## Anionic Polymerization of Alkyl Cyanoacrylates: In Vitro Model Studies for In Vivo Applications

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ABSTRACT: Alkyl cyanoacrylates were polymerized anionically in water medium at different levels of pH. The effect of pH on the molecular weight and softening points of the polymers was studied. Alkyl cyanoacrylates were also polymerized in suspension by using the combination of methanol–water, and also by using different catalysts such as triethylamine, diazabicyclooctane, and diazabicycloundecane. The poly(cyanoacrylates) thus obtained were characterised by infrared and nuclear magnetic resonance spectroscopy. The molecular weight, molecular weight distribution, and the softening points of the polymers were determined. These studies would help in more effective usage of cyanoacrylates as bioadhesives under varied physiological conditions. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 336–344, 1999

Key words: anionic polymerization; alkyl cyanoacrylate; bioadhesive; biomaterial

## **INTRODUCTION**

Polymers play an important role in biomedical applications<sup>1-4</sup> such as in making artificial organs, implants, catheters, in controlled drug delivery,<sup>5-8</sup> stopping internal bleeding,<sup>9-11</sup> joining ruptured tissue<sup>12,13</sup> etc. due to their biocompatibility and biodegradability.

Polymeric adhesives especially those based on alkyl cyanoacrylates<sup>14–16</sup> are used extensively for joining tissues, as haemostatic agents, in healing wounds, and for sealing fistulae. Cyanoacrylates are also used in surgeries,<sup>17–22</sup> in specialised microsurgeries in sealing the corneal perforations,<sup>23–27</sup> etc. They have become very popular in all these applications due to their instant polymerization anionically when in contact with citrated blood. They are also found to be biodegradable, biocompatible, nontoxic,<sup>28–30</sup> and nonallergenic. Alkyl cyanoacrylates are used to stop internal bleeding. Internal bleeding could occur anywhere along the entire length of the gastrointestinal tract (GT). The environment varies to a great extent in different parts of the GT in terms of acidity, basicity, enzymes secreted, etc. Therefore, it is observed that the cyanoacrylates are used in different surgeries successfully under drastically dissimilar environments.

In our recent study<sup>31</sup> conducted by using n-butyl-2-cyanoacrylate on human patients, it was seen that a success rate of 90% was achieved for complete obliteration of gastrointestinal bleeding. The reason for the reoccurance of bleeding in the rest of the 10% patients was not clearly understood.

Therefore, keeping the above facts in view, an attempt has been made to understand the nature of poly(alkyl cyanoacrylates) obtained under different conditions of polymerization such as the pH of the medium, the type and concentration of initiating radicals and different catalysts. Such work seems to have been not reported in literature so far.

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Buffer Solution	Volume of Stock Solution A in ml	Volume of Stock Solution B in ml	pH
1	10.2	39.8	3.00
2	19.3	30.7	4.00
3	25.7	24.3	5.00
4	32.1	17.9	6.00
5	43.6	6.4	7.00

 Table I
 The Composition of Buffer Solutions

## **EXPERIMENTAL**

#### **Materials**

All the monomers, namely, methyl cyanoacrylate, ethyl cyanoacrylate, s-butyl cyanoacrylate, and noctyl cyanoacrylate were prepared in this laboratory.<sup>32–34</sup> Methanol (high-performance liquid chromatography grade) (Spectrochem, Bombay, India); citric acid (BDH, Bombay, India); sodium phosphate dibasic (dihydrate) (Loba Chemie, Bombay, India); diazabicyclo[2.2.2]octane (DABCO); and diazabicyclo[5.4.0]undec-7-ene (DBU) (Fluka Chemika, Switzerland); triethylamine (TEA) (s.d. Fine Chemicals, Bombay, India), and sodium hydrogen carbonate (NaHCO<sub>3</sub>) (E-Merck India Limited; Bombay, India) were used as received. Double distilled water (DDW) was prepared by distilling it twice over potassium permanganate.

## Preparation of Citrate Phosphate Buffer Solutions<sup>35</sup>

Two stock solutions were prepared. Stock solution "A" (0.1M solution of citric acid) was prepared by dissolving 21.01 g of citric acid in 1000 ml of DDW. Stock solution "B" (0.2M solution of dibasicsodium phosphate) was prepared by dissolving 35.6 g of dibasic sodium phosphate in 1000 ml of DDW. These two solutions "A" and "B" were mixed in different proportions (Table I) and diluted to 1000 ml to give the buffer solutions of the desired pH.

