Novel Bioactive Molecules

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CHAPTER 1

CNS DRUGS USING NANOTECHNOLOGY: A KINETIC STUDY

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Abstract : The development of BBB targeting technologies is a very active field of research and development. One goal is to develop chemically modified derivatives of CNS drug (Alrazolam) chemically modified nanoparticulate of drugs, capable of crossing biological barriers, in particular the BBB. Nanocarrier drug delivery involves targeting CNS drugs enclosed in a particular PVP- 44000 polymer. Drug nanoparticles have been shown to increase bioavailability and enhance drug exposure for oral and parenteral dosage forms. Nanoparticles of clonazepam were prepared by nanoperception method. PVP-44000 greatly enhanced the solubility of poorly water soluble drug. i.e. alprazolam. Drug release fellow zero order kinetics with fickinian diffusion.

(Keywords: Bioavailability, Alprazolam (ALZ), Drug delivery, Polymeric surfactants, Release kinetics)

Introduction

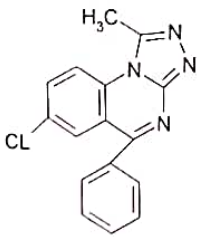
Alprazolam belongs to the class of benzodiazepine with anxiolytic, muscle relaxant, an anticonvulsant property which is generally used as a hypnotic and as a tranquilizer[1-3]. It is most frequently prescribed in the therapy of anxiety as being relatively safe with mild side effects. It has no appreciable solubility in water at physiological pH. It is rapidly and completely absorbed after oral administration, with peak levels in plasma occurring within 1-2 h after oral administration[4]. The predominant metabolites in human plasma are α'-hydroxyalprazolam, 4-hydroxyalprazolam and α'-benzophenone. The pharmacological activity of α'- hydroxyalprazolam and 4-hydroxyalprazolam is about 60% and 20% less than that of ALZ, respectively, and the benzophenone is essentially inactive[5-6]. ALZ was found to be highly photolabile and special care should be taken to avoid light exposure during its storage and handling. Drug photo stability constitutes an important current subject of investigation because the photodegradation process can result in the loss of potency of the drug and also in adverse effects due to the formation

of minor toxic degradation products[7-8]. A large number of analytical and pharmacological techniques for the determination of some benzodiazepines and their metabolites have been reported[9-12] especially in biological fluid and pharmaceutical formulations[13]

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years; however it can occur at any time[14-20]. PVP-44000 was chosen as the carrier polymer for the preparation of drug-polymer composite particles. PVP-44000 is a water-soluble synthetic polymer included in inactive ingredients list and widely used in pharmaceutical applications. It has been used as a binder, dispersion aid, film plasticizer, crystal growth retarder, anti-irritant, stabilizer for enzyme and heat sensitive drugs, antitoxic agent, solubility enhancer, viscosity modifier and carrier in controlled release applications for preparing drug-polymer composite particles[21-28].

The most important objectives to prepare composite particles are protection and stabilization of the active substance, increasing the bioavailability of poorly soluble molecules by improving solubility and design of controlled release formulations of the drugs with high water solubility to reduce adverse side effects and improve therapeutic efficacy[29-38]. Most controlled drug delivery systems focus on the production of drug-loaded polymeric particles by incorporation or encapsulation of drug within a polymer. There are several techniques to produce drug-loaded polymeric particles such as emulsion/solvent evaporation, phase separation, spray-drying, freeze-drying and interfacial polymerization. Excessive solvent use and disposal, thermal and chemical degradation of products, trace residues, inter-batch particle size variability and multistage processing are the main drawbacks of these methods.

Structural formula



IUPAC Name - 8-Chloro-1-methyl-6- phenyl-4H-S-triazol [4, 3- α] [1, 4] benzodiazepine.

Molecular Formula - $C_{17}H_{13}ClN_4$

Molecular Weight - 308.76

Protein binding- 80% primarily to Ibumin.

Bioavailability - 90%

Materials and Methods

Methanol (Lab-Scan 99.8%) was used as the solvent to dissolve ALZ and mixed CTAB - PEG400, Diazepam pure drug was obtain from INTAS Pharm. LTD. Ahmadabad (Gujarat) as gift sample with 99.99% w/w assay value and was used without further purification. PEG400-CTAB was kindly supplied by BDH. Methanol (Merck, 99.9), NaH2 P04 (Merck, min. 99) and Mill Q water were used for HPLC analysis. HCl (Merck, 37) and deionizer water were used for dissolution tests.

