Publications & Presentations
Formulation development and systematic optimization of solid lipid nanoparticles of quercetin for improved brain delivery

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Abstract

Objective This study aims at formulating solid lipid nanoparticles (SLNs) of quercetin, a natural flavonoid with established antioxidant activity, for intravenous administration in order to improve its permeation across the blood–brain barrier into the CNS, and eventually to improve the therapeutic efficacy of this molecule in Alzheimer’s disease.

Methods The SLNs of quercetin were formulated using Compritol as the lipid and Tween 80 as the surfactant through a microemulsification technique, and optimized employing a 3² central composite design (CCD). Selection of the optimized SLN formulation, using brute-force methodology and overlay plots, was based on its efficiency of entrapping quercetin inside the lipophilic core, particle size, surface charge potential and ability of the SLNs to release the entrapped drug completely. The optimized formulation was subjected to various in-vivo behavioral and biochemical studies in Wistar rats.

Key findings The optimized formulation exhibited a particle size of less than 200 nm, 85.73% drug entrapment efficiency and a zeta potential of 21.05 mV. In all the in-vivo behavioral and biochemical experiments, the rats treated with SLN-encapsulated quercetin showed markedly better memory-retention vis-à-vis test and pure quercetin-treated rats.

Conclusions The studies demonstrated successful targeting of the potent natural antioxidant, quercetin, to brain as a novel strategy having significant therapeutic potential to treat Alzheimer’s disease.

Keywords Alzheimer’s disease; blood brain barrier; design of experiments (DoE); flavonoids; memory enhancement

Introduction

Alzheimer’s disease is a progressive age-related neurodegenerative disorder with distinct neuropathological features. About three percent of world’s population between the age of 65 to 74 years, and nearly half of the population aged 85 years or older, is inflicted with this disease. In this condition, amyloid-beta (Aβ) accumulates as plaques in the extracellular space of the gray matter and in artery walls as cerebral amyloid angiopathy, and tau protein accumulates as neurofibrillary tangles within neurons. The other neuropathological features of Alzheimer’s disease include neuronal loss, synaptic depletion, Hirano bodies and granulovacuolar degeneration. Various strategies have been developed to prevent or mitigate the progression of Alzheimer’s disease. Despite the medical need for an effective therapeutic treatment, the pace of progress towards this goal has been painstakingly slow. Current therapies for Alzheimer’s disease, such as the cholinesterase inhibitors and NMDA receptor antagonists, provide moderate symptomatic delay of the disease without arresting the disease progression. Accordingly, newer approaches for the disease management are the acute need of the hour.

Flavonoids, a class of secondary plant metabolites, have recently gained wide attention because of their antioxidant, anti-inflammatory, antiplatelet and other beneficial properties. Quercetin, a natural flavonoid molecule, has a long history of consumption as a part of the human diet, and is found in fruits, vegetables, wine and tea. It has been postulated to act as a novel neuroprotectant by mitigating the increased levels of reactive oxygen species produced by normal mitochondrial activity, which accelerate the neurodegenerative processes of Alzheimer’s disease. In this regard, quercetin has been documented to be a more potent antioxidant and radical scavenger than vitamin C, vitamin E and...
Formulation of optimized quercetin SLNs

Sanju Dhawan et al.

β-carotene. Besides, this flavonoid has been shown to improve spatial learning and memory in D-galactose-treated aging in mice. These valuable effects of quercetin, however, are thwarted because of its limited penetration into the CNS. Although a few attempts have been made to formulate various drug delivery systems of quercetin-like microemulsions and nanoparticles, no studies have been reported on enhancing the brain permeability of the drug. Accordingly, this investigation aimed to formulate solid lipid nanoparticles (SLNs) of quercetin for intravenous administration to improve its permeation across the blood-brain barrier into the CNS and eventually, to improve its therapeutic efficacy in Alzheimer’s disease.

Materials and Methods

Materials

Quercetin was purchased from M/s Sisco Research Laboratories (Maharashtra, India). Compritol 888 ATO was a gift from Central Drug House Pvt. Ltd (New Delhi, India). All other reagents were of analytical grade and were used as received.

Formulation of solid lipid nanoparticles

Compritol (quantity varied as per the experimental design) was heated to its melting point (i.e. 70–75°C) and quercetin (50 mg) was dispersed thoroughly in the molten lipid to form a homogenous dispersion. Water (6 g) and Tween 80 (quantity varied as per the experimental design) were mixed separately and heated to the same temperature as the lipid dispersion. After the two phases became isothermal, the aqueous phase was poured into the lipid phase under magnetic stirring to obtain a clear homogenous microemulsion, which was then poured into 100 ml of cold water, and stirred at 1500 rev/min in an ice-bath for 40 min to obtain a fine dispersion of the SLNs. The dispersion was freeze-dried using a lyophilizer (Alpha, 2–4 LD plus; Martin Christ, Osterode am Harz, Germany) to obtain a fine powder. The lyophilized powder was subsequently re-dispersed in 1% Tween 80 (v/v) solution or normal saline solution (0.9% w/v NaCl) to obtain Tween 80 coated or uncoated particles, respectively. Various SLN formulations were prepared employing varying concentrations of Compritol and Tween 80, keeping the amount of quercetin constant. By studying the total drug content, particle size, drug release profile and drug entrapment efficiency of the above-mentioned formulations, limits and ranges for Compritol and Tween 80 were set for the subsequent optimization studies using a two-factor central composite design (CCD).

Experimental design

A CCD (with $\pi = 1$) using three levels each of the two factors (i.e. Compritol and polymer Y (i.e. Tween 80), was adopted for further investigations as required by the design, and the factor levels were suitably coded. Table 1 summarizes an account of the 13 experimental runs studied employing a total of nine formulations. All the studies were conducted in triplicate, and the formulation at central point (0, 0) was studied in quintuplicate.

In-vitro drug release

Drug release studies from SLNs were performed in the solvent mixture of phosphate-buffered saline (pH 7.4) and methanol (80:20, v/v) using the dialysis bag method. Quercetin-SLN suspension equivalent to 2 mg of drug was placed in the bags, which were then suspended with the help of a thread in a conical flask containing 200 ml of dissolution medium (37 ± 1°C) stirred at 100 rev/min. A sample of the dissolution medium (2 ml) was periodically withdrawn at each time interval and immediately replaced with the same volume of fresh dissolution medium to maintain the sink condition. An analogous study was also performed with an equal amount of pure quercetin in its solution. Quercetin in the sample solution was analysed at 257 nm by a previously validated UV-Vis spectrophotometric method taking $E^\infty_{1%}$ as 663 and molar extinction coefficient, $\varepsilon$, as 19690.4.

