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GRAFTING OF POLY(VINYL PYRROLIDONE) ONTO GELATIN AND ITS APPLICATION AS SYNTHETIC PLASMA EXPANDER

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Abstract—Grafting of *N*-vinyl pyrrolidone onto gelatin is reported using α,α′-azobisisobutyronitrile as initiator. The effects of various variables like initiator concentration, monomer concentration, time and temperature are reported. These variables appreciably affect the percentage grafting, grafting efficiency and molecular weight of the grafted chain. The graft copolymers were characterized by i.r. spectroscopy and ninhydrin tests. Sulphathiazole was coupled to these graft copolymers using a diazotization technique. Blood compatibility of these graft copolymers of different side chain molecular weight was found to be satisfactory.

INTRODUCTION

Application of polymers in medicine and surgery is an important and fascinating field of polymer science [1–3]. Numbers of polymers have been prepared and characterized for their use as biomaterials including blood compatible materials *viz.* plasma expanders etc. [4, 5]. Tailor-made polymers are playing a key role in various medical applications [6]. Of the materials developed for biomedical use, synthetic plasma substitutes are important. Synthetic plasma expanders are commonly used in cases of extensive bleeding or burns or shock where much plasma has been lost, the blood thickens and circulation ceases. Restoration or normal volume can be achieved by using a synthetic plasma expander [5]. The role of a plasma expander is simply to maintain the correct water balance between the blood and tissue and to allow the continued transport of nutrients and gases. Solutions of poly(vinyl pyrrolidone) (PVP), dextran, hydroxyethyl starch, gelatin and alginates are quite frequently used to avoid the risk of hepatitis [7–10]. It is very well known that the homopolymer of PVP and gelatin have drawbacks as plasma expanders. PVP, when used in large quantities for continuous therapy, is not metabolized and is retained indefinitely in the body (liver, spleen etc.) giving rise to adverse side effects. On the other hand, transient swelling of kidneys has been noticed when pure gelatin solution was used as a plasma expander. Further it was established that the pure gelatin solution exhibits variation in viscosity upon prolonged storage [11, 12].

We now report grafting of PVP onto gelatin using AIBN as an initiator. It is expected that some of the defects mentioned above could be overcome by the modification of gelatin. It is presumed that the resulting copolymer exhibits additional new properties by eliminating drawbacks such as variation in the viscosity upon storage and avoiding excess usage of PVP.

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EXPERIMENTAL

Materials

Gelatin (Riedel), *N*-vinyl-2-pyrrolidone (NVP) (Fluka), α,α′-azobisisobutyronitrile (AIBN), (Merck) and sulphathiazole (Sigma, U.S.A.) were used. Acetone, diethyl ether, isopropyl alcohol and all other reagents were of reagent grade.

Purification of monomer

NVP was distilled under reduced pressure and the middle fraction of the distillate was used.

Grafting procedure

Graft copolymerizations were carried out in a three necked 250 ml flask fitted with condenser and gas inlet. An isopropanol–water mixture (1:1, v/v) was used as a solvent. In a typical experiment 2.5 g of gelatin were dissolved in the solvent. N_2 was bubbled for 30 min to expel O_2 and then the required amounts of monomer and initiator were added. The contents were stirred at a rate of 120 rpm and the reaction was allowed to proceed. The products were poured into excess of acetone to precipitate graft copolymers which were collected on sintered crucibles, washed thoroughly with acetone and dried at 40° *in vacuo*.

Extraction of homopolymer

The homopolymer (PVP) was removed by extracting the crude graft copolymers with acetone in a Soxhlet apparatus for 72 hr. The homopolymer was then precipitated from acetone by pouring into excess diethyl ether. It was filtered and dried over P_2O_5 in a vaccum desiccator. It was weighed for the determination of grafting efficiency.

Percentage grafting (PG) and grafting efficiency (GE) were calculated using the formulae:

$$PG = \frac{\text{weight of the graft copolymer} - \text{weight of gelatin}}{\text{weight of the gelatin}} \times 100$$

$$GE = \frac{\text{weight of the grafted side chain polymer}}{\text{weights of the grafted side chain polymer} + \text{homopolymer}} \times 100$$

Infra-red spectra

I.R. spectra of homopolymer, gelatin and graft copolymer were obtained in a Perkin–Elmer Model 337 grating i.r. spectrophotometer.

Ninhydrin test

The graft copolymers were treated with ninhydrin prepared by the procedure of Moore and Stein [13] after digestion with pronase using the method of Rao *et al.* [14].