Determination of λ_{max} using solvent [Methanol]

The pure form of diazepam was accurately weighed 10mg and dissolved in 100 mL of methanol (stock solution 100 µg/mL). The stock solution was further diluted 1.0 mL in 100 mL aqueous methanol to give a concentration of 1 µg/mL. The absorption spectra were obtained with Elico 164 UV-Visible double beam spectrophotometer a scan range of 200-400 nm and determine the maximum absorbance of drug at λ_{max} 222 nm.

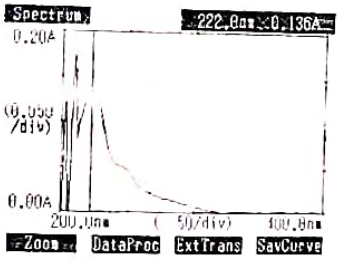


Fig. 1: Determination of λ_{max} ALZ

Solubility measurements:

The solubility of ALZ in different fluids such as distilled water, CTAB, PEG-400, mixed CTAB + PEG 400, PEG- 4000, PVP- 44000, mixed PEG4000 + PVP 44000, (at their CMC

values) was determined by placing 50 mg of ALZ in 15 mL of each fluids. The mixtures were shaken (37°C ± 0.5°C) on a magnetic stirrer. 5.0 mL aliquot was withdrawn at 10 min. interval and filter immediately using a 0.22 µm syringe filter. The saturation solubility measurements were assayed through ultraviolet absorbance show table-1. The pure form of ALZ was accurately weighed 10mg and dissolved in 100 mL of medium (PVP-44000 CMC 9×10^{-5} M). Stock solution 100 µg/mL. The maximum absorbance of drug find at λ_{max} 222nm.

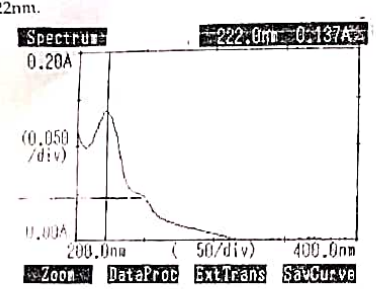


Fig. 2: Determination of λ_{max} using medium

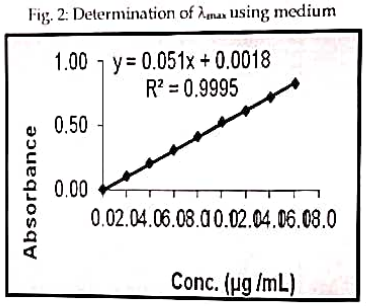


Fig 3: Determination of calibration curve by using medium.

<u>In *Vitro* dissolution study:</u>

1. Apparatus: Electrolab TDT - 08L USP apparatus.
2. Dissolution Media: PVP-44000 at cmc (9×10^{-5} M)
3. Rotation speed: 100 rpm.

4. **Preparation of ALZ standard solution:** 10 mg ALZ standard was weighed precisely, put in 100 mL volumetric flask and made up to the mark with dissolution media.

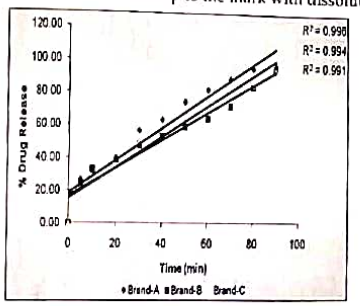


Fig. 4: Plot for zero order

5. **Test preparation:** Dissolution testing was performed on tablets containing 10 mg ALZ in 9×10^{-5} M PVP-44000 (37°C ± 0.5°C) using paddle method (USP apparatus- II) at 100 rpm. Sample of 5 mL were withdrawn at regular time intervals, replaced by fresh medium and spectro-photometrically analyzed at λ_{max} 222 nm after filtration through 0.45µm syringe filter. All dissolution tests were performed in triplicate.

