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ORIGINAL ARTICLE

Preparation and Evaluation of Ocular Inserts Containing Norfloxacin

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Abstract: Norfloxacin is a poorly water soluble drug, and to improve its solubility it was complexed with b-cyclodextrin (BCD). Several ocular patches/inserts of norfloxacin-b-cyclodextrin were prepared in hydroxypropyl methyl cellulose (HPMC) matrix. The influence of rate controlling membranes made of ethyl cellulose (EC) alone and in combination with polyvinyl pyrrolidone K30 (PVP K30) in different proportions on drug release kinetics was studied. The data were subjected to regression analysis. Various physical characteristics of the films were evaluated. In vitro release studies were carried out in a fabricated flow through cell. All the films prepared were found to be uniform in thickness, and the partition coefficient of norfloxacin and its betacyclodextrin complex was 0.048 and 0.853, respectively. I.R. spectra revealed complexation of norfloxacin with b-cyclodextrin. In vitro results revealed that 2 patch/insert formulations, V1 and V2, followed perfect zero order kinetics release (n = 1), and 3 formulations, V3, V4 and V5, released the drug by super case II kinetics (n > 1). The study confirmed the improved solubility of norfloxacin when complexed with b-cyclodextrin and that it can be delivered through films made of HPMC matrix cast with EC alone or with a combination of PVP K30. It was also observed that increasing the proportion of PVP K30 into EC increased the rate of release of norfloxacin.

Key Words: Ocular insert, norfloxacin, beta-cyclodextrins.

Introduction

The eye as a portal for drug delivery is generally used for local therapy against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of the drug, which is not intended. The unique anatomy, physiology and biochemistry of the eye render this organ impervious to foreign substances, thus presenting a constant challenge to the formulator to circumvent the protective barriers of the eye without causing permanent tissue damage.

Most ocular treatments like eye drops and suspensions call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. These dosage forms are easy to instill but suffer from the inherent drawback that the majority of the medication they contain is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow and lacrimo-nasal drainage. Therefore only a very small fraction of the instilled dose is absorbed by the target tissue for this reason, concentrated solutions and frequent dosing are required for the instillation to achieve an adequate level of therapeutic effect (1). One of the new classes of drug delivery systems, polymeric film ocular drug delivery systems, which are gaining worldwide accolade, release drugs at a pre-programmed rate for a longer period by increasing the precorneal residence time.

Norfloxacin is a hydrophobic broad spectrum antibacterial with a half-life of 3 to 4.5 h frequently used in ocular infections, and is insoluble in water (2). There are only a few ocular inserts available on the market, made of ethylene vinylacetate (EVA) (3-5) as a rate controlling membrane. Likewise, ethyl cellulose (EC) is also an excellent film-forming polymer but the films of EC alone are brittle. It offers more resistance to the diffusion

of drug molecules, and is less explored as a polymer for ocular delivery of drugs. The current literature indicates that none are made of hydrophilic monolithic systems containing norfloxacin. Hence this investigation was taken up to study the drug release kinetics of norfloxacin from a hydrophilic, monolithic reservoir system of HPMC cast with rate controlling membranes made of EC and PVP K30. With the addition of PVP K30 to EC, the films of EC become resilient and do not break easily and it was ascertained that the drug diffusion might improve (6-11). It was aimed to prepare ocular films containing norfloxacin with better solubility and longer duration of action delivering the drug in zero order kinetics.

Since the drug has poor aqueous solubility it was required to modify the solubility characteristics of norfloxacin. Therefore in this work an attempt was made to improve the solubility of the norfloxacin by forming an inclusion complex with β-cyclodextrin (BCD). Hydroxy propyl methyl cellulose (HPMC) and polyvinyl pyrrolidine-K30 (PVP K30) are hydrophilic polymers whereas EC is a hydrophobic polymer and all these polymers show excellent film forming ability. It was also considered to modify the characteristics of EC. Hence, in this investigation the complex dispersed in HPMC matrix was cast with rate controlling membranes made of either EC alone, or in combination with PVP K30. The effect of ethyl cellulose polymer as a rate controlling membrane alone and in different combinations with PVP K30, i.e. EC:PVP K30; 8:1; 4:1; 2:1 and 1:1 ratios, on drug release kinetics was investigated.