Table 1 Composition of various solid lipid nanoparticle formulations prepared as per the experimental design

<table>
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<tr>
<th>Formulation code</th>
<th>Trial No.</th>
<th>Coded factor levels</th>
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</table>

Translation of coded levels in actual units

<table>
<thead>
<tr>
<th>Coded Level</th>
<th>X1</th>
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<td></td>
<td>4</td>
<td>6</td>
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</table>

The raw data obtained from the in-vitro dissolution study were analysed using ZOREL software. This software has in-built provisions for applying the correction factor for volume and drug losses during sampling, calculating the values of percent drug released, rate of drug release and log fraction released at varied times. Using the software, the values of kinetic constant (k) and diffusional release exponent (n) were determined. Based on the phenomenological analysis, the type of release, whether Fickian, non-Fickian (anomalous) or zero-order, was predicted.

Drug entrapment efficiency

Drug entrapment efficiency was determined using the dialysis membrane method by allowing the unentrapped drug to diffuse through the membrane placed inside the sink medium (i.e. methanol). The unentrapped drug (i.e. drug diffused out of the membrane) was quantified by analysing it spectrophotometrically at 257 nm using a previously validated method taking $E^\infty_{1%}$ as 652 and molar extinction coefficient, $\varepsilon$, as 19690.4.
Particle size determination
The particle size distribution of all the nine SLN dispersions was observed using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) to access the size range and uniformity of particle size distribution in the formulation.

Zeta potential determination
The zeta potential of all the nine formulations was determined using a Zetasizer (Malvern Instruments Ltd, Worcestershire, UK). The instrument was operated at a constant temperature of 25°C using a clear disposable zeta cell.

Optimization data analysis
The response variables considered for systematic optimization were particle size, drug entrapment efficiency, amount of drug release in 20 h (ReLo) and zeta potential. Design Expert software ver. 6.0 (Stat-Ease, Minneapolis, MN, USA) was employed to fit full second-order polynomial equations with added interaction terms to correlate the studied responses with the examined variables. The prognosis of optimum formulations was conducted in two stages: first, a feasible space was located and second, an exhaustive grid search was conducted to predict the possible solutions. The optimized formulation was also located by overlay plot option of the Design Expert software, while ‘trading off’ of the responses.

Electron microscopic examination
The optimized formulation was viewed under a transmission emission microscope (TEM, H7500; Hitachi, Tokyo, Japan) to observe the surface morphology of the particles.

Stability studies
The SLN samples were stored in a refrigerator (i.e. at 2-8°C) and at 25°C/65% RH to assess the storage stability of optimized formulation and ascertain the storage conditions. Samples were periodically withdrawn at monthly intervals for four months and examined for their particle size, entrapment efficiency and drug release characteristics.

In-vivo studies
Animals
Male Wistar rats, 180–200 g, procured from Central Animal House, Panjab University, Chandigarh were used in the investigation. The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Indian National Science Academy guidelines for the use and care of animals.

Drugs and treatment schedule
Aluminium chloride solution and the optimized quercetin SLNs were freshly prepared at the beginning of each experiment. For oral administration, aluminium chloride was dissolved in drinking water and for intravenous administration, lyophilized SLNs were dispersed in normal saline or 1% v/v Tween 80 solution. The dose of quercetin for rats was calculated employing Equation 1, taking Km, factor for humans and rats as 37 and 6, respectively.¹²⁰

\[
\text{Human Dose} = \text{Animal Dose} \times \frac{\text{Animal } \text{Km}}{\text{Human } \text{Km}} \quad (1)
\]

Before experimentation, rats were randomized into the following seven groups, each group consisting of four rats.

- Group I: Naive rats
- Group II: Control (distilled water p.o. + vehicle for quercetin i.v.)
- Group III: Aluminium chloride (100 mg/kg p.o.)
- Group IV: Quercetin (4.41 mg/kg i.v.) dissolved in 70:30 v/v mixture of PEG 200 and DMSO
- Group V: Aluminium chloride (100 mg/kg p.o.) + quercetin (4.41 mg/kg i.v.) dissolved in vehicle
- Group VI: Aluminium chloride (100 mg/kg p.o.) + Tween 80 coated SLNs i.v. (equivalent to 4.41 mg/kg of quercetin)
- Group VII: Aluminium chloride (100 mg/kg p.o.) + uncoated SLNs i.v. (equivalent to 4.41 mg/kg of quercetin)

The study was carried out for a period of eight weeks.

Spatial navigation task
The acquisition and retention of a spatial navigation task was evaluated using a Morris water maze.¹²¹ Rats were trained to swim to a visible platform in a circular water-pool (180 cm in diameter and 60 cm in height) located in a test room.¹²² The rats received a training session consisting of four trials on day 52, 53, 54 and 55. The latency to find the escape platform was recorded to a maximum of 90 s. Twenty-four hours after the last training (i.e. on day 56), the rats were released randomly at one of the edges facing the wall of the pool and tested for retention of response.

Elevated plus maze paradigm
The elevated plus maze consisted of two opposite black open arms (50 cm × 10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls.¹²³ Acquisition of memory by the rats was tested on the 44th day from the start of aluminium chloride administration. The time taken by the rat to move from the open arm to the closed arm was recorded as the initial transfer latency (ITL). Rats were allowed to explore the maze for 20 s after recording the ITL and were made to return to the home cages. If the rat did not enter the enclosed arm within 90 s, it was pushed back into one of the enclosed arms and the ITL was recorded as 90 s. Retention of memory was assessed by placing the rat in an open arm. The retention latency was noted on day 45 and day 56, termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.¹²⁴

Assessment of gross behavioral activity
Gross behavioral activity was observed using digital photoautocounter at the end of every 15 days for a total of 60 days after the initiation of aluminium chloride treatment.¹²⁰

Biochemical assessment
Biochemical tests were carried out 24 h after the last behavioral test (i.e. on 57th day). Rats were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 4000 rev/min for 15 min

\[
\text{Human Km}_{\text{Human}} = \frac{\text{Animal Km}_{\text{Human}}}{\text{Animal Km}_{\text{Human}}} \quad (1)
\]
and samples of supernatant were used for estimating lipid peroxidation, glutathione levels and nitrite levels in brain tissue.\(^{23,25,26}\)

**Statistical analysis**

The behavioral and biochemical assessment data were analyzed by one-way analysis of variance, followed by one-tailed Student’s \(t\)-test.\(^{15,27}\) \(P < 0.05\) was considered as statistically significant.

