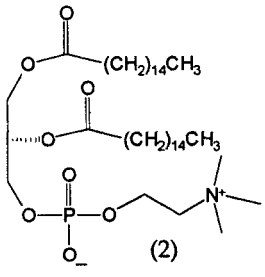
Many currently used medical devices are made of hydrophobic polymers. PC may be incorporated onto such materials by coating naturally occuring phospholipids, for example dipalmitoyl phosphatidyl choline (DPPC) (2), from an ethanolic solution. When coated, dramatic improvements in biocompatibility, both *in vitro* and *in vivo* have been observed on polymers such as PVC and polyethylene.

Phosphatidyl cholines containing diacetylene groups in the acyl chains (eg. 3) have been shown to form crosslinked coatings when irradiated with UV or γ–rays.



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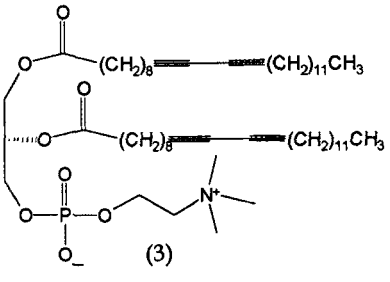
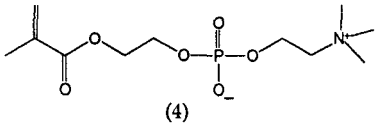


(3)

A further development has been to develop linear polymers containing the features of a natural phospholipid, ie. a PC group and straight chain alkyl groups. These polymers have many points of interaction with the hydrophobic surface, are achiral and by varying the ratio of monomers (n and p), different properties may be achieved. Accordingly, polymers (6a) have been prepared from co-monomers such as M-PC (4) and lauryl methacrylate (5), with varying ratios of n and p, which have proved to be highly effective surface coatings for conferring biocompatibility.[4,6,7]



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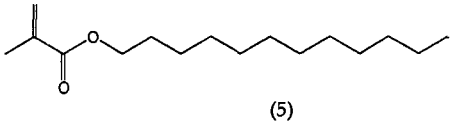

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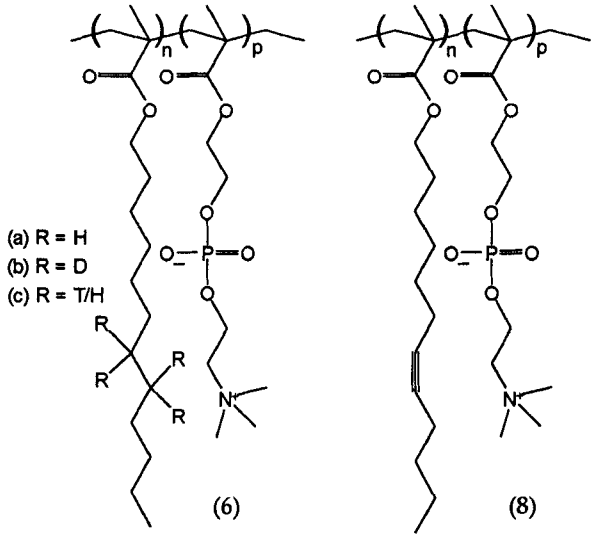
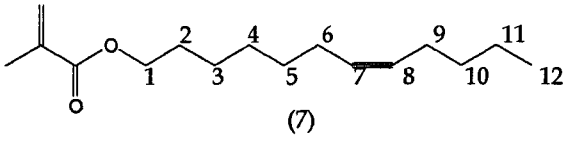


In order to accurately determine the stability of these polymers on various substrates, it was decided to prepare radiolabelled derivatives. Methacrylate monomers readily polymerise *via* a radical mechanism, which can be initiated by radioactive decay. Furthermore, the preparation and manipulation of radiolabelled monomers is not easy on a small scale. It was therefore decided to prepare an unsaturated polymer which could be subsequently reduced with tritium gas to give the labelled product. The use of ^{1}H and ^{3}H-nmr enables full characterisation to be achieved.



