IJPSR (2013), Vol. 4, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 September, 2012; received in revised form, 22 December, 2012; accepted, 29 December, 2012

IN VITRO AND *IN VIVO* EVALUATION OF DICLOFENAC POTASSIUM LYOPHILIZED ORALLY DISINTEGRATING TABLETS

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Keywords:

Diclofenac Potassium, Freeze-drying, Orally disintegrating tablets, *In vivo* absorption, Bioavailability, Dissolution rate

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QUICK RESPONSE CODE

IJPSR: ICV- 5.07

Website: www.ijpsr.com

ABSTRACT

Diclofenac Potassium, a sparingly soluble non-steroidal anti-inflammatory drug, was taken as candidate for decreasing the onset of action time and increasing its bioavailability by overcoming its first pass metabolism. Diclofenac Potassium orally disintegrating tablet (ODT) formulations were developed using lyophilization technique. The freeze dried tablet formulations were prepared by freeze-drying an aqueous solution of Diclofenac Potassium, matrix former, filler, and an anti-collapse. The tablets were evaluated from both compendial and non-compendial criteria (i.e. uniformity of weight, uniformity of content, friability, in vitro disintegration time, in vitro dissolution, wetting time, in vivo disintegration time, moisture analysis and scanning electron microscopy. The best formula results showed that lyophilized ODT disintegrated within few seconds and showed significantly faster in-vitro dissolution rate of Diclofenac Potassium in comparison with commercially available immediate release tablet Diclofenac Potassium tablet (Cataflam®). The in-vivo evaluation for the best formulation (LD#11) was performed in comparison with the immediate release tablet Diclofenac Potassium tablet (Cataflam® 50 mg). A randomized crossover design was adopted in the comparative bioavailability study and was done on a sample of four healthy human volunteers. Statistical analysis revealed significant difference between the Cataflam immediate release tablet and Diclofenac Potassium ODT (LD#11) regarding the following pharmacokinetic parameters: C_{max} and T_{max} ($p < 0.05$); while insignificant difference regarding $t_{1/2}$, $AUC_{(0-24)}$, $AUC_{(0-\infty)}$, and mean residence time (MRT) ($p > 0.05$). The relative bioavailability of the Diclofenac Potassium ODT (LD# 11) was 101.09% relative to the immediate release tablet (Cataflam®) taken as reference product. Though a significant decrease in the time of onset of action; however no significant increase in the relative bioavailability was observed.

INTRODUCTION: Over a decade, the demand for development of orally disintegrating tablets (ODTs) has enormously increased as it has significant impact on the patient compliance. Orally disintegrating tablets offer an advantage for populations who have difficulty in swallowing. It has been reported that Dysphagia (difficulty in swallowing) is common among all age groups and more specific with pediatric, geriatric population along with institutionalized patients and patients with nausea, vomiting, and motion sickness complications [1 & 2].

Orally disintegrating tablets with good taste and flavor increase the acceptability of bitter drugs by various groups of population. As a single unit dosage form that disintegrates in the oral cavity rapidly (usually in few seconds), ODTS enhance compliance and overcome difficulty in swallowing by pediatric and geriatric patients [3].

Recent data from the WHO forecast for Egypt a sharp increase in life expectancy until 2050. Although this is a positive trend; however old ages (geriatrics) poses a great risk for developing chronic diseases accompanied by different pains. The use of fast acting dosage forms with high bioavailability of drugs especially as Non-steroidal anti-inflammatory for pain relieving would be highly favored.

Diclofenac Potassium, the selected NSAID drug, is highly subjected to first pass metabolism which make it our candidate to overcome this problem [4]. Thereafter this could enhance the drug bioavailability with lower adverse effects through pre-gastric absorption from the mouth, pharynx and oesophagus [5]. Diclofenac Potassium is eliminated via metabolic transformation; therefore, an ODT of Diclofenac Potassium that is partially absorbed through the oral mucosa directly enters the systemic circulation may result in an increase in the fraction of drug reaching the systemic circulation. This would result in a rapid onset of action via a more comfortable and convenient delivery route than the intravenous route.

MATERIALS AND METHODS:

Materials: Diclofenac Potassium was supplied by Sinochem, China. Mannitol was supplied by SPI Pharma Inc, USA. Lactose was supplied by Meggle GmbH, Germany. Maltodextrin was supplied by Grain Processing Corp., USA. Gelatin, glycine, sodium chloride and potassium chloride were received from Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt. The water used was distilled de-ionized water. All other chemicals were reagent grade and used as received. Cataflam® 50 mg (Novartis, Egypt) was used as a reference tablet in in-vivo studies.

Preparation of ODTs: A (3^3) Full factorial design for the freeze dried formulae was adopted to determine the effect of filler type, matrix former type and matrix former concentration. Diclofenac Potassium ODTs were prepared using gelatin (hydroxypropylcellulose or xanthan gum) as a matrix former, a sugar alcohol (lactose monohydrate or mannitol or maltodextrin) and glycine as an anti-collapse The matrix former was used in three different concentrations (1, 3 and 5% w/v), while the three sugar alcohols and glycine were used at a concentration of 2% 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 (hydroxypropylcellulose or xanthan gum) was first dissolved in distilled water at about 40°C to obtain the required concentration. Mannitol or (lactose or maltodextrin) and glycine were then added to the gelatin solution in the pre-determined concentration. An accurately weighed amount of Diclofenac Potassium powder was dispersed in the prepared aqueous solution using a magnetic stirrer to result in a dose of 50 mg Diclofenac Potassium per 1 ml. One milliliter of the solution 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 hours. The frozen tablets were placed in a lyophilizer for 24 hours using a Novalyphe-NL500 Freeze Dryer with a condenser temperature of -45°C and a pressure of 7 x 10^{-2} mbar. These formulations were evaluated. 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 (29% relative humidity) at room temperature until further use.

Characterization of ODTs:

1. **Uniformity of Weight:** 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) [6].