**Results**

**In-vitro drug release**

The in-vitro drug release profile of the formulations, prepared as per the experimental design, is depicted in Figure 1. Formulations SA, SB, SC and SG exhibited a high burst release. A summary of the dissolution parameters (Table 2) shows that the value of diffusional release constant, \(n\), varies between 0.2823 and 0.5649, indicating that the type of drug release varies between Fickian and non-Fickian behaviour. Quercetin in its solution form, however, exhibited an \(n\) value of 0.7329, indicating non-Fickian behaviour. The values of the kinetic constant, \(k\), showed a declining trend with an increase in the concentration of Tween 80 at all the levels of Compritol studied. Maximum extent of drug release was observed at the lowest levels of both the ingredients.

**Drug entrapment, particle size and zeta potential determination**

The values of percent drug entrapment, particle size and zeta potential of the formulations, prepared as the experimental design are shown in Table 3. Formulations SA, SB, SC and SG exhibited low drug entrapment values. Formulations SB, SC and SG were observed to possess high mean values of particle size (i.e. \(>2\mu m\)) too.

**Exploration of polymer mechanism using response surface methodology (RSM)**

In all, eight coefficients \((\beta)\) were calculated, with \(\beta_i\) representing the intercept, and \(\beta_{ij}\) representing the coefficients of various quadratic and interaction terms (Equation 2).

\[
Y = \beta_0 + \sum\beta_i X_i + \sum\beta_{ij} X_i X_j + \sum\beta_{iii} X_i^3
\]

Table 4 lists the coefficient values of polynomial equations along with their statistical parameters for the studied response variables. To study the effect of the two independent factors (i.e., Compritol and Tween 80), 3-D response surface graphs and 2-D contour graphs were constructed.

At lower levels of Tween 80, there was a marginal decreasing trend in the values of particle size, as the amount of Compritol increased from lower to intermediate levels (Figure 2). Tween 80 exhibited significant positive influence on particle size at lower levels of Compritol, the effect being slightly negative but less significant at higher levels of the lipid. The slanting lines of the corresponding contour plot also confirmed the same.

As depicted in Figure 3, entrapment efficiency showed an umbrella-like asymptotic curve with an increased concentration of Compritol (i.e. the value of entrapment efficiency first increases nonlinearly and then decreases marginally with an increase in the concentration of Compritol). The corresponding contour plot depicted highest entrapment efficiency at the intermediate levels of both the polymers.

Figure 4 depicts a linear decline in the value of \(R_{10}\) with an increase in the concentration of Compritol. However,
**Table 4** Coefficient values of polynomial equations with their statistical parameters for the studied response variables

<table>
<thead>
<tr>
<th>Coefficient code</th>
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<th>Zeta potential</th>
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</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001.

**Figure 2** Response surface graph showing the influence of Compritol and Tween 80 on particle size of solid lipid nanoparticles.

**Figure 3** Response surface graph showing the influence of Compritol and Tween 80 on entrapment efficiency of solid lipid nanoparticles.

**Figure 4** Response surface graph showing the influence of Compritol and Tween 80 on Rebo of solid lipid nanoparticles.

**Figure 5** Response surface graph showing the influence of Compritol and Tween 80 on zeta potential of solid lipid nanoparticles.

Tween 80 did not seem to exert any significant effect on the values of Rel20, barring a slight increase at the higher levels of Compritol.

At low levels of Tween 80, a characteristic inverted ‘U’-type trend increase in the values of zeta potential with a shift from lower to intermediate levels of Compritol was observed (Figure 5). However, a somewhat declining trend was observed at lower levels of Compritol. The curved lines of the corresponding contour plot depicted the highest value of zeta potential at intermediate levels of both the polymers.

Two feasible regions were selected as per the following criteria:

- Size < 300 nm; entrapment > 81%;
- Rel20 > 74.5%; zeta potential > 15.5 mV

An exhaustive grid search was then conducted within the selected feasible regions to further narrow-down the region of optimal formulation. Based on the final grid search, the formulation containing 384 mg of Compritol and 5.76 g of Tween 80 was selected as the optimal SLN formulation. The selection of the optimum formulation was based on minimization of particle size below 200 nm to facilitate brain targeting, maximization of entrapment efficiency and Rel20, and maximization of zeta potential to avoid coalescence of particles. The said formulation exhibited a particle size of 159 nm, entrapment efficiency of 85.73%, Rel20 of 77.09%, and zeta potential of 21.05 mV.

To validate the search for optimal formulation, a region was marked in the overlay plot (Figure 6) corresponding to...
optima, and corresponding responses were predicted for the optima. In this investigation, the optima searched by brute-force methodology (feasibility and grid search) and overlay plot came out to be quite identical.

**Electron microscopic examination**

The TEM images of the optimal SLN formulation are shown in Figure 7. As depicted in the image, the particles possessed uniform shape. The size of all particles was found to be less than 200 nm.

**Stability studies**

The formulation stored in refrigerated conditions did not exhibit any significant change in its particle size, drug entrapment efficiency and drug release characteristics after four months of storage. The formulation stored at 25°C/65% RH, however, exhibited increased particle size from the erstwhile nanometer range to micrometer range along with a significant decrease in drug entrapment efficiency. The results of the stability studies are summarized in Table 5.

**In-vivo studies**

**Spatial navigation task**

In the spatial navigation task, the naive, control and quercetin per se group of rats quickly learned to swim directly to the platform in the Morris water maze. Aluminium chloride-treated rats showed an initial increase in escape latency, which declined with continued training during the acquisition of spatial navigation task. The rats that received pure quercetin along with aluminium chloride showed slight improvement in their behaviour. In contrast, concomitant administration of quercetin formulated as SLNs with aluminium chloride significantly (P < 0.00001) decreased the time taken to reach the platform in the pre-trained rats as compared with aluminium chloride-treated rats (Figure 8).

