Development and evaluation of diclofenac sodium thermorevesible subcutaneous drug delivery system

Fazli Nasir [a], Zafar Iqbal [a,*], Jamshaid A. Khan [a], Abad Khan [b], Fazli Khuda [a], Lateef Ahmad [a], Amirzada Khan [a], Abbas Khan [a], Abdullah Dayoo [c], Roohullah [a]

[a] Department of Pharmacy, University of Peshawar, Peshawar-25120, Pakistan
[b] Department of Pharmacy, Abdul Wali Khan University Mardan, Ambar Campus, Pakistan
[c] Faculty of Pharmacy, Sindh University Jamshooro, Sindh, Pakistan

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ABSTRACT

The objective of current work was to develop and evaluate thermoreversible subcutaneous drug delivery system for diclofenac sodium. The poloxamer 407, methyl cellulose, hydroxypropyl methyl cellulose and polyethylene glycol were used alone and in combination in different ratios to design the delivery system. The physical properties like Tsol–gel, viscosity, clarity of solution and gel were evaluated. The in vitro release of the drug delivery system was evaluated using membrane less method and the drug release kinetics and mechanism was predicted by applying various mathematical models to the in vitro dissolution data. Rabbits were used as in vivo model following subcutaneous injection to predict various pharmacokinetics parameters by applying Pk-Summit software. The in vitro and in vivo data revealed that the system consisting of the poloxamer 407 in concentration of 20% (DP20) was the most capable formulation for extending the drug release and maintaining therapeutic blood level of DS for longer duration (144 h). The data obtained for drug content after autoclaving the solutions indicate that autoclaving results in 6% degradation of DS. The data also suggested that the studied polymers poloxamer, MC and PG are good candidate to extend the drug release possessing a unique thermoreversible property.

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1. Introduction

The application of the polymers in pharmaceutical sciences particularly in drug delivery systems and formulations is well known (Arnott et al., 1974). Many polymers show a decrease in solubility as their hydrophobicity increases upon temperature change. In such type of polymer solutions, three types of interactions are observed; (i) between the molecules of polymer, (ii) between water and polymer molecules and (iii) between the water molecules. This phenomenon is called hydrophobic effect (Gong et al., 2009a; Kang et al., 2006; Mulik et al., 2009; Tyagi et al., 2004). As a result of the increase in hydrophobicity the polymer chains are linked by physical reversible linkage, and gels can therefore return to solution after the temperature stimulus, causing gelation is removed (Kang et al., 2006). These formulations may sustain delivery of the drug for long period of time improving the patient's compliance.

Pluronic (poloxamer) is a group of nonionic surfactants consisting of polyethylene oxide (PEO) and polypropylene oxide (PPO) copolymers. The thermal gelation of pluronic is a result of micellization which is due to the dehydration of hydrophobic poly propylene oxide blocks and hydration of poly ethylene oxide chains (Juhasz et al., 1989). Methyl cellulose is a synthetic methoxy derivative of cellulose. Methyl cellulose aqueous solution in concentration of 1–10% by weight forms a thermorevesible gel (Sarkar, 1979). HPMC is synthetic propylene glycol ether of methyl cellulose. Aqueous HPMC solution forms thermo-reversible gel on heating (Masae et al., 2001).

Diclofenac sodium 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid) a phenylacetic acid derivative is nonsteroidal anti-inflammatory agent used as analgesic and anti-rheumatic (Brogden et al., 1980; Catella-Lawson et al., 2001; Reynolds and Parfitt, 1989; Sallmann, 1986; Todd and Sorkin, 1988).

2. Materials and methods

2.1. Materials

Diclofenac sodium (DS) purity 99.30% (manufactured by Suzhou Ausun Chemical Company Limited), methyl cellulose (MC) 15 cps, hydroxypropylylmethyl cellulose (HPMC, Methocel E5) manufactured by Dow Chemical and polyethylene glycol (PEG 6000) manufactured by Clariant GMBH were the kind gifts of Medicraft

Pharmaceuticals Pvt. Ltd., Peshawar. Voren injection (DS) manufactured by Asian Continental Pharma, was purchased from local market. Methanol, acetonitrile, phosphoric acid, and triethylamine (HPLC grade), pluronic F-127 (poloxamer406), were purchased from Sigma–Aldrich (Oslo, Norway). Purified water was prepared using a Millipore ultra-pure water system (Milford, USA).

White New Zealand rabbits were purchased from the Department of Pharmacy University of Peshawar. The procedure for use and care of animals for this study were approved by the Ethical Committee of Department of Pharmacy University of Peshawar.

