Pharmacokinetics and *In Vitro* and *In Vivo* Correlation of NN-dimethylaminocurcumin (NNDMAC) Loaded Polycaprolactone Microspheres in Rats

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**Abstract**

**N**-**N**-**d**i**m**-e**t**h**y**l-**a**m**i**n**o**-**c**u**r**c**u**m**i**n** (NNDMAC), a novel curcumin analogue, has demonstrated significant hepatoprotective activity after oral administration. The objective of this investigation was to determine the pharmacokinetics of NNDMAC after the administration of its microsphere formulation. Additionally, it was aimed to determine the *in vitro in vivo* correlation (IVIVC) with the microsphere formulation. NNDMAC biodegradable microspheres were prepared using solvent evaporation technique by taking polycaprolactone as the polymer. A suitable release study based on the volume of distribution of NNDMAC was selected. *In vitro* release of the drug was determined. For *in vivo* studies, the microsphere formulation was injected by IP route. Pharmacokinetic properties of microsphere-encapsulated NNDMAC were determined and a comparison with i.v. solution form of NNDMAC was made. Pharmacokinetic analysis was performed using KINETICA and non-compartmental parameters were determined. Concentrations of the drug in plasma were determined by HPLC. IVIVC was established according to Drewe and Guitard (degree A). *In vivo* drug release into the systemic circulation was determined using Wagner-Nelson method. Results indicated that, when NNDMAC formulations were administered by IP route, mean residence time (MRT) and the area under the curve (AUC) were significantly higher (P<0.05) and maximum concentration (C<sub>max</sub>) of NNDMAC was lower than that of the free form. T<sub>max</sub> was same with both the administrations. The results obtained in the present study showed that microsphere encapsulated NNDMAC provides prolonged and effective plasma concentration after IP administration. The microsphere formulation sustained the release of the active for 9 days *in vitro* as well as *in vivo* in this rat model. Good IVIVC was achieved when the release medium selected was based on the volume of distribution of the drug.

**Key words**: NNDMAC, microspheres, sustained release, pharmacokinetics, IVIVC

**Introduction**

Curcumin and its analogues have been the subject of several pharmacological studies. Most of these studies were conducted with an intention to unravel their therapeutic potential and exploit the chemical structure for clinical use. Several bioactivities for curcumin and its synthetic analogues including their use in the cancers, tumors, alzheimers disease, inflammation, malaria, bacterial infections, neurological disorders, etc. have been reported (1). The analogues of curcumin were mainly...
synthesized to increase the poor bioavailability of curcumin, its stability as well as solve the problems associated with its synthesis (2). Curcumin is of particular interest for a variety of pharmacological applications. Most studies involving its use do not obtain pure samples. Extractions from the natural product, turmeric, are the most common sources of curcumin. Purification is accomplished using extensive chromatographic extraction and is a very labor intensive endeavor that does not provide a very pure material suitable for pharmacological use. As an alternative to the extraction, synthesis of curcumin has been attempted. The synthesis of curcumin involves the use of relatively expensive components that require intensive removal of impurities that require treatment for their disposal (3). Other methods for the synthetic production of curcumin from the starting products vanillin and 2,4-pentanedione involve the use of tri-butyl borate, boron oxide, and butylamine in a hydrolysis reaction with N,N-dimethylacetamide as a solvent and recrystallization using acetonitrile. In this second approach, there are problems associated with the recovery, waste disposal and toxicity. Because of these problems with the synthesis of curcumin, synthesis of curcumin analogues was also accomplished as an alternative. Analogues of curcumin were synthesized using a variety of approaches (2). After synthesis, these compounds were screened for a variety of activities. Our group has synthesized several of 1,7-diaryl-1,6-heptadiene-3,5-diones, and in particular curcumin and its analogues. NNDMAC is one such analogue which possessed hepatoprotective, antidepressant and anti-inflammatory activities (4,5,6). Its chemical structure is shown in the Figure 1. We previously developed a biodegradable microsphere formulation, a parenteral depot system, for NNDMAC (6). The purpose of this study was to investigate the pharmacokinetics of NNDMAC microspheres in a rat model and also determine the IVIVC with the formulation. The data from this study adds knowledge to our quest to improvise therapy against several diseases where in curcumin analogues are useful. There are several advantages with biodegradable parenteral depot system (7). With these types of biodegradable parenteral depot systems, there is a possibility of patenting clinically successful drugs after incorporating them into newer drug delivery systems without infringing the original drug or formulation patents. Further, the development in the concepts and techniques of controlled release drug delivery systems coupled with the increasing expense bringing new drug entities to market, has encouraged the development of this new drug delivery system. It is also easy to deliver the novel, genetically engineered pharmaceuticals, i.e. peptides and proteins to their site of action without incurring significant immunogenicity or biological inactivation with this new drug delivery system. The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and physiological parameter inherent in selected route of administration. Thus, parenteral biodegradable depot microspheres is the attractive dosage form to be tested to enhance the pharmacokinetic properties as well the pharmacodynamic activity with selected curcumin analogues.