**Elevated plus maze paradigm**

In the elevated plus maze task, mean ITL on day 20 for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm within 90 s. Following
training, naïve, control and quercetin per se treated rats entered the closed arm quickly and mean retention transfer latencies (1st RTL and 2nd RTL) to enter closed arm on days 45 and 56 were shorter as compared with the ITL on day 44 for each group, respectively. In contrast, aluminium chloride-treated rats performed poorly throughout the experiment and did not show any change in the mean retention transfer latencies on days 45 and 56 as compared with pre-training latency on day 44, demonstrating that chronic administration of aluminium chloride induced marked memory impairment. Chronic administration of quercetin-loaded SLNs following aluminium chloride administration significantly decreased the mean retention latencies on days 45 ($P < 0.005$) and 56 ($P < 0.0001$) as compared with pure quercetin administration, indicating enhancement of the anti-Alzheimer’s potential of quercetin on being formulated as SLNs. The results are depicted graphically in Figure 9.

Assessment of gross behavioral activity

In this series of experiments, the gross behavioral activity as measured by the mean scores of locomotor activity for each rat was relatively stable and showed no significant variation. The mean scores in naïve, control and aluminium chloride-treated rats did not show much change. Chronic administration of quercetin or SLNs of quercetin also had no significant effect on the locomotor activity as compared with naïve rats throughout the study period ($P > 0.1$).

Biochemical estimation

Chronic administration of aluminium chloride caused marked increase in free radical generation and significant rise in brain MDA, nitrite levels and depletion of reduced GSH. Further, there was less alteration in the brain MDA level, nitrite level and reduced GSH level due to quercetin per se treatment as compared with naïve rats. However, simultaneous chronic quercetin-loaded SLN administration to aluminium chloride-treated rats significantly prevented ($P < 0.00001$) the increase in MDA, nitrite levels and depletion of reduced GSH (Table 6).

Discussion

SLNs are considered to be safe and effective carriers for a variety of applications like topical delivery of drugs, pulmonary delivery and enhancement of efficacy of anticancer drugs. Their potential in brain targeting of therapeutic moieties is well established and widely reported in literature.
Among stearic acid, Compritol and palmitic acid, Compritol was selected as the lipidic carrier owing to better drug entrapment efficiency and lower particle size of the SLNs prepared using Compritol. Among Brij 78, Tween 80, Tween 40 and Lutrol F-68, Tween 80 was selected as the surfactant owing to better acceptability of Tween 80 through the intravenous route, better drug entrapment efficiency and ability to target SLNs to brain owing to its hydrophilic nature. Besides, earlier studies have also demonstrated successful formulation of SLNs using Compritol as the lipdic carrier and Tween 80 as the surfactant.\(^{11,13-16}\)

A CCD for two factors at three levels with \(\alpha = 1\), equivalent to a 3\(^2\) factorial design (FD), was chosen as the experimental design. This is considered to be an effective second-order experimental design associated with a minimum number of experiments to infer individual variables (main effects) and their second-order effects\(^{15,17}\) and has been successfully implemented for systematic optimization of various drug delivery systems.\(^{11,15-17}\) Further, this design has an added advantage of determining the quadratic response surface, not estimable using an FD at two levels.\(^{18,19}\)

The high burst release observed with Formulations SA, SB and SC can be attributed to the presence of un-entrapped drug present on the surface of SLNs, as the amount of lipid used in these formulations was not sufficient to entrap the drug completely. Further, Formulation SG also exhibited a high burst release, which can again be attributed to the presence of un-entrapped drug as the amount of surfactant used in this formulation was too low to support the high quantity of Compritol. The results of drug release pattern are in consonance with earlier findings where a combination of Fickian and quasi-Fickian behaviours has been proposed for release of drugs from SLNs.\(^{11,15-17}\)

The values of \(k\) indicated a significant change in the polymer characteristics with change in the surfactant levels. Further, the low percent drug entrapment observed in Formulations SA, SB, SC and SG can also be attributed to the reasons mentioned above. The large particle size of SB and SC was observed due to the presence of an excess of Tween 80 compared with the low amount of lipid in these formulations. This excess of hydrophilic surfactant made the particles coalesce, which was further supported by the low zeta potential of these formulations.\(^{11,15-17}\)

The high particle size of Formulation SG can also be attributed to the low zeta potential.

Quite high values of \(R^2\) of the MLRA coefficients for all four responses, ranging between 0.9983 and 0.9999, vouch high prognostic ability of the RSM polynomials. Statistically, the models for all the response variables were found to be highly significant using analysis of variance (\(P \leq 0.0001\)). The closeness in the magnitudes of adjusted \(r^2\) and predicted \(r^2\) to the actual model \(r^2\), and the low coefficient of variance also suggest high goodness of fit of the postulated model to the data. The response surface and contour plots for particle size confirmed that to attain a particular particle size, higher levels of Tween 80 have to be complemented with lower levels of Compritol and vice-versa. Further the \(U^1\)-type response surface curve depicting a curvilinear increase in the values of zeta potential with an increase in the concentration of Tween 80 unequivocally vouch for the presence of some type of interaction between the lipid and the surfactant. The optimum formulation searched by brute-force methodology and overlay plots came out to be identical indisputably vouching the efficient location of optimal formulation.

TEM studies of the optimized formulation showed uniform particle shape and a size of \(<200\) nm, which is considered imperative for ideal drug transport into the CNS.\(^{12,14}\) Stability studies indicate that storage at room temperature (i.e. \(25^\circ\)C) leads to decreased entrapment and increased particle size attributable to coalescence of the erstwhile nanoparticulate system to microparticulate one. Thus refrigeration (i.e. \(2-8^\circ\)C) was found to be the preferred storage condition for SLNs. Earlier findings have also indicated that \(4^\circ\)C is the best storage temperature for SLNs.\(^{10,14,46}\)

Experimentally, it has been demonstrated that chronic exposure to aluminium not only causes neurological signs that mimic progressive neurodegeneration but also results in neurolitamensous changes in the hippocampus, cerebral cortex, brain stem and spinal cord and biochemical changes which are seen in Alzheimer’s disease.\(^{12,15,46}\) Besides, increased concentration of aluminium in the brain has also been observed in neuritic deposits, plaques and neurofibrillary tangles in the brain in Alzheimer’s disease. It has been reported that aluminium accumulates significantly in the hippocampus following its chronic exposure. Aluminium gains access to the brain via specific high affinity receptors for transferrin that are expressed in the blood–brain barrier. Upon entering the brain it affects the slow and fast axonal transports, induces inflammatory responses, inhibits long-term potentiation and causes synaptic structural abnormalities, resulting thereby in profound memory loss. At the molecular level, aluminium influences DNA topology, gene transcription and cellular energy metabolism. It induces misfolding and self-aggregation of highly phosphorylated cytoskeletal proteins, such as neurofilaments or microtubule-associated proteins and A\(_3\)42 which are implicated in Alzheimer’s disease. Besides, induction of aluminium-induced neurotoxicity is known to be safer than other Alzheimer’s disease models, which account for higher mortality owing to the surgical intervention for intra-cerebroventricular administration of neurotoxins.\(^{12,15}\) Hence, in this investigation, it was decided to induce Alzheimer’s disease through oral administration of 100 mg/kg of aluminium chloride.