Viscosity measurements

The graft copolymers were hydrolysed with 6 N HCl at 105–108° *in vacuo* for 24 hr. The resulting solutions were dialysed against water to remove amino acids derived from hydrolysed gelatin backbone. The dialysate was evaporated to obtain a viscous (PVP) liquid which was precipitated in excess diethyl ether. The grafted side chains were then separated by filtration and dried *in vacuo*.

The viscosities of the grafted PVP side chains isolated by the above procedure were determined using an Ubbelohde type suspended level dilution viscometer at 30° using methanol as solvent. The intrinsic viscosity $[\eta]$ in dl/g was then obtained by extrapolation of a plot η_{sp}/C vs C to infinite dilution. The weight-average molecular weights were then calculated using the equation as given by Levy and Frank [15]

$$[\eta](\text{dl/g}) = 23 \times 10^{-5} \bar{M}_w^{0.65}$$

Coupling of sulphathiazole to graft copolymers

Sulphathiazole (100 mg) were dissolved in 17.5 ml of a mixture of acetone and 2 N HCl. This solution was then diazotized at 2° with 0.03 g sodium nitrite. The diazonium salt was added to a cold solution of 5 g gelatin or gelatin graft copolymer in 50 ml water and the pH was adjusted to 9 with dilute alkali. After one hour the solution was dialysed against distilled water and adjusted to physiological viscosity (1–1.2 dl/g).

Blood compatibility of graft copolymers

Graft copolymers with various side chain molecular weights coupled with sulphathiazole were sterilized in an autoclave at 110 lb for one hour. These solutions were injected (1 ml) intravenously into the blood stream of albino rats. The dose rate was increased by 1 ml per day and the maximum dose given was 5 ml. In control animals, pure gelatin and PVP solutions were injected. After the required time, the animals were sacrificed and blood was collected from heart venipuncture and estimated for urea content using standard procedures [16].

Microscopic studies

Blood collected from control and experimental animals were centrifuged. The plasma was subjected to microscopic studies and changes in the shape of blood cells were observed.

RESULTS AND DISCUSSION

In order to obtain a synthetic plasma expander of high blood compatibility, in the present investigation, NVP has been grafted onto gelatin using AIBN as an initiator. It was thought that some of the drawbacks associated with the two separate polymers (gelatin and PVP) could be overcome by the use of graft copolymers. All the graft copolymerization reactions were carried out in a homogeneous medium using isopropanol–water mixture to facilitate the grafting reaction, using AIBN as initiator.

The most important characteristic of a plasma expander is its molecular weight. If the molecular weight is too high, it will not be metabolized and will be deposited in the liver, spleen and other organs. With a view to discover the optimum molecular weight range of grafted PVP chains for use as a plasma expander, some of the reaction conditions for graft copolymerization were varied. Since the molecular weight of the grafted side chains depend on the monomer concentration, initiator concentration, temperature and time of the grafting reaction, the effects of these factors on the grafting reaction were investigated. The effects of the above parameters on the structure, composition of the copolymer and the molecular weight of side chains were investigated.

Effect of initiator concentration

Table 1 shows that the percentage grafting increased up to a certain level of initiator concentration and then decreased. The maximum in percentage grafting has been observed at $[\text{AIBN}] = 6 \times 10^{-3}$ mol l^{-1}. It has been presumed that, up to the critical initiator concentration, all the radicals produced from the initiator are utilized in producing growing monomer radicals as well as homopolymer radicals. After this limit, the radicals are mostly involved in recombination and other termination processes and hence the decrease in percentage grafting and grafting efficiency. A similar trend has also been observed by Rao *et al.* [17] in the grafting of poly(methyl methacrylate) onto collagen.

The molecular weights of grafted sidechains decreased slightly with increased initiator concentration initially and then remained more or less constant; this effect may be attributed to the number of grafting sites reaching a constant value. Hence there was an increase in molecular weights of the grafted chains.

Effect of monomer concentration

Table 2 shows that, with increase in monomer concentration, the percentage grafting and grafting efficiency gradually increased and then decreased after a certain monomer concentration. The decrease in grafting efficiency may be attributed to the par-

Table 1. Effect of initiator concentration in graft copolymerization of NVP onto gelatin

[AIBN] × 10^3 (mol l^{-1})	PG (%)	GE (%)	Molecular weight ($\bar{M}_w \times 10^{-4}$)
2	20.4	79.9	9.73
4	50.1	95.0	8.45
6	65.1	93.1	7.88
8	30.5	81.4	7.80
10	28.3	90.1	6.46

Gelatin = 2.59 g; [Monomer] = 0.449 mol l^{-1}. Time = 3 hr. Temperature = 70°. Total volume = 100 ml.