2. **Uniformity of Content:** Ten randomly selected tablets from each formula were individually assayed for drug content uniformity. The drug in

ODTs was assayed by dissolving each tablet in 250 ml simulated saliva fluid (pH=6.8). The solution was then filtered, properly diluted, and the absorbance was spectrophotometrically measured at λ_{max} = 282 nm for Diclofenac Potassium. Each individual tablet content must be between 90 -110 percent of the average content and the tablet formulation fails to comply with the test if more than one individual tablet content is outside these limits or if one individual content is outside the limits of 75 -125 percent of the average content.

3. **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, de-dusted and accurately re-weighed. The percentage loss in weight was calculated and taken as a measure of friability [7].

4. ***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 ZT3-3 disintegration tester (Erweka, Germany) [8]. 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).

5. ***In-vivo*** **Disintegration Time:** The *in-vivo* disintegration time of the prepared ODTs was evaluated in four 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 four 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 [9].

6. **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 [10].

7. **Moisture Analysis:** The tablets were analyzed for their residual moisture content after lyophilization using Karl Fischer titrator (Metrohm, Switzerland). Each tablet was pulverized, inserted in the titration vessel containing dried methanol (Karl–Fischer grade) and titrated with Kombititrant reagent (Merck, Germany) after a stirring time of 3 min. Results are presented as mean value ± SD (n = 3).

8. ***In-vitro*** **Dissolution Studies:** The dissolution profiles of Diclofenac Potassium in ODTs compared with Cataflam® tablet were determined in a dissolution tester (Erweka DT-700 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, 5, 7, 10, 15 and 30 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 282 nm after appropriate dilution.

Cumulative amount of drug dissolved in the preparations was calculated using calibration equation. Dissolution tests were performed in six vessels per formulation (n = 6). The market product, Cataflam, was also tested in the same way for comparison purposes using simulated intestinal fluid without enzymes at pH 6.8.

9. **Scanning Electron Microscopic (SEM) analysis:** Surface morphology and cross-sections of selected tablet formulations were examined using Jeol JSM-6400 scanning electron microscope (Tokyo, Japan). Cross-section samples were prepared by cutting a thin slice of the tablet using a scalpel.

In-vivo absorption studies:

1. **Study design:**

A. **Subject Population:** Four healthy male volunteers (ages between 23 and 36 years; mean age, 30 ± 2.2 years) participated in the study. All were within 10% of their ideal body weights (weights, 75 to 93 kg; mean weight 84 ± 10.4 kg and heights, 162 – 183 cm, mean height, 174 ± 5.7 cm). Health status of the volunteers was confirmed by complete medical history, physical examination and laboratory analysis for complete hematological and biochemical examination, all these were carried out at baseline. None of the volunteers had any history of drug or alcohol abuse, nor did they have any acute or chronic gastrointestinal, cardiac, vascular, hepatic or renal disease.

The subjects were instructed to take no drugs for 1 week prior to and during the course of the study; no concurrent medication was allowed during the course of the study. No consumption of nicotine was permitted 12 hours before and 24 hours after drug intake, moreover, on each test day, coffee, tea, and cola beverages were withheld from subjects 12 hours before the administration and till the blood sampling was completed. Each subject read, understood, and signed an informed written consent. All subjects were informed about the risks and objectives of the study.

B. Study Design: The study was performed to compare the pharmacokinetics of Diclofenac Potassium 50 mg ODT formula (LD#11) and the reference, Cataflam® 50 mg (Novartis) using non-blind, two treatments, two periods, randomized cross over design. Under this design half of the subjects were given the immediate release treatment first and the ODT treatment second and the other half were given the treatments in the opposite order. The study was approved by the Research Ethics Committee at Faculty of Pharmacy, Cairo University.

The subjects were instructed to take no medicines for one week prior to and during the course of the study. The subjects were received in the facility at 7 am of the day of study after an overnight fast as instructed before the study. From this time on they remained at the study site under controlled dietary and liquid intake until the end of the study day. No food was allowed for four hours after dosing. The washout was one week. The subjects were under medical supervision during the study and were watched for any adverse events such as gastrointestinal disturbances, nausea, vomiting, diarrhea or allergic reactions.

For the ODT, one tablet equivalent to 100 mg was given to each subject. The ODT was administered orally without water, and each subject was asked to keep the ODT in the mouth for few minutes until completely dissolved in the saliva, then water was allowed after 30 minutes (Treatment A). The immediate release tablet, Cataflam® 50 mg tablet was ingested with 200 ml of water (Treatment B).

C. **Collection of Blood Samples:** Venous blood samples were collected in heparinized glass tubes before administration of the dosage form ay 0, 5, 10, 15, 30, 45 minutes, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after administration of the Diclofenac Potassium ODT and Cataflam tablet. All samples were collected and plasma was immediately separated from the blood cells by centrifugation at 6000 rpm for 10 minutes and stored at -20°C until analysis.

D. Analytical procedure for determination of Diclofenac Potassium in plasma:

a) **Assay Method Description and Chromatographic conditions:** A simple, rapid, specific and reliable HPLC assay of Diclofenac Potassium in human plasma has been developed. Reversed phase chromatography was conducted. The analysis was done on Shimadzu LC Prominence 20 connected with PDA detector; using mixed column ODS/Cyano; ACE, (100 x 4.6 mm, 5 µm). The mobile phase was isocratic consisted of Methanol: 50 mM potassium dihydrogen phosphate buffer, in ratio of (50 : 50 v/v) and was delivered to the system at a flow rate of 1.5 ml/min, with an injection volume of 20 µl and the detection wavelength (λ_{max}) was 282 nm. Diazepam was used as internal standard. All assays were performed at ambient conditions.

b) **Preparation of stock and working standard solution:** Stock solution of Diazepam (internal standard) solution was prepared by dissolving 50 mg of Diazepam in 100 ml methanol; then sonication for 5 minutes (500 µg/ml). The working internal standard was prepared on each day of analysis by diluting the stock solution to contain (1.5 µg/ml).