The results of the spatial navigation test and elevated plus maze paradigm suggest that aluminium chloride caused significant cognitive impairment in rats. However, chronic treatment with quercetin-loaded SLNs in aluminium chloride-treated rats was able to reverse the deleterious neurodegenerative effects of aluminium chloride. Earlier studies report improved efficacy of metal chelators in Alzheimer’s disease on being formulated as nanoparticles.\(^{14,15,46}\) Nanoparticles, having some metal-chelating activity of their own, also significantly improve the brain permeation of drugs encapsulated in their lipidic matrices. In this study too, the antioxidant potential of quercetin was significantly enhanced by formulating it as SLNs. The results are in consonance with literature where nanoparticles coated with Tween 80 have been employed for brain targeting of a variety of drugs.\(^{19,43}\) However, in this study, Tween 80 is an integral part of the formulation system. Hence, SLNs without an additional coating of Tween 80 performed better than those.
coated with the surfactant. This was because an additional coating of Tween 80 caused the particles to coalesce, thus increasing their particle size and hindering their entry into the CNS. Further, better regulation of lipid peroxidation, as signified by constant levels of malondialdehyde in rats treated with SLN-encapsulated quercetin vis-à-vis the disease (test) rats, and maintenance of glutathione and nitrite levels in the brain homogenates, ratified the application of quercetin-loaded SLNs in reversal of aluminium-induced neurotoxicity.

Conclusions
The limited permeability of many therapeutic molecules across the blood–brain barrier can be improved by employing several techniques, like administering them through the intraventricular or intrathecal route or formulating them as nanoparticulate systems. As a lot of patient non-compliance is associated with the intraventricular/intrathecal routes, this study aimed at formulating SLNs of quercetin, a potential antioxidant, to target it to the CNS in the treatment of Alzheimer’s disease. It was a Herculean task to attain the required drug entrapment, particle size, zeta potential and drug release characteristics in the formulation using blends of Compritol and Tween 80 because of the diverse nature of these polymers. Only systematic studies using DoE optimization could surmount this hiccup of balancing the response parameters. The choice of experimental design (i.e., a 2-factor CCD) was found to be highly appropriate, as it can detect any non-linearity in factor-response relationship with minimal expenditure of developmental effort and time. The optimized formulation exhibited marked behavioral improvement in memory retention in rats with aluminium-induced dementia. Besides, the maintenance of lipid peroxidation, glutathione and nitrite levels in the brain homogenates of these rats also corroborated successful targeting of quercetin-loaded SLNs into the CNS. Hence, the significant reversal of aluminium-induced neurotoxicity achieved by employing quercetin-loaded SLNs indicates the immense potential of SLNs as a platform technology to target various natural and synthetic molecules to the brain to improve their efficacy in various CNS disorders.

Declarations
Conflict of interest
The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding
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References
Development and Validation of a Spectrofluorimetric Method for the Estimation of Rivastigmine in Formulations

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Kapil et al.: Spectrofluorimetric Method for the Estimation of Rivastigmine

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A rapid, sensitive, simple, and cost-effective spectrofluorimetric method was developed for the estimation of rivastigmine in bulk and pharmaceutical formulations. The relative fluorescence intensity of rivastigmine was measured in triple distilled water at an excitation wavelength of 220 nm and an emission wavelength of 289 nm. Linearity range was found to be 100 to 4000 ng/ml. The method was validated for various parameters as per the ICH guidelines and USP requirements. The detection and quantitation limits were found to be 20.5 and 62.1 ng/ml, respectively. The results demonstrate that the procedure is accurate, precise, and reproducible, while being simple and rapid too. The results were found to be in good agreement with the label claims.

Key words: Detection limit, fluorescence spectrophotometry, fluorimetry, linearity, quantitation limit, validation

Rivastigmine has been available as the drug of choice for the symptomatic treatment of moderate to severe Alzheimer’s disease. More recently, it has been indicated in mild to moderate dementia associated with Parkinson disease too. The usage of rivastigmine has been approved in capsule and liquid form in several countries like US and UK since 1997. Rivastigmine is a dual inhibitor of acetylcholinesterase and butyrylcholinesterase. Its efficacy is dose-related, with daily oral doses ranging between 6 mg and 12 mg[5]. Owing to its numerous clinical advantages, there has been a spurt in the number of publications on rivastigmine, esp. on its formulation aspects12'41.

Increasing popularity of rivastigmine, therefore, necessitates the development of a simple analytical method for its estimation in bulk and formulations, and during dissolution runs. UV/Vis spectrophotometry, in this context, is of limited utility because of its non-specific λ_max i.e., 221 nm, a region of high spectrophotometric interference. High performance liquid chromatography (HPLC) methods utilizing UV and fluorescence detectors are reported in literature15 61 for estimation of rivastigmine in dissolution release media and in biological fluids like plasma and serum. The chromatographic techniques, however, demand a lot of time, cost and expertise in their operation.

The objective of the present study, therefore, was to develop a simple, sensitive, rapid, precise, accurate, effective and cost-effective analytical method for estimation of rivastigmine in pharmaceutical formulations and during in vitro dissolution studies of its formulations. Further, the study would embark upon the validation of the developed methodology as per the ICH guidelines[17] and USP requirements^81.

Rivastigmine hydrogen tartarate was obtained ex gratia from M/s Sun Pharma Ltd, Vadodara, India and M/s Cipla Pharma Ltd, Mumbai, India. Marketed brand (Rivamer 1.5 mg, Batch no. GK71822, Sun Pharma Ltd, Kartholi, J&K, India) was employed as the reference. All other chemicals and reagents were of analytical grade and were employed as such. All the fluorescence measurements were conducted on a spectrofluorimeter (Hitachi F 2500, Japan) equipped with a Xenon arc lamp, preloaded with a data interpreting software (FL Solutions ver. 2.0).