A co-polymer (8) of M-PC (4) and dodec-7-yn-1-yl methacrylate (7), where n : p was 1.6 : 1 , was reduced using deuterium gas over Wilkinson's catalyst at room temperature for six hours. The deuterated polymer (6b) was precipitated and analysed by ^{1}H - nmr, which indicated that there was no residual unsaturation, and by ^{2}H -nmr (Fig 1) which showed that all the isotope was in the aliphatic portion of the molecule.

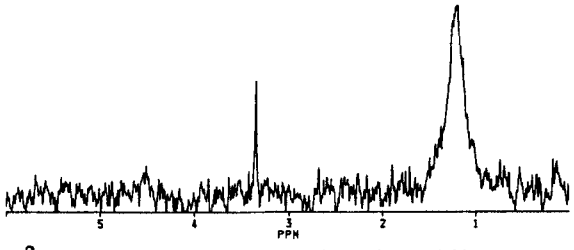


Fig 1. ^{2}H - nmr spectrum of deuterated copolymer (6b)

A mixture of tritium : hydrogen (1:6), was used under the same conditions to give a tritiated polymer (6c) which was analysed by ^{1}H and ^{3}H - nmr (Figs 2 and 3). These showed that the reaction had gone to completion and that there was no unsaturation due to residual acetylenes or olefins from partial reduction. All the tritium was located in the aliphatic portions of the polymer. The specific activity of the isolated material was 977 MBq/mg (26.3 mCi/mg), 427 GBq/mmole, (11.5 Ci/mmole).

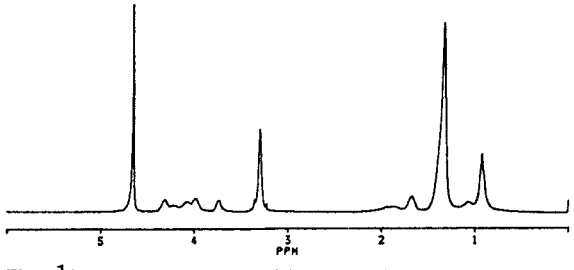


Fig 2. ^{1}H - nmr spectrum of tritiated copolymer (6c)

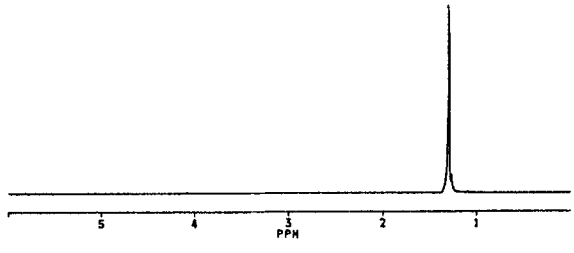
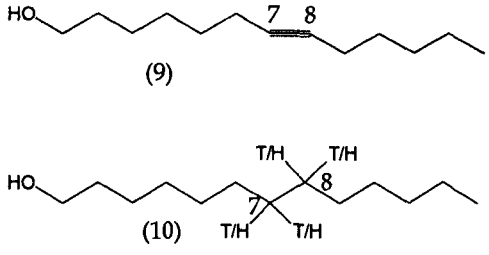


Fig3. ^{3}H - nmr spectrum of tritiated copolymer (6c)

As the proton (and triton) signals corresponding to positions [3-11] overlap in the nmr spectra, the extent of scrambling of the isotope from positions 6 and 7 was unknown. The use of lanthanide shift reagents in conjunction with ^{3}H-nmr has been described previously, enabling the exact labelling patterns in alkyl alcohols to be established [8]. To determine the labelling pattern in the polymers, dodec-7-yn-1-ol (9) was tritiated under identical conditions to those used for the

polymer. The [^{3}H]-dodecanol (10) was analysed by ^{1}H and ^{3}H nmr spectroscopy (Figs 4 and 5). Europium tris(dipivaloyl malonate) (11) was added to the solution to give a ratio of salt to alcohol of 1:2. The resulting conjugate (12) was analysed by ^{1}H and ^{3}H-nmr (Figs 6 and 7). The results are summarised in Table 1.