6. **Time point:** Dissolution amount was measured separately at 05, 10, 20, 30, 40, 50, 60, 70, 80 and 90 minutes.

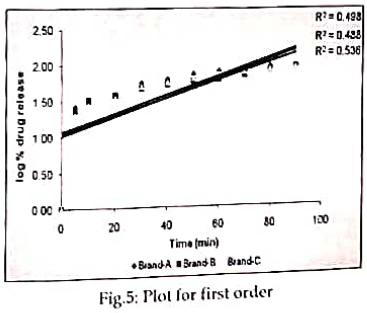


Fig.5: Plot for first order

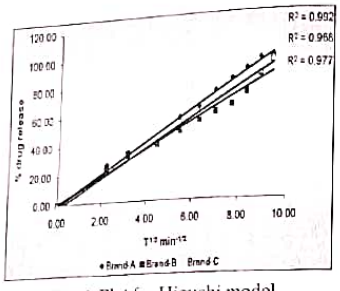


Fig. 6: Plot for Higuchi model

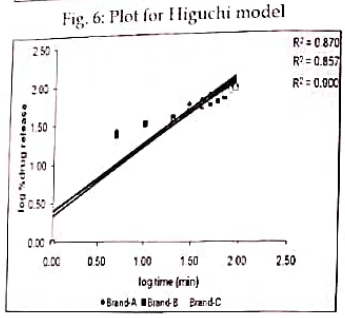


Fig. 7: Plot for Korsmeyer model

Preparation of Nanopraticles Method:-

The polymer is dissolved in a water miscible organic solvent (or solvent mixture) and added to an aqueous solution, in which the organic solvent diffuses. Particle formation is spontaneous, because the polymer precipitates in the aqueous environment. According to the current opinion, the Marangoni effect is considered to explain the process, solvent flow, diffusion and surface tensions at the interface of the organic solvent and the aqueous phase cause turbulences, which form small droplets containing the polymer.

Subsequently, as the solvent diffuses out from the droplets, the polymer precipitates.

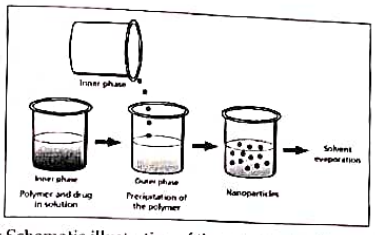


Fig. 8: Schematic illustration of the nanoprecipitation process

ALZ and PVP-44000were co precipitated to prepare drug-polymer composite particles. The effects of process parameters such as pressure, temperature, solution concentration. Drug-polymer solution injection rate and polymer/drug ratio on the particle of ALZ - PVP-44000 composite micro particles. Generally, very light, voluminous and fluffy powders as typical of the nano-precipitated samples were obtained in all experiments.

Results and Discussion

The morphologies of the samples are generally spherical or slightly agglomerated spheres.

It is interesting that when ALZ and Mixed PVP-44000 were precipitated together, spherical particles of smaller sizes were obtained while larger and plate-like particles were obtained when ALZ or PVP-44000 was precipitated alone. The precipitation mechanism and rates could be different in co precipitation experiments compared to precipitation of original substances alone. For the precipitation ALZ alone, this may be caused by inhibition effect of polymer on drug nuclei. When ALZ was precipitated alone the growth mechanism of nuclei may be more dominant causing formation of large and irregular-shaped particles. As known, polymer generally inhibits particle growth of some substances by blocking surface of particles resulting in increased surface area and smaller particle size. Thus, precipitation of the drug with polymer may weaken the growth of drug nuclei resulting in reduced particle sizes and different morphologies compared to ALZ precipitation alone.

When PVP-44000 is precipitated alone the precipitation rate may be slower. The precipitation rate is related to the solubility of the materials. Solution concentration, super saturation concentration and rate. PVP-44000is more soluble in methanol than ALZ (the maximum solubility of ALZ in methanol is about 80 mg/ml, for PVP-44000it is more than 500 mg/ml). So when PVP-44000 is precipitated alone, the time to reach super saturation would be longer, which indicates lower super saturation, nucleation and precipitation rates. This would also lower mass transfer and solvent removal rate, and

make reorganization of the polymer chains during precipitation more difficult preventing formation of discrete particles and causing agglomeration, and even formation of films or fibers. In co precipitation experiments, precipitation rate of PVP-44000 may be higher because of its interaction with ALZ causing formation of spherical particles instead of large and irregular-shaped particles.

