RESEARCH ARTICLE

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Solubility Enhancement of Candesartan by Polyamidoamine Dendrimers

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Abstracts: The present study was aimed for solubility enhancement of candesartan (CND) by dendrimers. The aqueous solubility of CND was measured in the presence of dendrimers in distilled water. The effect of variables (pH condition, concentration of dendrimer, temperature) has been studied via implementation of Box-Behken design of experiment. The order in which the dendrimers increased the solubility at a constant pH condition was G3 > G0. Results revealed that the solubility of CND in the dendrimer solutions was proportional to dendrimer concentration. The influence of dendrimer solution pH on the solubility enhancement of CND suggests that it involves an electrostatic interaction between the carboxyl group of the CND molecule and the amine groups of the dendrimer molecule. The solubility of CND was inversely proportional to the temperature under which the experiment was performed. Polyamidoamine (PAMAM) dendrimers have the potential to significantly enhance the solubility of poor water soluble drugs, CND.

INTRODUCTION

Drug delivery systems have developed the medicine by significantly improving the therapeutic efficacy and minimizing the side effect of various drugs. [1-3] Unique structures and properties of dendrimers have generated auspicious new platforms for drug delivery. A typical dendrimer consists of three basic components: a central core from which the polymeric branches emanate; repeat units that determine microenvironment of the interior and in turn solubilization ability of dendrimer; and terminal surface groups which are responsible for performance of dendrimers in solution. Polyamidoamine (PAMAM) dendrimers (Figure 1) are founded on an ethylenediamine core (C), branched units (B) and constructed from methyl acrylate and ethylenediamine with surface amine group (S). [4] Moreover, PAMAM dendrimers are biocompatible, non-immunogenic and water-soluble which makes them appropriate for drug delivery systems. [5-7] Duncan and co-workers [8] have successfully prepared conjugates of PAMAM dendrimers with cisplatin, a potent anticancer drug to improve nonspecific toxicity and poor water solubility. Candesartan (CND) (Figure 2) is an angiotensin type I receptor blocker antihypertensive agent. Low water solubility (0.00771 $g \cdot L^{-1}$) and high hydrophobicity (log P 6.1) of CND are might be the reason for their poor oral bioavailability (15%). In light of these, the present investigation was carried out to increase the solubility of hydrophobic drug, CND, by PAMAM dendrimers with simultaneous implementation of Box-Behken design of experiment to evaluate influence of variables like pH, concentration and temperature on saturation solubility.

MATERIAL AND METHODS

Materials

Candesartan was obtained as a gift sample from Torrent Research Center (Gandhinagar, India). Generation 0 (G0) and Generation 3 (G3) polyamidoamine (PAMAM) dendrimer were kindly gifted from Dendritech Inc. (USA). Double distilled water was used throughout the study. Other ingredients used were of analytical grade and were used as such without further modifications.

Methods

Selection of Dendrimer Generation

In order to study the effect of dendrimer generation on drug complexibility, G0 and G3 were utilized. The basic difference between these two generations is total no. of functional group attached to the core moiety. Dendrimers generation refers to the number of repetitive branching cycles that are accomplished during its production. Each consecutive generation results in a dendrimer approximately double the molecular weight of the prior generation. If the branching reactions are executed onto the core molecule three times, the resulting dendrimer is considered a third generation dendrimer (G3). Briefly, an excess amount of drug was added to various concentrations of each dendrimer generation at constant temperature 25°C and pH 7 followed by subsequent incubation in an orbital shaking incubator (Remi Labs, India) for 24 h. Aliquots were filtered through a 0.45 µm cellulose acetate filters (Sartorious Inc., US) and consequently analyzed for CND content at 297 nm using UV-VIS spectrophotometer (Shimadzu 1700, Japan) by a previously validated UV method ($R^2 = 0.9957$).

Experimental Design

The present study was conducted as per Box-Behken design of experiment. In this design, three factors (pH, concentration and temperature) with three levels were probed to investigate the main effects and interaction of the two factors on a single response (saturation solubility) (Table 1). The main purpose of the replication runs was to increase the precision and to minimize experimental error [9]. A third order quadratic model incorporating interactive and polynomial terms was used to evaluate the response.

