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Research Article

EXTENDED DOXORUBICIN HYDROCHLORIDE RELEASE FROM DEGRADABLE GELATIN-DIVINYL ESTER (DVE) INTERPENETRATING POLYMER NETWORKS (IPN)

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ABSTRACT

A degradable interpenetraing polymer network (IPN) composed of gelatin (hydrophillic) and Divinyl ester (DVE) (hydrophobic) polymers was developed [1] to investigate the doxorubicn hydrochloride (Dox) release characteristics. Dox, a potent anticancer antibiotic was successfully loaded by diffusion method and release profile was studied. IPNs showed lower burst release compared to hydrogels in 36 h. The release rate and the percent cumulative release was extended 6 days to 10 days when DVE to gelatin ratio was increased from 0.3:1 to 0.7:1 which is desirable for cancer targeting. Increasing glutaraldehyde concentration reduced the amount of free amino group present which played a considerable role in release of doxorubicin hydrochloride as well.

Keywords: Doxorubicin hydrochloride, DVE

INTRODUCTION

Though hydrogels [2] are widely used as drug delivery systems, hydrogels of single polymer are not really suitable for sustained/controlled release of potent, low molecular weight and water soluble drugs. So evolution of new hydrogels to achieve desired properties for particular treatment has gained a great deal of attention. One such kind of system is IPN [3,4], especially of synthetic and natural polymers. However, IPN hydrogel implant made of hydrophillic gelatine and poly (acrylic acid) from our group showed 1500 % swelling in 24 h and released 60 % of the drug loaded in initial burst release itself [5]. In the present study, PAA was replaced by hydrophobic, degradable divinyl ester synthesized from poly (caprolactone diol) [1] to get lower burst and extended drug release of doxorubicin hydrochloride which is desirable for cancer targeting. Such system would release the drug by diffusion as well as simultaneous degradation which avoid follow up surgery. Thus, IPNs based on gelatin and divinyl ester (DVE) have been synthesized to study the doxorubicin hydrochloride release.

Divinyl ester (DVE), gelatin A of 300 bloom (Sigma, Germany), glutaraldehyde 25 % solution (Thomas Baker, Mumbai, India), azo-bis-isobutyrylonitrile (AIBN) (G.S. chemicals, Bombay, India), Tween 20 (Sigma, Germany), Trinitrobenzenesulfonic acid (TNBS) (Sigma, Germany), Doxorubicin hydrochloride (Sigma, USA), DL valine (Fluka, Germany) Trichloro acetic acid (Sigma, Germany). All the chemicals were used as received.

Preparation of hydrogel, semi-IPN and full-IPNs using gelatin and DVE

The elaborate procedure for synthesis and characterization of DVE and polymer sample preparation has been reported elsewhere [1]. In brief, DVE in mehanol was emulsified with aqueous gelatin solution (Tween 20 as surfactant), DVE was polymerized and self-cross-linked in the presence of gelatin chains by radical initiator AIBN, for overnight to form sIPN films, then washed, cut into circular discs and cross-linked with glutaraldheyde to form IPNs. Overnight dried gelatin films were cross-linked using glu solution for 6 h to produce homopolymer gelatin discs. The composition and designation of samples were given in Table 1 (Column A-D).

Determination of free amino group

Free primary amino groups present in the hydrogels/semi-IPN/full-IPN films were determined using TNBS method suggested by Levy et al [6] with slight modification. Briefly, all samples (10 mg each) except semi-IPNs in which gelatin were non-cross-linked were swollen in PBS (pH 7.4) for 24 hours at 37ºC and washed with distilled water before treating with TNBS solution. The absorbance of TNP-valine complex was measured and free amino groups were calculated as described by Levy et al.