Stock solution of Diclofenac Potassium was prepared by dissolving 50 mg Diclofenac Potassium in 100 ml methanol; then sonication for 5 minutes (500 µg/ml). The working solution was prepared on each day of analysis by diluting the stock solution with methanol to give serial dilutions containing 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 µg/ml of Diclofenac Potassium; which were shaken well and filtered over 0.45 µm syringe filter and injected onto HPLC.

c) **Plasma sample preparation for determination of Diclofenac Potassium:** The extraction procedure was applied in the preparation of plasma samples and standards, where 1 ml of each human plasma was transferred into 15 ml tube fitted with polyethylene cap. 1 ml of internal standard working solution and 1 ml methanol were added. The mixture was vortexed for 2 minutes and centrifuged at 6000 rpm for 30 minutes. The upper layer was transferred to another tube and filtered through 0.45 µm syringe filter. A 20 µl volume of the supernatant was injected onto the HPLC column. Concentrations of Diclofenac Potassium in unknown samples were calculated with reference to the prepared calibration curve. Retention time of Diclofenac Potassium was 6.6 minutes.

d) **Preparation of *in-vivo* standard calibration curve:** For calibration curve, plasma standards were prepared by spiking 1 ml of blank plasma with 1 ml of the internal standard working solution and appropriate volumes of Diclofenac Potassium working solution to produce concentrations ranging from (0.25, 0.5, 1, 2, 3, 4 and 5 µg/ml). The spiked plasma standards were processes as described above. The calibration curve was obtained by plotting chromatographic peak area ratios (Diclofenac Potassium/Diazepam) against the corresponding nominal Diclofenac Potassium concentration added. Samples were prepared and injected on the same day.

e) **Sample calculation:** The unknown sample concentration was calculated from the following formula: $C = [(R + Y) / S]$*; w*here C is Diclofenac Potassium concentration, R is the peak area ratio (drug/internal standard), S is the slope of the calibration curve and Y is the Y-intercept.

f) **Pharmacokinetics Calculations:** Pharmacokinetic parameters from plasma data following administration of the two treatments were estimated for each subject by using a computer program, WinNonlin® software (version 1.5, Scientific consulting, Inc., NC). The plasma concentration–time data were evaluated, and the following pharmacokinetic parameters were calculated:

C_{max} (µg/ml): it was determined as the highest observed Diclofenac Potassium concentration during the 12 hours study.

T_{max} (hours); it was taken as the time at which C_{max} occurred.

AUC_{0-12} (µg.hr/ml)***;*** was determined as the area under the plasma concentration-time curve up to the last measured time point calculated by the trapezoidal rule.

$AUC_{0-\infty}$ (µg.hr/ml): it was determined as the area under the plasma concentration-time curve up to the last measured time point calculated by the trapezoidal rule plus the residual area calculated as the concentration of the last measured time point divided by the elimination rate constant. Where $AUC_{0-\infty} = AUC_{0-12} + C_t/k$; and C_t is the last measured concentration at the time t, and k is the terminal elimination rate constant estimated by log-linear regression analysis on data visually assessed to be a terminal log-linear phase.

$t_{1/2}$ is apparent terminal half life and was calculated as ***$t_{1/2}$ =*** 0.693/k plasma half life.

MRT is the mean residence time and was calculated AUMC/AUC

f_{rel} is the relative bioavailability and was calculated as (AUC_{ODT} / AUC_{IR}) X 100.

g) **Statistical analysis of the Pharmacokinetic parameters:** Statistical evaluation of C_{max}, t_{max}, $t_{1/2,}$ AUC_{0-12}, and $AUC_{0-\infty}$ data by one way ANOVA statistical test using SPSS® 11.0 software (SPSS Inc., Chicago).

RESULTS AND DISCUSSION:

1. **Characterization of Diclofenac Potassium ODTs:** All the prepared tablets showed acceptable weight variation with relative standard deviation ranged from 1 - 5% for the different formulations and the mean percent of Diclofenac Potassium content in ODTs was found to be more than 90% for all formulations. All tablets showed residual moisture content of no more than 3%, indicating that the lyophilization process was efficient in removing water from the tablets. Friability studies showed that tablets formulated with 3% and 5% matrix former showed acceptable percentage weight loss, within the acceptable range for tablets (less than 2%) except (LD#22) containing mannitol and 5% hydroxypropylcellulose. On the contrary, all the formulations containing 3% hydroxypropyl cellulose were friable.

On the other hand, tablets formulated with 1% matrix former were friable except those containing 1% gelatin. The decreased mechanical properties of ODTs formulated with 1% matrix former could be attributed to the fewer number of crosslinks formed between the matrix former strands as the concentration decreases. It has been reported that increasing the matrix former concentration usually results in a more extensive and rigid 3D network after freeze-drying due to increase in the number of matrix fibers forming crosslinks and interchain H-bonds, thereby resulting in an increase in the overall hardness of the tablets [11].

The mean drug content, percent friability, wetting time, *in-vitro* disintegration time and *in-vivo* disintegration time for the prepared ODTs are listed in **table 1**. It was also observed that tablets formulated with mannitol showed lower weight losses compared to tablets formulated with lactose and maltodextrin. This is in accordance with previously reported results in which mannitol as filler produces a stiff homogenous cake that improves the appearance and the hardness of the lyophilized product [12]. *In-vitro* disintegration studies showed that ODTs containing maltodextrin showed longer disintegration times compared to ODTs containing mannitol and lactose. This may be attributed to the matrix forming effect of maltodextrin that synergies the binding property of the matrix former, so hindering the rate of disintegration [13, 14, & 15].

ODTs prepared using 5% matrix former showed statistically significantly longer disintegration times compared to ODTs prepared using 3% and 1% matrix former. These results indicate that increasing the matrix former concentration in the tablets results in the formation of more cohesive and stable gels that are less likely to break up or dissolve easily in water. These results are further confirmed by wetting time experiments in which tablets containing higher matrix former concentration shows significantly higher wetting times compared to tablets containing of lower matrix former concentration. Short wetting time is indicative of the highly porous nature of the tablet matrix.

In-vitro disintegration results are also in accordance with friability results in which harder tablets showed longer disintegration times. Results show that *in-vivo* disintegration times were shorter when compared to corresponding *in-vitro* disintegration times for all formulations. This may be probably because of the gentle movement of the tablet in the mouth and hence gentle mechanical stress on the tablet. This is in accordance with the results obtained by Ciper and Bodmeier in a study on the preparation of a fast disintegrating capsule for administration in the oral cavity [16].