Materials and Methods

Norfloxacin (NOR) was obtained as a complimentary sample from Torrent Pharmaceuticals, Mumbai, and betacyclodextrin (BCD) from Sun Pharma Limited, Ahmednagar. Hydroxy propyl methyl cellulose (HPMC), ethyl cellulose (EC), and polyvinyl pyrrolidone K30 were all from the Central Drugs House, Mumbai. Glycerol and dibutyl phthalate (DBP) were from Qualigens Fine Chemicals, Mumbai.

In the present work, norfloxacin was estimated using a double beam UV spectrophotometer (Hitachi U-2000, Japan) at 272.5 nm. The formation of complexation between norfloxacin and β-cyclodextrin by neutralization, was studied by Fourier transform–infra red spectroscopy (Model Shimadzu FTIR 8000 series). Isotonic phosphate buffer saline (IPBS) of pH 7.4 was prepared in distilled water.

The in vitro data were analyzed by a zero order kinetics equation as well as Korsemeyer's equation (12) to understand the release profile and release mechanism. When a graph of the cumulative percentage of the drug released from the matrix against time is plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration. The rate of release of the drug can be described mathematically as follows:

Rate of release = $(dC_s/t) = k$

where C_s = concentration of the drug present in the matrix, k = rate constant and t = time. Since Cs is a constant, and x = amount of drug released described as

dx / dt = k integration of the equation yields

x = k t + constant

A plot of x versus t results in a straight line with the slope = k. The value of k indicates the amount of the drug released per unit of time and the intercept of the line at time zero is equal to the constant in the equation. The curves plotted may have different slopes, and hence it becomes difficult to exactly pinpoint which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, in vitro data were also analyzed using Korsemeyer's equation.

Korsemeyer et al. used a simple empirical equation to describe general solute release behavior from controlled release polymer matrices:

$m_t/m_\infty = k * t^n$

where m_t/m_∞ = fraction of drug released, k = kinetic constant, t = release time and n = the diffusional exponent for drug release.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (zero order) (case II transport); n = 0.5 for Fickian diffusion; and when $0.5 < n < 1$, diffusion and non-Fickian transport are implicated. Lastly, when $n > 1.0$ super case II transport is apparent. 'n' is the slope value of log m_t/m_∞ versus log time curve.

Preparation of inclusion complex by neutralization method (13)

Norfloxacin (NOR) and β-cyclodextrin (BCD) in a molar ratio of 1:1 were separately dissolved in 0.1 N NaOH, mixed and stirred for half an hour. 0.1 N HCl was added to this solution dropwise until the solution attained pH 7.5, when the complex precipitated. The precipitate was washed with distilled water until it was free of chloride ions.

The monolithic drug reservoir patches were prepared from aqueous solutions of HPMC with inclusion complex of BCD containing norfloxacin. The films were cast by the mercury substrate method (14). A Teflon ring was placed in a pool of mercury, and then the matrix solution containing the drug was loaded onto this ring. It was allowed to dry uniformly under ambient conditions. An area of 0.502 cm^2 containing 2 mg of norfloxacin was used for all the studies.

Preparation of rate controlling membranes

The rate controlling membranes were prepared from EC alone (V1) and also with EC: PVP K30 in different proportions. Four different proportions of EC:PVP K30 were modeled, i.e. 8:1(V2), 4:1(V3), 2:1(V4) and 1:1 (V5) ratios. Weighed quantities of the polymers or their ratios were solubilized in distilled water with continuous mixing. The matrix solution such prepared was pipetted and poured onto a Teflon ring placed in a mercury pool. The matrix was dried constantly under ambient conditions. In all the films dibutyl phthalate (DBP) 15% w/w was incorporated as a plasticizer.

Evaluation of ocular films:

The above films were evaluated for the thickness of each film using a micrometer of sensitivity of 0.001 mm (Mitutoyo, Japan). The average of 10 readings was taken. The mean thickness, standard deviation and percent coefficient of variation were calculated. The octanol:water partition coefficient (15) was determined for the drug and also its complex with BCD. A drug solution of 50 µg/ml was prepared in distilled water and 25 ml of this solution was taken in a separating funnel and shaken with an equal volume of octanol for 10 min and allowed to stand for 1 h. Then the aqueous phase was separated, and centrifuged for 10 min at 2000 rpm. The aqueous phase was assayed before and after partitioning, using a UV spectrophotometer to get the partition coefficient. Triplicate readings were taken and the average was calculated. A similar experiment was carried out with an equivalent amount of NOR-BCD complex. In vitro diffusion studies were performed in a fabricated flow through assembly using IPBS 7.4 as media. Norfloxacin was extracted from the film into a solution of IPBS 7.4 and after extracting the drug for 24 h the drug content was determined. The average of triplicate readings was taken. Films of 0.502 cm^2 in area were used from each formulation.