Invitro drug loading, calculation of percent loading and drug release studies

Dox loading was carried out by swelling known weight of discs (10 mg each) in 1 mg/ml stock solution of doxorubicin hydrochloride in phosphate buffer saline (pH 7.4) for 24 h at 37º C. Percent dox loading was determined by UV spectroscopy at 254 nm using a standard curve prepared with 5-25 µg/ml dox. Formula used was

$$\% \text{ dox loading} = \frac{\text{Amount in stock} - \text{Amount remaining after loading}}{\text{Amount in stock}} \times 100$$

Ten mg discs (in triplicate) were immersed in 2 ml PBS buffer (pH 7.4), incubated at 37±1º C and 50 rpm in a shaking water bath to study dox release. At specific time intervals, 0.5 ml of the buffer medium was withdrawn and 0.5 ml of fresh buffer was replaced. Amount of dox in the medium was determined immediately using UV spectrophotometer at 254 nm later on cumulative amount and percent released was calculated.

RESULTS AND DISCUSSION

Determination of free amino groups

From Figure 1a, it can be seen that IPNs ($D_{x0.3}G_x$) have free amino group inbetween sIPNs ($D_{0.3}G_x$ and $D_{x0.3}G$). Though the gelatin chains are uncross-linked sIPN Dx0.3G (marked *) showed less free amino groups due to the leaching of most of the gelatin chains on washing and centrifugation. Figure 1b shows the amount of free amino groups present when DVE ratio to gelatin was increased from 0.3 to 0.7 g. sIPN ($DG_{x0.03}$) showed higher free amino groups than IPNs. This was because of the accesible free amino groups due to free DVE. Further, free amino group value decreased when DVE increased from 0.3 g to 0.7 g in IPN ($D_xG_{x0.03}$) suggests increasing cross-linking density. These results indicate increasing glu and DVE concentration may extend the drug release by increasing the cross-link density and swelling.

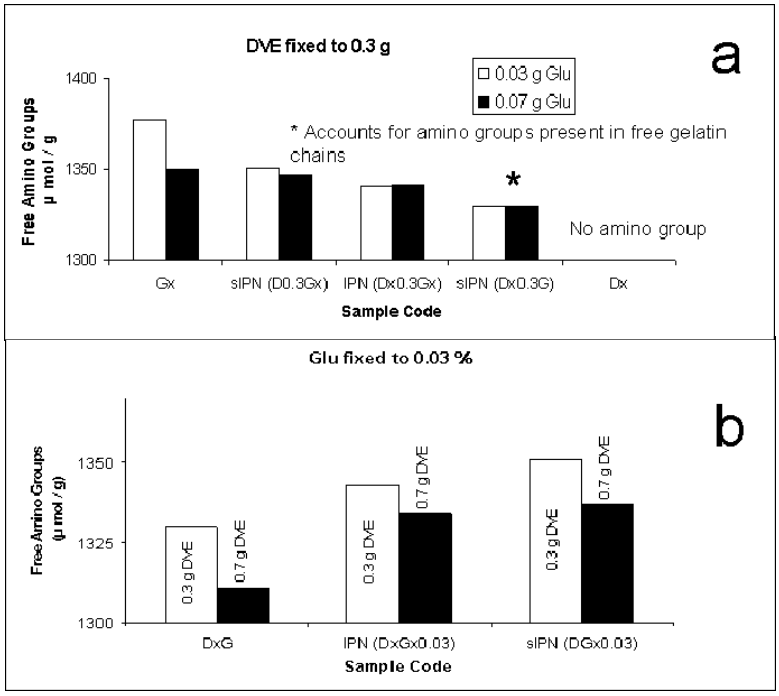

Fig. 1: The amount of free amino groups present in various samples determined by TNBS analysis

Drug loading efficiency

Figure 2 shows the percent drug loading in each sample and corresponding cumulative percent drug release. In all the samples drug loading was observed from 69-78%. It can be seen from the graph (Figure 2a) that increasing Glu concentration slightly decreases the percent drug loading. This indicates though the loading was dependent on Glu concentration to a small extent, Glu concentration and the composition of the formulations have minimal effect on drug loading. Since there is no role of DVE, drug loading was diffusion dependent. However, the percent drug release showed a significant difference in the formulation due to varying composition and cross-linker concentration.