## Anionic Polymerization of Alkyl Cyanoacrylates at Different pH

Ethylcyanoacrylate was anionically polymerized at pH ranging from 3.00 to 7.15 in citrate phosphate buffer medium keeping the monomer to medium ratio 1:50. This high dilution has been used in these experiments in order to control the pH of the medium accurately. In a three-necked round bottom flask fitted with a stirrer, condenser, and dropping funnel, 100 ml of the buffer solution was taken and maintained at 30°C. The monomer ethyl cyanoacrylate (2.0 g) was taken in a dropping funnel and was added dropwise over a period of 1 h to the well stirred buffer medium. The initial formation of polymer was observed by the appearance of turbidity. The contents were stirred for a period of 3 h in order to complete the polymerization. The buffer solution containing finely dispersed polymer was then filtered, washed, and dried in a vacuum oven (10 mmHg) at 45–50°C.

In some cases, the dispersed polymer could not be separated from the buffer medium by filtration. In such cases the polymer was separated by centrifuging at 5000 rpm for 40 min. In few cases low molecular weight polymer was formed which was tacky in nature. In these cases the polymer was dissolved in acetone and reprecipitated using methanol. The properties of the polymers thus obtained are given in Table II.

## Anionic Polymerization of Alkyl Cyanoacrylates in Methanol–Water Combinations

Ethyl cyanoacrylate was polymerized in DDW, DDW-methanol combinations, and methanol at 30°C while keeping the monomer to medium ratio 1:10 in all the cases. Here the dilution has been

Table IIThe Molecular Weight and Softening Ranges of Poly(ethyl cyanoacrylates) Obtained byAnionic Polymerization at Different pH

Polymer	pH of Polymerising Medium	Weight Average Molecular Weight (Mw)	Number Average Molecular Weight (Mn)	Molecular Weight Distribution (Mw/Mn)	Softening Range in °C
1	3.00	617	570	1.08	60–90
2	4.06	8344	7677	1.09	80-140
3	5.15	25954	19354	1.34	120 - 240
4	6.25	19672	14538	1.35	120 - 230
5	7.40	19554	14069	1.39	110 - 225

Polymer	Polymerization Medium <sup>a</sup>	Weight Average Molecular Weight (Mw)	Molecular Weight Distribution (Mw/Mn)	Softening Range in °C
1	$\begin{array}{c} \mathrm{H_{2}O:MeOH}\\ \mathrm{100:00} \end{array}$	8779	1.04	70–110
2	$\mathrm{H_{2}O}:\mathrm{MeOH}$ 75:25	8835	1.01	80-130
3	$\mathrm{H_{2}O}:\mathrm{MeOH}$ 50 : 50	9218	1.05	85-130
4	$ m H_2O: MeOH  m 25:75$	9399	1.04	90-135
5	$\begin{array}{c} \mathrm{H_{2}O:MeOH}\\ \mathrm{00:100} \end{array}$	9517	1.10	120–150

Table IIIThe Molecular Weights and Softening Ranges of Poly(ethyl<br/>cyanoacrylate)Polymerized in Methanol-Water Combinations

<sup>a</sup> H<sub>2</sub>O, water; MeOH, methanol.

Polymer	Polymer/ Polymerization Media <sup>a</sup>	Weight Average Molecular Weight (Mw)	Molecular Weight Distribution (Mw/Mn)	Softening Range in °C
1	PMCA/ H <sub>2</sub> O : MeOH	7847	1.04	109–172
	100:0			
2	PMCA/	8340	1.03	114 - 174
	$H_{2}O$ : MeOH			
	50:50			
3	PMCA/	8951	1.02	120-180
	$H_2O: MeOH$			
	0:100			
4	PsBCA/	9586	1.05	75 - 110
	$H_2O: MeOH$			
	100:0			
5	PsBCA/	9825	1.06	80 - 150
	$H_2O: MeOH$			
	50:50			
6	PsBCA/	10181	1.06	85 - 120
	$H_2O: MeOH$			
	0:100			
7	PnOCA/	14471	1.06	100 - 230
	$H_2O: MeOH$			
	100:0			
8	PnOCA/	14730	1.04	110 - 230
	$H_2O: MeOH$			
	50:50			
9	PnOCA/	15120	1.07	115 - 240
	$H_2O: MeOH$			
	0:100			

Table IVThe Molecular Weights and Softening Ranges of Different Poly(alkylcyanoacrylates)Prepared in Methanol-Water Combinations

 $^{\rm a}\,{\rm H}_2{\rm O},$  water; MeOH, methanol.