Description of open flow through assembly (16,17)

Since there was no specific official method prescribed for in vitro studies of ocular film/inserts, we fabricated an open flow through assembly, simulating the conditions of the ocular cavity, in our laboratory for the purpose of this investigation (Figure 1).

A 2 ml glass tube open at both ends was used as an in vitro diffusion cell. Two fluted glass adapters were fused at both open ends so that one formed the inlet and the other fluted end was used to withdraw samples. The inlet

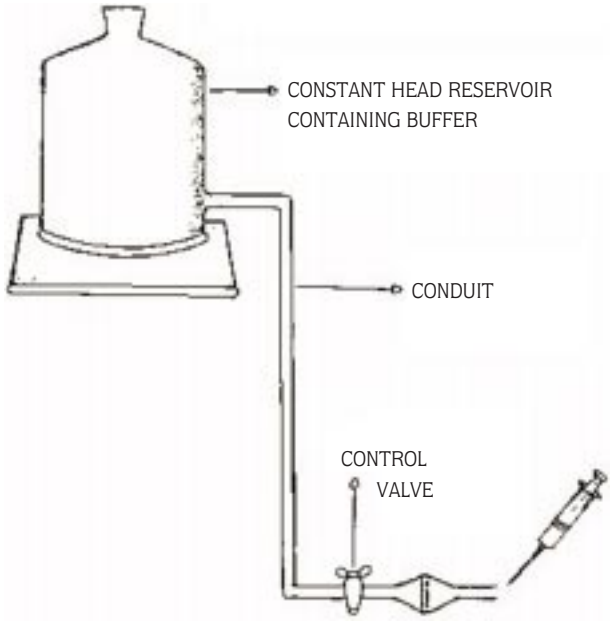


Figure 1. Schematic diagram of open flow through assembly.

end of this tube was connected to a reservoir containing IPBS pH 7.4. The head of the reservoir was kept constant. Flexible PVC tubing was connected from this reservoir to the cell, in which 2 ml of buffer was maintained constant. The rate of flow of buffer was controlled with a valve and adjusted to 0.166 ml/min. Taking 25 readings initially, the setup was validated and the standard deviation (0.166 ± 0.03 ml) and percent coefficient of variation were observed to be minimum; hence, the setup was used throughout the work.

IPBS pH 7.4 was put into the reservoir. A small volume of fluid was allowed to drain away, so as to remove any entrapped air bubbles in the cell. An ocular patch was stuck onto a thin small, circular, teflon disc, so that only one surface was exposed to the diffusion fluid. This disc was steadily inserted into the cell containing 2 ml of fluid. The temperature of the fluid was kept at 35 ± 1 °C constantly. At regular intervals the diffusion fluid was taken to analyze for drug content using a UV spectrophotometer. Simultaneously a blank was performed under similar conditions as described, with a drug devoid film. Triplicate readings were taken and the average was calculated and tabulated.

Results and Discussion

The drug content in each film formulation was found to be almost the same as for theoretical norfloxacin (2 mg) added to the monolithic film (Table 1). The thickness of the patches was fairly uniform and consistent, as indicated by their low standard deviations (Table 2). The partition coefficient was 0.048 and 0.853 for pure drug and the NOR-BCD complex, respectively, indicating a fair increase in the aqueous solubility of the drug. Figures 2 and 3 represent the IR spectra of norfloxacin and its BCD complex, respectively. The spectrum of norfloxacin shows an absorption band for C = O at 1730 cm^{-1} as the fraction of the –COOH group, whereas the IR spectra of the complex does not show any absorption band in that region, i.e. 1650 cm^{-1} to 1750 cm^{-1}. This clearly indicated that the complex between the norfloxacin and β-cyclodextrin is formed via the C = O group of norfloxacin and the hydrogen of the secondary hydroxyl group of the β-cyclodextrin. The IR spectrum of the norfloxacin shows a sharp peak at 2400 cm^{-1}, which indicates only one –OH group.

Table 1. Drug content of norfloxacin.