Various dissolution media viz. distilled water, 0.1N HCl, phosphate buffer (pH 6.8) and normal saline, alone and in combination with different organic solvents, in various proportions, were employed based on the sensitivity, ease of sample preparation, drug solubility, cost and applicability of the method employed. The relative fluorescence intensity (RFI) of rivastigmine was measured at an excitation wavelength (i.e., activating wavelength, λ_a) of 220 nm and an emission wavelength (i.e., fluorescence wavelength, λ_c) of 289 nm. Fig. 1 depicts a scan of the emission fluorescence of the drug obtained at the λ_c of 220 nm. The slit width for excitation and emission was kept as 10 nm. The photo-multiplier tube voltage was set at 700 V. Primary stock solution of 1000 μg/ml of rivastigmine hydrogen tartarate was prepared in triple distilled water (TDW). Secondary

![Fig. 1: Emission scan of rivastigmine at the excitation wavelength of 220 nm](image-url)
stock solution of 10 μg/ml of drug was prepared in TDW using aliquots of primary stock solution. For preparation of different drug concentrations, aliquots of secondary stock solution were transferred into a series of 10 ml standard flasks and volume was made up with TDW. A total of 12 different concentrations (100, 150, 200, 250, 300, 400, 500, 1000, 2000, 2500, 3000 and 4000 ng/ml) of rivastigmine were prepared for constructing a standard calibration curve and their RFI was recorded against blank.

To establish linearity of the proposed method, six separate series of solutions of the drug in the selected medium were prepared from the stock solution and analyzed. Least square linear regression analysis was conducted on the obtained spectrofluorimetric data using MS-Excel 2007 spreadsheet software. Different concentrations and their relative fluorescence intensities are shown in Table 1. At all the drug concentration levels studied, the values of standard deviation (SD < 7.8%) and the relative standard deviation (RSD < 3.9%) were found to be quite low, indicating high repeatability. The values of predicted concentrations were nearly matching with that of the nominal observed concentrations.

Linearity of the method was confirmed by plotting the ratio of response: concentration (i.e., sensitivity) vs. log of concentration. The linearity in the selected medium (TDW) was found to range between 100 and 4000 ng/ml. The graphical plot between sensitivity (response/amount) and log concentration also exhibited linearity in the said range, as depicted in fig. 2. Rivastigmine solutions (200 ng/ml) were prepared in the selected medium with and without common excipients (lactose, starch, methylcellulose, hydroxypropylmethylcellulose). All the studied solutions were scanned for their emission spectra at a fixed λmax of 220 nm and investigated for the change in emission spectrum, if any. The emission spectrum of rivastigmine was not found to alter in the presence of these common excipients in the selected medium.

The spectrophotometric method at a λmax of 221 nm, on the contrary, was found to be quite non-specific for the drug, as various excipients and solvents also absorb significantly in the said region.

To determine the accuracy of the proposed method, different quality control solutions, i.e., low (LQC: 150), medium (MQC: 500), and high (HQC: 2500 ng/ml) were prepared independently from stock solution and analyzed (n=6). Accuracy was assessed as the percentage relative error and mean percentage recovery. The magnitudes of prediction error (i.e., bias) values ranged between -0.18 and 0.16% for the three concentration levels studied (Table 2), unequivocally vouching high accuracy of the

<table>
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<tr>
<th>Drug concentration (ng/ml)</th>
<th>Mean relative fluorescence intensity (±SD)</th>
<th>% RSD</th>
<th>Predicted drug concentration (ng/ml)</th>
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<tr>
<td>100</td>
<td>47.3±0.66</td>
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<td>150</td>
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</table>
methodology employed. Further, as indicated in the table, the high mean % recovery values (nearly 100%) and the corresponding low standard deviation values (<0.98%) observed during the studies also corroborated high accuracy of the method.

Repeatability was determined using different levels of drug concentrations (as above during determination of accuracy), prepared from independent stock solution and analyzed (n=6). Inter-day variation and intra-day variation, and analyst variation were studied to determine intermediate precision of the proposed method. Different levels of drug concentrations (in triplicate) were prepared at two different times in a day and studied for intra-day variation. The identical protocol was followed on three different days to study inter-day variation (n=18). Different analysts prepared different solutions on different days. The RSD of the predicted concentrations from the regression equation was taken as the value of precision. In the repeatability study, the RSD values ranged between 0.331 and 0.983% (Table 2). At all the three studied concentration levels, precision showed satisfactory levels. Intermediate precision expresses within-laboratory variation on different days and by different analysts. Results of intermediate precision study and RSD values for each set at all the three levels are enlisted in Table 3. In all the cases, low magnitude of RSD (<1.00%) observed in the studies construe excellent repeatability and intermediate precision of the method.

The values of detection limit (DL) and quantitation limit (QL) of rivastigmine by the proposed method was calculated using the standard calibration curve as 3.3 g/5 and 10 a/5, respectively, where, 5 is the slope of the calibration curve and a is the standard deviation of the response. The values of DL and QL for rivastigmine were found to be 20.5 and 62.1 ng/ml, respectively. Evidently, this indicated excellent sensitivity of the method even at sub-microgram levels. In contrast, the values of DL and QL for spectrophotometric method were found to be quite high, i.e., 0.93 and 2.82 µg/ml, respectively.

Robustness of the proposed method was determined by changing pH of the media by ± 0.2 units and analyzing stability of drug in the selected medium at room temperature for 10 h. Three different concentrations (LQC, MQC and HQC) were prepared in different pH media and mean percentage recovery was determined. Robustness was found to be quite high, as the variation of pH of the selected media by ± 0.2 did not have any significant effect on RFI values. Mean percentage of recovery (=SD) was found to be 100.37% (±1.29). Drug solution in the selected medium exhibited no spectrofluorimetric change(s) for 10 h, when kept at room temperature.

The proposed method was also evaluated by estimation of rivastigmine in the pharmaceutical formulations. Extraction of drug from the formulation, or otherwise, was considered unnecessary; hence was not employed. Twenty capsules (Rivamer R...
1.5 mg) were weighed and emptied on a butter paper. Amount of the powder equivalent to 1.5 mg of rivastigmine was taken, dissolved in the selected medium and filtered. The solution was diluted suitably to prepare a concentration of 1.5 μg/ml of drug. This primary stock solution was filtered through Whatman” filter paper and the filtrate was further diluted to prepare a solution of 150 ng/ml of rivastigmine. The RFI value of the solution, thus prepared, was observed to estimate the total rivastigmine content in the formulation. The assay values of three samples of Rivamer” capsules ranged between 98.23 and 101.76%. Assay values (1.47-1.52 mg) of formulations were found to be quite close to the label claim of 1.5 mg. This corroborated that the interference of excipient matrix is insignificant in the estimation of rivastigmine by the proposed method. The whole process of assay involved the expenditure of minimal time (sparing a few min) and money. In contrast, HPLC procedures for the routine drug analysis require a great deal of developmental effort and expenditure in terms of solvents, columns, guard columns, productive and non-productive time etc. Hence, the spectrofluorimetric method vouches its undisputed efficacy, both in terms of time and cost.