TABLE 1: CHARACTERIZATION OF THE 3^3 FULL FACTORIAL DESIGN DICLOFENAC POTASSIUM FREEZE DRIED FORMULAE

Formula	X_1	X_2	X_3	Friability (%)	Wetting time (Seconds) *	*In vitro*-Disintegration time (Sec) *	*In vivo*-Disintegration time (Sec) *	Residual moisture (%) ***	Drug content (%) **
LD #1	-1	-1	-1	0.9%	20 ± 1.34	15 ± 0.71	10 ± 0.68	2.43 ± 0.41	103.82% ± 2.16
LD #2	0	-1	-1	1.21%	13 ± 0.24	10 ± 0.68	7 ± 0.81	1.94 ± 1.34	93.55% ± 1.23
LD #3	+1	-1	-1	0.72%	10 ± 0.78	5 ± 0.34	5 ± 0.73	2.16 ± 0.94	92.76% ± 1.56
LD #4	-1	0	-1	100%	15 ± 0.47	10 ± 0.89	6 ± 1.28	1.43 ± 1.35	94.67% ± 1.64
LD #5	0	0	-1	100%	11 ± 1.23	7 ± 0.63	5 ± 1.27	1.84 ± 0.79	91.28% ± 1.72
LD #6	+1	0	-1	100%	8 ± 1.34	5 ± 0.69	5 ± 1.39	2.54 ± 1.69	106.1% ± 1.83
LD #7	-1	+1	-1	10.51%	44 ± 0.24	40 ± 0.74	35 ± 0.64	1.76 ± 0.84	93.99% ± 2.17
LD #8	0	+1	-1	10.23%	38 ± 0.78	35 ± 1.44	30 ± 0.62	1.68 ± 0.69	95.49% ± 2.35
LD #9	+1	+1	-1	7.23%	37 ± 0.47	30 ± 0.92	24 ± 0.68	1.34 ± 1.83	97.92% ± 1.43
LD #10	-1	-1	0	Zero	37 ± 1.28	30 ± 0.67	23 ± 0.41	1.84 ± 0.69	98.82% ± 1.59
LD #11	0	-1	0	Zero	28 ± 1.27	23 ± 0.94	18 ± 0.73	1.68 ± 1.56	93.98% ± 2.38
LD #12	+1	-1	0	Zero	21 ± 1.39	14 ± 0.52	10 ± 1.28	2.34 ± 1.87	100.15% ± 2.23
LD #13	-1	0	0	100%	19 ± 0.64	15 ± 0.49	10 ± 0.27	1.54 ± 1.69	92.57% ± 2.24
LD #14	0	0	0	100%	14 ± 0.62	10 ± 0.68	7 ± 0.39	1.13 ± 1.35	93.19% ± 1.31
LD #15	+1	0	0	100%	11 ± 0.68	7 ± 0.63	5 ± 0.64	1.26 ± 1.48	101.31% ± 1.38
LD #16	-1	+1	0	Zero	420 ± 0.41	400 ± 0.69	390 ± 0.62	1.96 ± 0.48	102.67% ± 2.12
LD #17	0	+1	0	Zero	410 ± 1.28	390 ± 1.73	374 ± 0.61	1.68 ± 1.56	92.67% ± 2.12
LD #18	+1	+1	0	Zero	383 ± 1.27	375 ± 0.68	360 ± 0.68	2.94 ± 0.87	106.21% ± 1.38
LD #19	-1	-1	+1	Zero	114 ± 1.39	103 ± 0.93	93 ± 0.41	1.49 ± 1.69	102.04% ± 1.82
LD #20	0	-1	+1	Zero	93 ± 0.64	82 ± 0.68	74 ± 0.73	1.22 ± 1.35	93.54% ± 1.83
LD #21	+1	-1	+1	Zero	88 ± 0.62	80 ± 0.49	71 ± 1.28	0.98 ± 1.56	94.97% ± 1.51
LD #22	-1	0	+1	1.43%	324 ± 0.68	300 ± 0.63	288 ± 0.27	1.54 ± 0.87	91.98% ± 1.68
LD #23	0	0	+1	Zero	293 ± 0.41	270 ± 0.68	253 ± 0.39	1.94 ± 1.69	93.98% ± 1.75
LD #24	+1	0	+1	Zero	276 ± 1.28	260 ± 0.67	244 ± 0.64	1.69 ± 0.48	101.13% ± 1.34
LD #25	-1	+1	+1	Zero	721 ± 7.76	700 ± 8.91	769 ± 7.16	0.89 ± 0.56	92.56% ± 2.98
LD #26	0	+1	+1	Zero	745 ± 8.33	705 ± 7.98	700 ± 9.67	1.33 ± 0.87	94.88% ± 2.98
LD #27	+1	+1	+1	Zero	749 ± 9.34	710 ± 9.79	694 ± 11.8	0.69 ± 1.69	102.44% ± 2.98

* Data are mean values (n = 6) ± S.D. ** Data are mean values (n = 10) ± S.D. *** Data are mean values (n = 3) ± S.D

Independent variables	Levels		
	Low	**Medium**	**High**
X_1 = Filler Type	Mannitol	Lactose	Maltodextrin
X_2 = Matrix former type	Gelatin	Hydroxypropylcellulose	Xanthan gum
X_3 = Matrix former conc.	1	3	5
Transformed values	**-1**	**0**	**+1**

2. ***In-vitro*** **Dissolution Studies:** The cumulative Diclofenac Potassium dissolved as a function of time from ODTs compared to the market product Cataflam® is illustrated in **table 2**. Remarkable differences in the shape of the dissolution profiles of the prepared ODTs and the commercial tablet were observed. The percentages of drug dissolved from the best freeze dried ODT formula (LD#11) was 100.28%, compared to only 2.08% for Cataflam, after 1 minute. These results indicate that the process used to prepare the ODTs greatly enhanced the extent and rate of dissolution of Diclofenac Potassium from the prepared tablets (LD#11).