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (1)$$

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Table 1: Variables and their Levels

Coded Values	Actual Values		
	X_1[a]	X_2[b]	X_3[c]
-1	4	5	25
0	7	7.5	32.5
1	10	10	40

[a]X_1 - pH, [b]X_2 - concentration of dendrimer (mg·mL^{-1}), [c]X_3 - temperature (°C)

Table 2: Composition of Box-Behken Design Batches

Batch	Variable Levels in Coded Form			Saturation Solubility (mg·mL^{-1}) ± SD (n=3)
	X_1[a]	X_2[b]	X_3[c]	
CD1	-1	-1	0	11.4 ± 1.43
CD2	-1	0	-1	13.5 ± 1.23
CD3	-1	0	1	12.9 ± 2.23
CD4	-1	1	0	14.2 ± 2.45
CD5	0	-1	-1	14.9 ± 3.01
CD6	0	-1	1	14.1 ± 0.45
CD7	0	1	-1	18.1 ± 3.56
CD8	0	1	1	17.4 ± 3.92
CD9	1	0	-1	21.6 ± 3.41
CD10	1	0	1	19.2 ± 0.45
CD11	1	1	0	22.1 ± 1.54
CD12	1	-1	0	18.8 ± 2.43
CD13	0	0	0	15.4 ± 1.64
CD14	0	0	0	15.3± 0.47
CD15	0	0	0	15.8± 0.93

[a]X_1 - pH, [b]X_2 - concentration of dendrimer (mg·mL^{-1}), [c]X_3 - temperature (°C)

Table 3: Testing the Model in Portions

		for Saturation Solubility			F_{cal} = 5.67 F_{tab} = 8.94
Regression	DF[a]	SS[b]	MS[c]	R^2	DF = (6,3)
FM[d]	10	135.64	13.56	0.9970	
RM[e]	4	242.46	121.23	0.9870	
Residual					
FM	3	0.40	0.13		
RM	8	1.76	0.22		

[a] DF - degree of freedom, [b] SS - sum of squares, [c] MS - mean of squares, [d] FM - full model, [e] RM - reduced model

Where, Y_i was the dependent variable, b_0 was arithmetic mean response of the thirteen runs and b_i was the estimated coefficient for factor X_i. The main effects (X_1, X_2 and X_3) represent average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3 and X_1X_3) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2, X_2^2 and X_3^2) were included to investigate nonlinearity. Data was further analyzed by MICROSOFT EXCEL® 2007 for regression analysis. Analysis of variance (ANOVA) was implemented to assure no significant difference between developed full model and reduced model. Contour plots were plotted to study response variations against two independent variables using Design Expert® 7.1.6 (STAT EASE) demo version software.

RESULTS AND DISCUSSIONS

The high density of amino groups and special structure of PAMAM dendrimers may be anticipated to have potential applications in enhancing the solubility of low water soluble drugs. Drugs like nifedipine [10], ibuprofen [11], nicotinic acid [12], ketoprofen [13] and furosemide [14] has been already been investigated with dendrimer for improvement in solubility.

Selection of Dendrimer Generation

Higher generation dendrimers also have more unprotected functional groups on the surface, which can be used to tailor the dendrimer for a given application. Figure 3 clearly demonstrates that G3 was able to solubilize more amount of CND as compared to G0. This might be attribute to larger surface area and more functional groups provided by G3. Hence, G3 was selected as optimized generation for further investigation.

Experimental Design

Experimental design is widely exercised for controlling the effects of parameters in many processes. Its usage reduces number of experiments, using time and material resources. Moreover, the analysis performed on the results is easily

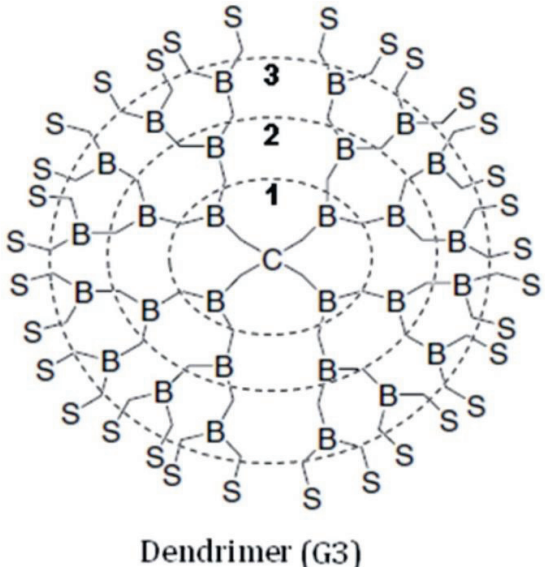

Figure 1: Schematic diagram of PAMAM G3 dendrimer, G1 (1), G2 (2), G3 (3), ethylenediamine core (C), branched units (B) and surface amine group (S)

