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Formulation and Evaluation of Oxcarbazepine Suspension: *In vitro/In vivo* Correlation

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Authors' contributions

This work was carried out in collaboration between all authors. Author APR designed the study and wrote the protocol. Author HV run experiments and performed the statistical analysis, wrote the first draft of the manuscript. Both managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Oxcarbazepine was formulated as suspension in order to perk up the palatability, augment bioavailability of the product, furthermore extended to estimate good degree of in vitro in vivo correlation (IVIVC).
Study Design: Formulation and Evaluation of Oxcarbazepine Suspension: In Vitro/In Vivo Correlation
Place and Duration of Study: Birla Institute of Technological Sciences- Hyderabad, India between July 2011 to January 2012
Methodology: Oxcarbazepine had poor aqueous solubility, thus the solubility was increased by hydrotropes then formulated into suspension using taragum as viscosity enhancer. Further validity of dissolution study was extended by In vitro/In vivo correlation using level A method.
Results: These suspensions were observed for in vitro dissolution profile and studied for in vivo pharmacokinetic profile, from the obtained values, a level A IVIVC modeling was observed, interestingly from the results attained suspension showed almost 98% drug release (FDR) and 85% drug absorbed (FDA) in 90min. Fascinatingly, from the correlation between FDR and FDA, slope and regression co-efficient obtained was near to 1.0

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indicated good linearity.
Conclusion: In conclusion, a point-to-point link from the level A which was a keystone of acceptable and reliable correlation was achieved.

Keywords: Oxcarbazepine; suspension; dissolution study; pharmacokinetics; IVIVC.

1. INTRODUCTION

Oxcarbazepine is 10,11-dihydro-10-oxo-5*H*-dibenz(b,f)azepine-5-carboxamide derivative of carbamazepine with low (0.08g/L) aqueous solubility [1] and 1.31 partition co-efficient. It belongs to iminostilbene category of antiepileptics and act on convulsions by post tetanic potentiation of synaptic transmission, also act on neuropathy by sodium channel blockade and calcium channel blockade mechanism and act on bipolar disorder by decreasing abnormal electrical activity in brain. It is absorbed from Gastro Intestinal region and 40% of the drug is bound to plasma proteins. It is mainly metabolized by glucuronic acid conjugation and as excreted by the kidney. A pharmaceutical suspension is a coarse dispersion in which internal phase (insoluble solid particle) is dispersed uniformly throughout the external phase (aqueous/ oily liquid). Development of suspension is generally to augment the solubility of Active Pharmaceutical Ingredient (API), for better organoleptic properties and to make certain the higher bioavailability of drug. The use of taragum as a viscosity enhancer [2] which has an acceptable daily intake (ADI) of 13g/kg and is GRAS listed [3]. The aspiration in observing pharmacokinetics is to quantitatively account for the amount which has entered the body from the instant of administration until it has been completely cleared [4]. Generally pharmacokinetic parameters are not measured directly but are determined experimentally from a set of dependent (concentration) and independent (time) variables. In vitro/ In vivo Correlation (IVIVC) can be established from four different levels of correlation [5]. Among all the correlations, level A was most suggested for accuracy on poorly water soluble drugs [6, 7]. In the current study, the possibility of developing a level A correlation between percent drug released and percent drug absorbed for oxcarbazepine suspension was investigated.

2. MATERIALS AND METHODS

Oxcarbazepine active pharmaceutical ingredient (OXC-API) and Taragum were obtained as a gift sample from Novartis, Mumbai, India and Exandal corp, UK resp. Urea was purchased from Finar Chemicals Ltd., Ahmedabad, India, Sodium citrate, sodium saccharin, sodium benzoate and Ascorbic acid were purchased from Fischer Scientific, UK. Sodium alginate was purchased from FMC biopolymer, USA. Remaining all other solvents used in HPLC was procured from Loba Chemie Ltd., Mumbai, India.

2.1 Formulation of Oxcarbazepine Suspension

Firstly oxcarbazepine solubility was enhanced by formulating into solid dispersion [8] using hydrotropic agents such as urea, sodium acetate and trisodium citrate. Later all ingredients were weighed and passed through 40. At first taragum and sodium alginate were dissolved in water before 24h. Later sodium citrate and sodium benzoate were added and dissolved and then drug blend was added to the solution. Ascorbic acid, sodium saccharin and fruit flavor were added to the prepared suspension which further entire solution was passed through colloidal mill.