![Chemical Structure of NNDMAC](image)

**Fig. 1:** Chemical Structure of NNDMAC

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Materials and Methods

The required aromatic aldehyde p N N dimethylamino benzaldehyde was obtained from Merck. Benzene was purchased from Universal laboratories. Column silica gel was purchased from Finar chemicals limited. HPLC grade methanol and acetonitrile were purchased from Merk specialties. Methanol, ethyl acetate, and n-Hexane were purchased from Finar reagents. Acetone, benzene and toluene were purchased from Universal laboratories. Polycaprolactone was purchased from Sigma-aldrich, Germany. Ethanol LR, ethyl acetate, tween 80 and dichloromethane were purchased from Finar reagents. Benzene purchased from Universal laboratories was used. To conduct in vitro drug release studies, magnetic stirrer and cyclo mixer from Remi Equipments Pvt. Limited were used. A SL 164 Elico Double Beam UV-Vis Spectrophotometer was used to analyze the samples. HPLC from Cyberlabs was used for analysis of all the plasma and serum samples.

Male Wister rats (100 to 150 gms, 5 to 6 weeks old) purchased from animal center of Mahaveera enterprises, Hyderabad were used in this study.

Fabrication of NNDMAC Microspheres

Microspheres of NNDMAC using biodegradable polycaprolactone as the polymer were fabricated using emulsion-solvent evaporation method. Dichloromethane was taken as organic phase in which polymer (400mg) and drug (200mg) in a ratio of 2:1 were dissolved (20ml). This organic phase was added to the aqueous phase containing tween 80 as surface active agent (1% w/v solution) drop by drop while the aqueous phase was kept for stirring on a magnetic stirrer. Stirring was continued till complete evaporation of dichloromethane occurred. As the organic phase evaporates precipitation of the polymer and drug occurs due to which drug gets entrapped in the polymer and acid solution and stirred for one hour to get a solid mass. Thus obtained mass was filtered and washed with water. This crude drug was purified and separated by column chromatography using 60-120 mesh TLC grade silica gel. The column was filled with silica gel of mesh size 60 to 120 and wet packing method was followed. The reaction product mixed with silical gel was loaded on top of the column and column was run with a mixture of n hexane and ethyl acetate (75:25) of 500 ml volume. The pure product was subsequently eluted by running the column with a mixture of methanol and benzene (50:50). The elutant was allowed to air dry. It was recrystallized by subsequent solubilization in benzene followed by methanol to get pure product. The purity of the compound obtained was confirmed using HPLC. A HPLC procedure employing a C-18, 100 X 4.6 column, SPD-10A UV-Vis detector, LC-10 AD pump and C-R7A Plus integrator was used. HPLC grade methanol and water in the ratio of 70:30 was taken as the mobile phase. The detection wavelength was 425 nm. Further, the structure was confirmed using NMR.