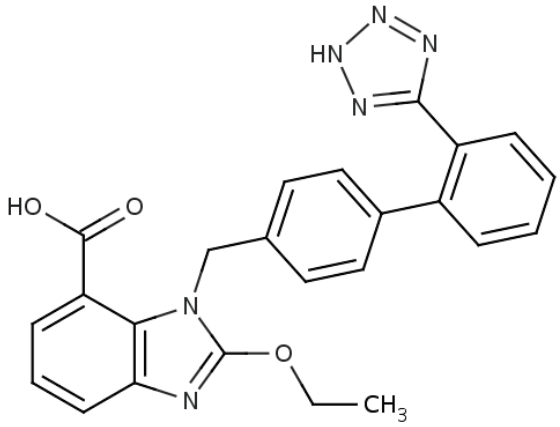

Figure 2: Structure of Candesartan

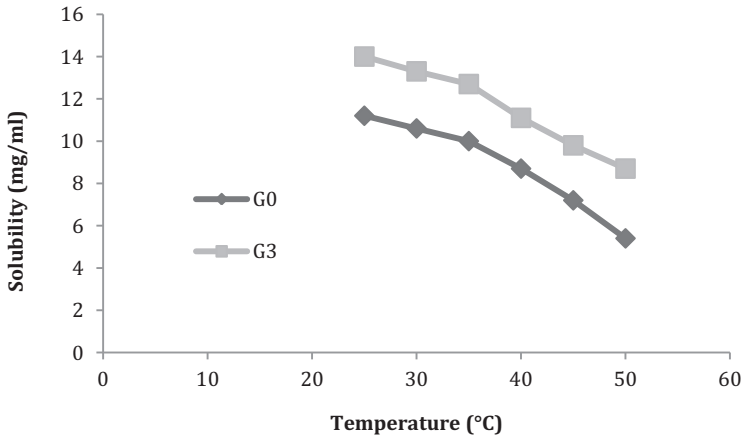

Figure 3: Effect of dendrimer generations on solubility of CND

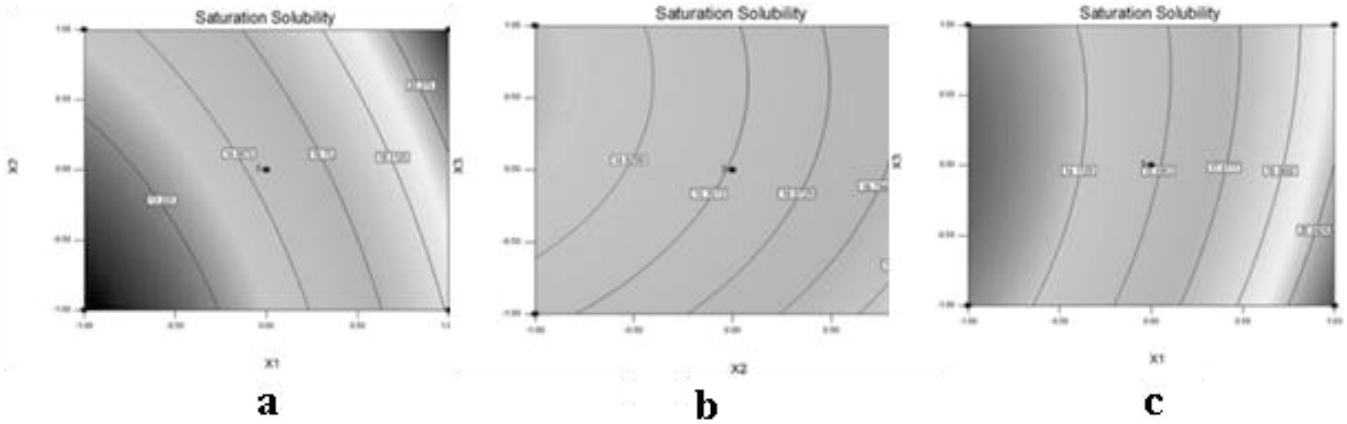

Figure 4: Contour plot of pH (X_1) and concentration of dendrimer (X_2) on the saturation solubility of CND (a), contour plot of concentration of dendrimer (X_2) and temperature (X_3) (b), contour plot of pH (X_1) and temperature (X_3) on saturation solubility of CND

understood and experimental errors are minimized. Statistical methods measure the effects of alteration in operating variables and their mutual inter-actions on process by means of experimental design way. Preliminary investigations of the process parameters revealed that factors like pH (X_1), concentration of dendrimer (X_2) and temperature (X_3) had significantly influenced saturation solubility of CND. The results of saturation solubility for 15 batches (CD1 to CD15) displayed a wide variation (11.4 to 22.1 mg·ml^{-1}) (Table

I). The data clearly indicates that all three factors (concentration of dendrimer (X_2) and temperature (X_3)) strongly influence the selected response (saturation solubility). The fitted polynomial equations (full and reduced model) relating to the response, saturation solubility are shown in below.