Table 2. Effect of monomer concentration in graft copolymerization of NVP onto gelatin

[NVP] (mol l^{-1})	PG (%)	GE (%)	Molecular weight ($\bar{M}_w \times 10^{-4}$)
0.224	26.2	84.0	6.22
0.337	49.8	89.4	7.11
0.449	65.1	93.1	7.88
0.775	36.0	93.1	12.20
1.00	19.2	87.1	13.00

Gelatin = 2.5 g; [AIBN] = 6×10^{-3} mol l^{-1}. Time = 3 hr. Temperature = 70°. Total volume = 100 ml.

ticipation of initiator radicals in graft copolymerization rather than homopolymerization. As the monomer concentration is increased, more monomer units would compete for the initiator radicals resulting in increase of rate of hompolymerization. Hence, there was decrease in percentage grafting and grafting efficiency. The molecular weights of the isolated PVP grafts were found to increase with increase in monomer concentration, an effect which may be due to the increase of chain length after the number of grafting sites has reached a constant value.

Effect of time

The percentage grafting and grafting efficiency gradually increased with time and then levelled off. This result may be attributed to the fact that the free radicals formed initially on the polymeric backbone contribute more for grafting reaction, whereas with increase of time, some of the free radicals and the macro radicals might be involved in homopolymer formation. The molecular weights of PVP side chains are more or less constant with increase of time with only a slight increase. These results are in agreement with the investigations of other workers [18].

Effect of temperature

With increase in temperature of the grafting reaction, the percentage grafting and grafting efficiency increased up to a certain level and then decreased. It was established that more grafting sites will be created by frequent chain transfer of growing radicals to the backbone at higher temperatures resulting in an increase of percentage grafting and grafting efficiency. Further increase in temperature decreased the percentage grafting; this effect can be attributed to the involvement of growing radicals in termination processes. Increase in temperature not only facilitated the chain transfer process, but also accelerated homopolymer formation thereby decreasing the grafting efficiency.

Table 3. Effect of time on graft copolymerization of NVP onto gelatin

Time (min)	PG (%)	GE (%)	Molecular weight ($\bar{M}_w \times 10^{-4}$)
60	25.0	88.8	
90	33.0	88.8	6.86
120	47.6	93.6	7.80
150	65.1	91.1	7.88
180	59.4	92.2	9.15

Gelatin = 2.5 g; [AIBN] = 6×10^{-3} mol l^{-1}; [NVP] = 0.4488 mol l^{-1}. Temperature = 70°. Total volume = 100 ml.

Table 4. Effect of temperature on graft copolymerization of NVP onto gelatin

Temperature (°C)	PG (%)	GE (%)	Molecular weight ($\bar{M}_w \times 10^{-4}$)
50	38.8	87.8	5.88
60	44.8	91.1	6.96
70	65.1	93.1	7.88
75	25.0	84.5	6.38

Gelatin = 2.5 g; [AIBN] = 6×10^{-3} mol l^{-1}; [NVP] = 0.4488 mol l^{-1}. Time = 3 hr. Total volume = 100 ml.

Characterization of graft copolymer

Purification of graft copolymer. The ungrafted homopolymer was readily removed from graft copolymer by extracting with acetone. For comparison, physical mixtures of gelatin and PVP have been extracted with acetone and PVP was completely extracted.

Treatment of isolated grafts with ninhydrin reagent

The grafts isolated after pronase digestion have been treated with ninhydrin reagent giving the characteristic blue colour normally associated with the presence of amino acid. In the case of homopolymer extracted from a physical blend, no blue colour was produced indicating that the grafting of the polymer to the amino acid residues in gelatin has occurred.

Proof of grafting

Proof of grafting has been provided by i.r. spectra of samples of graft copolymer. In Fig. 1, the i.r. spectra of gelatin, homo-(PVP) and (PVP)-grafted gelatin are given. The i.r. spectrum of pure gelatin shows the presence of amide absorption bands at 1550 and 1660 cm^{-1}, whereas the spectrum of gelatin/PVP graft copolymer showed characteristic absorption bands of PVP [19] at 1290 cm^{-1} (C—N groups) and 1700 cm^{-1} (>C=O) in addition to the bands of gelatin. However, there was overlapping of >C=O absorptions of gelatin and PVP (1660–1700 cm^{-1}) whereas the band due to amide **II** can be seen in the graft copolymer at 1550 cm^{-1}. The i.r. spectra clearly indicated grafting of PVP onto gelatin.