In a nutshell, the proposed method was found to be sensitive, simple, rapid, accurate, precise and inexpensive for routine analysis of rivastigmine in bulk, pharmaceutical formulations and during dissolution studies of oral formulations. The sample recoveries in all the investigated formulations were in good agreement with their respective label claims, indicating non-interference of excipients during the spectrofluorimetric estimation of drug.

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A simple, effective and cost-effective method for preparing mucoadhesive films

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INTRODUCTION

Mucoadhesive films have lately gained immense attention owing to their widespread use in sublingual (1), ophthalmic (2), vaginal (3), periodontal (4), gastrointestinal (5), transdermal (6) and most importantly, buccal (7, 8) drug delivery. Such films have several unique merits over other bioadhesive systems like tablets and gels (9, 10). Mucoadhesive films are generally preferred over mucoadhesive tablets because of the flexibility and comfort associated with the former. In addition, they can circumvent the relatively short residence time of the mucosal gels, which are easily washed away and removed by body exudations like saliva, tears or vaginal secretions. Moreover, the films also help in protecting the wound surface, thus helping to reduce pain and treat the disease more effectively, esp. during local delivery (9).

Formulation procedure of these bioadhesive films invariably encompasses the preparation of hydrogels employing polymers like sodium carboxymethylcellulose (CMC), caromers or hydroxypropylmethylcellulose (HPMC). These hydrogels are subsequently dried to form thin mucoadhesive films usually by pouring into petri-dishes (11) or using film-moulds (12). However, the former method seldom guarantees the uniformity of film thickness while the later is an expensive technique requiring specialized mould design. Herein, we present simple, effective, time-effective and cost-efficient and reproducible method of preparing uniform-thickness mucoadhesive films from polymeric hydrogels.

MATERIALS

Microscopic glass slides were purchased from M/s SD Fine Chemicals (Mumbai) and soda-lime glass plates were procured from a local vendor. A standard acrylic adhesive, Fevikwik® (Pidilite Industries, Mumbai, B No. 8907H) was procured from a local vendor. The polymers,
Buccoadhesive Films for Once-a-day Administration of Rivastigmine: Systematic Formulation Development and Pharmacokinetic Evaluation

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ABSTRACT
Context: Rivastigmine, an anti-Alzheimer’s drug, suffers from major predicaments like low oral bioavailability, severe GI adverse effects related to rapid fluctuations in drug plasma levels, and high frequency of dosing.

Objective: The present investigation aims at developing buccoadhesive films capable of delivering the drug in vivo in a sustained manner. Augmentation of drug bioavailability by the avoidance of first-pass effect through the buccal route and reduction in GI side effects would be other key advantages of this system.

Methods: Buccoadhesive films of rivastigmine were systematically designed and evaluated for in vitro drug release, ex vivo buccal permeation and ex vivo buccoadhesive strength. Optimal composition of the polymer blends was rationally chosen using a central composite design and overlay plot. In vivo pharmacokinetic studies were carried out in rabbits, and attempts were made to establish in vitro/ in vivo correlations (IVIVC).

Results: Besides possessing the requisite drug release regulation, the optimized formulation exhibited excellent buccoadhesion, and buccal permeation. Pharmacokinetic studies indicated extension of plasma drug levels and level A of IVIVC was successfully established.

Discussion: Excellent buccal bioadhesion and transmucosal permeation, coupled with drug release control, ratify the potential of the optimized formulation to deliver the drug in a controlled and site-specific manner. Successful establishment of IVIVC substantiated the judicious choice of in vitro dissolution media for simulating the in vivo conditions.

Conclusion: Besides unraveling the polymer synergism, the study helped in developing an optimal once-a-day buccoadhesive drug delivery system exhibiting excellent trans-buccal permeation and buccoadhesive characteristics with improved bioavailability potential.
ABSTRACT

Rivastigmine, a drug extensively prescribed for treatment of Alzheimer’s disease, suffers from major predicaments like high frequency of dosing and severe GI adverse effects related to rapid rise and fall in drug plasma levels. The present investigation, therefore, aimed at developing GI retentive floating-bioadhesive formulation for delivering rivastigmine in a sustained manner at the desired site of absorption. Effervescent floating-bioadhesive hydrophilic matrices were systematically formulated using CCD and evaluated for in vitro drug release, floatation and ex vivo bioadhesive strength. Optimal composition of polymer blends systematically chosen using brute-force methodology, overlay plots and desirability function exhibited excellent bioadhesive and floatational characteristics besides possessing adequate drug release control. Pharmacokinetic studies were carried out in rabbits, and various levels of IVIVC were established. In vivo gamma scintigraphic studies in human volunteers ratified the gastroretentive characteristics of the optimized formulation with retention time of 5 h or more. Thus, besides unraveling the polymer synergism, the study helped in developing an optimal once-a-day gastrointestinal retentive drug delivery system exhibiting excellent swelling, floating, and bioadhesive characteristics.
Developing oral drug delivery systems using formulation by design: vital precepts, retrospect and prospects

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Introduction: Over the past few decades, the domain of drug formulations has metamorphosed from the conventional tablets and capsules to advanced and intricate drug delivery systems (DDS), both temporal and spatial. Formulation development of the oral DDS, accordingly, cannot be adequately accomplished using the traditional 'trial and error' approaches of one variable at a time. This calls for the adoption of rational, systematized, efficient and cost-efficient strategies using 'design of experiments (DoE)'. The recent regulatory guidelines issued by the key federal agencies to practice 'quality by design (QbD)' paradigms have coerced researchers in industrial milieu, in particular, to use experimental designs during drug product development.

Areas covered: This review article describes these principles of DoE and QbD as applicable to drug delivery development using a more apt expression, that is, 'formulation by design (FbD)'. The manuscript describes the overall FbD methodology along with a summary of various experimental designs and their application in formulating oral DDS. The article also acts as a ready reckoner for FbD terminologies and methodologies. Select literature and an extensive FbD case study have been included to provide the reader with a comprehensive portrayal of the FbD precept.

Expert opinion: FbD is a holistic concept of formulation development aiming to design more efficacious, safe, economical and patient-compliant DDS. With the recent regulatory quality initiatives, implementation of FbD has now become an integral part of drug industry and academic research.