Polymer	Catalyst	Concentration in g/20 ml	Weight Average Molecular Weight (Mw)	Molecular Weight Distribution (Mw/Mn)	Softening Range in °C
1	TEA	$2.60 imes10^{-2}$	13898	1.00	190–245
2	TEA	1.00	13839	1.00	175 - 238
3	DABCO	$1.25 imes10^{-8}$	4587	1.10	75-130
4	DABCO	$2.50 imes10^{-8}$	3318	1.04	75–95
5	DABCO	$3.75 imes10^{-8}$	3590	1.10	75 - 120
6	DABCO	$5.00 imes10^{-8}$	4462	1.14	75-150
7	DBU	$3.75 imes10^{-7}$	36961	1.03	130 - 235
8	DBU	$6.25 imes10^{-7}$	39441	1.01	127 - 200
9	DBU	$12.50 imes10^{-7}$	37924	1.02	145 - 214
10	DBU	$18.75 imes10^{-7}$	37656	1.02	140 - 205
11	DBU	$25.00 imes10^{-7}$	37657	1.03	140 - 220
12	DBU	$37.50 imes10^{-7}$	38303	1.01	150 - 200
13	NaHCO <sub>3</sub>	$6.25 imes10^{-7}$	34390	1.01	140 - 200
14	NaHCO <sub>3</sub>	$12.50 imes10^{-7}$	35044	1.01	140 - 205
15	NaHCO <sub>3</sub>	$18.75 imes10^{-7}$	35045	1.01	145 - 205
16	$NaHCO_3$	$25.00 imes10^{-7}$	35261	1.01	150 - 211

Table V The Molecular Weights and Softening Ranges of Poly(ethyl cyanoacrylates) Prepared by Using TEA, DABCO, DBU, and NaHCO $_3$  as Catalysts

reduced from 1:50 to 1:10, because the high dilution did not affect the polymerization. The polymerization was carried out in the same manner as described earlier.

The other alkyl cyanoacrylates such as methyl, s-butyl, and n-octyl cyanoacrylates were also polymerized in a similar manner and the results are given in Tables III and IV. The poly(cyanoacrylates) thus obtained were characterised by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy.

# Polymerization of Ethyl Cyanoacrylate by Using Different Base Catalysts

Ethyl cyanoacrylate was anionically polymerized in presence of catalysts such as TEA, DABCO and DBU at 30°C while maintaining the monomer to aqueous catalyst medium ratio 1 : 10. The amount of catalyst used in each polymerization run is shown in Table V. The poly(ethyl cyanoacrylate) thus obtained was filtered washed and dried in a vacuum oven.

The polymers obtained by all the three techniques described earlier were characterised by their softening points and molecular weights.

## Characterization

#### Infrared (IR) Spectroscopy

The IR spectra of the polymers were taken on KBr pellets by using Nicolet fourier transfer (FT)-IR 740 spectrophotometer.

#### NMR Spectroscopy

The NMR spectra of the polymers were recorded on a Gemini 200 MHz NMR unit.

#### Softening Range

Softening ranges of the polymers were determined by using Buchi-510 melting point apparatus. The rate of heating was maintained at 0.2– 2.0°C/min.

#### Molecular Weight

The number average (Mn) and weight average (Mw) molecular weight, and molecular weight distribution of the polymers were determined by eluting 1.0% solution of the polymer in tetrahydrofuran, through styragel packed columns by using polystyrene standards on a Shimadsu gel permeation chromatography analyser.

## **RESULTS AND DISCUSSION**

### Synthesis of Poly(ethyl cyanoacrylate) at Different pH

The molecular weights and softening range of the poly(ethyl cyanoacrylates) synthesized at different pHs are shown in Table II.