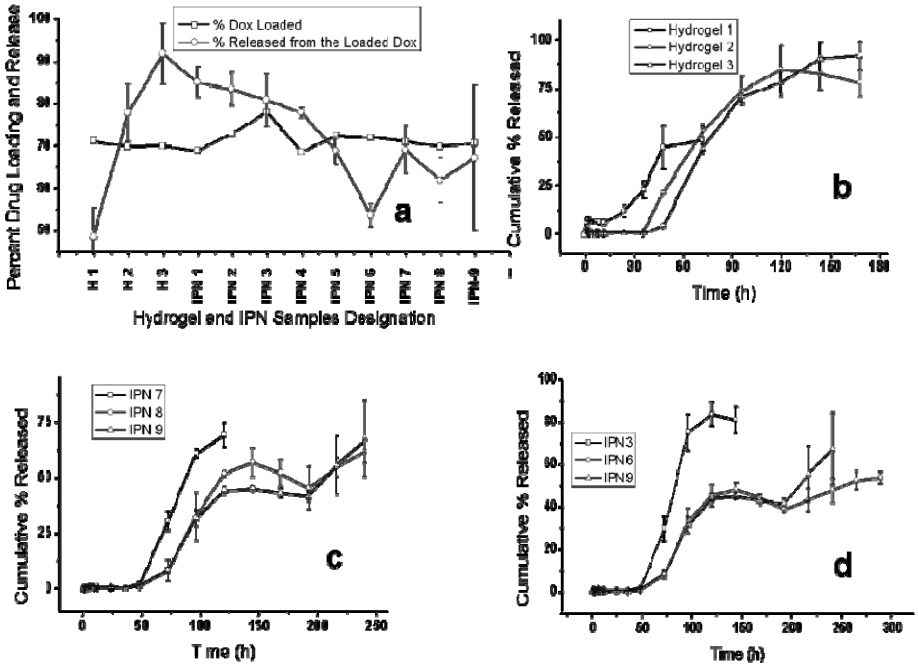

Fig. 2: Dox loading with respect to formulations and dox release profiles obtained from different hydrogel and IPN samples

Invitro doxorubicin hydrochloride release from hydrogels and full-IPN discs

This study was aimed to quantify the percentage cumulative release of doxorubicin hydrochloride from the hydrogels and full-IPNs. Figure 2 b-d sugggests sustained doxorubicin hydrochloride release for atleast 10 days by some of the full-IPNs. All the samples showed two phase burst release profile. Hydrogels-1 showed upto 45 % release in phase 1 burst whereas full-IPN-1 showed only 25 % and sustained for 2-3 days. The second phase burst exhibited higher drug release for 3-5 days and later provided sustained drug release upto 10 (full-IPN-8, 9) to 12 days (full-IPN-6). Cumulative amount and percent released from the hydrogels and IPNs were given in Table 1 (Column E & F). It shows 84.95 ± 3.68 % of the loaded doxorubicin was released from full-IPN-1 which was the highest and 53.69 ± 2.81 % release, which was the lowest released from full-IPN-6. The first phase initial burst could be due to the release of surface immobilized dox and the second phase burst could be due opening of the pore channels due to swelling and thus due to the increase in solute diffusion coefficient. However, hydrogel-3 (cross-linked with 0.07% Glu) had shown 91.75 ± 7.03 % release of loaded dox which was higher than hydrogel-1 (48.57 ± 6.98 %) (cross-linked with 0.03 % Glu). This phenomena is opposite to the full-IPNs, was probably due to the cross-linking of dox with free amino groups of gelatin [7].

