Radiolabeling Biopolymers and Synthetic Polymers for Precise Quantification

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Statement of Purpose:

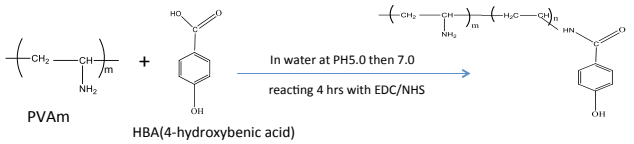
Precise quantification of biopolymer and synthetic polymers is very important for biomaterials research. Radio-labeling and gamma counter readings provide 50~100 times greater sensitivity than that of UV and florescence determination methods. The radioiodine labeling method has been well established for protein quantification through iodination of phenol groups of tyrosine within the protein structure[1]. The aim of this work is to modify and label biopolymers such as hyaluronic acid (HA) for studying its interaction with phenolboronic acid(PBA) and synthetic polymers such as polyvinyl amine (PVAm) electrolyte for biosensor paper preparation and PLGA-PNIPAAm block copolymer for ophthalmic drug delivery by incorporation of a phenol group into these polymer chains. Thus, these polymers can be labeled using ICL method to achieve precise quantification. The method allows not only accurate quantification, but also ease of imaging to investigate the distribution of the polymer.

Methods:

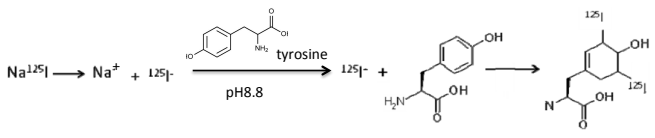
HA and PVAm were conjugated with 4-hydroxphenyl acrylamide and 4-hydroxybenic acid respectively using the EDC coupling reaction (Scheme 1) to introduce phenol groups into the polymers. Phenol groups were incorporated into PLGA-PNIPAAm chains through copolymerization with 4-hydroxphenyl acrylamide. After synthesis, all of the above 3 modified polymers were dialyzed using 3500 MWC regenerated cellulose membrane to remove free iodine and other unreacted reagents. NMR was used to confirm the addition of phenol groups to these polymers.

ICl method labeling (Scheme 2) was conducted under pH 8.8, regulated using 2M glycine buffer.

Gamma counting (Wizard 3 1480 Automatic Gamma Counter, Perkin-Elmer) allows accurate quantification of these I-125 labeled molecules. The radiolabeled polymers were also applied in different biomaterial studies.



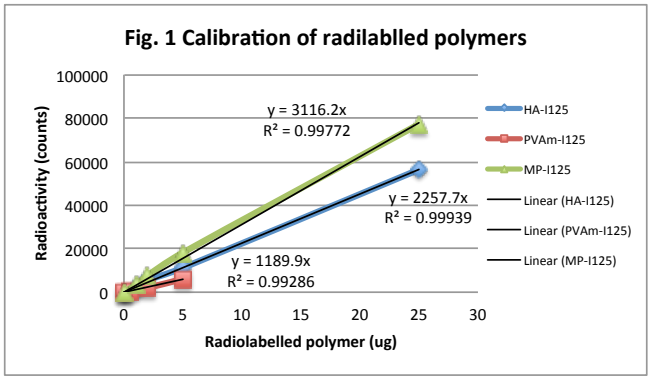
Scheme 1: EDC reaction to incorporate phenol groups to PVAm polymer chain.



Scheme 2: Mechanism of ICl radiolabeling

Results:

New chemical shifts at 6.8~7.8 corresponding to hydrogen elements in phenol group in NMR spectra of the modified polymers provided evidence of successful modification for all three polymers. At optimized conditions, incorporation of phenol groups ranged 4-8% (mol) and was confirmed by NMR quantification calculations. Very linear calibration curves were obtained as shown in Fig. 1. R^2 values were greater than 0.99, indicating that a good correlation and precise measurement can be achieved. The lower limit of detection was about 1~5ug for these polymers using such a method.



Study of I 125 labeled HA and PBA modified hydrogel surface suggested that HA may interact with PBA surfaces to reduce end of day eye dryness and discomfort of contact lens wearers; PVAm labeled with I 125 was used to examine correlation of its amount and increased adhesion. Influence of PVAm to bonding strength between two cellulose surfaces was successfully and quantitatively measured. Drug loaded PLGA-PNIPAAm micelles were applied to the eye rabbit eye and distribution in different tissues was tracked and quantified.

Conclusions:

Phenol groups were incorporated in 3 typical biopolymer and synthetic polymers and these modified polymers were successfully radio-labeled using the iodination method. A reliable and precise quantification method was established and further strongly supported their application in our biomaterial studies. It is believed that these modification methods can be extended to other polymers containing carboxyl acid, amine or vinyl group for radiolabeling and quantification.

References:

1.John L. Brash, Protein surface interactions and biocompatibility: A forty year perspective, Proteins at interfaces III State of the art, pp277-300, ACS Symposium series, Vol.1120, 2012

2. Gopal B. Saha, Radiopharmaceuticals and methods of radiolabeling, Fundamentals of nuclear pharmacy, pp80-108, Springer-Verlag, New York, 1992