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Research paper

In vitro and in vivo evaluation of nimesulide lyophilized orally disintegrating tablets

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ABSTRACT

Development of a lyophilized orally disintegrating tablet (ODT) that enhanced the in vitro dissolution and in vivo absorption of nimesulide (NM), a drug with poor solubility and poor bioavailability, is presented. The ODTs were prepared by freeze-drying an aqueous dispersion of NM containing a matrix former, a sugar alcohol, and a collapse protectant. In addition, different disintegration accelerators were tested. The influence of formulation parameters on the disintegration time and in vitro dissolution of NM from ODTs along with other tablet characteristics was investigated. Results obtained from disintegration and dissolution studies showed that lyophilized ODTs disintegrated within few seconds and showed significantly faster dissolution rate of NM compared to the plain powder drug and NM in commercially available immediate release tablet Sulide®. The ODTs were also examined using differential scanning calorimetry, X-ray diffraction, and scanning electron microscope. Stability results, after 12-month storage of selected ODTs at 25 °C and 60% relative humidity, were satisfactory. The extent of absorption of NM from a selected ODT when compared to an conventional immediate release tablet as a reference after administration of 100 mg oral dose of NM was determined in healthy subjects using a randomized cross-over design. In this study, the rate of absorption of NM from ODT was faster than that from the reference tablet, had a significantly higher ($p = 0.012$) peak plasma concentration, and shortened time to C_{max} by 1 h ($p = 0.029$). The extent of absorption expressed by AUC was 62% larger when compared to the commercially available tablet.

1. Introduction

Clinically, non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed preparations. Nimesulide (NM), a preferential COX-2 inhibitor, shows high anti-inflammatory, antipyretic and analgesic activity with moderate incidence of gastric side effects and a high therapeutic index [1]. NM belongs, according to the biopharmaceutic classification system (BCS), to Class II drugs with poor solubility and high permeability [2,3]. Class II drugs suffer from low bioavailability following oral administration of traditional dosage forms. NM is virtually insoluble in aqueous systems (solubility 0.01 mg/ml) [4]. The very poor aqueous solubility and wettability of NM gives rise to difficulties in the pharmaceutical formulation of oral or injectable solutions and leads to a variable bioavailability. Several studies have been carried out to increase the aqueous solubility of NM such as by complexing NM with β-cyclodextrin [5,6] or by incorporating it within a NM-L-lysine–β-cyclodextrin complex [4]. An enhanced dissolution of NM from crystals prepared by solvent change (ethanol to water, 1:1) in the presence of Tween 80 (1%) has also been reported [7]. In all these studies, in vivo testing in human volunteers was not reported.

In this study, ODTs containing NM were prepared by a freeze-drying technique in order to improve the dissolution rate and oral bioavailability of NM. NM is largely eliminated via metabolic transformation [8]; therefore, an ODT of NM that is partially absorbed through the oral mucosa directly enters the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism of the liver, which may result in an increase in the fraction of drug reaching the systemic circulation and also result in a rapid onset of action via a more comfortable and convenient delivery route than the intravenous route.

2. Materials and methods

2.1. Materials

Nimesulide was kindly supplied by Alkan Pharma, Egypt. Sorbitol and mannitol were kindly supplied by Roquette Pharma, France. Gelatin, glycine, Tween 20, Tween 80, sodium chloride and potassium chloride were received from Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt. Polyethylene glycol (PEG 400, PEG 4000 and PEG 6000) and polyvinyl pyrrolidine (PVP K25, PVP K30 and PVP K 90) were purchased from Fluka AG (Buchs, Switzerland). The water used was distilled de-ionized water. All other chemicals were reagent grade and used as received. Sulide® 100 mg (Alkan, Egypt) was used as a reference tablet in in vivo studies.

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2.2. Preparation of ODTs

Nimesulide ODTs were prepared using gelatin as a matrix former, a sugar alcohol (sorbitol or mannitol) and a collapse protectant (glycine). Gelatin was used in three different concentrations (1%, 2% and 3% w/v), while the two sugar alcohols and glycine were used at a concentration of 0.886% w/v. The percentage of sugar alcohol and glycine used was optimized during the formulation process to result in a strong and elegant tablet that could be handled with ease. Gelatin was first dissolved in distilled water at about 40 °C to obtain the required concentration. Sorbitol (or mannitol) and glycine were then added to the gelatin solution in the predetermined concentration. An accurately weighed amount of NM powder was dispersed in the prepared aqueous solution using a magnetic stirrer to result in a dose of 50 mg NM per 1 ml. One milliliter of the suspension was then poured in each pocket of a PVC blister pack with a diameter of 13 mm and a depth of 3 mm resulting in a dose of 50 mg per tablet. The tablet blister packs were then transferred to a freezer at −22 °C and kept in the freezer for 24 h. The frozen tablets were placed in a lyophilizer for 24 h using a Novalyphe-NL 500 Freeze Dryer with a condenser temperature of −45 °C and a pressure of 7×10^{-2} mbar. The best of these formulations (based on tablet properties) was taken forward to the next stage which involved the addition of a water-soluble surface active agent or polymer in order to improve disintegration time and/or friability. These disintegration accelerators were sodium lauryl sulphate (SLS); three grades of PEG, namely PEG 400, PEG 4000 and PEG 6000; three grades of PVP, namely PVP K25, PVP K30 and PVP K90; and two grades of Tweens, namely Tween 20 and Tween 80. All of these were added at a concentration of 1% w/v except SLS, which was added at 0.05% w/v. The detailed composition of the prepared ODTs is presented in Table 1. The prepared ODTs were kept in tightly closed containers in desiccators over calcium chloride (0% relative humidity) at room temperature until further use.