Keywords: design of experiments, drug delivery, experimental design, product development, quality by design, response surface methodology, systematic optimization

Expert Opin. Drug Deliv. [Early Online]

1. Introduction

In an endeavor to combat various pathological states, drugs have been administered through various possible routes. Oral intake, amongst these routes, has unambiguously been the most sought after by the patients and manufacturers alike ⁷. Development of an effective oral drug delivery system (DDS), however, invariably involves rational blending of diverse functional and non-functional polymers and excipients. Optimizing the formulation composition and the manufacturing process of such a drug delivery product to furnish the desired quality traits is, therefore, a Herculean task. The traditional approach of optimizing a formulation or process essentially involves studying the influence of one variable at a time (OVAT), while keeping all others as constant. Using this OVAT approach, the solution of a specific

Chapter 1


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**Abstract**

Invariably, the design of an effective and cost-effective oral drug delivery system (DDS) aims at delivering the drug over extended periods of time to a chronically ill patient. Paralleled with aggressive research in the domain of extended release (ER) oral DDS, its industrial demand has been escalating exponentially with an alarming number of marketed product launches lately. The concept of ER formulation involves drug release from a polymeric material in a precise and engineered manner. This drug release is usually controlled by several physical processes, including polymer permeation by dissolution media, diffusion of dissolved drug from the matrix and erosion of the matrix material. Essential to the development of a successful ER DDS, therefore, is the comprehension of drug release kinetics as influenced by several physical, chemical and geometrical parameters of the formulation device. The quantitative interpretation of the drug dissolution profile is facilitated by a set of generic equations which mathematically translate the profile as a function of these multiple parameters. The quintessence of ER drug delivery to yield an impeccable therapeutic effect, accordingly, is modulation and modeling of its drug release kinetic potential. Mathematical modeling, coupled with numerical simulation, provides useful guidance and insight to the pharmaceutical product development scientist on ER, largely reducing the experimentation needed to obtain the desired drug release profiles. In the last a few decades, a number of mathematical models have been hypothesized by various researchers to analyze drug release.

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INTRODUCTION

The endeavor to target the drug molecules effectively to apt receptor sites, and consequently, elicit the desired therapeutic effect, has led to the emergence of diverse routes of drug delivery. Despite the options of such routes, oral intake has unambiguously been the most sought after by the patients and manufacturers alike. From the patient perspectives, its status is primarily a consequent of the wide acceptability of this “natural” route, better safety, low cost of therapy, ease and convenience of this non-invasive method. Besides, the simple, flexible and inexpensive development and manufacturing of oral drug products, coupled with the unproblematic availability of raw materials, equipment and technology, and simple regulatory approval account for their popularity amongst the manufacturers too. Accordingly, despite tremendous strides made in novel non-oral drug delivery systems, more than one-half of the drug formulations available commercially today, across the globe, are the oral ones.

Development of an oral formulation usually starts with the establishment of a theoretical drug release profile for the formulation based on desirable target blood concentration. Based upon the previous experience and the information available in the literature, various prototype formulations utilizing different excipients and processes are developed, and are characterized for drug pharmacokinetics. The drug release profiles are further analyzed to compute the rate and extent of absorption of drug.
INTRODUCTION

Design and development of an immaculate drug product or pharmaceutical process usually involves multiple objectives under its ambit. For decades, this task has been endeavored through trial and error, supplemented by the previous experience, knowledge and wisdom of the formulator. A product development scientist, accordingly, always used to remain in a dynamic environment, taking drug delivery challenges in stride time and again. These challenges arose invariably as a result of escalating competitiveness among manufacturers to improve efficacy and cost-effectiveness of products, rapidly changing compendial and regulatory specifications for drug delivery devices, and increasing quality consciousness among physicians as well as patients. Furthermore, while optimizing such formulations, there was always a constraint on time, resources, and materials. Hence, it was important for a pharmaceutical scientist to use effective methodology to develop products in a timely manner without sacrificing quality. Development and modification of a formulation was carried out by the analysis of its composition and influence of process factors on dosage form characteristics, changing any one single factor at a time (COST).

Using this COST approach, the solution of a specific problematic property could be achieved, but attainment of the true optimum composition or process could never be guaranteed (Schwartz et al., 1973; Doornbos and Haan, 1995). However, despite applying the best knowledge, skills, and wisdom to achieve the said goal, the outcome was not easily ascertainable. The formulator could either hit the bull’s eye quickly or miss the target altogether even after arduous workouts. The final product would, thus, be satisfactory but sub-optimal, as a better formulation might still exist for the studied conditions (Araujo and Brereton, 1996; Singh et al., 2005c). This conventional COST approach of drug formulation development suffers from several pitfalls, as enumerated in Box 1.
Alzheimer's Disease: 
Pathology and Treatment Strategies

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Human brain is a complex organ responsible for directing almost every activity of human body. Apart from regulating basic life-sustaining functions like respiration, excretion, etc, brain is responsible for speech, behavior and memory also (Figure 1).

Figure 1: The human brain

Alzheimer’s disease (AD) is a progressive and degenerative disorder of the CNS that ultimately results in the loss of cognitive function. It is a major public health issue affecting one in every ten persons over the age of 65. Particularly, in the group of individuals over 85, one in every three tends to suffer from dementia. As our aging population is expanding rapidly, the financial and emotional toll of AD continues to grow. Clinically, AD is characterized by a progressive mental deterioration and significant changes in personality and thought depending upon the speed of the intellectual decay.

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Pathology of AD

Alois Alzheimer, in 1907, described a “peculiar disease of the cortex”, on the basis of “strange alterations of the neurofibrils” and “foci which are build up by a peculiar
Abstract

Being the most delicate organ of the body, human brain is protected by a Blood-Brain Barrier (BBB) which restricts the entry of most medicinal agents and potentially toxic substances into the brain. Nevertheless, the process for new drug development for the treatment of Central Nervous System (CNS) disorders has not kept pace with the progress in molecular neurosciences, as most of the new drugs discovered are unable to cross the BBB effectively. Accordingly, the scientists across the globe are lately engaged in developing newer strategies to deliver drugs to CNS for the treatment of various threatening brain disorders. A few of such approaches have indeed been found to have immense potential in trespassing BBB, eventually to bio-distribute to the desired CNS sites. The current review article presents a brief outline of the anatomy and physiology of BBB in general, and diverse strategies utilized to deliver drugs to the CNS, in particular.