Formulation	Actual amount of drug	% Drug content*
V1	1.9481	97.41
V2	1.9446	97.23
V3	1.9298	96.49
V4	1.9334	96.67
V5	1.9372	96.86

* → Average of 3 readings

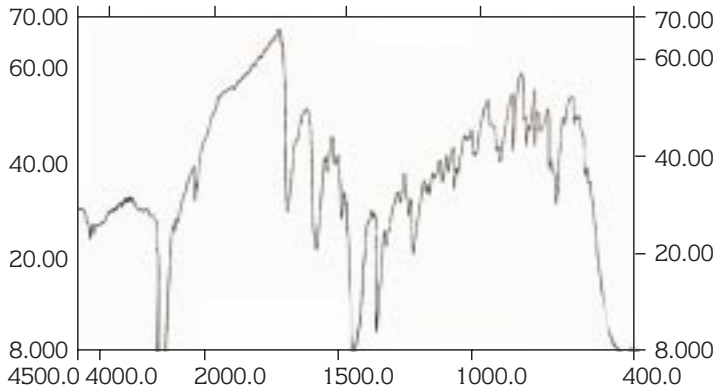
Table 2. Thickness, slopes and regression values of zero order and Korsemeyer models.

Formulae	Thickness* (μm)	Zero order		Korsemeyer	
		k	r	n	r
V1	71.1 ± .0037	0.4199	0.988	1.00	0.986
V2	71.0 ± 0.0030	0.4897	0.978	1.00	0.979
V3	73.3 ± 0.0050	0.7284	0.999	1.10	0.977
V4	74.1 ± 0.0044	0.8170	0.979	1.11	0.950
V5	75.8 ± 0.0034	0.9224	0.978	1.15	0.961

* → Average of 10 readings

In vitro release studies

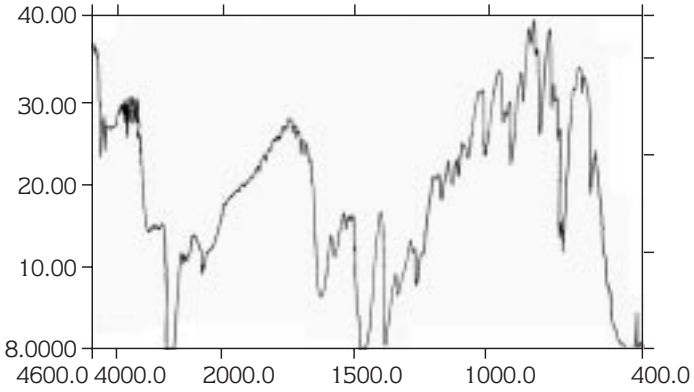
The basic in vitro data obtained are tabulated in Table 3. Initially the drug release from the HPMC matrix film containing drug complex was studied. The study showed first order release kinetics with a rate constant of $3.983*10^{-3}$ (r = 0.9476); nearly 90% of the drug was released within 3 h. This means that the film is to be applied several times a day to the conjunctiva. Therefore, to reduce frequency of administration and to control the drug release for several days, further films, with EC and PVP K30, were prepared in different ratios. In controlled drug delivery zero order is the most preferred kinetics of drug release. Therefore films of EC were modeled to release the drug in zero order mode. The zero order plots of V1, V2, V3, V4 and V5 were found to be fairly linear, as indicated by their high regressional value (Figure 4). Therefore, it was ascertained that the drug release from V1, V2, V3, V4 and V5 could follow either near zero or zero order kinetics. The zero order curves alone are not sufficient to predict zero order since each curve, albeit straight, has a different slope. Hence to confirm the exact mechanism of drug release from the films, the data were computed and graphed according to Korsemeyer's equation, as shown in Figure 5. Regression analysis was performed (18) and the regression value 'r' suggested



* Footnote :

----------PARAMETERS OF SPECTRUM----------26/11/08 11:28:53 - - -
MEASURING MODE ; %T
RESOLUTION ; 4.0 Cm^{-1}
ACCUMULATION ; 40
AMP GAIN ;AUTO
DETECTOR ; DETECTOR 1 (2.8 mm/sec)
APODIZATION ; HAPP-GENZEL
REMARKS ; NOR
ANALYST ; SS

Figure 2. I.R. spectra of norfloxacin.



* Footnote :

----------PARAMETERS OF SPECTRUM----------26/11/08 12:28:53 - - -
MEASURING MODE ; %T
RESOLUTION ; 4.0 cm^{-1}
ACCUMULATION ; 40
AMP GAIN ;AUTO
DETECTOR ; DETECTOR 1 (2.8 mm/sec)
APODIZATION ; HAPP-GENZEL
REMARKS ; NOR -BCD
ANALYST ; SS

Figure 3. IR spectra of norfloxacin- ß-cyclodextrin complex.