2.3. Characterization of ODTs

2.3.1. Uniformity of weight

The test was carried out according to the European pharmacopoeia [9]. Twenty tablets, from each formula, were individually weighed and the mean of tablet weights was calculated. Results are presented as mean value ± standard deviation (SD).

2.3.2. Tablet friability

Twenty tablets, from each formulation, were accurately weighed and placed in the drum of friabilator (Erweka type, GmbH, Germany). The tablets were rotated at 25 rpm for a period of 4 min and then removed, dedusted and accurately re-weighed (EP 2000). The percentage loss in weight was calculated and taken as a measure of friability.

Table 1
Composition of NM ODT formulations.

Formulation	Gelatin (%w/v)	Sorbitol (%w/v)	Mannitol (%w/v)	Glycine (%w/v)	Disintegration accelerators (%w/v)	NM (%w/v)
G1	1	0.886		0.886		5
G2	1		0.886	0.886		5
G3	2	0.886		0.886		5
G4	2		0.886	0.886		5
G5	3	0.886		0.886		5
G6	3		0.886	0.886		5
G7	2		0.886	0.886	0.05% SLS	5
G8	2		0.886	0.886	1% PEG 400	5
G9	2		0.886	0.886	1% PEG 4000	5
G10	2		0.886	0.886	1% PEG 6000	5
G11	2		0.886	0.886	1% PVP K25	5
G12	2		0.886	0.886	1% PVP K30	5
G13	2		0.886	0.886	1% PVP K90	5
G14	2		0.886	0.886	1% Tween 20	5
G15	2		0.886	0.886	1% Tween 80	5

2.3.3. In vitro disintegration time

Disintegration times of the prepared ODTs were determined with six tablets in distilled water kept at 37 ± 0.5 °C using a DST-3 disintegration tester (Logan Instruments Corp., NJ, USA) according to EP (2002) specifications. The disintegration time was defined as the time necessary for the ODT to completely disintegrate until no solid residue remains or only a trace amount of soft residue remains on the screen. A digital stopwatch was used to measure the disintegration time to the nearest second. Only one ODT was analyzed at a time in order to ensure utmost accuracy. All results are presented as mean value ± SD ($n = 6$).

2.3.4. In vivo disintegration time

The in vivo disintegration time of each of the prepared ODTs was evaluated in six human volunteers after giving informed written consent. The volunteers had no history of hypersensitivity to NSAIDs. Prior to the test, all volunteers were asked to rinse their mouth with distilled water. Each of the six subjects was given a coded tablet. Tablets were placed on the tongue and immediately the time was recorded. They were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. Immediately after the last noticeable mass had disintegrated, the time was recorded. The subjects were asked to spit out the content of the oral cavity after tablet disintegration and rinse their mouth with distilled water. The swallowing of saliva was prohibited during the test, and also saliva was rinsed from the mouth after each measurement. The test results are presented as mean value ± SD [10].

2.3.5. Wetting time

Ten milliliters of distilled water containing eosin, a water-soluble dye was placed in a Petri dish of 10 cm diameter. Tablets were carefully placed in the centre of the Petri dish and the time required for water to reach the upper surface of the tablet was noted as the wetting time. The test results are presented as mean value of three determinations ± SD [11].

2.3.6. Moisture analysis

The tablets were analyzed for their residual moisture content after lyophilization using Karl Fischer titrator (Veego Matic-MD, Veego Instruments Corporation, India). Each tablet was pulverized, inserted in the titration vessel containing dried methanol (Karl–Fischer grade) and titrated with Hydranal Composite 5 reagent (Riedel-de-Haën, Seelze, Germany) after a stirring time of 3 min. Results are presented as mean value ± SD ($n = 3$).

2.3.7. In vitro dissolution studies

The dissolution profiles of NM in ODTs compared with the plain drug were determined in a dissolution tester (Pharma Test Dissolution Tester, Germany) following the USP paddle method. All tests were conducted in 900 ml simulated saliva fluid without enzymes (SSF) at pH = 6.8. The dissolution medium was maintained at a temperature of 37 ± 0.5 °C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 50 mg. At specified time intervals (1, 2, 3, 5, 7, 10, and 15 min), 3 ml of dissolution medium was withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume. Samples were filtered through 0.45 μm millipore filter and assayed for drug content spectrophotometrically at 393 nm after appropriate dilution. Cumulative amount of drug dissolved in the preparations was cal-