Synthesis and Characterization of NNDMAC

A mixture of acetyl acetone (0.01 mole), p N N dimethyl benzaldehyde (0.02 moles), boric acid (0.01 mole), in dimethyl formamide (10 ml), was taken into a round bottom flask (RBF) and few drops of diethanolamine and acetic acid mixture was added. The mixture was then refluxed in a mantel for 16 hours at 150°C temperature. The reaction was monitored by TLC (Thin Layer Chromatography) for the confirmation of the product. After 16hrs of reflux the reaction mixture was poured into a 10% acetic acid solution and stirred for one hour to get a solid mass. Thus obtained mass was filtered and washed with water. This crude drug was purified and separated by column chromatography using 60-120 mesh TLC grade silica gel. The column was filled with silica gel of mesh size 60 to 120 and wet packing method was followed. The reaction product mixed with silical gel was loaded on top of the column and column was run with a mixture of n hexane and ethyl acetate (75:25) of 500 ml volume. The pure product was subsequently eluted by running the column with a mixture of methanol and benzene (50:50). The elutant was allowed to air dry. It was recrystallized by subsequent solubilization in benzene followed by methanol to get pure product. The purity of the compound obtained was confirmed using HPLC. A HPLC procedure employing a C-18, 100 X 4.6 column, SPD-10A UV-Vis detector, LC-10 AD pump and C-R7A Plus integrator was used. HPLC grade methanol and water in the ratio of 70:30 was taken as the mobile phase. The detection wavelength was 425 nm. Further, the structure was confirmed using NMR.
stirring results in size reduction as well as spherical particle formation.

**In vitro Drug Release Studies**

A dialysis membrane was used for the release study. The release medium, PBS (7 ml) was taken into the receiver compartment. Release medium was designed based on the volume of distribution. The volume of distribution of this drug in rats was ~ 7 ml (5). This has been selected so as to obtain good *in vitro – in vivo* correlation. The donor compartment was immersed into the receiver compartment so that the edge just touches the receiver compartment. A 100mg of the microparticles were dispersed in 2 ml of PBS and placed in the donor compartment and of this suspension 1 ml was used in the release studies. The percentage loading of the drug was found to 70% and as a reason, the 50 mg of microsphere suspension used in the release studies contained 35 mg of the drug. The rpm of the system was maintained using magnetic stirrer and bead. Samples (1 ml) were removed from the receptor compartment and replaced with fresh medium immediately. The samples were then analyzed for the drug. A n=3 was used in the study and the data is reported as mean ± S.D. The release studies were also conducted with the pure drug so as to show the sustained release of the drug from the microspheres. This set of release studies were exactly similar to that conducted using the microspheres, excepting the use of pure drug in this case. The amount of the drug taken in the release studies was also the same (35 mg). Before selecting the wavelength to be used in the analysis of the compound, a UV spectrum of the compound in PBS and methanol was generated. The UV spectrum of the compound in the release medium was also generated. Based on the results and the sensitivity, the samples were analyzed using a UV-visible spectrophotometer at 425 nm wavelength.