Drug Loading Analysis

Drug content of particles was determined by HPLC. MeOH+0.2 mol/l NaH_2PO_4 (55:45, v/v) was used as mobile phase. Mobile phase through the degasser (Agilent 1100 G 1379 A), pump (Waters 600) and column (Kromasil 100-5C18. 250 mm×4.6mm i.d., 5 µm particle size, Hichrom) at injection rates of 0.7-0.8ml/min. Samples were injected in to the column via Rheodyne injector (7725i, 20 µl loop).

The drug loading of the particles was expressed as the ratio of the mass of loaded drug to the mass of sample:

$$\% \text{ drug loadin} = \frac{\text{mass of loaded drug}}{\text{Total mass of the particle}} \times 100$$

XRD Analysis

XRD analyses were performed at' ambient temperature with diffraction angles from 10° to 75° with CuKα radiation at operating parameters of 40 mA and 45 kV. Phase identification of sample was performed using' the International ICDD (Centre for Diffraction Data) data base which is available in Expert High Score Plus analysis in Pan Analytical XRD equipment.

FT-IR analysis

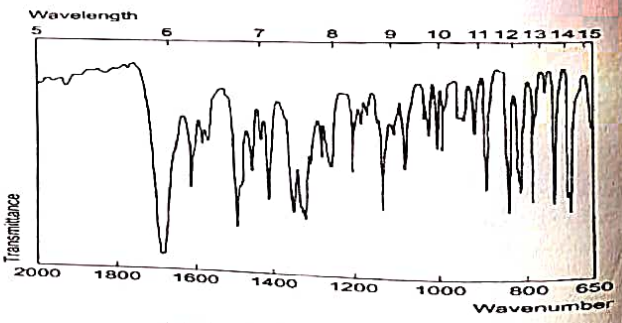


Fig.9: Reference IR spectrum of ALZ

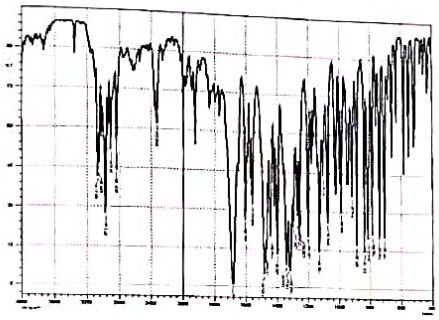


Fig.10: IR spectrum of pure ALZ

DSC Analysis- The thermal analyses were performed with a DSC (Perkin Elmer DSC-4000). Thermo grams were measured by heating the sample from 35 to 200°C at a rate of 10°C/min.