It is established that alkyl cyanoacrylates polymerise by anionic polymerization. The reac-

## Step 1

Dissociation of polymerisation medium to produce anion.

$$A-B \longrightarrow A^{\oplus} + B^{\Theta}$$

Step 2







Stability of carbanion due to resonance



Scheme 1 Polymerization of an alkyl cyanoacrylate in presence of an anion.

tions that an alkyl cyanoacrylate undergoes during its polymerization in presence of an anion are shown in Scheme 1. The structural features responsible for the reactivity of alkyl cyanoacrylates are the two strongly electron withdrawing groups on the



**Figure 1** Infrared spectra of poly(methyl-2-cyanoacrylate). (a) Polymerized in DDW; (b) Polymerized in methanol.

 $\alpha$ -carbon atom. These groups reduce the electron density on the  $\beta$ -carbon atom which renders this position susceptible to nucleophilic attack. The lack of substitution on the  $\beta$ -carbon atom also enhances its nucleophilic susceptibility. The  $\beta$ -carbon atom hence becomes so susceptible that even weak bases like water can initiate polymerization. Therefore an anion can easily attack the  $\beta$ -carbon atom and create a carbanion at the  $\alpha$ -carbon atom as shown in step 2 of Scheme 1. The nitrile and the ester groups stabilise the carbanion formed at the  $\alpha$ -carbon atom and hence further attack on another monomer is possible to continue in a similar way to give the polymer as shown in steps 3 and 4 of Scheme 1.

From Table II, it is observed that with an increase in pH from 3.00 to 7.40 there is a drastic increase in weight and number average molecular weights from 617 to 19,554 and from 570 to 14,069, respectively. This is because under acidic conditions the anions available for initiation of polymerization are very few and the cations (i.e.,  $A^+$  as seen in step 2 of Scheme 1) are abundantly available to terminate any propagating species and, hence, the molecular weight in acidic pH is very low. However as the pH increases, the number of anions freely available increases and, hence, the chances of termination are less. Therefore, there is a scope for the chain to grow further and hence the molecular weights are higher.

There are two aspects to be considered at higher pH conditions: (i) A larger number of anions are present and hence a larger number of sites for initiation of polymerization are available for the chain growth. (ii) A larger number of cations ( $A^+$  ions in step 2, Scheme 1) are neutralised by larger number of anions present and, therefore, the probability of termination is reduced.

As the pH of the polymerization medium increases from 3.00 to 5.15 the second aspect mentioned above is more predominant and, hence, there is an effective increase in molecular weight and at the same time the first aspect also exists which is evidenced by an increase in the molecular weight distribution. Furthermore, at higher pH from 5.15 to 7.40 there is an excess of anions even after the neutralisation of the cations. Hence, there is an increase in the rate of polymerization and the first aspect is predominant. Therefore, there is an effective decrease in molecular weight and an increase in molecular weight distribution.

It can also be seen that from pH 3.00 to 7.40 the molecular weight distribution increased due to the increase in available anions for the initiation.

#### Effect of Methanol–Water Mixtures as Medium of Polymerization

When ethyl cyanoacrylate was polymerized in DDW; methanol and DDW combinations; and



Figure 2 <sup>1</sup>H NMR spectrum of methanol initiated poly(ethyl-2-cyanoacrylate).

methanol, it was found that as the percentage of methanol in the medium is increased there is a small but distinctive increase in the molecular weight of the polymer (Table III). This can be explained by the fact that methanol is more basic than water; therefore, as the percentage of methanol increased the percentage basicity increased. Because of this the stability of the carbanion in the propagating species (step 3 in Scheme 1) increased resulting into the formation of higher molecular weight species.

Similar results were also observed even with the other alkyl cyanoacrylates (Table IV). The presence of the methoxy group (1120 cm<sup>-1</sup>) at the beginning of the polymer chain due to the anionic polymerization in methanol and its absence in the polymer polymerized in water was confirmed by IR (Fig. 1). A peak at around 1120 cm<sup>-1</sup> indicated the presence of methoxy (—OCH<sub>3</sub>) group for the polymer polymerized in methanol (Fig. 1a) and the presence of the broad peak at 3600 cm<sup>-1</sup> (Fig. 1b) indicated the presence of hydroxy group for the polymer polymerized in DDW.