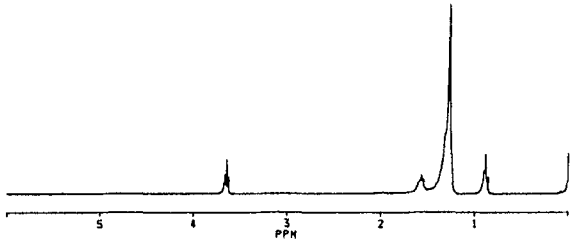


Fig4. ^{1}H - nmr spectrum of tritiated dodecanol (10)

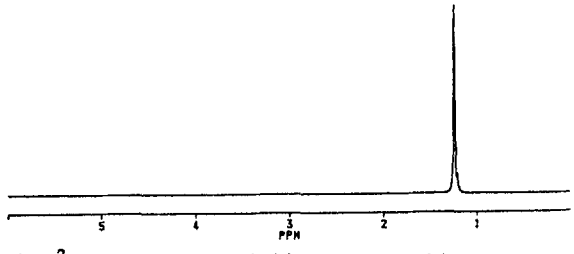


Fig5. ^{3}H - nmr spectrum of tritiated dodecanol (10)

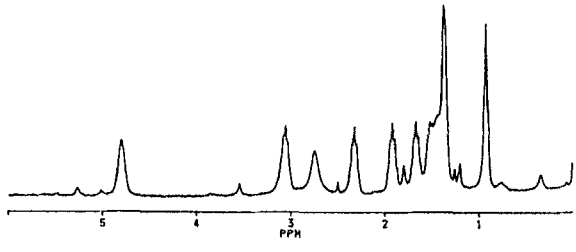


Fig6. ^{1}H - nmr spectrum of tritiated dodecanol conjugate(12)

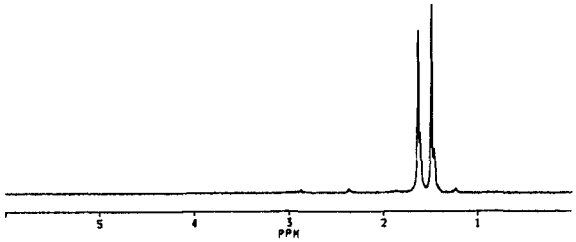
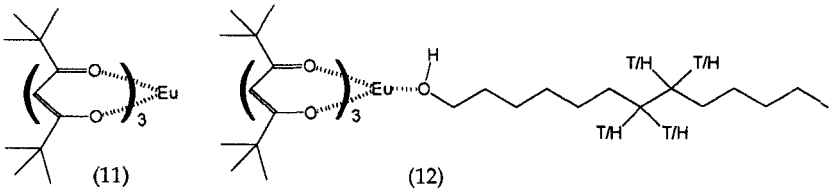


Fig7. ^{3}H - nmr spectrum of tritiated dodecanol conjugate(12)

TABLE 1: ^{1}H and ^{3}H-nmr data for experiments with lanthanide shift reagent

position	^{1}H-nmr chemical shift, δ	^{3}H-nmr chemical shift, δ	relative intensity, %
1	12.70		
2	6.72		
3	4.80		
4	3.04		
5	2.31		
6	1.91		
7	1.67	1.60, 1.63	48.6
8	1.51	1.46, 1.49	48.6
9 to 11	1.37		
12	0.90		



(11) (12)

It can be seen that minimal isotope migration has occurred from the expected positions, and that the tritium has been evenly added across the triple bond. Evidence of doubly labelled species can be observed as small peaks 0.02 ppm upfield of the major lines. It is unlikely that the acetylenic polymer (8) would be reduced to give a different distribution of isotope.