Table 1: Sample code, composition and drug release details of the samples prepared

Sample code	Hydrogel / IPN composition	DVE : Gelatin ratio	Glu concentration (Wt %)	Cumulative amount Released (µg) ± SD	Cumulative percent released (µg) ± SD
A	B	C	D	E	F
Hydrogel-1	$G_{x0.03}$	0.0 : 1	0.03	278 ± 40.00	48.55 ± 06.98
Hydrogel-2	$G_{x0.05}$	0.0 : 1	0.05	438 ± 37.49	77.86 ± 06.66
Hydrogel-3	$G_{x0.07}$	0.0 : 1	0.07	517 ± 39.57	91.75 ± 07.02
IPN-1	$Dx_{0.3}Gx_{0.03}$	0.3 : 1	0.03	471 ± 20.45	84.95 ± 03.68
IPN-2	$Dx_{0.3}Gx_{0.05}$	0.3 : 1	0.05	490 ± 23.47	83.43 ± 03.99
IPN-3	$Dx_{0.3}Gx_{0.07}$	0.3 : 1	0.07	507 ± 39.00	80.84 ± 06.21
IPN-4	$Dx_{0.5}Gx_{0.03}$	0.5 : 1	0.03	430 ± 06.56	77.89 ± 01.18
IPN-5	$Dx_{0.5}Gx_{0.05}$	0.5 : 1	0.05	401 ± 18.67	68.89 ± 03.20
IPN-6	$Dx_{0.5}Gx_{0.07}$	0.5 : 1	0.07	311 ± 16.32	53.69 ± 02.81
IPN-7	$Dx_{0.7}Gx_{0.03}$	0.7 : 1	0.03	395 ± 32.07	69.10 ± 05.61
IPN-8	$Dx_{0.7}Gx_{0.05}$	0.7 : 1	0.05	348 ± 29.87	61.89 ± 05.31
IPN-9	$Dx_{0.7}Gx_{0.07}$	0.7 : 1	0.07	382 ± 97.27	67.20 ± 17.11

D represents DVE and G represents Gelatin

Mark x indicates crosslinking of the corresponding polymer. Numerical subscription of D indicates the amount of DVE in g used. Numerical subscription of G indicates the weight percent of glutaraldehyde used for crosslinking.

Effect of Glu and DVE on percent drug release

The effect of cross-linker (Glu) percentage on rate of drug release was shown in Figure 2b. For fixed amount of DVE (0.7g), increasing Glu concentration decreased the rate of release from full-IPNs. Full IPN 7 exhibited 69% of drug release in 120 h but almost same percentage of dox (67%) was released from IPN 7 in 240 h.

It shows when the concentration of the Glu was varied only to small extent (0.03 to 0.07%), the rate of release was extended significantly. Similarly, a drastic decrease in rate of dox release from hydrogels was observed when Glu was increased from 0.03 to 0.07 % (Figure 2a). This was because of the decrease in the solute diffusion coefficient due to the increased cross-linking density. These results show the role of glu concentration in extending the drug release. The effect of amount of DVE on drug release rate was shown in Figure 2c. The drug release rate from the full-IPN was controlled by (i) hydrophobic nature and (ii) molecular weight of DVE. Low molecular weight of DVE (Mn = 630) along with gelatin cross-linking with glutaraldehyde made the system closely cross-linked and less porous which sustained the drug release for about 10 days in case of full-IPNs 8 and 9.

This result was in contrast to hydrophillic full-IPN made of gelatin-poly (acrylic acid) (PAA) [2] which showed 60 % initial burst release of an antibiotic for the treatment of Osteomylities. But potent drugs like dox needs a slow burst in cancer treatment, especially on post surgical treatment, where normal cells also present in the vicinity of the tumor cells. Thus Replacing PAA by hydrophobic DVE showed initial maximum burst of 25 % (full-IPN-1) in 48 h and extended dox release for 10 days which would make the IPN system suitable for cancer targeting.

CONCLUSION

IPN hydrogels were successfully developed by emulsion technique. Free amino group determination showed that increasing glutaraldehyde concentration extended the drug release for some extent. However increasing the hydrophobic DVE ratio to gelatin from 0.3 g to 0.7 g extended the drug release for upto 10 days. In conclusion, the hydrophillic-hydrophobic IPN synthesized may find application as sustained drug delivery vehicle for passive cancer targeting.

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