2.2 Evaluation of Suspension

2.2.1 Appearance

Appearance of suspension was observed physically.

2.2.2 Determination of viscosity

Brookfield Viscometer (Spindle LV model) was used to measure viscosity. 1mL of fluid was taken in cup and bob was allowed to completely immerse in the cup. Viscometer was switched on, run it till indicator shifted from red light to green light. Viscometer was allowed to run in both ascending and descending modes (10-100rpm) at 5min interval and 1min data collection interval time.

2.2.3 Determination of sedimentation volume

20mL of the solution was taken in 50mL glass measuring cylinder. The suspension was dispersed thoroughly by moving upside down for three times and volume of sedimentation was noted (V_0). Later suspension was allowed to settle for 24h and volume was read (V_u) and calculated using $F=V_u/V_o$.

2.2.4 Assessment of re-dispersibility

Suspension was allowed to settle in measuring cylinder. Mouth of the cylinder was closed and was inverted through 180° and number of inversions required for restoration was noted. If uniformity attained in one inversion, then it has 100% redispersibility. Every additional inversion decreases the percentage of ease of redispersibility by 5%.

2.2.5 Estimation of drug content

1mL of solution was dissolved in 10mL of methanol. Further it was diluted with 0.75% SLS for OXC determination. If any supernatant appears, then filtered using Whatmann filter paper no.1 then observed for absorbance.

2.2.6. Dissolution study

Dissolution test was carried out using USP type II (paddle) apparatus for 1h. The stirring was kept at 25rpm, 0.75% SLS and 0.1N HCl as a dissolution medium (900mL) for OXC and temperature was maintained at 37 ± 1ºC. 5mL of samples were collected at regular time intervals of 0, 5, 10, 15, 20, 30, 45 and 60min and were assayed spectrophotometrically at 256nm for OXC with a triplicate number of experiments was performed.

2.2.7 Stability of suspension

Electrokinetic properties of suspension from Zeta potential is measured by diluting the suspension with distilled water. The zeta potential was measured in triplicates in multinodal mode using the Malvern zetasizer inspection system.

2.3 *In vivo* Study

2.3.1 Experimental design

Albino rats (National Institute of Nutrition, Hyderabad, India) of either sex, weighing 180-210g were selected [9]. Animals were maintained under standard laboratory conditions. The experimental protocol has been approved by Institutional Animal Ethical Care Committee (IAEC) of BITS-PILANI, Hyderabad (No: IAEC/RES/06/03) and animals were maintained as per CPCSEA guidelines. Human dose was extrapolated to animal dose using USFDA dose calculator [10]. In the study design for pharmacokinetics assessment a number of six Wistar rats were selected for drug administration with three animals for each formulation.

2.3.2 Assessment of pharmacokinetic data and data analysis

All the animals in every group were administered drug with 1ml of polyethylene glycol (vehicle). Blood was collected from the retro-orbital sinus after anesthetizing animal with anesthetic ether. 0.1mL of 2.8% sodium citrate was used as an anticoagulant. Blood samples were taken at regular time intervals from 0h till 12h following drug administration. Plasma was separated by centrifugation and stored at -20ºC until further analysis. Plasma OXC (oxcarbazepine) and MHD (active metabolite of oxcarbazepine) concentration [11] was determined using a validated High Performance Liquid Chromatography (HPLC) method with minor modifications. Briefly, HPLC system consisted of Waters auto sampler (Water Co., Massachusetts), a Waters 2691 separation module pump, Waters 2487 dual lambda UV detector operated at 210nm. The stationary phase was Waters symmetry C18 reverse-phase column (150mm*4.6mm, 5µm). Mobile phase used was 5mM of pH 7 buffer/ acetonitrile (55:45) at a flow rate of 0.8mL/min. The pharmacokinetic parameters such as AUC_{0-12}, $AUC_{0-\infty}$, C_p and K_E were evaluated along with the amount drug absorbed, calculated through wagner-Nelson model using Try kinetica PK-PD version 5.0 program.