**Pharmacokinetic Study**

The study was conducted in rats after getting approval from ethical committee constituted for this project in Vaagdevi College of Pharmacy, Warangal, AP, India. Male wistar rats (250 g) were purchased from Mahaveer Enterprises, Hyderabad. Animals were maintained in an air-conditioned room at 22 ± 2° C and relative humidity of 45-55% under a 12 h light:12 h dark cycle. The animals had free access to standard food pellets and water was available ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Vaagdevi College of Pharmacy, Warangal (Registration No: 1047/ac/07/CPCSEA) and constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSEA), India. After quarantine period, rats were divided into two groups (n=4). One group was administered drug solution via i.v. route while other group was injected with biodegradable microspheres intraperitoneally. A 35 mg of the drug dissolved in sterile PEG 400 was used in the i.v. solution administration. A 400 mg of the NNDMAC microspheres were dispersed in 4 ml of normal saline and a 500 µl of this suspension containing 35 mg of the drug were injected into each rat. Blood samples (0.5 ml) were collected from retro orbital sinus of rat eye under anesthesia at intervals of 0.25, 0.5, 0.75, 1, 3, 6, 12, and 24 hrs in case of i.v. solution. For microparticular system along with above time intervals samples were also collected after 4 and 9 days. The blood samples so collected were added to a series of graduated micro centrifuge tubes containing 0.3 ml of sodium citrate solution (4% w/v in water). All the samples were centrifuged at 3000 rpm for 10 minutes and plasma was separated into other micro centrifuge tube by using micro pipette and stored in deep freeze. The drug was extracted.
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from the plasma by adding 500 µl of ethyl acetate, and vortexed on cyclo mixer for 20 min. The organic phase was separated and collected into another micro centrifuge tube and allowed to air dry by keeping the lid of the tube open for 24 hours. These dried tubes were stored in deep freeze until HPLC analysis was performed. HPLC analysis samples were reconstituted with 50 µl of mobile phase (methanol: water, 70:30) and analyzed at 230nm wavelength. The wavelength was selected based on the sensitivity and specificity of the compound in a HPLC UV-Vis detection. The HPLC method was validated for inter day and intraday variability’s. From the plasma data various pharmacokinetic parameters were determined.

**In Vitro In Vivo Correlation**

There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C (8). Here the correlation was established according to Drewe and Guitard basing on degree A. The parameters compared were cumulative absorption profile to that of in vitro dissolution profile i.e. correlation of the amount of drug dissolved to that of respective fraction of dose absorbed ($T_{50}$). Cumulative amount of the drug absorbed was calculated using Wagner-Nelson method approximating the kinetics of the drug to one compartment open model. According to Wagner-Nelson method, the cumulative amount of drug released from the microspheres into the systemic circulation in a rat was calculated as given below:

$$A_b/A_{b^\infty} = (C_p + K[AUC]_0^t) / K[AUC]_0^\infty$$

Where $A_b$ is the cumulative amount released at any time, $A_{b^\infty}$ is the dose administered, $C_p$ is the plasma concentration at any time, $K$ is the elimination rate constant and AUC is the area under the curve. $K$ was also determined in this study in another set of rats where NNDMAC was administered via i.v. route. To determine the IVIVC with the formulation, the percentage of NNDMAC dissolved from microspheres was plotted on X-axis and the corresponding NNDMAC absorbed in the rats from the microspheres was plotted on the Y-axis and this graph was fitted to a straight line and the correlation coefficient was determined. This correlation indicates the strength of IVIVC of this study.

**Data Analysis**

All data in this study were presented as mean or mean±SEM. Data were analyzed by t-test. Significance was recognized at $P < 0.05$.