From the NMR spectra (Fig. 2) the absence of unpolymerized monomer was confirmed by the absence of two distinct singlets at  $\delta$  6.5 and 7.1 ppm, which are attributed to the olefinic protons of the cyanoacrylate monomer as shown below.



Furthermore, the structure of the repeating unit of the polymer was confirmed by the chemical shifts of the protons of the repeating unit as shown in Figure 2. A singlet at  $\delta$  3.45 ppm signifies the protons of the methoxy anion (at one end of the polymer chain) which initiated the polymerization.

For water-initiated polymer, the peak at  $\delta$  3.45 ppm is absent. The protons of hydroxy (-OH) anion is seen at  $\delta$  3.8–3.9 (Fig. 3).

Therefore it is seen that when there are more than one anion (—OH and  $-OCH_3$ ) in competetion for initiation of polymerization then the more basic anion ( $-OCH_3$ ) is responsible for initiation and further as the percentage of the more basic



Figure 3 <sup>1</sup>H NMR spectrum of DDW initiated poly(ethyl-2-cyanoacrylate).

anion increases the molecular weight also increases.

# Synthesis of Polyethylcyanoacrylates by Using Different Catalysts

The basic catalysts, triethylamine (TEA); diazabicyclo-[2.2.2]octane (DABCO); diazabicyclo[5.4.0] undec-7-ene (DBU) and sodium hydrogen carbonate (NaHCO<sub>3</sub>) are used for the anionic polymerization of ethyl cyanoacrylate. TEA is a strong base, whereas DABCO and DBU (strongly hindered amines) are weak bases.

Because of the higher basicity of TEA, there is large excess of anions in the medium (pH 12.4) and the initiation sites are thus large in number. The monomer gets polymerized instantaneously in less than 2 min when TEA was used as catalyst. Therefore, a lower molecular weight polymer is obtained.

When DBU is used as catalyst in the medium higher molecular weight polymers are obtained because of its relatively lower basicity than that of TEA (Table V).

The above catalysts namely TEA, DABCO, and DBU have been used for a model study only, and being toxic are not recommended for use *in vivo*. However biocompatable catalysts are very strongly recommended. One such example is that of NaHCO<sub>3</sub> the results for which are shown in Table V. It can be seen that when NaHCO<sub>3</sub> is used the molecular weights of the polymers are very high and that they increase with an increase in the concentration of the catalyst. Also it is

observed that the increase in molecular weight without and with NaHCO<sub>3</sub> from 19,554 (pH 7.4 Table II) to 35,261 (Concentration:  $25.00 \times 10^{-4}$ , Table V) is drastic and would, therefore, help in controlling internal bleeding efficiently.

## CONCLUSIONS

It can be concluded that the rates of polymerization of alkyl cyanoacrylates are higher in higher pH environments. The molecular weights of the polymers obtained are low in highly acidic medium (pH 2.0-4.0). Therefore internal bleeding occurring in the stomach where the pH is on the acidic side (between 1.0 and 3.0) can be stopped using alkyl cyanoacrylate in combination with a weak base (biocompatible) catalyst which will enhance the rates of polymerization and increase the molecular weight of the polymer. Thus, not only will the bleeding be stopped faster, but also the degradation rates of the polymer will be reduced drastically due to its higher molecular weight.<sup>37</sup> Therefore, the probability of reoccurance of bleeding at the same place is reduced, and also the products of degradation, namely, cyanoacetate and formaldehyde will be released in smaller quantities and at slower rates, thus keeping them in metabolizable limits.

Therefore, depending on the environment, the desired type and quantity of catalyst can be used to enhance or reduce the rate of polymerization and the molecular weight of the polymer. The bases, namely, TEA, DABCO, and DBU used in the present study are a model study and are, therefore, not recommended for use *in vivo*. However biocompatible and biodegradable catalysts like NaHCO<sub>3</sub> are strongly recommended. Studies on similar lines that use of other biocompatible catalysts are in progress in our laboratory.

This study, therefore, helps in using the alkyl cyanoacrylates more effectively under different physiological conditions, to stop internal bleeding.

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