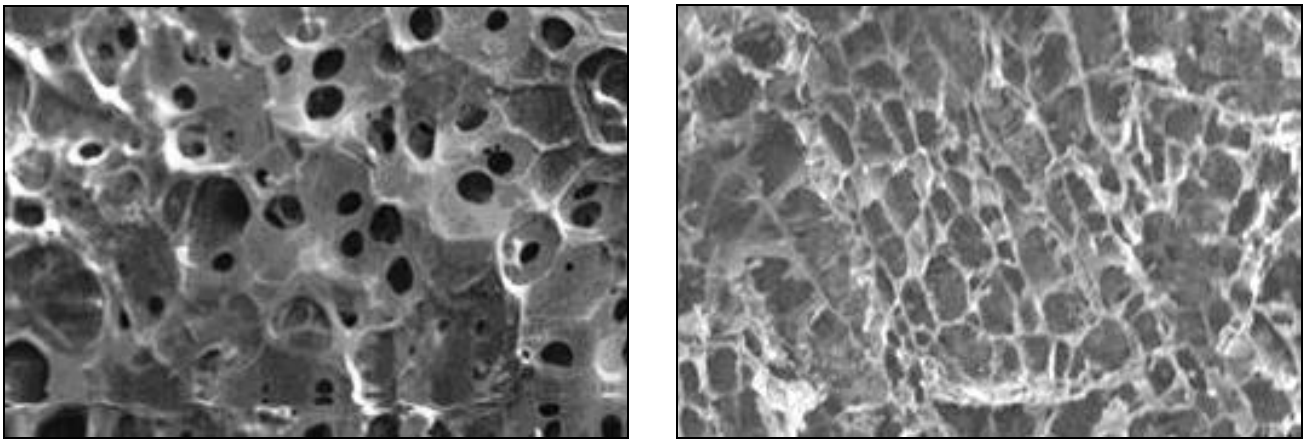
ODTs containing 3% gelatin and lactose showed faster dissolution rates when compared to the other ODTs formulations. These results correlate well with disintegration and wetting time testing results, where increasing the gelatin concentration resulted in longer disintegration and wetting times. Also (LD#11) tablet formulation containing lactose showed faster drug release than the corresponding formulations containing mannitol and maltodextrin. The percentage of drug dissolved from all ODT formulations was almost 100% after 15 min compared to only 44.05% for Cataflam tablets.

TABLE 2: PERCENTAGE OF DICLOFENAC POTASSIUM DISSOLVED FOR THE FREEZE DRIED ODTS FORMULAE

Time	Percentage Diclofenac Potassium dissolved													
minute	Cataflam	LA #1	LA #2	LA #3	LA #4	LA #5	LA #6	LA #7	LA #8	LA #9	LA #10	LA #11	LA #12	LA #13
1	2.08	82.98	100.01	58.78	16.42	22.24	23.45	10.84	12.34	12.22	80.08	100.28	34.95	52.00
2	6.98	93.78	100.02	78.22	22.56	33.11	34.27	16.54	18.67	17.94	90.17	100.27	52.04	75.32
5	13.24	100.01	100.03	99.96	34.18	42.66	43.06	25.21	27.89	26.21	100.01	100.42	68.36	93.96
7	17.98	100.03	100.02	100.00	39.89	49.89	48.00	32.24	34.84	35.43	100.01	100.20	76.68	100.05
10	25.06	100.07	100.01	100.00	47.74	52.14	51.49	40.98	44.78	46.98	100.00	100.27	91.18	100.00
15	44.05	100.02	100.03	100.02	56.88	67.11	66.91	51.92	56.22	55.75	100.02	100.23	100.40	100.03
30	76.92	100.08	100.07	100.00	81.93	87.18	86.78	70.88	74.28	73.23	100.02	100.33	101.29	100.00

Time	Percentage Diclofenac Potassium dissolved													
minute	LA #14	LA #15	LA #16	LA #17	LA #18	LA #19	LA #20	LA #21	LA #22	LA #23	LA #24	LA #25	LA #26	LA #27
1	54.98	36.19	1.39	2.66	3.02	76.75	80.45	50.11	12.18	18.175	6.75	0	0	0
2	77.32	58.32	2.55	4.04	4.45	91.34	92.22	72.44	14.76	28.13	14.76	0	0	0
5	99.96	96.96	3.11	5.81	5.23	95.63	97.54	95.46	31.13	50.98	48.85	1.12	1.09	0.98
7	100.05	100.97	4.88	6.54	6.33	98.92	100.01	100.01	38.08	58.86	57.00	3.21	2.78	1.70
10	100.00	100.70	6.92	7.58	7.00	100.03	100.06	100.00	49.45	79.45	80.07	5.31	3.01	2.31
15	100.03	101.07	7.71	8.71	8.71	100.03	100.04	100.02	68.86	91.22	100.06	6.22	5.55	3.95
30	100.00	100.53	10.01	12.68	10.12	100.02	100.04	100.03	101.18	101.18	100.03	7.33	6.12	5.80

3. **Scanning Electron Microscopic (SEM) Analysis:** Scanning electron micrographs of the surface and cross-section views of ODTs (LD#11) is shown in **figure 1**. The micrographs show the highly porous nature of the prepared lyophilized tablets, which appears in both the surface and the inner structure. The highly porous nature of the tablets explains the rapid penetration of water, which results in rapid wetting, disintegration, and dissolution in the oral cavity. The micrographs show that ODT (LD#11) contains larger and more diffuse pores (especially from the cross-section view) which might explain the very fast *in-vitro* and *in-vivo* disintegration as well as short wetting time.