**Results**

The drug was synthesized successfully using protocol followed in this study. After synthesis, the structure of NNDMAC was confirmed using NMR. The NMR results are as follows: 7.6-7.8(m, 8H, Ar); 6.1(d, 2H, HC=CH); 4.9(d, 2H, HC=CH); 3.3(s, 2H, CH$_2$); 2.2(s, 12H, N(CH$_3$)$_2$). A UV-Vis spectrum was generated so as to identify the wavelength to be used in the assay of the compounds. The spectrum indicated two different $\lambda_{max}$ values which were 230 nm and 425 nm. The drug assay at 425 nm was used to analyze the in vitro release samples while the assay at 230 nm was used in the HPLC. This is because the sensitivity of the assay was higher at 230 nm in a UV-Vis detector used in the HPLC and the specificity of the assay was higher at 425 nm when a UV-Vis spectrophotometer was used. At 230 nm, there was a significant interference from the degradation products of the polymers in the in vitro release studies with Pharmacokinetics of Microspheres in Rats
that of the drug and this interference was not noted at 425 nm, suggesting more specificity of the assay at 425 nm in a UV-Vis spectrophotometer. Thus, this wavelength was also used in the assay of the drug after synthesis. In this case, the starting material used in the synthesis also demonstrated a $\lambda_{\text{max}}$ at 230 nm. The assay at 230 nm in HPLC for plasma samples was used because of the higher sensitivity when compared to that at 425 nm. The HPLC method used was validated for interday and intraday variabilities. The results of intra and inter-day variation of NNDMAC at three different concentrations levels (Level 1, Level 2 and Level 3) were determined. The data indicates that the maximum %relative error at Level 1, Level 2 and Level were 1.8, 1.72 and -1.99, respectively while the maximum % relative standard deviation was 1.5, 1.9 and 1.4 respectively indicating that the method has acceptable accuracy and precision. Also, the calculated t-values were lesser than the tabulated t-value of 4.3 for $\alpha = 0.05$ at two degrees of freedom. This indicated that the experimental values were not significantly different from the nominal which reflected the accuracy of the method. Further, one way ANOVA was performed to get estimates of within and between day variability. The calculated F value was lesser than the tabulated F ($\alpha = 0.01$) of 10.92 indicating that the inter-day variability was not significantly different from the intra day variability at 1% level of significance.

The drug release in vitro from the microspheres was sustained for 9 days (Figure 2). A 99.8% drug was released during this time. The rest of the drug could be the drug irreversibly bound to the polymer. The release of the drug from pure samples was also investigated. A 100% of the entire pure drug taken at similar quantities was released within 48 hours under similar conditions. This confirms the sustained release of the drug from the microsphere formulations. Plasma concentration vs time curve after single IV bolus solution and IP formulation (microsphere) administration are shown in the Figure 3. Drug concentration started to be detected in the plasma from 1 hour onwards. Drug release was sustained in vivo after the microsphere administration. After IP administration of the microspheres, the noncompartmental parameters, area under the concentration time curve (AUC), MRT, $t_{\text{max}}$, and $C_{\text{max}}$ were determined using KINETICA. All the noncompartmental PK parameters for the i.v. bolus administration and microsphere administration are shown in Table 1. After administration NNDMAC microsphere formulation via the IP route, mean residence time (MRT) and the area under the curve (AUC) were significantly higher ($P<0.05$) and maximum concentration ($C_{\text{max}}$) of NNDMAC was lower than that of the free form. $T_{\text{max}}$ for both the administrations was the same. To determine degree A IVIVC, the amount of the drug absorbed was determined using Wagner-Nelson. The cumulative percentage of drug dissolved and

![Fig. 2: In Vitro Drug Release from NNDMAC Microspheres](image)

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PK Parameter | IV Solution Administration | IP Microsphere Administration
---|---|---
Cmax (µg/ml) | 2.4 ± 0.2 | 3.2 ± 0.3
Tmax (h) | 1 ± 0.2 | 1 ± 0.1
AUC (µg*h/µl) | 0.025 ± 0.01 | 0.055 ± 0.005
MRT (h) | 9.3 ± 2 | 131.47 ± 3
R² | 0.997 | 0.954

Table 1 Pharmacokinetic Variables of NNDMAC after single IP and IV Administration of Microspheres and Solution (Mean±SEM)

Fig. 3: Plasma Log(Conc) vs Time Profile of NNDMAC After IV Administration of Solution (—– ) and IP Administration of the Microsphere Formulation (—– —–)

Cumulative fraction of drug absorbed were compared. The graphical analysis confirms a good degree of correlation (r² = 0.982) (Figure 4). Thus, it can be concluded that good IVIVC was obtained when the volume of distribution was used as the volume of the release study.