Table 3. In vitro release data of V1, V2, V3, V4 and V5.

Time (h)	Log time	Cum% drug release	Log m_t/m_∞ x 10^3	Cum% drug release	Log m_t/m_∞ x 10^3	Cum% drug release	Log m_t/m_∞ x 10^3	Cum% drug release	Log m_t/m_∞ x 10^3	Cum% drug release	Log m_t/m_∞ x 10^3
0	0.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	-0.3010	0.18	0.307	0.28	0.477	0.33	0.545	0.47	0.602	0.34	0.562
1	0.0000	0.39	0.642	0.62	0.778	0.68	0.855	0.87	0.954	0.90	0.987
2	0.3010	0.84	0.974	1.18	1.176	1.40	1.166	2.24	1.380	2.35	1.401
4	0.6020	2.00	1.348	3.22	1.544	3.19	1.523	7.02	1.879	6.86	1.760
8	0.9030	5.05	1.750	6.62	1.857	6.66	1.842	14.09	2.260	12.70	2.080
12	1.0791	7.91	1.953	8.53	1.973	9.57	1.999	19.04	2.400	18.40	2.220
24	1.3802	12.90	2.157	13.38	2.167	16.56	2.237	33.25	2.680	38.25	2.613
36	1.5563	18.75	2.319	20.66	2.356	25.03	2.417	44.55	2.760	50.26	2.731
48	1.6812	25.65	2.455	28.25	2.492	32.34	2.528	55.38	2.850	63.74	2.835
60	1.7781	33.62	2.573	34.07	2.574	42.12	2.643	66.04	2.853	67.89	2.862
72	1.8573	39.05	2.638	41.40	2.658	51.77	2.732	71.14	2.888	75.31	2.907
84	1.9242	46.30	2.712	57.12	2.793	62.25	2.812	78.38	2.920	83.08	2.950
96	1.9822	51.82	2.760	65.70	2.859	71.04	2.870	83.50	2.950	89.66	2.983
108	2.0334	57.52	2.806	70.68	2.891	79.35	2.918	88.88	2.990	93.18	3.000
120	2.0791	61.89	2.838	73.78	2.910	87.00	2.958	92.59	3.000		
132	2.1205	65.68	2.865	76.41	2.925	95.79	3.000				
144	2.1583	69.68	2.889	79.10	2.940						
156	2.1931	72.80	2.908	81.14	2.951						
168	2.2253	76.03	2.927	83.60	2.964						
180	2.2552	78.86	2.943	85.45	2.974						
192	2.2833	82.32	2.961	85.70	2.988						
204	2.3093	85.25	2.977	90.73	3.000						
216	2.3344	87.47	2.988								
228	2.3579	89.86	3.000								

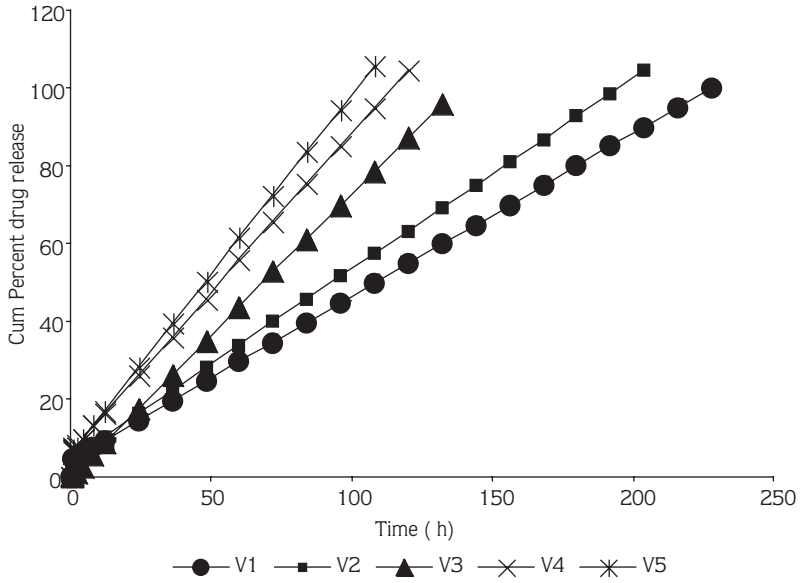


Figure 4. Comparative zero order kinetic plots of different formulations V-1, V-2, V-3, V-4, V-5.
V1 → EC rate controlling membrane
V2 → PVP K30 : EC ; 8:1
V3 → PVP K30 : EC ; 4:1
V4 → PVP K30 : EC ; 2:1
V5 → PVP K30 : EC ; 1:1