Mechanism of grafting

At first, initiator radicals are formed by the decomposition of initiator and primarily attack monomer creating a radical centre. Monomer radicals in turn abstract hydrogen from the backbone and chain transfer takes place leading to grafting [20, 21].

$$(CH_3)_2\underset{\displaystyle CN}{\underset{|}{C}}-N=N-\underset{\displaystyle CN}{\underset{|}{C}}(CH_3)_2 \rightarrow (CH_3)_2\dot{\underset{\displaystyle CN}{\underset{|}{C}}}$$

AIBN

$$(CH_3)_2\dot{\underset{\displaystyle CN}{\underset{|}{C}}} + mM \rightarrow (CH_3)_2\underset{\displaystyle CN}{\underset{|}{C}}-\dot{M}m$$

(homopolymer)

$$(CH_3)_2\underset{\displaystyle CN}{\underset{|}{C}}-\dot{M}n + \text{gelatin-H} \rightarrow \text{gelatin}^{\cdot}$$

(chain transfer)

$$\text{gelatin}^{\cdot} + mM \rightarrow \text{gelatin } M^{\cdot}m \text{ (grafting)}$$

where $\dot{M}m$ = monomer radical

Since we have used AIBN as initiator for grafting, the amino group of gelatin backbone might have been involved in the initiation. A similar mechanism

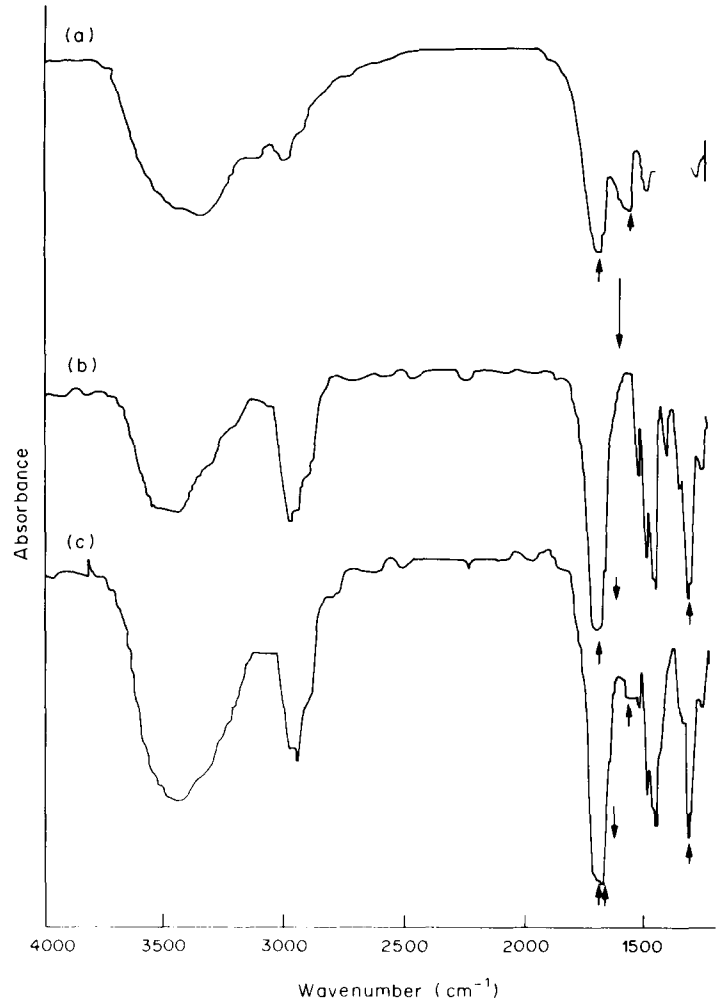
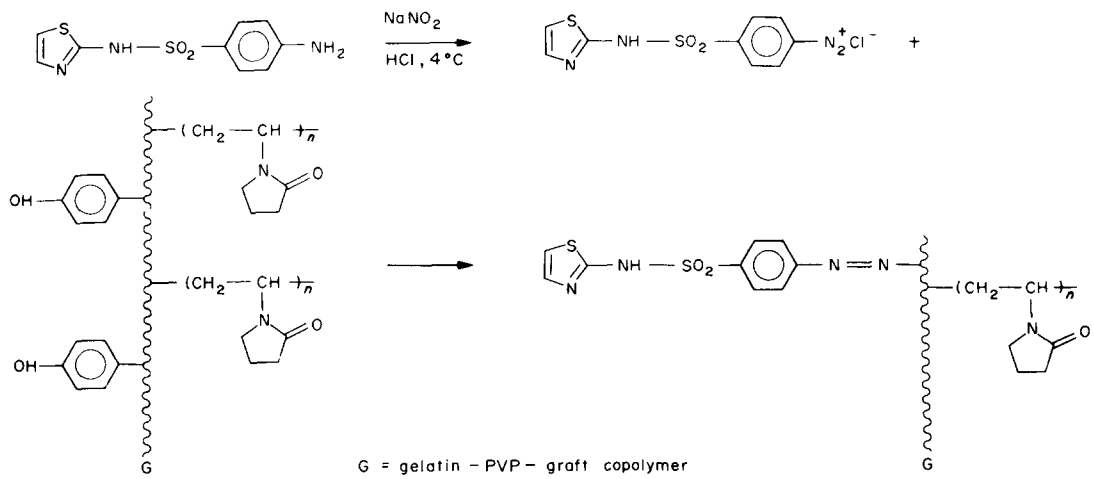