2.4 *In vitro In vivo* Correlation

Out of four modeling methods, level A is mostly used and the typical mathematical process of developing a level A IVIVC [12] involves assessment of cumulative percent drug released from in vitro dissolution studies then obtained Area under Curve using the trapezoidal rule. Application of the deconvolution of in-vivo plasma profile by a model independent method such as the Wagner-Nelson method to estimate the In vivo percent drug absorbed from the cumulative area under curve, followed by comparison if In Vivo fraction of drug absorbed on Y axis to In Vitro fraction of drug dissolved on X axis. Further linear correlation between FDR and FDA were established for pooled mean data of formulations from $Y=aX+b_0$ where Y is FDA; X is FDR; a and b_0 are regression parameters. For the model r was determined where r=1 indicates a linear relationship with good In vitro/ In vivo correlation.

3. RESULTS AND DISCUSSION

The drug blend of solid dispersions consists of 150mg of OXC-API and 500mg of mixture of urea, sodium citrate and sodium acetate thus has enhanced solubility of up to 0.5mg/mL was observed. For further formulation of suspension, these solid dispersions were used and given in Table 1. 4mL of suspension was made for the single dose formulation with 150mg of the drug.

Table 1. Formulation table of oxcarbazepine suspension

Formulation ingredients (mg)	F1	F2	F3	F4	F5
OXC blend	750	750	750	750	750
Tara gum	20	20	40	40	27
Sodium alginate	20	40	20	40	40
Sodium citrate	20	20	20	20	40
Sodium benzoate	5	5	5	5	5
Ascorbic acid	5	5	5	5	5
Sodium saccharin	180	160	160	140	132
Flavor	qs	qs	qs	qs	qs
Distilled waster (mL)	4	4	4	4	4

All the prepared formulations were elegant in appearance. All the evaluation parameters were as shown in Table 2.

Table 2. Evaluation responses of suspensions

Parameters	F1	F2	F3	F4	F5
Appearance	Light pink color	Light pink color	Light pink color	Light pink color	Light pink color
Viscosity (cP)	100	106	190	181	237
Sedimentation volume (F)	0.07	0.66	0.26	0.76	0.91
Redispersibility	90	85	90	95	95
Drug content (%)	99.8	98.7	99.9	98.1	99

As acceptable range is F=1 or 0.9 for 1h, only F5 showed 0.91. The initial volume (V_o) was 20mL for the suspension, the sedimentation volume after 24h was taken as Vu which gives sedimentation volume of 0.91. The sedimentation volume (F) of F5 was 0.94 as sedimentation volume after 24h due to higher concentration of Sodium citrate and viscosity enhancers, the particles flocculate easily. However viscosity of all formulations was found out to be 100-240cP. Higher viscosity is due to the presence of high concentration of viscosity enhancer. The drug content for all the formulations was between 98-100%. From viscosity studies, the formulated suspension showed non-Newtonian flow type of behavior. Dissolution studies are one of the most widely used techniques in the characterization of drugs and in quality control of drug dosage forms [13]. The dissolution study as of oxcarbazepine suspension was as shown in Table 3 with excellent release profiles.

Table 3. Dissolution data of oxcarbazepine suspension

Time (min)	F1	F2	F3	F4	F5
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
5	29.85 ± 0.12	49.61 ± 0.32	22.83 ± 0.83	36.88 ± 0.17	48.73 ± 0.61
10	50.65 ± 0.25	64.37 ± 0.51	40.08 ± 0.31	55.52 ± 0.62	66.56 ± 0.28
15	67.89 ± 0.19	78.50 ± 0.87	63.44 ± 0.52	70.99 ± 0.82	78.94 ± 0.85
20	80.28 ± 0.52	88.68 ± 0.91	82.89 ± 0.19	89.52 ± 0.63	85.61 ± 0.52
30	87.81 ± 0.89	90.93 ± 0.12	91.34 ± 0.33	92.25 ± 0.45	92.11 ± 0.43
45	90.92 ± 0.67	93.14 ± 0.27	94.46 ± 0.68	93.14 ± 0.51	97.96 ± 0.14
60	96.65 ± 0.55	96.66 ± 0.83	97.55 ± 0.46	96.66 ± 0.43	98.06 ±0.65

F represents different formulations; Values are expressed as mean ± SD, n=3

All the formulations showed almost 80% of drug release in first 30min and nearly 96% of drug release in 60min. Commencing dissolution studies, all the formulations showed similar drug release profiles and the best formulation of F5 was chosen based on its flow properties with good sedimentation volume, re-dispersibility. Viscosity behavior (Fig. 1) at different rates of speed, viscosity decreases with the rate of shear indicating the shear thinning flow behavior of the fluid.