Discussion

Optimal design of controlled release systems requires a thorough understanding of pharmacokinetics of drug with and without the delivery system (9). Also the comprehension of in vitro in vivo correlation (IVIVC) of the delivery is essential to better tailor the delivery system for the future needs (8,10). The development of new injectable drug delivery systems has received considerable attention over the past few years. This interest has been sparked by the advantages this delivery system possess, which include ease of application, localized delivery for a site specific

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action, prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effect common to most forms of systemic delivery and improved patient compliance and comfort. Thus, we developed NNDMAC microspheres and in this study, investigated the pharmacokinetics and IVIVC of the active. The developed formulation can be used where in the curcumin analogues can be used for therapeutic purposes. The pharmacology of curcumin and its analogs is complex, with extensive metabolic conversions involved in the activation, inactivation and elimination of the drug. It is cleared via glucuronidation. These drug properties also contribute to the marked heterogeneities in efficacy observed with curcumin and its analogs. Hence, drug carrier technologies represent a rational strategy to improve pharmacokinetics thereby enhancing the pharmacodynamics of the drug. After IP administration of the microspheres, drug concentration reached $C_{\text{max}}$ within a longer time ($P<0.05$) than that of solution forms. The results are similar to studies performed with other drugs, administration routes and species(11). The results suggest that the absorption of NNDMAC after microsphere administration from injection sites was slower. In this study, it was determined that the $C_{\text{max}}$ of NNDMAC with microsphere administration was lower than that obtained out of injecting solution. After IP administration of microsphere encapsulated drugs, which acted as a local depot, there was a slower release, lower $C_{\text{max}}$, and long-lasting concentrations of active agent in the plasma compared with administration of the free form. After IP administration of microsphere formulation, the low $C_{\text{max}}$ and plasma concentrations may cause a reduction in dose-dependent side effects of the drug (12). In the present study, MRT of NNDMAC from microspheres was longer than those of the drug obtained out of injecting a solution. These results suggest that microsphere encapsulated drug formulations provide longer effective concentrations in plasma. The microspheres of this study were prepared using a biodegradable polymer polycaprolactone. Polycaprolactone (PCL) is a biodegradable polyester with a low melting point of around 60°C and a glass transition temperature of about “60°C (13). PCL is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) and has therefore received a great deal of attention for use as an implantable biomaterial. In particular it is especially interesting for the preparation of long term implantable devices, owing to its degradation which is even slower than that of polylactide. PCL is an Food and Drug Administration (FDA) approved material that can be used in the human body as (for example) a drug delivery device. A variety of drugs have been encapsulated within PCL beads for controlled release and targeted drug delivery.