Fig. 1. Infra-red spectra of (a) gelatin, (b) PVP and (c) gelatin–PVP graft copolymer.

was proposed by other investigators when MMA was grafted onto wool using AIBN as initiator [21].

The grafting efficiency was found to be very high (88–92%) in this system, whereas the amount of homopolymer obtained was rather low (10–12%). This result clearly indicated that chain transfer of radicals to gelatin is more prominent than transfer to solvent (isopropanol–water mixture).



Scheme 1. Coupling of sulphathiazole to gelatin–PVP graft copolymer.

Biological evaluation of graft copolymers

In order to test the efficacy of gelatin–PVP graft copolymer as plasma expander, a 5% aqueous solution of graft copolymer has been intravenously administered into 3-month-old albino rats. Since molecular weight plays an important role during its action as a plasma expander, graft copolymers of various side chain molecular weights have been tested. It has been reported that blood loss is always associated with infections. To prevent this side effect, sulphathiazole, an antibacterial agent, has been coupled to gelatin–PVP graft copolymer. Since gelatin–PVP graft copolymer contains phenolic-OH groups, it is advantageous to couple sulphathiazole by diazotization. The reactions involved during coupling are shown in Scheme 1.

The aqueous solutions of graft copolymers containing sulphathiazole were dialysed against water to remove unreacted sulphathiazole and low molecular weight components present if any. In order to find the biological tolerance of the experimental animal to the graft copolymer solution, the dose rate of administration has been increased by 0.1 ml per day and the maximum dose has been given as 5 ml to observe blood compatibility.

The pyrogenic properties have been investigated in experimental animals. The body temperature of the animals before and after administration of aqueous solutions have been noted and found to increase by only 0.4°.

The antigenic property of polymer solution has also been studied and no antibodies have been detected. Anaphylactic reaction has been noticed in control animals after 34 days of administration (where gelatin and PVP were administered). However, even after 48 days of administration, no anaphylactic reaction was noticed in animals in which graft copolymer solution was injected.

Of the serological methods, agglutination tests were employed. A drop of the polymer solution was mixed with a drop of the serum of the experimental animals. A control experiment was carried out by using serum and physiological saline. The results were read under a microscope after 5, 10, 15 and 20 min. In no case was agglutination observed.

To verify preliminarily the possibilities of polymer deposition in the liver, spleen, lungs and bone marrow of the rats, the animals were sacrificed 10 days after the test period and activity of the above organs was measured after homogenization. In no case after a single application could any specific activity be detected indicating that the infused polymer is not deposited in the living system.

However, Table 5 shows that, with the increase of molecular weight, the urea content of the blood was marginally increased; this effect may be attributed to the complex biological reactions in the living system. This complex biological mechanism can be explained according to the Krebs scheme [22]. The amino acid arginine (present in gelatin) is decomposed by the enzyme arginase to yield one molecule of urea and one of ornithinine. Ammonia and carbon dioxide combine with ornithinine to form citrulline. The addition of a further molecule of ammonia results in the formation of arginine which takes part in the cycle again. The enzyme arginase which is essential in this cycle is abundantly available in the mammalian body (rat liver). The increase in urea content with increase of grafted PVP chains might be due to longer retention of PVP in the liver during metabolization. Further, it has been conclusively proved by experiments with animals that the formation of urea occurs only in the liver [22].

Table 5. Influence of molecular weight of grafted PVP on blood compatibility

Molecular weight ($\bar{M}_n \times 10^{-4}$)	Urea (mg/100 ml of blood)	Shape of the red cells
6.46	9.16	Normal
7.88	8.27	Normal
8.45	11.50	Normal
9.15	14.40	Normal
12.20	12.90	Slightly wrinkled
13.00	16.00	Moderately wrinkled

In conclusion, it is observed that incorporation of hydrophilic polymer (PVP) onto gelatin meet the requirements of the synthetic infusion solutions thereby eliminating the side effects caused by the two individual polymers *viz.* gelatin and PVP.

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