SURFACE VIEW **CROSS-SECTION**

FIGURE 1: SEM GRAPH OF DICLOFENAC POTASSIUM FREEZE DRIED FORMULA (LD#11)

4. **Assessment of Pharmacokinetic Parameters:** The study was completed by the four volunteers who were included in the pharmacokinetic analysis. The volunteers tolerated very well the two treatments and did not complain of any adverse effects during the course of the study. No signs of GI disturbances or allergic reactions were observed from any of the volunteers during the study

5. ***In-vivo* absorption studies:** The plasma mean concentrations of Diclofenac Potassium following administration of an oral dose (50 mg) of the selected ODT formula (LD#11) and Cataflam® tablet to four healthy volunteers are shown in **tables 9 and 10 and figures 7 and 8**; respectively. **Figure 9** shows the comparative mean plasma concentration of Diclofenac Potassium following administration of an oral dose (50 mg) of Cataflam® IR tablets and ODT (LD#11) to four healthy volunteers.

a. **Peak plasma concentration (C_{max}):** The peak plasma concentration (C_{max}) of Diclofenac Potassium following the administration of Cataflam® tablets has a mean of 2.1545 ± 0.0435 µg/ml ranged from 2.11 – 2.214 µg/ml. The peak plasma concentration (C_{max}) of Diclofenac Potassium following the administration of selected ODT formula (LD#11) has a mean of 2.5 ± 0.11 µg/ml ranged from 2.401 – 2.642 µg/ml as shown in **tables 11 and 12**. ANOVA showed significant differences (ρ = 0.001) as shown in **table 13**.

b. **Time of peak plasma concentration (T_{max}): Tables 11 and 12** showed that the mean time taken to peak plasma concentration for *(T_{max})* following administration of Cataflam® tablets was 1.25 ± 0.00 hours, while it was 0.25 ± 0.00 hour following administration of selected ODT formula (LD#11). T_{max} is significantly different (ρ < 0.05).

c. **Area under the curve (AUC_{0-12}) and ($AUC_{0-\infty}$):** The area under the plasma concentration curve AUC_{0-12} and $AUC_{0-\infty}$ of Diclofenac Potassium following the administration of Cataflam® tablets have means of 207.88 ± 0.4 µg.hr/ml (ranged from 7.285 – 8.18 µg.hr/ml) and 8.962 ± 0.265 µg.hr/ml (ranged from 8.6615 – 9.292 µg.hr/ml), respectively. On the other hand, the area under the plasma concentration curve AUC_{0-12} and $AUC_{0-\infty}$ of Diclofenac Potassium following the administration of selected ODTs formula (LD#11) have means of 8.344 ± 0.352 µg.hr/ml (ranged from 8.0891 – 8.8395 µg.hr/ml) and 9.060 ± 0.606 µg.hr/ml (ranged from 8.4683 – 9.7317 µg.hr/ml), respectively as shown in table (11 and 12). ANOVA showed a significant difference between the two formulae Cataflam® and ODT (LD#11) (ρ = 0.777) as shown in table 14.

d. **Elimination half life ($t_{1/2}$):** The elimination half life ($t_{1/2}$) of Diclofenac Potassium following the administration of Cataflam® tablets has a mean of 5.32 ± 1.83 hours ranged from 3.12 – 7.58 hours, where the elimination half life ($t_{1/2}$) of Diclofenac Potassium following the administration of selected ODT formula (LD#11) has a mean of 4.81 ± 0.57 hours ranged from 4.00 – 5.33 hours as shown in table (11 and 12). The elimination half life of the ODT was determined to be statistically not different relative to the mean half-life estimate following administration of IR tablets (p = 0.612) as shown in **table 15**.

e. **Mean Residence time (MRT):** The mean residence (MRT) of Diclofenac Potassium following the administration of Cataflam® tablets has a mean of 3.865 ± 0.153 hours ranged from 3.667– 4.04 hours, where the mean residence (MRT) of Diclofenac Potassium following the administration of selected ODT formula (LD#11) has a mean of 3.921 ± 0.12 hours ranged from 3.831 – 4.09 hours as shown in table 11 and 12. Statistical comparison of MRT parameter indicated insignificant difference (p = 0.581) as shown in **table 16**.

According to the mean plasma levels of the four subjects completing the study, the relative bioavailability (f_{rel}) of the test formula was found to be 101.09% based on the mean ($AUC_{0-\infty}$) compared to that of the reference standard product**.** Diclofenac Potassium was detected in plasma as soon as the 5 minutes sampling time in the four subjects following the administration of the ODT indicating very rapid absorption by this route of administration.

On the contrary, Diclofenac Potassium did not appear in plasma until the 15 minutes sampling time in the four subjects following administration of the IR tablet.

Based on these results, it can be concluded that the high rate and same extent of bioavailability obtained from the ODT formula may be attributed to rapid absorption and faster elimination of Diclofenac Potassium from the buccal mucosa. Because of the nature of the study design and the small number of subjects in this study, the results can only be considered preliminary and further studies with a larger number of subjects under different conditions such as varying conditions of ingesting ODTs with water and food intake should be conducted.

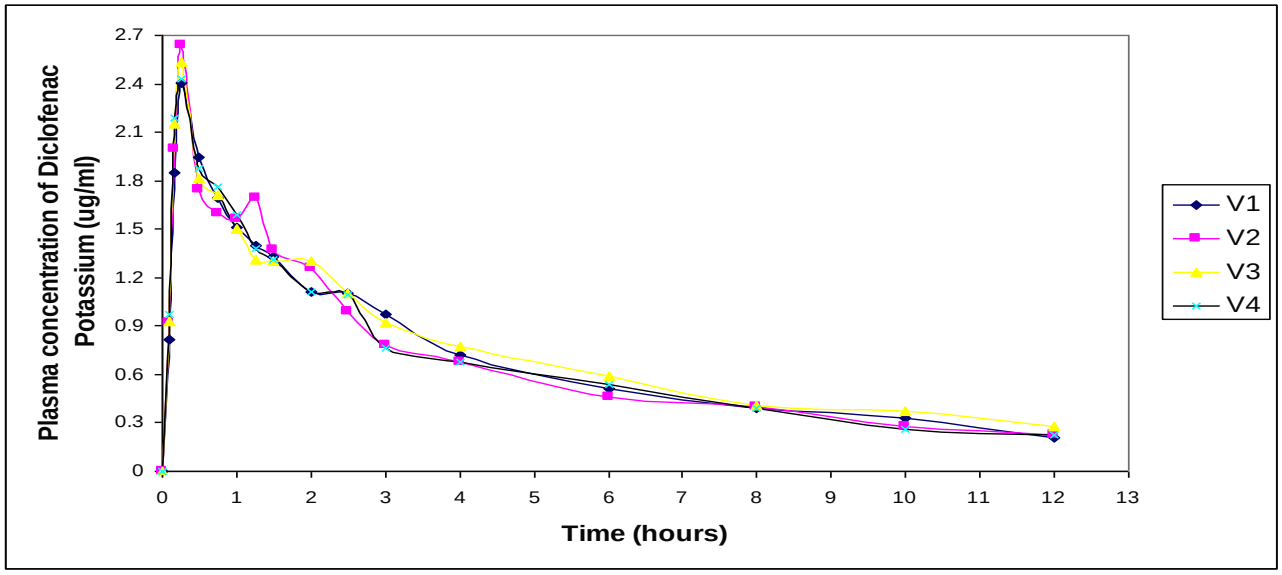


FIGURE 2: INDIVIDUAL PLASMA CONCENTRATION OF DICLOFENAC POTASSIUM FOLLOWING ADMINISTRATION OF AN ORAL DOSE (50 MG) OF THE SELECTED ODT FORMULA (LD#11) TO FOUR HEALTHY VOLUNTEERS.