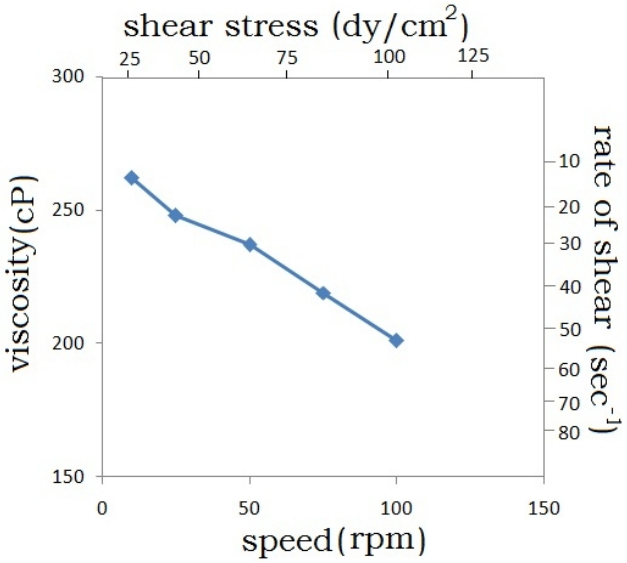

Fig. 1. Influence of rotational speed, shear stress and rate of shear on viscosity of optimized formulation

In a non-Newtonian system, one point determination is virtually useless in characterizing the flow properties of the system. Thus multipoint instrument that operates at a variety of rates of shear was opted where tackiness, slip and spreadability are easy to measure. Zeta potential of F5 from Fig. 2 estimates the stability of systems containing dispersed particles, this was considered to be the flocculated suspension as the zeta potential of particle is -28.9. Particles with higher zeta potential show high stability of colloidal systems, as the obtained zeta potential was within the range +30 to -30mV we can ensure that the suspension has good stability.

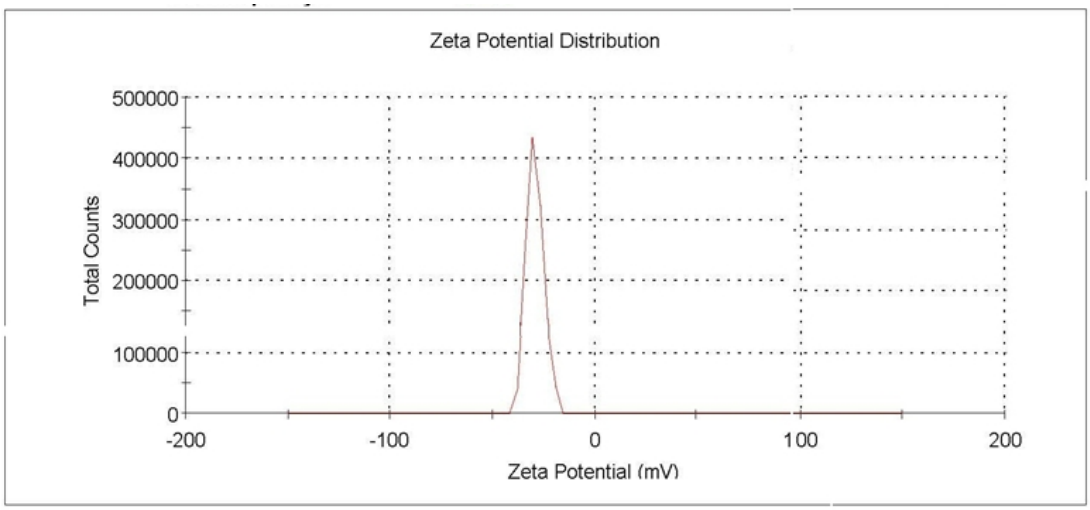

Fig. 2. Zeta potential of oxcarbazepine suspension

Few electrolytes, sodium alginate a macromolecule electrolyte and sodium citrate a strong electrolyte (organic deflocculant) act as deflocculating agents. At positive zeta potential, reducing the electric barrier between the particles, as evidenced by a decrease in the zeta potential and the formation of a bridge between adjacent particles so as to link them together in a loosely arranged structure thus maximum flocculation occurs and will persist until the zeta potential has become sufficiently negative for deflocculation. The study was conducted till 12h and observed for plasma drug concentration. The maximum concentration reached was about 20.7µg/mL at 4h as shown in Table 4.