Full model polynomial equation:

$$y = 15.4 + 3.71X_1 + 1.57X_2 - 0.56X_3 + 0.95X_1^2 + 0.27X_2^2 + 0.45X_3^2 + 0.12X_1X_2 + 0.02X_2X_3 - 0.45X_1X_3 \quad (2)$$

Reduced model polynomial equation:

$$y = 15.9 + 3.71X_1 + 1.57X_2 - 0.56X_3 + 0.73X_1^2 \quad (3)$$

The polynomial equations can be utilized to draw conclusions after considering magnitude of coefficients and mathematical sign (positive or negative). The coefficients b_1, b_2, b_3 and b_{11} were found to be significant ($P<0.05$) and thus, they were retained in the reduced model. Table III shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors. The high values of correlation coefficients (R^2) for saturation solubility indicate a good fit. The critical value of F for saturation solubility was at $\alpha = 0.05$ which was equal to 8.94 (DF = 6, 3). Since the calculated value (F = 5.67) was less than critical value (F = 8.94) hence, it may be concluded that the interaction term b_{12}, b_{23}, b_{13} and the nonlinearity terms b_{22} and b_{33} not contribute significantly to predict saturation solubility and hence can be omitted from the full model. The change in saturation solubility as a function of X_1, X_2 and X_3 is depicted in the form of counter plot (Figure 4) based on Box-Behken experimental design.

High levels of both X_1 and X_2 and low levels of X_3 were found to be favorable for higher saturation solubility. Multiple linear regression analysis revealed that coefficients b_1 and b_2 are positive and b_3 is negative. This indicates that as pH (X_1) and concentration of dendrimer (X_2) increased, saturation solubility increased which was not the case for temperature (X_3) since with increase in it saturation solubility decreased.

Effect of pH Condition on the Solubility of CND in PAMAM Dendrimer Solutions

Solubility profiles of CND measured in the presence of PAMAM third generation dendrimers at pH 4, 7, and 10. The solubility of CND was found to be pH dependent. The solubility of CND in PAMAM dendrimer solutions was highest at pH 10, lower at pH 7, and lowest at pH 4. The improvement in solubility is due to of an electrostatically interaction between the surface amine groups of dendrimer molecule and the carboxyl group of CND. Evidence for this is seen from the solubility of CND in dendrimer solution over a range of pH values (Figure 4). CND, a weakly acidic molecule (pK_a 8.15) will not fully ionized at low pH value and hence cannot freely interact electrostatically with the dendrimer molecule. The highest solubility of CND in dendrimer solutions at high pH was because of complete ionization of CND at pH 10, as a result, CND freely interact electrostatically with the dendrimer molecule.

Effect of PAMAM Dendrimer Concentration and Temperature on the Solubility of CND

In the presence of PAMAM dendrimer solubility of CND in the dendrimer solutions increased in an almost linear manner with an increase in concentration of PAMAM dendrimer value (Figure 4). This was most probably due to the increase in the number of surface amines group and internal cavities that are available to interact with CND molecules. Owing to this specific and interesting property of PAMAM dendrimers, the cavities in PAMAM dendrimers can keep small guest molecules inside and make dendrimers suitable for enhancing the solubility of hydrophobic drug molecules such as CND in aqueous solutions. Also, there are tertiary amines in these internal cavities, which could interact with the atoms of the CND molecules by hydrogen bond formation. Moreover, PAMAM dendrimers have primary amines on the surface, which could interact electrostatically with the carboxyl group in the CND molecules. The results of third critical factor (temperature) were totally opposite than the other two factors (concentration and pH) (Figure 4). The probable reason for this is inability of dendrimer to form a complex with drug at higher temperature.

CONCLUSIONS

Different generations (G0 and G3) of PAMAM dendrimers have the potential to significantly enhance the solubility of poorly water-soluble drugs such as candesartan. The drug solubility was found to be increased with pH of the solution and concentration of the dendrimer while it was decreased at high temperature. Both observations are evidence of interactions between the surface amine groups of dendrimer molecule and the carboxyl group of candesartan. Further, extensive *in-vivo* studies are required to conform enhancement in bioavailability.

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