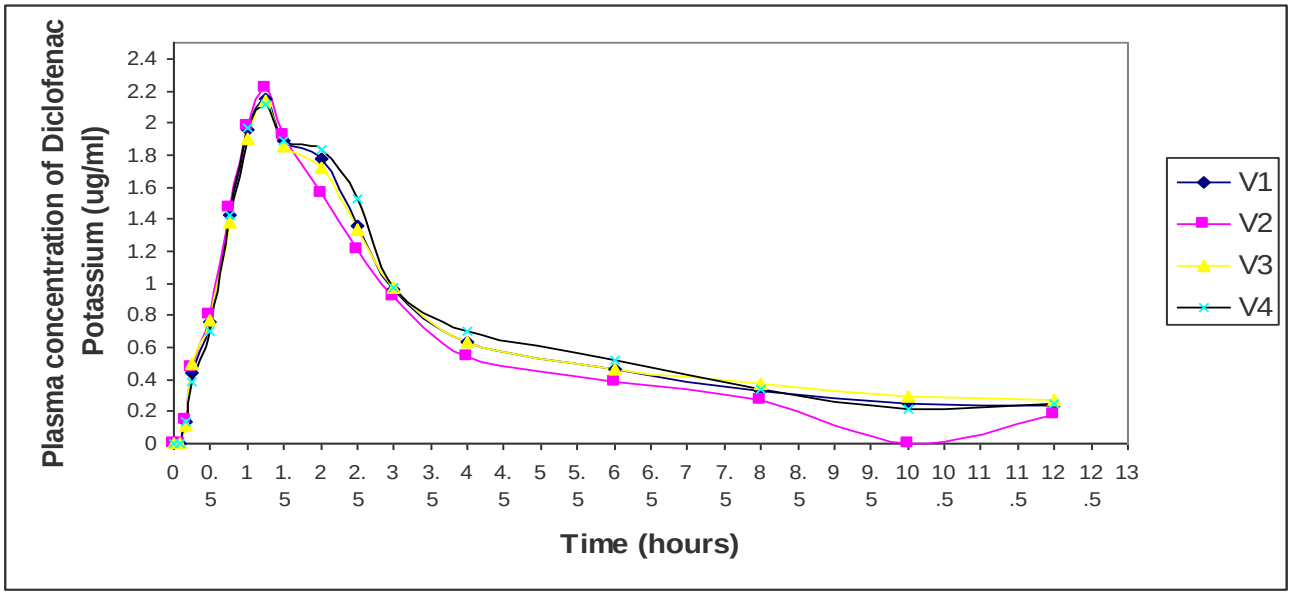


FIGURE 3: INDIVIDUAL PLASMA CONCENTRATION OF DICLOFENAC POTASSIUM FOLLOWING ADMINISTRATION OF AN ORAL DOSE (50 MG) OF THE SELECTED MARKET PRODUCT, CATAFLAM®, TO FOUR HEALTHY VOLUNTEERS

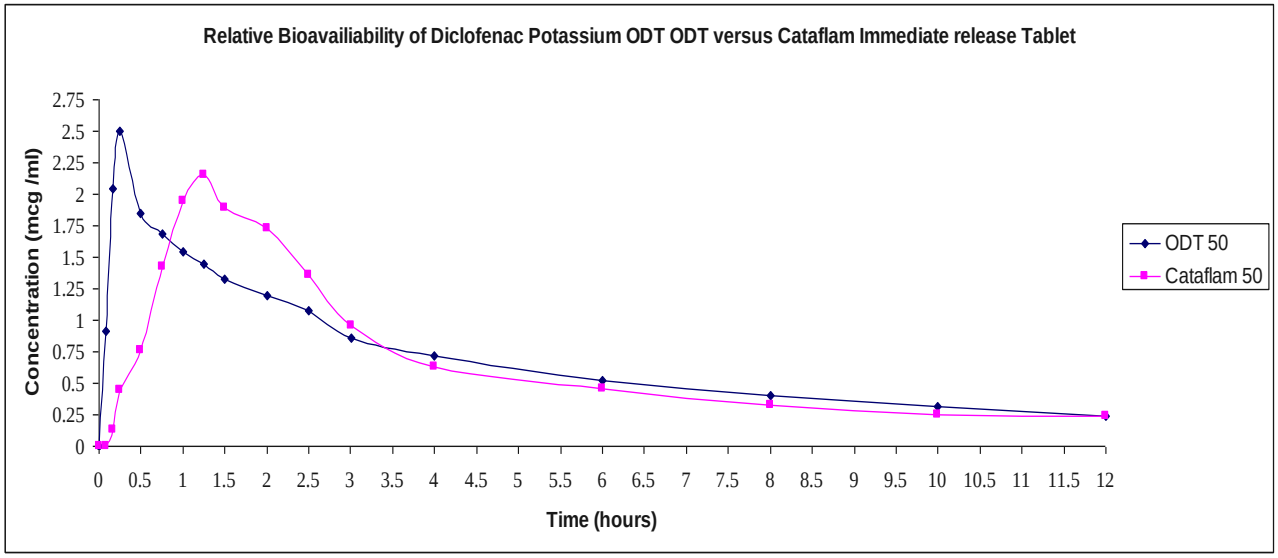


FIGURE 4: MEAN PLASMA CONCENTRATION OF DICLOFENAC POTASSIUM FOLLOWING ADMINISTRATION OF AN ORAL DOSE (50 MG) OF CATAFLAM® IR TABLETS AND ODT (LD#11) TO FOUR HEALTHY VOLUNTEERS.