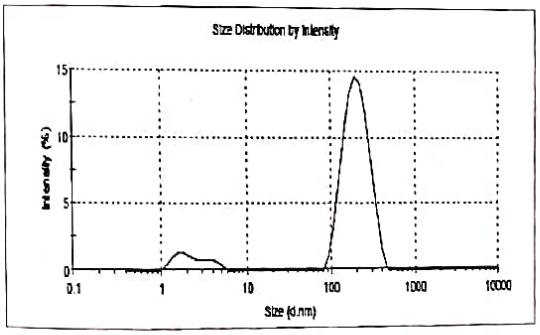


Fig 11: Size distribution of Alprazolam

Particle Size Analyses

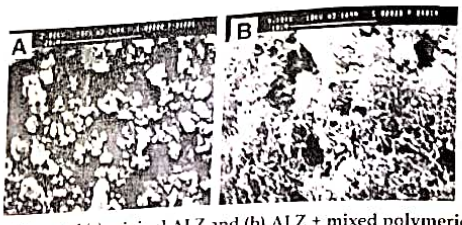


Fig: -12- SEM image of (a) original ALZ and (b) ALZ + mixed polymeric surfactant

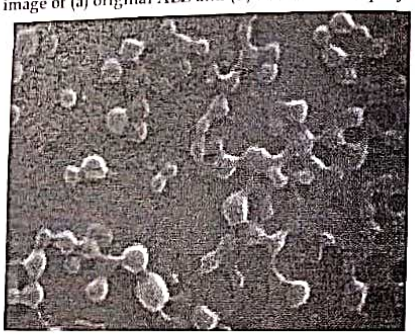


Fig- 13- SEM Image of Alprazolam

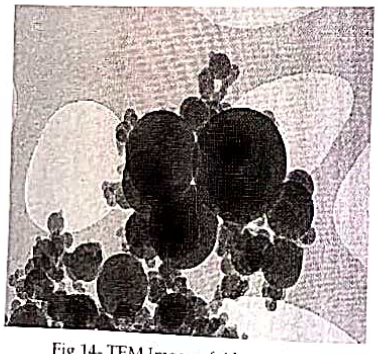


Fig 14- TEM Image of Alprazolam

A dissolution study of dosage forms necessitates modifications in the dissolution medium to increase the solubility. ALZ is a lipophilic compound and is practically insoluble in water. ALZ is weak base, and it is expected that ALZ would dissolve much more completely and rapidly in a solution with low pH. However, the addition of surfactant is a reasonable approach, which if implemented correctly, can approximate the gastrointestinal fluid condition.

In order to compare the effects of various kinds of surfactants on the dissolution profiles of ALZ with limited water solubility, solubility study was conducted using media containing cationic, anionic or nonionic surfactant at their CMC's values and water. Polymeric surfactant PVP-44000 greatly enhances the solubility of ALZ than anionic and cationic surfactant. On the basis of solubility data, dissolution media was selected and the results indicate that extent of dissolution of ALZ is significantly dependents on the polymeric surfactant used. The values of release exponent (n), kinetic constant (k) calculated from different kinetics models i.e., zero- order, first- order, Higuchi model and Korsmeyer- Peppas model are presented in tables -2. As observed from the table, correlation coefficient (r^2) of all formulation was high enough to evaluate the drug dissolution behavior using equations. In most of the cases it was revealed that the release kinetics of ALZ from the tablet appeared to follow release kinetics of Zero order ($r^2 > 0.991$-0.996) as well as Higuchi release kinetics ($r^2 > 0.968$-0.992) but zero order release kinetics predominates.

The Korsmeyer-Peppas model is used to analyze drug release from pharmaceutical dosage forms when the release mechanism is not well known or when more than one type of release phenomena is involved. The exponent, termed the release exponent *n*, was studied by Peppas and coworkers to characterize different drug release mechanisms from thin films. for the cylindrical tablets, $0.45 \leq N$ corresponds to fickian diffusion mechanism $0.45 < 0.89$ to non-fickian transport, and $n > 0.89$ to case II (relaxational) transport and $n > 0.89$ to super case II transport. To Portion of the release curve, where $mt/M\infty < 0.6$ should, be used. In the current study, the value of release rate exponent (n), ranged between 0.39 – 0.40. For this case of follow fickian diffusion.

The result for scanning microscope 200 nm.

Table: **1 - Solubility study of ALZ in different fluids**

S. No.	Sample (each fluid at their CMC values)	Wt. of drug in mg	Overall volume in mL	Abs. at λ_{max}- 285 nm	Solubility increase in fold
1.	ALZ + Distilled Water	50	15	0.564	6.13
2.	ALZ + CTAB	50	15	0.682	7.41

3.	ALZ + PEG-400	50	15	0.470	5.10
4.	ALZ+CTAB+ PEG 400	50	15	0.522	5.67
5.	ALZ + PEG-4000	50	15	0.290	3.23
6.	ALZ + PVP-44000	50	15	1.112	12.08
7.	ALZ + PEG 4000 + PVP 44000	50	15	0.092	1

Table 2: Regression (R^2) value of kinetic models

S. No	Bra-nds	Zero order	First order	Higuchi model	Korsm- eyar Peppas model	'n'exponen t
1.	A	0.996	0.498	0.992	0.870	0.40
2.	B	0.994	0.488	0.968	0.857	0.39
3.	C	0.991	0.536	0.977	0.900	0.39

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Published & Printed By :

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Web: www.nityapublications.com
Email : info@nityapublications.com
Mob. : +91-9009291840

MRP Rs.

ISBN 978-93-94894-48-8