A combination of ^{3}H nmr with the use of lanthanide shift reagents enables these labelled polymers to be used with a high degree of confidence.

EXPERIMENTAL

Equipment

Nuclear magnetic resonance spectra were obtained on a Bruker AC300 spectrometer. Deuterium spectra were obtained at 46.1 MHz and tritium spectra were obtained at 320 MHz.

[^{2}H] M-PC/lauryl methacrylate copolymer

M-PC/dodec-7-yn-1-yl methacrylate copolymer (8) (20 mg) was dissolved in a mixture of benzene (0.45 ml) and ethanol (0.15 ml) and degassed under reduced pressure. This was repeated several times, being flushed with helium between degassing. Wilkinson's catalyst (tris(triphenylphosphine) rhodium (1) chloride) (5 mg) was added and the solution again degassed before stirring under an atmosphere of deuterium (5 ml) for six hours, by which time no further gas was being taken up.

After removing the excess gas, acetone (2 ml) was added and the solution allowed to stand until precipitation was complete. The solution was removed and the residue washed with acetone (x 2) and dried under vacuum to give the labelled polymer(12 mg). The product was analysed by ^{1}H and ^{2}H-nmr in $CDCl_3/CD_3OD$.

[^{3}H] M-PC/lauryl methacrylate copolymer

M-PC/dodec-7-yn-1-yl methacrylate copolymer (8) (20 mg) was dissolved in a mixture of benzene (0.45 ml) and ethanol (0.15 ml) and degassed under reduced pressure. This was repeated several times, being flushed with helium between degassing. Wilkinson's catalyst (tris(triphenylphosphine) rhodium (1) chloride) (5 mg) was added and the solution again degassed before stirring under an atmosphere of tritium and hydrogen (1:6, 5 ml, 74 GBq, 2 Ci) for six hours, by which time no further gas was being taken up.

After removing the excess gas, acetone (2 ml) was added and the solution allowed to stand until precipitation was complete. The solution was removed and the residue washed with acetone (x 2) and dried under vacuum to give the labelled polymer (3 mg , 2.92 GBq , 79 mCi) . The product was analysed by ^{1}H and ^{3}H-nmr in $CDCl_3/CD_3OD$.

[7,8-^{3}H] Dodecan-1-ol

Dodec-7-yn-1-ol (20 mg) was dissolved in a mixture of benzene and ethanol (3:1, 0.6 ml) and degassed under vacuum with helium. Wilkinson's catalyst (5 mg) was added and the solution

again degassed. The solution was stirred under an atmosphere of tritium and hydrogen (1:6, 5 ml, 74 GBq, 2 Ci) for sixteen hours, by which time no further gas was being taken up. The solvents were evaporated and the residue purified by preparative tlc on silica, eluting with dichloromethane:ethyl acetate (1:1), to give the product(15.2 mg, 82 μmole, 76% yield, 13.5 GBq 365 mCi, 18.5% radiochemical yield). The specific activity was 165 GBq/mmole (4.5 Ci/mmole), 888 MBq/mg (24 mCi/mg). The product was analysed by ^{1}H and ^{3}H-nmr in $CDCl_3$.

[7,8-^{3}H] Dodecan-1-ol / Europium tris(dipivaloyl malonate) complex

[7,8-^{3}H] Dodecan-1-ol (8 mg, 7.1 GBq, 192 mCi) and dodecan-1-ol (25mg) were dissolved in deuterochloroform (1.0 ml). A portion (0.25 ml) was removed and europium tris(dipivaloylmalonate) (15 mg, 0.022 mmole) was added and the mixture gently warmed until all the solid had dissolved. The solution was placed in a 3mm Teflon nmr tube and analysed by ^{1}H and ^{3}H-nmr in $CDCl_3$.

ACKNOWLEDGEMENT

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