TABLE 3: SUMMARY OF DICLOFENAC POTASSIUM ODT 50 MG TABLET *IN-VIVO* INTERPRETATION

	V1	V2	V3	V4	Mean	SD
T_{max}	0.25	0.25	0.25	0.25	0.25	0
C_{max}	2.40	2.64	2.53	2.43	2.50	0.109
AUC_{0-24}	8.35	8.095	8.84	8.09	8.34	0.352
$AUC_{0-\infty}$	8.47	8.634	9.41	9.73	9.06	0.606
$t_{1/2}$	4.00	5.06	5.33	4.85	4.81	0.575
MRT	3.93	3.83	4.09	3.84	3.92	0.119

TABLE 4: SUMMARY OF CATAFLAM 50 MG TABLET *IN-VIVO* INTERPRETATION

	V1	V2	V3	V4	Mean	SD
T_{max}	1.25	1.25	1.25	1.25	1.25	0
C_{max}	2.15	2.214	2.14	2.11	2.15	0.044
AUC_{0-24}	7.97	7.285	8.07	8.18	7.87	0.404
$AUC_{0-\infty}$	8.87	8.66	9.02	9.29	8.96	0.265
$t_{1/2}$	5.43	5.158	7.58	3.12	5.32	1.826
MRT	3.88	3.667	4.04	3.86	3.86	0.152

TABLE 5: THE MEAN PHARMACOKINETIC PARAMETERS OF DICLOFENAC POTASSIUM AFTER ADMINISTRATION OF 50 MG ODT (LD#11) AND IMMEDIATE RELEASE (IR) TABLET TO FOUR VOLUNTEERS

Parameter	ODT (LA# 10)	IR tablet	Statistical test
C_{max} (µg/ml)	2.5 ± 0.11	2.15 ± 0.044	p = 0.001
T_{max} (h)	0.25 ± 0.00	1.25 ± 0.00	p = 0.00
$AUC_{(0-24)}$ (µg*h/ml)	8.344 ± 0.352	7.88 ± 0.4	p = 0.777
$AUC_{(0-\infty)}$ (µg*h/ml)	9.061 ± 0.61	8.963 ± 0.265	p = 0.777
$T_{1/2}$ (h)	4.811 ± 0.57	5.32 ± 1.83	p = 0.612
MRT (h)	3.92 ± 0.12	3.86 ± 0.153	p = 0.581
Relative Bioavailability (f_{rel}) = 101.09%			

Data are mean value ± S.D

CONCLUSIONS: We demonstrated that an orally rapidly disintegrating tablet of is a promising formulation resulting in more rapidly dissolved Diclofenac Potassium and more effectively absorbed into the blood stream with significantly faster onset of action when compared to standard immediate release oral dosage form. The study suggests that ODT (LD#11) formulation developed in this work may be an alternative to conventional formulations of Diclofenac Potassium.

REFERENCES:

1. Lindgren S. and Janzon L. Dysphagia: Prevalence of swallowing complaints and clinical finding. Med Clin North Am, 1993; 77: 3 - 5.
2. Sastry S.V., and Nyshadham J.R., Fix JA. Recent technological advances in oral drug delivery: A review. Pharm Sci Technol Today, 2000; 3: 138 - 45. Pubmed.
3. Danckwerts M.P.: "Intraoral Drug Delivery: A Comparative Review." American Journal of Drug Delivery, 2003; 1(3): 171-186.
4. Hinz B., *et al.* Bioavailability of Diclofenac potassium at low doses. *Br J Clin Pharmacol* 2005; 59: 80 - 4.
5. Chaudhari P., Chaudhari S. and Kolhe S.: Formulation and evaluation of fast dissolving tablets of famotidine. *Indian Drug,* 2005; 42: 641 - 649.
6. British Pharmacopoeia 2012, Appendix XII G; Uniformity of Weight; Ph. Eur. method (2.9.5).
7. British Pharmacopoeia 2012, Appendix XVII G; Friability of Uncoated Tablets; Ph. Eur. method (2.9.7).
8. British Pharmacopoeia 2012, Appendix XII A; Disintegration Test for Tablets and Capsules; Ph. Eur. method (2.9.1).
9. FDA, Guidance of Industry: Food-effect Bioavailability and Bioequivalence Studies, 2002.
10. Sherimeier S. and Schmidt P.C.: Fast dispersible ibuprofen tablets, Eur. J. Pharm. Sci., 2002; 15: 295 - 305.
11. Djagny K.B., Wang Z., and Xu S. Gelatin: A valuable protein for food and pharmaceutical industries, Crit. Rev. Food Sci. Nutr., 2001; 41: 481 - 492.
12. Stella V.J., Umprayn K, and Waugh W.N.: " Development of parentral formulations of experimental cytotoxic agents I: rhizoxin (NSC – 332598)." *Int. J. Pharm.,* 1988; 43: 191 - 199.
13. Corveleyn, S. and Remon, J.P.: Maltodextrins as lyoprotectants in the lyophilisation of a model protein, LDH. Pharm. Res., 1996; 13: 146–150.
14. Muyldermans G. and Vanhoegaerden R.: Gelatin-maltodextrin intercations and synergies application in 25% low fat spreads. Gums and stabilizers for the food industry, 1991; 429 - 439.
15. Corveleyn S. and Remon J.P.: International Journal of Pharmaceutics, 1997; 152, 215 - 225.
16. Ciper M. and Bodmeier R. "Preparation and characterization of novel fast disintegrating capsules (Fastcaps) for administration in the oral cavity" *Int. J. Pharm.,* 2005; 303, 62 - 71.

How to cite this article:
Degwy MAAEAAED, Tayel S, El-Nabarawi MA and Randa El Rehem TA: *In vitro* and *in vivo* evaluation of Diclofenac potassium Lyophilized Orally Disintegrating Tablets. *Int J Pharm Sci Res*. 2013; 4(1); 248-259.