that the curves were fairly linear. Slope values were computed from the graph. The 'n' values suggest that the films V1 and V2 follow perfect zero order kinetics (n = 1.0) whereas the ocular films V3, V4 and V5 follow a super case II transport mechanism (n > 1.00), possibly owing to chain disentanglement and swelling of hydrophilic polymer. The zero order rate constants, slope value 'n' and their respective 'r' values are given in Table 2. Initially it was theorized that incorporating a portion of hydrophilic polymer PVP K30 into EC might increase the diffusion of drugs from the HPMC matrix. In this study we obtained increasing rate constant values (k), which confirmed our theory. This is probably because the PVP K30 reduces the resistance offered by the EC film alone, and by increasing pores and/or their diameter the drug diffuses with less resistance.

Hence it could be ascertained that a hydrophobic polymer like EC can be modeled with a hydrophilic polymer like PVP K30 to release by zero order, limiting to a proportion, which in this case is proved to be 8:1. Therefore, to develop potential ocular applications that can sustain the drug level for several days, EC:PVP K30; 8:1, can be used.

Conclusions

Norfloxacin is a broad-spectrum antibacterial drug with a half-life of 3.0 to 4.5 h. It is also less soluble in water. The drug is successfully being used in the treatment of many eye infections. Therefore, it was chosen as a model drug for this study. The solubility of norfloxacin was improved by complexing it with BCD using a neutralization technique.

In this work, different rate controlling membranes were prepared from ethyl cellulose alone and in combination with PVP K30, i.e. 8:1, 4:1, 2:1 and 1:1. The effect of drug release kinetics was investigated. The films were evaluated for their thickness. An in vitro study was carried out in a fabricated open-flow through assembly.

All 6 formulations were found to be smooth, transparent and flexible. Thickness was fairly uniform as indicated by their low coefficient of variation. In vitro release studies revealed that the patches V-1 and V-2 follow perfect zero order kinetics release. Increasing the amount of PVP K30 in the rate controlling membrane increases the rate constant 'k' and the ocular films V3, V4, and V5 follow super case-II transport, possibly owing to chain disentanglement and swelling of hydrophilic

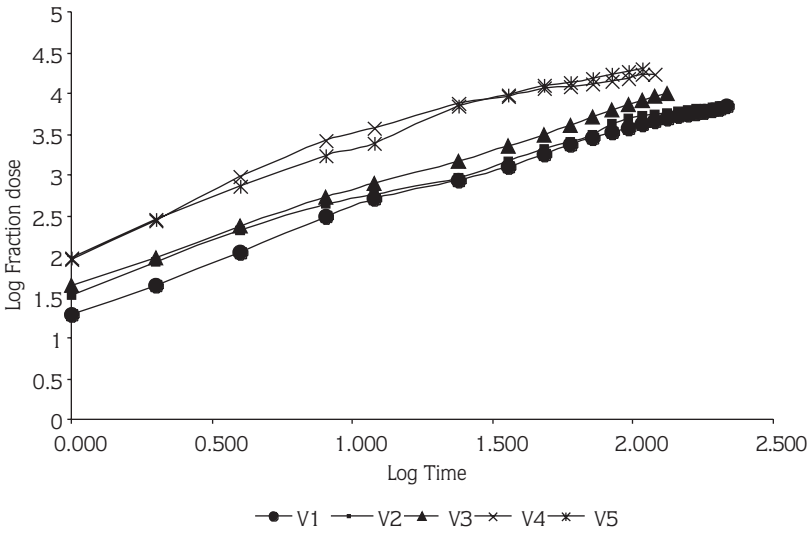


Figure 5. Korsemeyer data curves of V1, V2, V3, V4, and V5.
V1 → EC rate controlling membrane
V2 → PVP K30 : EC ; 8:1
V3 → PVP K30 : EC ; 4:1
V4 → PVP K30 : EC ; 2:1
V5 → PVP K30 : EC ; 1:1

polymer. However, addition of PVP K30 to EC is limited to 8:1 proportion, to release the drug by zero order. As the amount of EC is increased, duration of release also increases without significantly altering the rate of drug release.

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