2.2. Instrumentation

Perkin-Elmer HPLC system (Norwalk, USA), consisted of a pump (series 200), on-line vacuum degasser (series 200), auto-sampler (series 200), Peltier column oven (series 200), linked by a PE Nelson network chromatography interface (NCI) 900 with UV/VIS (series 200). The whole HPLC system was controlled by Perkin-Elmer Total chrom Workstation Software (version 6.3.1). Centrifuge (Centurion. Scientific Ltd.), Shaking water bath B.S.11 Lab Companion (Jelo Tech Korea), pH meter (Hanna instruments 8417, USA) and Autoclave HS-60 (Hansuin Medical Co. Ltd., Korea).

2.3. Preparation of diclofenac sodium in situ gel formulations

The drug and polymers were accurately weighed according to the composition for the formulations as shown in Table 1. Diclofenac sodium 5 mg/ml solutions with different polymers alone and in polymers combination were prepared using cold method (Schmolka, 1972; Wei et al., 2002). The polymer was dispersed in cold water with continuous stirring, the dispersion was then stored in refrigerator for 24 h to obtain clear polymer solution, DS was then added to this solution and dissolved with continuous stirring.

The final solutions were sterilized by autoclaving at 121 °C, 15 psi for 20 min and were evaluated for their physicochemical properties i.e. Tsol–gel, viscosity, clarity, drug content, in vitro drug release and in vivo pharmacokinetic parameters.

2.4. Measurement of Tsol–gel DS formulations

The Tsol–gel of the formulations was measured by tube inversion method (Gilbert et al., 1987; Vadnere et al., 1984). 2 ml of each solution was transferred to a 5 ml test tube in a digital Shaking water bath maintained at gelation temperature and sealed with a parafilm. The solutions were equilibrated for 5 min to effect the gelation. The gelation of the solutions was verified by inverting the test tube at 90°. Measurements were performed in triplicates and Student's t-test at $p < 0.05$ was performed for statistical significance.

2.5. Measurement of steady shear viscosity of diclofenac sodium formulations

The steady shear viscosity before and after autoclaving was measured using cone and plate viscometer. A 0.5 ml sample of the solution was applied to the lower plate of the viscometer. The viscosity was taken using spindle 52 at 37 ± 0.1 °C at a shear rate

ranging from 5 to 400 rpm. All samples were analyzed in triplicate and for statistical significance Student's t-test at $p < 0.05$ was performed.

2.6. Clarity of the formed gel of DS formulations

The clarity of the DS solution and formed gels before and after autoclaving of the formulations was observed visually at 5 °C, 25 °C and 37 °C.

2.7. Drug content of DS formulations

All of the samples were analyzed in triplicate for the drug content using HPLC method (Nasir et al., 2011) before and after autoclaving. Only samples with drug content within $100 \pm 10\%$ of labeled amount were accepted.

2.8. Determination of in vitro drug release from DS formulations

The in vitro drug release was determined using membrane less dissolution model (Chandrashekhar, 1998). From each preparation, solution containing DS (2 ml) was transferred into tubes (ca. ≈ 10 ml). The solutions were equilibrated for 5 min to effect the gelation in digital shaking water bath maintained at 37 ± 1 °C. Phosphate buffer pH 7.4 (2 ml), used as dissolution medium, was poured slowly on the surface of the gel not to disturb the surface layer. Whole of the dissolution medium was collected as a sample after predefined time intervals i.e.0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24, 36, 48, 72, 96 and 120 h depending upon formulation that remain intact for the specified period of time. As soon as samples were collected fresh dissolution medium (2 ml) was added to the test tube. The samples were suitably diluted and analyzed for diclofenac content using HPLC method (Nasir et al., 2011).

2.9. Drug release kinetics

The data obtained from in vitro experiments was fitted to various mathematical models to assess the drug release kinetics.

2.9.1. Zero order kinetic model
$$Q_t = Q_0 + K_0 t \tag{1}$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K_0 is zero order release constant.

2.9.2. First order kinetic model
$$\log Q_t = \log Q_0 + \frac{K_1 t}{2.303} \tag{2}$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution and K_t is first order release constant.

2.9.3. Higuchi model
The model relates cumulative drug release versus square root of time as shown in Eq. (3).

$$Q = K_H \sqrt{t} \tag{3}$$

Table 1
Composition of diclofenac sodium thermoreversible gel.

S. no.	Formulation	Diclofenac sodium (mg)	PL F127 (mg)	MC (mg)	PEG (mg)	Distilled water
1	DP18	5.0	180	–	–	qs to 1 ml
2	DP20	5.0	200	–	–	qs to 1 ml
3	DPM15/3	5.0	150	30.0	–	qs to 1 ml
4	DMPG1.5/10	5.0	–	15.0	100.0	qs to 1 ml
5	DMPG3/2	5.0	–	30	20.0	qs to 1 ml