The second objective of this study was to evaluate the IVIVC with the microsphere formulation. A predictive IVIVC can empower in vitro dissolution as a surrogate for in vivo bioavailability/biotheraquelance (8,10). IVIVCs can decrease regulatory burden by decreasing the number of biostudies required in support of a drug product. Additionally, IVIVC is also helpful in the product development including the development of depot microspheres. The development of an IVIVC is a dynamic process starting from the very early stages of development program through the final step. Different types of IVIVCs are used in the regulatory terminology. These include assumed IVIVC, retrospective IVIVC, and prospective IVIVC. An assumed IVIVC is essentially one that provides the initial guidance and direction for the early formulation development activity. Thus, during stage 1 and with a particular product concept in mind,
appropriate in vitro targets are established to meet the desired in vivo profile specification. This assumed model can be the subject of revision as prototype formulations are developed and characterized in vivo, with the results often leading to a further cycle of prototype formulation and in vivo characterization. Out of this cycle and in vivo characterization and, of course, extensive in vitro testing is often developed what can be referred to as retrospective IVIVC. With a defined formulation that meets the in vivo specification, Stage 2 commences. At this stage based on a greater understanding and appreciation of defined formulation and its characteristics, a prospective IVIVC is established through a well defined prospective IVIVC study. Once the IVIVC is established and defined it can be then used to guide the final cycle of formulation and process optimization leading into Stage 3 activities of scale-up, pivotal batch manufacture, and process validation culminating in registration, approval and subsequent post-approval scale-up and other changes. In this study, a part of retrospective IVIVC was accomplished based on the volume of the distribution to be the volume used in the release studies. It is taken under the assumption of one definition of volume of distribution and the definition is “It is the hypothetic volume of the body compartment in which the drug is distributed”. Although this definition is still controversial it would most likely benefit our study in establishing the retrospective IVIVC we aimed at. Thus, the release was performed in 7 ml of PBS based on the volume of distribution (5). The aim of such a study is to obtain good in vitro – in vivo correlation. Ideally, physiological conditions at the site of administration should be taken into account when selecting the in vitro dissolution/release test conditions. The complexity of the release mechanism of some novel/special dosage forms and the lack of knowledge about the conditions under which release occurs in vivo make it difficult to design physiologically based tests in all cases, but it should be possible to conceive a test that can detect the influence of critical manufacturing variables, differentiate between degrees of product performance, and to some extent characterize the biopharmaceutical quality of the dosage form. As the release mechanism and site of application vary dramatically among the novel/special dosage forms, the experimental test conditions have to be tailored according to the conditions at the site of administration (eg, temperature of the test) and the release mechanism (eg, chewing gums will require different agitation rates than suspensions). Within a given category, it may be necessary to have product type-specific dissolution tests (eg, separate tests for lipophilic and hydrophilic suppositories), and in some cases for products containing the same drug and administered in the same type of novel/special dosage form but with a different release mechanism (analogous to the range of tests available in the USP for theophylline extended release dosage forms). Several studies also used volume of distribution to be volume to be used in the release medium especially for sustained release dosage forms. In this study also, we used similar approach.

The correlation was established according to Drewe and Guitard basing on (degree A) i.e., the comparison of cumulative absorption profile and cumulative in vitro release profile was made. A level A correlation of in vitro release and in vivo absorption could be obtained for individual plasma level data by means of the Wagner and Nelson method. This type of evaluation of in vivo absorption was previously applied for drug delivery systems. To develop level A correlation the estimation of the in vivo absorption or dissolution time course is performed using an appropriate deconvolution techniques such as Wagner-Nelson procedure or Loo-Riegelman.
method or numerical deconvolution for each formulation and subject. Wagner-Nelson and Loo-Riegelman methods are both model dependent in which the former is used for a one-compartment model and the latter is for multi-compartment system. However, Wagner-Nelson method is less complicated than the Loo-Riegelman as there is no requirement for intravenous data. However, misinterpretation on the terminal phase of the plasma profile may be possible in the occurrence of a flip-flop phenomenon in which the rate of absorption is slower than the rate of elimination. To avoid complicated calculations which are bound by regression parameters and make the analysis simpler, we used Wagner-Nelson method in this study. Further, the presence or absence of flip-flop in the pharmacokinetic data was also verified using i.v. bolus data. Additionally, these techniques represent a major advance over the single-point approach in that these methodologies utilize all of the dissolution and plasma level data available to develop the correlations. Good IVIVC was observed in this retrospective IVIVC study. The correlation made according to the volume of distribution is suitable for NNMDAC microsphere. The same methodology should be investigated for other drugs so as to further add a speck of knowledge to this type of establishment of IVIVC.

Conclusion

In conclusion, when microsphere formulations are compared to solution, formulation demonstrated a lower Cmax, higher MRT and provides effective and prolonged plasma concentration in the body after IP administration. In addition, when NNMDAC was administered in a microsphere entrapment form it had long duration of activity threshold, this result may be beneficial for the diseases in which NNMDAC is useful. Good IVIVC was obtained when the volume of distribution was used as the volume of the release study.

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activity of some novel curcumin analogs (communicated).