Table 4. Plasma concentration profile of oxcarbazepine suspension

Time (h)	Plasma MHD concentration (µg/mL)
0	0.0 ± 0.00
0.25	15.7 ± 0.25
0.5	16.5 ± 0.26
1	17.3 ± 0.15
1.5	18.6 ± 0.25
2	19.6 ± 0.1
3	20.17 ± 0.2
4	20.7 ± 0.2
6	19.7 ± 0.1
8	18.7 ± 0.15
10	17.6 ± 0.17
12	15.9 ± 0.2

Values are expressed as mean ± SEM, n=3

The pharmacokinetic parameters obtained were maximum plasma concentration 19.7µg/mL, 20.7µg/mL for OXC alone and OXC suspension resp. AUC_{0-t} were 209 h* µg/mL and 222 h* µg/mL for OXC alone and OXC suspension resp. where are basic pharmacokinetic parameters such as K_E had no significant alteration such as 0.0339h-1 and 0.034 h-1 for OXC alone and OXC suspension resp. The percent drug absorption *in-vivo* depends on two

important factors, solubility and intestinal permeability as given by Guidance for Industry, FDA [14]. Level A provides a linear correlation with percent drug released from dissolution studies to percent drug absorbed from animal plasma studies. The percent drug released *vs* percent drug absorbed was studied for 90min where 98% of the drug was released from in vitro dissolution studies to 85% of drug absorbed from in vivo studies under level A correlation to obtain point-to-point correlation between dissolution studies and drug absorption studies was successful in achieving the favored target of good correlation as given in Table 5 and Fig. 3.

Table 5. Data representing *in vitro in vivo* Correlation for oxcarbazepine suspension

Time (h)	Percent drug released	Percent drug absorbed
0.25	78.94	65
0.5	92	77
1.0	98	83
1.5	98.12	85
Level A *IVIVC* modeling		
Slope	0.993	
Intercept	13.64	
R	0.989	

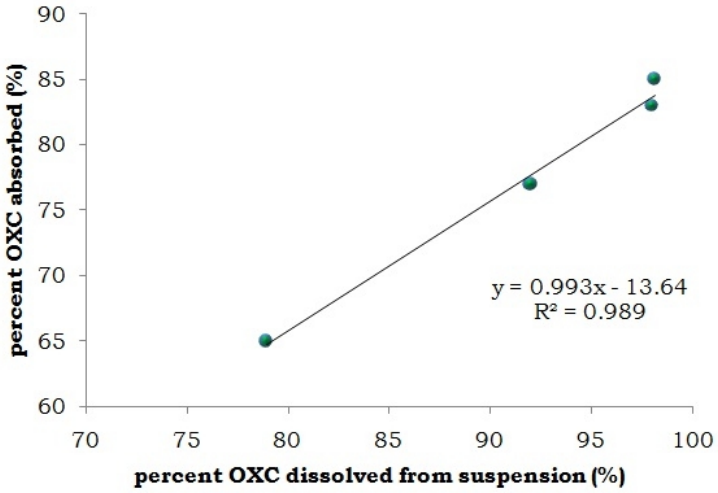

Fig. 3. Level an *In vitro-In vivo* modeling of oxcarbazepine suspension

Thus the dissolution test may serve as proxy for bioequivalence studies [15] and an appropriate dissolution condition based on in vivo performance could be adapted for routine and in-process quality control of oxcarbazepine suspension [16]. It was interesting, therefore, to explore if the condition of dissolution of this study, which is very akin to what is proposed by thFDA, correlates with serum plasma profiles already obtained by performing *In-vivo* studies for immediate release dosage forms [17]. The level A modeling showed very good linear correlation between percent drug dissolved and percent drug absorbed for oxcarbazepine suspension. 98% Percent drug dissolved was used designed for the study along with 85% of drug absorbed from plasma studies. Amazingly, slope and regression co-efficient obtained for the correlation was nearer to 1.0 indicating good correlation from level

A modeling. A point-to-point link from level A which is a keystone of an acceptable and reliable correlation was achieved.

4. CONCLUSION

In conclusion, a point-to-point link from level A which is a keystone of an acceptable and reliable correlation was achieved. From the results of the current study, *IVIVC* developed by level A correlation makes oxcarbazepine dissolution profile meaningful, as it allows predicting in-process quality control of oxcarbazepine suspension.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the BITS ethics committee.

All authors hereby declare that all experiments have been examined and approved by the BITS ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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